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October 10-13, Kemer, Antalya, Türkiye

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Welcome to the Turkish & Italian Joint International Animal Reproduction Congress

We are delighted to present the **Book of Abstracts** for the **TIAR-2024 Turkish-Italian Joint International Congress**, held from October 10-13, 2024. This prestigious event brought together leading scientists and researchers from around the world, showcasing 73 oral presentations, 47 poster presentations, and 15 invited talks, 16 of which were delivered by young researchers. This congress fostered a vibrant environment for knowledge exchange, inspiring new collaborations and breakthroughs in animal reproduction science.

Researchers from 14 different countries gathered in the historic surroundings of Kemer, contributing to the rich and expanding literature in animal reproduction. Young researchers had the opportunity to meet, share their scientific perspectives, and spark new research ideas. The congress concluded with hope and anticipation for the next meeting in Messina, Italy, for TIAR-2025. We are honored to have hosted this dynamic and knowledge-filled congress, and we extend our sincere gratitude to all participants, our organizing team, and sponsors.

The high-quality research presented at this congress will form the foundation of a **Special Issue in Frontiers in Veterinary Science**, providing a comprehensive overview of the latest advancements and future directions in animal reproduction science. This Special Issue will showcase innovative and impactful research in areas such as companion animal reproduction, food animal reproduction, equine reproduction, reproductive biotechnologies, and herd health management.

We invite researchers to submit their original research articles or review papers to this Special Issue. We welcome submissions covering clinically relevant reproductive physiology and pathology, new gamete preservation technologies, advancements in reproductive management for small and large animals, and broader topics such as innovative teaching techniques in reproduction. Researchers who could not attend the congress are also encouraged to contribute their most significant findings to this collection.

Please submit your manuscripts via Frontiers in Veterinary Science.

We look forward to your valuable contributions and to advancing the field of animal reproduction together.

With sincere love and respect,

The TIAR-2024 Committee

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Advancing induced anhydrobiosis for cell and gamete preservation

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Citation
Palazzese, L., Loi, P. Advancing induced anhydrobiosis for cell and gamete preservation.

Abstract
The freeze-drying of rooster sperm was first described alongside cryopreservation, but while cryopreservation became the standard for cell and gamete storage, freeze-drying stagnated. Recent research has renewed interest in non-cryogenic storage methods, especially after demonstrating the full fertility of freeze-dried mouse sperm. Eliminating liquid nitrogen (LN) for preservation would dramatically reduce costs and the environmental impact. Funding agencies are now supporting this multidisciplinary field involving engineers, biologists, and physicists. Over the past decade, significant progress has been made in water elimination techniques, including alternatives to freeze-drying, using molecules from anhydrobiotic organisms like tardigrades. These advancements have enabled the controlled dehydration of sperm from livestock and endangered species, as well as somatic cells like fibroblasts. This review highlights these breakthroughs, emphasizing their potential for biodiversity conservation, assisted human reproduction, and even space travel.

Advancing Induced Anhydrobiosis for Cell and Gamete Preservation

The freeze-drying of rooster sperm, explored initially with cryopreservation, eventually stagnated as cryopreservation became the standard for gamete storage. Recently, interest in non-cryogenic storage methods has revived, especially following the demonstration of full fertility in freeze-dried mouse sperm. Avoiding liquid nitrogen (LN) for preservation would significantly reduce costs and environmental impact, making preservation more accessible. This multidisciplinary field now attracts funding from agencies supporting engineers, biologists, and physicists working together. Over the past decade, advances in water elimination techniques, inspired by molecules from anhydrobiotic organisms like tardigrades, have allowed for the controlled dehydration of livestock sperm and somatic cells, such as fibroblasts. This review examines these breakthroughs and their implications for biodiversity conservation, human reproduction, and even space travel.

Cryopreservation Alternatives and Water Extraction

Long-term preservation of viable biomaterials remains vital across scientific fields, with rising interest in conserving germplasms (sperm, oocytes, cells) for applications in human reproductive medicine, livestock production, and wild species conservation. Effective storage methods for eukaryotic cells emerged in the early 20th century, focusing on freezing, vitrification, and freeze-drying (lyophilization). While lyophilization faced structural integrity challenges, it remains a key area of interest [1]. Freezing methods evolved with the advent of mechanical freezer technology and LN availability, making cryobiobanking a widely-used, well-mastered technique. However, the cost, energy consumption, and carbon footprint of these methods restrict their use primarily to affluent countries with adequate LN production and energy infrastructure [2]. Many developing countries lack access to these techniques.

A breakthrough came in 1998 with the successful birth of offspring from freeze-dried mouse sperm, showcasing an alternative to conventional freezing [3]. While freeze-dried sperm are nonviable and immobile upon rehydration, the issue of motility loss was addressed through Intracytoplasmic Sperm Injection (ICSI), demonstrating that nuclear and cellular viability need not align. Despite this initial success, subsequent progress was limited, with only sporadic follow-up reports [4].

Water removal remains challenging as it often damages cell membranes and chromatin. Early experiments with anucleate cells, like platelets (which lack nuclei), provided proof of principle by simplifying structural preservation [5]. The complex challenge of dehydrating mammalian cells has deterred funding, requiring interdisciplinary collaboration beyond embryologists and involving engineers and physicists to develop effective methods [6]. After promising results with somatic cells, the first offspring cloned from freeze-dried mouse somatic cells was born in 2022 [7]. Mouse models, valued for reliability and efficiency, enabled rapid testing, with the technique involving embryonic stem cell (ESC) lines created from blastocysts cloned from freeze-dried somatic cells. These ESCs served as donor nuclei for Somatic Cell Nuclear Transfer (SCNT) [7]. However, reliance on ESC derivation restricts the method's use beyond mice, as native ESCs remain largely experimental in species such as pigs, sheep, and cattle [8].

European funding agencies are now supporting this interdisciplinary field, fostering collaboration among biologists, embryologists, engineers, physicists, and computational chemists. Small mammals, such as mice [3], rats [9], and hamsters [10], have produced offspring from freeze-dried sperm, but in large mammals (cattle [11], pigs [12], sheep [13]), embryo development stops at the pre-implantation stage. Nevertheless, the multidisciplinary approach has helped clarify preservation challenges in large mammals.

For freeze-drying to truly replace LN usage, it must eliminate LN entirely. Conventionally, sperm samples were frozen directly in LN before vacuum initiation [14]. However, milder freezing conditions (-50°C) can sufficiently preserve freeze-dried sperm in optimal condition [15]. Freeze-drying media commonly include calcium chelators like EDTA and EGTA to inhibit endonucleases [16]. Trehalose has further improved DNA integrity preservation, notably in ram [15] and stallion [17] sperm, and expanded freeze-drying's applicability to cats. Cat sperm, microwave-dried with trehalose, sustains in vitro development to the morula stage even after international shipping [18]. Trehalose also aids dehydration of cat oocyte germinal vesicles (GVs), suggesting potential for oocyte preservation at 11% relative humidity [19].

Studies recommend preserving freeze-dried mouse sperm at -20°C or -80°C [20]. Alternative methods, like spin-dry or vacuum-dry encapsulation, show similar blastocyst formation rates for samples stored at room temperature and 4°C, but research underscores the need for species-specific methods as findings in mice may not transfer to other mammals [13].

Multidisciplinary Advances and Strategies

The Pv11 animal cell line from *Polypedilum vanderplanki*, known for extreme desiccation tolerance, exemplifies anhydrobiosis triggered by elevated trehalose levels [21]. Molecules found in Pv11 cells, including late embryogenesis abundant (LEA) proteins [22] and trehalose transport channels, offer innovative strategies for cryopreserving mammalian sperm. Trehalose, typically unable to permeate cell membranes, enters cells via trehalose transporter 1 (TRET1) [23]. Transient expression of the TRET gene in sperm, via mRNA transfection, potentially enhances dehydration tolerance. Screening identified gene g4064, responsible for trehalose transport, enabling trehalose incorporation into cytoplasm [25]. This approach may facilitate dehydration in somatic cells and sperm with exogenous mRNA vectors, enhancing anhydrobiosis tolerance [21].

Another area of research includes the development of custom freeze-dryers for sperm preservation. Laboratories currently use equipment designed for drug production, but spin-dry methods [13] have shown gentler preservation results, prompting interest in species-specific freeze-dryers.

Infertility care disparities persist between developed and developing countries due to access and socio-cultural differences. Despite IVF advances, accessibility remains limited, with LN scarcity in regions like northern Africa further restricting reproductive care [26,27]. Freeze-drying sperm offers an alternative but still necessitates phenotypic assessments, follow-up studies, and molecular analyses like transcriptomic and methylome profiling to ensure safety and efficacy in clinical use.

Conclusion

WHO data indicate over 180 million couples in developing countries face infertility, with limited access to fertility treatments [28]. Freeze-drying sperm, though requiring ICSI for fertilization, could make IVF more accessible, particularly as ART technologies have seen minimal use in wildlife conservation, except in cases like the black-footed ferret and giant panda [4]. Integrating lyophilization with cryopreservation offers promising storage alternatives, though more research is needed on rehydration processes. Freeze-dried spermatozoa preserved in an anhydrous state demonstrate exceptional DNA stability, withstanding extreme conditions, including high radiation [30]. Mouse sperm, freeze-dried and stored on the ISS for nine months at -95°C, showed minor DNA damage yet similar fertility rates as ground-stored controls, indicating space radiation tolerance [30].

Future research on space-preserved sperm may pave the way for lunar storage, akin to the Svalbard Global Seed Vault, potentially in Moon lava tubes shielded from radiation and temperature extremes [33]. As advances extend preservation from months to years [3, 13, 31], freeze-drying may ultimately outpace traditional cryopreservation for long-term storage, with tardigrade-inspired methods continuing to set new benchmarks [32].

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Veterinary reproductive management and AI of dairy cattle – From science to solutions

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Mee, J.F. Veterinary reproductive management and AI of dairy cattle – from science to solutions.

Introduction

Veterinary reproductive management and AI of dairy cattle has played a key role in improving herd fertility (Mee, 2007). This role is highly varied across dairy industries internationally. Veterinary practitioners may operate as a farmer-employed vet, a milk cooperative-employed vet, a pharmaceutical company-employed vet or, more commonly, as a private veterinary practitioner (PVP). The role of each of these vets differs in relation to improving herd fertility. The farmer-employed vet has the greatest impact as they are much more involved in all aspects of herd fertility management from AI-ing cows right through to calving cows (Mee, 2004a). A milk cooperative-employed vet or a pharmaceutical company-employed vet acts in a consultative role to the farm-employed or private vet, but is not involved in day-to-day reproductive management on the dairy farm. However, when a problem of poor reproductive performance is reported they are called in to trouble-shoot a solution. The private vet may interact with and drive the farm reproductive management programme through development of standard

operating procedures (SOP), ad hoc or routine postpartum, pre-breeding and pregnancy checks, fertility data analysis and provision of reproductive pharmaceuticals in addition to routine herd health and nutritional management. The provision of these services differs between seasonal and non-seasonal dairy industries (Mee, 2010).

Given this diversity of roles, the impact of the vet on herd fertility performance is also highly variable. And this refers only to the role of the vet on-farm. Vets can have an equally influential role in the development of the national genetic selection index used to breed more fertile daughters in the future, in regulation of semen and embryo importation and in control of nationally-important infectious disease affecting cow fertility, e.g. brucellosis (Eşki et al., 2021). In addition to the vet's input in reproductive management, there are many non-veterinary service providers who also contribute to herd fertility performance, in particular, nutritionists, AI company staff, lay ultrasound scanner operators and increasingly, agri-technology company staff.

National dairy cow fertility trends

All of these service providers operate within an environment where, nationally, dairy herd fertility is declining, static or improving. Dairy farmers may be oblivious to such secular trends, even on their own farms, a feature of 'farm-blindness' (Mee, 2020). These background trends are also generally not known to the vets providing herd fertility services, as they are often not recorded and reported or published in the national veterinary press. In the absence of such information the apparent effectiveness or ineffectiveness of the vets' role in herd fertility may be under- or over-estimated. Hence reporting of such national trends is recommended.

From the published literature we can conclude that, in general, and where data are accessible (not in all countries), dairy cow fertility (genotypically and phenotypically) globally (primarily in Holstein-Friesians) began to decline around 1980 (Pryce et al., 2014). This phenomenon occurred in diverse dairy industries (both confinement; Norman et al., 2009 and pasture-based

systems; Mee, 2004b) indicating that the decline was animal-related rather than management/enterprise type-related, though management system does affect reproductive performance (Mee, 2012). This decline continued for years depending on the mitigation measures implemented in different countries at different times to varying effectiveness. The nadir of dairy cow fertility occurred in the 2000s. Since then dairy cow fertility has begun to improve and in many countries (e.g. Ireland; Figure 1) continues to do so today (Ramsbottom, 2020). However, there are exceptions to this general pattern. In some countries dairy herd fertility data have not been reported nationally so no trends can be established (e.g. Italy, Turkiye). In other countries where data have been analysed, a decline in dairy cow fertility has been debated (e.g. Canada; Bousquet et al., 2004; LeBlanc, 2010). Across countries the definition of dairy cow 'fertility' also varies depending on the reproductive metrics recorded so direct comparisons between countries may only be possible in general terms that, overall reproductive performance has changed or not; some metrics may have improved, e.g. submission rate, while contemporaneously others, e.g. conception rate, have not changed.

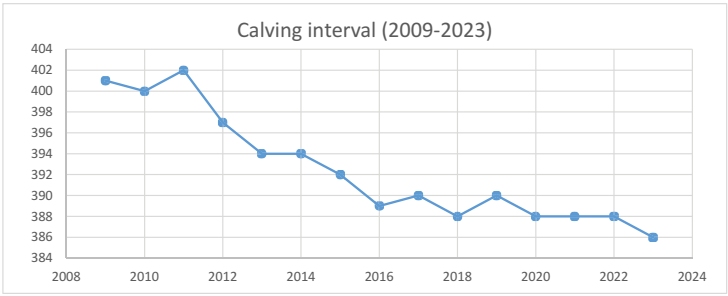

FIGURE 1
Secular trends in calving interval (days) in Irish spring-calving dairy herds nationally over 15 years (2009-2023) (ICBF, 2024)

How did dairy cow fertility improve and what role has the vet played?
Given the global extent of the problem of declining dairy cow fertility there have been numerous approaches to halting this decline and eventually improving dairy cow fertility and the vet has played a key role in this change (Mee, 2020). This improved dairy cow fertility paradigm has recently been described as the 'high fertility cycle' (Middleton et al., 2019, Fricke et al., 2023). The key drivers of the high fertility cycle are initiated epigenetically during the transition period when cow metabolic status influences the quality of the oocytes that will ovulate during the subsequent breeding season (Britt, 1991). Thus, the PVP has a critical role in managing the fertility cycle by promoting and delivering i) improved dry and transition period health and nutrition [in particular, body condition scoring (BCS) management], ii) better estrus detection (non/automated), iii) effective synchronisation (and re-synchronisation)-timed AI (including sexed-semen) programmes and iv) a greater emphasis on (in particular, cow) fertility in national selection indices. Integral to all of these strategies is good vet-client communication (Sheldon et al., 2006) and where feasible, implementation of fertility extension programmes (Brownlie et al., 2015).

Improved transition period health and nutrition
The 'Britt hypothesis' clearly demonstrated that ameliorative measures aimed at improving dairy cow fertility need to be implemented during the dry period, not just at the point of insemination. Good dry period nutrition not only benefits the ovum but also the fetus and the next generation calf (Mee, 2023). These ameliorative measures may include diet formulation, feed accessibility and cow comfort. The vet has a key role in monitoring the nutritional status of the dry cows and the health of postpartum cows. For the former, while blood metabolic profiling can be used by the vet, more widespread adoption of farmer-implemented BC scoring is likely to have greater impact at industry level. The role of the vet in this is in setting up a BCS monitoring schedule and SOP and in interpreting the ensuing data. This has been shown to be critical to ensuring cows enter the high fertility cycle after calving (Middleton et al., 2019). Given that the majority of metabolic disorders and cow culling occurs in the immediate postpartum period (Mee,

2024a), routine veterinary monitoring of affected cows pre- and post-partum is warranted.

Better estrus detection

One of the characteristic of the decline in dairy cow fertility was a decline in both the duration and the intensity of estrus as milk production increased (Lopez et al., 2004), particularly in confinement systems where estus expression is poorest (Palmer et al., 2012). This resulted in farmers complaining that they found it increasingly difficult to detect cows in estrus in order to AI them. This initially stimulated a growth in simple estrus detection aids (e.g. tail paint, 'scratch cards'...) to assist farmers in detecting estrus. However, as the problem deteriorated and agri-technology improved, automated methods of estrus detection were successfully developed (Santos et al., 2022) which allow farmers to access estrus data on their mobile phones. Today the latter technologies are beginning to replace traditional estrus detection aids in confinement and even in pasture-based dairy systems where estrus expression tends to be better (Mee and Boyle, 2020). This has now resulted in a plethora of data-driven estrus detection devices on modern dairy farms primarily based on activity monitors. The vet has a role in advising farmers on the pros and cons of the various devices and on their integration into the herd reproductive management programme.

Effective synchronisation timed AI programmes

A parallel response to the decline in estrus activity over time was to rely more heavily on insemination without detection of estrus – timed AI (TAI) programmes. Vets have been highly influential in the ongoing development of these programmes and their on-farm implementation. Numerous, complex synchronisation/re-synchronisation programmes now exist which can be tailored to individual farm management requirements for both nulliparous heifers and lactating cows (Sartori and Consentini, 2024). These programmes can both increase the submission rate and the conception rate and hence improve herd pregnancy rate. Latterly, there has been substantial

growth in the use of sexed semen both in nulliparae and in lactating cows and in some countries increased use of beef semen in dairy cows. These are both in response to the growing issue of low value male (Jersey) dairy calves. In addition to these improvements in synchronisation of insemination with induced ovulation, the farmer-employed vet (and in some countries also the PVP) has a major role to play in the AI process itself as there can be significant variation in conception rates between AI operators, whether professional or DIY (do-it-yourself = farmers), (Buckley et al., 2003, Sahin et al., 2022). While the value of traditional training/retraining in AI procedures is well known (Dalton et al., 2021, Sahin et al., 2022), recent advances in bovine simulators may offer a more animal welfare-friendly approach to updating AI skills, even for veterinarians (Azuaga Filho et al., 2023). As important as retraining is, it is equally important to analyse the risk factors associated with reduced inseminator performance. This variation between AI inseminators may be even more important in dairy industries where the primary objective is to increase milk output per cow per day, without parallel genetic selection to improve cow fertility.

Fertility in national selection indices

While the relative contributions of non-genetic factors to the improvement in dairy cow fertility have been debated (Lucy, 2023), there is general consensus that the move away from simple uni-trait genetic selection indices (for milk production) to multi-trait selection indices which place emphasis on both milk production and fertility (as well as other traits) has been a major contributory factor to improved cow fertility. Multiple examples of this development of functional genetic selection indices now exist internationally, generally with annual updates on weightings apportioned to each trait. This has allowed dairy industries select for increased milk production in parallel with selection for improved cow fertility by using, for example, the Irish Economic Breeding Index (EBI; Figure 2), (Ring et al., 2021).

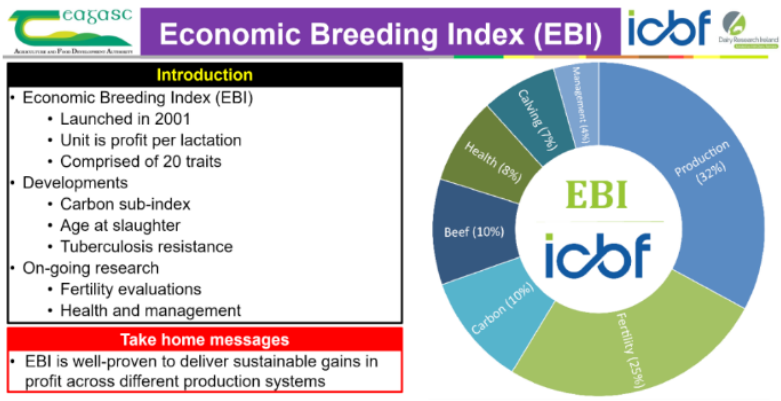

FIGURE 2
The Irish Economic Breeding Index (EBI) including a Fertility Sub-index with a weighting of 25%

Veterinary reproductive management

One of the major influencers in improving dairy herd fertility is the farm's veterinary practitioner. However, the knowledge, attitudes and practices (KAPs) of practitioners can be highly variable. A current international survey of veterinary reproductive management across European countries has illustrated contrasting veterinary KAPs (some examples shown in Table 1). For example, practitioners in Turkiye are much more likely to treat anovulatory anestrus (AA) with trace elements than in Ireland. This particular differences may reflect local nutritional conditions but many of the other differences in KAPs suggest that there may be other factors responsible. These probably include local accepted KAPs amongst practitioners, educational foundational KAPs and continuing professional development (CPD) influences. Irrespective of the causes of these differences they indicate that dairy cows/herds with similar reproductive problems may be treated differently depending on the prevailing national practitioner reproductive management KAPs. Similar heterogeneity has been reported for calving management internationally (Mee et al., 2023). While heterogeneity is to be welcomed in veterinary practice it is best implemented where evidence-based KAPs underpin such variation.

TABLE 1. Veterinary reproductive management knowledge, attitudes and practices (KAPs) in Turkiye and in Ireland (% of respondents) (Mee, Terkin and Pereira, unpublished data, 2024)

Veterinary reproductive KAPs (n=10)	Turkiye (%)	Ireland (%)	Difference %
AA treatment with trace elements	55.9	16.1	40
RFM case def >24h	32.4	67.7	35
Routine post-partum check Day 1	23.5	0	24
FOC Dx by abnormal estrus +/- US	76.5	53.2	23
Clinical endo treatment with i/u AB	44.1	64.5	20
RFM manual removal	38.2	58.1	20
Puerperal metritis treatment with i/u AB	35.3	48.4	13
Clinical endo case def >40 DIM	38.2	27.4	11
RFM treatment with i/u AB	29.4	21	8
Puerperal metriti case def enlarged uterus	52.9	58.1	6

AA = anovulatory anestrus; RFM = retained fetal membranes; case def = case definition; FOC = follicular ovarian cyst; US = ultrasound; endo = endometritis; i/u = intrauterine; AB = antibiotics; DIM = days in milk.

Future challenges

In some countries the decline in dairy cow fertility is no longer the existential challenge it once was (*'Where will it end*?' – Lucy, 2001), (though the gap between nulliparous and pluiriparous fertility continues to exist) and dairy cow longevity has now become the new 'wicked problem' (Dallago et al., 2021). But other adjacent issues have arisen which vets and all industry stakeholders still need to address. These include promulgation of more widespread use of sexed semen for lactating dairy cows, dealing with consumer push back against modern breeding technologies (Britt et al., 2021), integration of genomic data in genetic selection, growing rates of inbreeding depression (Guinan et al., 2023, Sen et al., 2021), successful adoption of constantly changing agri-technologies (Mee, 2024b) and how to make best use of the new 'AI' (artificial intelligence) to improve dairy herd fertility in the future (De Vries et al., 2023).

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Global reproduction control guidelines: The difficult choice of whether or not and how to do gonadectomy in companion animals

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Citation
Romagnoli, S. Global reproduction control guidelines: the difficult choice of whether or not and how to do gonadectomy in companion animals.

Abstract
The control of reproduction in companion animals, particularly dogs and cats, has evolved from a routine procedure to a complex decision-making process influenced by advancements in veterinary medicine, client expectations, and ethical considerations. In the past, spay-neuter procedures were widely recommended to control pet populations and prevent unwanted breeding. However, recent studies have revealed potential health risks, such as an increased incidence of tumors, following surgical gonadectomy. These findings, coupled with the availability of new reproductive technologies, have led to an exploration of alternative methods, including ovary-sparing surgeries, vasectomies, and medical interventions.

Globally, pet ownership has significantly increased. For example, Australia has 6.3 million dogs and 5.3 million cats, Europe hosts 92.9 million dogs and 113.5 million cats, while the US reports 76.8 million dogs and 58.3 million cats. Similarly, in South America, Brazil has 32.9 million dogs and 14.2 million

cats, and Argentina has 10.3 million dogs and 4.6 million cats. China is home to 51.2 million dogs and 65.4 million cats. This rise in companion animals has elevated the complexity of reproductive control, with veterinarians now facing more nuanced questions from pet owners, particularly around alternative procedures and long-term health impacts.

While pets in private homes benefit from individualized reproductive control, stray and shelter animals are typically gonadectomized for cost-efficiency and population management. The rise of alternatives to traditional surgeries presents a potential shift in shelter management, though cost-effectiveness remains a concern. Additionally, ethical debates surrounding methods like trap-neuter-return (TNR) highlight the complexity of managing stray populations.

Veterinarians must now navigate a variety of reproductive control methods, taking into account owner preferences, breed predispositions, and the specific needs of shelter animals. Despite the growing availability of alternatives, traditional gonadectomy remains the predominant approach in both private practice and shelter settings due to its efficacy and affordability. The future of reproductive control in companion animals lies in balancing these advancements with ethical considerations and client expectations.

Management techniques for non-neutered dogs in Norway

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Citation
Engeland, I. V. Management techniques for non-neutered dogs in Norway.

Abstract
In Norway, it is a legal requirement that dogs must not be neutered. The ban on castration is based on a combination of climatic, ethical, and cultural factors and the potential side effects of castration. Dog owners are responsible for having control over their dogs all the time. Veterinarians are critical in advising how dog owners can manage their non-neutered dogs. Techniques like training, socialization, leash walking, health monitoring, and hormonal treatment ensure non-neutered dogs a good quality of life while controlling the dog's sexual instinct.

Introduction

While castration is common globally, Norway has limited use of the practice. Norwegian law emphasizes animal welfare, banning routine castration without medical or behavioral justification due to ethical and environmental considerations. This approach is supported by Norway's climate, where cold winters encourage indoor housing and lessen stray animal issues, contrasting with areas where stray dogs pose public health risks. In Norway, stray animals are rare, and found animals are typically rescued or rehomed (1, 2).

Ethical and Cultural Considerations

Norwegian animal welfare policies respect the animal's natural state, minimizing surgical interventions that alter behavior or cause pain. The castration ban reflects a cultural respect for animal integrity, treating unnecessary castration as ethically questionable. Castration, which permanently alters the body and potentially the behavior, is deemed unnecessary unless crucial to the animal's welfare or functionality (3, 4, 5).

Side Effects of Castration

Castration can prevent certain diseases and manage behavior but has side effects, including surgical risks and post-surgical complications. Castration may slow metabolism, increasing obesity risks, and has been associated with diseases like diabetes, hypothyroidism, and immune conditions (4, 5). Behavioral impacts vary; while aggression may reduce, castration may also induce anxiety or lower activity. Early castration, particularly, raises risks of orthopedic problems like hip dysplasia (6). These potential effects underscore Norway's preference for non-surgical management of sexual behavior.

Legal Framework

The Norwegian Animal Welfare Act of 2010 (§ 9) mandates that surgical procedures must benefit animal health and well-being, not merely convenience (1). Routine castration is prohibited without clear medical reasons, ensuring that unnecessary pain or behavioral change is avoided. Non-medical castrated animals are barred from shows and competitions unless an exemption from a veterinarian is provided (7).

Accepted Exceptions for Castration in Animals

While the law generally bans castration, exceptions are allowed for farm animals and pets under specific circumstances. For example, male pigs are castrated to prevent meat taint, and horses used for riding are castrated for safety. Cats, traditionally excluded, are now neutered to control the stray population, as stray cats often suffer in harsh winter climates.

Non-Surgical Management Techniques

1. Training and Socialization

Responsible dog ownership includes managing the behavior of non-neutered dogs through early training and socialization, emphasizing good manners, social skills, and obedience. Training addresses behaviors like aggression or marking without surgery. Physical exercise, interactive games, and mental stimulation are essential for non-neutered dogs to prevent frustration or aggression, especially if nearby dogs are in heat (8, 9).

2. Leash Walking

Norway requires dogs to be leashed for half of the year to protect people and wildlife, and even during the other months, leash walking is encouraged for control. This policy aligns with Norway's laws for responsible dog handling, supporting safe, controlled interactions for non-neutered dogs.

3. Health Monitoring

Regular veterinary check-ups are crucial for non-neutered dogs, which face risks of conditions like testicular cancer or prostate issues in males and mammary tumors or pyometra in females. Health screenings, typically conducted during annual vaccinations, allow for early detection of hormone-related conditions, such as alopecia or seborrhea, which may affect non-neutered dogs more often.

4. Managing the Heat Cycle

Female dogs require close supervision during their heat cycle to prevent unwanted breeding. Isolating females from males during heat periods, using dog diapers, and selecting isolated walking areas reduce the risk of accidental mating. Leash use and controlled interactions prevent potential issues with male dogs drawn to a female in heat.

5. Hormonal Treatments

When non-surgical methods cannot prevent unwanted pregnancies, hormonal treatments provide a temporary, reversible solution. Deslorelin acetate, a hormone-releasing implant, is widely used in Norway to manage

sexual behavior in dogs. By suppressing testosterone, this implant reduces aggressive and sexual behaviors for up to a year, offering an effective non-surgical alternative (10, 11). Progestogens also help manage reproductive cycles but carry potential side effects, making veterinary supervision essential (5).

Ethical, Medical, and Behavioral Justifications for Neutering

Exceptions to the castration ban include medical conditions, such as cancer or severe behavioral issues, where castration supports the animal's well-being. Conditions like testicular cancer or prostatic disease justify neutering to protect the animal's health. Veterinarians assess and document the justification for each castration, ensuring the procedure aligns with the law's welfare-focused standards (12, 13).

Conclusion

Norway's castration ban embodies a commitment to respecting animals' natural states, only allowing castration when medically necessary or behaviorally warranted. This policy, rooted in ethical principles, depends on responsible pet ownership and the guidance of veterinarians. Non-surgical management techniques, including training, leash walking, health monitoring, managing heat cycles, and hormonal treatments, ensure that non-neutered dogs maintain a high quality of life. Norway's approach to animal welfare challenges dog owners and veterinarians to responsibly handle non-neutered pets, ensuring animals live according to their natural states without unnecessary interventions.

Keywords: non-neutered, castration, dog, ethic, training, hormones

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Effect of neutering on general health in dogs and cats; Post neutering complications and owner challenges

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Citation
Bastan, I. Effect of neutering on general health in dogs and cats; Post neutering complications and owner challenges.

Abstract
Gonadectomy is an important reproductive management tool employed in many countries and a commonly recommended veterinary procedure. However, the necessity, and in particular the timing of gonadectomy in dogs and cats is still controversial. Epidemiologic research has identified that gonadectomy confers a mixture of beneficial and harmful outcomes depending on age at neutering breed and sex. In recent decades, concerns have been raised regarding possible long-term health problems including obesity, urinary incontinence, bladder stones, hypothyroidism, diabetes mellitus, hip dysplasia, cruciate ligament rupture, behavioural changes, cognition problems, as well as several forms of cancer. Health issues related to gonadectomy can be a financial and emotional burden for pet owners. The objective of the present review was to bring to light the most recent literature, summarize it and discuss the findings focusing on the risks and benefits of neutering in dogs and cats.

Introduction

Gonadectomy, or more generally referred to as "neutering," is a common procedure in many countries to control reproduction in dogs and cats. Neutering of dogs and cats is promoted for population control, preventative healthcare, and behaviour modification (1). Gonadectomy is an effective treatment and prevention of androgen-induced diseases in male cats and dogs, such as benign prostatic hyperplasia (BPH), prostatitis, testicular tumours (2-4). In female dogs and cats neutering prevents and treats potentially pyometra and reduces the incidence of mammary tumours (5-8). However, gonadectomy is reported to increase the risk of some musculoskeletal degenerative diseases (e.g., hip dysplasia, cranial cruciate rupture), obesity and associated diseases, several forms of cancer (e.g., osteosarcoma, lymphoma, cardiac hemangiosarcoma), immune-mediated diseases and cognitive dysfunction syndrome (9-15).

The necessity of gonadectomy in dogs and cats is still controversial. This is mainly because gonadectomy confers a mixture of benefits and adverse effects that depend upon the age at neutering, sex, species and breed. Thus, the purpose of this review is to compile and present information in a clear and concise manner, addressing the indication of neutering, it's possible risks and benefits.

Risk and Benefits for health

Neoplasia

Mammary tumors are the most frequent neoplasia in female dogs (16,17). Delayed spaying increases the risk of developing mammary tumors (18-20). Intact female dogs are at four times greater risk of developing mammary tumors, compared to those spayed before two years of age (20,21). The protective effect of spaying at an early age is a 91 and 86% reduction in development of mammary tumors in cats spayed before 6months and 7 to 12months, respectively (22).

Neutering is associated with risk of developing splenic and cardiac hemangiosarcoma. Studies have identified that spayed females had a five times greater risk of developing hemangiosarcoma than intact females and

that neutered male had a 2.4 times greater risk than intact males (23,24). A study on osteosarcoma in several breeds was found a 2-fold increase in occurrence in neutered dogs relative to intact dogs (25). In relation to other types of tumours, such as lymphoma, recently Bennett et al. identified and quantified host risk factors for lymphoma in a large population of Australian dogs (26).

Animal behaviour

Behavioral problems are an important reason for relinquishment of pet dogs and cats by owners (27,28). The impact of neutering on behavior is complex, with both positive and negative effects reported (29,30). Non-sexually dimorphic behaviours, such as fear-based aggression, are not affected by gonadectomy (30). Gonadectomy reduces reproductive behaviors by eliminating estrus-related actions in females and reducing roaming, mounting and urine marking in dogs (31-33). Castration of male cats is deemed very effective in the case reducing roaming, spraying, fighting and aggression in free-roaming female cat (34,35).

Several studies of dogs referred for treatment of behaviour problems have identified a higher proportion of spayed than intact females among animals exhibiting aggression (36,37). Early age gonadectomy has been reported to increase noise phobia (32,33).

Obesity

Almost all studies agree that neutered cats and dogs are more likely to be overweight or obese than intact cats and dogs (38-41). Gonadectomy induces obesity through two main pathways: increased appetite and a lowered metabolic rate. In intact dogs, eating suppresses the release of gastrointestinal hormones like cholecystokinin and glucagon, leading to a feeling of fullness. However, within a week post-neutering, food intake increases by 20% and remains high. This rise could be attributed to the activation of luteinizing hormone receptors in the gastrointestinal tract post-gonadectomy, potentially boosting the release of cholecystokinin and/or glucagon (42).

Skelatel system

Sex hormones play a role in controlling the elongation of the bones. Dogs that undergo prepubertal gonadectomy (before 6 months of age) experience delayed growth plate closure (43). Most studies have found that neutered dogs have a significantly higher risk of developing orthopedic disorders than intact dogs. The most common orthopedic problems are cranial cruciate ligament rupture, hip dysplasia, elbow dysplasia, and osteoarthritis (44-46). Additionally, neutered dogs are more prone to obesity, complicating the relationship between neutering and osteoarthritis (47).

Immune system

Sundburg, *et al*. evaluated the possible association between neutering and diseases associated with immune function compromise in dogs. The authors concluded that neutered dogs had a significantly greater risk of hypoadrenocorticism, autoimmune haemolytic anaemia, atopic dermatitis, hypothyroidism, inflammatory bowel disease and thrombocytopenia than intact dogs with neutered females being at greater risk than neutered males for all but hypoadrenocorticism and autoimmune haemolytic anaemia (48).

Urinary system

The risk for urinary incontinence (UI) is low in intact bitches (49) .UI after spaying can occur immediately or up to 10 years after surgery, about 75% of the bitches become incontinent within 3 years after gonadectomy (42). The risk of UI influenced by various factors such as body weight or breed and time of spaying (49).

In a large study of male and female cats, gonadectomy and obesity were risk factors for the development of feline lower urinary tract disease (FLUTD) (50) but other previous studies showed no difference (51,52). The risk factors for FLUTD differ across countries due to geography, obesity, season, diets, and cats' lifestyle. Therefore, further research on the subject is needed.

Conclusions

Gonadectomy confers a mixture of benefits and adverse effects that depend upon the age at neutering, sex, species, and breed. Neutering has tangible benefits such as lowering the incidence of reproductive cancers

and increasing lifespan. In contrast, neutering appears to increase risks of several cancers, obesity, musculoskeletal degenerative diseases and immune-mediated diseases. It is impossible to predict the precise outcome of neutering for any individual given the numerous and interacting etiologic factors involved in most serious behavioural and medical conditions. Any decision to neuter a particular pet must include consideration of individual circumstances and the values and goals of the owner as well as the risks and benefits identified by epidemiologic data.

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Advancements in equine reproductive management

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Citation
Mari, G. Advancements in equine reproductive management.

Abstract
Managing the reproduction problems of mares and stallions properly can enhance their fertility, whereas mismanagement can reduce fertility even in normal mares and stallion. The economic repercussion of reproductive failure and costs involved in attempting to improve reproductive efficiency are considerable, and identify areas of strength and, equally, areas of weakness is paramount to ensure efficacy of interventions in relation to reproductive efficiency. Assisted reproductive techniques and perinatal intensive care play a relevant role, but the main focus of this presentation will be on reproductive management for getting a mare pregnant, either by natural mating or artificial insemination. As implied by the title, the aim of this review is to offer the most up-to-date information on the diagnosis and treatment of the main causes of reduced reproductive fertility, with particular attention to those that can be applied in a clinical practice. In addition, the areas of research under investigation will be presented, hopefully providing future advancements in understanding molecular mechanisms of major reproductive male and female diseases.

Studies on increasing pregnancy in buffaloes by modifications to ovsynch protocol

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Citation
Alkan, S. Studies on increasing pregnancy in buffaloes by modifications to ovsynch protocol.

Abstract
There are native Anatolian Buffaloes and imported Italian Buffaloes in Turkey which are both Mediterranean Buffaloes. Buffaloes are mainly reared for milk production in Turkey. So far, buffalo rearing system have totally been "Village Style" without any scientific administrations. The owners of these type of farms hardly get convinced to have their buffaloes treated or administered. So, buffaloes with reproductive problems directly go to slaughter. We planned a long-lasting field study to treat mild metritis in buffaloes that have been named as "Residual Metritis". We adopted intrauterine antibiotic therapy to the oosynch programme and controlled these therapies with Lugol's Solution which has no side-effects like antibiotics, and we called this application "Lugo synch". With this long-lasting field trial, we compared the Lugo-synch Programme with antibiotic therapy. The results have been encouraging. We concluded that, Lugo-synch can be a preferable alternative to antibiotics with the advantages of being cheaper, easy administration, effectiveness and does not spoil milk.

Comparative clinics in postpartum: Innovative diagnostics and therapeutics

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Citation
Rizzo, A., Stelletta, C., Mariella, J., Tekin, K. Comparative clinics in postpartum: innovative diagnostics and therapeutics.

Abstract
This mini-review focuses on the comparative postpartum clinical practices across four species: bovine, equine, small ruminants (sheep/goats), and canine. The review synthesizes key findings in the management of postpartum disorders, such as metritis, retained placenta, and mastitis, and specie-specific disorders highlights innovative diagnostic techniques and therapeutic approaches. By comparing these practices, the review aims to identify commonalities and species-specific challenges while also discussing recent advancements in reproductive health management. This comparative analysis is crucial for developing more effective and species-appropriate therapeutic protocols, ultimately improving postpartum outcomes in veterinary practice.

The effect of some cryoprotectant mixtures during the cryopreservation of drone semen

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Citation
Kaya , A., Uysal , O., Akyol, N. The effect of some cryoprotectant mixtures during the cryopreservation of drone semen.

Introduction and aim

The initial successful study on the cryopreservation of drone semen was conducted using the dimethyl sulfoxide (DMSO) and it is still widely used today (1). In the subsequent years, the efficacy of glycerol (GLY), dimethylacetamide (DMA), ethylene glycol (EG) has been researched (2, 3). Nevertheless, the desired success has not been achieved due to cytotoxic, genotoxic, and hyperactivation effects induced by cryoprotectant-related factors, in addition to cold shock (4-6). A study on the toxicity of cryoprotectants revealed that the mixture of DMSO and EG exhibited lower toxicity than the individual use of each cryoprotectant (7). Similarly, in other species has been demonstrated that the use of cryoprotectant mixtures is more effective (8, 9). In the study, semen samples of drones

were cryopreserved using diluents containing DMSO, DMA, EG, and GLY alone or in combination with each other. After thawing parameters such as motility (MOT), plasma membrane integrity (PMI), hypo-osmotic swelling test (HOST), mitochondrial membrane potential (MMP), and acrosome integrity (AI) were examined. The study investigated the effectiveness of different cryoprotectant mixtures in the long-term preservation of drone semen.

Methods

A total of 100 µl of semen was collected from drones of several colonies in each trial and mixed. Semen was diluted with a Tris-citrate solution containing 10% DMSO, EG, DMA, and GLY, and 5% mixtures of each pair, to create groups with 100 × 10^6/ml spermatozoa in each group. All groups were cryopreserved using a programmable freezing machine and after thawing were evaluated for spermatological parameters (M, PMI, HOST, MMP, and AI). The differences between the groups were determined using One-Way Analysis of Variance (ANOVA) and Post-hoc Tukey tests with the help of SPSS v.26 statistical software.

Results

According to the obtained data, significant differences were found between the groups for all parameters ($P \leq 0.05$). In terms of motility, the most successful groups were the DMSO and DMSO-EG groups. Regarding PMB, the DMSO, DMSO-EG, and EG-GLY groups demonstrated success. In terms of HOST, the DMA, DMSO-GLY, and EG-GLY groups demonstrated superior performance. About MMP, the EG group exhibited the most significant activity. In terms of AI, the EG, GLY, DMSO-EG, DMA-GLY, DMA-EG, and GLY-EG groups exhibited the most favorable outcomes.

Discussion and conclusions

The findings of the study suggest that the utilization of cryoprotectant mixtures may be a viable alternative to the use of a single cryoprotectant in the cryopreservation of drone semen. The DMSO-EG and EG-GLY association in particular gave better results compared to the other groups. It is thought that this situation arises from the differences in the mechanisms of action of cryoprotectants (10, 11). It is believed that the synergistic effect

of these two substances is beneficial in the cryopreservation of drone semen. Similarly, cryopreservation of drone semen with mixtures of DMSO and trehalose has been shown to improve post-thawing parameters (12). In conclusion, the study has demonstrated that different cryoprotectant mixtures have a positive effect on spermatological parameters in the cryopreservation of drone semen. However, there is a need to further develop this study with in vivo experiments.

Keywords: Cryopreservation, dimethyl sulfoxide, drone semen, ethylene glycol, honey bee

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Is the gene expression of placentitis different from that of placental edema in the mare?

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Citation
Lanci , A.L., Mariella , J., Baldassarro, V.A., Cescatti, M., Ellero, N., Burato, V., Giardino, L., Castagnetti, C. Is the gene expression of placentitis different from that of placental edema in the mare?.

Introduction and aim

Diagnosis of placentitis in the mare is based on ultrasonography and clinical signs (premature lactation and/or vulvar discharge) [1,2], but in clinical practice it is not always possible to differentiate placentitis from placental edema [3]. Despite their prevalence, most published gene-related research has focused on experimental models of placentitis rather than clinical cases

[3]. The aim of the present study was to evaluate the gene expression pattern of the chorionic surface of the fetal membranes after their expulsion in mares with placenta edema and in mares with confirmed spontaneous placentitis compared to normal ones.

Methods

Pregnant mares hospitalized for attended parturition at the Veterinary Teaching Hospital of the University of Bologna were enrolled. At admission, a clinical evaluation and transrectal ultrasonography were performed. When an increase in the Combined Thickness of the Uterus and Placenta (CTUP) was observed, a cervical swab was performed to obtain a bacterial culture. Treatments received during pregnancy (anti-inflammatory drugs, antibiotics) were recorded. After parturition, a macroscopic and histopathological examination of the placenta was performed in all mares. Diagnosis of placental edema or placentitis was confirmed after macroscopic and histopathological examination of the placenta and with the results of the bacterial culture respectively.

Data and samples were available for 23 mares divided in 4 groups: H Group, 10 mares with normal pregnancy and eutocic delivery, that did not receive any treatment and gave birth to healthy foals. E Group, 6 mares with placental edema that did not receive any treatment. Ensaid Group, 4 mares with placental edema that received non-steroidal anti-inflammatory drug (nsaid). P Group, 3 mares with confirmed placentitis that received antibiotics and anti-inflammatory drug.

A placental sample uniform in size (2 × 2 cm) was collected near the umbilical cord attachment and was manually separated into two portions: chorion and allantois. Chorion samples were stored at -80 °C.

All samples were homogenized, and total RNA isolation was performed using RNeasy Microarray Tissue Mini Kit (Qiagen, Hilden, Germany, Cod. 73404) by the automated extractor QIAcube Connect. Total RNA was eluted in RNase FreeWater, and using a spectrophotometer (Nanodrop 2000, Thermo Scientific, Waltham, MA, USA), absorbance values at 260, 280 and 320 nm

were measured. For the reverse transcription to generate the cDNA, the RT^2 first strand kit was used, with 1 µg of pooled RNAs from each experimental group. The mRNA expression was analyzed using the PCR array for the horse angiogenesis growth factor (PAEC-072Z), including 84 genes related to growth factors, cytokines and chemokines, pro-angiogenic factors and angiogenesis inhibitors, five different housekeeping genes and different quality controls. For the analysis, the Geneglobe software was used, selecting the whole housekeeping genes set for the first normalization. A second normalization was used to calculate the relative expression (expressed as Fold Regulation, FR) compared to a normalizer control group, using the stringent Cut-off of FR $\pm$ 3 to select the significant regulations.

Results

Based on the first normalization only, using the average Ct of the housekeeping genes for each experimental group, a clusterization analysis was performed, grouping the different samples depending on the expression levels of all the genes included in the array. Depending on this analysis, the two placental edema groups (E and Ensaid) have been included in the same cluster, with the control group (H) as the nearest. The P group resulted the most different from all the other groups.

Considering the FR analysis in comparison with the control group (H), the E group showed only 1 upregulated gene (*CCL15*) and the Ensaid group four (*BTG1*, *CCL15*, *CCL2*, *IL6*). As expected from the clusterization analysis, the P group resulted the most regulated compared to the control, showing 8 upregulated (*ANGPT2*, *CCL15*, *CCL2*, *CXCL14*, *FST*, *THBS1*, THBS2, *TIMP1*) and 2 downregulated (*NPPB*, *SPINK5*) genes.

Discussion and conclusions

For the first time, chorionic gene expression of placentitis was compared to placental edema. Based on the present results, placentitis was associated with several genes involved in pro-inflammatory activity and in premature placental separation, which frequently occurs. Genes such as *CCL15*, *CCL2* and *CXC14*, involved in the regulation of inflammatory processes, as suggested by Robles et al. [4], could play a role in premature delivery

induction and myometrial activation caused by placentitis. The upregulation of *TIMP1* in placentitis could be attributed to the high expression levels of extracellular matrix (ECM) components, including collagen and proteoglycans, and low protease degradation, as suggested previously [5]. Although the causes and pathogenesis of placental edema remain to be clarified, these findings can improve the knowledge about placentitis by identifying its distinct gene expression profile which is different from the one of placental edema. Further investigation could be done on the genes involved in ECM remodeling components such as matrix metalloproteinases and on cytokines and circulating proteins in the blood of mares with placentitis and placental edema. Being able to differentiate these two different conditions could have an important impact in reducing the use of antibiotics in clinical practice.

Keywords: mare, chorion, gene expression, placentitis, placental edema, mRNA, cDNA.

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Investigation of some reproductive parameters in zebrafish

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Citation
Mart , F.,Herdoğan , M., Çay, H.A., Inanç, M.E., Innal, D., Güngör, Ş. Investigation of some reproductive parameters in zebrafish.

Introduction and aim

This study aimed to determine the average spermatological parameters in zebrafish and the number of oocytes, fertilization rate in female zebrafish, developmental stages of lavrae, and number of lavrae.

Methods

Commercially purchased zebrafish were kept under laboratory conditions at MAKU Faculty of Veterinary Medicine, Reproduction and Artificial Insemination Clinic Building. In this study, zebrafish were mated. Eggs were collected and examined under stereo microscope to determine the

number of eggs and fertilization rate. Fertilization rate was assessed at day 1 post fertilization (dpf) and expressed as percentage of viable embryos. The number of hatched larvae was expressed as the percentage of live larvae at 3 dpf. After mating, male zebrafish were anesthetized and their length and weight were measured. The testes of the fish were then dissected, the testes were weighed, and semen samples were taken and analyzed for motility, concentration and abnormal spermatozoa. Sperm viability (SYBR14-PI) and high mitochondrial membrane potential (HMMP) were also analyzed by flow cytometry.

Results

The average number of oocytes released because of mating was 160.8±45.89, the fertilized ratio was 75.34±6.61%, and the ratio of viable larvae was 70.64±8.19%. The zygote was observed at 0-0.5 hours post-fertilization (hpf). The cleavage period was observed at 1-2 hpf, followed by the blastocyst stage at 2-6 hpf. The segmentation stage was observed at 10-24 hpf. On average, the larvae hatched at 3 dpf.

The mean body weight (g), length (mm), and testicle weight (g) of male zebra fishes were 0.55 ±0.01; 38.16±0.11, and 0.0091±0.0013, respectively. The morphology integrity of spermatozoa was 67.80±2.62%, 10.80±1.79% sperm head abnormalities, 3.80±1.34% neck abnormalities, and 17.6±2.77% sperm tail abnormalities. Average motility 73.39±3.86%, sperm concentration 140±22.60x10^6, viability rate 84.81±1.94%, and HMMP was determined as 53.38 ± 3.41%.

Discussion and conclusions

The results of this study provide valuable information on zebrafish reproduction and sperm quality and are largely consistent with other findings in the literature. The onset of zebrafish sperm motility has been described to be associated with a decrease in intracellular K+ (1). It has also been reported that zebrafish sperm motility is prolonged by deionized saline (2). In one study (3), deionized water or sperm activating saline solutions were used to activate zebrafish motility and motility lasted 120 s and 300 s, respectively, while in this study motility lasted 480 s. It is thought that the difference in

motility times in this study is due to the activation solutions used. When sperm collection methods were evaluated by Jing and Huang (4), sperm motility was found to be 89%, 90% and 65% with dissection without crushing, abdominal massage and dissection with crushing, respectively, and collection with abdominal massage was shown as the best method. In our study, the sperm motility obtained using the cutting method was found to be 74.73% and was like the previous studies. For sperm concentration, dissecting without crushing had a mean of 65×10^6 spermatozoa/ml and the abdominal massage method had a mean of 79 $\times10^6$ spermatozoa/ml, whereas in our study it was $140 \pm 22.60 \times 10^6$ spermatozoa/ml. Other studies have reported that the average number of oocytes in zebrafish typically ranges from to 100-200. For example, researchers have reported that an average of 150 oocytes were released in zebrafish (5), which agrees with the findings of the present study. The fertilization rate also varies between 70-80% in similar studies (6), indicating that the obtained rate of 75.34% is consistent with the literature. Similar embryonic developmental stages have been reported (6). The zygote, cleavage, and blastocyst stages are consistent with the present study, and the hatching of larvae at 3 dpf is consistent with known data on the zebrafish developmental process. In similar studies, the body weight and length of zebrafish were reported to be between 0.4-0.6 g and 35-40 mm, respectively (1). The results of this study are generally consistent with findings in the literature. In similar studies, zebrafish sperm motility was reported between 70-80% (7). Sperm concentrations and viability rates have also been previously reported (8). These results support the findings of the present study and provide reliable data on the reproductive biology of the zebrafish. Such studies will help us better understand the reproductive biology of zebrafish and optimize its use as a model organism in biomedical research.

Keywords: Zebrafish, Reproductive, Sperm, Oocyte, Larvae

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Interaction of parity and age on uterine arteries in periparturient healthy mares: preliminary study

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Introduction and aim

Doppler ultrasonography of the uterine arteries is a common diagnostic method to evaluate uterine blood flow and vascularization in the equine species. Maternal age and parity are known to affect horse reproduction; but they may also act as confounding factors, as nulliparous mares are usually young, while older mares are mostly multiparous. Unlike maternal age, parity appears to have a non-linear effect [1]. This study aims to evaluate whether the interaction of parity and age influence the diameter, cross-sectional area and resistance index of the uterine arteries, in pre- and post-partum mares with normal pregnancies and eutocic deliveries.

Methods

Two-dimensional pulsed-wave (PW) Doppler flow ultrasonography of both uterine arteries was performed, using Esaote Mylab Alpha with a 5-10MHz linear probe. This study included healthy mares hospitalized for attended parturition at a Veterinary University Hospital. Transrectal US was performed at various time points: every 5-10 days from admission to parturition (Tpre −30, -20, -15, -10, -5, 0) and within 72h post-partum (Tpost). Diameter (mm), cross-sectional area (cm^2), and resistance index, expressing uterine impedance, of the gravid uterine artery (GUA) and non-gravid uterine artery (NGUA). Mares were divided in four groups according to age (≤10 years Young-Y; >10 years Old-O) and parity (primiparous-P; multiparous-M): YP, YM, OP, OM. Pre-partum time points were then paired for data analysis: -30/-20, -15/-10, -5/0. Differences in all parameters between times (Tpre) within each group, between groups at each time (Tpre, Tpost) and between GUA and NGUA at each time were estimated using non-parametric tests.

Results

Sixty light breed mares were included: 10/60 Italian Saddlebred, 2/60 PSA, 3/60 Quarter Horse, 45/60 Standardbred. YP (n=11) had an average age of 6 (4-9) years, YM (n=19) had an average age of 8 (5-10) years and an average parity of 3 (2-4), OP (n=6) had an average age of 16 (12-22) years, OM (n=24) had an average age of 14 (11-24) years and an average parity of 6 (2-12). For the NGUA, there were no differences in diameter and area across different time points within each group. However, the resistance index was significantly lower in the YM ($p<0.001$), OP ($p=0.018$), and OM ($p<0.001$) groups at Tpre-30/-20, -15/-10, -5/0 compared to Tpost. In the YP, the resistance index did not differ across time points. For the GUA, no differences in diameter were found, but the area was higher at T-5/0 than at Tpost in the YM group ($p=0.004$). The resistance index was significantly at Tpre-30/-20, -15/-10, -5/0 than at Tpost in the YP ($p=0.002$), YM ($p<0.001$), OP ($p=0.028$), and OM ($p<0.002$) groups. Analyzing all parameters among the groups at each time point for the GUA, no differences in diameter and resistance index were observed, while the area was significantly lower at Tpre-5/0 in YP compared to YM ($p=0.011$) and OM ($p=0.015$). For the NGUA: at Tpre-5/0 the diameter and area were significantly lower in YP compared to YM ($p=0.026$

and p=0.026, respectively) and OM (p=0.003 and p=0.005). At Tpre-15/-10, diameter and area were significantly lower in YP compared to OP (p=0.022 and p=0.032, respectively) and OM (p=0.004 and p<0.001), with area also lower in YP compared to YM (p=0.035). At Tpre-30/-20, the area resulted lower in YP compared to OP (p=0.037) and OM (p=0.008). No differences in all parameters were found between GUA and NGUA except for resistance index in OM, which was significantly lower in GUA (p=0.024).

Discussion and conclusions

In the present study, resistance index declined as expected after parturition. Both diameter and cross-sectional area were influenced by age and parity, with lower values observed in the YP group. This suggests that increasing age and/or parity leads to structural and functional degeneration in the reproductive tract, impacting embryo, placenta, fetal intrauterine growth and foal health [1]. Previous studies have shown that diameter of the uterine artery is affected by parity, while age influenced resistance index during pregnancy [2]. Our findings indicate no differences between GUA and NGUA, suggesting that non-gravid horn, not holding fetal hind limbs, is essential for full fetal development, especially in late pregnancy [3]. After parturition, a rapid uterine involution and a decrease of uterine artery blood flow occur, with an increase in the resistance index [4] that was also observed in this study. Since the OP group is the smallest, but still the most particular and the least studied, future goals will be to increase the number of animals in this group, evaluating whether the parameters of the uterine arteries can give indications for the use of a reproductive assisted technique such as ovum pick-up or embryo transfer in these mares instead of a pregnancy. Furthermore, this preliminary study will allow to have normal reference values, which can be compared with those of mares with different periparturient pathologies, in order to be able to discriminate between these and to be able to intervene promptly.

Keywords: Mares, peripartum, uterine arteries, color doppler, uterine blood flow, parity, age

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Hemosiderin-laden macrophages: The iron curtain in canine mammary tumours

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Introduction and aim

The term "immune contexture" in oncology refers to the type, functional orientation, density, and location of adaptive immune cells in tumours [1]. Canine mammary tumours (CMTs) are the most common neoplasms in intact female dogs. Considering its role as a translational model for human breast cancer and the promotion of immunotherapy also in veterinary medicine, different studies focused on the CMT immune contexture. Of the various cells involved in cancer, tumour-associated macrophages (TAMs) are implicated in all stages of cancer from tumorigenesis to metastasis outgrowth. They plastically change their immune response according to

local and systemic signals with an antineoplastic (M1) or a protumoral (M2) phenotype [2]. Among their different role, they are also involved in iron metabolism. Hemosiderin-laden macrophages (HLMs) sequester iron as hemosiderin, a ferritin protein aggregates, to prevent depletion of iron and maintain levels of cytotoxic free iron. HLMs are described in both human and canine mammary tumours, however, their function is still not completely understood [3-4]. This study aims to evaluate the distribution of HLMs in CMTs in relation to other inflammatory population groups, such as tumour infiltrating lymphocytes (TILs) or tertiary lymphoid structures (TLSs). The expression of some protumoral and proangiogenic molecules was also evaluated.

Methods

CMT samples presenting HLMs (n=50) were retrieved from the archives of the Unit of Veterinary Pathology of the Department of Veterinary Sciences of Messina. All the cases were reclassified according to the most recent classification and grading [5-6] and evaluated in haematoxylin & eosin (H&E) for the HLMs location. Prussian blue, Meguro stain and modified Meguro stain were performed to confirm the presence of iron. Immunohistochemistry was carried out for Macrophage Marker (MAC/387), CD204, EPO, EPOR, and HIF-1α. To distinguish TILs from TLSs, CD21 antibody was used to mark dendritic cells, present only in TLSs.

Results

In H&E stain, HLMs were mainly organized in conspicuous groups variously located. Prussian blue, Meguro stain and modified Meguro stain revealed the iron deposits in HLMs with blue, brown and purple colour, respectively. CD21 antibody marked several follicular dendritic cells in both the outer and inner parts of inflammatory cell groups with a follicular-like pattern, recognized as TLSs. HLMs were interspersed or in the marginal area of TILs and TLSs indifferently. HLMs were negative for Macrophage Marker (MAC/387) and positive for CD204 antibody, and variably for EPO, EPOR and HIF-1α. In proximity to necrotic or solid growth pattern areas, also the neoplastic cells expressed EPO, EPOR and HIF-1α.

Discussion and Conclusions

The role of macrophages in recycling iron is well known, and HLMs are always identified in the liver and spleen. Their role in neoplasia is poorly understood, even if in human medicine they are recognized as "iron curtain" for their disposition in conspicuous groups in the stromal microenvironment [7]. The positivity for CD204 and the negativity for the Macrophage marker (MAC/387) allow to identify HLMs as M2 macrophages. The proneoplastic role of M2 cells is related to the production of several molecules that promote tumour progression and survival through mechanisms such as neoangiogenesis. HLMs express VEGF and its receptor, molecules that stimulate the proliferation of endothelial cells contributing to the stromal infiltration by inflammatory cells [4]. EPO and its receptor have proliferative, antiapoptotic and angiogenic roles with a loop of autocrine and paracrine stimulation influencing macrophagic polarization towards M2 phenotype [8]. Therefore, considering its expression in HLMs, EPO could lead to M2 phenotype in HLMs with the production of protumoral molecules. Additionally, the expression of neoangiogenic molecules is linked to the expression of HIF-1α [4,8]. HIF-1α positivity was found in HLMs, supporting the hypothesis that they could be involved in neoplastic survival, contributing iron both to neoplastic cells and the inflammatory microenvironment [7], such as TILs and TLSs. In conclusion, HLMs could act as an interfacial boundary mediating immune and metabolic response as an iron curtain also in CMTs, potentially representing a new prognostic or therapeutical marker.

Keywords: hemosiderin-laden macrophages, canine mammary tumours, tumour microenvironment, immune context, tertiary lymphoid structures

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The endometrial microbiome: New insights into equine endometritis

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Introduction and aim

In veterinary clinical literature, the uterus of a healthy mare has been considered sterile or to have a transient non-resident microbiota (1). However, this opinion was based on the classical culture-based approach, which can miss the great microbial diversity present in a specific environment (2). With the advent of the non-culture-based sequencing techniques targeting the 16S rRNA gene, the dogma of a sterile uterus has been challenged and it has been shown that the uterus of healthy mares hosts a resident microbiota (3). The aim of this study is to characterise the uterine microbiome of mares with endometritis using 16S rRNA sequencing and to compare it with the classical microbial culture technique.

Methods
Five mares with a history of subfertility, clinical signs of endometritis and a positive microbial culture test were included in this study. During the follicular phase, together with the swab for bacteriological culture, a double-guarded cytobrush was collected for the analysis of microbiome. DNA was then extracted from the cytobrush samples using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek). PCR amplification of the variable region V1– V2 of the bacterial 16S rRNA gene was performed. The amplicons were sequenced on the Illumina MiSeq platform and the DNA sequencing data were then analysed to assess the composition of the equine microbiome and compared with the results of the classical culture method.

Results
According to the bacteriological culture test, the pathogens isolated were *Streptococcus equi subs zooepidemicus* in four mares and *Escherichia coli* in one. After metagenomic sequencing, ASVs belonging to 14 bacterial phyla and 124 genera were found. The most represented Phylum was Firmicutes, followed by Proteobacteria, Actinobacteriota, Bacteroidota and Cyanobacteria. At Genus level, the most abundant genera were: *Streptococcus*, *Escherichia-Shigella*, *Corynebacterium*, *Arcanobacterium*, *Porphyromonas*, *Staphylococcus* and *Pseudomonas*. Of the four mares diagnosed with streptococcal infection by bacteriological culture, the genus *Streptococcus* accounted for 79% and 99% of the relative abundance in two mares, but only 3.4% and <1% in the other two, according to the sequencing results. One of these, however, showed a relative abundance of *Corynebacterium* of 44%. Furthermore, *Escherichia* dominated the microbiome of the last mare with 99% of the relative abundance.

Discussion and conclusions
With the advent of the 16S rRNA amplicon sequencing technique, a deeper understanding of not only the presence or absence of bacteria, but also the composition, diversity and abundance of the uterine microbiome can been achieved, providing valuable insights into the diagnosis and treatment of endometritis. This is the first study to describe the endometrial microbiome of mares with endometritis. The results of 16s sequencing analysis confirmed

the results of bacteriological culture, as the microbiome of most mares was dominated by *Streptococcus* and *Escherichia,* causing a status of dysbiosis. However, the relative abundance of the pathogens was highly variable: in mares classified as positive according to the culture technique, the relative abundance of the isolated pathogen ranged from 99% to <1%. This result is probably due to the limitation of the bacterial culture, which does not take into account the large bacterial diversity in the uterus. Another explanation could be due to the sampling technique, as uterine swabs or brushes only sample a small segment of the uterus and therefore focal infections may have been missed, leading to different results between the two techniques. In summary, the results of this preliminary study suggest that the endometrial microbiome of mares with endometritis is in a state of dysbiosis dominated by a few pathogenic bacteria. The lack of diversity and richness of the endometrial microbiome has previously been associated with endometritis in cows (4,5).

Keywords: endometritis, mare, horse, endometrial, microbiome, bacteria.

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In vitro developmental potential of wild European mouflon oocytes after short or long-term storage

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Introduction and aim

The European mouflon (*Ovis aries musimon*) is a wild native species resident in Sardinia Island (Italy) since the Neolithic age (1-3). In 1955, due to poaching and habitat loss, some animals were transferred to the nearby Giglio Island to avoid their extinction (1-3). The life on the island functioned as a time capsule, conserving ancestral genetic traits (1). Reproductive biotechnologies can safeguard these invaluable and irreplaceable animal genetic resources, through gamete conservation and *in vitro* embryo production (IVEP). In the present study, the *in vitro* developmental potential of cumulus-oocyte complexes (COCs), retrieved from the ovaries of ovariectomized mouflons, was evaluated after short- (overnight holding in Earle's/Hank's salts medium, EH medium) or long-term storage (cryopreservation through vitrification) in field conditions and assessed for nuclear maturation after *in vitro* maturation (IVM) in laboratory. Furthermore, *in vitro* oocyte maturation rate was correlated with the ovarian cycle stage.

Methods

After ovariectomy (Institutional review board approval n. 20/2022 University of Bari Aldo Moro), immature COCs were subjected to EH treatment (n. 23 COCs from 3 donors) (4) or vitrification (n. 26 COCs from 8 donors) (5) in field conditions. The choice of the storage strategy was conditioned by the surgery schedule, as for EH treatment it was necessary to not exceed 24 hours from collection to arrival at IVM laboratory. After short or long-term storage followed by IVM, nuclear chromatin configurations were assessed (X2 test). Serum estrogens levels were compared between subjects in follicular *vs* luteal phase (Unpaired Student's t test). Correlations with *in vitro* oocyte maturation rate were analyzed (X2 test). Statistical significance was considered at $p<0.05$.

Results

Both short and long-term storage technique allowed meiosis resumption with the achievement of the metaphase II stage, without statistically significant differences between them (19.0%, 4/21 vs 19.2%, 5/26). Estrogens levels in the follicular phase were significantly higher than those in the luteal phase (11.1 ± 2.6 pg/mL *vs* 4.2 ± 0.6 pg/mL, $p<0.001$). Oocyte nuclear

maturation rates did not differ between follicular and luteal phases (13%, 3/23 *vs* 25%, 6/24).

Discussion and conclusions

Overnight holding in EH medium and vitrification have the potential to preserve mouflon oocyte meiotic competence as highlighted by meiosis resumption up to metaphase II stage after IVM. However, short- and long-term storage techniques as well as IVM still need to be optimized in both domestic and wild species, as insurance against future calamities. In the present study, estrogen levels were measured for the first time in mouflon and were found to be in line with those of domestic sheep (6). Estrogens concentration in follicular microenvironment, from which COCs were isolated, did not influence the developmental potential of mouflon oocytes, possibly because they were exposed, in our *in vitro* experimental conditions, to 17-β-estradiol at micromolar concentration during IVM culture.

Keywords: mouflon; oocyte; field conditions; EH holding; vitrification; *in vitro* maturation (IVM); estrogens; bioenergetic-oxidative status.

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Exploring the impact of diacetyl and barberry on reproductive parameters in male wistar rats: A preliminary study

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Herdoğan, M., İnanç, M.E. Exploring the impact of diacetyl and barberry on reproductive parameters in male wistar rats: A preliminary study.

Introduction and aim

Diacetyl, a by-product of lactic acid fermentation, is a natural component of fermented foods and is used in many packaged foods as a flavor enhancer to produce a butter-like taste. Studies have shown that diacetyl ingestion leads to uncontrolled protein acetylation (1), alters cell membrane structure (2), increases reactive oxygen species production, and reduces antioxidant activity (3). Additionally, diacetyl is associated with cellular DNA damage and apoptosis (3). The absence of specific criteria for this artificial butter flavoring in foods means the daily intake of diacetyl by consumers is unknown. Barberries, widely distributed and native to Türkiye, have been studied for their anti-inflammatory, antimicrobial, antioxidant, and other pharmacological properties, and are used for medicinal purposes and as food (4). This study aims to investigate the effective doses of diacetyl on male reproductive health and spermatological parameters and the effective dose of Barberry (*Berberis crategina* DC.) on these parameters.

Methods

In this study, seven groups of 30 animals were formed to determine the effective doses of diacetyl and barberry. The control group received only water, with doses of 25, 150 and 300 mg/kg for diacetyl (DA) and 150, 300 and 600 mg/kg for barberry (B) administered to the other groups respectively. All animals were administered oral gavage daily for 42 d. Throughout the experiment, body weight, water consumption, and feed intake of the animals were recorded. At the end of the experiment, animals were euthanized by cervical dislocation under anesthesia. Spermatozoa were obtained from the right epididymis by trimming and motility, concentration, and abnormal spermatozoa were examined. Furthermore, mitochondrial reactive oxygen species levels, lipid peroxidation levels, nitrosative stress levels, plasma membrane and acrosome integrity, and viability parameters were analyzed using flow cytometry. The weights of the testis, epididymis, vesicula seminalis, and prostate were recorded and calculated as relative organ weights. The left testis and epididymis were excised for histopathological examination.

Results

DA150 and DA300 groups had increased water consumption compared to DA25, B150, B300 and control groups ($p < 0.001$). Furthermore, the DA300 group exhibited elevated feed intake compared to all other groups, except for the DA150 group ($p < 0.001$). Considering spermatological findings, DA300 and DA150 groups had the highest lipid peroxidation levels ($p < 0.001$), with DA300 showing the lowest viability ($p < 0.05$). Nitrosative stress was higher in B600 group compared to B150, B300 and control ($p < 0.05$). Spermatozoa concentration in the DA300 group was lower than in the DA25, B600, control ($p < 0.05$), and B300 ($p \leq 0.001$) groups. The higher morphological integrity was observed in the B300 group compared to the DA25, DA150, and DA300 groups ($p < 0.05$). Sperm head abnormalities were higher in all DA groups than in the control and B groups ($p < 0.05$). Histopathological examination revealed that the seminifer tubule diameter was larger in all DA groups than in the control and B300 groups ($p<0.001$). Additionally, non-diffuse germ cell exfoliation was observed in the DA300 group.

Discussion and conclusions

No study has been found with diacetyl or barberry in terms of spermatological parameters. Bawazir (5) reported tubular atrophy, deformed Sertoli cells, oedema in the inter-tubular space, and hyperemia in vessels among testicular histopathology findings in rats exposed to 25 mg/kg DA for 2 and 4 weeks. In this study, oedema and hyperemia appeared in all groups, including the control. According to Creasy and Chapin (6), these findings are consistent with the normal testicular histopathology. The study indicated that DA exposure negatively affected spermatological parameters, particularly at a 300 mg/kg dose. Additionally, 300 mg/kg B yielded better spermatological results than the other B groups and the control group for some parameters. This preliminary study suggests that doses above 300 mg/kg DA may produce more significant results in reproductive toxicology studies.

Keywords: Diacetyl, Barberry, Spermatologic parameters, Reproduction.

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Effect of uterine ozone insufflation on mares affected by chronic endometritis

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Introduction and aim

Endometritis is one of the main causes of subfertility in mares and causes huge economic losses in the equine breeding industry. The onset of an endometrial inflammatory response occurs physiologically following each reproductive event with the aim of eliminating semen and bacteria introduced into the uterine lumen; the persistence of this condition beyond 48 hours after breeding lead to the pathological condition of persistent breeding-induced endometritis (PBIE). If not controlled, PBIE can hesitate in a chronic state, characterized by degenerative changes and fibrosis of the endometrium [1]. Given the lack of response to traditional therapies, especially in chronic forms, alternative therapies such as ozone therapy (O_3) have recently been proposed [2][3].

The present study aimed to describe uterine O_3 effects in mares affected by chronic endometritis in terms of pregnancy rates and endometrial biopsy (EB) findings.

Methods

Eleven commercial mares (N=11), aged between 10-20 years, barren for at least one year were included in the study. All the mares were followed by the same veterinarian specialized in equine reproduction and had an history of unsuccessfully conventional treatments.

During the first estrous cycle, the animals underwent EB followed by O_3 treatment; in the second estrous cycle, EB was repeated, and artificial insemination (AI), with fresh, chilled or frozen semen from different commercial stallions, according to the breeding schedule of the mare, was performed. Pregnancy diagnoses were performed 14 days after ovulation and, if positive, monitored by ultrasound until parturition. Treatment consisted in the insufflation of the uterus with O_2-O_3 gas mixture (volume: 300 ml; [O_3]: 40 µg/ml) for 3 consecutive days through an AI pipette and 60 ml syringes filled with gas obtained by an ozone generator.

Samples collected from EB underwent i) histopathological grading according to the Kenney & Doig 1986 classification, ii) Picrosirius Red Staining for histological visualization and quantification of collagen fibers, and iii) endometrial gene expression analysis of markers of either fibrosis (Matrix Metallopeptidase 2 [MMP2], Matrix Metallopeptidase 9 [MMP9], Metallopeptidase Inhibitor 1 [TIMP1], Metallopeptidase Inhibitor 2 [TIMP2]) and local innate immune response (Defensin Beta 4B [DEFB4B], Lysozyme [LYZ], Secretory Leukocyte Peptidase Inhibitor [SLPI]).

Fisher exact test was used to compare pregnancy rates and histopathological grades before and after O_3 treatment, while Wilcoxon test was employed for either fibrosis (Picrosirius Red Staining) and gene expression analyses (pre- vs post- O_3 treatment).

Results

After O_3 treatment, 10/11 were pregnant at 14 and 60 days ($P<0.001$), 9/11 at 90 days ($P=0.002$) and 9/11 carried the pregnancy to term and gave birth to a live foal ($P=0.002$). Histopathological grade according to Kenney & Doig improved after O_3 treatment in 5/11 mares (NS). The amount of fibrous tissue in terms of collagen fibers was reduced after O_3 treatment in 8/11 mares with no statistical significance ($P>0.05$). No differences in the level of expression of all the genes considered (MMP2, MMP9, TIMP1, TIMP2, DEFB4B, LYZ, and SLPI) were found between before and after O_3 treatment.

Discussion and conclusions

In conclusion, intrauterine O_3 treatment for chronic endometritis was able to improve pregnancy rates in barren mares. Further studies in a larger number of animals are warranted to confirm these results and better describe the effects of O_3 on uterine quality.

Keywords: endometritis, endometrium, ozone, subfertility, mare, gene expression

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Post-partum ovarian recovery in cinisara cattle

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Salvatore, M., Stefania, D.G., Valentina, P., Gabriele, M., Vito, B., Santo, C. Post-partum ovarian recovery in cinisara cattle.

Introduction and aim

The postpartum period, in cattle, is characterised by a variable anoestrus time. Generally, in most cows, the follicular growth resumes within 7-10 days. This is associated with a transient increase in FSH hormone that occurs around 3-5 days after calving [1]. In dairy cattle with good management, the first ovulation usually occurs at about 15 days postpartum. However, beef cows with good body condition scores normally have 3 follicular waves before the first ovulation postpartum, which occurs at about 30 days. Nevertheless, beef cows in poor body condition may have as many as 10 waves and ovulate for the first time at about 100 days [1]. Several factors influence the number of anovulatory cycles. Generally, the first ovulation is silent (i.e., no oestrus behavioural), followed by a short cycle in more than

70% of cases. Dual-purpose cows should have an intermediate situation, although they are often reflected more in beef cattle [2]. The Cinisara cattle is a native Sicilian dual-purpose breed raised in semi-extensive conditions. The calving period is generally between fall and spring; however, she can calve round-year. Traditionally, the bull is removed on the 8 of December. Anecdotally, it is reported that this breed after calving becomes pregnant very early and can have an inter-partum of less than 365 days (two calves per year) [3]. This study aimed to investigate the postpartum period in the Cinisara cow to ascertain its precocity of ovarian recovery through ultrasound and blood progesterone concentration assessment.

Methods

This study included 20 three-year-old Cinisara cows and received the positive approval of the ethics committee of the Department of Veterinary Sciences of Messina. The bovines were healthy in their third lactation. They came from two herds (same management) in the Cinisi area (Palermo, Sicily). All historical data were acquired to calculate the inter-partum (more than 600 calvings). During the investigation, all animals were milked and suckled the calf at the same time. Clinical and ultrasound examinations were performed every 3 days until 51 days after calving. The experiment was designed after the calvings of September. Blood samples were collected for progesterone (P4) assay (Speed reader, Virbac). The ultrasound exam was performed to record and measure the dominant follicle diameter (>8 mm) and corpus luteum (Draminski iScan 2, 7.5 Mhz linear probe). At about 100 days post-partum an ultrasound exam was performed to confirm and stage eventual pregnancies, measuring the foetal biometry [4].

Results

The first dominant follicle's onset was observed 10 to 14 days post-partum, without oestrus behaviour. Only 2 cows (group 1) ovulated with the formation of a short corpus luteum (9 days), followed by a manifest heat about 20-22 days after parturition, followed by a regular lutein phase (18 days; P4=5-6 ng/ml). In 4 cows (group 2) the first follicular wave was anovulatory and the first ovulation was at 20-22 days without oestrus signs. The corpus luteum was short and was followed by more manifest heat

at about 31-33 days after parturition with a regular luteal phase. The first follicular waves were anovulatory in 12 cows (group 3), a follicle ovulated about 30 days after calving with a short corpus luteum, followed by more evident heat at about 40 days after parturition. At pregnancy diagnosis, 12 cows were pregnant with a staged pregnancy between 30 and 60 days (median 45 days). Cross-referencing staging and calving date, the calving-conception interval (considering only pregnant cows) had a median of 75 days, corresponding to a median inter-partum of 355 days. The retrospective documentary survey revealed an average inter-partum value of 390 days, corresponding to a delivery-conception interval of about 100 days.

Discussion and Conclusions

The results of this study demonstrated a good grade of precocity in some Cinisara cows, placing themselves in an intermediate situation between dairy and beef cows. The inter-partum value is excellent, in line with other dual-purpose cows with medium productivity. Furthermore, the data suggest that Cinisara has a fast ovarian recovery that traces some of the best dairy breeds in some individuals. This is related to a certain precocity in conception by a fair percentage of cattle. In agreement with previous observations, Cinisara cows may be mounted as early as 30 days after calving but generally become pregnant in later heats [3]. However, Cinisara can concept before 100 days after calving and have an inter-partum of less than 1 year. It is not uncommon to find Cinisara cows with two calves (one newborn and one about 1 year old) born in the same year. The precocity of postpartum ovarian recovery and the short calving-conception interval are typical features of dairy cows, which are generally not nutritionally stressed, more rarely than grazing beef cows, but always with good feed supplementation and BCS value [1,2]. Although breeders generally give food integration during postpartum, this breed feeds mainly on pasture and decidedly very poor pasture. Precocity is, likely, an inherent characteristic of this native Sicilian breed.

Keywords: bovine, ultrasound, follicular wave, progesterone, first ovulation

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Is the FOXL2 gene mutation involved in the development of granulosa cell tumours in equines?

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Palmieri, V., Giambrone, G., Vullo, C., Marino, G., Enrico, M.G., Catone, G. Is the FOXL2 gene mutation involved in the development of granulosa cell tumours in equines?.

Introduction and aim

Granulosa-theca cell tumour (GTCT) is a significant cause of infertility in mares, representing for 85% of ovarian tumours and approximately 2.5% of all neoplasms in horses [1]. In women, granulosa cell tumours (GCT) comprise about 5% of ovarian neoplasms and 70% of ovarian sex cord-stromal tumours. Human GCTs are categorized into adult and juvenile types, distinguished by clinicopathologic features. The adult type, the most common, occurs in peri- or post-menopausal women, while the juvenile type, affects prepubertal girls and young women [2]. In the adult type, a somatic "missense" mutation in the FOXL2 gene (402C□G; C134W) is present in 97% of cases but is absent in the juvenile type [2]. Adult GCTs exhibit a variety of histological patterns from well-differentiated to

poorly differentiated. Well-differentiated patterns include microfollicular, macrofollicular, trabecular, insular, solid tubular, and empty tubular. "Call-Exner bodies" are typical. Adult GCTs contain round to oval cells with characteristic "coffee-bean" nuclei, few mitotic figures, mild nuclear atypia, scant cytoplasm and often luteinized cells. Juvenile GCTs share the macroscopic appearance with the adult type but differ significantly histologically, exhibiting a follicular or solid diffuse pattern with larger luteinized granulosa cells containing hyperchromatic or markedly bizarre nuclei and high mitotic activity [3]. This study aimed to explore if this categorization is possible in equines.

Methods

12 cases of equine GTCT were obtained by unilateral ovariectomy, using a flank-assisted laparoscopic technique. Ovaries were classified based on their macroscopic and microscopic appearance. For histopathology specimens were fixed in formalin and embedded in paraffin blocks. Slides were evaluated by experienced veterinary and human pathologists. GTCTs were classified according to the most prevalent pattern [4]. Additionally, a molecular test for the FOXL2 gene mutation was performed. The DNA was extracted using a commercially available kit, from 12 samples of formalin-fixed paraffin-embedded (FFPE) tissue. PCR was used to amplify the DNA region of interest before Sanger sequencing.

Results

The median age of the mares was 11.6 years (3-25 years). All mares presented infertility problems; 8/12 exhibited stallion-like behaviour, while the remaining mares showed anoestrous or abnormal oestrous behaviour. All cases showed unilateral ovarian enlargement and a hypotrophic contralateral ovary. Macroscopically, the neoplastic ovary had an ovoid or spherical shape with a smooth surface, enclosed by a fibrous capsule. The cut surface revealed a multicystic structure in 9 cases and a solid structure in 3 cases. Cystic structures contained serous or serohaematic material, surrounded by solid connective septa of varying thickness and a greyish-white or yellowish colour. Histological patterns included follicular (macrofollicular, microfollicular, or macro-microfollicular) in 6/12 cases, solid (insular, tubular,

and diffuse) in 2/12 cases, and mixed in 4/12 cases. The cell population was characterized by neoplastic granulosa cells in all cases and luteinized cells in 12/12 cases. Additionally, 6/12 cases presented "Sertoli-like" elements and 12/12 cases exhibited a fibro-thecal component. Call-Exner bodies were present in only 1/12 cases. Mitotic activity was low in all cases. The FOXL2 amplification and sequencing demonstrated a missense point mutation at position C135W, with a cytosine for guanine, in only one case (8.3%).

Discussion and conclusions

Comparing the morphological features observed in these cases with the two subtypes of the tumour in women, pathologists agreed that mare cases exhibit greater homology with the adult type of the human tumour regarding growth patterns and low mitotic activity. Even the age of onset of equine GTCTs (median of 11.6 years) is similar to the human adult type. The typical features of the juvenile form in women were not observed in any equine cases, including the case in a 3-year-old mare, which could be age-equivalent to the juvenile form in women. Only 1/12 equine cases presented the classic Call-Exner bodies, suggesting these structures are not so typical in equine tumours. In contrast to the adult type, equine GTCT seems to not carry constantly the FOXL2 mutation (1 case, 8.3%). In horses, the FOXL2 gene consists of 378 amino acids, compared to 376 amino acids in women, explaining why the amino acid change occurs at position C134W in women and C135W in mares. The lack of mutation of FOXL2 in most cases would suggest a juvenile-type similarity. Likely, equine GTCTs cannot be separated into juvenile or adult types. FOXL2 mutation cannot be used as a diagnostic marker [5] lacking in most of the tumours and it has probably a limited role in the genesis of these tumours in this species.

Keywords: Mare, ovary, neoplasm, histopathology, gene mutation

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Reproductive health effects in the male dog following oral lepidium meyenii supplementation

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Zappone, V., Gattuso, D.T., Tomasella, C., Cavallo, C., Cristarella, S., Pastore, S., Troisi , A., Quartuccio, M. Reproductive health effects in the male dog following oral lepidium meyenii supplementation.

Male infertility can have serious financial implications for dog breeders. A major threat to semen quality, both in vivo and in vitro, is the reactive oxygen species (ROS) accumulated by spermatozoa during spermatogenesis and steroidogenesis. Sperm function is not impaired when ROS and antioxidant levels are in balance, as this ensures that no significant damage occurs [1]. However, metabolic oxidative stress caused by excessive ROS production

or low antioxidant status, or both, can lead to impaired sperm function. Therefore, ensuring low levels of ROS is crucial for proper fertilisation, particularly for capaCitation, hyperactivation and the acrosomal reaction [1]. A promising approach to improve semen quality and male reproductive health is the use of antioxidants that regulate ROS levels. *Lepidium meyenii* (Maca) is a plant that has become popular due to its antimicrobial, antioxidant and anti-inflammatory activities [2]. Maca is rich in valuable nutrients and secondary metabolites such as macamides, alkaloids and glucosinolates. Macamides and glucosinolates reduce free radicals and protect cells from oxidative stress [3]. The aim of this study was to assess the effects of oral supplementation of *Lepidium meyenii* on the quality of canine semen and its storage under refrigerated conditions at 5°C, and on serum levels of testosterone and canine prostate-specific esterase (CPSE). Forty male dogs were enrolled in the study. Subjects with semen parameters incompatible with adequate reproductive capacity and who had experienced at least one reproductive failure in the 6 months prior to the study, either by natural mating or artificial insemination, were classified as subfertile (n=20). Subjects with semen parameters consistent with adequate fertility and a normal reproductive history in the 6 months prior to the study, with a numerically representative litter from both natural mating and artificial insemination, were classified as normo-fertile (n=20). The subjects in the subfertile (n=20) and normofertile (n=20) groups were further divided into a control group (SC group, n=10; NC group, n=10) and a treatment group (ST group, n=10; NT group, n=10). Dogs in the treatment groups received Maca in their diet in a capsule formulation (75 mg/kg), while the control groups received placebo (starch) capsules. Three semen samples were collected from each subject at three points in the semen cycle: immediately before the start of oral supplementation (T_0), after 31 days (T_{31}), and after 62 days (T_{62}). Blood samples were collected at the time of sperm collection and used to assess testosterone and CPSE concentrations. Sperm collection was performed after removal of the extragonadal reserve to minimise defects in sperm stored in the epididymis. The ejaculate was fractionated by discarding the third fraction and immediately assaying the first two fractions. Semen was evaluated for volume, concentration, motility, morphology and membrane integrity. An aliquot of the fresh semen analysed above was centrifuged

and the supernatant seminal plasma removed to optimise storage at 5°C. The remaining pellet was diluted appropriately with CaniPRO™Chill10 extender. Samples were immediately refrigerated and stored at 5°C. Total motility, progressive motility and membrane integrity were assessed at 3 (T_3), 24 (T_{24}), 48 (T_{48}) and 72 hours (T_{72}). The results of the study indicate that oral supplementation of 75 mg/kg Maca extract in dogs results in an overall improvement in male reproductive health, with beneficial effects on both seminal parameters and serum testosterone and CPSE levels. In fact, sperm parameters such as ejaculate volume, total sperm count, motility, morphology, and membrane integrity improved significantly. Furthermore, semen parameters evaluated after storage at 5°C showed less decline over time in sperm samples from subjects who had received the oral Maca supplementation compared to those from subjects in the control groups. Finally, an increase in serum testosterone levels and a decrease in CPSE levels were also observed. In conclusion, the results of the present study indicate that oral supplementation of Maca as part of the diet of male dogs resulted in significant improvements in their reproductive health. The observed positive effect on male canine fertility suggests that the use of Maca as a dietary supplement could be a promising strategy to improve semen quality and support reproductive health in the canine population.

Keywords: Lepidium meyenii, Maca, Dietary supplementation, Canine semen, Cooled semen, Testosterone levels, CPSE levels.

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Artificial insemination success in cattle: Efficiency analysis of techniques used in estrus detection

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Introduction and aim

Cattle breeding is a vital component of Turkey's agricultural sector and significantly contributes to the economy and rural livelihoods. Increasing reproductive efficiency is crucial for sustainable production. Artificial insemination (AI) offers genetic improvement, disease control, and better reproductive performance, but its success depends on accurate estrus detection.

This study investigates the efficiency and effectiveness of AI and estrus detection techniques in cattle breeding. The primary objective is to improve cattle population management and increase pregnancy success rates through a comparative analysis of estrus detection methods such as behavioral cues, vaginal examination, and ultrasonography. By achieving these objectives, the study aims to improve reproductive management practices and overall productivity by providing veterinarians with up-to-

date data on estrus detection and artificial insemination in cattle breeding in Turkey.

Methods

The study was conducted using surveys and interviews with 23 veterinarians from various farm sizes: family-type (1-10 cattle), small (11-60 cattle), and medium/large (61+ cattle). Data on estrus detection methods and AI practices were collected and analyzed. Specific cattle at each farm were examined for health status, signs of estrus, and AI applications. The survey included questions on estrus detection techniques, AI programs, semen manipulations, hormonal treatments, and herd genetics.

Results

The survey revealed diverse usage and effectiveness of estrus detection methods. Behavioral signs were the most commonly used method (90%), followed by vaginal examination (75%) and ultrasonography (60%). Among these, ultrasonography showed the highest accuracy rate at 95% ($p < 0.05$). The study found that AI performed 12-18 hours after detecting estrus had an 80% success rate ($p < 0.05$). Additionally, cattle in optimal health conditions had an AI success rate of 85% compared to 50% for cattle with health issues. Water baths at 30-37 °C were commonly used for sperm thawing (62%, $p < 0.05$). These results highlight the importance of accurate estrus detection and optimal health conditions for improving AI success rates in cattle.

Discussion and Conclusions

The findings indicate potential for improving estrus detection and AI techniques. Combining different detection methods can enhance accuracy and improve AI success rates. According to Alves et al. [1], the accuracy of estrus detection methods in dairy cows can significantly impact AI outcomes, suggesting that precise detection methods are crucial for success. Developing strategies to refine AI techniques and creating educational materials for cattle breeders and veterinarians can bridge the knowledge and practice gaps. Barth [3] highlights that education and proper training of breeders play a vital role in improving AI success rates. Increasing the use of technological devices that provide objective and accurate results

will be effective in AI success. Barbosa et al. [2] found that technological advancements in estrus detection, such as ultrasonography, have shown higher accuracy rates compared to traditional methods. Future studies should focus on integrating advanced technologies such as smart herd management systems and smartphone applications to further improve estrus detection and reproductive management. Jemal and Lemma [5] emphasize the importance of integrating modern technologies to enhance the accuracy and efficiency of AI practices.

Keywords: Artificial Insemination, Cattle, Estrus, Estrus Detection, Reproductive Efficiency.

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Artificial insemination in small and large dog breeds: Retrospective study

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Citation
Kahraman, D., Güngör, Ş., İnanç, M.E., Herdoğan, M., Çay, H.A., Mart, F., Ata, A. Artificial insemination in small and large dog breeds: Retrospective study.

Introduction and aim

The practice of artificial insemination in dogs, which was first performed approximately 250 years ago and resulted in the successful production of offspring, remains a relevant topic in veterinary medicine (1). Retrospective evaluation of past practices is of great value, both to enable physicians to evaluate their practices and to contribute to the literature. This study aimed to evaluate the spermatological parameters and pregnancy rate of artificial

insemination in dogs brought to the Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Animal Reproduction and Artificial Insemination Clinic, between 2016 and 2024.

Methods

In the study, 12 small (6 Pomerianan, 1 Yorkshire Terrier, 1 Shih Tzu, 3 French Bulldog) and 8 large (2 Anatolian Shepherd Dog, 2 Mixed breeds, 1 German Shepherd, 1 Great Dane, 1 Chow Chow, 1 English Bulldog) breed dogs were used. The breeds were classified according to their weight, with small breeds < 25 kg and large breeds > 25 kg. All male dogs were between 1 and 7 years old. All female dogs were between 1.5 and 6.5 years old. Anamnesis and vaginal smear methods were used to determine the estrus period in female dogs. In addition to general information, participants were asked for details about the first instance of proestrus bleeding, the presence of a male dog, observations of mating behavior, and the history of the female and male dogs about the fertility of healthy offspring. In the vaginal smear method, samples were taken from the vagina of the female using a cotton swab stained with giemsa dye. Female dogs with a keratinized superficial cell density of $\geq$ 70% were artificially inseminated. The female dogs that were determined to be in estrus were inseminated intravaginally with fresh semen obtained from same breed male dogs by digital manipulation technique. Before insemination, spermatological examinations (motility, density, and spermatozoa concentration) were performed. The collected semen was drawn into a syringe and connected to an artificial insemination catheter by using an adjuvant. On the insemination stand, the females were lifted by their hind legs when their front side down. The artificial insemination catheter was considered at a 45-degree angle from the vulva. Semen was deposited in front of the posterior cervix. The female was kept with her hind legs in lifted for 15 min to allow the semen to pass into the uterus more quickly.

Results

After artificial insemination, information about births was requested from the owners of dogs brought to the clinic. In total, the pregnancy outcomes of 20 dogs were recorded: 13 females became pregnant and gave birth, while seven did not conceive. The average age of the dogs was 3.35$\pm$0.36

years. The mean age of the small-breed group was 2.9±0.36 years, while that of the large-breed group was 4±0.70 years. A statistically significant difference was observed in terms of volume (0.94±0.51 vs 3.38±0.78 ml) ($p < 0.05$) and concentration (169.12±63.27 vs 176.75±25.01 $\times 10^6$/ml) ($p < 0.01$) between small-breed and large-breed dogs, respectively. Furthermore, a positive correlation was observed between male age and semen volume ($p < 0.05$). The pregnancy rate was 66.66% in small-breed dogs and 62.5% in large-breed dogs; however, no statistically significant difference was observed between the breeds in terms of pregnancy rate ($p>0.05$). Nevertheless, a positive correlation was detected between pregnancy with motility and semen concentration, as well as between concentration and motility ($p<0.05$). No statistically significant difference was found between the spermatological parameters of pregnant and non-pregnant animals. However, the average motility values of pregnant animals were 79.08%, concentration 247.36 $\times 10^6$/ml, and volume 1.50 ml.

Discussion and conclusions

In the examinations conducted using fresh dog semen from the animals presented to our clinic for artificial insemination, differences in density and volume were observed in small and large breeds, as expected from a spermatological perspective. In dogs, the total number of spermatozoa in the ejaculate ranges from 400 $\times 10^6$ to > 1.000 $\times 10^6$, and is related to body weight. As a general rule, dogs should produce ~10 $\times 10^6$ spermatozoa/lb body weight (2-3). This finding was consistent with our results. A similar study reported that dog size affects semen volume and concentration. Dogs of larger sizes exhibit greater concentrations and volumes than smaller dogs (4). In conclusion, age and size can affect the main spermatological parameters, which should be considered during routine semen evaluation.

Keywords: Artificial İnsemination, Dog, Retrospective, Pregnancy, Fresh Semen

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iNOS and TSHR localization in follicles and corpus luteum during development in Bruna cow ovaries

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Fauci, D.L., Aragona, M., Aronica, V., Fazio, E., Medica, P., Cravana, C. iNOS and TSHR localization in follicles and corpus luteum during development in Bruna cow ovaries.

Introduction and aim

Numerous studies on both animal and human ovaries have demonstrated that thyroid-stimulating hormone receptor (TSHR) and inducible nitric oxide synthase (iNOS) play a crucial role in regulating ovarian functions such as folliculogenesis, oogenesis, ovulation, puberty onset and the lifespan of the corpus luteum (CL) (1-6). This study aimed to examine the immunolocalization of TSHR and iNOS in the ovarian follicles and in the corpus luteum (CL) to determine if their expression is influenced by their different developmental stages in the cow's ovary.

Methods

Ten ovaries were collected from 5 Bruna cows. The estrus cycle phases have been determined post-mortem (7). Three of them were cyclic (follicular, early luteal and late luteal phases), aged between 12-24 months, two were acyclic (prepubertal and in anestrus, 10 months and 8 years old, respectively) and the ovaries have been classified based on the presence or absence of the CL, in CL+ and CL- ovaries. The immunohistochemical (IHC) processing for iNOS and TSHR has been performed. The evaluation of the peroxidase reaction has been done by quantifying the intensity of staining and classifying it by gradation (from negative to strongly positive).

Results

Our results confirmed the presence of TSHR and iNOS in various structures of the bovineovary in their different developmental phases. Also, the primordial, primary, secondary, terziary follicles within the same ovary showed up a different immunolocalization. The immunostaining was present or absent in both the germinal and somatic follicular components with different combinations. Specifically, follicles in the same developmental stage within the same ovary either expressed or did not express TSHR and iNOS, indicating phases where expression was silenced. The iNOS reaction was strongly present also in the wall of corpora atretica, suggesting a role during the follicle atresia processes by a crosstalk with the inflammatory infiltrate. Furthermore, the research revealed the immunolocalizaton of TSHR and iNOS in the CL, in which the expression changed based on the different developmental phase: the protein immunolocalization was strongly present in the large luteal cells during the late diestrus with a decrease in the subsequent estrus phases until a total silencing for the TSHR in the corpus albicans.

Discussion and Conclusions

Our results may indicate that these proteins play a critical role in the exit from dormancy and, consequently, in the development or atresia of follicles and oocytes. Inaddition, their different expression in the different CL stages may imply for these proteins a putative involvement in the growth, maintenance, and regression of bovine luteal tissue. The findings validated the role of the iNOS-nitric oxide (NO) and TSHR pathway in regulating the dynamic vascular processes associated with the morpho-functional changes in the bovine ovary.

Keywords: gonad, bovine, luteal tissue, immunohistochemistry, atresia, dormancy

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Dirofilaria repens in the testis of six dogs

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Giorgio, S.D., Napoli, E., Benedetto, G.D., Sfacteria, A., Gaglio, G., Brianti, E., Marino, G. Dirofilaria repens in the testis of six dogs.

Introduction and aim

Dirofilaria repens (Spirurida: Onchocercidae) is the causative agent of canine subcutaneous dirofilariosis (1). Adults of *D. repens* localize in the subcutaneous tissues and fasciae and most of the infected animals are asymptomatic though dermatological disorders have been described sporadically (2). Also, the presence of *D. repens* adults in different districts regarded as unusual such as in the testes (3,4) have been reported. In humans, *D. repens* subcutaneous migration can cause local swellings with variable final locations, being male and female gonads frequently reported (5). Some authors suggested that the genital localization as well as the mammary glands could be related to chemotactic stimulus caused by hormones produced by these organs (6). The present study aims to report six cases of *D. repens* in the canine testis.

Methods

Between December 2020 and January 2022, six canine cases (median age 48 months) with the presence of parasites in the testes were found. They were all incidental findings coming from different area of Sicily (Italy): Syracuse (n. 4), Catania (n. 1) and Agrigento (n. 1). No scrotal or testicular

enlargement or inflammation were suspected clinically. Orchiectomy was performed using a closed castration technique and during the surgery, the presence of whitish worms on the surface of the testicle within the *tunica vaginalis* layers was noticed. After that, the dogs underwent an extensive parasitology examination and blood samples (K3EDTA) were collected to perform a modified Knott's test (7). Parasites were fixed in 70% ethanol for morphological identification. Testes were fixed in 10% formalin and processed for histological examination.

Results

Thirteen adult nematodes (5 males and 8 females), clinical signs attributable to subcutaneous dirofilariasis as well as skin alterations were not observed. All the retrieved nematodes were morphologically identified as *D. repens*. The male worms were shorter than the females, showing a ML and MW of 65 ± 3 mm (range 62 - 68 mm) and 3.8 ± 1 mm (range 3.7 to 3.9 mm), respectively. The female ML and MW were 138 ± 8 mm (range 130 - 146 mm) and 4.8 ± 1 mm (range 4.7 - 4.9 mm), respectively. The nematodes were characterized by a rounded whitish body and distinct longitudinal ridges on the external cuticle surface were observed. In both males and females, the anterior extremity was rounded with a simple buccal capsule without marked lips, and small cephalic papillae. The esophagus was not clearly differentiated in muscular and glandular region and connected to the intestine by a simple valvular junction. The caudal extremity of the males was rolled in a spiral and featured by a conical shape. Circulating microfilariae were found in 5 out of the six positive dogs. The microfilaremia ranged from 10 to 500 mfl/mL and in all cases the microfilariae were identified as *D. repens*. There were not gross or histological inflammatory or degenerative changes in the testicular parenchyma of infected dogs.

Discussion and conclusions

Testicular localization of *D. repens* in dog is regarded as uncommon, indeed, so far, only 3 cases have been described in Europe (3,4,8) and 5 in India (9). Many reports have speculated on the causative reasons of testicular localization in human patients such as the lower temperature of this area, as well as a specific tropism due to the higher concentrations of sexual

hormones (10). In this report all adult parasites were localized within the *tunica vaginalis* and in any case the parasites were found in the testicular parenchyma, unlike what observed by Demiaszkiewicz (2013) in a dog from Mazovian province. As regards the presence of circulating microfilariae, only one of the six *D. repens* adult positive dogs scored negative for the Knott's Test. The present study confirms the previous reports of *D. repens* in dog testes and considering the zoonotic potential of this parasite, it is important to provide active surveillance in areas where *D. repens* is present to prevent the presence of reservoir or the spreading of the infection in case of dog movements between endemic and non-endemic areas.

Keywords: nematodes, dirofilariasis, *tunica vaginalis*, genital localization, castration.

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Impact of atrazine on the function and structure of bovine epididymal spermatozoa

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Introduction and aim

In the 20th century, the increase in the world's population was accompanied by the need for food supply, which led to the adoption of intensive agriculture using pesticides such as atrazine (ATZ). Despite its efficacy in controlling weeds, ATZ persists in both ground and surface water, even in areas where it has not been used for many years. This raises environmental and health concerns since ATZ is classified as an endocrine disruptor(1). It has been linked to alterations in endocrine and reproductive functions across various mammalian species. Specifically, ATZ alters the testis endocrine function, including testosterone release which may contribute to the damage observed in Leydig and Sertoli cells, which are crucial for

spermatogenesis(2). However, the direct effects of ATZ on the later stages of spermatogenesis, particularly on sperm stored in the epididymis, are not understood. Understanding these effects is essential for developing strategies to mitigate the risks posed by ATZ. This study aims to investigate the impact of ATZ exposure on bovine sperm function and viability, specifically, to assess whether bovine spermatozoa at different stages of epidydimal maturation (head, body and tail) exhibit varying sensitivities to this pesticide. A range of assays was conducted to evaluate parameters of sperm function and viability including mitochondrial activity and oxidative stress-related markers.

Methods

Six pairs of bovine testes (aged>12 months) were obtained from a local abattoir and stored overnight at 5ºC. The epididymis was then isolated from the testis and the epididymal head, body and tail were dissected to allow spermatozoa from each compartment to be collected separately. The sperm suspensions obtained were assessed for subjective total motility (epididymal tail), concentration and total number of sperm (Neubauer) and viability of spermatozoa (Eosin-Nigrosin). In addition, the JC-1 dye was used to evaluate Mitochondria Membrane Potential (MMP). Fluorescent intensity was measured using a Cytation3 Imaging Reader (BioTek-Instruments, USA) and the ratio of the fluorescent intensity of JC-1 aggregates to monomers was used as an indicator of MMP. After evaluation, a total of $30x10^6$ sperm from each epididymal compartment were exposed to three different concentrations of ATZ: 0.1µM, 1µM and 10µM. These concentrations were guided by data from other relevant experimental model studies(3). ATZ was dissolved in DMSO (0.01% maximal concentration) and was found to have no deleterious effect on sperm in the current study. Sperm incubation was performed in Tris-citrate-fructose medium with or without ATZ at 37°C for 2 and 4 hours. After 2 and 4 hours of incubation, motility (tail), viability and MMP of the SPZ were reevaluated. In addition, aliquots of the samples before exposure to ATZ and at the end of the 2 and 4 hours of incubation were centrifuged for 5 minutes at 500g. At the end of centrifugation, the supernatant was collected, transferred into a new tube and immediately stored at -80ºC to preserve its integrity for subsequent analyses. The stored samples were subjected to metabolomic analysis using Nuclear Magnetic

Resonance (NMR), to comprehensively profile the metabolic changes. The Ferric Reducing Antioxidant Power (FRAP) assay was used to quantify the overall antioxidant capacity of the media post-incubation. Statistical analysis was performed using GraphPad-Prism 8 (GraphPad-Software, San Diego, USA) and an ANOVA model using concentration, time and their interaction as fixed effects was performed. When differences were observed, a Post hoc comparisons were performed using Scheffé test. For the MMP data, all values in the fluorometric assessment were analyzed fold variation to the control (control DMSO) before analysis. Data are expressed as Mean±SD. For all analyses, $P<0.05$ was considered significant.

Results

No significant differences ($p>0.05$) were observed in the viability and MMP of spermatozoa from the epididymal head, body and tail between the experimental groups incubated with and without ATZ for 2 or 4 hours. Similarly, there were no differences ($p>0.05$) in the motility of sperm from the epididymal tail between the groups incubated with and without ATZ for 2 or 4 hours. However, the incubation duration itself had a significant impact on sperm viability, independent of ATZ exposure. Specifically, extending the incubation time from 2 to 4 hours significantly decreased ($p<0.05$) sperm viability in samples obtained from the epididymal head (58.6±11.8% to 50.8±16.3%) and body (61.9±11.7% to 49.4±15.4%), but not from the epididymal tail (75.3±9.3% to 70.4±13.1%). These findings suggest that while ATZ exposure did not directly affect the measured parameters of sperm viability, MMP, and motility within the 2 to 4-hour incubation period, the length of the incubation itself affected sperm viability, particularly in the earlier stages of epididymal maturation.

Discussion and Conclusions

Results indicate that exposure to ATZ did not impact sperm motility (tail), viability or MMP in any epididymal compartment, regardless of the incubation time (2 or 4 hours). These findings suggest that the conventional markers may not be sufficient to detect subtle or early effects of ATZ on epididymal spermatozoa. To address this limitation, additional markers are being studied to provide a more comprehensive assessment of ATZ's effects. Specifically,

metabolomic analysis using NMR to reveal alterations in biochemical pathways and identify potential biomarkers of early ATZ toxicity. Furthermore, oxidative stress levels induced by ATZ exposure may be pivotal as it is a critical factor in sperm health. This approach will enhance our understanding of ATZ's impact on reproductive health and contribute to the development of more sensitive and specific biomarkers for assessing environmental toxicants in agricultural settings.

Keywords: Atrazine, spermatozoa, bovine, toxicology

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Sperm quality is improved in SLC (Single Layer Centrifugation) technique using ovicoll colloid

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Introduction and aim

Fertility is a versatile phenomenon, including not only male but also female factors. Traditionally, many researchers have attributed the fertility of breeding rams to sperm quality, while Lavanya Goodla (1) have largely disregarded the contribution of the other major component of the ejaculate the seminal plasma. Choosing the best spermatozoa from the rest of the ejaculate is one approach to enhancing sperm quality. Rodriguzes-Martinez, claims that a new technique for sperm choice has been developed that selects good quality spermatozoa and also casts out them from the seminal plasma (2). Oxidative stress has a well-known bad effect on ram fertility, with ROS (reactive oxygen species) impairing sperm production, DNA integrity, motility and membrane. ROS, which are metabolites of oxygen and necessary for the continuity of life, cause disorders in cell functions. Oxidative stress causes a decrease in the antioxidant protection mechanism and an increment in reactive oxygen species, leading to deterioration of reproductive function (3). Not only capaCitation factors, which is the period

that the sperm spends in the female genital tract in order to gain fertilization ability (4) but also other substances such as sperm motility inhibiting factors (5) have a detrimental impact on sperm motility in vitro. Some species including bull and stallion is more resistant to freeze than the cell membrane of ram spermatozoa on account of its composition, with the viability and quality of ram spermatozoa low after the freeze-thaw process. This, along with the specific form of the sheep cervix, are the main causes of low fertility in sheep when intracervical insemination is used. The aim of this analysis was to investigate the effect of a single layer colloid centrifugation technique on the quality of texel ram semen post- thawing.

Methods

The colloid utilized in centrifugation was Ovicoll and the thawed sperm concentration, motility, morphology and live/dead ratio, were assessed before and after SLC (single layer colloid centrifugation) and flow cytometry was used to, which further assess SLC samples for live/dead ratio, reactive oxygen species and mitochondrial membrane potential. For this study, the semen of six individual Texel rams were tested with or without Ovicoll for motility, viability and morphology under the microscope. CellRox for reactive oxygen species, live/dead sperm viability and JC-1for mitochondrial potential were also analyzed using a Sony SH800 device. The paired sample t-test and Wilcoxon were performed in SPSS 27 to test reactive oxygen species, live/dead sperm viability, and JC-1for mitochondrial potential, motility, concentration and morphology.

Results

The study found that Ovicoll had a significant positive effect on sperm concentrations, live:dead ratio and morphology and motility. Ovicoll also significantly increased the reactive oxygen capacity of the live sperm, which is a negative and undesirable effect. In JC-1 analysis, no alterations in the membrane structure of mitochondria were detected linked to the Ovicoll treatment. A sequin from our first ram was used to calibrate the Sony SH800 device. Therefore, some analysis results such as reactive oxygen species, live/dead sperm viability, and JC-1 may have been affected. Also, these results

may have been affected since egg yolk was not used and a sequin was used for calibration.

Discussion and Conclusion: It is extremely important to examine the spermatological characteristics of the male material used in breeding and to take into account the results. If spermatological characteristics are not within the desired parameters fertility will be negatively affected. In sheep that are raised in a flock, semen production and quality are very important regardless of the yield direction of the breeding rams. Morrell et. al (6) found that single layer centrifugation technique, through the process of species-specific colloid formulations, gave stallion. Morrell et al. (7) used 17 beef bull semen straws and 20 dairy bull semen straws in their study. They found that membrane integrity(46% and 40%) and normal morphology (87% and 76%) and high respiratory activity(52% and 12%) were higher for dairy bulls than beef bulls. Trevizana et al. (8) used the JC-1 dye to determine the mitochondrial membrane potential in their study of 40 bulls. They found high mitochondrial activity in young bulls, at a rate of 81.7$\pm$9.4%.

To conclude SLC technique remarkably developed the post-thaw longevity and grade of ram spermatozoa, utilizing Ovicoll beneficial.

Keywords: flow cytometry, ram spermatozoa, reactive oxygen species, single layer colloid centrifugation, Ovicoll,

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Effects of favipiravir T-705 on rat testicular tissue

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Ömür, A.D., Uçak, G., Erbaş, E., Özkanlar, S., Kara, A., Celep, N.A., Akarsu, S.A., Karahanli, A., Kamer, B. Effects of favipiravir T-705 on rat testicular tissue.

Introduction and Aim: Favipiravir is a broad-acting antiviral agent used in the treatment of viral diseases such as COVID 19 (1). This drug found to penetrates many tissues and organs in the body and associated to pathological changes while male reproductive toxicity studies are limited

(2). The present study is aimed to investigate the effects of favipiravir on the testicles during and after treatment, focusing on oxidative stress, apoptosis, spermatological parameters, and histopathological changes.

Methods
42 male Sprague-Dawley rats were divided into six groups, with 7 rats per group. 3 groups served as control groups and received physiological water by oral gavage, while the other 3 groups served as experimental groups and were administered 200 mg/kg Favipiravir by oral gavage. For both the control and experimental groups, 7 rats were sacrificed on the 14th, 28th, and 50th days of administration. The effect of favipiravir on epididymal sperm quality was subjectively determined as a percentage by scanning three different microscope fields. MDA level, a marker of oxidative stress, was measured by ELISA. Real-time PCR and western blot were used to measure inflammation.

Results
A decrease in spermatological parameters was observed in all experimental groups compared to the control groups. The lowest epididymal spermatozoon density and motility values were detected in the experimental group sacrificed on the 50th day ($p<0.001$). DNA damage, acrosomal damage, abnormal spermatozoa rate, and dead/live spermatozoa percentage were highest in the group sacrificed on the 50th day ($p<0.001$). Favipiravir administration caused an increase in the level of MDA, inflammation, and apoptosis, in rat testis. Histological examination revealed testicular histoarchitexture changes, narrowing and defects in seminiferous tubules.

Discussion and Conclusion
In conclusion, Favipiravir administered to rats by oral gavage at a dose of 200 mg/kg decreased epididymal sperm quality and caused changes in testicular histology, oxidative damage, apoptosis, and inflammation.

Keywords; Favipiravir, oxidative stress, rat, sperm, testis.

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The oxidative state improvement of dairy cows in post-partum

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Introduction and aim

The intense metabolic pressure, during the peri-partum, results in a considerable production of Reactive Oxygen Species (ROS), which is not adequately neutralized by antioxidant systems (1). The aim of the study was to evaluate reproductive performance and oxidative status of cows after the administration of vitamin association, during the last 3 weeks of pregnancy. There were also evaluated the colostrum quality and the immune status of calves.

Methods

The study (ethics committee number 1/19) involved 20 Italian Fresian cows at the end of pregnancy. The cows enrolled were multiparous, aged between 3 and 8 years and with an inter-partum period between 365 and 385 days. The animals were randomly divided into 2 groups: Treated Group (T) composed of 10 cows treated with 10 ml/head of an association containing retinol acetate 100,000 I.U., cholecalciferol 25,000 I.U., alpha-tocopherol acetate 100 mg (Adecon® - Fatro, Italy) and Control Group (C) composed of 10 cows, treated with 10 ml saline (NaCl 0.9%). In all cows the administrations were performed at -21, -14, -7 days before calving.

The calving, pathologies in the immediate postpartum and the reproductive parameters were evaluated. Calves underwent a clinical examination to assess their health status.

An aliquot of colostrum was taken within 2 h after birth to evaluate the quality by means a refractometer. All cows underwent to blood samples from the coccygeal vein in serum vacutainer tubes at 21 days before calving date (T-21) and 21 days after calving (T+21), to evaluate oxidative status. In calves, 24 h after birth blood samples were taken to evaluate oxidative status and IgG concentrations.

The serum obtained was stored in 1.5 mL eppendorf at -20°C until analytical determinations were made. ROS were assayed by colorimetric reaction capable of assaying reactive oxygen metabolites (dROMs) (2). BAP (Blood Antioxidant Potential) was determined with the BAP test using a photometric system (3) and the IgG concentrations were evaluated by refractometer.

Results

The treated cows showed the first postpartum heat earlier (40.29$\pm$15 days) than the C group (46$\pm$ 13.78 days) and consequently they had a reduced calving interval conception (100.14$\pm$42.22 vs 107.86$\pm$56.47 days); however these data were not statistically significant. A statistically significant difference ($p<0.05$) was observed for both BAP and dROMs concentrations, in cows Group T compared to Group C, at T+21. The same result about oxidative

status was obtained in calves at 24 h after calving. The concentrations of IgG in the serum of calves showed statistically significant differences in Group T than C, while in the colostrum there were no differences.

Discussion and conclusions
In conclusion, the administration of antioxidants in the prepartum provides useful support to both cows and calves, to improve oxidative status and the immune system.

Keywords: Dairy cows; Postpartum; ROS; Antioxidant; Colostrum

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Seminal plasma extracellular vesicles as potential influencers of male fertility: The dog as a study model

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Introduction and aim

It is well understood that several sperm properties such as metabolism, motility, membrane functionality, ability to bind to zona pellucida, and fertilizing capacity are influenced by the epididymis: during it transit, the spermatozoa undergo constant remodeling of their membrane with sequential addition and removal of molecules. Epididymis and prostate secrete soluble molecules and extracellular vesicles (EVs) that are categorized as epididymosomes and prostasomes. These EVs are present in seminal plasma and contain several proteins regulating sperm maturation, motility, sperm-zona pellucida binding, and fertilization [1]. In addition, EVs contain microRNA (miRNAs) that have an important role in meiosis, mitosis, and spermatogenesis contributing to the mechanisms involved in male fertility [2].

The lack of some EV proteins has been associated with impaired sperm function [3]. The study of EVs would seem a valuable approach to detecting the expression profile of miRNAs and proteins, identifying useful non-invasive diagnostic biomarkers for male reproductive diseases.

In veterinary medicine, EVs from seminal plasma of bull with proven fertility improved the *in vitro* fertilizing abilities of low-fertility bulls [4]. These results underline the influence of plasma EVs on sperm physiology and fertility, but also the possibility that EVs might have therapeutic potential too.

In the human household, dogs share the same environment, and are exposed to the same household contaminants of humans; in fact, many studies suggest that dogs exhibit the same range of reproductive abnormalities as humans [5]. Such trends demonstrate the importance of dogs as a sentinel species for environmental influences on human reproductive health.

Nowadays, no comparative studies on the composition of plasma EVs in fertile and no-fertile dogs have been carried out. For these reasons, this study is focused on the domestic dog as an indicator of the reproductive health of humans and consists of a preliminary EV characterization isolated from sperm of 3 dogs considered fertile and 3 dogs considered hypofertile based

on their reproductive history. Assuming that any difference between fertile and hypofertile individuals could be attributable to miRNAs contained in EVs, the present study characterized the miRNA cargo in EV sperm of fertile and hypofertile dogs.

Methods

Three ejaculates of each dog (once a week) were used to isolate EVs through ultracentrifugation at 100,000 g for 60 minutes at 4°C. The pellet of EVs was resuspended in 200 µl of PBS and stored at -80°C until further analyses.

EVs were characterized by NanoSight, Western blotting, flow cytometry, and transmission electron microscopy according to MISEV2018 guidelines [6].

Then, all types of EVs were used for RNA isolation. MiRNA libraries were prepared using the TruSeq Small RNA Library Preparation kit. Small non-coding RNA libraries were sequenced, and data analyzed by an external service. *Canis familiaris* miRNAs available at MirBase (http://www.mirbase.org/) were used to identify known miRNAs.

Results

EV characterization showed no differences between the two categories of dogs but confirmed EV isolation.

In the EV sperm, 288 miRNAs were identified and distributed into: 21 miRNAs exclusively in the EV sperm of fertile dogs, 29 miRNAs exclusively in the EV sperm of hypofertile dogs, and 238 miRNAs detected in both groups. Among these 238 miRNAs, nine showed a difference in relative expression levels between hypofertile and fertile individuals, with a statistical significance of 10%. Moreover, our data showed that miR-216b was more abundant in the EVs of hypofertile dogs.

Discussion and conclusion

MiR216b is important for early embryo development, but when it has to be present in limited amounts. Indeed, low level of miR-216b in spermatozoa resulted in a low level of this miRNA also in zygotes and embryos that,

obviously, had a higher relative level of its target gene K-RAS. High expression of K-RAS resulted in a greater rate of cleavage and increased cell number in the blastocysts, compared to zygotes and embryos obtained from spermatozoa with high miRNA-216b levels [7].

The identification of miR-216b may suggest new implications of vesicle-derived miRNAs in influencing sperm miRNA content in fertility regulation.

Keywords: dog, semen, extracellular vesicles, microRNA, hypofertility

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Hyaluronic acid/collagen i-enriched 3d-microbeads fabrication and matching to ovine oocyte IVM

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Mastrorocco, A., Michele, F.D., Cacopardo, L., Martino, N.A., Dell'Aquila, M.E. Hyaluronic acid/collagen i-enriched 3d-microbeads fabrication and matching to ovine oocyte IVM.

Introduction and aim

Three-dimensional in vitro maturation (3D-IVM) is a promising approach to improve IVM efficiency as it could prevent cumulus-oocyte complex (COC) flattening and preserve its structural and functional integrity. We recently developed a 3D-IVM protocol, in the sheep, in which COCs were encapsulated in alginate microbeads enriched with Collagen I (CI), thus recreating the physiological 3D follicular environment with a component of ovarian extracellular matrix (ECM). After IVM in those conditions, lamb oocytes showed increased maturation rate and improved bioenergetic status (1). The aim of this study is to insert a second main ECM component,

hyaluronic acid (HA), in the microbead fabrication system, in order to create a more animal-reliable in-vitro model and to test the suitability of these HA/CI-enriched microbeads on maturation rate of prepubertal lamb oocytes.

Methods

Two experiments were conducted for assessing 1) microbead morphological characterization (size and shape) and stability under culture conditions and 2) the effect of HA/CI enriched 3D-IVM system vs standard 2D-IVM (controls) on oocytes maturation. Empty- (Exp. 1) and COC-including microbeads (Exp. 2) were generated as previously reported (2). In Exp. 1, alginate microbeads were fabricated with 0.0004% CI and two HA concentrations, 2 and 4 mg/ml following indications from other tissue and cell systems (3,4). Microbead features were characterized acquiring brightfield images immediately after fabrication and after maintenance in simulated IVM condition (over 24 hours at 38.5 $^{\circ}$C under 5% CO_2 in air). Images retrieving dimensions (radius) and the shape descriptor "aspect ratio" were analyzed using the Analyze Particles tool (ImageJ) (2). After IVM, cumulus expansion was evaluated and denuded oocytes were stained with Hoechst 33258, fixed in 3.8% formaldehyde solution in PBS and observed under epifluorescence microscopy to assess their meiotic stage (Chi-square test: significance at $P<0.05$) (1). In each experiment, three replicates were performed. In Exp. 1, a mean number of 15-20 empty microbeads were produced per condition, thus containing 2 or 4 mg/ml HA. In Exp.2, each replicate consisted of 25 COCs cultured under 2D-IVM and 7-10 COCs cultured in HA/CI-enriched microbeads per condition.

Results

In exp. 1, 50 microbeads were analyzed for each HA concentration. With both HA concentrations we got reproducible microbead size of 1051.33±118 µm in radius and roundness shape, suitable for inclusion of ovine COCs. Empty microbeads, produced with both examined HA concentrations, remained stable in shape and size when incubated in simulated IVM condition and no broken beads were observed at any culture time. In exp. 2, a total of 126 COCs were analyzed. After IVM, cumulus expansion rate was preserved in all tested conditions. In detail, after culture in microbeads with 2 mg/ml

or 4 mg/mL HA, cumulus expansion rate was 85% and 80%, respectively compared with 85% after 2D-IVM. By comparing 3D-IVM in HA/CI-enriched microbeads versus 2D-IVM, the addition of 2 mg/ml HA significantly increased the maturation rates (20/26, 77% vs 42/78, 54%; $P<0.05$). On the contrary, a downward trend in the maturation rate was recorded after COCs culture in microbeads fabricated with 4 mg/ml HA (9/22, 41% vs 42/78, 54%; NS).

Discussion and conclusions

These preliminary data indicate the potential of Alginate/CI/HA hydrogel microbeads as a bio-responsive matrix adapt at supporting oocyte IVM. Moreover, these data allow us to consider that hyaluronic acid can contribute to improve microbeads quality but that its concentration must be properly evaluated in order to avoid excessive hydrogel stiffness, because the HA molecules not only binds with water but also interacts with the alginate reinforcing the polymeric network that can compromise the adequate oocyte maturation. Future focus will be on using computational model to predict viscoelastic descriptors of alginate/CI hydrogel in the presence of different HA concentrations. Additionally, further studies should pivot toward investigating the hydrogel's capacity to yield to cytoplasmic and molecular oocyte maturation.

Keywords: 3D, IVM, hyaluronic acid, collagen I, extracellular matrix

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The Immunohistochemical expressions of p16 and Ki67 in canine seminomas

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Introduction and aim

Seminoma, testicular cancer, originates from germ cells, usually grows slowly, and has a lower tendency to metastasize (1). Seminomas in humans are divided into two subtypes: Classical Seminoma (CS) and Spermatocytic Seminoma (SS) (2). In recent years, this WHO classification has also been made in canine seminomas (3). p16 is a tumor suppressor protein (CDKN2A gene) that prevents progression into S phase of cell cycle and is a cyclin-dependent kinase inhibitor. While Ki67 is expressed in all stages of cell proliferation, G1 and S are less abundant. While it is maximally detected in G2 and mitosis, it is absent in G0 for Ki67(4, 5). The aim of the study was to immunohistochemically determine the expression of p16 and Ki67 antibodies in types of canine seminomas.

Methods
Testes of 30 male dogs of different breeds and ages, diagnosed with seminoma and surgically removed or collected from autopsy, were used in the study. The samples were routinely stained with Hematoxylin Eosin and PAS. As for immunohistochemistry, immunoperoxidase staining (Thermo Scientific UltraVision Large Volume Detection System Anti-Polyvalent, HRP / ABC-P) was performed using p16 and Ki67 antibodies. 3-Amino-9-ethylcarbazole (AEC) as chromogen solution was applied. All findings were evaluated under a light microscope (Leica DM 4000) and photographed (Leica DFC-280). The detected positivity was graded semiquantitatively according to its prevalence and severity in ten different microscope fields at x40 magnification.

Results
Seminomas were classified as SS (n:23), CS (n:5) and CS/SS (n:2) according to their subtypes. Carcinoma in situ (CIS) formation was detected in 3 of the CSs. All CSs (100%) were positive for p16 antibody. While SSs were positive in 8 cases (35%), all CS/SSs were negative. Although Ki67 was negative in all CIS formations of CSs (n:3, 100%), positivity was detected in CSs without CIS (n:2, 40%). While SSs were positive in 14 cases (61%), all CS/SSs were positive (n:2, 100%).

Discussion and Conclusions
Grieco et al. (6) stated that the morphological, histochemical and immunohistochemical properties of dog and human CS and SS are similar. Similar findings were detected in this study. In humans, 620 cases of seminoma were negative with p16 antibody in 90.3% and positive in 9.7% (7). In this study, the positivity was found to be 13/30 (43%), and it was determined that the rate may be higher in animals than in humans. The high rate of p16 detected in CSs showed that it worked actively as a tumor suppressor gene. However, with Ki67, the cases of SS showed high proliferation, confirming that they have a higher malignancy than CS. In conclusion, p16 and Ki67 expressions showed helpful for determining the proliferative activity in classifications of the canine seminomas.

Keywords: Ki67, p16, Seminoma, Testis, Tumor.

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Could curcumin be an effective option for freezing ram semen?

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Citation
Balci, N., Ucar, S.E., Senlikci, H., Arici, R., Yagcioğlu, S., Sandal, B., Ozdas, O.B., Sandal, A.I. Could curcumin be an effective option for freezing ram semen?.

Introduction and aim

It is a well-known fact that the sheep population in our country holds a critical position in the livestock area. This study aims to determine the efficacy of curcumin, which possesses antioxidant, anti-inflammatory and antimicrobial effects, against structural-functional changes that may occur in semen during freezing processes. Reactive Oxygen Species (ROS) generated to a much greater extent than normal during the freezing process negatively

affect the fertilization abilities of spermatozoa. While controlled ROS production plays a physiological role, high ROS production unbalanced by the cellular antioxidant defense system can lead to oxidative stress. The data obtained from current study are expected to serve as a reference for other studies on the effects of curcumin on spermatozoa.

Methods

In the study, five Kivircik breed rams were used. The care and feeding of the rams were conducted within the Department of Reproduction and Artificial Insemination at Istanbul University-Cerrahpasa. Semen samples were collected from each ram using an electroejaculator, twice a week totaling 10 times, outside the breeding season. The pooled samples were divided into four equal volumes and gradually diluted with a Tris extender containing 5% glycerol and 20% egg yolk in a controlled manner. The four groups were formed as follows: Tris (Control), Tris+5 mM Curcumin (C5); Tris+10 mM Curcumin (C10); Tris+15 mM Curcumin (C15). Diluted semen was placed at 50 million in each straw. The spermatozoa were frozen using a CryoBio System. Initially, the spermatozoa were cooled from 26°C to 5°C at a rate of 0.3°C/min and then equilibrated at 5°C for 60 minutes. Subsequently, they were further cooled from 5°C to -20°C at a rate of 0.5°C/min, followed by rapid freezing from -20°C to -150°C at a rate of 30°C/min. After waiting for 5 minutes at -150°C, spermatozoa were stored in liquid nitrogen tanks at -196°C [1]. Data were expressed as means $\pm$ standard deviations (SD). Normality was assessed using the Shapiro–Wilk test and homogeneity of variances was examined using Levene's test. If the assumptions of normality and equal variances were satisfied, one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was employed for data analysis. Alternatively, if normality and equal variances assumptions were not met, the Kruskal-Wallis test followed by Dunn's multiple comparisons test was utilized. Statistical analyses were carried out using IBM© SPSS© Statistics version 29 (IBM© Corp., Armonk, NY, USA). Results with p-values below 0.05 were considered statistically significant.

Results

In current study of the antioxidant activity of curcumin in cryopreservation of ram semen the frozen thawed semen samples' evaluated parameters were reported in Table 1, 2. The thawed semen samples were analyzed for motility, progressive motility, average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head (ALH), beat cross frequency (BCF), straightness (STR), and linearity (LIN) values [2] using Computer Assisted Sperm Analysis System (CASA System). No statistical significance was detected. Assesment with flow cytometry for acrosome integrity, membrane integrity, high mitochondrial activity and DNA damage [3] also revealed no statistical significance.

Discussion and Conclusions

The analysis conducted after thawing showed no statistical differences in sperm parameters, acrosome integrity, membrane integrity, high mitochondrial activity and DNA damage parameters among the different concentrations of curcumin used. [4,5]. Current literature review indicates that curcumin has not been employed in storage of ram semen. It is believed that the data obtained will serve as a reference for other studies on the effects of curcumin on spermatozoa.

Keywords: Sperm, freezing, curcumin, DNA damage, acrosom integrity

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Investigation of the effect of carazolol use on fertility before artificial insemination in cattle

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Citation
Bakır, O., Ata, A. Investigation of the effect of carazolol use on fertility before artificial insemination in cattle.

Introduction and aim

Beta-blockers were initially developed in 1960 to reduce the heart's capacity by blocking the effects of the sympathetic nervous system that can cause a rapid heartbeat. Beta-blockers bind to beta receptors and prevent stress hormones from triggering a reaction. The strength of the tonic activity determines the specific drug effects by blocking beta receptors (1). Beta-blockers reduce cardiac stress by slowing the heart rate and decreasing the contractile force of the heart muscles (2). During artificial insemination in female animals, in the treatment of endometritis by combining with other drugs in cows, as well as during semen collection procedures from males, and in the treatment and prevention of retentio secundinarum cases caused by a difficult birth, this has been extensively researched (3,4). Carazolol is a beta 2-adrenoreceptor blocker frequently used in the veterinary field during artificial inseminations. This study aimed to investigate whether the use of Carazolol affects the pregnancy rate just before artificial insemination in cows.

Methods

The study used 150 Holstein cows obtained from the Burdur/Çeltikçi district and villages. Cows with at least one calving, no reproductive problems, 45-60 days after calving, and 3-4 years of age were included in the study. Artificial insemination (AI) was performed at least 12 hours after the onset of estrus. AI was performed with commercially proven bull semen (007HO12236, BAYONET-ET, ST genetics, Navasota, USA). The study groups are briefly described as follows: Holstein cows were divided into 3 equal groups (Group-I 1 mg Carazolol/100 kg body weight, n=50; Group-II placebo group, 2 ml 0.09 Physiological Serum /100 kg body weight, n=50; Group-III control group, without any treatment, n=50). Motility was subjectively assessed by scanning at least 7 microscope fields with a phase contrast microscope with a heating table. Viability (SYBR-14/PI), plasma membrane and acrosome integrity (PMAI) (FITC-PNA/PI), mitochondrial membrane potential (JC-1), mitochondrial reactive oxygen species level (MROS) (MITOSOX RED/PI), and lipid peroxidation levels (BODIPY/SYBR-14), were measured by flow cytometer. A pregnancy examination was performed by rectal palpation. Conception rates were analyzed using a chi-square test and given as %.

Results

Motility was 60.83%, viability levels 67.79%, PMAI levels 67.57%, high mitochondrial membrane potential (HMMP) 18.11%, low mitochondrial membrane potential (LMMP) 81.87%, mitochondrial reactive oxygen species level (MROS) (MITOSOX RED/PI), MROS levels 3.18%, lipid peroxidation levels (BODIPY/SYBR-14) 22.79%. The conception rate was determined as 74% (37/50), 62% (31/50), and 60% (30/50) in group I, group II, and group III respectively.

Discussion and conclusions

Pancarci et al. (5) reported a conception rate of 64.8% (243/375) in their study of 375 cattle treated with Carazolol. Similarly, Hammerl and Rüsse (6) found a conception rate of 75.3% (260/345) in their study of 345 cattle treated with Carazolol. In another study conducted by Panowsky (7), 66.1% (600/397) of the 397 heads of cattle treated with Carazolol became pregnant. Kırşan et

al. (8) reported that conception rate was achieved in 97% (97/100) of cows in the group treated with Carazolol before artificial insemination, compared to 78% (78/100) in the control group. Bostedt et al. (9) found that 70.5% of the 85 cows in the GnRH group in their study were diagnosed as pregnant by rectal examination. The conception rate was 70.7% in the Carazolol-treated group and 61.5% in the control group. Although the differences between these conception rates were not statistically significant, these treatments have a positive effect on increasing the pregnancy rate. In cows with delayed ovulation, the conception rate was 63.2% in cows treated with Carazolol, 64.3% in cows in the GnRH group, and 45.4% in the control group. Our study produced similar conception rate results, yet no statistical difference was found. The conception rate was determined as 74% (37/50), 62% (31/50), and 60% (30/50) in group-I, group-II, and group-III respectively. These conception rates were very similar to the pregnancy rates obtained by Bostedt et al. (9), who found that 70.5% of 85 animals treated with GnRH were found to be pregnant by rectal examination, while this rate was 70.7% in the group treated with Carazolol and 61.5% in the control group. According to these conception rates, an increase in the pregnancy rate, which was not statistically different, was achieved with the use of Carazolol during artificial insemination applications in Holstein cows.

Keywords: Beta-blocker, Carazolol, Cow, Artificial Insemination, Fertility

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Effects of triton X-100 pretreatment of lyophilized ram sperm on embryo developmental competence

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Citation
Ustuner, B., Yagcıoglu, S., Nur, Z., Alcay, S., Demir, K., Gokce, E., Ozturk, G.B., Toker, B., Sagirkaya, H., Soylu, M.K., Birler, S., Pabuccuoglu, S. Effects of triton X-100 pretreatment of lyophilized ram sperm on embryo developmental competence.

Introduction and aim

In in vitro fertilization (IVF) procedures, formation of female and male pronuclei occurs synchronously, while this synchronization is not possible in ICSI procedure (1). Mechanical injection step of ICSI induces the formation of the female pronucleus earlier than the male pronucleus. It has been reported that sperm plasma membrane delays oocyte activation and decondensation of sperm chromatin after injection of spermatozoon and harms male-female pronuclei synchronization (2). The spermatozoon with an intact acrosome structure containing many hydrolyzing enzymes is injected into the oocyte during ICSI, which does not occur during the natural fertilization process and can damage the cytoplasmic components of the oocyte (3). In previous researches 26.3% and 75% acrosome integrity rates were observed in lyophilized ram and bull semen respectively (4,5). In this study, it was aimed to determine the effect of destruction of lyophilized and frozen-thawed ram sperm plasma and acrosomal membrane with Triton X-100 on development of embryos produced by ICSI.

Methods

Ten Kivircik rams were used. Collected semen was pooled and divided into two equal aliquots as lyophilization (L) and freezing (F) groups. Semen was diluted with Tris-based extender to a final concentration of approximately $100x10^6$. Semen of lyophilization group (L) was diluted to $100x10^6$ (spermatozoa/glass vials) with TCM 199 including 10% fetal calf serum and each sample in cryotubes was lyophilized using Freeze-Dryer (Labconco, Kansas City, U.S.) (4). The effect of Triton X-100 on percentage of plasma membrane integrity, acrosome integrity and chromatin integrity of lyophilized (L and LTX-100) and frozen-thawed (F and FTX-100) semen were evaluated.

Ovaries from adult ewes were collected from a local abattoir. The slicing method was used to obtain the cumulus-oocyte complexes (COCs) and then they were matured in M199. In vitro matured oocytes were randomly divided into five groups as follows and experiments were replicated for 4 times; Group 1: Intracytoplasmic injection with L sperm Group 2: Intracytoplasmic injection with LTX-100 sperm Group 3: Intracytoplasmic injection with F sperm Group 4: Intracytoplasmic injection with FTX-100 sperm Group 5: IVF with fresh sperm. Lyophilized and frozen sperm groups were treated with 0.1% Triton X-100 for 1 min at 38.5°C in 5% CO2 (LTX-100 and FTX-100 groups respectively). Lyophilized and frozen sperm groups with or without Triton X-100 treatment were used for ICSI and then oocytes were activated in HSOF with 5 mg/mL ionomycin for 5 min. They were then rinsed with HSOF several times before cultured in fertilization medium for 20 h. In all groups, the presumptive zygotes were cultured in a SOFaa medium for 7 days. For IVF, matured oocytes were washed with fertilization media and then transferred into a fertilization medium. After sperm separation, oocytes were fertilized with motile sperm of a concentration of $1x10^6$/mL for 20 h at 38.5 °C in humidified air with 5% CO2. IVF and ICSI procedure was done as originally described by Gómez et al. (1998). The cleavage and blastocyst rates were determined at 48 and 144 h respectively.

Results

The plasma membrane integrity of the frozen-thawed group was significantly higher than the FTX-100 group ($P < 0.05$). Acrosome integrities of L and LTX-100 groups were completely compromised and the acrosome integrity of the FTX-100 group was significantly lower than the F group ($P < 0.05$). The chromatin integrities of L and F groups were higher than the Triton X-100 treated groups ($P < 0.05$). ICSI with L, LTX-100, F and FTX-100 semen groups produced similar cleavage rates ($P > 0.05$). The cleavage rate after IVF was significantly higher than lyophilized ICSI outcomes ($P < 0.05$). There were not any significant differences among all the groups regarding blastocyst numbers ($P > 0.05$)

Discussion and conclusions

Olaciregui et al. (6) reported that the rate of blastocyst development after ICSI with lyophilized semen and frozen semen in sheep was 25.6% and 24.5%, respectively. In our study, blastocyst development rates obtained with lyophilized and frozen semen were 32.8% and 28.4%, respectively, and these results were higher than in this study. Pretreatment of lyophilized and frozen semen with Triton X-100 did not affect embryonic cleavage and blastocyst development rates. In conclusion, the data presented here confirms that ram sperm can be effectively lyophilized and injected into oocytes to initiate embryo development. Also, it was concluded that Triton X-100 application is not very necessary.

Keywords: Intracytoplasmic sperm injection (ICSI); lyophilization; sheep; embryo development.

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Effects of nicotinic acid and folic acid combination supplemented extender on the freezability of ram semen

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Citation
Yilmaz, M.M., Cimen, T., Huraydin, O., Gokce, E., Karakci, D., Aktar, A., Nur, Z., Ustuner, B. Effects of nicotinic acid and folic acid combination supplemented extender on the freezability of ram semen.

Introduction and aim

Cryopreservation can preserve sperm fertility in appropriate extenders, but during this process it causes structural, biochemical, functional and molecular distruption in spermatozoon. Due to the low cholesterol/

phospholipid ratio of the ram spermatozoon plasma membrane, they are more sensitive to sudden temperature changes (1). Reactive oxygen species(ROS) one of the causes of oxidative stress(OS), disrupt membrane fluidity and inducing lipid peroxidation which leads to loss of motility, a decrease in mitochondrial membrane potential due to OS causes sperm DNA fragmentation and ultimately cell apoptosis (2,3). It has been reported that supplementing antioxidant substances to ram semen freezing extenders has positive effects on post-thaw spermatological characteristics by eliminating the negative effects of ROS (4,5). Nicotinic acid, which has a strong antioxidant capacity by stabilizing free radicals and inhibiting lipid peroxidation, and Folic acid, which can protect cell membranes and DNA against free radical damage with its lipid peroxidation inhibition capability, are among these antioxidants (6,7).

The aim of this study was to determine the post-thaw spermatological characteristics (motility, acrosome, plasma membrane functional and DNA integrity, and mitochondrial membrane potential),antioxidant parameters[(MDA(Malondialdehyde),TAC(Total antioxidant capacity)] of ram sperm frozen with the supplementation of nicotinic acid and folic acid combination.

Methods

Semen collected from five Merino rams, aged between 2 and 4 years, in the non-breeding season, using an electro-ejaculator. Collected semen samples with a mass activity of 3 or more on the scale, at least 70% motile, and a concentration of $1x10^9$ were pooled and diluted with a Tris-egg yolk-glycerol extender by two-dilution method. The pooled ejaculates were divided into four equal aliquots according to control and antioxidant groups (Control, 10 mM NA with no FA(NA10), 10 mM NA+50 nM FA(NA10FA50) and 20 mM NA+50nM FA(NA20FA50). Diluted semen samples were cooled to 5°C, followed by a 2-hour equilibration process at 5°C. The equilibrated semen was frozen with a freezing machine and stored in liquid nitrogen at -196°C for at least one month until post-thaw evaluation. All the semen parameters were evaluated twice during the study: first at the fresh stage and at the post-thaw stage. Semen samples were examined by the specified methods for

motility/kinematics [(Computer-assisted sperm analyzer-CASA)], acrosome integrity[(Fluorescein lectin staining assay-conjugated pisum sativum agglutinin (FITC-PSA)], plasma membrane functional [(Hypoosmotic swelling test-(HOST)] and DNA integrity[(Terminal-deoxynucleotidyl-transferase-mediated-dUTP nick-end labelling-(TUNEL)], and mitochondrial membrane potential[5.5', 6,6'-tetrachloro-1, 1', 3,3'-tetraethyl- benzimidazole carbocyanine iodide-(JC-1)], antioxidant parameters [MDA-(ELISA kit),TAC-(Colorometric kit)]. Data obtained from the study were analysed using SPSS (SPSS 26 for Windows; SPSS, Chicago, IL).

Results

Fresh sperm was adversely affected by the freezing process in terms of all spermatological parameters ($P<0.001$). Comparing the TM evaluated by CASA, although there were no statistical differences among the groups, the highest motility was detected in the NA10FA50 with 53.34% and the lowest motility was detected in the control group with 43.44%. There were no statistical differences in terms of plasma membrane and DNA integrity, MDA and TAC rates among the groups ($P>0.05$). Although the lowest acrosomal damage in terms of acrosome integrity was obtained in the NA10FA50, it was only statistically different from the control group ($P<0.05$). Mitochondrial membrane damage was significantly highest in the control group compared to all antioxidant groups ($P<0.05$).

Discussion and conclusions

Studies reported that on the freezing of human sperm that the NA10FA50 dose group of NA and FA combination has a positive effect on sperm parameters (8). The conclusion of this study suggest that the addition of 10 mM NA or a combination of 10 mM NA and 50 nM FA to the extender had a positive effect on the freezability of ram semen.

Keywords: ram semen, cryopreservation, nicotinic acid, folic acid

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Polyester plastination of romanov sheep female reproductive system

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Citation
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Introduction and aim

Plastination is an innovative preservation technique that has revolutionized anatomical studies by producing durable and odorless specimens (1). Plastination offers significant advantages for anatomical education and research, providing long-lasting specimens that maintain structural integrity and detail (2). This study focuses on the polyester plastination technique to preserve the female genital system of Romanov sheep, aiming to create high-quality cross-section models for veterinary anatomy.

Methods

Four female Romanov sheep genital systems were collected post-mortem and subjected to a series of steps including fixation, dehydration, forced impregnation with polyester resin, and curing (3). The specimens were initially fixed with a proper position in a formaldehyde solution, followed by

dehydration in graded cold acetone baths (4). The dehydrated specimens were then immersed in a polyester resin mixture under vacuum to ensure thorough impregnation (5). Finally, the specimens were cured and hardened to complete the plastination process (6).

Results

The plastinated specimens retained good anatomical detail and structural integrity, providing clear visualization of the female reproductive anatomy. The resulting models were durable, lightweight, and free from the odor typically associated with preservation methods. Polyester plastination proved to be an effective technique for preserving the female genital system of Romanov sheep.

Discussion and conclusions

The plastinated specimens are valuable resources for veterinary education, offering enhanced durability and clarity compared to traditional preservation methods (7). While cross-sectional plastination samples have demonstrated utility in education, they may be constrained by prolonged processing times and the utilization of expensive chemicals during sample collection (8). However, the long-term usability of plastinates can prevent this cost. This technique holds promise for broad application in anatomical studies, improving the quality of teaching and research in veterinary medicine.

Keywords: Plastination, Polyester, Romanov sheep, Reproductive system, Sectional anatomy

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Locoregional anesthesia in standing equine laparoscopic ovariectomy: A 2003-2023 literature review

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Vullo, C., Catone, G., Marino, G., Gugliandolo, E., Miloro, R., Giambrone, G. Locoregional anesthesia in standing equine laparoscopic ovariectomy: A 2003-2023 literature review.

Introduction and aim

Standing laparoscopic ovariectomy (LO) under sedation is the preferred method in equids performed with a combination of sedation along with analgesia of the ovary, which may be achieved through various techniques and anaesthetic agents [1]. This systematic review aims to evaluate studies published between 2003 and 2023 in the equine veterinary literature in which quality of analgesia with loco-regional anaesthesia during LO was assessed.

Methods

This systematic review was conducted according to the "Preferred Reporting Items for Systematic Reviews and Meta-Analysis" (PRISMA) guidelines for systemic reviews. A systematic literature search was performed, including articles relating to equine LO. PubMed, Web of Science, and Scopus were used as databases. For all databases, the search strings were the following searched using the following terms: [(laparoscopic ovariectomy OR gonadectomy) AND (horse OR mare OR equid OR donkey OR mule) AND (locoregional anaesthesia OR pain OR analgesia OR analgesia assessment OR analgesic drugs)]. Only English-language peer-reviewed papers published between 2003 and 2023 were considered. Specifically, articles included in the study discuss standing LO in equids in which quality of analgesia correlated to locoregional anaesthesia during or after LO was assessed. Eligibility of studies was assessed following the objectives modified from "PICOs": Population: equids receiving ovariectomy; Intervention: standing LO; Outcome: degree of analgesia obtained with locoregional anaesthesia.

Results

Among 51 studies collected from databases only 5 papers were considered eligible for this review. Specifically, all the studies included are original articles. Considering the anaesthesia, 2/5 articles evaluated only injective anaesthesia, 1/5 only epidural and 2/5 association of injective and epidural analgesia. The pain evaluation was effectuated intraoperatively in 4/5 studies and postoperatively in 1/5 studies. The scale pain used were the visual analogue scale (VAS) (3/5), composite pain scale (CPS) and Horse Grimace Scale (HGS) (1/5), a system modified from Sampaio et al. [2] and Schauvliege et al. [3] (1/5).

Discussion and conclusions

This systematic review aimed to investigate studies evaluating quality of analgesia with locoregional anaesthesia in standing LO from 2003 to 2023. Over the last 20 years, it was possible to identify only 5 manuscripts that were eligible to be included in this systematic review.

Farstvedt et al. showed that mesovarian injection of lidocaine was associated with significantly lower pain responses, compared with intraovarian injection. VAS was used to describe the severity of pain [4].

Pezzanite et al. proved that pain scores were improved in liposomal bupivacaine-treated horses in a dose-dependent matter relating to the local slow release of anaesthetic from liposomal encapsulated bupivacaine. Pain was evaluated in the postoperative period with CPS and HGS [5].

Although commonly used, the direct injection of drugs in the ovarian pedicle could be associated with some disadvantages. To avoid this, Koch et al. considered that the application of topical mepivacaine provided intraoperative analgesia similar to the injection of mepivacaine into the ovarian pedicle. The intraoperative pain was evaluated with VAS [6].

In a study by Vullo et al. [7], epidural anaesthesia allowed successful ovarian manipulation in six mules, while this was not completely tolerated in two mules. Analgesia, depth of sedation, and ataxia were scored during surgery using a scoring system modified by Sampaio et al. [2] and Schauvliege et al. [3].

According to Virgin et al., continuous IV detomidine infusion may provide a safe, easily adjustable alternative to caudal epidural detomidine with similar analgesic effects for standing LO. VAS was used to score intraoperative pain [8].

In conclusion, although some works use only one between injection and epidural anaesthesia, the best results would seem to be given by an association between these two techniques. Specifically, mesovarian injection anaesthesia appears to be superior to ovarian anaesthesia, but topical mesovaric anaesthesia could also be a possible alternative. In addition, in cases where epidural cannot be applied, continuous IV administration of detomidine would lead to equivalent results in pain management.

Keywords: standing laparoscopic ovariectomy, equids, locoregional anaesthesia, pain management, analgesia

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Evaluation of bovipure® and equipure® for colloid centrifugation of dromedary camel spermatozoa

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Introduction and aim

The obtainment of high-quality sperm ensures the preservation of long-term genetic material and to promote advanced assisted reproductive technologies, however, semen processing techniques for Dromedary camels' (*Camelus dromedarius*) (DC) ejaculates, are limited (1). Colloid centrifugation

(CC) allows the selection of highly motile spermatozoa improving the sperm kinematic parameters and reducing the bacterial load (2-3). The beneficial effects of CC have been previously tested in the DC species (4), however, a colloid preparation specific for the DC species is not currently available on the market. The aim of the present study was, therefore, to evaluate two commercial colloid preparations: Bovipure® and Equipure® (NidaCon, Sweden), for the CC of dromedary camel spermatozoa.

Methods

Dromedary camel ejaculates (n=6) were collected by the camel semen collection kit method (5) and diluted with tris-citrate fructose buffer (4) with the addition of 3% bovine serum albumin (TCF-BSA) to reduce viscosity. Aliquots (two mL) of semen samples, diluted to about 30 x 10^6 spermatozoa per mL, were carefully layered on two mL of three different colloid preparations previously placed in 15 mL falcon tubes: Equipure®, Equipure® 70% (stock solution diluted with 30% TCF-BSA), and Bovipure® 70% (diluted with 30% TCF-BSA), respectively. The tubes were centrifuged at 300 g for 20 minutes at room temperature (4), the supernatant was removed, and the pellet was suspended again in two mL of TCF-BSA.

The samples were evaluated, before and after colloid centrifugation, with a computer assisted sperm analyzer (Ivos II, Hamilton Thorne, USA) previously set for the DC species (6). Sperm concentration, percentages of motile and progressively motile spermatozoa, total motile and progressive sperms, percentages of rapid progressive, medium progressive, and slow spermatozoa were calculated. Sperm kinematic parameters: average path velocity (μm/s) (VAP); straight line velocity (μm/s) (VSL); curvilinear velocity (μm/s) (VCL); amplitude of lateral head displacement (μm) (ALH); and beat cross frequency (Hz) (BCF)) were also recorded for motile and progressively motile spermatozoa, respectively. The pre-colloid and post-colloid (Equipure®, Equipure® 70%, Bovipure® 70%) data were analyzed through one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graphpad Prism5 statistical software. Statistical significance was set for $p<0.05$ and data were expressed as mean $\pm$ standard deviation (SD).

Results

The colloid centrifugation reduced the sperm concentration from 25.82 ± 3.08 x 10^6 spermatozoa to 20.24±4.15, 19.64±3.70 and 8.32±2.14 spz x 10^6/mL in samples centrifuged with Equipure® 70%, Bovipure® 70%, and Equipure®, respectively. Statistical differences were noticed between Pre-colloid *vs* Equipure® ($p<0.001$), Pre-colloid *vs* Bovipure® 70% ($p<0.05$), Equipure® 70% *vs* Equipure® ($p<0.01$) and Bovipure® 70% *vs* Equipure® ($p<0.01$). The number of motile spermatozoa decreased from 14.77±0.37 to 8.69±0.92, 6.40±0.77 to 4.04±0.77 spz x 10^6 in samples centrifuged with Equipure® 70%, Bovipure® 70%, and Equipure respectively. Significant differences were found comparing Pre-colloid with Equipure ($p<0.01$) and Pre-colloid with Bovipure® 70% ($p<0.05$). The total number of progressive spermatozoa did not significantly change (Pre-colloid: 5.25±0.24, Equipure® 70%: 4.31±0.46, Equipure®: 2.63±0.41, Bovipure® 70%: 2.36±0.36). No statistical differences were detected regarding sperm kinematic parameters although Equipure® 70% and Equipure® induced a VCL increase in both motile and progressive spermatozoa.

Discussion and conclusions

The tested commercial colloid centrifugation provided a sperm recovery rates from 22 to about 60% with Equipure and Equipure® 70%, respectively. Recovery rates from 26.1±5.2% to 35.4±6.9% were previously observed by using SLC on dromedary camel ejaculates, with the same centrifugation regime (4). The number of motile and progressive spermatozoa markedly decreased only with Equipure®; the dilution with TCF-BSA might have increased the recovery rates helping the sperm passage through the solution Equipure 70%. It would be interesting to evaluate the effect of different TCF dilution also with different amount of BSA, in order to evaluate the effect of the latter compound on sperm recovery rates and motility pattern. The kinematic parameters showed a positive trend related with the sperm velocity (Particularly about VCL) but most probably the low number of observations did not allow the detection of significant differences.

These preliminary data show that colloids, and particularly Equipure® diluted with 30% TCF-BSA, might be used for dromedary camel spermatozoa. Proper dilution rate and centrifugation regimen shall be established for

the DC ejaculates, as well the *in vitro* and *in vivo* fertility of the obtained spermatozoa.

Keywords: Camelidae, Ejaculates, Recovery rate, Motility, Progressive motility, Kinematic

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Evaluating canine laser fluorescent reader for progesterone in cow serum and whole milk

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Introduction and aim

Serum, plasma, or milk progesterone (P4) concentration in dairy cattle is useful for determining the stage of the estrous cycle and may be applied to determine pregnancy. Even though P4 levels during pregnancy are higher than during diestrus, estimating the presence or absence of a functional (P4-secreting) corpus luteum (CL) is often sufficient in clinical practice (1). The CL was defined as functional when serum or plasma P4 levels >1 ng/mL (2), Radio Immuno Assay (RIA) is considered the gold standard for evaluating P4 in mammalian serum, while the Virbac SpeedReader (SR) is a laser-induced fluorescent quick reader (15 minutes/sample) validated to measure serum P4 concentration in dogs, with a lower detection limit of 1 ng/mL (2,3). The aim of this study was to test if SR is comparable to RIA in correctly measuring

when serum and milk P4 in dairy cows is above or below 1 ng/mL, and if there are differences in P4 concentration between diestrus and pregnant animals.

Methods

This study was approved by the Ethical Committee of Pisa University (approval 18/2023) after informed consent was obtained from the animal supervisor. Between March and June 2024, 26 samples of 10 ml of blood and milk, were collected from 18 dairy cows stabled at the CIRAA dairy farm of Pisa University during routine health and reproductive evaluations. Animal reproductive status (absence of an active corpus luteum, diestrus, or pregnancy) was evaluated by transrectal ultrasound (Easi-Scan:Go; IMV, L'Aigle, France). After sampling, tubes were left at room temperature for about 30 minutes to allow blood coagulation and were then immediately centrifuged at 1500g for 10 minutes at 20°C. Serum was divided into two aliquots and frozen at -20°C, while milk was frozen without centrifugation. A serum sample from each collection was sent to the Department of Veterinary Medical Sciences of Bologna University on dry ice to measure P4 by RIA (4). The second serum and the milk sample from each collection were thawed at 20°C and analyzed using the SR, following the instructions for dog serum. Statistical analyses were performed using Jamovi statistical software, with significance set at $P<0.05$. The inter-assay coefficient of variation (CV%) was calculated from 5 measurements of the same sample for SR serum and milk P4 concentrations of a pregnant, a diestrus, and an estrus cow. Linear regression was used to compare values over 1 ng/mL between serum RIA and SR serum and milk P4 concentrations. The Shapiro-Wilk test was used to evaluate the normality of distribution of serum RIA, serum, and milk SR concentrations for values >1 ng/mL. The Student's T-test was used to evaluate differences between pregnancy and diestrus P4 concentrations for serum RIA, serum, and milk SR concentrations for values >1 ng/mL. **Results**

Out of 26 samplings, 7 were performed in diestrus, 9 in pregnancy (above 28 days), and 10 in the absence of active corpora lutea. The CV% was always smaller than 13.4%, and 10/10 and 9/10 samplings performed in the absence of an active corpus luteum resulted in <1 ng/mL for serum RIA and serum and milk SR analyses, respectively. Serum and milk SR with (P4) >1 ng/mL were

linearly related to RIA (y = 0.2713*y + 0.2007; R^2 = 0.92; P<0.001; y = 0.2469*x + 0.9416; R^2 = 0.68; P<0.001). Distributions of serum RIA, serum, and milk SR concentrations for values >1 ng/mL were normal (P>0.1), and there were significant differences between pregnant and diestrus non-pregnant cows with (P4) >1 ng/mL for serum RIA (13.5±3.7 vs 5.9±2.0; P<0.01), serum SR (3.8±1.2 vs 1.8±0.5; P<0.001), and milk SR (4.3±1.3 vs 2.4±0.9; P=0.006).

Discussion and Conclusions

We chose RIA as the reference assay in this study because it is considered the gold standard for determining the P4 concentration in bovine plasma. The SR results for animals in diestrus and estrus resulted in a CV% under 15%, so the method can be considered valid for assessing the P4 concentration when it's >1 ng/mL. The main limitations of SR are that P4 concentrations are 3 times lower than those measured by RIA, and this assay is not able to detect P4 values under the aforementioned concentration of 1 ng/mL. This shows that this instrument provides more qualitative than quantitative results. However, qualitative results can be valid for bovine heat detection and non-pregnancy diagnoses, especially in marginal areas where veterinarians cannot consistently be on the farm. These results indicate that SR can be a useful instrument for evaluating CL functionality, even though it does not reflect the actual concentration of this hormone in the serum and milk of cows. Larger sample sizes will be necessary to validate this method.

Keywords: cow, endocrinology, progesterone, pregnancy, cycle stage

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The effect of rainbow trout seminal plasma-cysteine combinations on ram semen cryopreservation

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Citation
Kurt, E.G., Üstüner, B. The effect of rainbow trout seminal plasma-cysteine combinations on ram semen cryopreservation.

Introduction and aim

Rainbow trout seminal plasma (RTSP) stands out for its rich content, consisting of saccharides, antioxidants, proteins, and other ingredients. Approximately 13% of RTSP proteins are considered as marine specific (1,2). Ustuner et al. (2016) have shown that these RTSP components enhance the success of semen cryopreservation (3). Also it is known that RTSP is rich in methionine fractions although is quite poor in cysteine fraction (2). Cysteine and methionine work synergistically to enhance semen cryopreservation by participating in the synthesis of hypotaurine and taurine (4). This study aims to evaluate the effects of the cysteine-RTSP combination against to cryo-capaCitation and incubation resilience of ram semen in non-breeding season.

Methods

Five separate extenders were used to evaluate the effect of the RTSP-cysteine combinations. The groups were named according to the extender contents: the control group (C) contained only the base extender; CC included 1 mmol cysteine added to the control group; RTSP1, RTSP10, and RTSP15 had 1%, 10%, and 15% RTSP and 1 mmol cysteine added to the control group, respectively.

For semen cryopreservation, ten rams housed under the same conditions at Faculty of Veterinary Medicine (Bursa, TURKEY) were used. Semen was collected from the rams using electroejaculation during the non-breeding season. Fresh semen samples with +++ mass activity, 80% motility, and approximately $1x10^9$ spt/ml volume were pooled. The pooled semen was divided into five separate volumes according to group number. After each group of semen was underwent two-step dilution, semen was cryopreserved with programmable freezing machine (3). Frozen-thawed semen was incubated at five hours for examining the incubation resilience. Post-thaw semen evaluation was performed separately at three time point (0^{th}, 3^{rd}, 5^{th} hours of incubation). Evaluating the post-thaw semen parameters; CTC, Rh123-PI and TUNEL staining methods were used for detected acrosome status, mitochondrion status and DNA fragmentation level respectively (6,7).

Results

The lowest spermatozoon motility levels obtained from control group at all the incubation times ($P<0.05$). The lowest and highest uncapacitated spermatozoon rates were obtained from C and RTSP15 groups at all the incubation times ($P<0.05$). Also the highest acrosome reacted spermatozoon rates obtained from Control group at all of the incubation times ($P<0.05$). According to the Rh123-PI and TUNEL examinations, the RTSP10 group had the highest functional mitochondria, living spermatozoon, and DNA integrity rates at all incubation times ($P<0.05$).

Discussion and Conclusions

Considering our data, it is observed that either pure cysteine or cysteine-RTSP combinations protect motility values of frozen-thawed ram semen. Acrosome status determination is important for the assessment of fertility success of frozen-thawed semen. The existence of both the highest number of uncapacitated spermatozoon and the lowest number of capacitated and also acrosome-reacted spermatozoon rate is expected for optimal fertility. Considering the results of the CTC staining, it is observed that the addition of cysteine alone to the semen extender does not change the capacitated, uncapacitated and acrosome reacted spermatozoon rate compared to the control group at all incubation times. This indicates that cysteine is insufficient to protect ram sperm against cryo-capaCitation lonely. However, it has been observed that the cysteine-RTSP combination provides better protection to ram sperm against cryo-capaCitation. The protective effect of RTSP against cryo-capaCitation may depend on its lipoprotein content.

Reactive oxygen species (ROS) primarily target the mitochondria -the center of energy metabolism of cells- in spermatozoa (7). Therefore, mitochondrial defects can quickly occur in response to ROS. Cysteine as an antioxidant has a protective effect on sperm mitochondrial function. Additionally, various antioxidants present in RTSP are known to protect spermatozoa against to ROS. Current study demonstrated that the combination of RTSP and cysteine exhibits a synergistic effect, providing higher protection for the mitochondrial status of ram sperm.

When examining the TUNEL evaluation data, the first notable observation is that the DNA fragmentation rate statistically increases progressively during the incubation period only in the control group. In other groups, the DNA fragmentation rate increased numerically during the incubation period, but this increase was not statistically significant. These data clearly show that the RTSP-cysteine combination protects ram sperm against DNA fragmentation due to both cryopreservation and incubation resistance.

Keywords: Cryopreservation, Cryo-capaCitation, Incubation Resilience, Rainbow Trout Seminal Plasma, Ram semen.

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Relationship between 1proakap4 and morphology, total and progressive motility in canine fresh semen

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Citation
Siena G, Milani C, Contiero B, Maenhoudt C, Robiteau G, Slimani S, Sergeant N, Tiret L, Fontbonne A. Relationship between 1proakap4 and morphology, total and progressive motility in canine fresh semen.

Introduction and aim

ProAKAP4 is the precursor of AKAP4, a structural protein of sperm flagellum. ProAKAP4 concentration reflects the long-lasting motility of spermatozoa, being also involved in their capaCitation and fertility. In dogs, immunoassay to quantify sperm proAKAP4 is suggested as an innovative parameter to consider in semen quality evaluation (1). The aim of our study was to evaluate the relationship between proAKAP4 concentration and total (TM) and progressive motility (PM) as well as sperm morphology. Semen parameters (concentration, TM and PM) and proAKAP4 variations in semen samples from dogs collected twice within the same day were also assessed.

Methods

Fourteen male dogs of 12 different breeds, 1 to 14-year-old and 6.9-95 kg bodyweight underwent semen collection at least twice during the same day and/or on different days (1-36 days). Sperm concentration (SpermaCue) and morphology of spermatozoa (Spermac®, Minitube, Germany) were assessed. TM and PM were assessed using CASA (Ceros II, Hamilton Thorne Inc., USA). ProAKAP4 concentration assay was performed on the spermatic fraction using an ELISA sandwich kit (Dog 4MID® Kit, 4BioDx, France). The time interval between two semen collections was classified as: first semen collection (T0), semen collection performed within the same day (T0.5), and after ≥1 day (T1). Correlation between proAKAP4 and TM, PM, and morphology was performed using Spearman rank correlation (r). Morphological abnormalities of spermatozoa were classified as normal spermatozoa (%), detached heads (%), presence of cytoplasmatic droplets (%) as well as head, acrosomal, tail, and intermediate piece abnormalities (%). A non-parametric paired Wilcoxon test to verify the difference between TM, PM as well as concentration of spermatozoa at T0 and T0.5 was performed for the 8 included dogs collected at T0 and T0.5. To verify a difference in the proAKAP4 results between times of collection (T0 and T0.5), a statistical analysis was performed using a mixed repeated model considering the animal effect as a mixed and repeated factor, and time as a fixed factor. Correlation among TM, PM, proAKAP4, and semen concentration in ejaculates collected twice from the same dogs during the same day (T0 and

T0.5) was also calculated using Spearman rank correlation (r). Significance was set as $P < 0.05$.

Results

During the whole duration of the study (T0, T0.5 and T1), no correlation was found between proAKAP4 results and any sperm morphology parameter. A weak correlation was found between proAKAP4 values and TM (r=0.458, P=0.005). In contrast, PM and proAKAP4 were not significantly correlated (r=0.191, P=0.477). Concerning samples collected during the same day (T0 and T0.5), a correlation was found between TM and proAKAP4 results (r=0.636, P=0.01) as well as between semen concentration and proAKAP4 values (r=0.568, P=0.024). No differences in proAKAP4 concentration, TM, PM, and semen concentration were found at T0 and T0.5 in ejaculates from the same dogs collected twice within the same day (sample size n=8) (P=0.829, P=0.07, P= 0.727, P=0.727).

Discussion and conclusions

Our results confirmed the correlation between proAKAP4 values and TM reported in the literature in different species. Whereas, in our study, proAKAP4 values were not correlated to PM and % of abnormal tails (1, 2). In clinical practice, it is common to collect semen samples from the same dog during the same day, especially for cryopreservation. For this reason, we assessed variations in sperm motility, sperm concentration, and proAKAP4 concentration in different ejaculates from the same dogs collected twice within the same day. In accordance with what was reported in previous literature, no differences were found in sperm motility between T0 and T0.5. In contrast, in our study, no difference was found in semen concentration between T0 and T0.5 (3). Furthermore, in our study, no significant difference in proAKAP4 values was found among different ejaculates from the same dogs collected twice within the same day. Further studies on a larger sample size are needed to analyse the relationship between proAKAP4 concentration and other semen evaluation parameters in the canine species, as well as proAKAP4 variations in different ejaculates collected from the same dogs at different time points.

Keywords: proAKAP4, semen analysis, morphology, total motility, progressive motility, semen concentration, biomarker, dog

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The effects of JWH-018 on testicular tissue: A focus on iNOS expression

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Citation
Yücel-Tenekeci, G., Mutluay, D. The effects of JWH-018 on testicular tissue: A focus on iNOS expression.

Introduction and aim

The aim of this study was to investigate the effect of JWH-018, a synthetic cannabinoid commonly known as 'Bonzai' or 'Jamaica', on spermatogenesis on the expression of inducible Nitric Oxide Synthase (iNOS) as well as to assess the histopathological changes in testicular tissue.

Methods

Thirty male CD1 mice were used in this study. JWH-018 was initially dissolved in absolute ethanol (1%) and then brought to the final volume with 0.9% saline (99%). The solution was administered to the animals by intraperitoneal injection at a volume of 1 ml/kg body weight. The mice were divided into six different groups (n=5 per group) according to the treatment protocol: Control groups 1 and 2 (C1, C2) received 0.9% NaCl solution; Ethanol groups 1 and 2 (E1, E2) received ethanol (1%) only; and JWH-018 groups 1 and 2 (JWH1, JWH2) were treated with 0.3 mg/kg JWH-018 (1) (Cayman Chemical, Ann Arbor, MI). All animals in the JWH1 and JWH2 groups were injected with the relevant dose of JWH-018 once a day for 9 days, whereas the Control groups and Ethanol groups were administered 0.9% NaCl and 1% absolute

ethanol, respectively. Animals in groups C1, E1, and JWH1 were sacrificed 2 days after the last injection. Mice in groups C2, E2, and JWH2 did not receive any treatment for 45 days following the last injections (1). Testicle samples were fixed in Bouin's solution, processed routinely, and embedded in paraffin. Sections of 4-5 µm thickness were cut from the paraffin blocks and stained with Masson's Trichrome, Hematoxylin-Eosin, and Periodic Acid-Schiff (PAS). Histopathological assessment was conducted using the Cosentino histological rating method (2). Additionally, degenerative changes and other alterations, such as disorganized germinal epithelium and desquamating germ cells, were noted. To grade these changes, 100 seminiferous tubules were examined, and the number of tubules exhibiting these changes was counted. The number of residual or apoptotic-like cells observed in degenerative changes was scored semi-quantitatively. Immunohistochemical staining was performed on the paraffin-embedded sections, and the immunoreactivity of iNOS was evaluated semi-quantitatively.

Results

In the histological rating, mouse testicles from all groups were determined to be grade I with regular germ cell arrangement. In the JWH1 group, 5 out of 100 seminiferous tubules exhibited degenerative changes and desquamative germ cells. In these tubules, the germinal epithelial layer was either thin or irregularly arranged, often reduced to a single layer. According to the assessment of the presence of residual/apoptotic-like cells, the control and ethanol groups were rated as mild, the JWH1 group as severe, and the JWH2 group as moderate. In PAS staining, elongated spermatids, nuclear membranes of round spermatids, and apoptotic-like cells showed positive staining. iNOS immunopositivity was observed as membranous and granular in almost all Leydig cells and was scored as severe. According to the prevalence of the distribution, iNOS immunopositivity was scored as moderate in elongated spermatids and mild in spermatocytes.

Discussion and Conclusions

Nitric Oxide (NO) is a highly reactive compound that can act as a physiological messenger or a toxin in various tissues, with the induced isoform being referred to as iNOS. In the event of tissue damage, relevant

pro-inflammatory mediators induce iNOS activity and gene expression (3,4). High concentrations of NO can lead to cell death (5,6). In our study, the prevalence of iNOS positivity in testicular tissue was determined in comparison to the cells, but no significant difference was observed between the groups. The histopathological response characterized by degeneration, desquamative germ cells, and residual germ cells observed in this study was consistent with findings from other toxicopathological studies (7,8). Histopathologically, the observation of degenerative changes in the experimental groups, albeit in a small number of seminiferous tubules, indicated the potential damage caused by JWH-018. Additionally, the increase in residual/apoptotic cells in the experimental groups supported this notion. Therefore, although there was no significant change in iNOS positivity, it is suggested that JWH-018 can induce apoptosis.

Keywords: iNOS, JWH-018, spermatogenesis, synthetic cannabinoid, testis

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Classification of livestock breeds based on post-thaw spermatological parameters using random forest algorithm

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Citation
Boran, H.K.N., Özen, D., Tirpan, M.B., Daşkin, K.T.O., Akçay, E. Classification of livestock breeds based on post-thaw spermatological parameters using random forest algorithm.

Introduction and aim

Livestock production is a fundamental aspect of global agriculture, with ongoing efforts directed towards optimising breeding strategies to enhance productivity and sustainability (1,2). The significance of understanding and

classifying breeds based on semen quality parameters lies in its potential to enhance genetic selection, reproductive efficiency, disease resistance, and market relevance within the livestock industry (3,4). This study aims to classify livestock breeds based on post-thaw spermatological parameters of semen, utilising the random forest algorithm.

Methods

A total of 500 semen samples from Simmental (n=200), Holstein (n=200) and Charolais (n=100) breeds were analyzed at Ankara University Andrology Laboratory in 2023 using a CASA device. Each sample underwent comprehensive analysis to assess post-thaw spermatological parameters. The dataset was preprocessed to address missing values, normalize data, and remove outliers. A random forest algorithm was used to process the dataset and obtain reliable results. The original dataset was randomly divided into the training, validation and testing set with 70:15:15 ratio, respectively. Training set is used to train the models, while test set is used for testing the examined model for performance comparison purposes. Parameter tuning was carried out with the application of a five-fold cross-validation technique on the training set. The following performance metrics were calculated for the model: accuracy, precision, area under the curve (AUC), and F1 scores. All calculations in were performed with R version 4.2.2 using R Studio (version: 2022.07.2+576).

Results

Progressive Motility, VAP, VSL, VCL and STR were the most influential variables to models predictive performance. The accuracy, precision, AUC, and F1 scores for Charolais, Holstein and Simmental breeds were, 0.85, 0.92, 0.82; 0.92, 0.81, 0.75; 0.85, 0.98, 0.93; 0.67, 0.89, 0.79, respectively.

Discussion and conclusion: In conclusion, this study demonstrates the feasibility and effectiveness of utilizing post-thaw spermatological parameters of semen for classifying livestock breeds in the production industry. The random forest algorithm proved capable of accurately distinguishing between breeds based on semen quality profiles, offering valuable insights for breeders and producers (5). By incorporating machine

learning techniques into breed classification practices, we can enhance genetic selection, improve reproductive efficiency, and ultimately contribute to the sustainable development of the livestock industry (6).

Keywords: Bull semen, CASA, kinetic parameters, motility parameters, bull breeds

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Cryopreservation of ram semen with mangiferin additive tris based extender

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Citation
Tuna, F., Ocal, E., Yüksel, R., Sandal, A.I., Arici, R., Yagcioğlu, S., Sandal, B., Ozdas, O.B., Senlikci, H. Cryopreservation of ram semen with mangiferin additive tris based extender.

Introduction and Aim: Ram semen is less resistant to freezing processes compared to bull semen. High levels of ROS cause oxidative stress, protein modification, lipid peroxidation and DNA damage (1). For this rationale, researchers are investigating the efficacy of antioxidants. In this experiment, it was aimed to observe the effect of mangiferin (2), whose antimicrobial, antiviral, anti-inflammatory and antioxidant effects have been proved before.

Methods:5 Kivircik rams kept in Istanbul University-Cerrahpaşa Veterinary Faculty were used. Semen was collected twice a week with an electroejaculator. Pooled semen was divided into 4 groups. Tris and 20% egg yolk was prepared by adding 5% glycerol, 25µM, 50 µM and 100 µM mangiferin. Four groups were formed as Control, MGF25, MGF50, MGF100 (3).

The diluted semen was place into straws (0.25 mL) and adjusted $50x10^6$ spermatozoa. Then frozen with CryoBio System. The protocol started at 26 °C and cooling to 5 °C at 0.3 °C/min, then to -20 °C/min at 0.5 °C/min then to -150 °C/min at 30 °C/min. Finally, the straws were plugged into liquid nitrogen (4). After thawing motility, progressive motility, average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head (ALH), beat cross frequency (BCF), straightness (STR), wobble coefficient (WOB), and linearity (LIN) values were evaluated by computer assisted sperm analysis system (CASA). Acrosome-membrane integrity, mitochondrial activity and DNA damage were evaluated by flow cytometry.

Statistical analysis was performed using IBM© SPSS© Statistics version 29 (IBM© Corp., Armonk, NY, USA). Shapiro–Wilk tests were used to determine the distribution of the dataset and Levene's test for homogeneity of variances. When normal distribution and homogeneity of variance conditions were met, one-way analysis of variance (ANOVA) test was used, otherwise Kruskal-Wallis test was used. When a significant difference was detected between groups, ANOVA was followed by Dunnett's multiple comparison test and Kruskal-Wallis test was followed by Dunn's multiple comparison test. $P<0.05$ was considered statistically significant.

Results

Thawed semen samples' evaluated parameters were evaluated. There were no statistical differences related with sperm motility and kinematic parameters. Significant differences were detected for the mitocondrial activity and acrosome integrity. Mitochondrial activity was statistically significant to the advantage of MGF 50, while the least acrosome integrity was observed in MGF100, no difference was detected in membrane integrity and DNA damage.

Discussion and Conclusions

There were no studies about mangiferin on the freezing of ram semen. The protective effect of mangiferin was observed on mitochondrial activity parameters, while acrosome integrity was lowest in the MGF100, suggesting that the high dose might have a toxic effect. Saxena et al. (5) investigated the effects of Mangiferin extract on bull semen after cryopreservation and reported that it improved semen parameters only to a limited extent, while it deteriorated aggressively when added at 1.5% (5). This is in parallel with the acrosome integrity results of MGF100. Further studies are required for definitive results on the efficacy of mangiferin.

Key words: Sperm, freezing, DNA damage, acrosom integrity, mitochondrial activity

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Extracellular vesicles isolated from sheep oviductal cells improve the quality of sheep embryos

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Faris, I.F.M., Yağcıoğlu, S., Ersoy, N., Bozkurt, G.B.Ö., B.T., Taşlı, P.N., Şahin, F., Pabuccuoğlu, S., Birler, S. Extracellular vesicles isolated from sheep oviductal cells improve the quality of sheep embryos.

Introduction and aim

The oviduct provides an appropriate micro-environment for gamete maturation and transport, and the direction of spermatozoa toward the oocytes conducting successful fertilization and sustaining early embryonic development. The interaction of signals such as EVs between the embryo and the mother environment is crucial to successful embryo development.

This study focused on sheep oviductal epithelial cell-derived-EVs' effects on in vitro embryo production in sheep. The quality of in vitro produced embryos is essential in improving pregnancy rates. The aims of the current hypothesis were, firstly, to obtain a monolayer cell culture of sheep oviductal epithelial cells (SOEC) to collect conditioned media. Then, extracellular vesicles were isolated from the conditioned oviductal cell culture media by sequential ultracentrifugation, quantified, and characterized by the nano-tracking analysis. After the isolation of SOEC-EVs from SOEC-conditioned media, they were supplemented into the embryo culture medium and investigated their effects on the development and quality of in vitro produced sheep embryos.

Methods

Two methods were used for the isolation of sheep oviductal epithelial cells. In the first method (scraping), oviducts were cut longitudinally. Cells were scraped by a glass slide and seeded. The other method was the flushing technique. The oviductal lumen was flushed with 1 mL of DMEM supplemented with FCS (10%) in a petri dish. Healthy and light-colored clumps of cells were collected and seeded. The medium was substituted every 48 hours until the cells reached 90% confluency. Then, the medium was discarded, replaced by DMEM supplemented with EV-depleted FCS (10%), and incubated for 24 hours. After the incubation, the conditioned media were collected. Centrifugation was started at 300 g for 10 minutes to remove the debris and dead cells. The supernatant was centrifuged at 2000 g for 20 minutes to pellet apoptotic bodies. Then the supernatant was centrifuged at 10.000 g for 30 minutes and 20.000 g for 40 minutes to remove the large vesicles; the last centrifuge (ultracentrifuge) was achieved at 100.000 g for 60 minutes. After the ultracentrifugation, the

supernatant discarded, and the tubes were washed with 1 mL PBS to dissolve the isolated EVs. Nano Tracking Analysis (NTA version 3.4 Build 3.4.4) was used to characterize and quantify the concentration and the size of a single EV. The examination of SOEC-EVs' effects on the development and quality of embryos was performed by supplementing the isolated EVs into embryo culture media. Presumptive sheep zygotes were cultured in SOFaa supplemented with SOEC-EVs in different concentrations: Low EV ($9.8x10^6$ EVs/50µL), Medium EV ($49x10^6$ EVs/50µL), and High EV ($98x10^6$ EVs/50µL). For Control group no EVs added. The cleavage rates and the blastocyst development were evaluated at Day 2 and Day 8, respectively.

Results

The SOEC-EVs concentration and mean size of a single EV obtained by the flushing method were assessed as $3.46x10^9$ particles/mL and 200 nm, respectively. The SOEC-EVs obtained by scraping method were assessed as $9.80x10^9$ particles/mL and 184 nm as concentration and mean size of a single EV, respectively.

Similar cleavage rates were obtained in Control, Low EV, Medium EV, and High EV groups (68.4%, 64.7%, 66.0%, 59.5%, respectively). For blastocyst development, although in all EV added groups had higher rates (23.4%, 33.5%, 29.1% and 26.8%, respectively), the differences were not important statistically ($p>0.05$). The total cell number of blastocysts ($\pm$SEM) in Control, Low EV, Medium EV and High EV groups were counted at Day 8 blastocysts as 142.8 ± 11.9, 298.3 ± 36.1, 225.3 ± 15.4 and 279.4 ± 15.3, respectively. The qualities of blastocysts were much better in EV-added groups than in the control ($p<0.05$).

Discussion and conclusions

This research accomplished the first definition of sheep oviductal epithelial cells derived-EVs, demonstrating the impact of SOEC-derived EVs as modulators of the embryo–oviduct relations. The results indicate that SOEC-EVs improve blastocyst quality in sheep. Future research is needed to investigate the cargo contents of EVs and to determine how the effects of EVs on in vitro developed embryos work in sheep.

Keywords: Sheep oviductal epithelial cells; extracellular vesicles; in vitro fertilization; blastocyst quality.

Angiogenesis and oxidative stress pathways: A gene expression study in roe deer testis

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Troisio, I., Zannoni, A., Vannetti, N.I., Hausz, B.L., Bacci, M.L., Ventrella, D., Elmi, A. Angiogenesis and oxidative stress pathways: A gene expression study in roe deer testis.

Introduction and aim

Many mammals which have been evolving in non-equatorial regions have developed mechanisms to synchronize reproduction with the environmental cycle to optimize reproductive success. Animals with a seasonal reproductive cycle, as roe deer *(Capreolus capreolus)*, respond to environmental signals through melatonin, responsible of pulsatile secretion of GnRH which

regulates the secretion of gonadotropic hormones (LH and FSH) and the initiation of reproductive activity. During the mating season (mid. July-mid. August), there is an increase in sperm production, testosterone, and spermatic plasma (1). The regulation of seasonal reproduction is influenced by testosterone, gonadotropins, and plasma proteins, which modulate sperm maturation and sperm integrity.

Angiogenesis and the Vascular Endothelial Growth Factor (VEGF) play a fundamental role in spermatogenesis, promoting the growth and development of testicular tissues. Oxidative stress, instead, mediated by ROS can negatively affect male fertility, causing DNA damage, apoptosis, and epigenetic alterations. This can compromise the quality of sperm, the survival of germ cells and furthermore can activate apoptotic pathways, compromising spermatogenesis (2). The aim of the study was to investigate the variation in the expression of genes associated to the oxidative stress and angiogenesis pathways.

Methods

For research purposes this study analyzed 18 samples of mature male roe deer testicles, obtained during the 2018 hunting season in the Bolognese Apennines: 9 have been hunted between June 1st and July 15th (pre-mating period); the remaining half between August 15th and September 30th (post-mating period). The expression of genes of the oxidative stress and angiogenesis pathways have been studied by quantitative Real Time PCR (qPCR) allowing the simultaneous analysis of the expression of 84 genes (RT2 Profiler PCR Array, Qiagen) for each pathway (Caw Angiogenesis or Caw Oxidative stress) in order to assess variations of one group pool compared to the other. Then to validate data, a qPCR of selected genes was performed in duplicate on each animal, using as housekeeping genes: actin beta (ACTB) and 14-3-3 protein zeta/delta (14-3-3□) (YWHAZ).

Results

Genes, whose expression varied by at least 3 times, have been identified to verify the array data on individual animal. In particular, among genes involved in oxidative pathway, extracellular superoxide dismutase (SOD3)

and scavenger receptor class A member 3 (SCARA3) are up regulated about 8 times for the former ($p < 0.0001$) and about 3 times for the latter ($p= 0.0018$) in the post-mating samples. Instead in the angiogenesis pathway there are evidence of downregulation of expression of Leptin (LEP) and Thrombospondin2 (THBS2) confirmed by validations on each individual.

Discussion and Conclusions

This study is still ongoing, nevertheless provides a basis for a deeper understanding of reproductive activity of the roe deer, a poorly studied wild species. For instance SOD3 and SCARA3, belong to the oxidative pathway and both play an important role in protecting cells from oxidative stress as reported in the epididymis in the same species (3). Further analysis geared towards understand the role of genes which appear to be down or up regulate in both pathways needed, to gain insight on roe deer reproduction.

Keywords: Gene expression, Roe deer, Angiogenesis, Oxidative stress, Rutting Season, Testicular Physiology

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Bovine congenital defects recorded by veterinary practitioners using a mobile phone app. – A national study

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Citation
Mee, J.F., Murphy, D., Curran, M. Bovine congenital defects recorded by veterinary practitioners using a mobile phone app. – A national study.

Introduction and aim

Congenital defects may be defined as abnormalities of structure and function present at birth (Mee, 2021) but which may not be recognised in some cases until animals are older. They are reported to occur at a low frequency (<1%) in cattle but are under-diagnosed due to submission and reporting biases in (passive) surveillance programmes. One of the perspectives on congenital defects rarely documented is that of veterinary practitioners, apart from cases/case series. Veterinary practitioners are uniquely placed to detect congenital defects first-hand on farms and to provide a high quality professional diagnosis. In order to determine the potential for veterinary practitioners to contribute to surveillance of congenital defects, a nation-wide study was set up with the largest veterinary practice group in Ireland.

Objectives
The objective of the project was to field-test a mobile phone app designed for use by veterinary practitioners to collect clinical case data during routine farm visits.

Methods
In total, 59 vets in 28 veterinary practices across Ireland participated over three years (2021-2023). Information and images were collected during dairy and suckler farm visits (mainly calvings) onto a mobile phone using Typeform. The questionnaire consisted of 15 questions; three photos could be collected/case.

Results
In total, 191 cattle with congenital defects were recorded. The three most commonly recorded individual defects were intestinal atresia (24.1%), Schistosomus reflexus (20.4%) and ankylosis (6.8%); multiple defects were recorded in 13.1% of cases. Intestinal atresia included atresia of the intestines (43) and atresia ani (3). Multiple defects most commonly affected the musculoskeletal system (22/25 cases) and of these, those involving ankyloses (11) and palatoschisis (6) were the most common.

Discussion and Conclusions
This study highlighted two congenital defects of cattle which are often considered as sporadic in occurrence and of low prevalence. Based on the findings reported here, both intestinal atresia and Schistosomus reflexus need to be viewed in a new light as clinically important bovine congenital defects warranting preventive strategies at farm and national levels. The results of this study also indicate that veterinary practitioner surveillance using mobile phone apps could be expanded to include other clinically important conditions and in other countries.

Keywords: Congenital defects, veterinary practitioners, intestinal atresia, schistosomus reflexus

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The relationship of some hormone and biochemical parameters levels with fertility in repeat breeder cows

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Citation
Yenilmez, K., Gökçe, E. The relationship of some hormone and biochemical parameters levels with fertility in repeat breeder cows.

Introduction and aim

The aim of the present study was to determine the relationship between Anti Müllerian Hormone (AMH), insulin, Non-esterified Fatty Acids (NEFA), Beta Hydroxy Butyrate (BHB), glucose, thyroid hormones (T3, T4 and TSH), and progesterone concentrations and conception in Repeat Breeder cows.

Methods

In the study, 60 RB cows, which were housed in the same farm under the same care, feeding and reproductive management, and which were determined by ultrasonographic examination to be non-pregnant and free of gynecological problems despite insemination at least 3 times, were used. These animals were divided into two groups as those who became pregnant after the 4th insemination (Group I, n: 40) and those who did not become pregnant (Group II, n: 20). A 10 ml blood sample was taken from the tail vein of all animals during estrus. These animals were inseminated 12 hours after the onset of estrus with semen from the same bull with proven fertility and

by the same technician. On the 9th and 17th days after insemination, blood samples were taken from the tail vein for progesterone analysis. Sera of the blood samples were separated and stored at -80 degrees Celsius until analysis. The levels of triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), AMH and insulin were measured by ELISA and serum progesterone, NEFA, BHBA and glucose were determined by autoanalyzer. Pregnancy examination was performed by ultrasound on the 45th day after insemination.

Results

According to the results of the comparison between pregnant (Group I, n: 40) and non-pregnant (Group II, n: 20) RB cows, there was no statistically significant difference in AMH, NEFA, BHBA, TSH, T3, T4, Insulin and glucose concentrations ($p>0.05$). Progesterone concentration was significantly higher in pregnant RB cows ($p<0.05$).

Discussion and conclusions

In conclusion, AMH, NEFA, BHBA, TSH, T3, T4, Insulin and glucose concentrations were not found to be effective on fertility in RB cows.

Keywords: Anti Müllerian Hormone, Cow, Repeat Breeder

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Joint Efforts for the Development of a Sustainable Sheep Breed Germplasm Cryobank in Southern Italy

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Introduction and aim

In Southern Italy, native sheep breed rearing is strongly linked to regional gastronomic, historical and cultural heritage. Among them, Gentile di Puglia (GdP) breed, is prized for wool, meat and milk production in addition to disease and hostile condition resilience [1]. However, wool market crisis, transhumance abandonment and indiscriminate crossbreedings are threatening with extinction GdP, thus making necessary to preserve it for Southern Italy community and worldwide biodiversity [1]. This study focuses on sustainable and circular economy principles to preserve and enhance GdP breed. Genetic improvement strategies (wool quality assessment) alongside biotechnological (oocyte cryopreservation) and clinical reproductive approaches (on-farm reproductive monitoring) were explored. This combined approach aims to valorise and conserve GdP by developing a germplasm cryobank ensuring its survival and promoting biodiversity in a sustainable manner

Methods

Wool samples were collected in the dorsal region of adult GdP sheep (106 ewes and 16 rams) during shearing. Fiber diameter (FD) was read individually in 5 replicates using FibreLux® MicroMeter [2]. Herd overall fertility was assessed through 5 pregnancy diagnoses in 3 years on controlled mated sheep, performed through transabdominal approach using an 8-10 MHz convex probe. The observation of a fluid-filled uterus with placentomes and at least one fetus were considered pregnancy positive signs [3]. GdP pre-pubertal lamb immature cumulus-oocyte complexes (COCs) underwent vitrification through Open Pulled Straw (OPS, Minitube) (n. 65 COCs) or Rapid-i™ Kit (Vitrolife) (n. 60 COCs), in 3 independent runs [4]. Fresh COCs were used as control for each vitrification group (n. 70 and 76 COCs, respectively). Vitrified and fresh COCs underwent in vitro maturation (IVM) and nuclear chromatin evaluation (X2 test) [5]. Metaphase II (MII) oocytes were assessed for qualitative (X2 test) and quantitative bioenergetic-oxidative parameters (Unpaired Student's t test) [5]. Statistical significance at $p<0.05$

Results

The mean wool FD was 26.27±1.25 µm in ewes and 26.01±1.03 µm in rams. Pregnancy rates (58.6%, 136/232; 65.4%, 51/78; 51.8%, 157/303; 30.8%, 137/445 and 43.3%, 142/328) resulted far from the ideal situation with more than 85% of pregnant animals. COC vitrification through OPS or Rapid-i™ Kit reduced the maturation rate compared to fresh ctrl (14%, 8/59 vs 61%, 41/67; $p<0.00001$ and 10%, 6/61 vs 44%, 30/68; $p<0.0001$, respectively). The rate of MII oocytes showing perinuclear/subcortical mitochondrial distribution pattern, indicator of cytoplasmic maturity, did not differ between each vitrification group and its fresh control (50%, 4/8 vs 54%, 22/41 and 50%, 3/6 vs 83%, 25/30, respectively). Vitrification through OPS increased mitochondrial membrane potential compared to fresh ctrl (156.5±135.3% vs 100±39.5%; $p<0.05$) whereas no differences were observed through Rapid-i™ Kit (76.8±8.3% vs 100±46.8%). Intracellular ROS levels (OPS: 79.2±33.9% vs 100±28.7%; Rapid-i™ Kit: 50.2±18.6% vs 100±103.3%) and overlap coefficient (OPS: 99.4±18.7% vs 100±25%; Rapid-i™ Kit: 76.4±19.5% vs 100±37.7%) did not vary in both groups compared to fresh ctrl

Discussion and conclusions

Wool FD measurement showed that the Merino-type features are retained, although falling in the larger micron ranges, thus setting the stage for genetic improvement strategies. On-farm reproductive monitoring revealed suboptimal pregnancy rates, highlighting the need to integrate traditional reproductive management with advanced reproductive biotechnologies. COC vitrification reduced oocyte nuclear maturation compared to fresh ctrl. Nevertheless, mature oocytes gained cytoplasmic maturity, indicating promising developmental competence. In terms of sustainability and circular economy, these findings underline the importance of combining clinical and biotechnological assisted reproduction approaches with genetic improvement strategies, to support GdP and other local sheep breed conservation and valorization in Southern Italy. By optimizing reproductive efficiency and wool quality, economic viability and environmental sustainability of sheep farming can be enhanced. This integrated approach not only preserves local breed genetic heritage but also promotes a circular

economy by ensuring that every aspect of the breeding process contributes to the overall sustainability and resilience of the agricultural ecosystem

Keywords: Gentile di Puglia sheep breed; conservation strategies; pregnancy; wool quality; vitrification; oocyte in vitro maturation; circular economy

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Use of smartpill® device to evaluate uterine parameters in repeat breeder dairy cows

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Citation
Frattina, L., Carbonari, A., Burgio, M., Cicirelli, V., Rizzo , A. Use of smartpill® device to evaluate uterine parameters in repeat breeder dairy cows.

Introduction and aim

The aim of this study was to measure uterine pH, temperature and pressure in repeat breeder cows at heat, using SmartPill® motility testing system (Medtronic-Milano-Italia), a novel device normally used in human gastroenterology diagnostics. These parameters were compared with healthy cows.

Methods

All procedures were approved by the ethics committee of the University of Bari "Aldo Moro" (protocol No. 1/2020). The device is a wireless motility capsule that consists of a rigid polyurethane shell and measures 26.8 mm in length and 11.7 mm in diameter. It contains sensors for pH, temperature and pressure. The capsule was manually inserted into the uterus through the cervix of experimental cows, 10 RB and 10 healthy (HB) cows, at heat, and monitored for 15 minutes. The receiver was positioned over the hindquarter and real-time measurements of temperature, pH and intraluminal pressure were recorded. Potential behavioral signs of pain were also monitored during the experiment. The data set was objected to analysis of variance (ANOVA) using the Generalized Linear Model (GLM) by SAS software.

Results

No differences in temperature were observed between groups. HB cows had higher pH values of mean, while the median data, the first and third quartiles, minimum and maximum were higher in RB than HB cows ($p<0.05$). For intraluminal pressure, values in HB were higher than RB ($p< 0.0001$) and the statistical analyses detected significant differences for minimum and first quartile.

Discussion and conclusions

The device is non-invasive and not stressful for the cows: at no time the pain score exceeded the threshold requiring analgesic intervention. This study confirmed that RB syndrome is associated with an alteration in the uterine microenvironment, in particular pH and intrauterine pressure levels (1,2). pH changes in RB cows can be found in subclinical endometritis, which alters the physio-chemical properties of the uterine microenvironment. The data obtained in this study shown that the RB cows had a higher frequency of contractions, tendentially even stronger, but of shorter duration, than HB cows. These alterations of contractility could be not functional for sperm transport. Further studies should clarify whether these changes are primary or secondary causes for the development of the syndrome.

Keywords: Dairy cow; Repeat Breeder; SmartPill; Uterine envirorment; Contractility

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Low-dose megestrol acetate: Efficacy, safety, and estrus interval in cats for reproductive suppression

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Pereira , M.C., Grassi , A., Pipan , M.Z., Romagnoli, S. Low-dose megestrol acetate: Efficacy, safety, and estrus interval in cats for reproductive suppression.

Introduction and aim

Megestrol acetate (MA) is a synthetic analog of progesterone originally formulated as a contraceptive for humans. Nowadays, its current application lies within veterinary medicine for estrus suppression in both bitches and queens, through blockage of the hypothalamic-pituitary-ovarian axis (1). Estropill™, MSD is the commercial MA product in, marketed in Italy in syrup formulation. Historical use of different protocols, including excessive dosages of MA and careless patient selection have been associated with endocrine, uterine and mammary complications (1). This study aims to investigate the efficacy and safety of a low-dose MA treatment in suppressing heat in queens and intact male behavior and fertility in tomcats, and the interval between end of treatment and ovarian activity resumption in treated queens.

Materials and Methods

A total of 21 post-pubertal, heathy, privately-owned intact cats, from which 18 females and 3 males were presented to the veterinary teaching hospitals of the universities of Padova, Italy and Ljubljana, Slovenia for short-term control of the reproductive function. All animals were submitted to a a) complete clinical and b) reproductive examination (including vaginal cytology for females and examination of penile mucosa for males), c) hematology (complete blood count, biochemistry and progesterone [P4] assay), d) urinalysis and e) reproductive ultrasound before being enrolled in the study, to ensure the general and reproductive health status of the animal before treatment. Inclusion criteria were absence of past reproductive problems, vaginal cytology excluding estrus and serum P4 concentration below 2.0 ng/ml. Queens with higher P4 values, which indicate ovulation, saw their treatment postponed for the necessary amount of time to rule out pregnancy and avoid P4 overexposure (from to 20-40 days depending on initial P4 value and date of last heat). Subjects were treated orally with 11.5 µg/kg (approximately 5 drops/kg/) of Estropill™ every 24 hours. Treatment duration was decided in accordance with the owners' request and the subjects were divided into 3 groups considering treatment length: G4 - 4 months (4 females and 1 male), G5 – 5 months (3 female and 2 males) and G6 – 6 months (11 females). Monthly check-ups were conducted repeating procedures a), b) and e), while a) to e) were carried out again post-treatment. Queens were reexamined at the first signs of heat post-treatment and underwent vaginal cytology to confirm estrus. Weight gain and time until reproductive activity resumption were statistically compared between groups resorting to the Student *t* test for paired samples and one-way ANOVA test, respectively.

Results

The 21 enrolled cats with a mean age of 2.4 ± 1.67 years, ranging from 9 months to 6 years, and weight of 3.76 ± 1.03 kg (range 2.3 - 6.2 kg) belonged to 9 different breeds (British Shorthair n=5, European shorthair n=4, Maine Coon n=4, Sphynx n=3, Siamese n=1, Ragdoll n=1, Persian n=1, Norwegian Forest cat=1 and Bengal n=1). Seventeen/18 treated queens consistently exhibited behavioral and cytological anestrus. One queen, however,

displayed vocalization, lordosis, rubbing and increased affection during treatment. Two/3 tomcats' penile spikes and marking behavior disappeared completely after 3 months of MA treatment, being present again by 100 days post-treatment cessation. The third tomcat demonstrated persistence of spikes throughout treatment (although a reduction in size was noticed) never completely ceasing urinary marking and sexual behavior, which eventually led to a successful pregnancy.

The queens took an average of 50.11±17.1 (range 23-77) days to resume cyclicity after treatment. No significant effects of season of the year when treatment finished or treatment duration (42.3±30.1, 49.3±10.21 and 52.5±15.5 days 4, 5 and 6 months, respectively) were observed. One queen, who ended the 4-month MA cycle in August, showed the first signs of heat after 184 days, for which she was considered an outlier and therefore not included in this statistical analysis. Clinical, laboratory and ultrasound parameters during monthly checkups remained unchanged, except for increased appetite which resulted in weight gain. Body weight increased throughout treatment in all groups, being significant in G4 (p-value=0.021) and G6 (p-value=0.0004). Furthermore, most queens (7/10 for which body weight data was available) experienced non-significant weight loss from treatment end to first manifestation of heat signs.

Discussion: The use of MA has been surrounded by controversy; however, this study demonstrates that minimal dosages of this drug produce the desired contraceptive effect in queens. The female who exhibited estrus signs during therapy showed an important weight gain (32%), probably leading to an underdosing of MA. The queen who took longer to resume cyclicity was housed in an outside enclosure which possibly hinders frequent and thorough heat detection. The male who never responded to treatment and a female who fully did, mated at the queen's first heat, at 27 days post-treatment, resulting in conception and kittening of 4 healthy cats, which confirms the reversibility of contraception with MA.

Conclusion: The low dose MA treatment is a safe option for controlling reproduction activity and is highly effective in suppressing cyclicity in adult queens for up to 6 months. Cyclicity resumes on average about 7 weeks after treatment cessation. Results in tomcats do not endorse the use of MA as an effective contraceptive in tomcats and deem further investigation. No side effects were observed besides the increase in body weight, that resulted in underdosing in 1 subject. This suggests that the dosage of 11.5 µg/kg/day is close to the minimum effective dose of the drug, thus contributing to a greater safety of treatment.

Keywords: Megestrol acetate, feline contraception, queen

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Heat stress in dogs: Influence of oxidative status and sperm quality

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Introduction and aim

The vitality and functionality of spermatozoa are important for the success of canine reproduction. Alterations in motility and structure of spermatozoa induce a decrease of reproductive function to failure of fertilization. In several species, heat stress (HS) and increased Reactive Oxygen Species (ROS) are known to have a deleterious effect on sperm quality and functionality due to lipid oxidation on sperm membranes [1]. This study aimed to evaluate the effects of environmental HS on canine quantitative and qualitative ejaculate parameters.

Methods

This study received ethics committee approval (Prot. 656/18). Twenty dogs were enrolled for this study, in 2022, precisely from May to August. Dogs after enrollment were divided in two groups of 10 subjects each one. The first group was kept in a closed environment with controlled humidity and air conditioning system (thermoneutrality group: TN). The second was held outdoors and thus under the influence of the ambient weather conditions (heat stress group: HS). All owners were provided with a temperature and humidity monitoring system placed within the indoor space for the TN

group and in the outdoor area for the HS group, for the all duration of the study. Data loggers were set to record temperature and humidity on an hourly basis. Semen and blood samples were collected at 30-day intervals, starting from May (T0). Semen was analyzed by the Computer Assisted Sperm Analyzer (CASA) that was set up for canine semen specific parameters. For the evaluation of the oxidative status on blood samples, d-ROMs (reactive oxygen metabolites) and BAP (biological antioxidant potential) concentrations were obtained by the means of a photometric analytical system (FREE Carpe Diem®). During each control time for semen and blood sampling, the data loggers were downloaded to calculate the Temperature Humidity Index (THI). For all parameters the analysis of variance (ANOVA) was performed and significance was set at $p < 0.05$.

Results

ROS and BAP levels and sperm quality parameters indicated important variations due to the effects of environmental heat stress. The parameters of oxidative stress were significative increased in HS groups (TN 75.88 UCARR vs HS 155.00 UCARR: $p<0.0)1$, while antioxidants were reduced (T90 TN 2281.11 mmol/L vs HS 1445.99 mmol/L: $p < 0.01$).

Discussion and conclusions

The results obtained in this study shown that there was an imbalance between the production of metabolites of oxidative processes and the activity of the anti-oxidant system in dogs of the HS group. This was indicative of oxidative stress [2]. Regarding ejaculate parameters, a significant increase in tail abnormalities (bent and rolled tail) was found in semen samples from HS dogs. Indeed, cell apoptosis was considered to be the main consequence of HS, followed by metabolic and structural abnormalities in sperm [3]. To the best of our knowledge, no specific data on the effects of environmental heat stress on semen parameters in canine species are yet reported in the bibliography. This currently does not allow us to corroborate these results, but they can be used as preliminary data for further future studies on this topic.

Keywords: Dog; Sperm quality; Heat Stress; Reactive Oxygen Species; Antioxidant.

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Inducing pregnancy in hair goats by the female effect outside the breeding season

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Özmen, M.F., Cirit, Ü. Inducing pregnancy in hair goats by the female effect outside the breeding season.

Introduction and aim

Estrus synchronization (ES) methods are used to prevent the economic losses and plan reproduction. Hormonal and non-hormonal ES methods can be used together or separately. Methods based on the use of progesterone and PMSG can be applied successfully at any time of the year (1). Non-hormonal methods can also be used widely in the field such as additional feeding protocols (flushing), male effect and adjusting the light exposure time (1, 2). In this study, the effects of mixing the estrus induced goats with goats that had not been treated outside the breeding season on estrus and pregnancy rates were investigated.

Methods

The study was carried out outside the breeding season (at the end of April) using a total of 32 female hair goats and 6 billy-goats, aged between 2 and 4. Goats were divided into 2 groups. The females in the first group (n: 16 goats, 3 billy-goats) were inserted with vaginal sponges containing progesterone for

11 days. On the 12th day, 500 IU PMSG was applied along with the sponge removal. No treatment was applied to male goats. Within 36 hours after sponge withdrawal, all goats showed estrus and were mated. The mated estrous goats joined the 2nd group immediately (n: 16 goats, 3 billy-goats). No treatment was applied to the second group. Female goats in the 2nd group were observed for 7 days. Goats showing heat were mated with 3 billy-goats and pregnancy examination was performed by ultrasound after 30 days. After 150-160 days, the births of the pregnant goats were followed and the number of offspring was recorded.

Results

The all 16 goats in Group 1 showed estrus and were mated. In Group 2, 9 of the 16 female animals showed estrus within 4 days and were mated ($P<0.05$). It was determined that 13 goats in group 1 and 9 goats in group 2 were pregnant ($P>0.05$). In total, 15 lambs were born in group 1 and 13 lambs were born in group 2. Multiple pregnancy rates in group 1 and 2 were 25% and 37.5%, respectively ($P>0.05$).

Discussion and Conclusions

In our literature review, we could not find any study similar to our study on hair goats. Although the results of the female-female effect during anestrus season seem surprisingly good, the fact that the number of animals in the study was limited should also be kept in mind. As a result of our study, in hair goats;

1) By using the female effect, estrus synchronization methods may be applied less costly and successfully outside the mating season.

2) It was concluded that new studies involving more animals are needed.

Keywords: Estrus, Female Effect, Goat, Pregnancy.

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Effects of mitoquinone and caffeic acid supplemented extender on the freezability of ram semen

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Citation
Yilmaz, M.M., Cimen, T., Ustuner, B. Effects of mitoquinone and caffeic acid supplemented extender on the freezability of ram semen.

Introduction and aim

Cryopreservation can preserve sperm fertility in approciate extenders, but during this process it causes structural, biochemical, functional and molecular distruption in spermatozoon. Due to the low cholesterol/phospholipid ratio of the ram spermatozoon plasma membrane, they are more sensitive to sudden temperature changes (1). Reactive oxygen species (ROS) one of the causes of oxidative stress (OS), disrupt membrane fluidity and inducing lipid peroxidation which leads to loss of motility, a decrease in mitochondrial membrane potential due to OS causes sperm DNA fragmentation and ultimately cell apoptosis (2,3). It has been reported that supplementing antioxidant substances to ram semen freezing extenders has positive effects on post-thaw sperm characteristics by eliminating the negative effects of ROS (4,5). Among these antioxidants include Mitoquinone, one of the mitochondrial-targeted antioxidants, and caffeic acid, which

reduces free oxygen radicals by preventing the consumption of free radical scavenging enzymes (6,7).

The aim of this study was to determine the post-thaw sperm characteristics (motility, acrosome, plasma membrane and DNA integrity, and mitochondrial membrane potential), antioxidant parameters (MDA (Malondialdehyde), and total antioxidant capacity of ram sperm frozen with the supplementation of mitoquinone and caffeic acid.

Methods

Semen was collected from five Merino rams, aged between 2 and 4 years, in the non-breeding season, using an electro-ejaculator. Collected semen samples with a mass activity of 3 or more on the scale, at least 70% motile, and a concentration of $1x10^9$ were pooled and diluted with a Tris-egg yolk-glycerol extender by the two-step dilution method. The pooled ejaculates were divided into seven equal aliquots according to control and antioxidant groups (Control, Mitoquinone(M) 100 nM, 150 nM, 200 nM; Caffeic Acid(CA) 50 µM,100 µM,150 µM). Afterwards, diluted semen samples were cooled to 5°C within 1 hour. Subsequently, a 2-hour equilibration process was carried out at 5°C. The equilibrated semen was frozen using a freezing machine. The frozen straws were stored in liquid nitrogen at -196°C for at least one month until post-thaw evaluation. All the semen parameters were evaluated twice during the study: first at the fresh stage and at the post-thaw stage. Semen samples were examined by the specified methods for motility/ kinematics [(Computer-assisted sperm analyzer-CASA)], acrosome integrity [(Fluorescein lectin staining assay -conjugated pisum sativum agglutinin (FITC-PSA)], plasma membrane functional [(Hypoosmotic swelling test-(HOST)] and DNA integrity [(Terminal-deoxynucleotidyl-transferase-mediated-dUTP nick-end labelling-(TUNEL)], and mitochondrial membrane potential [5.5', 6,6'-tetrachloro-1, 1', 3,3'-tetraethyl- benzimidazole carbocyanine iodide -(JC-1)], antioxidant parameters [MDA-(ELISA kit),TAC-(Colorometric kit)]. Data obtained from the study were analysed using SPSS (SPSS 26 for Windows; SPSS, Chicago, IL).

Results

Fresh sperm was adversely affected by the freezing process in terms of all spermatological parameters (P<0.001). The post-thaw motility of M200, CA100 and CA150 antioxidant groups was greater than the control group (P<0.05). No differences were observed among all groups including control group in terms of post-thaw kinematic parameters (P>0.05), except ALH, STR and WOB values (P<0.05). The post-thaw plasma membrane integrity of M groups was compared with CA groups; the CA100 group, which had the lowest plasma membrane integrity among the antioxidant groups, was observed to be statistically different only from the M200 and CA150 groups (P<0.05). In the evaluation of post-thaw acrosome membrane damage, all the antioxidant groups were lower than control group, except CA50 and CA100 groups (P<0.05). No difference was observed among all groups including control group in terms of post-thaw DNA fragmentation, MDA and TAC values. For the post-thaw mitochondrial membrane potential, all groups except the CA50 group were determined to be superior to the control group statistically (P<0.05).

Discussion and conclusions

In the study, the effects of mitoquinone and caffeic acid on the sperm freezing of different species, it was seen that the high-dose MitoQ and CA had positive effects on sperm parameters (6,7). Low doses of antioxidants could not protect sperm motility from the negative effects of cryopreservation. In conclusion, when evaluated in terms of spermatological and antioxidative parameters, mitoquinone and caffeic acid could be used successfully for ram semen cryopreservation.

Keywords: ram semen, cryopreservation, mitoquinone, caffeic acid

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Differential expression of AQP1 and AQP3 in ovarian tissue of bitches during anestrus and diestrus

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Introduction and aim

Aquaporins (AQP) are a class of small hydrophobic integral membrane proteins that play a role on water movement (1). The water and ion flow across the cell membrane is important in the aspects of regulation in a variety of cellular processes including the uterus and ovaries (2, 3). Aquaporin expressions can be regulated by sex steroid hormones (4,5). Limited data is available on the expression patterns of AQPs in the uterus however, no data is available for ovarian tissue in bitches. Thus, the aim of this study was to evaluate the effect of diestrus and anestrus period on the uterine and ovarian aquaporin gene expression in female dogs.

Methods

A total of 24 healthy dogs presented for elective ovariohysterectomy were enrolled in the study (n:24). Dogs were separated into two groups according to their sexual cycle as diestrus (n:12) or anestrus (n:12) by using vaginal cytology as previously described (6). Routine ovariohysterectomies were performed (7) following animal care guidelines. Then uterine and ovarian tissue samples were collected for further analysis of gene expression of *AQP1* and *AQP3*. Collected samples were placed in RNA later solution. Then, samples were frozen in liquid nitrogen and stored at -80 °C. RNA isolation was performed according to the modified Trizol method (8). Following purity and concentration measurements, the sample integrities were checked by agarose gel electrophoresis. Following DNase I treatment, cDNA synthesis was performed from the samples. Expression levels of the *AQP1* and *AQP3* genes were determined using a SYBR Green I dye containing kit. The qPCR protocol consisted of initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 60 sec. *GAPDH* and *RPS15* housekeeping genes were used. The primers used for gene amplification of were designed for the present study using PrimerBLAST (NCBI) and Primer3Plus. The expression levels of target genes were determined using the 2^-ΔΔCt method, and changes were compared using a Student's t-test.

Results

The uterine aquaporin expressions were similar among groups. However, ovarian *AQP1* expression upregulated nearly 6-fold in the anestrus group. Moreover, *AQP3* was upregulated 3-folds in ovarian tissues of anestrus bitches.

Discussion and conclusions

AQP water channels may contribute to the fluid trafficking in the uterine tissues by transcellular pathways (5). As previously described by Aralla et al., (5) and supported by the present study, *AQP1* uterine expression was not affected from the sexual cycle period. Also, it has been reported that progesterone treatment did not affect the *AQP3* expression in mares (9). Similar mechanisms might have roles on the uterine *AQP* expression. On the

other hand, the reason for the lack of gene expression differences between uterine aquaporins may be the design of the study groups. In human, ovarian origin *AQP1* and *AQP3* expressions showed differences according to the phase of sexual cycle. *AQP1* and *AQP3* expressed in theca and granulosa cells (10). Thus, the ovarian structures such as follicles and granulosa cells might have role on ovarian *AQP* expressions, however, it is not clear why the inactive period of ovarian tissue showed increased *AQP1* and *AQP3* expressions in bitches. Further studies should be conducted to determine the role of altered *AQP* expressions in canine ovaries.

Keywords: Anestrus, aquaporin, canine, diestrus, gene expression, ovary, uterus.

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Non-Surgical uterine flushing procedure in sheep using Wallace Sure Pro® catheter

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Citation
Önder, N.T., Şahin, O., Kılıç, M.C., Demir, M.C., Gökdemir, T., Yıldız, S., Kaçar, C., Öztürkler, Y. Non-Surgical uterine flushing procedure in sheep using Wallace Sure Pro® catheter.

Introduction and aim

The limited use of non-surgical uterus flushing in sheep can be attributed to the complex and changing nature of the sheep cervix (1,2). Different speculums, tocolytic agents, and catheters are utilized for the application

of transcervical techniques in small ruminants (1-5). The Wallace Sure Pro® catheter is utilized for human embryo transfer procedures (6,7). In our study, we assessed the effectiveness of uterine flushing using the Wallace Sure Pro catheter because of its thin structure and compatibility with the 1 mm Bakes Rosebud urethral catheter.

Methods

In this study, 10 Tuj breed sheep between the ages of two and five were used. Total duration, cervical transit, and flushing durations were recorded throughout the study. The volume of fluid obtained through flushing was quantified as a percentage relative to the total volume of fluid given. The tails of each sheep were fixed and their perineal areas were sanitized. The overall duration was recorded following the contact of the speculum with the vulva. The cervix was retracted to the vulva. The cervical transition was conducted using a 1mm Bakes rosebud urethral sound which's handle was cut. Once the cervical transition was finished, the Wallace Sure Pro® catheter was inserted through the catheter and the sound was extracted. Subsequently, a b-flow flow regulator was attached to the catheter. A separate hose was attached to the opposite side of the B-flow flow regulator in order to allow the use of the vertical embryo recovery filter system. A cannula with a gauge size of 18 was inserted into the injection port of the B-flow flow regulator. The 3-Way Stopcock T-Connector was inserted into the plastic part of the cannula. A syringe filled with Lactate Ringer's solution was attached to the side of the cannula. The central pathway was oriented in an upward direction, and a syringe with a capacity of 50-60 ml, without a plunger, was positioned in this location to create negative pressure within the uterus. Lactate Ringer's solution was used through a second syringe to inject a total of 400 ml of fluid, 20 ml at a time, into the uterus. The fluid obtained from the flushing procedure was gathered in a beaker and subsequently transferred into a graduated cylinder for the purpose of measurement.

Results

The study found that the average total duration was 34.85±1.61 minutes (mean ± standard error), the cervical transition period was 66.80±16.27 seconds, the flushing duration was 26.65±1.28 minutes, and the average amount of flushed fluid was 96.63±0.46%.

Discussion and conclusions

Upon analyzing various studies, the amount of fluid recovered after flushing in sheep ranges from 84% to 99% (1,3). The fluid recovery rate of 96.25% was considered in line with findings from other studies. The study found that the addition of a negative pressure source to the flushing system allowed the fluid to be discharged rapidly. Consequently, it has been determined that uterine flushing procedures are effective and readily applicable using the human embryo transfer catheter Wallace Sure Pro®, which we believe is widely accessible to numerous researchers.

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Keywords: Sheep, Ewe, Transcervical, Uterus flushing, Wallace Sure Pro.

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Effects of polystyrene nano/microplastic (nmps) on in vitro exposed cumulus-oocyte complexes

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Introduction and aim

One of the main concerns related to plastic pollution regards the impact that nano-microplastics (NMPs) can have on human and animal health. They have been shown to accumulate in the gastrointestinal tract of various animal species, favoring the development of inflammatory and oxidative processes, genotoxicity and mutagenicity. Recently, NMPs have been

detected in the human placenta (1) and in the ovaries and uterus, negatively affecting the fertility of mice (2). It has been observed that NMPs cause the development of numerous morpho-functional alterations of the female reproductive organs, through the induction of oxidative stress. To date, there is no experimental evidence demonstrating the effects of NMP on cumulus-oocyte complex (COC). Therefore, the aim of the present study was to evaluate the effects of increasing concentrations of polystyrene NMPs on COCs, exposed during in vitro maturation (IVM), on meiosis resumption and redox/oxidative status of ovine oocyte.

Methods

COCs from the ovaries of slaughtered prepubertal lambs (4/6-months old) underwent in vitro maturation (IVM) in a TCM199-based medium under 5% CO_2 in air at 38.5°C for 24h (3) in presence of polystyrene NMPs beads (PS-NMPs) of 50 nm in diameter at different concentration (5, 50 or 100 µg/ml); five replicates were performed. Oocytes cultured in absence of beads were used as controls. Following IVM, cumulus cells were removed and oocytes were analyzed for redox/oxidative status by confocal laser scanning microscopy after labeling of reactive oxygen species (ROS) with 2',7'-dichlorodihydrofluorescein diacetate (3). Oocytes were then fixed in paraformaldehyde, mounted on glass slide, stained with Hoechst 33258 for meiotic stage evaluation and those showing the metaphase II (MII) plate with the first polar body (PB) were destined to oxidative status assessment by laser scanning confocal microscopy (3). Discrete data were analyzed by Chi-square test while continuous data were analyzed by ANOVA test.

Results

A total of 411 COCs were cultured for IVM. Treatment with PS-NMPs significantly reduced the percentages of MII-PB in the 50 µg/ml (35/113; 31.0%) and 100 µg/ml (30/94; 31.9%) culture conditions, compared to controls (43/91; 47.3%, $P < 0.05$), whereas, no differences was found for the 5 µg/ml condition (39/113; 34.5%). Furthermore, oocytes exposed to the highest PS-NMPs examined concentration (100 µg/ml) showed a reduction of metaphase I (MI) rate (10/94; 10.6% vs 22/91; 24.2%, $p < 0.05$) and an increase in germinal vesicle (GV) percentage (38/94; 40.4% vs 15/91; 16.4%,

$p < 0.001$), compared with controls, respectively. Regarding the effects of PS-NMPs on the oocyte redox/oxidative status, the ROS intracellular levels, expressed as arbitrary densitometric units (ADU), were significantly increased in all experimental conditions in which COCs were exposed to PS-NMPs compared with controls (1111±485 for 5 µg/ml with $p < 0.001$; 1272±580 for 50 µg/ml with $p < 0.01$, 1108±447 for 100 µg/ml with $p < 0.01$; 847±333 for controls).

Discussion and Conclusions

Our results demonstrate that high concentrations of PS-NMPs induce an alteration of meiotic processes with an evident damage to the ability of oocytes to resume meiosis in vitro until reaching nuclear maturity, as a fundamental prerequisite for oocyte fertilization. The damage suffered by oocytes is triggered by oxidative stress of which NMPs have also been shown to be the cause in other cellular systems (2). In our study we verified that even the lowest tested concentration of 5 µg/ml PS-NMPs is able to increase ROS levels. Further studies will be necessary to test the effects of PS-NMPs on fertilization events and embryonic development of ovine oocytes exposed in vitro.

Keywords: oocyte in vitro maturation, nano/microplastics, polystyrene beads, sheep, oxidative stress.

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Effect of diluent replacement on liquid storage of canine semen at +4c

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Introduction and aim

It is aimed to compare extend the storage period of dog semen in terms of liquid storage conditions without being subjected to cryopreservation. In this study, the effects of liquid storage of canine spermatozoa under different storage conditions at 4 °C. More specifically, the objective was to investigate the effects of renewing the extender or adding to it an energy source during cold storage of dog semen for 9 days on sperm motility, viability and acrosome integrity.

Methods

Four clinically healthy dogs of unknown fertility but presenting normal spermatological parameters were used in the study: two alabai, one rottweiler and one crossbreed. The dogs ranged in weight from 30 to 80 kg and in age from 3 to 5 years. Semen (eight ejaculates per dog) was obtained by digital manipulation and collected in glass collectors warmed to 37°C and pooled from 4 dogs at 3-day intervals. After collection, individually and pooled semen was evaluated in terms of concentration, motility, viability, and acrosome integrity[1]. Concentration and motility values were determined using the Makler counting chamber according to the World Health Organization (2021) handbook[2]. All ejaculates used for this study presented at least 200 million/ml sperm. Sperm viability was determined according to the eosin-nigrosin staining method and the percentage of abnormal acrosome was assessed by the hancock wet fixation method. Pooled semen was diluted 1:3 in tris:citrate:10% egg-yolk extender and then divided into 3 equal aliquots (final volume was 10mL for each group), one for each experimental group: control, Energy source (ES) and Replacement of culture (RCM) media[3]. Sperm samples were then cooled to 4ºC and its quality assessed, every 24 hours for 9 days, in terms of motility, viability, and acrosome integrity as described above. Every day all experimental groups were centrifuged 300g for 10min before the evaluation. All experimental groups vortexed gently after the centrifugation and practiced the evaluations. Subsequently, in the ES group 1 mM Glucose was added to the diluted semen and in the RCM group the supernatant was gently removed and replaced with tris-citrate-egg yolk extender from the stock solution.

Results

Sperm motility ended on the 6th day in the control group, on the 8th day in the ES group and on the 10th day in the RCM group ($p<0.05$). While the percentage of motile sperm on the 6th day was 0% in the control group, it was 20% in the ES group and 50% in the RCM group ($p<0.05$). Whereas the viability on the sixth day was 1% in the control group, it was 15% in the ES group and 27.5% in the RCM group ($p<0.05$). The percentage of abnormal acrosome on the 6th day was 8% in the control group, 18% in the ES group and 14% in the RCM group ($p<0.05$). Although the addition of the energy

source to the medium used for liquid storage of canine semen ensured the continuation of sperm motility, it caused a significant decrease in the percentage of abnormal acrosome and dead sperm compared to the control group ($p < 0.05$).

Discussion and conclusions

The results in this study indicate that dog spermatozoa can be successfully stored in 4 °C for 9 days. Sperm motility deteriorated in Control, ES and RCM groups during the 48h of liquid storage, although cold storage of canine semen for 24h did not significantly reduce functional of semen. Replacing the present extender with fresh extender prolonged sperm life and motility during storage. Motility persisted longer in the ES and RCM groups compared to the control group for 9 days. The percentage of viable sperm was higher in the ES and RCM groups compared to the control group on all experimental days. From day 3 onwards, acrosome damage increased significantly in the ES group. The percentage of abnormal acrosomes was lower in the RCM group compared to the control. Semen quality continuously decreases during cold storage at 4 °C.

In conclusion, renewal of the medium used for liquid storage of canine semen was significantly beneficial in preserving sperm motility.

Keywords: Canine Semen, Liquid Storage, Replacing media

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Pathological conditions in the male reproductive system of dogs: A 14-year retrospective study

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Ahlat, O., Demirtaş, O.B. Pathological conditions in the male reproductive system of dogs: A 14-year retrospective study.

Introduction and aim

In veterinary medicine, it is essential to examine the male reproductive system lesions and diseases clinically and pathologically (1, 2). Evaluation of clinical diagnoses with histopathology reports has an important place in the treatment of diseases. Clinical diagnoses alone may not be sufficient. If treatment is not administered properly, serious problems may arise, such as infertility. This study aimed to present the pathologies encountered in the tissues of the male reproductive system of dogs submitted to the Department of Pathology, Faculty of Veterinary Medicine, Ankara University between 2010 and 2023.

Methods

Data in the histopathology reports of a total of 70 dogs were evaluated. These data were categorized according to anatomical locations, including the testes and epididymis (n: 38), the scrotum (n: 7), the prostate (n: 6), and

the penis (n: 17) and prepuce (n: 8). Pathological conditions in these sites were defined under three main headings: inflammatory, non-inflammatory changes and neoplasms. In addition, the age and breed distributions of the dogs were also analyzed.

Results

Among the testicular neoplasms (73.7%), seminoma, interstitial (Leydig) cell tumor, Sertoli cell tumor, and mixed germ cell-sex cord-stromal tumor were diagnosed. Orchitis and/or periorchitis (13.2%) were necrotic, hemorrhagic, purulent and pyogranulomatous. Chronic orchitis and fibrosis were described in one dog. Non-inflammatory changes (21.1%) included degeneration, interstitial hemorrhage, necrosis, and atrophy. Fibrinonecrotic epididymitis, chronic epididymitis, degeneration of the epididymis with foreign body granulation tissue, intraluminal cysts, papillary hyperplasia, interstitial edema, and hemorrhage were reported in five dogs. Ulcerative dermatitis, phlegmon, fibrinonecrotic hemorrhagic inflammatory change, subacute dermatitis, chronic inflammatory granulation tissue, hemorrhage in the dermis, hypertrophy in the muscles, and mast cell tumor were observed in the scrotum. Subacute prostatitis and chronic inflammatory granulation tissue, papillary cystic hyperplasia, cysts, adenocarcinoma, and fibromyxosarcoma were reported in the prostate. There was transmissible venereal tumor (TVT), squamous cell carcinoma (SCC), mast cell tumor, and subacute balanoposthitis in the penis and prepuce. Additionally, papilloma, sweat gland adenoma, subacute dermatitis, ulcerative dermatitis, vascularization and hyalinization of collagen, necrosis and hemorrhage were described in the penis. Hepatoid adenoma/carcinoma and fibroma were also diagnosed in the prepuce. These pathological conditions were found in dogs between the ages of 0-5 (25.4%), 6-10 (35.8%), and 11-16 (38.8%); mostly in Terrier (18.8%), Mixed (15.6%), Golden Retriever (10.9%).

Discussion and Conclusions

While it was pointed out that interstitial cell tumor, seminoma and Sertoli cell tumor are very common testicular tumors in dogs, seminoma was the most common one encountered in the study. The finding that these tumors were mostly seen in mature-aged dogs and cryptorchid testes was particularly

consistent with the literature (1-5). Testicular degeneration is a known condition in older dogs**.** The study concluded that testicular degeneration may be related to aging. Orchitis has been reported incidentally in dogs (3). Morphological diagnoses suggest that orchitis may occur due to bacterial infection. Mast cell tumors are common in scrotal tumors (6, 7). Similarly, in the study, mast cell tumor was detected in the scrotum. Based on the literature (1, 3) it can be inferred that scrotal dermatitis may be generally related to self-licking, self-trauma and physicochemical injury. Important primary tumors of the penis and prepuce include papilloma and TVT (1-3). In this study, TVT and SCC were remarkable. Although prostatic disorders (hyperplasia, prostatitis, cysts, carcinoma) are very common in male dogs (1-3, 8, 9), the examined tissue was insufficient to conclude in this study. Besides, breed predisposition could not be determined precisely because the tissues were removed from very different breeds and the number was again, insufficient.

Keywords: dog, male, pathology, reproductive, retrospective.

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The effect of nanoparticle addition to diluted dog semen on storage at 4°c

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Introduction and aim

The demand for assisting reproduction by artificial insemination in pet animals is increasing day by day and this application is becoming routine. However, the fact that not all dogs give semen regularly and are not accustomed to this method is a disadvantage for the application. The aim of this study was to use the method of short-term storage of semen from male dogs at +4°C to provide the opportunity to inseminate the female dog in the estrous cycle during the ovulation period with a single ejaculate. To achieve this, specific doses of nanoparticles will be added to the sperm extender to try to achieve the target (1–3).

Methods

In the study, sperm was collected from four fertile Kangal breed male dogs, aged 24-48 months, housed at the Sivas Cumhuriyet University Kangal Research and Breeding Center. Semen was obtained from the dogs by digital manipulation method. Semen was collected twice a week for a total of 4 times. In order to eliminate individual differences, the semen samples were pooled. Initially, sperm parameters were checked after each

semen collection. Motility rate >80%, abnormal spermatozoon rate <20% and spermatozoon concentration >250x10^6 were determined. The sperm samples diluted with Tris-egg yolk extender were then divided into 7 equal parts (4). While one of the samples was kept for the control group, zinc nanoparticle 50 µg/ml (Z1), 100 µg/ml (Z2), 200 µg/ml (Z3) and activated carbon nanoparticle 50 µg/ml (C1), 100 µg/ml (C2), 200 µg/ml (C3) were added to the diluent respectively. Motility rates, live/dead rates, abnormal spermatozoon rates were checked every 8 hours starting from the 0th hour (5).

Results

When the short-term stored samples were evaluated, it was found that the total motility loss was the lowest in the groups in which 200 µg/ml activated carbon nanoparticle was added compared to the others ($p<0.05$). In addition, when zinc nanoparticle 50 µg/ml was added to the diluent, total motility loss was found to be less when compared with the control group ($p<0.05$). When the morphological examination results were evaluated, it was observed that there was no significant difference between the groups ($p>0,05$).

Discussion and conclusions

While direct references on nanoparticles added to dog semen extenders are limited, the existing literature on additives and nanoparticles in semen preservation provides a foundation for further exploration into the potential benefits of nanoparticles in dog semen extenders (6–11). As a result, it was determined that the addition of activated carbon and zinc as nanoparticles to the reconstituted semen obtained from kangal breed dogs showed longer motility, but the group showing sufficient motility was the group in which only 200 µg/ml activated carbon nanoparticles were added.

Keywords: Kangal Dog, Semen, Short-Term Storage, Nanoparticles, Activated Carbon, Zinc.

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MAPKAP1 gene identified by a GWAS is associated with days open in Holstein heifers

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Introduction and aim

Genetic selection aimed at increasing milk production has inadvertently led to decreased fertility in dairy cattle due to antagonistic relationships between production and reproduction traits. To counter this and improve fertility, it's essential to include fertility traits in breeding programs (1). Dairy cattle of extended period from calving to conception (days open) experience reduced annual milk and fat production, lower reproductive efficiency, increased costs, decreased profitability, and potential health risks (2). This study aimed

to estimate heritability and to identify candidate genes related to days open of Holstein first calving heifers reared in Çanakkale province of Türkiye.

Methods
A total of 460 first lactation records and genotypes of 376 animals were used in the genome-wide association analysis. Genotyping of the animals were provided by using Illumina BovineSNP50K beadchip. After quality control, 42444 SNPs remained for the analysis. Calving year and season were included as fixed effects in the model. Outlier phenotypic records ($\pm$3 standard deviation) were excluded from the data before analysis. Variance component estimation and single-step GWAS analysis were performed with the restricted maximum likelihood (REML) using WOMBAT (3) software.

Results
The heritability (0.21$\pm$0.09) for days open was estimated by using a genomic relationship matrix. Chromosome-wide Bonferroni threshold (3.42 * 10-05) were considered for statistical significance to eliminate the false positives. We have found only one single nucleotide polymorphism (SNP) exceeding the significance threshold. This SNP is located on the intron of the MAPKAP1 gene on chromosome 11.

Discussion and conclusions
The MAPKAP1 gene is a member of the mTORC complex whose expression is known to increase during the embryonic stage of mammals (4). This gene was also reported to be hub-genes associated with high fertility in beef cattle (5). As a result, MAPKAP1 gene is suggested as a candidate gene for days open in Holstein heifers, indicating a potential region for reproductive efficiency.

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Keywords: Holstein heifers, fertility, days open, GWAS, SNP, MAPKAP1 gene

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Co-relation between oxidative stress and uterine microbiota in postpartum dairy cows

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Introduction and aim

Postpartum cows often experience uterine infections that can impair reproductive health and productivity. Oxidative stress (OS), indicated by an imbalance between reactive oxygen species (ROS) and antioxidants, may play a critical role in these infections by affecting the uterine microbiota [1]. The uterine microbiota in cows with purulent vaginal discharge (PVD) is characterized by an imbalance of microbial communities, often involving opportunistic pathogens that can compromise uterine health. Understanding the dynamics of these microbial populations are essential for developing therapies. Metagenomic sequencing reveals decreased diversity and increased Bacteroides, Porphyromonas, and Fusobacterium in postpartum cows [2,3]. This study aims to elucidate the correlation between OS markers

in blood samples and the uterine microbiota, particularly the prevalence of uterine pathogens in PVD cows, using endometrial samples from postpartum dairy cows.

Methods

Endometrial and blood samples were collected from 16 Holstein cows (PVD=8 and Healthy=8) at 21, 28, and 35 days postpartum (dpp). PVD cows was diagnosed using the gloved hand method, based on the presence of mucopurulent vaginal discharge with a mucus score ≥ 2, while cows with a mucus score < 2 were considered healthy [4]. OS markers, including reactive oxygen metabolites (d-ROM), antioxidants (OXY) and oxidative status index (OSI), were evaluated using blood samples via photometric determination of plasma thiols (Free Radical Elective Evaluator, Diacron International, Grosseto, Italy). For the analysis of uterine microbiota, endometrial samples were collected using cytobrushes. DNA was extracted and quantified, and the 16S rRNA gene sequences were analyzed using metagenomics. Statistical analyses were performed using RStudio (version 3.6.3), including Pearson correlation. Mixed linear regression models, accounting for repeated measurements, were fitted to assess the effect of PVD versus healthy cows.

Results

At 21 dpp, PVD cows exhibited significantly higher serum d-ROMs (116 ± 3.1 vs. 75 ± 2.5 UCarr (Carrtelli units)) and OSI (0.23 ± 0.015 vs. 0.19 ± 0.012) levels, and lower OXY levels (395 ± 22.6 vs. 520 ± 21.2 µmol/L) compared to healthy cows ($P < 0.05$). No significant differences were observed at 28 and 35 dpp. Regarding microbiota diversity analysis, at 21 dpp, PVD cows showed significant differences in alpha diversity (Shannon's and Simpson's indices) and beta diversity at the genera level compared to healthy cows ($P < 0.05$). PVD cows exhibited higher relative abundances of Fusobacterium, Porphyromonas, Trueperella, and Parvimonas, and lower relative abundances of Proteobacteria, Bacteroidota, and Firmicutes compared to healthy cows. Temporal changes analysis revealed that at 28 and 35 dpp, PVD cows maintained higher relative abundances of Fusobacterium and Porphyromonas, with significant differences in beta diversity compared to healthy cows ($P < 0.05$). Correlation analysis indicated a strong positive

correlation ($r = 0.68$, $P < 0.01$) between d-ROM levels and the relative abundance of pathogens like Fusobacterium and Porphyromonas, suggesting that higher OS is associated with increased prevalence of these pathogens. Significant negative correlations were observed between OXY levels and pathogen abundance, indicating that higher antioxidant levels are associated with a lower prevalence of Trueperella ($r = -0.54$, $P < 0.05$).

Discussion and Conclusions

OS plays a crucial role in various pathological conditions affecting reproduction particularly during the postpartum period when antioxidant levels decrease, leading to an imbalance between prooxidants and antioxidants [5]. This imbalance, marked by increased d-ROM levels, is exacerbated by the metabolic stress associated with the onset of lactation, milk production and increased microbial diversity. The uterus of PVD cows often contains higher quantities of Porphyromonas, Fusobacterium, and Trueperella, which are typically associated with uterine inflammation [6]. Positive correlations indicate that higher OS relative to antioxidant capacity favors pathogen growth. The results showed a significant relationship between OS and uterine microbiota in PVD cows. Elevated OS markers at 21 dpp correlated with lower microbial diversity and a higher prevalence of specific pathogens, such as Fusobacterium, Trueperella, and Porphyromonas, suggesting that OS may impair immune function, creating a favorable environment for pathogen growth. This study emphasizes the critical interplay between OS and uterine microbiota in postpartum cows with PVD. In conclusion, controlling OS through targeted interventions (e.g., antioxidant therapies or nutritional additions) could potentially improve uterine health and reproductive performance. Additionally, investigating alternative therapies, such as prebiotics, probiotics, postbiotics, and herbal therapeutics, as viable options for preventing and treating uterine infections in dairy cows holds promise for sustainable and effective interventions. Further research is necessary to explore mechanistic pathways and develop effective strategies for managing OS in postpartum dairy cows.

Keywords: uterine disease; postpartum cows; oxidative stress; antioxidants; uterine microbiota

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Hypochlorous effects on uterus and ai success postpartum in cattle

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Simsek, S., Simsek, A., Kizil, S., Bastan, I., Ozkan, S., Sahin, D., Korkmaz, F., Ergiden, Y. Hypochlorous effects on uterus and ai success postpartum in cattle.

Introduction and aim

Extending the calving interval in cattle can lead to substantial economic losses, emphasizing the importance of efficiently managing the puerperal process. Diseases affecting cows during the puerperal period can also negatively impact reproductive performance. Uterine infections and diseases, as outlined by Noakes (1) and Ocal and Kalkan (2), can adversely affect this process, leading to prolonged calving intervals and decreased fertility. Commercial treatments are generally preferred to address these issues, but

they often entail high costs and may disrupt the natural uterine flora. One such substance is hypochlorous acid (HOCl), a compound produced by neutrophils during the immune response and phagocytosis. This research aimed to monitor the stages of uterine involution in postpartum Holstein cattle and to evaluate the impact of applying HOCl solution to the uterus. Specifically, the study investigated how HOCl affects the endometrial flora and the optimal timing for artificial insemination (AI).

Methods

The study involved 20 pregnant Holstein cattle from the International Livestock Research and Training Center Directorate (UHAEM) Cattle Breeding Branch. The cattle were divided into three experimental groups: Group I (Control): 5 Holsteins with no treatment. Group II (Shawm Control): 7 Holsteins receiving an isotonic solution. Group III (HOCl): 8 Holsteins receiving hypochlorous acid (HOCl). For Group III, the HOCl solution, an isotonic fluid, was administered intrauterine 15 days postpartum (Pp 15) at a volume of 150 ml, with two applications 24 hours apart. To evaluate the effects of the treatments, several assessment methods were employed: Rectal Examinations: Used to assess the condition of the reproductive tract. Ultrasonographic Examinations: Provided images of the uterus to monitor its involution. Vaginal Discharge Scores: Assessed the characteristics of vaginal discharge. Cervical Mucus Viscosities: Measured the consistency of cervical mucus. Additionally, uterine wash samples were collected from the animals on days 21 ± 2 postpartum for total bacterial count analysis. This analysis included the evaluation of both aerobic and anaerobic bacterial populations to understand the impact of treatments on uterine flora (3).

Results:

Rectal Examinations: No abnormal conditions were observed among the different groups, indicating that the overall reproductive health of the animals was similar across groups.

Ultrasonographic Examinations: The cervix uteri diameter was found to be smaller in the HOCl-treated group compared to the control groups at 30 days postpartum. This suggests that HOCl treatment may influence uterine involution, potentially leading to a quicker return to normal cervix size.

Microbiological Examinations: In the HOCl group, microbiological analysis revealed the presence of Escherichia coli (E. coli), Staphylococcus spp., and Streptococcus spp., but at low densities. This indicates that HOCl treatment did not lead to significant microbial proliferation and was relatively benign in terms of bacterial flora.

Estrus Detection: The electrical resistance of the vaginal mucosa in the HOCl group was closest to the reference value for determining estrus, which is between 180-320 x 10 units/1 ohm. The HOCl group's measured resistance was 328 x 10 units/1 ohm, suggesting that these animals were in a suitable estrus range.

AI Readiness: The HOCl group showed an optimal Spinbarkeit/Elongation (cm) size of 23.0 $\pm$ 0.8 cm. This measurement is indicative of favorable conditions for AI, suggesting that HOCl treatment did not adversely affect the uterine environment or the timing for successful insemination. Overall, the study results indicate that HOCl treatment may positively influence uterine involution and maintain appropriate conditions for reproductive success without significantly disrupting uterine microbial balance or estrus detection parameters.

Discussion and conclusions

Based on the project results, HOCl was administered intrauterine postpartum and proved to be a natural and non-irritating solution for the cattle. HOCl is the preferable treatment solution compared to others that might cause more significant disturbances or side effects. The use of HOCl in cattle effectively addresses the negative impacts of uterine infections that can occur during the postpartum period. By mitigating these infections, HOCl contributes to a healthier uterine environment. HOCl treatment was associated with more favorable uterine involution, as indicated by the smaller cervix uteri diameter

observed in the HOCl group compared to the control groups. HOCl supports more efficient recovery and normalization of the uterus after calving. The optimal timing for AI was improved in the HOCl group, as evidenced by appropriate vaginal mucosa resistance values and suitable cervical mucus elongation. HOCl application can increase the likelihood of successful AI by ensuring the uterine conditions are optimal timing for AI. Overall, the HOCl solution helps mitigate reproductive issues related to uterine health and positively influences uterine retention. HOCl contributes to better reproductive performance and potentially higher productivity in cattle breeding operations. In summary, the findings of this project demonstrate that HOCl is an effective and beneficial treatment for postpartum cattle, helping to manage uterine infections, supporting uterine involution, and affecting the endometrial flora and the optimal timing for AI.

Keywords: Endometrial Flora, Uterus Involution, Hypochlorous, Holstein, Artificial Insemination

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L-2-oxothiazolidine-4-carboxylate enhances bovine oocyte development by reducing oxidative stress

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Introduction and aim

As the world population reached 7 billion people, it is necessary to ensure the sustainability of animal and agricultural resources with modern biotechnological methods to meet the global food need. The livestock sector provides approximately 34% of global food protein, and cattle breeding has become an industry in developed world countries. In cattle, there are studies showing that the addition of cysteine to the in vitro maturation medium (IVM) improved blastocyst formation rates by increasing intracellular GSH level and reducing ROS level (1, 2). Cysteine, which acts as an antioxidant, is highly toxic when used directly in high concentrations. For this reason, increasing intracellular cysteine production using L-2-Oxothiazolidine-4-carboxylate (OTC), a precursor of cysteine, may provide a significant advantage. In studies where OTC was used orally in animals and humans, it was observed that it significantly increased GSH levels in retinal pigment epithelial cells compared to other cysteine precursors (3). The aim of the study was to examine the effects of OTC addition to bovine IVM medium on GSH and ROS levels and maturation-related gene expressions in oocytes.

Methods

Bovine ovaries taken from the slaughterhouse were brought to the laboratory in a 0.9% NaCl at 30-35°C. Oocyte-cumulus complexes were collected from the follicles with a diameter of 2-8 mm on the ovaries. Oocyte-cumulus complexes with at least three compact layers of cumulus cells surrounding them and a homogeneous cytoplasm were selected. OTC was added to IVM medium at 0 (Control), 1, 2 and 4 mM concentrations and incubated for 24 hours with a temperature of 39°C, 5% CO_2 and 95% humidity. Then, nuclear maturation, apoptotic activity, GSH and ROS analyses were performed. GPX, MARF1, SOD1 and SIRT2 gene expression changes in oocytes were examined. ANOVA followed by Tukey HSD test was performed to compare differences between the groups.

Results

When nuclear maturation rates were examined after the IVM period, it was observed that there was a significant difference between the control group and OTC groups in terms of MII rates ($p<0.05$) and that OTC groups gave lower values as the concentration increased (92.9%, 70.0%, 68.2%, 57.1%, respectively). In the apoptosis analysis, it was observed that the OTC 2 mM concentration gave the highest value non-apoptotic oocyte values, and that OTC 1 and 2 mM concentrations significantly reduced apoptosis rates compared to the control group, although there was no statistically significant difference between all groups: (88.1%, 97.5%, 100.0%, 82.9%, respectively, $p>0.05$). While no significant difference was observed between the groups in the GSH absorbance analysis, when ROS levels were examined, it was observed that there was a significant difference between the Control and OTC groups ($p<0.05$) and that the OTC groups significantly reduced ROS levels (61.9%, 40.85%, 35.71%, 39.36%, respectively). When gene expressions in oocytes were examined, it was observed that GPX, MARF1, SIRT2 expressions were up-regulated while SOD1 expression was down-regulated in OTC groups compared to the control group.

Discussion and conclusions

Developing animal husbandry and ensuring the sustainability of animal products is an important strategy to meet the increasing food needs of our country and the world market. In this context, studies are ongoing to provide an in vitro environment similar to in vivo conditions. It is known that Reactive Oxygen Species (ROS) are necessary at basal levels in cell metabolism, but excessive increases in the amount of ROS in vitro cause delays in embryonic development. To reduce ROS levels in vitro, it is known that the addition of enzymatic and non-enzymatic antioxidants to media positively affects embryo development (4).

In this study, it was observed that significant results were obtained in OTC groups compared to Control group by interpreting the analyses performed on oocytes supported by gene expressions. Despite the low Metaphase II rates, the up regulation of MARF1 and SIRT2 expressions emphasizes that OTC supplementation supports nuclear and cytoplasmic maturation. While

OTC supplementation was observed to reduce ROS levels in ROS analysis, down-regulation in SOD1 expression also supports this situation. Although no difference was observed between the groups in GSH analysis, it is important to see up-regulation in GPX expression in OTC groups.

In conclusion, the addition of 2 mM OTC to the IVM medium in cattle reduces apoptosis rates in oocytes, protects against oxidative stress and supports oocyte development.

Keywords: bovine; in vitro oocyte maturation; OTC; nuclear maturation; oxidative stress.

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Seminal plasma-derived EVs preserve ram semen against cold shock and improves sperm parameters

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Introduction and aim

Assisted reproductive techniques are important biotechnological tools to solve reproductive problems in humans and animals. To determine a male's fertility potential, beside the structure of the sperm produced, the structure of the accompanying seminal plasma is also important (1). Extracellular vesicles (EVs) are like a nano-sized cargo system surrounding by a double membrane and carrying of nucleic acids, proteins, lipids, and small molecules. While some biomolecules in seminal plasma circulate freely, some are encapsulated in EVs to protect them from inactivators such as proteases and nucleases. Proteomics analysis of seminal plasma EVs was revealed that sheep seminal plasma EVs are rich in protein structure (2). The aim of this study was to investigate the effects of EVs obtained from ram seminal plasma on the cryopreservation of ram semen.

Methods

To obtain seminal plasma EVs, semen was collected from 6 rams (located at the Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine Farm) twice a week for 4 weeks with an artificial vagina. After serial centrifugation of seminal plasma, EVs were obtained from the seminal plasma by ultracentrifuge method. Nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) analyses were performed to determine the concentration and characterization of the EVs.

For cryopreservation procedure, semen was collected from 4 rams with an artificial vagina twice a week for 5 weeks. Seminal plasma-derived EVs were added as 0 (Control), 1x (EV-1), 4x (EV-2) and 16x (EV-3) according to the sperm concentration ($80x10^6$ spermatozoa per straw) and kept at room temperature for 30 min. Then the semen was frozen according to the protocol and stored in liquid nitrogen. After thawing, motility and kinematic

parameters, viability, membrane integrity, mitochondrial activity and oxidative stress analyses were performed.

Results

There were significant differences between the groups in terms of motility and progressive motility compared to Control ($p<0.05$), and the highest values were given by the EV-2 group (40.93%, 50.87%, 64.13%, 58.03% and 20.23%, 26.21%, 34.49%, 29.94%, respectively) after thawing. In the viability analyses, it was seen that the EV groups gave higher rates of live spermatozoa compared to Control ($p<0.05$; 64.16%, 72.12%, 72.98%, 71.53%). Although there were no significant differences between the groups in terms of the ratio of spermatozoa with intact membranes, the ratios were observed to be higher in EV groups ($p>0.05$; 57.98%, 61.70%, 63.38%, 64.69%). All EV groups showed higher mitochondrial activities compared to Control ($p<0.05$; 62.00%, 70.36%, 67.51%, 67.98%). Although no significant differences were observed between the groups, decreases in oxidative stress were observed in EV groups compared to Control ($p>0.05$; 68.47%, 58.40%, 59.54%, 62.80%).

Discussion and conclusions

Long-term preservation of semen in mammals is an active topic that is constantly being studied by researchers. Even the most effective freezing and thawing methods used today are below the standard.

The total protein concentration of ram seminal plasma is related to the characteristics of the semen and is an important tool for interpreting fertility and quality (3). Many studies support that seminal plasma protects spermatozoa against cold shock damage by adding it to the diluent (4). Also in pig, it was seen that seminal plasma-derived EVs added to semen maintain sperm motility and plasma membrane integrity after 10 days of storage at 17°C (5).

When the results of this study were examined, it can be concluded that seminal plasma-derived EVs contributes to the prevention of damage during cryopreservation of ram semen. It was observed that EVs increased motility, progressive motility and live spermatozoa rates. Also, they reduced oxidative

stress experienced during cold shock and protected spermatozoa against membrane damage. It was thought that fertilization capacity may also increase due to the increased mitochondrial activity.

In conclusion, addition of seminal plasma-derived EVs to the ram semen at a ratio of 4x to the sperm concentration before freezing contributed to the preservation of spermatozoa against cold shock.

Keywords: seminal plasma; extracellular vesicles; ram semen; sperm cryopreservation; sperm parameters.

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Transposase-enhanced cytoplasmic injection as an alternative for the production of transgenic sheep

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Introduction and aim

The production of transgenic sheep has proven difficult since its original application by Hammer and co-workers in 1985 (1). Pronuclear microinjection (PNI) of in-vivo produced sheep zygotes resulted in the integration of the linear transgene into 0.10% of the live born sheep which did not express the human growth hormone (hGH) transgene when driven by the mouse metallothionein-I promoter. A more recent result for PNI injection of zygotes derived from South Australian Merino Sheep resulted in six live born lambs being transgenic out of 413 injected single-celled zygotes (1.45% ei) (2). In other words, there was no improvement in transgenesis rates by PNI between 1991 and 2010.

We employed transposase based transgenesis (3) because it is less cumbersome than producing lentiviruses, it is safer and cheaper to produce the single construct plasmids and it results in efficient transgenic animal (TA) production. This coupled to the large transgene transfer capacity which

the piggyBac transposase affords, rendered it applicable to explore whether it would be effective in the implemention of sheep transgenesis. So, in this study we aimed to compare pronuclear injection (PNI) vs cytoplasmic injection (CTI) techniques in in vivo produced sheep zygotes by using piggyBac transposase based, self-inactivating, single construct plasmids containing both the transposon and transposase in the same construct (pmhyGENIE-3). We used in vitro produced sheep zygotes for pronuclear injections as well.

Methods

Donor Kivircik ewes (n=5) were synchronized and superovulated. Thirty-six hours after sponge withdrawal estrous was detected by teaser ram and all donor animals in estrous were mated twice 12 h apart by using healthy Kivircik rams. One-cell stage embryos were obtained approximately forty hours after GnRH injections by oviduct flushings via laparatomy. In vitro production of sheep zygotes were described elsewhere (4). For PNI, zygotes were centrifuged in 13.000 g for 15 min to visualize pronuclei. All zygotes used were injected with 10 ng/µL of pmhyGENIE-3 plasmids containing a mammalian selection cassette (MSC) as previously described (3). After injections of the gene construct by transposase enhanced PNI (te-PNI), healthy (not lysed) embryos (te-CTI- and te-PNI-in vivo; 12 and 19 embryos, respectively) were immediately transferred into oviducts of recipient animals (n: 2 and 3, respectively). In vitro produced embryos (te-PNI-in vitro) were cultured for 3 days in SOF medium. Cleaved embryos were examined for EGFP expression, and EGFP expressing embryos (n: 65) transferred into oviducts of recipient animals (n: 11).

Results

Of the sixty five in vitro produced embryos which were injected with pmhyGENIE-3 and transferred to eleven pseudopregnant ewes, only one ewe delivered a lamb which was not transgenic by epiflurescence. For the nineteen in-vivo produced embryos treated with te-PNI and transferred into three pseudopregnant ewes, only one of them delivered at term to a set of twin lambs, one of which (5.3%) was transgenic by epifluorescence. Of the twelve in-vivo produced embryos who were CTI treated and transferred into

two pseudopregnant ewes, a single ewe gave birth to twins, one of which was transgenic by epifluorescence, representing a transgenic efficiency of 8.3%.

Discussion and conclusions

Sheep are very important animals for transgenesis studies both as model for human diseases and bioreactors for valuable therapeutics. However transgenic efficiency is still not satisfactory. In this study we used cytoplasmic injection technique as an alternative to PNI. Since PNI technique needs ultracentrifugation and very skilled operator, the loses of embryos after injection are more likely. It can be suggested that the use of cytoplasmic injection of the transposase-mediated gene construct in in vivo produced zygotes provides us with both ease of implementation and transgenic efficiency.

Keywords: sheep; piggyBac; transgenesis; pronuclear injection, cytoplasmic injection

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Frequency of multioocyte follicles in dogs with different age groups

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Citation
Karabulut, T.S.F.T., Sönmez, K., Enginler, S., Gürgen, H.Ö., Öztürk, N. Frequency of multioocyte follicles in dogs with different age groups.

Introduction and aim

The origin of multicyte follicles (MOFs) is unknown. They have been reported to occur in dogs, rabbits and cats. MOFs are observed more frequently in neonatal and young animals than in adult animals [1]. In the female dog, MOFs with 2–17 germ cells per follicle have been reported. Scientific knowledge about the application of biotechnology in the breeding of pets has increased. This knowledge benefits both domestic dogs and endangered wild canids [1, 2]. The low efficiency of in vitro maturation of canine oocytes from antral follicles limits the use of reproductive biotechnologies. The use of oocytes from preantral follicles would provide an alternative source of follicles, as they are available in greater quantity and at all stages of the animal's reproductive life. Biotechnologies used in reproduction, use oocytes

from antral follicles. Advances in the in vitro culture of preantral follicles and the maturation process of oocytes in preantral follicles would improve the results of reproductive biotechnology and provide a large number of oocytes from the same animal in the future [2]. The aim of this study was to determine the presence and frequency of MOFs in dogs.

Methods

Ovaries were obtained from bitches undergoing ovariohysterectomy at the Veterinary Teaching Hospital of the Istanbul University-Cerrahpaşa. The bitches were spayed for routine ovariohysterectomy or pyometra. Fourty-eight bitches of different breed from 6 months to 13 years were involved in the study. Reproductive status and cycle stage of bitches (prepubertal/postpubertal) and were recorded before the surgeries.

Both ovaries were fixed in the formalin solution (10%) after immediately surgeries. The ovaries were bisected longitudinally, embedded in paraffin wax, sectioned at 5 mikrometer and stained with haematoxylin and eosin. From each ovary only two longitudinal section were evaluated and MOF numbers were recorded.

Results

Multioocytes were observed in 16 cases out of a total of 48 cases. There is variability between the right and left ovaries, MOFs seen more frequently in right ovaries. In dogs <3 years MOFs was detected in 20.83% of the animals and in dogs >7 years MOFs was detected in 12.5%.

Discussion and conclusions

It is reported the presence of MOFs, in the ovaries of bitches. Prepubertal bitches had more primordial multioocyte follicles, and more multioocyte follicles at the secondary stage compared to adult dogs. Future studies are needed to ascertain between the fertility and existence of MOFs.

Keywords: Multioocyte follicule, canine, IVF, fertility, infertility, one health.

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The ovarian expression of sperm acrosome associated 3 protein in bitches

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Introduction and aim

Fertility is a complex process and infertility can have many causes. Sperm protein reactive with antisperm antibodies (SPRASA) is a protein that has been discovered in the past as a target of autoantibodies in infertile men. In the literature, SPRASA has been shown to be expressed in ovarian follicles, corpora lutea and oocytes of female dogs by immunohistochemistry [1]. Female mice immunized with SPRASA were profoundly infertile after timed mating, and the ones that became pregnant had reduced fetal viability. The results suggest that SPRASA plays a role in female fertility [1, 2]. SPRASA was initially thought to have testis-specific expression restricted to the acrosome. The function of SPRASA is unknown, but preliminary data suggest

that SPRASA may play an important role in fertilization. SPRASA remains localized in the equatorial region of the sperm after binding to the oolemma, supporting its role in oocyte binding and fusion. An antiserum reactive with SPRASA inhibits the binding of acrosome-reacted human sperm to hamster oocytes [1, 3].

It is estimated that there are four million stray dogs in Turkey. There is a need for non-surgical contraception in dogs because ovariohysterectomy is resource-intensive. For this reason, we conducted this study to confirm the expression of SPRASA in normal ovarian tissue in female dogs in order to find a humane solution for controlling the stray dog population in the future.

Methods

One hundred ovaries from fifty bitches were obtained after routine ovariohysterectomy (OVH) at Istanbul University-Cerrahpaşa Teaching Hospital, Faculty of Veterinary Medicine. The bitches were undergoing OVH for elective spay or pyometra. No gross abnormalities of the reproductive tract were detected at the time of surgery. Bitches of different breeds aged 6 months to 13 years were included in the study. The reproductive status (prepubertal/postpubertal) and cycle stage were recorded.

Ovarian tissue was fixed in formalin, embedded in paraffin, sectioned on loaded slides (5 µm) and subjected to routine immunohistochemistry (IHC). For the IHC procedure, antibodies were diluted with reducing diluent: Atlas antibodies: 1:10 dilution (Anti SPACA3 Polyclonal Antibody #HPA 023633, Stockholm, Sweden).

Results

Positive results were found mainly in the nucleus of granulosa cells. The expression of SPRASA was found in the oocyte nucleus, granulosa cells, ooplasm and theca cells with different intensity depending on the individual. No histological abnormalities were detected on the slides stained with hematoxylin and eosin.

Discussion and Conclusions
Our results are consistent with those of other authors [1, 2]. The possibility to confirm the presence of SPRASA from canine ovaries could be a preliminary step to develop an immunocontraceptive vaccine in population control of dogs in the future.

Keywords: Canine, female dog, SPACA3, non-chirurgical contraception, population control, stray dogs, one health.

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The effect of gnrh on reproductive performance at the end of the season in nulliparous assaf ewes

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Citation
Kara, U., Acar, D.B. The effect of gnrh on reproductive performance at the end of the season in nulliparous assaf ewes.

Introduction and aim

Small ruminants are the major mainstay of animal sourced foods in developing and transitioning countries. However, sheep and goats are short-day, seasonally polyestrous animals with a limited reproductive season in a year. Therefore, estrous synchronization and breeding control help to improve reproductive performance (1,2). The sheep breeders prefer maximum prolificacy for flock productivity. Increased reproductive efficiency in the flock positively affects economic parameters, annual milk and meat production, and farm profitability (3). Studies on the enhancement of reproductive productivity of nulliparous ewes are still ongoing, and the reproductive management efficiency of these animals still needs to be defined (4). Based on this uncertainty, the present study aims to determine the efficacy of GnRH administration at the beginning of synchronization protocol in nulliparous Assaf sheep at the end of the breeding season.

Methods

The study was carried out at the end of the breeding season (from the beginning of December to the end of the parturition period) on a single commercial farm. The animals' body condition score ranged from 2.5 to 3.5 (on a 1-5 scale), and they were between 7 and 9 months old. Blood samples were collected at the beginning of the study (day 0) from all ewes and after intravaginal sponge removal (day 7) from synchronization groups for progesterone analysis. The nulliparous ewes were randomly divided into three groups. Ewes in Group I (n=49) received an intravaginal sponge and were injected with GnRH (4 µg of buserelin acetate) at the time of sponge insertion (day 0). On the day of sponge removal (day 7), all ewes received d-cloprostenol and 500 IU eCG. The rams were joined into the herd for one ram for four ewes on day 8, and the onset of the estrous of ewes was noted according to symptoms. In Group II (n=45), the same treatment was received except for GnRH injection on day 0. Group III (n=23) was designed as a control group, and any hormonal treatments were not administered, and the rams were joined. Pregnancies were confirmed on day 25 by transrectal ultrasonography and on day 45 by transabdominal ultrasonography. Estrus response, pregnancy rates, embryonic death, aborts, parturition rates, twinning rates, fecundity, prolificacy, and fertility rates were noted. Vaginal discharge characteristics after sponge removal were observed and scored.

Results

The mean serum progesterone concentrations of Group I, Group II, and Control on Day 0 were 1.98, 1.75, and 3.09 ng/ml, respectively. The vaginal discharge scores were statistically different between score 1 and score 2 in the synchronization groups ($P<0.05$). However, the vaginal discharge characteristics and scores did not affect embryonic mortality and pregnancy rates significantly ($P>0.05$). The estrus rates were similar Group I and Group II (93.87% and 95.55%, respectively) ($P>0.05$) but higher than the control group (56.52%) ($P<0.05$). The estrous duration and estrus onset were also insignificant between hormone treatment groups ($P>0.05$). The pregnancy rates on day 25 were higher in the Group I and Group II (79.59% and 93.33%, respectively) than in the control group (52.17%). The fertility rate was

significantly higher in the synchronization groups than in the control group ($P<0.05$). The highest fecundity and prolificacy rates were found in Group II.

Discussion and conclusions

Ano-Perello et al. (5) reported that GnRH treatment in progesterone-based synchronization protocols increases estrus response and reproductive efficiency in ewes by improving follicular turnover. On the contrary, our results showed that the GnRH administration did not positively affect estrus response and other reproductive performances in nulliparous Assaf ewes. A different breed, Segurena meat ewes, was treated in the study by Ano-Perello et al. (5). Also, well management and environmental conditions could affect the results. The vaginal drainage, pH, and microbiota could be failed during intravaginal progesterone usage in sheep. The researchers reported that the long-term treatments give decreased pregnancy rates than the short-term treatments in accordance with vaginal discharge characteristics (6). On the other hand, there are contrary results that the vaginal discharge wasn't affected the conception rates (7). In the present study, the pregnancy rates were similar in the synchronization groups with different vaginal discharge scores.

In conclusion, the GnRH administration on progesterone-based protocol did not positively affect the reproductive values at the end of the season in the nulliparous Assaf ewes. It was concluded that the economic burden and workload should be evaluated during the planning of hormone usage and treatment protocol.

Keywords: Assaf ewe, GnRH, progesterone based synchronization, fertility, vaginal discharge.

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Gonadectomy of mouflons (ovis aries) in the field

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Introduction and aim

The overpopulation of wild animals may cause the destruction of the vegetation. Gonadectomy is a useful procedure for birth control and may help in controlling animal populations (1). The aim of this study (approval number 20/2022) is to describe an innovative technique of gonadectomy in mouflons, using a suitable anesthetic/analgesic protocol and an innovative device in field conditions.

Methods

Twenty-eight mouflons (8 male and 20 female) were neutered, in the Natural Park of the Tuscan Archipelago. For all animals, the anesthesiologic protocol was performed using xylazine (0.1 mg/kg) and an association of tiletamine/zolazepam (4 mg/kg), mixed in the same siringe and

injected in the brachiocephalic muscle. After 10 minutes, propofol (2 mg/kg) was administered intravenously and anesthetic maintenance was performed with isoflurane (2). Butorphanol (0.5 mg/kg) was administered intravenously immediately after the start of anaesthetic gas administration and an intramuscolar injection of benzilpenicilline (10,000 UI/kg) and dihydrostreptomicine (12.5 mg/kg) was injected at the same time to provide antibiotic coverage. The animals were continuously monitored through multiparametric monitor and all surgeries were performed by the same surgery staff.

For the male, at the level of the scrotal neck, an anterior approach was performed with a 3 cm incision of the median raphe of skin and dartos. Then the tunica vaginalis proper was incised and the testis were exteriorized. The animals were randomly divided in two groups: in the first (C group) was used the classic ligation with absorbable suture thread for ligation of the spermatic cord; in the second (E group) was used Caiman® device, employed for clamping the spermatic cord and cutting in one stroke. All procedures were performed for both testicles. Subsequently, for all animals, the skin was sutured with detached U-shaped stitches.

The females were ovariectomized, 10 subjects via the left flank (F group) and 10 subjects via midline (M group), using in both groups the Caiman® device. In animals of the F group, a vertical skin incision, on the left flank, on the paralumbar fossa, close to the iliac wing, was performed. All muscular layers were punctured with the scalpel and muscle fibers were separated down to the peritoneum, which was held with the forceps, punctured with the scalpel, and cut with scissors. The surgeon grasped and exteriorized the uterus, locating the ovaries. The Caiman® clamp was placed at the base of the left ovary, and ovariectomy was performed. The same procedure was repeated on the right ovary, using the same surgical approach. After removal of the ovaries, peritoneum, abdominal muscle and skin were closed with absorbable sutures. Ovariectomy of the M group was carried out similarly but through a midline access. The incision was performed cranial to the udder, at about 10 cm from the umbilical scar. The surgical procedures were like those described above.

For all mouflons, both males and females, the surgical times, the intraoperative nociceptive response, and the intraoperative complications were detected to compare the effects of the two techniques. At the end of the surgery, in all animals, subcutaneous injection of triidrate amoxicilline (15 mg/kg) and intramuscular injection of ketoprofen (0.3 mg/kg) were administered as the antibiotic and anti-inflammatory coverage for the post-operative period.

All the subjects were observed for 5 h post-surgery and the day after by the veterinary staff evaluating behavioral changes, as reluctance to move, reduced feed intake, and changes in posture. After that, the animals were released in a large enclosure and monitored by park operators for one week. Surgical times, intraoperative nociceptive response, postoperative pain, and the frequency of complications were compared in all groups, using T student.

Results

For all animals, no intra or post-operative complications were reported, and they were neutered without side effects. The results of evaluated parameters were similar in two groups, in both males and females.

Discussion and conclusions

These results show the suitability of the anesthesiologic/analgesic protocol used during gonadectomy in field, both in male and females. In the male groups, the use of Caiman® has induced a uniform compression of the blood vessels comparable to the classic ligation with absorbable suture thread. As regard the female, the two different approaches have produced similar results for the evaluated parameters. However, the postoperative control of the healing of animals was easier in animals neutered from the flank.

In conclusion, the described anesthetic/analgesic protocol and surgical procedures were suitable for neutering of male and female mouflons in the field conditions.

Keywords: Mouflon, gonadectomy, anesthetic/analgesic protocol, Caiman® device, flank approach, midline approach

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Reproductive effects of long-term bakuchiol use in male rats

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Citation
Koşal, V. Reproductive effects of long-term bakuchiol use in male rats.

Introduction and aim

Bakuchiol (BAK), with the chemical formula [(1E,3S)-3-ethenyl-3,7-dimethyl-1,6-octadien-1-yl] phenol, is a prenylated phenolic monoterpene extracted from the fruit of Psoralea corylifolia L., which belongs to the Leguminosae family. Research has demonstrated that BAK possesses various pharmacological effects, including antioxidant, antibacterial, anti-inflammatory, anti-aging, anti-cancer, and estrogen-like properties. BAK is frequently used in Far Eastern countries to increase male reproductive system functions. This study aimed to investigate the effects of long-term BAK administration on reproductive parameters and oxidative stress in male rats (1-4).

Methods

18 male Albino Wistar rats were randomly divided into 3 groups. 1 mL of physiological saline daily administered by oral gavage for 6 weeks in Control group. Group I received a daily dose of 10 mg/kg BAK per rat, Group II received a daily dose of 50 mg/kg BAK per rat by oral gavage for 6 weeks. Rats were sacrificed by exsanguination method under anesthesia. Motility, density, and abnormal spermatozoon rates were examined in semen samples taken by epididymis puncture. Levels of Malondialdehyde (MDA), Nitric Oxide

(NO), and Catalase (CAT) in testicular tissue were analyzed using the Real-time PCR method.

Results

As a result of the study, the sperm motility rates were determined as 76.66% in the Control group, 55% in Group I, and 46.66% in Group II. It was determined that the motility rate significantly decreased in the groups treated with BAK ($p<0.001$). No difference was observed between the groups in terms of density. The abnormal sperm rates were 13.16% in the Control group, 41.83% in Group I, and 47.5% in Group II. The increase observed in the groups treated with BAK (Group I and Group II) was statistically significant ($p<0.001$). Abnormal spermatozoa heads were found to be disrupted hook-shaped, flattened, and had an arrowhead appearance. As a result of PCR analysis from testicular tissue, an increase in MDA and NO levels and a decrease in CAT level were detected in the BAK applied groups ($p<0.001$).

Discussion and conclusions

It is reported that BAK has strong antioxidant properties and does not cause physiological problems (5-7). These studies differ from the presented study in terms of factors such as different target organs, working with cell cultures, and generally being limited to a single daily dose of BAK. In this study, it was determined that long-term BAK administration negatively affected sperm parameters and increased oxidative stress in male rats. In a similar study, Takizawa et al. (8) found parallel results due to long-term BAC use. They also reported that long-term BAK use inhibits spermatogenesis, causes degeneration in Leydig cells and pituitary-testicular axis disruption.

The effects of long-term BAK application on the male reproductive system need to be examined in more detail.

Keywords: Bakuchiol, Testis, Sperm, Rat, Oxidative stress.

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Breed-specific ultrasound equation for predicting parturition in english staffordshire bull terriers

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Citation
Bertero, A., Ponzio, P., Cucuzza, A.S., Rota, A. Breed-specific ultrasound equation for predicting parturition in english staffordshire bull terriers.

Introduction and aim

Predicting the parturition date in the bitch can be complicated, especially if ovulation date is unknown. Ultrasound examination of the conceptuses can be a valuable aid, as various embryo-fetal parameters have been correlated with gestational age (1). The application of specific equations based on the measurement of fetal biparietal diameter has proven to be a particularly reliable method (2).

Despite the practicality and undeniable advantages of this technique, there are still limitations mainly due to the formulas used. These are influenced not only by the dog size, but also by the breed, especially if certain conformational characteristics are present. In the light of this, different equations have been developed, particularly for breeds characterized by distinct morphological features (i.e. Chihuahuas, German Shepherds, etc.) (3-5). The English Staffordshire Bull Terrier is a popular breed that, having a

characteristic very large skull, could deserve a specific equation for predicting birth date based on fetal biometrics. Therefore, aim of this study is to assess fetal biparietal diameters in pregnant English Staffordshire Bull Terrier bitches by ultrasonography in order to develop a formula to calculate the expected parturition date.

Methods

Five pregnant English Staffordshire Bull Terrier bitches were included in the study. Ultrasound examinations were performed weekly, starting from the second half of pregnancy. The estimated day of LH-surge was known through plasma progesterone determination (Enzyme Linked Fluorescent Assay, MINI VIDAS®, bioMérieux Italia S.p.A, Florence, Italy).The fetal biparietal diameter (BP) was measured considering the outer limit of the greater distance between the parietal bones of the skull. For each bitch, the average values of all fetal BPs observed during each ultrasound examination were calculated. These data were retrospectively correlated by simple linear regression with the "days before parturition" (DBP), calculated on the date of delivery (day 0).

Results

The mean age of the bitches was 4.4±1.7 years (mean ± standard deviation), range 3 to 6.5 years. Twenty-seven alive puppies were born from the 5 pregnancies. The mean size of the litter was 5.8±1.1 puppies (range 5-7). At least 3 fetuses were evaluated in each ultrasound examination. BP was significantly correlated to the gestational age ($r = 0.99$; $P<0.0001$) and the following equation "y = ax+b" was obtained through linear regression, where "y" are the "days before parturition", "x" is BP expressed in mm, "a"=-1.321 and "b"=38.16, with a high coefficient of determination ($R^2=0.97$; $P<0.0001$).

Discussion and conclusions

BP can be easily obtained by ultrasonography and is very useful for predicting parturition date, but the influence of breed on this parameter is a subject of debate and concern. Therefore, to achieve adequate prediction accuracy, breed-specific equations should be developed and applied. Our formula showed high reliability, although it should be tested in its practical application

to calculate the expected date of delivery in English Staffordshire Bull Terrier bitches.

Keywords: Ultrasound, fetal biometry, gestational age, pregnancy, dog, biparietal diameter, English Staffordshire Bull Terrier.

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Multiparametric ultrasound of the prostate of normozoospermic dogs

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Introduction and aim

Ultrasonography is an important tool in canine reproduction enabling non-invasive evaluation of the reproductive system. A multiparametric ultrasound approach consists of B-mode, Doppler, Elastography and Contrast-enhanced

Ultrasound (CEUS) evaluation and can provide comprehensive information regarding the integrity, vascularization, stiffness and perfusion of the tissue under study (1). To date, no studies are available regarding a multiparametric ultrasound assessment of the prostate of normozoospermic dogs. Hence, the present study aims to perform a standardized evaluation of the canine prostate to establish normal values.

Methods

Eight intact healthy male dogs aging from 1 to 8 years were included. Ultrasound evaluation was performed using the ACUSON S2000 (Siemens, Germany) machine using a linear multifrequency transducer. B-mode evaluation assessed prostatic morphology, size and volume (cm^3). Doppler assessment was performed on the Cranial, Subcapsular, Parenchymal and Caudal regions of the prostatic artery to obtain the Systolic Peak Velocity (SPV in cm/s), End-diastolic Velocity (EDV in cm/s), Time-average Maximum Velocity (TAMAX in cm/s), Time-average Mean Velocity (TAMN in m/s), Resistance Index (RI) and Pulsatility Index (PI). Shear-wave Elastography (SWE) was performed using the Virtual Touch Tissue™ Imaging Quantification (VTTIQ) software. A colored elastogram was obtained and then nine electronic calipers were placed within the prostatic parenchyma, three in the ventral, three in the central and three in the dorsal portion. Shear-wave velocities (SWV in m/s) in each ROI and a total prostatic mean SWV were calculated. CEUS evaluation was performed by intravenously injecting 0.03 mL/kg of a second-generation contrast medium (SonoVue®, Bracco, São Paulo, Brazil) followed by 5 mL of saline solution and a 90s video was recorded. Quantitative assessment was performed using a dedicated software (Contrast Dynamics™, Siemens, Germany). Five random ROIs were placed within the parenchyma to obtain Peak Intensity (Peak in %), Time to Peak (TTP in s), mean transit time (MTT in s), area under the curve (AUC in %) and the Average Number of Pixels (Pixels). Semen collection was performed by digital manipulation, and sperm-rich fraction was evaluated. Volume, subjective evaluation of sperm motility and velocity, sperm concentration (improved Neubauer chamber), sperm morphology (eosin-nigrosin stained smears), and seminal plasma concentration of alkaline phosphatase (AP) were assessed. Criteria for normozoospermia were established as follows:

progressive motility > 70%, velocity > 3, total sperm output > 200 x 10^6 spermatozoa/mL, morphologically normal sperm cells (MNS) > 70% and AP > 5000 U/l. (2) Quantitative data were expressed as mean ± standard deviation (SD). Significance was set at $p < 0.05$.

Results

On B-mode ultrasound, mean prostatic measurements were: length 3.28 ± 0.29 cm; height: 2.2 ± 0.46 cm; width: 3.15 ± 0.30; volume: 10.72 ± 2.74 cm^3. On Doppler assessment, only the Cranial portion of the prostatic artery was consistently assessed in all 8 patients, being the most significant to be evaluated (SPV: 20.24 ± 8.33 cm/s; EDV: 4.69 ± 1.73 cm/s; TAMAX: 7.33 ± 1.88 cm/s; TAMN: 3.89 ± 1.3 cm/s; RI: 0.74 ± 0.08; IP: 2.08 ± 0.89), whereas the Subcapsular and the caudal portion was measured just in 7 patients, and the parenchymal one in 6. Prostate SWV was similar in the Ventral (2.59 ± 0.46) and Central (2.55 ± 0.45) parenchymal portions, differing in the Dorsal portion (2.85 ± 0.30). However, mean prostatic stiffness calculated with all nine measurements (2.66 ± 0.40) was still similar to the Central and Ventral stiffness. On CEUS, mean perfusion parameters were: Peak: 26.12 ± 9.74; TTP: 22.1 ± 4.32 s; MTT: 28.05 ± 3.75 s; AUC: 699.4 ± 248.7 %; Pixels: 408.9 ± 65.55. On seminal evaluation, all patients presented normal parameters (mean volume: 1.65 ± 0.51 mL; total sperm output: 288.312 ± 69.845 x 10^6 sptz; progressive motility: 86.25 ± 8.763%; velocity: 4.25 ± 0.7; AP: 24648 ± 16095 U/l; MNS: 81.94 ± 7.169%).

Discussion and conclusions

A multiparametric assessment of the canine prostate is feasible, however, Doppler assessment of all portions of the prostatic artery might not be always possible in all patients, therefore, assessment of the Cranial portion could be prioritized. Prostatic SWV varies according to the depth of evaluation using VTTIQ and measurements of the Ventral and Central regions are more representative of the overall prostatic stiffness. Even though no standard sperm output parameters have been correlated to conception rate and fertility in dogs, the results of the present study may be considered borderline, based on the normozoospermic reference range established. This finding may be influenced by the divergent age of the dogs. Moreover,

the quantitative ultrasonographic data of this study could be considered important for future investigations on the abnormal prostatic conditions and for dogs with abnormal sperm parameters to understand if prostatic sonographic findings can be different in these patients.

Keywords: Doppler Ultrasound, Elastography, Contrast-enhanced Ultrasound, Canine.

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Meta-analysis of the prevalence of neospora caninum from cattle and water buffalo in Türkiye

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Özbakış-Beceriklisoy, G., Koç-İnak, N., Özen, D. Meta-analysis of the prevalence of neospora caninum from cattle and water buffalo in Türkiye.

Introduction and aim

Neospora caninum (N. caninum), is a apicomplexan parasite with a wide host range. It was first recognized in dogs in Norway in 1984 (1) then formally described as a new species in 1988 (2), and has since become a serious disease affecting mostly cattle and dogs worldwide. Abortions and neonatal mortality present significant challenges in livestock operations, with neosporosis being a leading cause of abortion in cattle (3). Reproductive failure results in substantial economic losses in cattle, making the prevalence rate particularly significant. This study aimed to determine the prevalence of Neospora caninum in aborted cattle tissues and the blood sera of cattle and water buffalo in Türkiye using meta-analysis.

Methods

Search strategy: A comprehensive literature search was performed on Scopus, Web of Science, PubMed, and Google Scholar databases from 29/04/2024 to 13/05/2024. The search strategy used combinations of words and keywords in English and Turkish, including Neospora, Neospora caninum, Cattle, Water Buffalo, Türkiye, Turkey, and their synony "Inclusion and exclusion criteria: The criteria for inclusion in the study are listed as follows: [1] Having an original research article and a case report published in a peer-reviewed journal, [2] The study was published between 01/01/2000 and 01/01/2024 [3] The language of the article is English and Turkish. Exclusion criteria from the study are listed as follows: [1] The study is not an original research article and case report, [2] Irrelevance of the study, [3] Duplicated publications.

"Meta-analysis: The meta-analysis was performed using Stata v.18 software (StataCorp LP, College Station, TX), with the level of significance set at $p<0.05$. The data were first subjected to Freeman-Tukey transformation (double arcsine transformation) before the meta-analysis so that the data followed an approximately normal distribution. Then, a random effect meta-analysis with transformed proportions using Der Smonian Leard estimator was implemented. These were then back-transformed to prevalence rates to facilitate interpretation of the outcomes and confidence interval (CI). Cochran's Q test and I2 test statistic were used to assess heterogeneity. A random effects meta-regression analysis was performed to assess the heterogeneity of effect between studies using Region and material as moderators. The forest plot was used in the visual presentation of the findings. Begg test and Funnel plots were used to assess potential publication bias.

Results

Included studies: The electronic database yielded 38 articles. After duplicates were removed, 36 remained, of these, 35 articles conducted on 12322 cattles, 92 water buffalos and 276 aborted cattle fetuses were selected for full-text evaluation following a review of the title and abstract). In total, 78 prevalence data were included in the qualitative and quantitative synthesis.

Study characteristics: The sera/aborted materials of suspected animals were sampled from seven geographical regions of Türkiye. Fourteen aborted materials and 63 blood samples' data were detected. Aborted cattle fetuses were evaluated with Molecular techniques (PCR, Nested PCR, qPCR) and Histopathological analysis (IHC, IF). The serum samples were assigned with Serological techniques (ELISA, c-ELISA, iELISA, IFA, Immunocomb, Rapid Test Kits).

Main outcomes: The study revealed a prevalence of 9.1% (95% CI: 6.6-11.7%). The 95% confidence interval for the overall estimate, along with the z-test statistic of 11.23 and the p-value of <0.001 suggest that was statistically significantly different from 0 for the overall prevalence of Neospora. No small study effect was observed for the outcome. The results of the I2 statistic indicated a high degree of heterogeneity for all three outcomes, with values of 95.89%. While the inclusion of moderators such as Material and Region in the meta-regression did result in a reduction in heterogeneity, the level of heterogeneity remained relatively high for all three outcomes.

Discussion and Conclusions

In a recent meta-analysis study, the global prevalence of N. caninum infection in aborted cattle fetuses was determined to be 35% via serological and 43% molecular methods. The same analysis also revealed the rate of prevalence of N. caninum infection in cattle that experienced abortion was 47% (4). The prevalence of Neospora caninum seropositivity in water buffalo was determined as 48.4% across thirteen countries (5). According to the current findings, the prevalence of N. caninum infection in cattle and water buffalo was recorded 9.1% in Türkiye. This low prevalence could be related to the inclusion of asymptomatic or healthy animals in the conducted studies.

When evaluating abortion cases, it is very important to analyze neosporosis along with other factors that cause abortions in cattle and to evaluate the results. In addition to abortion cases in cattle, a nationwide epidemiological study is needed to determine the real prevalence of neosporosis in Türkiye, which causes serious economic losses such as persistently infected calves being born, reduced milk yield, and premature culling. Furthermore,

multidisciplinary studies are required and will play a crucial role in the epidemiology and the solving of neosporosis.

Keywords: Infertility, Farm Reproduction, Neosporosis, Neospora caninum, Turkey.

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Radiographic imaging in the pregnant bitch: risks, advantages and disadvantages

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Abstract
Radiographic imaging is a critical tool in both human and veterinary medicine, with innovations reducing radiation risks significantly. While highly regulated and researched in human pregnancy, the use of X-rays in pregnant bitches remains controversial.

X-rays in the last week of pregnancy in the bitch are widely used to determine the number of fetuses. Researchers have also shown that measurement of the fetal head and pelvimetry in the canine species are useful to determine the risk of dystocia. Up until today no studies have investigated the possible impact of X-rays on fetuses and embryos in the canine species. This review examines the benefits and risks of radiographic imaging in the late stages of canine pregnancy, compared to ultrasound, amidst growing breeder concerns about its safety and necessity.

Introduction

The discovery of radiographic imaging changed medicine forever and may be considered one of the keystone moments of todays advanced medicine. Its discovery allowed better diagnosis and advancement of different specialties of human medicine. The repeated exposure to high values of radiation without any type of protection resulted in radiation illness, neoplastic disorders and others (1). The effect on gonads, embryos and fetuses have been noted much later. In veterinary medicine radiography was introduced in 1920 (2) and improvements of technique and safety developed for human medicine have been adapted in veterinary medicine. It has to be taken into account that X-rays were available much earlier than ultrasound examination which made it the only technique which allowed a "look inside" the body (2). Radiographic imaging in the pregnant bitch has been an important tool for small animal practitioners but breeders' opinion regarding this practice is changing.

Literature Review

Improvements of radiographic techniques, reduction of radiation and the introduction of protective measures decreased the side effects of X-rays (1). The International Commission on Radiological Protection (ICRP) advises to consider the three following principles: justification, optimization and dose limitation (1). Radiographic imaging in pregnant women is performed solely in cases in which the benefits of radiographic imaging exceeds the possible side effects. Nowadays, X-rays in veterinary medicine is most frequently used for diagnosis of orthopedic disorders and evaluation of thoracic organs (2). Evaluation of abdominal organs on the other hand is performed mainly via ultrasound examination (2). X-rays are static two-dimensional images which need to be obtained in correct position of the patient to allow correct interpretation of the image. Ultrasound on the other hand is a dynamic process which allows not only the evaluation of organic structure but also some aspects of their functionality (e.g., intestinal peristalsis, cardiac activity and others) (2). It is therefore considered the superior imaging technique in many specialties, orthopedics excluded. Furthermore, ultrasound examination does not pose a danger of radiation to the operator or the patient. In pregnant bitches, ultrasound examination

is used for pregnancy diagnosis as well as for pregnancy monitoring (2,3). The exam does not only allow determination of presence or absence of embryonic structures but allows also early determination of the viability of the embryos via measurement of the cardiac activity (3). X-rays in early stages of pregnancy are not useful for diagnosis as embryos and fetuses have no calcified structures until late stages of pregnancy. The presence of an enlarged uterus may be an indication for pregnancy yet the list of differential diagnosis for an enlarged uterus is long which makes x-ray not suitable for pregnancy diagnosis in any stage prior to fetal skeletal calcification. In late-stage pregnancy X-rays are performed to determine the number of pups (4). In this particular case radiography is the superior technique as counting the number of pups via ultrasound is highly error-prone (4).

Imaging Techniques and Their Application

Radiographic imaging is performed in lateral recumbency as well as in ventro-dorsal recumbency (4). Correct positioning is of great importance especially considering that evaluation of the relation between the pelvic opening and the diameter of the head of the pups (5–7) may be performed. This evaluation is useful in estimating the risk of dystocia due to relatively too large pups, especially in cases of singleton pregnancies or pregnancies in large or giant breeds with two or three pups. In certain bitches positioning them in correct ventro-dorsal recumbency may lead to a significant amount of stress. Studies so far have evaluated cortisol levels in the parturient animal and conclude that manipulation should be reduced to a minimum during the birthing process (8). No studies have yet established an effect of stressful events days before parturition. Although knowledge about this specific aspect is limited it may be presumed that the character of the bitch and her prior experiences with veterinarians have an important influence on the level of stress experienced. Bitches which are very frightened or stressed when presented to the veterinarian prior to pregnancy, will not react differently in late-stage pregnancy.

In veterinary medicine the absence of patient cooperation is of great importance as physical or pharmacological restraint is necessary to obtain high quality images. In absence of pharmacological restraint the exposition

to radiation of the operator has to be taken into consideration as well (2). Pharmacological restraint of a pregnant bitch in order to perform X-rays which are performed exclusively for informative reasons rather than out of diagnostic necessities has to be viewed critically.

Risk assessment

Radiation exposure of a single image, or in case of late-stage pregnancy up to three images, is low. At the moment of radiographic imaging in the pregnant bitch, organogenesis of the pups is completed. A growing number of breeders follow the hypothesis that as long as it hasn't been proven completely safe, it may be dangerous. Studies report that a dose of <50 mGy does not interfere with a pregnancy and does not influence its outcome in women (9). The moment in gestation in which embryos or fetuses are exposed to radiation is crucial yet reported threshold doses for permanent consequences on the level of the fetus is between 60 mGy and 1000 mGy (9). A single radiographic image without contrast enhancement of any sort may create a fetal exposure between <0.001rad (<0.01 mGy) and 0.62 rad (6.2 mGy) which are levels far beneath what has been reported to be dangerous for the fetus (10). No studies have been published regarding dangerous exposure levels in the domestic dog.

Breeders Concerns

Although X-rays in late-stage pregnancy have been widely used and well accepted by the community of breeders for decades, a shift in opinion over the last years was observable. A growing number of breeders consider the exam prior to birth of little to no use but consider the stress the bitch is put under to perform the exam as important. Breeders may also mention the dangers of radiation to the bitch, the fetuses, the gonads of the bitch and even the gonads of the fetuses as motive to not perform x-ray with the sole purpose of puppy count. On the other hand, breeders consider X-rays performed at the (presumed) end of birth useful to ensure the end of parturition with correct expulsion of all pups.

The scientifically proven impact of stress during the birthing process is a fact that is generally accepted in the community of breeders.

It may therefore appear contradictory that breeders may opt to transport the bitch and perform X-rays after the presumed conclusion of the birthing process while unknowingly transporting a bitch which still has pups in utero.

Although stress may be a valid concern of breeders regardless of our important lack of data regarding the impact of stress during pregnancy caused by a non-invasive procedure, the origin of the fear of possible negative effects of X-rays on the health of the bitch and the fetuses is difficult to understand. Stress during X-rays in a bitch in end-stage pregnancy may be evaluated empirically yet negative influences on gonadal health in particular may be considered as extremely rare or completely absent with methods available to researchers today. Determination of the impact of exposure to radiation during a low number of X-rays in pregnant bitches is extremely difficult. General health in itself and gonadal health in particular may be influenced by many environmental, genetic and other factors throughout the life of an animal. It would be nearly impossible to prove that the presence of mutations, genetic abnormalities, neoplastic disorders, fertility issues or complete infertility is connected to a radiographic study performed during late-stage pregnancy. Nevertheless, for researchers and specialists in small animal reproduction it would be important to understand breeders' doubts and concerns regarding radiographic imaging in the pregnant bitch in order to maintain open and professional communication.

The role of the veterinarian

Communication between the owner and the breeder are a very important part of a clinicians professional life yet frequently personal concerns of owners which are not or not yet scientifically proven may not be properly considered by some clinicians. Correct communication with and education of the owner improves owner's compliance and therefore patient treatment as owners which feel taken seriously are also more open to suggestions. Especially in the case of experienced breeders it may be difficult to change their point of view or to bring them to consider other points of views. Although X-rays in late-stage pregnancy may not be considered strictly necessary, in cases of singleton pregnancies or pregnancies with an unusual low number of pups for this breed may give information which is important

for an uncomplicated parturition. It is important to give the breeder all information possible on the topic yet also stating clearly that the scientific evidence may be scarce at the moment. Furthermore, if stress of the bitch is of the breeders main concern, clinicians should ensure a calm environment with the main aim to ensure the bitches well-being as well as reducing the manipulation and time of manipulation to the minimum necessary.

Conclusion

Radiography towards the end of pregnancy in the bitch is a well-established technique and still considered useful to this day. Although ultrasound examination is the superior technique for diagnosis and monitoring of the canine pregnancy the number of pups is determined more accurately with X-rays. Breeders fears and worries regarding the safety and usefulness of X-rays to determine number of pups need to be taken seriously and should not be dismissed although they may seem incoherent at times. Further studies are needed to understand this shift in breeders opinion and their worries regarding this diagnostic procedure. Although evaluation of negative impacts on gonadal health at this point in time may be considered nearly impossible, further research is needed to understand the stress bitches effectively experience during non-invasive procedures in late-stage pregnancy. Possible methods of evaluation of fetal stress during or immediately after the procedure should also be taken into consideration.

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Imaging of the canine prostate gland

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Abstract
The canine prostate is primarily assessed using B-mode ultrasound (US), which excels in evaluating gland dimensions, capsule integrity, and parenchymal changes. However, distinguishing benign from malignant conditions using B-mode US alone is challenging, necessitating advanced techniques such as Doppler, Contrast-Enhanced US (CEUS), Elastography, Computed Tomography (CT), and Magnetic Resonance Imaging (MRI). This review synthesizes current knowledge on these imaging techniques for assessing normal prostate, benign prostatic hyperplasia (BPH), prostatitis, prostatic cysts and prostatic neoplasia (PN). BPH and prostatitis show specific imaging features such as prostate enlargement and heterogeneity, with

cysts and abscesses. Prostatic neoplasia, though rare and predominantly found in neutered dogs, presents as prostatomegaly with irregular margins. Advanced techniques like CEUS and elastography showed promising results in differentiating benign from malignant lesions. CT and MRI provide critical insights into capsular distortion and regional involvement, enhancing diagnostic accuracy for canine prostate conditions.

Introduction

The canine prostate is an androgen-dependent ovoid-shape bilobed gland positioned at the bladder neck encircling the proximal urethra. B-mode ultrasound (US) represents the imaging modality of choice (1,2), since it allows an improved evaluation of the gland in terms of dimensions, integrity of the capsule and parenchymal changes (3,4). Nevertheless, the ability of US to differentiate benign from malignant conditions, remains still limited due to the overlapping findings (5). Hence, advanced techniques such as Doppler, CEUS and elastography US, CT scan and MRI have been investigated into the assessment of the prostate gland to overcome these limits (6). The purpose of the present review is to report current knowledge related to the available imaging techniques of the canine prostate gland.

Normal Prostate

Radiographs can be used to determine the size, shape, margins, location and the presence of prostatic mineralization. However, radiography cannot differentiate prostatic diseases (7).

On US, the prostate shows a hyperechoic capsule and a homogeneous echogenic parenchyma, with the urethra being located in the center of the gland and appearing as a hypoechoic line and circle in longitudinal or transverse plan, respectively (8). The gland size may be assessed by single measurements (4,9) or prostate volume (10) and is influenced by the size and age of the dog (11–13), as well as echogenicity (13). After castration, the prostate undergoes a volumetric and vascular involution, becoming an ellipsoid-shaped structure with a hypoechoic parenchyma (9,14–16), reaching the 80% of size reduction within 90 days since androgen deprivation (14,16).

Prostatic vascularization may be assessed by Doppler with different flow characteristics according to the sampling region (3,17) and age of the dog (13). However, its use in clinical practice remains limited due to the low reproducibility of the technique (13,18).

CEUS is an ultrasonographic technique involving the use of an intravenous contrast agent to highlight vascular supply and showed promising results in the evaluation of prostate perfusion in normal and diseased prostates (19–21).

From contrast injection, the prostate is enhanced in 10 seconds, reaching a peak around 30 seconds, and having a homogeneous wash-out phase. In longitudinal view, the contrast enhances first both prostatic cranial arteries and the capsule, then branching off homogeneously from the periphery to the center of the gland, towards the urethra. During the wash out phase, the gland homogeneously loses enhancement, except for the urethra, whose wash-out is longer when compared to prostate parenchyma (19). Prostatic enhancement after castration is lower and contrast parameters results to be different (16,22,23).

Data concerning the use of elastography US are scant and reports the use of strain and 2D-Shear Wave elastography in the assessment of the canine prostate gland (24,25). Normal prostates show an intermediate stiffness (24) characterized by low shear-wave velocities, with a softer tissue in case of healthy dogs (25), being not influenced by age (24).

The prostate can be readily identified in CT studies of the abdomen and pelvis. In intact dogs, on pre-contrast scans, it appears as an oval to spherical shape with symmetrical lobes in axial view, with soft tissue density and homogenous attenuation surrounding the urethra (26–28). Prostatic size assessment has been proposed by calculating a ratio of the prostatic measurements of length, width and height to the length of the sixth lumbar vertebra (29). Another study proposed a threshold to signal prostatic enlargement based on published ratios (26). Contrast medium administration

can enhance the visualization of the prostate, demonstrating homogenous enhancement, with the median septum becoming prominent (29).

A limited number of studies have been conducted to describe normal and abnormal findings of the canine prostate using MRI. Cho et al. performed a study regarding the normal prostatic morphology, reporting that the canine prostate is identified as a bilobed oval-shaped structure with a homogenous aspect on T1-weighted images (T1W) and heterogenous on T2-weighted images (T2W), with high and low signal radiation lines, and a low signal intensity from the prostatic capsule (30), similar to what had been reported by Willmitzer et al (31). The median septum cannot be identified in T1W, but can be identified in T2W, and T2W with fat saturation(30). The prostatic urethra shows a high signal intensity on T2W images, being significantly enhanced on post-contrast T1W images and T2W images with fat saturation. In addition, they report that post-contrast T1W images show inhomogeneous contrast enhancement of the gland, with central and radial striations (30,31).

Benign prostatic hyperplasia

Dogs affected by BPH may have an enlarged prostate gland with inhomogeneous prostatic parenchyma, due to the presence of hyperechoic foci with shadowing effects, representing mineralization and several parenchymal cysts, with variable dimensions, representing the prostatic ducts with accumulation of prostatic fluid (13,20).

An overall increase in prostatic vascular blood supply occurs in BPH- and prostatitis affected dogs, even though specific parameters calculated by using CDUS are not directly influenced (17).

On CEUS examination, benign conditions show a homogeneous enhancement with an increased pixel intensity due to the higher vascularization occurring in both BPH (15) and prostatitis (21,32). Avascular area representing cysts have an increased peripheral rim enhancement persisting in the wash out phase in case of abscesses (21).

On CT scan, BPH-affected glands present symmetrical or asymmetrical enlargement, heterogenous aspect both on pre- and post-contrast evaluation, associated with the presence of cystic structures distributed throughout the parenchyma and no damage to the prostatic capsule nor regional lymphadenopathy (26). Prostatic density in BPH does not change significantly in pre-contrast studies, but it has been shown to be slightly lower in post-contrast in the early arterial phase by Pasikowska et al. (26). Contradicting information was then reported by Vali et al (33), as they reported that attenuation parameters did not allow differentiation between normal and BPH-affected dogs. Therefore, CT assessment should consider qualitative and quantitative findings and confront the obtained information with the patient's history, clinical examination and laboratory findings.

No specific study has been performed to describe specific MRI findings of BPH in canines, although the assessment is often similar to the expected findings based on sonographic and CT features.

Prostatic and Paraprostatic cysts

Cysts are classified as either retention cysts or paraprostatic cysts (1,34). Retention cysts are parenchymal cavitating fluid-filled lesions, whereas paraprostatic cysts are located outside the prostate, being associated to remnants of uterus masculinus or Müllerian ducts (1,35). Very large cysts may resemble true paraprostatic cysts, making differentiation challenging, although the former is usually associated with other prostatic parenchymal changes typical of BPH, and the wide base of attachment/origin of the cyst may be found within the prostate (paraprostatic cysts are attached only by a thin stalk-like structure) (1). Large prostatic retention cysts or paraprostatic cysts have anechoic fluid, although they may become more echogenic and have obvious sediment consisting in mineralization with shadowing effect (7,36).

A canine paraprostatic cyst is a fluid-filled structure located outside the prostate gland but associated with its surrounding tissues (1). They may vary in size and appear as cystic lesions with a thick wall, echogenic content, that may occasionally get infected inducing steatites and edema (7,37). CT and

MRI may help for surgical planning and to determine their benign nature. Actually, they may pose diagnostic challenges due to their potential to resemble other cystic formations of neoplastic origin (38) .

Prostatitis

Prostatitis may occur together with BPH, therefore prostate may be increased in size, characterized by a hypoechoic band surrounding the prostatic capsule as a result of the prostatic oedema, especially in acute prostatitis (32). The parenchyma is usually heterogenous and in acute prostatitis has a hypoechoic appearance, which is followed in more chronic cases by increased echogenicity, with focal echogenic regions (7). Steatitis or peritonitis affecting the periprostatic fat causes a hyperechoic and hyperattenuating appearance (7). Prostatic mineralization may occur in prostatitis both in intact and neutered dogs (5,8,32). Prostatic abscesses may be present and could be distinguished by cysts by a hypoechoic content and a thicker wall. However, ultrasound guided aspiration of the cystic content is necessary to define the nature of the content (1,32,39).

CT features of prostatitis are often similar to those of BPH, but it has been reported that the length and attenuation of the medial iliac lymph nodes is significantly higher in patients with prostatitis (33). MRI findings of prostatitis have not been reported and its assessment is, once again, based on the expected features according to sonographic and CT features.

Prostatic neoplasia

PN is an uncommon condition in dogs accounting the 13% of the prostatic pathologies (1,40,41) and having a higher prevalence in neutered individuals (40,42,43). The differentiation between benign and malignant conditions may be challenging and, to our knowledge, there are no early predictive ultrasonographic signs of prostatic neoplastic changes. PN-affected prostates may be characterized by prostatomegaly, irregular margins and shape, and heterogeneous parenchyma, due to the presence of cysts or mineralization (5). Castrated dogs with prostatic mineralization have a high likelihood of having PN and may be detected by using both US, whereas

intact dogs with prostatic mineralization may be affected by non-neoplastic conditions (5).

The development of PN is in many cases associated with changes in the vascular supply to the prostate as well as changes to the prostatic parenchyma (8), even though data concerning the use of PWUS are not reported.

Trends for prostatic tumors have been reported in dogs concerning the ultrasonographic appearance e using CEUS, showing promising results in differentiating benign from malignant lesions (20). Prostatic neoplasia may present as single nodule or diffuse prostatic pathology. The single nodule may be evaluated and compared to the surrounding parenchyma. The diffuse pattern is characterized by an inhomogeneous enhancement with abnormal vessel diameter and orientation within the gland. Prostatic adenocarcinoma may present variable enhancement pattern, with an increased contrast intensity of the single nodule (20) or with hypoechoic lesions with potential anechoic areas representing necrotic tissue (21).

To our knowledge there is just one case report concerning the use of elastography in the detection of prostatic malignancies in dogs (44).

Few studies describing CT and MRI findings of prostatic neoplasia are available in the current literature. One study described both CT and MRI findings on a short number of cases and stated that on both imaging modalities, heterogenous appearance with capsular distortion, associated with cavitations, calcifications, strong enhancement on post contrast evaluation, regional lymphadenopathy and involvement of adjacent structures are features associated with neoplasia (45). The authors also report that MRI provides detailed information regarding involvement of the ureterovesical junction, trigone, post-prostatic urethra and neurovascular bundle compression or invasion, but CT might have a better sensitivity to identify mineral foci. Periprostatic fat streaking or fluid has also been described to be present in prostatic neoplasia, as well as ductus deferens and urethral invasion, bony changes consistent with hypertrophic osteopathy

and, infrequently bone lesions suggestive of metastases in the lumbar or sacral vertebrae (46).

Conclusions

Multimodal imaging is crucial for accurately diagnosing canine prostate conditions. While B-mode ultrasound is the primary modality, its limitations necessitate the use of advanced techniques like Doppler, CEUS, Elastography, CT, and MRI. These sophisticated imaging methods enhance diagnostic accuracy, ensuring comprehensive evaluation and effective management of canine prostate conditions, ultimately improving clinical outcomes and patient care.

Keywords: Prostate, Dog, Ultrasound, Computed Tomography, Reproductive Imaging

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Ozone insufflation & frozen AI: Effects on post-breeding uterine inflammation in mares.

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Introduction and aim

Endometritis represents the main cause of subfertility in mares and the third most common disease in horses. Breeding-induced endometritis (BIE) is a physiological reaction after mating, caused by the introduction of semen into the uterine lumen, especially when frozen semen is used [1]. In susceptible

mares, this reaction can persist beyond 48 hours after insemination leading to persistent breeding-induced endometritis (PBIE) and infertility [1].

The aim of this study was to evaluate if intrauterine ozone (O_3) insufflation, prior to low quality frozen-thawed semen artificial insemination (AI), has an effect on BIE in reproductively healthy mares.

Methods

Eight clinically and reproductively healthy mares aged 11-23 years, were included in the study. Animals were alternatively assigned to the treatment (T, N=8) or the control (C, N=8) cycle group in a crossover study design with a washout cycle between the two. During the first day of estrus (D0), uterine samples were collected by cytobrush for cytological examination, uterine edema (grade 0-5, [2]) and liquid accumulation (grade 0-4, [3]) were evaluated by US. Then, in T cycle, mares were submitted to intrauterine insufflation of a O_2-O_3 gas mixture (volume: 300 ml; [O_3]: 40 µg/ml) for 3 consecutive days while, in the C cycle, mares were left untreated. AI was performed 24 hours after ovulation induction, in both cycles, with 2 ml of frozen-thawed semen pooled from two stallions. With the aim of exacerbate the uterine reaction, poor quality semen (total subjective motility <20%) was employed. Six (t6), 24 (t24), 48 (t48), and 72 (t72) hours after AI, mares underwent US and uterine cytological examination to evaluate the degree and persistence of uterine inflammation after breeding.

Statistical analyses were performed using the Jamovi Software Version 2.4.7, to evaluate the number of PMN, uterine edema, and liquid accumulation. The Shapiro-Wilk test assessed the distribution normality of the T and C populations at each timepoint (D0, t6, t24, t48, t72 hours, respectively). Populations were not normally distributed therefore differences among treatments for each time-point (D0, t6, t24, t48, and t72) were analyzed using the Mann-Whitney U test.

Data were expressed as Median and interquartile range and differences were considered statistically significant with $P<0.05$.

Results
Uterine edema and PMN count were similar in the two cycle groups at D0 and raised at t6. The uterine edema and liquid accumulation significantly decreased from t6 until t72, without statistical differences between the T and C cycles. There were also no statistical differences between the number of PMN per 400x microscopic field for T vs C cycles (median, IQR; P>0.1)): 0.050, 0.125 vs 0.100, 0.200 (D0); 75.0, 194.0 vs 150.0, 213.0 (t6); 14.2, 38.6 vs 30.2 vs 36.7 (t24); 5.4, 5.47 vs 6.45, 5.45 (t48); 2.2, 5.75 vs 2.5, 8.53 (t72)

Discussion and conclusion: While an endometrial inflammatory response is physiological after breeding in mares, this can predispose to PBIE which is a pathological condition that results from this response continuing longer than 48 hours. Given the frequent failure of traditional treatments against uterine inflammation and the necessity to reduce the use of antibiotics, alternative therapies such as platelet-rich plasma (PRP) or O_3 uterine infusion are studied [4] [5]. O_3 insufflation treatment could therefore represent a possible low-cost and safe therapy to be used in mares affected by both BIE and PBIE. The aim of this study was to evaluate if intrauterine ozone ($O_{3)}$ insufflation, prior to frozen semen artificial insemination (AI), influences on BIE in reproductively healthy mares. To exacerbate the uterine reaction, very low quality frozen-thawed semen was employed, with very poor expectation of pregnancies: for this reason, the evaluation of pregnancy rates was not included in the analysis of the results.

In the conditions of this study, intrauterine O_3 treatment before AI had no effect on post-breeding uterine inflammation, evaluated by uterine edema, fluid accumulation, and the PMN concentration in the uterine lumen, comparing treatment and control cycles, in reproductively healthy mares. Studies testing different O_3 concentrations and/or including multiple diagnostic evaluations as well including mares susceptible to PBIE could give different results.

Keywords: ozone therapy, breeding-induced endometritis, post breeding-induced endometritis, artificial insemination, PMN, uterine inflammation, mare.

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Intrauterine infusion of mesenchymal stromal/stem cell–derived conditioned medium in problem mares

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Introduction and aim

Endometritis is considered one of the main causes of impaired fertility in mares [1]. Regenerative therapies, including stem cells and their derivatives, are being increasingly recognized as promising options for uterine treatment owing to their immunomodulatory properties [2-5]. This study tested the effect of Wharton's jelly mesenchymal stromal/stem cell–derived conditioned medium (WJMSCs-CM) on uterine response in problem mares.

Methods

Equine WJMSCs were isolated from 3 samples and frozen at passage 3. WJMSCs were thawed and maintained in culture medium (DMEM + 10% FBS) until 80-90% confluence, then the culture medium was replaced with serum-free Ringer Lactate. The CM was collected after 24 hours of starvation and cellular debris were removed by centrifugation at 3345 g for 30 minutes at 25°C and then stored at –80°C until use. Twelve mares, aged between 4 and 20 years, that had failed to conceive in the previous breeding season or after at least two insemination attempts within the same season were included. The mares were randomly assigned to control (CTR) or CM group. Estrous progression was tracked via transrectal ultrasonography; when a pre-ovulatory follicle appeared, ovulation was induced and, 24h later, mares were inseminated with cooled semen. CM group received an intrauterine infusion of 20 ml of WJMSCs-CM, whereas CTR group was treated with the same volume of Ringer Lactate, 7-8 hours after insemination with cooled semen. A low-volume flush (LVF) was conducted immediately before (PRE) and 12h after (POST) the intrauterine infusion. An aliquot of LVF was sent for bacteriology, by inoculation in Brain Heart Infusion broth and incubated aerobically at 37°C for 24h. In case of turbidity, broth sub-cultivation in Agar plates was performed for additional 24h at 37°C; arisen colonies were firstly screened by standard rapid techniques and then identified by Maldi Tof Ms. The LVF was centrifuged at 400 g for 5 min, and interleukin

(IL)-10 concentration was evaluated by ELISA (Assay Genie, Ireland) in supernatants; pellets were smeared and stained with Diff-Quick (Bio-Optica, Italy) for cytology: presence of 0–2, 2-5 and >5 polymorphonuclears/high power fields (PMNs/HPF) indicate no, moderate and severe endometrial inflammation, respectively. Pregnancy diagnosis was performed at 14 d and weekly confirmed until 60 d after ovulation.

Comparison of IL-10 concentrations and inflammation scores between groups and times was performed using the Wilcoxon test and the Median test, respectively. Pregnancy rates between groups for all mares and for mares positive for bacteria (CTR vs. CM) were compared using the Chi-square test. Differences were considered significant at $P < 0.05$.

Results

Positive bacteriology was detected in 5/6 and in 4/6 mares of CTR and CM groups, respectively; no differences were found between PRE and POST samples in both groups. The inflammation score ranged between moderate and severe in both groups before and after treatment, with no significant differences observed (CTR: PRE 2.3 ± 0.8 and POST 2.7 ± 0.5; CM: PRE 2.3 ± 0.8 and POST 2.7 ± 0.5). The IL-10 concentration in LVF differed ($P<0.05$) between PRE and POST only in CM group (1023 ± 158 vs. 1427 ± 188 pg/mL), and a difference between CTR and CM was found ($P<0.05$) in POST samples (1085 ± 342 vs. 1427 ± 188 pg/mL). No differences were found for pregnancy rates of the CM (4/6; 67%) and CTR (2/6; 33%) groups; while among the mares that were positive for bacteria, a difference ($P<0.05$) was found between CTR (1/5; 20%) and CM (3/4; 75%) groups.

Discussion and conclusions

The uterine infusion of WJMSCs-CM in mares, 7-8 hours after AI with chilled semen did not show clinical effects in endometrial inflammatory response or bacteriology results 12 hours after treatment. This could be due to the different timing of treatment and evaluation used in this work compared to similar studies [3-4]. Indeed, an anti-inflammatory effect of WJMSCs-CM was confirmed by the higher concentrations of IL-10, an essential cytokine for modulating the immune response. Moreover, the lack of difference in pregnancy rates between groups could be due to the low number of mares

involved in the study. Although CM showed no effects on uterine cultures at 12h bacteriology , the higher pregnancy rate in the mares positive for bacteria receiving CM treatment suggests a potential delayed antimicrobial effect of WJMSCs-CM. Further studies are necessary to confirm these results.

Keywords: equine, regenerative treatment, endometritis, umbilical cord, uterus.

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Flow cytometric sperm analysis of ant spermatozoa Lasius alienus (Hymenoptera: Formicidae)

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Introduction and aim

Ants are social insects belonging to the family Formicidae, which belongs to the order Hymenoptera. More than 12.000 species have been identified to date, with an estimated total of approximately 14.000 (1). Most ant colonies are composed of infertile females, which form the worker and soldier classes. In addition, there are fertile male drones and females called queens, which ensure reproduction in the colony (2). The objective of this study

was to examine the characteristics of spermatozoa of ants (Hymenoptera: Formicidae) in-vitro.

Methods

The study was carried out using semen obtained from 15 male *Lasius alienus* (Foerster, 1850) ants collected around the Reproduction and Artificial Insemination Clinic building of Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine. It was carried out using ant semen obtained from 15 male ants (drone) that came out spontaneously in the clinic and stuck to the entrance door glass or windowpanes to get out. Male ants were exposed to chloroform vapor, resulting in eversion of the endophallus and extraction of sperm into a 35 °C PBS solution via disruption of tissues on a glass petri dish using pressure and a scalpel. Spermatozoa were aspirated with a micro automatic pipette and diluted with 500 µL of PBS solution. After determining the presence and viability of spermatozoa under a microscope, they were kept in PBS solution for short-term storage. Plasma membrane and acrosome integrity (PMAI) (FITC-PNA/PI), viability (SYBR-14/PI), mitochondrial reactive oxygen species level (MROS) (MITOSOX RED/PI), lipid peroxidation levels (BODIPY/SYBR-14), peroxy-nitrite-mediated nitrosative stress (DHR/PI), intracellular calcium levels (FLUO-4/PI) and mitochondrial membrane potential (JC-1) were measured by flow cytometer every 24 h from 0 h to 96 h. In addition, sperm apoptosis levels (ANNEXIN V/PI) were measured at 0 h.

Results

At 0 h, PMAI levels were 69.87%, viability levels 72.82%, MROS levels 13.39%, lipid peroxidation levels 10.33%, nitrosative stress levels 10.02%, intracellular calcium levels 7.94%, high mitochondrial membrane potential (HMMP) 95.94%, early apoptotic sperm levels 7.11% and late apoptotic sperm levels 11.77%. At the 96th hour, PMAI was 59.80%, viability 53.30%, MROS levels 32.87%, lipid peroxidation levels 19.03%, nitrosative stress levels 16.63%, intracellular calcium levels 21.33% and HMMP 49.57%.

Discussion and conclusions

Information available in the literature on ant sperm is limited. In a study conducted in Leafcutter ants, the mean percentage of live sperm under fluorescence microscopy (SYBR-14/PI) was 36.95$\pm$1.98% for samples

collected from accessory testes and 62.54±2.12% for samples collected from the spermathecae of queens (3). Additionally, the viability of ant spermatozoa collected from the spermathecae of queens was evaluated by flow cytometry, and the results indicating that 98.6+1.5%, 98.4+2.8%, and 78.8+8% were present in three different groups, respectively (4). In another study conducted on *Linepithema humile* ants, when sperm viability was examined flow cytometrically, it was found to be 90.1 ± 5.4% in the nest without protein supplementation, while it was 88.7 ± 4.1% in the nest with protein supplementation (5). Many factors such as methodology, individual factors, colony size, and species differences may account for this difference between studies. Consequently, it was determined that the PBS solution could be employed for the short-term storage of ant sperm. As in other animal species, there is potential to make it available to producers and researchers by developing commercial preparations for the reconstitution of ant semen.

Keywords: Ant, Flow cytometry, Spermatozoa, Lasius, Hymenoptera.

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Prostatic diseases in dogs: retrospective study of 124 cases

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Introduction and aim

The diseases of the prostate gland are relatively common in male dogs especially intact males aged older than 6 years (1). Common prostatic diseases of dogs include benign prostatic hyperplasia (BPH), prostatitis, prostatic cysts, prostatic abscesses, and less commonly prostatic neoplasia (PN) (2, 3). Although information on prostatic disorders is abundant (1,4,5), there are not many population studies looking at the prevalence of these disorders in dogs (3,6). The aim of this study was to investigate the incidence of prostatic diseases, as well as evaluate the clinical relevance of the most commonly observed clinical signs associated with the different prostatic disorders in dogs admitted to the International Veterinary Hospital, Antalya.

Methods

Electronic medical records, from May 2020 to May 2024, of client-owned dogs from the hospital were searched to identify dogs diagnosed with benign prostatic hyperplasia, prostatitis, prostatic cysts, prostatic abscesses and prostatic neoplasia based on prostatic aspiration cytology, histopathology, prostatic aspirate or a positive bacterial culture. The medical records of included cases were reviewed, and data collected included signalment, clinical signs, and physical examination findings.

Results

One hundred twenty-four dogs met the inclusion criteria. Among dogs experiencing a prostatic disorder, most frequently recorded diseases were benign prostatic hyperplasia (n:78, 62.9%) and prostatitis (n:29, 23.4%), followed by abscesses (n:10, 8.1%), neoplasia (n:5, 4%), cysts (n:2, 1.6%). The most common four breeds diagnosed with prostate diseases were Golden Retrievers (n=18, 14.5%), Turkish Kangal Shepherd Dogs (n=17, 13.7 %), Labrador Retriever (n =14, 11.3 %), German Shepherd (n =15, 12.1%). Dogs presenting with prostatic neoplasia (12.8 $\pm$ 1.9 years) were older ($P < 0.05$) than those with BPH (7.4 $\pm$ 1.8 years), prostatitis (7.2$\pm$ 2.1 years), prostatic abscesses (8.6 $\pm$ 2.3 years), prostatic cysts (8.5 $\pm$ 1.5 years).

Discussion and conclusions

Weight loss and lethargy were clinical signs strongly associated with PN. Hematuria and urinary incontinence were more common symptoms in dogs with prostatitis compared to dogs with BPH. However, tenesmus was a significant clinical finding of BPH compared to prostatitis. Seven out of 10 dogs (70 %) diagnosed with prostatic abscessation, presented acute local and systemic signs. There was no statistical difference between the prevalence of asymptomatic dogs diagnosed with BHP and dogs diagnosed with prostatitis. Prostatic culture data were available for 34 dogs. Of the cases for which culture results were available, 17.6% (6/34) of cases had a negative bacterial culture. *Escherichia coli* was the most common bacterium cultured in 53.5% (15/28) of the dogs. It was cultured with *Proteus* species, in six cases (21.4%), with *Staphylococcus* species in four cases (14,2%) , with *Pseudomonas aeruginosa* in three cases (10,7%) with *Enterococcus* species

in two cases (7.11%). The mean age of the dogs experiencing prostatitis and BPH were 7.2 ± 2.1 and 7.4 ± 1.8, respectively, a slightly younger value than 8 years as previously reported (6,7). This could be due to the fact that general ultrasound examinations are performed yearly on dogs over the age of 5 at our hospital. Prostatitis and BHP were incidental findings in 27 % and 24 % of the dogs in this study, respectively. suggesting that the prevalence of both diseases is higher than what is observed clinically. Breed predisposition had already been described in German Shepherd (6) , Doberman Pinschers (6), Scottish Terrier (9) that we did not find here. The signalment and clinical presentation of dogs in the present study was similar to previous reports of dogs with prostatic disease (10,11,12). *E. coli* was the most common bacteria cultured in our study as already reported (13,14).

Key Words: Prostatic Diseases, dog, benign prostatic hyperplasia, prostatitis, prostatic neoplasia.

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A preliminary study of three boric acid doses to improve in vitro development of ovine embryos.

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Introduction and aim

Boron (B) is the 5th element in the periodic table and is found in nature as borax, boron, boric acid, colemanite, kernite, ulexite, and borate (4). It also plays a role in the absorption and balance of phosphorus, magnesium, and calcium in bones and joints (5, 10, 11). Although the biochemical mechanism of boron is not fully known, it is thought that it may be a metabolic regulator in cell membrane functions (12) and enzymatic system (5). In addition, it also has antibacterial and antifungal effects. There are a limited number of studies in the literature regarding animal embryo culture with boron or boric acid. The first study on embryo development in the literature found that 12-25 µM boric acid supported blastocyst development with the addition of mouse embryo culture (9). There were several studies on animal reproduction of boron for potential beneficial effects (1, 3, 6-8, 13). In addition, in a recent study, 20-250 µM/L boric acid as an antioxidant did not support embryo development after developing in vitro bovine embryo culture conditions (7). According to the latest research in the literature; after adding 2, 1 and 0.4 µg of boric acid to the in vitro culture medium for bovine embryo development, blastocyst development was 47%, 49%, 54%, respectively, and a significant difference was observed positively compared to the control group. In this study, the highest improvement was achieved especially in the low dose 0.4 µg boric acid group (13). In our study, boric acid at doses of $1.62x10^{-1}$ µM, $1.62x10^{-3}$ µM, $1.62x10^{-5}$ µM was added to the in vitro maturation medium of ovine oocytes obtained from the slaughterhouse and incubated for 24 hours to mature and reach the MI stage.

Methods

Mature oocytes were fertilized by thawing frozen spermatozoa and were removed from the fertilization medium after 22 hours. Embryos were placed in culture media containing boric acid at doses of $1.62x10^{-1}$ µM, $1.62x10^{-5}$ µM, and $1.62x10^{-7}$ µM and cultured for 7 days, and blastocyst rates were determined. All media in vitro oocyte an embryo handling technique were done according to Birler et al.2002 (2).

Results

According to our first findings cleavage rates of the groups respectively 0μM, $1.62x10^{-1}$ μM, $1.62x10^{-5}$ μM, $1.62x10^{-7}$ μM were 83%, 83%,75% and 79%. Blastocyst rates of the groups were 42%,23%,40% and 16%respectively. Degeneration rates were 45%, 21%, 45% and 34%.

Discussion and conclusions

In conclusion as a preliminary study, we have introduced boric acid to ovine embryo culture as a new supplement and $1.62x10^{-5}$ μM group gave us best results as the control group. The doses used in the previous studies. The results in ovine embryo culture but to improve ovine embryo in vitro culture systems further investigations should be done. We aim to add boric acid to ovine embryo culture from oocyte embryo maturation process to embryo culture stage to see improvement.

Keywords: Boric Acid, ovine, embryo, development, in vitro culture.

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Melatonin Modulates EREG, BMP15, and PGR gene expression in superovulated rat ovary tissue

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Citation
Yakan, A., Dalkıran, S., Özkan, H. Melatonin Modulates EREG, BMP15, and PGR gene expression in superovulated rat ovary tissue .

Introduction and aim

Superovulation is used in reproductive treatments and experimental studies. This method involves the use of exogenous gonadotropins to increase the number of oocytes completing development in each cycle, aiming for high yield of oocytes (1, 2). Studies have reported superovulation disrupts the redox balance in the ovary, leading to increased oxidative stress (3). The most common approach to mitigate the effects of oxidative stress is the use of antioxidants (4, 5). In this study, the effects of melatonin application on oxidative stress and follicle development in the ovary resulting from superovulation were investigated. For this purpose, the expressions of genes responsible for folliculogenesis, oocyte development, and health in ovarian tissue, including Epiregulin (*EREG*), Bone Morphogenetic Protein 15 (*BMP15*), and Progesterone Receptor (*PGR*) were examined.

Methods

Twenty-eight Wistar albino rats aged 10-12 weeks were used. Four groups were created, each containing an equal number of animals: Control, Superovulation (So), Superovulation+5mg melatonin (SoM5), and Superovulation+20 mg melatonin (SoM20). Superovulation was induced using Gonadotropin-Releasing Hormone (GnRH), Pregnant Mare Serum Gonadotropin (PMSG), and Human Chorionic Gonadotropin (hCG) (6). Beginning one week before the experiment, a single daily dose of melatonin was administered intraperitoneally to the SoM5 and SoM20 groups. At the end of the experiment, the rats were euthanized, and ovarian tissues were collected. Total RNA isolation from the tissues was performed using the TRIzol method. The isolated RNAs were then converted to cDNA. Subsequently, the expression levels of *EREG*, *BMP15*, and *PGR* were calculated using qPCR and the $2^{-\Delta\Delta Ct}$ method (7, 8). qPCR reaction conditions were set at 15 s at 95 °C, 60 s at 60 °C, and 30 s at 72 °C for 40 cycles, following a 10-min incubation at 95 °C (8). Actin Beta (*ACTB*) was used as the housekeeping gene.

Results

In the SoM20, the expression of the *EREG* showed an approximately 5-folds upregulation ($P<0.001$), while the *PGR* expressions exhibited approximately 7-folds increase ($P<0.001$) compared to the control. The *BMP15* expression levels showed similar expression patterns in groups. Moreover, correlation analysis revealed a significant positive correlation between *EREG* and *PGR* (r: 0.712; $P<0.001$).

Discussion and conclusions

EREG plays a role in the regulation of many biological processes such as inflammation, oocyte maturation, and cell proliferation (9). Similarly, PGR is a transcription factor initiates the transcription of genes necessary for oocyte release and maturation (10). The upregulation in *EREG* and *PGR* genes in the SoM20 suggested the potential effectiveness of melatonin in regulating Luteinizing hormone secretion and release. It was reported in a study that melatonin application showed dose-dependent differences in the *EREG* gene in the ovary (11). The lack of significant changes in *EREG* and

PGR expression in the SoM5 may be due to the dose-dependent effect of melatonin. In conclusion, a 20 mg dose of melatonin might have protective effects on ovarian health via molecular regulation of *EREG* and *PGR*. These findings suggest 20 mg melatonin application in superovulation practices could be therapeutically used to preserve ovarian health and support follicle development.

Keywords: *BMP15*, *EREG,* melatonin, *PGR,* superovulation.

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Ultrasonography and elastography of ovarian sex cord-stromal tumor (luteoma) in a dog: Case report

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Introduction and aim

Primary ovarian neoplasms are uncommon and have low incidence in domestic mammals (1), being reported in multiparous individuals or those with ovarian remnant tissues (2). Luteomas are extremely rare, exhibit benign behavior, and do not metastasize (3). Ultrasound is an important imaging tool in animal reproduction, aiding in the study of ovarian and uterine characteristics, contributing to the diagnosis of reproductive status, and identification of masses by providing information regarding the external and internal aspects of the analyzed structure, as well as its extent and location (4). Elastography is a relatively new non-invasive, ultrasound-based technique designed to measure tissue elasticity in situ classified as acoustic radiation force impulse (ARFI) and real-time shear velocity (RSV). In addition,

elastographic evaluations can be performed using the qualitative technique and the quantitative technique employing compression waves (5). The aim of this study was to describe the B-mode ultrasonographic and elastographic characteristics of an ovarian sex cord-stromal tumor (luteoma) in a female dog with an ovarian remnant.

Methods

A healthy, adult, spayed female Fox Paulistinha dog underwent ultrasonographic evaluation using the Versana Balance V2, GE ultrasound, with an 8.0 MHz multifrequency linear transducer, at a private veterinary diagnostic center.

Results

A hypoechoic, heterogeneous area with regular margins and defined contours was visualized caudal to the left kidney, in the left pedicle region, showing slight vascularity on color Doppler, measuring 1.96 x 1.45 cm. Immediately after B-mode ultrasound, elastography was performed using the esaote X75 ultrasound with specific software designed for tissue stiffness analysis, QElaXto® software using the 2D-SWE technique (Esaote®, Italy), previously validated for this application (6). Shear wave elastography mode revealed a non-deformable dark elastogram, and quantitative analysis showed an average shear wave velocity of 2.90 m/s, suggestive of malignancy. The patient was referred for laparotomy, and the excised tissue was subjected to histopathological analysis, resulting in a diagnosis of luteoma.

Discussion and conclusions

Luteoma is a rare neoplasm in dogs, and it is noteworthy to highlight the applicability of elastography. The reference value obtained in this study may provide significant diagnostic value associated with histopathology due to the similar cellular morphology of ovarian tumors, emphasizing the uncommon or underdiagnosed condition in veterinary medicine.

Keywords: canine, elastography, luteoma, neoplasm, reproduction, ultrasonography

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Effect of nanoparticles added to diluted ram semen on storage at +4 °C

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Citation
Aslan, M.D., Uslu, B.A. Effect of nanoparticles added to diluted ram semen on storage at +4 °C.

Introduction and aim

The aim of this study was to investigate the changes in the viability and motility of semen during short-term storage of ram semen at +4 $^{\circ}$C by adding zinc or vegetable activated carbon nanoparticles to semen diluent (1,2). Sheep breeding has an important place worldwide thanks to its advantages. In addition to increasing reproductive efficiency in sheep with genetic conservation, it is very important for the continuation of profitability and sustainability in terms of breeding (3,4).

Methods

24-month-old Kangal breed rams were used in the study. Semen was collected from 4 rams twice a week by electro-ejaculator method. This research was completed with 4 replications. In order to eliminate individual differences, collected spermatozoa were pooled and placed in a 33 $^{\circ}$C water bath. In the spermatologic examination, >90% motility, <10% abnormal spermatozoon rate and concentration >2x10^{9}/ml were determined. Semen

samples were diluted with Tris-egg yolk diluent and divided into 7 equal parts (5). While one of the samples was kept for the control group, 50 µg/ml (Z1), 100 µg/ml (Z2), 200 µg/ml (Z3) of zinc nanoparticle and 50 µg/ml (C1), 100 µg/ml (C2), 200 µg/ml (C3) of activated carbon nanoparticle were added to the diluent respectively (6). Motility rates, live/dead rates, abnormal spermatozoon rates were recorded every 8 hours starting from the 0th hour.

Results

When the short-term stored samples were evaluated, it was found that the total motility loss was the lowest in the groups in which 100 µg/ml and 200 µg/ml activated carbon nanoparticles were added compared to the others ($p<0.001$). In addition, when zinc nanoparticle was compared with the control group, total motility loss was found to be less ($p<0.05$). When the morphological examination results were evaluated, it was observed that there was no significant difference between the groups ($p>0.05$).

Discussion and conclusions

In recent years, there have been numerous studies where nanoparticles are added to semen extenders to improve spermatological parameters. Upon examining these studies, it is evident that despite using different nanoparticles, this approach offers a promising method for enhancing semen preservation and protection techniques(7–9). As a result, it was concluded that the addition of activated carbon and zinc as nanoparticles to reconstituted semen obtained from rams outside the vaccination season will provide longer motility.

Keywords: Ram, semen, short-term storage, nanoparticles, activated carbon, zinc.

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Spermatological and biochemical examination of New Zealand rabbit semen after thawing

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Citation
Öztürk, C., Döşeyici, M.E., Aksoy, N.H. Spermatological and biochemical examination of New Zealand rabbit semen after thawing.

Introduction and aim

The rabbit is seen as a valuable laboratory animal that can be used as a model to investigate phenomena related to human reproduction (1). In rabbits, the method of freezing rabbit semen is frequently used in experimental studies. The motility of rabbit sperm decreases after thawing (2). This is a significant disadvantage for the use of frozen semen (3) Cryopreservation of rabbit semen causes oxidative stress and shortens the life span of spermatozoa (4). Cryopreservation has a negative effect on spermatological parameters. In this study, the protective effectiveness of the additive was evaluated using spermatological parameters and biochemical (oxidative stress) methods.

Methods

In the present study, the effects of caffeic acid on rabbit semen were investigated by using the antioxidant substance trehalose as a positive control. In the experiment, 4 male rabbit were used. Ejaculates were

collected by artificial vagina method 2 times a week for 4 weeks. After pooling, semen was divided into four equal groups and diluted with diluents containing basic diluent + 6% DMSO + caffeic acid (25μM), basic diluent + 6% DMSO + caffeic acid (50μM), basic diluent + 6% DMSO + trehalose (positive control) (50mM) and basic diluent + 6% DMSO (Control). After dilution, semen samples were aspirated into straws (0.25 ml) and and allowed to equilibrate at +4°C for 1 hour. The samples were frozen in liquid nitrogen vapour (-110°C to -120°C) for 15 minutes and the straws were stored in liquid nitrogen (-196°C). Samples were thawed at 38 °C for 30 seconds and examined. Phase-contrast microscopy and fluorescence microscopy were used to evaluate spermatologic parameters. Total oxidant status (TOS) and total antioxidant levels (TAS) were measured using ELISA method. The results were expressed as mean±standard deviation and One-Way Analysis of Variance (ANOVA) was used for evaluation. Duncan's post hoc test followed. Differences at $P<0.05$ were considered statistically significant. Analyses were performed using SPSS 21 package program.

Results

The highest value of sperm motility values after thawed semen was reached in trehalose 50 mM group (46.25±1.25%) and there was a statistical difference with the control group (35.63±1.99%) ($p < 0.05$). Sperm membrane integrity (SYBR 14/PI) results were determined as 47.11±0.96%, 49.05±0.85%, 49.79±1.04% in caffeic acid 25 μM, caffeic acid 50 μM and trehalose 50 mM groups, respectively, and a statistical difference was found between the supplement groups and the control group (%39.01±1.21) ($p < 0.05$). In the results of acrosome integrity (FITC-PNA), the highest value was reached in trehalose 50 mM group (48.70±1.03%) and a statistical difference was observed with the control group (41.48±0.80%). Total antioxidant capacity (TAS mmol/L) and total oxidant level (TOS μmol/L) values were analyzed after thawing. Trehalose 50 mM group (1.86±0.52; 13.75±2.59) showed a statistical difference with the control group (0.55±0.11; 32.34±3.59) in both parameters ($p < 0.05$).

Discussion and Conclusions

Researchers have determined that trehalose has a positive effect on semen oxidative stress parameters (5,6). It was also determined that trehalose had a positive effect on the T-AOC activity of semen after thawing (7). The effect of trehalose on rabbit semen motility was similar to our study. (8). Examining the effects of caffeic acid on frozen-thawed boar spermatozoa, it also revealed beneficial effects on total and progressive sperm motility at a concentration of 100 µM (9). In conclusions, it was determined that caffeic acid added to the extender when freezing rabbit semen had a better protective effect at higher doses. It is thought that the trehalose group used as a positive control in the study showed higher protection than caffeic acid, and it may be useful to test caffeic acid in higher doses in future studies.

Keywords: caffeic acid, cryopreservation, fluorescent staining, oxidative stress, rabbit sperm,

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Prevalence of neospora caninum in bovine foetuses: Comparison of three diagnostic methods

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Citation
Hayes, C., Casey-Bryars, M., Innes, E., Markey, B., McAloon, C., Mee, J., Sanchez, C. Prevalence of neospora caninum in bovine foetuses: Comparison of three diagnostic methods.

Introduction and aim

Neospora caninum is widely accepted to be one of the major causes of bovine abortion worldwide. Both diagnosis and control of the parasite can be challenging. Knowledge of its prevalence and the performance of the diagnostic techniques available to detect it are vital. The primary objective of this study is to determine the animal-level prevalence of *Neospora caninum* in a cohort of bovine foetuses submitted to a veterinary diagnostic laboratory.

Methods

The foetuses enrolled in the study were voluntarily submitted to the Irish government Regional Veterinary Laboratory in Cork between September 2022 and February 2023. Thoracic fluid or blood, a fresh swab of the midbrain and fixed brain tissue were collected. Histopathological diagnoses were made by the individual research officer assigned to the case. *N. caninum* foetal fluid/serum antibody ELISA testing was carried out using the IDEXX *Neospora caninum* Antibody Test Kit. A PCR assay for *N. caninum* was carried out on the brain swabs using LSI VetMAX *Neospora caninum* Detection Kit (ThermoFisher)

Results

The number of foetuses enrolled in the study was 363. PCR results were available for all foetuses. Seventeen (4.7%) were *N. Caninum*-PCR-positive and three were inconclusive. Antibody ELISA results on blood or thoracic fluid were available for 326 foetuses. Four (1.2%) were positive and one was inconclusive. Histopathology results were available for 90 foetuses. Of these, five (5.6%) had lesions consistent with *N. caninum* infection and one was inconclusive. Twenty-one foetuses (5.8%) were categorised as positive by at least one of the three tests. Of the 17 PCR-positive foetuses, 14 had available antibody ELISA results and four had available histopathology results. Of these, one was antibody-positive and three were histopathology-positive. One of the four antibody-positive foetuses was PCR-positive. Histopathology results were available for two antibody-positive foetuses, only one of which was considered positive. Three of the five histopathology-postive foetuses were PCR-positive and one was antibody-positive. No foetus was positive in all three diagnostic tests.

Discussion and Conclusions

Agreement between the different diagnostic options for N. caninum was poor in this sample set, consistent with previous studies. The 5.8% overall positivity rate was lower than an apparent prevalence of between 9 and 23% reported internationally in the existing literature over the last two decades.

Keywords: *Neospora caninum*, bovine foetal death, bovine abortion, PCR, ELISA, histopathology

Settlement frequency stress on testis and the protective effect of oleuropein in Japanese quails

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Citation
Güngör, İ.H., Çelik, E., Bahşi, M., Karakoç, Ş., Türk, G., Çiftçi, M., Şimşek, Ü.G., Sönmez, M. Settlement frequency stress on testis and the protective effect of oleuropein in Japanese quails.

Introduction and aim

The coexistence of poultry is considered as the frequency of settlement, and this is highly influential on animal welfare and sexual performance (1-3). Stress negatively affects hormonal mechanisms, and sperm quality,

production and motility in poultry (4). Oleuropein is the most important natural phenolic compound found in olive leaves and fruit and belongs to the secoiridoid class in chemical structure (5-7). Stress factor occurs in Japanese quails due to the settlement frequency. In this study, it was aimed to determine the rate at which stress affects testicular and serum fatty acids and by this stress on testicular exocrine function is improved by adding oleuropein to the ration at different rates.

Methods

The 90 15-day-old Japanese quails were divided into two groups. In the first group, 36 quails were exposed to normal conditions (12 quails/cage) and in the second group, 54 quails were exposed to settlement frequency (18 quails/cage) to create stress factor. Then, these groups were divided into three subgroups among themselves, and experimental groups were formed by adding 0, 200 and 400 ppm oleuropein to the diets. Basal diet was applied to the control groups. The application continued for 43 days. At the end of the study, six quails (36 in total) in each group were slaughtered by cervical dislocation method. Blood samples were collected from the vena jugularis after slaughter. Testicular weight, gonado-somatic-index (GSI), the count of testicular spermatids and spermatozooa were also determined. Fatty acids in the blood serum fatty acid rates were detected.

Results

Settlement frequency stress caused a significant ($p<0.05$) decrease in spermatid and sperm count compared to the control group. Both doses of oleuropein increased stress induced decreases in spermatid and sperm counts when compared to the stress group. On the other hand, the spermatid count of the 400 ppm group, which was not subjected to any stress, was significantly higher than the control group ($p<0.05$). Settlement frequency stress did not affect palmitic, stearic, oleic, linoleic, arachidonic and docosahexaenoic acid levels compared to the control. Conversely, the addition of 200 ppm oleuropein to the diets of quails in the stress group resulted in a decrease in the levels of stearic acid from saturated fatty acids, as well as arachidonic and docosahexaenoic acids from PUFA, and an increase in the levels of oleic acid from monounsaturated fatty acids, when

compared to the stress only group. Similarly, the level of palmitic acid, one of the saturated fatty acids, in the blood serum of quails in the stress + 400 ppm group was found to be significantly decreased compared to the stress only group. Conversely, administration of 200 and 400 ppm oleuropein in the diet to quails in cages with normal settlement frequency caused a decrease in the level of palmitic acid, an important saturated fatty acid.

Discussion and conclusions

The results of this study clearly show that the increase in the frequency of settlement causes stress to the reproductive system by producing a decrease in testicular exocrine activity in Japanese quails. It was also revealed that the addition of oleuropein to the diet had a significant effect on the recovery of this activity of the testis. Oleuropein decreased the level of saturated (palmitic and stearic acid) and unsaturated (arachidonic and docosahexaenoic acid) fatty acids and increased the level of monounsaturated fatty acid (oleic acid) in the blood serum of quails subjected to settlement frequency stress, and these changes were found to be related to spermatids and sperm production in the testis. As a result, it is suggested that the addition of 200 - 400 ppm oleuropein to the diets of male Japanese quails exposed to settlement frequency is beneficial due to its positive effect on the exocrine function of the testis.

Keywords: Settlement frequency, stress, quail, testis, fatty acids.

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Analyzing motility and kinetic parameters using canonical correlation analysis

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Citation
Gül, E.B., Özen, D., Tırpan, M.B., Daşkın, A. Analyzing motility and kinetic parameters using canonical correlation analysis.

Introduction and aim

The use of frozen semen from bulls with high-yield characteristics is common in cattle breeding enterprises to increase animal productivity (1). It is crucial to evaluate the post-thaw quality of frozen semen through analyses that provide information on the motility, morphology, concentration and other characteristics of spermatozoa (1,2,3). This study aims to unravel the dynamic relationship between semen motility parameters and kinetic properties of Simmental breed bulls using a multivariate approach.

Methods

This study focused on quantitative research methods by conducting motility examinations, and analyses of kinetic parameters on 1556 frozen semen samples of the Simmental breed received at Ankara University Andrology Laboratory in 2023 using a CASA device (Computer-aided semen analysis). After thawing, a 5 µL semen sample was placed on a pre-warmed slide and covered with a coverslip (22 mm x 22 mm). The Sperm Class Analyzer v.4.2 software (Barcelona, Spain) and CASA system analyzed the samples by obtaining consecutive and digitalized photographic images in a 60-frame-per-second time-lapse. Motility parameters (Prog. motility, non-Prog. motility) and kinetic parameters (VCL, VSL, VAP, STR, LIN, WOB, BCF, ALH) were determined with a 10× magnification objective at 37 °C(4). For canonical correlation analysis, motility parameters were determined as the first variable set and kinetic parameters were determined as the second variable set. For each set, synthetic canonical covariates u and v were created, respectively. Canonical correlation analysis was used to determine orthogonal linear combinations of the variables within each set that best explain the variability both within and between sets.

Results

Two statistically significant canonical dimensions were initially generated, the first of which represented 85.53% of the variance shared by the two synthetic variables. Non-progressive motility significantly impacted the first set of motility parameters (u). Subsequently, the three variables with the highest influence on the second set of kinetic parameters were ALH, VAP, and BCF, respectively (v). Results showed that the most prominent contributors for kinetic parameters to predict motility (u) were LIN and ALH followed by VAP and BCF. The model showed that one unit increase in ALH resulted in a 1.64 unit increase in the covariate regarding the motility parameters (u) ($p<0.001$).

Discussion and conclusion: The results of this study have helped us understand the relationship between motility parameters and kinetic parameters, shedding light on two important canonical variables. It has been observed that non-progressive motility significantly affects the first set of motility parameters (u), which is consistent with previous studies suggesting

that non-progressive motility could be an important factor in the assessment of sperm quality(3,5).

The second set of kinetic parameters (v) appears to be predominantly influenced by ALH, VAP, and BCF. These parameters are used in sperm examination and are closely related to motility. Additionally, VAP and ALH parameters has been associated with fertilization capability in many animal species, including bulls(3,6,7).

In parallel, the research has revealed that an increase of one unit in ALH could lead to an increase of 1.64 units in the common variable related to motility parameters (u) ($p<0.001$). This also confirms the strong relationship between ALH and motility. Shojaei et al. (2012) compared the spermatological parameters of high fertility(HF) and low fertility(LF) bulls and reported that the motility, progressive motility and ALH values of sperm obtained from HF bulls post-thawing were higher, also these findings support this research(7).

Most studies on semen quality usually examine variables that may have multiple causes and multiple effects. Determining outcomes based on research that separately examines singular causes and effects may distort the complex reality of animal productivity. In conclusion, this research is expected to contribute to the practitioners and the literature in understanding the relationship between motility parameters and kinetic parameters of semen in order to increase the reproductive productivity of Simmental bulls.

Keywords: Bull semen, CASA, kinetic parameters, motility parameters, canonical correlation analysis.

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Prostatic neoplasia inducing ischemic priapism in a dog

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Citation
Spada, S., Castiello, E., Felice, D.D., Aires, L.N., Russo , M., Greco, A. Prostatic neoplasia inducing ischemic priapism in a dog.

Introduction and aim

Prostatic neoplasia, though relatively uncommon, can affect both intact and neutered dogs, with a higher risk observed in neutered individuals (1). Diagnosis of this condition poses challenges due to the absence of early specific blood and imaging markers associated with neoplastic changes (2). Symptoms typically manifest as an enlargement of the prostate gland, leading to compression of surrounding structures, inflammation, and potential secondary infections. This can result in severe symptoms such as rectal tenesmus, constipation, diarrhoea, dyschezia, haematuria, dysuria, and

stranguria (3). In this case report, a rare occurrence of prostatic neoplasia resulted in ischemic priapism due to venous outflow compression.

A 10-year-old intact male Weimaraner presented with lethargy, inappetence, mild hindlegs lameness, tenesmus, dyschezia, dysuria, and priapism. Despite previous treatment with Deslorelin acetate for prostatic cysts and benign prostatic hyperplasia six months earlier, clinical examination revealed severe pain upon rectal and penile palpation. On physical examination, the ensheathed penis was red and swollen, with caseous necrotic material on the tip of the urethral orifice. Urine and blood analyses were unremarkable except for a slight serum monocytosis.

On abdominal ultrasound the prostate was increased in size, with irregular margins and mild inhomogeneous echotexture scattered by hyperechoic foci with distal acoustic enhancement and the presence of an anechoic area (1.61 x 1.32 cm) non vascularized on colour doppler. Cranial to the prostate, irregular mild thickening of the urinary bladder trigone was detected. The medial iliac lymph nodes appeared increased in size and diffusely hypoechoic. The corpora cavernosa were congested and ischemic with low vascular signal detected by Colour Doppler and Contrast-enhanced ultrasound. No evidence of testicular diseases was detected.

A CT contrast total body scan was performed in order to better define the abnormalities detected on ultrasound and to rule out neoplastic and metastatic diseases. The urinary bladder was over-distended, associated with moderate left and mild right ureteral dilation. The urethra appeared characterized by severe dilatation of the trigone and the penile portion and by a stricture just caudally to the prostate. The prostate resulted to be caudally dislocated in the pelvic area, increased in size, irregularly shaped, with smooth margins, inhomogeneous contrast enhancement, mineral foci and showing severe adhesion to the colon. The caudal margins of the prostate gland were not well-defined and exhibited a severe infiltrative appearance, spreading to the membranous portion of the urethra and the corpus spongiosum. The bulbus glandis was swollen with ring enhancement. Furthermore, the os penis showed an irregular periosteal bone proliferation.

Additionally, ductus deferens invasion was detected, with bilateral thickening of a linear soft tissue structure arising from the craniodorsal surface of the prostate, running caudolaterally within the peritoneum towards the inguinal canal. Internal pudendal veins appeared enlarged, congested and mildly enhanced. Lungs and pleura appeared normal, with no sign of atelectasis, effusion or focal lesions. No signs of metastasis were detected in the liver, spleen, lymph nodes or vertebral bones. CT scan findings were suggestive of a neoplastic condition of prostatic origin, inducing a severe urethral dilation and obstruction of penile venous outflow, inducing then priapism. An ultrasound guided fine needle aspiration of the prostate gland was performed, confirming the diagnosis of prostatic adenocarcinoma. Due to the poor prognosis and bad general conditions, euthanasia was performed in accordance with the owner.

Discussion and Conclusions

Ischemic priapism, caused by penile outflow venous congestion and enhanced blood viscosity, may be often associated with neoplasia, heparin therapy, vasoactive drugs, and neurological conditions such as spinal cord injury and anaesthesia (4). This study underscores the rare but serious secondary effects of advanced prostatic neoplasia in dogs and emphasizes the challenges of ultrasound assessment in cases where visualization of the prostate is hindered. Early detection of prostatic malignancies is crucial, and CT imaging may serve as a valuable tool in investigating such conditions (5).

Keywords: Canine, priapism, prostatic neoplasia, ultrasound, computed tomography, reproductive imaging.

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The effects of proanthocyanidin on testicular toxicity in rats exposed to a glyphosate-based herbicide

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Avdatek, F., Kırıkkulak, M., Yeni, D. The effects of proanthocyanidin on testicular toxicity in rats exposed to a glyphosate-based herbicide.

Introduction and aim

Glyphosate (GLP)-based herbicides are extensively used in agriculture and non-agricultural areas. They disrupt the endocrine system, altering spermatozoa properties and causing oxidative stress by depleting antioxidants like glutathione (1,2). In rats, GLP reduces sperm motility, count and increases abnormal spermatozoa, leading to sterility (3). Proanthocyanidins PA from grape seed extracts are more effective in scavenging free radicals and preventing oxidative tissue damage than several vitamins (4,5). They exhibit multiple biological activities, including anti-inflammatory effects, and protect against ROS-induced male infertility by safeguarding spermatozoa DNA and membrane integrity (6). This study aims to evaluate the effects of PA on spermatozoa health, oxidative stress, DNA changes, testosterone levels, and testicular histology in male rats exposed to GLP.

Methods

This study was conducted with the approval of the Animal Experiments Local Ethics Committee of Afyon Kocatepe University dated 26.01.2021 and numbered 49533702/06. The animal material included 24 male 2.5-3-month-old Wistar Albino rats weighing 160-180 g. Control group (n=6) was given only standard rat food and drinking water. The PA group (n=6) was given PA at a dose of 400 mg/kg/day, and the GLP+PA group (n=6) was first given GLP at a dose of 787.85 mg/kg/day LD50/10, then PA was given at a dose of 400 mg/kg/day. The GLP group (n=6) was given GLP at a dose of 787.85 mg/kg/day LD50/10 via gastric gavage. The experimental part of the study lasted for 56 days. On the last day of the anesthetized with 10 mg/kg Xylazine HCl plus 50 mg/kg Ketamine HCl intramuscular. Sperm motility, hypo-osmotic swelling test (HOST) and spermatozoal DNA damage were determined according to the method described (1). Abnormal spermatozoon ratios in the sperm samples were analyzed using Giemsa staining (7). Malondialdehyde (MDA) concentrations in the testicular tissue samples (8), reduced glutathione (GSH) concentrations in the samples were evaluated (9). Blood testosterone samples analyses were evaluated using ELISA kit.

Results

Decreases in the GLP group and the increases in the PA and GLP+PA groups were statistically significant ($p<0.001$). The administration of GLP increased DNA damage compared to the control group, but the GLP+PA and PA applications reduced DNA damage ($p<0.001$). The analysis of testosterone levels indicated a statistically significant reduction in the GLP group compared to the other groups.

Discussion and conclusions

GLP induced increased spermiotoxicity and decreased motility were reported previously (10) similar to our study. PA has been shown to improve sperm count and motility following exposure to Zearalenone (ZEN) and prevent an increase in abnormal sperm ratios (11). Additionally, it has demonstrated protective effects against cadmium-induced testicular toxicity by enhancing sperm parameters and reducing oxidative stress (12). PA decreased lipid

peroxidation and oxidative stress in cisplatin-treated rats (13); and similarly, PA improved oxidative stress parameters in varicocele-induced rats (14).

In the current study, PA was found to effectively prevent the decline in spermatological parameters caused by GLP exposure and mitigate oxidative stress and toxicity in testicular tissue.

Keywords: Glyphosate, proanthocyanidin, rat, spermatozoa, testicular toxicity.

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Impact of intraepididymal platelet-rich-plasma injection on sterol and vitamin levels in ram semen

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Introduction and aim

Platelet-rich-plasma (PRP) is a plasma component obtained by centrifugation of whole blood, and contains a higher concentration of platelets than whole blood. PRP contains numerous growth factors and their isomers, including PDGF, VEGF, FGF, TGFβ and IGF (1). PRP is also rich in bioactive lipids (2). The epididymis is a vital organ of the male reproductive system where spermatozoa undergo significant biochemical changes, particularly in their membranes. These changes result in the maturation of spermatozoa, which gain motility and fertilization ability. The ability of spermatozoa to gain fertilization ability during epididymal transport is likely due to changes in membrane cholesterol. It has been proposed that cholesterol secreted from the epididymal epithelium contributes to the maturation of spermatozoa during epididymal transport (5). Furthermore, vitamins may interact with calcium homeostasis in the epididymis for sperm maturation and fertilization (6). Additionally, IGF1 has been reported to be positively correlated with vitamin D (7). The rationale for this study stems from several factors. Firstly, PRP is rich in bioactive lipids (2). Secondly, there is a relationship between growth factors and cholesterol (8) and vitamins (7). Thirdly, there is a role for cholesterol (4) and vitamins (6) in the maturation of spermatozoa. Fourthly, there is a paucity of data regarding the impact of intraepididymal PRP injection on semen cholesterol and vitamin levels, which play a pivotal role in the maturation of spermatozoa in adult rams. Consequently, the objective of this study was to investigate the impact of intraepididymal PRP administration on sterol and vitamin levels in ram semen.

Methods

In this study, 12 one-year-old Akkaraman rams were utilized. The animals were divided into two groups, with six animals in each group. Subsequently, the animals in the experimental group were administered 0.2 ml of

autologous PRP (approximately 150-200 million platelets) six times at 15-day intervals. The animals in the control group were injected with 0.2 ml of 0.9% NaCl (placebo) to induce stress associated with the injection procedure. The levels of cholesterol and other sterols (ergosterol, β-sitosterol, and Δ-sterol) and vitamins (α-tocopherol, Δ-tocopherol, retinol, retinol acetate, vitamins D_2, D_3, K_1, and K_2) in the semen obtained from each ram on six occasions at 15-day intervals were determined by HPLC method.

Results

The intraepididymal PRP injection had no significant effect on vitamins and sterols, with the exception of retinol acetate. The retinol acetate level in the PRP group was found to be significantly higher than in the control group ($P < 0.05$).

Discussion and conclusions

The results of this study demonstrate that intraepididymal injection of PRP increases the retinol acetate level in ram semen. Given the low serum retinol level in infertile males (9) and the involvement of vitamin A in the maturation process in the epididymis (10), it was concluded that intraepididymal administration of PRP to rams has made a significant contribution to the epididymal maturation process of spermatozoa by increasing the level of retinol acetate.

Keywords: Epididymis, platelet-rich-plasma, semen, sterol, ram, vitamin.

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Downregulation in miR-34b is associated with reduced progressive motility in arabian stallions

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Introduction and aim

Seminal plasma secreted by the testes, epididymis, and accessory sexual glands provides a nourishing and protective environment for sperm. The heterogeneous molecular composition of seminal plasma, including proteins, free nucleic acids, and inorganic chemical ions, is directly related to the regulation of sperm functionality (1). Sperm progressive motility is a

reliable function as a determinant in assessing sperm quality and predicting fertilization success (2). MicroRNAs (miRNAs), play a significant role in regulating gene expression (3). Because of their high conservation among species and resistance to environmental conditions, miRNAs have significant potentials as biomarker candidates (4). Studies on humans and farm animals indicate that miR-34b expression in seminal plasma plays active role in regulating sperm motility (5-6). The selection of breeding stallions involves various factors such as genetics, performance, and conformation highlighting the importance of developing non-invasive biomarkers for identifying fertile individuals (7). This study aimed to investigate the potential use of miR-34b in seminal plasma as a biomarker to determine the progressive motility level of semen in Arabian stallions.

Methods

Semen samples were collected from 14 adult Arabian stallions via artificial vagina. First, progressive motility of the semen samples was analyzed by a computer assisted semen analyzer (CASA, SCA, Microptic, Spain), objectively. Then, low progressive motility (LPM; <40%, n=7) groups and high progressive motility (HPM; >40%, n=7) groups were determined. Semen samples were centrifuged with 600 xg for 10 minutes to separate seminal plasma. RNA isolation from seminal plasma was performed according to the modified TRIzol method (8). Subsequently, Poly(A) tails were added to the obtained RNAs, followed by cDNA conversion (3). The miR-34b expression levels in the seminal plasma of the LPM and HPM groups were determined using qPCR. U6 was used as a housekeeping gene for calculating miR-34b expression levels, and fold changes were determined using the $2^{-\Delta\Delta Ct}$ method (9).

Results

The average progressive motility value in LPM group was 16.13%, while that in HPM group was 44.75%. Compared to HPM group, miR-34b expression downregulated approximately 10-folds (0,09$\pm$0,08) in the LPM group ($P<0.05$).

Discussion and conclusions

Studies have reported that the deficiency of miR-34b in transgenic animals reduces progressive sperm motility, leading to infertility (10). Research on humans and bulls has shown association between miR-34b expression and sperm motility (11-12). The results of this study have demonstrated a significant downregulation in seminal plasma miR-34b in Arabian stallions with low progressive motility. These findings represent the first study to examine the relationship between miRNAs in seminal plasma and sperm progressive motility in stallions. Based on findings, it is believed that miR-34b in seminal plasma might serve as a biomarker for sperm progressive motility in stallions. However, because of the multifaceted roles of miR-34b and the limited number of studies in stallions, further research is needed to expand and evaluate these findings in a broader context.

Keywords: Arabian stallion, fertility, miR-34b, progressive motility, seminal plasma.

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Expressions of selected inflammatory genes in ovary tissue of superovulated rats supplemented with melatonin

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Yakan, A., Dalkıran, S., Özkan, H. Expressions of selected inflammatory genes in ovary tissue of superovulated rats supplemented with melatonin.

Introduction and aim

Superovulation is widely used for treating reproductive defects and in reproductive research. This practice involves the exogenous administration of gonadotropins to stimulate the growth and maturation of ovarian follicles (1). Although superovulation is used for various purposes in reproductive procedures, it might have adverse effects on oocyte, ovarian, and embryo health (2,3). Some of these adverse effects are associated with oxidative damage to the ovary. Therefore, the use of antioxidants to reduce oxidative stress is becoming increasingly common (4). While melatonin is known as a potent antioxidant, its specific impact on reducing damage in the ovary during superovulation is not yet fully understood at the molecular levels. This study aimed to determine the changes at the selected oxidative stress and inflammation-related genes in ovary tissue of superovulated rats supplemented with Melatonin. In the study, Prostaglandin-Endoperoxide

Synthase 2 *(PTGS2),* interleukin 1 beta *(IL1B),* and tumor necrosis factor-alpha *(TNF-α)* genes were selected.

Methods

Twenty-eight female Wistar albino rats aged 10-12 weeks were divided into four groups: Control (Con), Superovulation (So), Superovulation+5mg melatonin (SoM5), and Superovulation+20mg melatonin (SoM20). Starting one week before superovulation, melatonin was administered to the SoM5 and SoM20 groups via intraperitoneal injection every day at 17:00 until the end of the experiment. Superovulation in the So, SoM5, and SoM20 groups was induced with 40 µg Gonadotropin-Releasing Hormone (GnRH), 300 IU/kg Pregnant Mare Serum Gonadotropin (PMSG), and 300 IU/kg Human Chorionic Gonadotropin (hCG) (5). At the end of the experiment, the rats were euthanized, Total RNA isolated from ovarian tissue using the TRIzol method was reverse transcribed into cDNA using a kit, and the expression levels of the *PTGS2, IL1B,* and *TNF-α* genes were determined by qPCR. Gene expression results were calculated as fold changes using the $2^{-\Delta\Delta Ct}$ method and Actin Beta *(ACTB)* gene used as housekeeping (6, 7).

Results

PTGS2 showed a significant upregulation in the Superovulated group compared with the Control, with approximately 56-folds in So, 55-folds in SoM5, and 96-folds in SoM20 ($P<0.05$; $P<0.01$; $P<0.001$, respectively). On the other hand, *IL1B* and *TNF-α* genes showed similar expression patterns in groups.

Discussion and conclusions

The *PTGS2* gene plays major role in the regulation of prostaglandin synthesis, follicular luteinization, oocyte release, and inflammation pathways (8). Downregulated *PTGS2* gene expression is associated with the inhibition of oocyte release and ovulation in females (8,9). Superovulation upregulates *PTGS2* expression levels, thereby supporting follicular luteinization (9). The relatively greater upregulation of *PTGS2* expression in the SoM20 group suggests that melatonin supports follicle development and luteinization in a dose-dependent manner. The negative effects that may occur in the ovarian

inflammation pathway due to superovulation may be slightly limited by using 20 mg/kg melatonin.

Keywords: *IL1B,* melatonin, *PTGS2,* superovulation, *TNF-α.*

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The effect of the addition of ginkgo biloba to semen diluents on the freezing of ram semen

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Citation
Güngör, İ.H., Cinkara, S.D., Cihangiroğlu, A.Ç., Kaya, Ş.Ö., Kaya, E., Acısu, T.C., Erişir, F.E., Yılmaz, Ö., Gür, S., Sönmez, M., Türk, G. The effect of the addition of ginkgo biloba to semen diluents on the freezing of ram semen.

Introduction and aim

The long-term storage of semen by freezing allows for the transportation and control of the sperm (1). However, ram semen is not resistant to freezing due to its membrane structure. Therefore, Ginkgo Biloba (GB), which is known to possess high antioxidant properties, was selected for the purpose of suppressing oxidative stress during the freezing of ram semen and improving the quality of the freezing process. The objective of this study was to investigate the potential of high doses of GB to induce a pro-oxidant effect and, at the same time, to ascertain whether low doses of the same substance could exert antioxidant properties.

Methods

Semen was collected from the rams by artificial vagina and pooling was performed. Pooled semen was combined with diluents containing different ratios of GB and thus experimental groups [Group 1: %4 GB, Group 2: %2 GB, Group 3: %1 GB, Group 4: %0.5 GB, Group 5: %0.25 GB ve Group 6: Control (%0 GB)] were formed. The aforementioned concentrations of GB were determined subsequent to the completion of our study on the short-term storage of semen (2). The reconstituted semen was subjected to a glycerolisation-equilibration process and then frozen in an automatic freezing device. After thawing, spermatological and kinematic analyses by CASA, viability, acrosomal damage and mitochondrial membrane potential analyses by flow cytometry were performed. In addition, membrane integrity was determined by HOS test. In addition, oxidative stress analyses and vitamin, fatty acid and cholesterol levels were measured.

Results

The results of the analyses indicated that the groups containing 0.25% and 0.50% GB exhibited an increase in total, progressive, rapid motility, vitality and high mitochondrial membrane potential compared to the control group. Conversely, the experimental group containing 4% GB demonstrated a detrimental effect on spermatozoa in the analyses. The oxidative stress analyses revealed that all experimental groups, with the exception of the 4% GB group, exhibited increased GSH levels in comparison to the control. Additionally, the 1% GB group demonstrated elevated GSH-Px levels, while

the 0.25% and 1% GB groups exhibited increased CAT levels and the 4% GB group exhibited elevated MDA levels in comparison to the control. In comparison to the control group, the 2% GB group exhibited a significant decrease in MDA levels and the 4% GB group exhibited a significant decrease in SOD levels. There was no significant difference in the levels of vitamins, fatty acids and cholesterol between the experimental and control groups.

Discussion and conclusions

The detrimental impact of GB extract on spermatozoon metabolism can be attributed to the pro-oxidant properties of quercetin, a constituent of GB, which induces oxidative stress in spermatozoa (3). The positive spermatological effects are believed to be attributable to the fundamental antioxidant characteristics of GB, including the reduction of free radical generation (6), the scavenging impact on superoxide and hydroxyl radicals, and the inhibition of reactive oxygen species production (4, 5, 7). Furthermore, it has been demonstrated that GB reduces the effects of apoptosis-related genes and free radical production in cells, increases antioxidant enzyme activity in tissues and cells, and acts as a protective agent against exogenous toxic stimuli (6). It was thus established that the incorporation of 0.25% to 0.50% GB extract into semen diluents in rams serves to safeguard spermatozoa against the deleterious effects of freezing and thawing, while also enhancing the quality of the freezing process due to its antioxidant properties. Consequently, it is advised that GB extract be included in ram semen reconstituents at the aforementioned rates.

Keywords: Ram, freeze storage, ginkgo biloba, oxidative stress, flow cytometer.

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Estimation of gestational age in bovine aborted and stillborn fetuses from dairy cows using morphometry

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Citation
Jawor, P., Mee, J.F. Estimation of gestational age in bovine aborted and stillborn fetuses from dairy cows using morphometry.

Introduction and aim

In addition to determining the timing and causes/s of mortality in cases of bovine abortion and stillbirth it can be important to determine the gestational age of the foetus/es. In the absence of accurate artificial insemination (AI) or natural service dating it can be difficult to estimate bovine fetal gestational age. The majority (95%) of bovine foetuses submitted for laboratory investigation are from the >120 days (1). Aspects of dentition and pilosity can assist during this period but are not sufficiently correlated with gestational age to be useful on their own. While simple formulae exist to predict gestational age, they generally only use a single metric, crown rump length (CRL), and lack data on R^2 and error statistics. Hence, the objective of this study was to use multiple foetal morphometrics to predict gestation length (GL) in aborted and stillborn fetuses from dairy cows.

Methods

Measurements were collected at necropsy from 1,295 single abortion/perinatal mortalities from Irish dairy cows which had recorded case histories including service date. The following foetal morphometrics were measured at necropsy: DD - digital diameter of the left fore fetlock using a metal calipers, CRL (crown rump length) was measured in three ways (the first two with a measuring tape and the third with an osteometric calipers):- Pollc - curved from the base of the tail to the crown, CRL eye - curved from the base of the tail to the eye, Str CRL - straight from the base of the tail to the crown, girth – measured behind the shoulder with a weighband, and body weight – recorded on a calibrated electronic weighing scales. Calf breed category was recorded in each case as: Je = Jersey or Jex sire or dam (n=394); Non-Je = other dairy breeds or crosses (n=450); Beef = beef sire on non-Je (n=142); un = unclear (n=10). Data from Je and Non-Je calves were used. The final dataset (844 records) was divided into train (80%) and test (20%) sets. Data analysis was performed using Python 3.9.7 and linear regression was performed with statsmodels package.

Results

Recorded gestation length (GL) varied between 128 and 316 d. Morphometric variables were highly autocorrelated (0.86-0.98), hence models with one morphometric variable and breed category were built. While breed category was statistically significant, it had little impact on the output values. sCRL (0.63), girth (0.61) and DD (0.61) and had the highest R^2 in train and test datasets combined with lowest mean absolute error (MAE), (mean difference in predicted days from GL) (10.3-10.8) and lowest percentage of mismatch cases (more than +/-24 days from GL), (9.5-10). Body weight had the poorest associations with GL (R^2 0.49, MAE 11.8, mismatch 13.0).

Discussion and Conclusions

Though all morphometrics were simple to measure in practice, all foetal morphometrics were, not unexpectantly, correlated, hence individual measurement models were constructed. Of these straight CRL had the highest and body weight models had the lowest predictive ability. Bovine gestation length, from the fourth month of pregnancy onwards, could be

predicted with different foetal morphometrics within approximately +10 days in dairy calves. This error term (10 d) is for the model, not individual calves, hence, in some cases, the difference for predicted gestation will be higher and in other cases lower. This variation could be related to errors in records of insemination, errors in measurements or differences in the rate of foetal development over the gestational horizon which included term and pre-term foetuses.

Keywords: Bovine foetus, dairy cows, gestation length, morphometry, crown rump length.

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Immunohistochemical presence of some aquaporins in stallion testicles

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Citation
Altun, K.Ç., Demirtaş, O.B., Uslu, E., Yazlık, M.O., Olğaç, K.T., Vural, S.A. Immunohistochemical presence of some aquaporins in stallion testicles.

Introduction and aim

Recent studies show that spermatozoa have high water permeability compared to other mammalian cell types (1). As spermatozoa leave the testicles and travel towards the female uterus and to the ova, changes occur in the osmolality of the fluids around them. Regulation of sperm volume in response to such osmotic changes is important for maintaining constant cell size for normal shape and function of the sperm tail. Since the water molecule is polar and the diffusion of polar compounds is prevented by lipid bilayers, non-selective water permeability through plasma membranes is very low. Aquaporins (AQPs) are water-selective channels that allow 10- to 100-fold higher capacity for transport of water across plasma membranes. It

is expressed in almost every organism and tissue. Aquaoporins are involved in the bidirectional transfer of solutes and water across cell membranes in response to osmotic and hydrostatic pressure or concentration gradients (2,3). Among mammalian cells, 13 members of the AQP family have been identified so far, each predominantly localized in different tissues, and most individual cell types have more than one AQP family member (1).

AQPs, according to their structure and permeability;

1) Orthodox aquaporins considered to be primary selective towards water: AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8

2) Aquaglyceroporins, which transport glycerol and other small neutral solutes in addition to water: AQP3, AQP7, AQP9 and AQP10

3) Subcellular or unconventional aquaporins with different evolutionary paths localized in intracellular membranes: AQP11 and AQP12; it is divided into three.

AQP1, AQP3, AQP7, AQP9 and AQP11 were expressed in sperm and testicles. But there is not enough information on this subject for stallions. The aim of this study is to contribute to the literature by investigating the presence of AQPs, which have just begun to be used, in testicular tissue by examining Aquaporin 1, Aquaporin 3, Aquaporin 7, Aquaporin 9, Aquaporin 11 in stallion testicles with immunohistochemical methods and to shed light on future studies.

Method

In the study, 5 Gemlik breed stallions, aged 3 -year-old, from a government institution were used. The testicles obtained from stallions after routine castration were stored at +4 °C and delivered to Ankara University, Faculty of Veterinary Medicine, Department of Pathology without delay. The samples were fixed in 10% buffered formalin solution and histochemical and immunohistochemical stainings were performed using Aquaporin 1, Aquaporin 3, Aquaporin 7, Aquaporin 9 and Aquaporin 11 markers.

Results

Macroscopically, the cross-sectional surfaces of the testicles were occasionally hemorrhagic and necrotic. Histopathologically, severe hyperemia and widespread necrosis were observed in all testicles. Aquaporin 1, Aquaporin 3, Aquaporin 7, Aquaporin 9 and Aquaporin 11 immunohistochemical staining revealed immunopositivity of varying severity, especially in the interlobar areas and/or germ cells (especially AQP 9 and AQP 11). However, no positivity was observed in the spermatozoa found in the ducts.

Discussion and Conclusions

Aquaporins are among the markers that have an important role in energy metabolism and water transport and whose effectiveness in fertilization during the vital period is being investigated. They are water-selective channels that allow high capacity for water transport across plasma membranes. It has long been known that spermatozoa have high water permeability compared to other cell types. They rapidly contract or swell when exposed to extreme osmolality ranges. By taking advantage of this swollen state, it is used in human medicine for fertility with the Western blot technique (4). It is an undeniable fact that in veterinary medicine, the research will make a significant contribution since there are many aspects open to evaluation in this context. Although studies on fertilization in the intraavital period have been conducted, there are few studies on the localization of these markers in testicular tissue (5). For this reason, it was aimed to show the distribution of AQPs in castrated testes in detail. Significant immunopositivity was observed, which cannot be ignored, due to the pores not closing completely due to rapid fixation of the tissues after castration.

Keywords: testicle, aquaporin, immunohistochemistry, stallion, castration.

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First embryo transfer in mares in Türkiye

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Citation
Gündüz , M.C., Kurban, İ. First embryo transfer in mares in Türkiye.

Introduction and aim

The first successful equine embryo transfer was reported in 1972 (1). Embryo recovery and transfer can be used to obtain multiple foals per mare per year, and to have mares that are unable to carry a pregnancy to term to produce a foal (2). The standard method of embryo collection in the mare is a non-surgical transcervical uterine lavage (3). A sterile catheter is inserted through the cervix, and uterine lavage is performed by using embryo flush medium that is then allowed to flow back out the uterus and to pass through an embryo filter. Contents of the filter are poured into a petri dish and examined. There are no reported data on cases of equine embryo transfer in Türkiye. In the present study, we aimed to have mares produce foals by embryo transfer.

Methods

We included a Friesian mare and a paint mare as donors and various mares as recipients in the study. The donor and recipient mares were synchronized by single injection of Cloprostenol (250 µg) (Estrumate, MSD). When mare had dominant follicle (≥ 35 mm) and substantial uterine edema, 3000 IU hCG

(Chorulon, MSD) was administered to induce ovulation. Recipient mares were selected and injected with ovulation induction agent to maintain synchrony window between +1 and −3 days from donor mares' ovulation. The mares were examined daily by using ultrasonography to confirm ovulation.

Semen was collected from the same Friesian stallion with known fertility and E-Z Mixin BF (ARS, USA) was used as extender. Sperm were counted immediately after collection with Makler Counting Chamber (Sefi-Medical Instruments, USA). The insemination dose contained 500 million progressively motile sperm in 30 mL of extended semen.

Embryos were collected between 7-8 days post ovulation. 32 Gauge French Foley catheter was used for uterine lavage. The tip of the Foley catheter was inserted manually through the cervix into uterine body. The balloon of the catheter was inflated just cranial to the cervix and the uterus was completely filled by gravity flow with Lactated ringer's supplemented with 1% Fetal Calf Serum. Uterine lavage was performed 3 or 4 times. After the completion of the lavages, 30 IU oxytocin was administrated intravenously and the uterus was massaged transrectally.

The flushing medium was filtered through a filter with a pore size of 75 µm allowing somatic cells and certain debris to pass through while retaining the embryos. The filter content was transferred to the petri dish marked with squares and the bottom of the filter was washed several times with additional flushing medium. The petri dish was examined under a stereomicroscope and any identified embryos were washed with flushing solution and placed in the holding medium (EquiHold, Minitube) before being graded microscopically for quality and freedom from abnormalities. Embryos were transferred to 0.25 ml straw placed between an air bubble and a second fluid column for ease of identification.

The recipient mares were not sedated unless necessary but premedicated with flunixin meglumine (Flumegline, Türkiye). Finally, the embryo was transferred into the uterine body of the recipient mare by using the non-surgical transcervical transfer method. Four days after transfer,

ultrasonography examination was performed to diagnose pregnancy. Pregnant mares were maintained on oral progesterone (Regumate, USA) until 60 days after transfer.

Results

Six uterine lavages were performed on the two mares (four on the Friesian mare, two on the paint mare). Three embryos were obtained. Our embryo recovery rate was 50% (three embryos from six collection efforts). Morphological assessment revealed that two embryos were graded as excellent (Grade 1) while one embryo was graded as good (Grade 2). Two of these were at the blastocyst stage while the remaining one was at the morula stage. Three embryos were transferred to recipients. In the mare receiving the morula embryo, embryonic resorption started ten days after transfer. Two of the three transferred embryos maintained successful pregnancy and delivered a healthy live foal (66% birth).

Discussion and conclusions

Assisted reproduction techniques have been in use for many years in Equine Reproduction. As one of these techniques, embryo transfer offers not only the advantage of producing multiple foals from a donor mare per year but also the advantage of overcoming the inability of donor mares to carry foals due to reproductive and musculoskeletal problems, as well as producing foals from donor mares competing in sports (4).

While Embryo transfer is widely used throughout the world, there have been no reports of embryo transfer in mares in Türkiye. Thus, to the best of our knowledge, this study describes the birth of foals through non-surgical transcervical embryo transfer in Türkiye for the first time ever. We are of the opinion that the use of embryo transfer technology would be extremely beneficial for the development Turkish Equine Breeding Industry.

Keywords: Embryo transfer, assisted reproduction, friesian, mare, foal, equine.

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Comparison of post-thaw sperm motility and kinetic parameters of different bull breeds

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Citation
Göçer, M., Özen, D., Olğaç, K.T., Akçay, E., Daşkın, A. Comparison of post-thaw sperm motility and kinetic parameters of different bull breeds.

Introduction and aim

In cattle breeding, the selection of breeds is linked to their yield characteristics. In Türkiye, there is a marked preference for specialized breeds in the livestock industry, with meat breeds such as the Belgian Blue and Charolais, versatile breeds like the Simmental, and dairy breeds exemplified by the Holstein being particularly favored. The use of cryopreserved semen in artificial insemination is a widespread reproductive practice to maintain and enhance these productivity traits. For cryopreserved semen to be used in artificial insemination, it must meet certain quality standards. These quality standards are controlled by evaluating the parameters of the spermatological

examination after thawing. Spermatological examination reveals essential insights for achieving reproductive success. The study aims to determine the motility values and kinetic parameters of cryopreserved semen (Simmental, Holstein, Charolais and Belgian Blue) by performing post-thaw spermatological CASA analysis to evaluate the statistical differences between the breeds by comparative analysis of the obtained data.

Methods

In this study, motility values (progressive mot, non-prog. mot) and kinetic parameters (VCL, VSL, VAP, STR, LIN, WOB, BCF, ALH) of a total of 1987 frozen semen (40 Belgian Blue, 101 Charolais, 293 Holstein, 1556 Simmental) were evaluated by CASA device. The data obtained were systematically recorded and pooled. Descriptive statistics for each variable were calculated and presented as "Mean±Standard Deviation". Prior to the hypothesis testing procedure, data was examined with Shapiro- Wilk test for normality and Levene test for homogeneity of variances, as parametric test assumptions. Data were subjected to one-way ANOVA (analysis of variance) to compare sperm parameters among breeds. Gabriel test was used after any significant result as a post hoc procedure. A probability value of less than 0.05 was considered significant, unless otherwise noted. SPSS 21 was used for statistical analysis.

Results

When the results of the study were analyzed, Charolais (64,66±0,98%) and Simmental (68,07±0,28%) showed similar results in terms of motility data, but Belgian Blue showed the highest motility rate (73,56±1,89%) and Holstein showed the lowest rate (58,72±0,99%) ($p<0,001$). Belgian Blue (54,1±2,03%), Simmental (47,6±0,29%), Charolais (42,53±1,06%) and Holstein (33,2±1,17%) were statistically different from each other in progressive motility rates ($p<0,001$). In VCL parameter, Holstein (71,54μm/s±1,45), Charolais (70,82μm/s±1,41) and Belgian Blue (67,11μm/s±1,14) showed statistically similar values. Simmental (81,77μm/s±0,39) showed no statistical relationship ($p<0,001$). In VSL parameter, Charolais (37,61μm/s ±0,83) was similar to Simmental (37,54μm/s±0,22) and Holstein (34,32 μm/s ± 0,98). Belgian Blue (45,78μm/s±1,17) showed different results from the other breeds ($p<0,001$). In the VAP

parameter, Simmental (52,23μm/s±0,25), Belgian Blue (53,85μm/s±1,14) and Charolais (48,6μm/s±0,8) showed similar results. Charolais (48.6μm/s±0.8) was similar to Simmental and Holstein (46.58μm/s±1.03) ($p<0.001$).

Discussion and conclusion

In these days, CASA device is used to make more objective measurements with the developing technology. Motility examination can be performed practically and reliably with CASA (1). Motility examination allows us to examine the characteristics of spermatozoa that are critical for fertility success (2). Ntemka et al. evaluated the end of solution parameters of semen of five cattle breeds (Holstein, Brown Swiss, Limousin, Belgian Blue, Blonde d' Aquitaine). Accordingly, no statistical difference was found in the motility, progressive motility, VSL, VCL and VAP rates of Belgian Blue and Charolais (3). These results are in parallel with the results obtained in this study. In the study by Hoflack et al. comparing Holstein and Belgian Blue breeds, only VSL parameter was similar between Belgian Blue and Holstein. For this study, only motility and progressive motility rates were similar with Belgian Blue. However, there was no similarity with Holstein breed (4). Muino R. et al. showed similarity with this study in the VCL parameter in a study conducted with Holstein breed (5). Shojaei H. et al. showed parallel results with our motility data in their study with Holstein breed (6). This difference in the results may have been observed due to the CASA software used, the version of the software and different straw sizes. In addition, frozen semen in Belgian Blue breed should be used more carefully after thawing because it is more sensitive to stress.

Keywords: Bull semen, CASA, kinetic parameters, motility parameters, bull breeds, ANOVA.

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Investigation of gene variants providing resistance to maedi-visna infection in sheep raised in Northern Cyprus

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Citation
Betmezoğlu, M., Akan, G., Tuncel, G. Investigation of gene variants providing resistance to maedi-visna infection in sheep raised in Northern Cyprus.

Introduction and aim

Small ruminant lentiviruses (SRLV) are a widely prevalent group of viruses from the Retroviridae family(1). These viruses are responsible for causing chronic, degenerative, and multisystem diseases. This viral infection in sheep is a serious and slowly progressing disease, leading to widespread interstitial pneumonia and ultimately death. The disease, known as Visna, is characterized by nervous symptoms. Visna and maedi are two distinct forms of the disease caused by the same virus species in sheep. Both diseases lead to fatal outcomes (2). An important feature of visna and maedi is their extended incubation period, lasting from 3-6 months to 5-6 years, earning them the label of slow virus infections. These infections are lifelong, with no current effective treatments or vaccines available (3). The early signs of the disease usually manifest after two years of age and commonly entail

a decline in body condition and the development of endurative mastitis (hard udder) (4). Disease progression is associated with serious clinical symptoms such as breathing difficulties, chronic fatigue, loss of motor control, and arthritis. Among adult sheep, significant transmission occurs both horizontally through the respiratory tract and vertically from dams to offspring through the ingestion of infected colostrum (3,5). According to the results of the studies, TMEM154 NC_019474.2:g.4860407G>A genotype has been reported that animals with the KK genotype are resistant to infection compared to those with the EE and EK genotypes (6)

The primary goal of this study is to MV in various samples from sheep breeds used in livestock activities in Northern Cyprus and to develop a PCR detection kit to identify the TMEM154 genotype linked to MV susceptibility rapidly. This innovative kit will significantly reduce the time and cost of MV testing, effectively preventing economic losses in sheep flocks in regions where MV outbreaks are prevalent. Additionally, it will pave the way for herd improvement programs to be initiated.

Methods

78 blood samples were collected from superior sheep breeds at handpicked farms. Blood samples were then promptly transported to DESAM Research Laboratories, ensuring their integrity through a maintained cold chain.
The genomic DNA extraction process was carried out using the advanced Thermo Fisher Scientific PureLink™ Genomic DNA Mini Kit, ensuring high-quality DNA yield from the samples contained in EDTA tubes. To accurately identify the NC_019474.2:g.4860407G>A variant in the TMEM154 gene, we meticulously crafted two distinct forward primers, a shared reverse primer, and a probe. These were custom-designed to ensure that the final nucleotide of the forward primer aligned with the variation region. Leveraging the chromosome sequence with reference code NC_019474.2 from the NCBI database, we employed the SnapGene program to fashion primer and probe designs specific to the mutation site. Rigorous validation was conducted by cross-checking the designed primers and probes in the NCBI Primer BLAST database to guarantee precision and to control for non-specific binding.
The DNA samples isolated from animals were used to optimize the designed

primers for the K35 allele and E55 allele. Following the completion of the optimization process, real-time PCR analysis was performed for all samples.

Results

In all genotyped samples from 17 herds affected by MV, the putative protective allele KK at amino acid position 35 of TMEM154 was observed at frequencies of 29.49% and the risk alleles EE 23.08%, EK 42.31%. A significant difference was found between the KK, EE and KE groups.

Discussion and Conclusions

MV is a widespread disease in the global sheep industry. The TMEM154 gene and its E35K amino acid mutation are widely recognized as crucial resistance or susceptible in sheep (6). As a result of this study, the low prevalence of the Visna Maedi resistant allele in the herds in Northern Cyprus indicates a potential increase in the incidence of the disease. Identifying animals with KK, EK, and EE genotypes and conducting breeding studies in the herds will help prevent decreased productivity due to this infection in livestock. Moreover, the PCR conditions, once designed and optimized as part of the project, will be developed into a kit. This will enable rapid resistance screening at the request of herd owners, providing a valuable tool for managing livestock health.

Keywords: Visna, maedi, genetic, TMEM154, resistance.

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Morphometric measurements in akbash shepherd dogs with pedigree registrations

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Topçu, B., Betmezoğlu, M. Morphometric measurements in akbash shepherd dogs with pedigree registrations.

Introduction and aim

Akbash is a cultural treasure of Turkey that originates from the Eskisehir province and is a valuable part of sheep and goat farming (1). It is curious about strangers but is also balanced, affectionate towards the people and animals it protects, and very agile in working with shepherds. The main purpose of this Project is to check the breedings are being done by the true criteria and to observe if the breed type is homogenized or not by comparing different dogs (2). By choosing to measure the dogs that live close provinces outside the area of origin can prove that Akbash has the potential of adapting to different climates and faunas which results of them to improve morphological traits that are fit to the type of breed or not (3). With this way we can think of a comparative inference and observe the outcomes of selection (4).

Methods

The study involved 46 dogs with pedigree registrations to ensure the specimens were purebred and to observe the results of selective breeding methods applied by the Turkish Kennel Club. Measurements were taken outside the area of origin to evaluate possible adaptive differences in morphology. The measurements were done using a tape measure according to the European Metric System, with nineteen body parameters recorded from each dog (5). For the Body Proportions there were three different parameters. For the Body Parameters of the ten different parameters have been measured. For the Head Parameters six different parameters have been measured. The data obtained were analyzed using the GraphPad statistical software program. The response variables of LW, HS, HR, BL, HGC, CD, and CC were determined by analyzing descriptive statistics for body dimensions using ANOVA and Student's T-Test. Additionally, the impact of sex, country, and age group was determined.

Results

At first glance, the differences between males and females indicate some sexual dimorphism.Comparing shoulder height and back length reveals a rectangular structure in the examined samples. The topline, crucial for the breed type, is appropriate, with the hip height slightly higher than the shoulder height. A notable aspect is the slightly wide chest, contributing to the Akbash's dynamic body structure and effortless gait, proving it to be a highly functional dog.The head is not excessively heavy; the skull length is slightly greater than its width, and the muzzle length is slightly shorter than the head length.It has been found to have a mesocephalic head structure. The analysis of exterior body measurements revealed statistically significant differences in the average height at withers and the average height of the back between male and female Akbash dogs ($P<0.05$). However, for all other variables, the differences between males and females were not statistically significant ($P > 0.05$).

Discussion and conclusions

One of the most notable aspects is that a dog of these dimensions possesses only a slightly wide chest. This characteristic contributes to the Akbash's freely moving forelimbs, dynamic body structure, and effortless gait. It proves that Akbash is a highly functional dog in every aspect (6). The shoulder height of the dogs in this project is higher compared to those in past studies in Eskişehir (2). This could be due to breeding programs aiming at homogenizing the breed type and changes in environmental factors. Despite parametric differences due to environmental factors and feeding patterns, the Akbash Shepherd Dog maintains its anatomical structure, coat quality, and character across various environments, working efficiently as a flock guardian. Given the FCI's recognition of the Kangal as a Turkish shepherd dog breed, it is crucial to establish the standards for the Akbash, another Turkish shepherd dog. By defining the morphometric characteristics of the population and conducting pedigree breeding in line with standards, Akbash dog breeding will satisfy the prerequisites for official recognition. The data obtained from this investigation will be an invaluable contribution to this process (2, 3). Genetic determination of the Akbash breed will be made with future genetic studies. Genetic research will be conducted on reproductive genetics and some diseases.

Keywords: Akbash, morphometrics, selective breeding, pedigree, ANOVA.

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Time-dependent changes in velocity-based subpopulations of post-thaw bull semen via CASA analysis

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Introduction and aim

The determination of progressive motility (PM) compared to total motility (TM) is very important in the evaluation of frozen bull semen after thawing and this value is also related to fertility (1, 2). PM is the ability of the spermatozoon to move rapidly and linearly. However, because each spermatozoon moves with different speed and linearity, very different subpopulations can be form in the semen (3). In advanced CASA systems, the PM rate is determined according to the velocity values (VCL, VAP, VSL) and linearity levels (LIN, STR and WOP) of the spermatozoa (4, 5). The alterations in the velocity and linearity values used in the motility analysis do not affect the TM. However, they can lead to significantly different values for the PM (6). Therefore, the knowledge of the rates of change in the subpopulation groups formed according to the reference values used in the determination of PM and the examination of levels of change in these rates according to the incubation time after thawing may provide a different perspective for post-thawing quality standards of frozen bull semen, which initially appear to have the same TM and PM values. The objective of this study was to investigate the time-dependent changes in the PM subpopulations, as determined according to different velocity and linearity values, in bull semen with similar quality characteristics after thawing.

Methods

Frozen semen from different bulls produced in a semen production center was extensively evaluated for quality after thawing. As a result of the investigations, frozen semen from four different bulls with similar initial TM (%85-87) and PM (%43-44) values after thawing were selected. Assessments were made at four different times: immediately after thawing, after 30 minutes, after 60 minutes and after 90 minutes. A total of 96 analyses (4 bulls x 6 straws x 4 incubation times) were performed, and the kinematic properties of 67,038 spermatozoa were evaluated using the CASA system (ISAS Proiser, SPAIN). Data were evaluated using three different clustering methods. Different subpopulation groups were created by determining PM ratios according to different VCL values (>150, >100, >50, >25 µm/s) and STR rates (%>90, >80, >70, >60%). In the other grouping, the data were divided into four subgroups according to the VCL (>50) and STR (>70) characteristics

of the spermatozoa. In addition, sub-populations were also created by means of a multivariate K-means cluster analysis using the VCL, VAP, VSL, LIN, STR, WOB, ALH and BCF values. The ratios of change in subpopulations of different semen samples were evaluated depending on the incubation time. A X2 test procedure was used to evaluate statistical differences in the percentages of spermatozoa assigned to different subpopulations.

Results

The initial levels of PM were similar immediately after thawing (between 43.1% and 44.9%). However, there were significant differences in PM rates between semen samples (between 34.5% and 44.6%) depending on the incubation period from thawing to 90 minutes. On the other hand, there were significant differences ($p<0.05$) among the bulls in terms of the rates of change the between VLC(+)/STR(+) (the proportion of sperm moving rapidly and progressively) and VLC(+)/STR(-) (the proportion of sperm moving rapidly but not progressively) subpopulation groups (between 27.9% and 43.4%) during the incubation period from thawing to the 90 minutes. Furthermore, the results of the cluster analysis indicate that the distribution rates of subpopulation groups (in particular, group 4, which exhibits a rapid and progressive sperm motility, and group 5, which displays hyperactive and progressive sperm motility) among bulls vary depending on incubation period from thawing to 90 minutes. ($p<0.05$).

Discussion and conclusions

In order to determine the quality standards of frozen bull semen, it is very important to determine post-thawing motility values and kinematic parameters using a CASA system. However, it is very important for the success of AI applications that these characteristics are maintained for a certain period of time after thawing. The results of this study showed that significant differences can occur in subpopulations of semen with similar quality characteristics immediately after thawing, depending on the incubation time from thawing to 90 minutes. Therefore, examining the time-dependent changes in post-thawing analyses by creating subpopulations may provide a more accurate determination of semen quality.

Keywords: Bull, frozen semen, CASA, subpopulation, incubation time.

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Cryopreservation of bovine semen: Shilajit's antioxidant effects

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Introduction and aim

During cryopreservation, the excessive production of reactive oxygen species (ROS) increases the rate of lipid peroxidation (LPO), which in turn leads to detrimental effects on spermatozoa functions, resulting in decreased fertilization capability (1,2). The spermatozoa plasma membrane contains significant amounts of PUFA (polyunsaturated fatty acids), which are easily oxidized by ROS and produce harmful agents toxic to cells. Additionally, during cryopreservation, spermatozoa are subjected to physical and biochemical stress, resulting in reductions in parameters such as motility, plasma membrane integrity, fertility capability, and sperm metabolism (3). To maintain normal cellular functions, a significant portion of ROS needs to be deactivated, as they can exert oxidative effects. Seminal plasma contains various antioxidant substances to protect spermatozoa against these oxidative effects (4). Antioxidants are compounds and reactions generally

known for eliminating, neutralizing, or counteracting the formation and effects of reactive oxygen species (ROS). In the study, Shilajit, an antioxidant substance added to sperm diluents, is primarily attributed to its physiological effects arising from the presence of bioactive dibenzo-alpha-pyrones, which act as carrier molecules for active components, along with humic and fulvic acids (5). The aim of this study is to preserve the spermatic parameters and fertilization capability in bulls regularly subjected to sperm collection, despite exposure to cold shock and oxidation following cryopreservation.

Methods

In our study, four bulls raised and housed at Egevet Bull Station were used. Sperm was collected from the bulls using artificial vagina. Steridyl® (Minitube) was used as the sperm extender. The extender was divided into five equal parts to create one control sample and four different experimental groups. While no additional substance was added to the extender in the control sample, Shilajit was added to the experimental groups in concentrations of 1%, 2%, 3%, and 4%, respectively. The extenders prepared according to the study groups were added to the sperm samples. To achieve homogeneity between the extender and sperm, an equilibration process was conducted for 3 hours at +4°C. After equilibration, the Minitube Quattro integrated filling device was used for pipetting. Sperm samples were filled into 0.25 mL straws and frozen using the Digitcool (IMV Technologies) device, bringing them to -196°C. The frozen straws were stored in liquid nitrogen at -196°C. Thawing of the frozen sperm straws was done in a water bath at 37°C for 30 seconds. Sperm motility was assessed using the CASA system (Sperm Class Analyser, Barcelona, Spain) and a heated stage phase contrast microscope. Motility, progressive motility, and kinematic parameters such as VSL, VCL, VAP, BCF, ALH, WOB, LIN, and STR were examined. Data were presented as the mean $\pm$ standard deviation and $p<0.05$ value was considered significant. motility and progressive motility among control, %1, %2, %3, %4 groups were analyzed by the Kruskal-Wallis test following the Mann-Whitney U test to define the diversity among the groups.

Results

The spermatological examinations conducted using the CASA system post-thawing revealed significant findings across the studied bulls. For Bull 1, comparisons between the control and experimental groups demonstrated statistically significant differences in motility ($p<0,001$), progressive motility ($p<0,05$), and kinematic parameters including VCL ($p<0,05$) and ALH ($p<0,05$). In the cases of Bull 2 and 3, significant differences were observed in both motility ($p<0,001$) and progressive motility ($p<0,05$) between the control and experimental groups. However, no statistically significant differences were identified ($p>0,05$) in the spermatological parameters of Bull 4. These results indicate that the addition of Shilajit in varying concentrations (1%, 2%, 3%, and 4%) to the sperm extender had significant effects on improving motility and progressive motility parameters compared to the control sample in all three bulls.

Discussion and conclusions

The present study demonstrates that supplementation of Shilajit in varying concentrations (1%, 2%, 3%, and 4%) within the sperm extender significantly improves post-thaw sperm motility and progressive motility in bulls. These findings underscore the potential of Shilajit as an effective additive in sperm cryopreservation protocols to mitigate oxidative stress-induced damage and enhance sperm quality. Future research focusing on the molecular mechanisms underlying Shilajit's antioxidant activity and its effects on fertility outcomes will provide valuable insights for its application in reproductive management strategies.

Keywords: Antioxidant, bull, cryopreservation, shilajit, sperm.

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Effect of dietary supplementation with salmon oil on semen quality in male dogs

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Introduction and aim

The possibility of artificial insemination for dogs is becoming increasingly popular, necessitating that breeders not only maintain the quality of sperm but also enhance certain qualitative indicators of it. Sperm quality parameters are influenced by numerous factors, including nutritional supplements. However, only a limited number of studies have been conducted on the efficacy of male fertility supplements, particularly fatty acids, in male dogs. Salmon oil, a source of omega-3 and omega-6 fatty acids, has gained interest for its potential effects on canine reproduction. Studies conducted by other authors suggest that fatty acids are an important factor for the proper functioning of the plasma membrane of sperm cells and play a decisive role in the course of fertilization. This is because they are responsible for the necessary fluidity and permeability of the membrane, which is necessary for reaching the oocyte and fusing with it during fertilization. Furthermore, the lipid composition of the sperm plasma membrane is a key factor influencing the following characteristics: sperm motility, resistance to ambient temperature, overall sperm viability and cell membrane integrity. These

factors can enhance canine fertility (sperm motility, viability, concentration, and morphology) and reproductive performance (1, 2). The aim of this study was to investigate the effect of liquid salmon oil as a short-term food supplement on semen quality parameters in dogs.

Methods

A total of six clinically healthy German Pomeranian Spitz dogs (2.3–3 years old) were included in this study. The study was conducted in a kennel where all dogs were housed under identical conditions and fed the same diet. The dogs were not used for breeding purposes for a period of three months prior to and during the course of the study. The salmon oil product was manufactured by Nature's Protection and used as a dog food supplement. The dogs were randomly assigned to two groups: a control group (N=3) and an experimental group (N=3). For a period of one month, the experimental group dogs received salmon oil (1mL/day) (100 g of this food supplement includes Omega-3 fatty acids - 14000 mg, EPA - 2000 mg, DHR - 3000 mg, DPR - 1000 mg, Omega-6 fatty acids - 11000 mg, Omega-9 fatty acids - 30000 mg), whereas the control group dogs were not given anything additional. Semen samples were collected before the study and one month after the start of the study. Semen samples were collected manually and transported to the laboratory for semen analysis within one hour. In the laboratory, all samples (N=12) were evaluated for semen volume, subjective sperm motility, sperm viability, concentration, and morphology. The volume of semen was quantified in milliliters in a measuring container. The motility of the spermatozoa was examined subjectively by visual inspection at 37 °C under phase-contrast microscopy using an Olympus BH2 microscope with a pre-warmed 37 °C stage (Olympus Optical Co., Ltd., Japan) and 400 × magnification. Sperm motility was analyzed on 5-µl aliquots of semen. Prior to analysis, the entire sample was incubated in a 37 °C water bath (Memmert, Germany) for 5 minutes before analysis. The percentage of progressive spermatozoa in three – five fields of the microscopic view was determined as a subjective motility. The viability of spermatozoa was determined by eosin-nigrosin staining (Minitube, Germany). The concentration and morphology of dog's semen were evaluated using conventional semen evaluation methods (3). Sperm concentration was assessed in Neubauer improved (Germany)

blood cell counting chamber. The total number of abnormal spermatozoa was determined in dry preparations, stained with SpermBlue (Microptic, Spain).

Results

The analysis of the research results indicated that the supplementation of dog food with salmon oil had a positive effect on certain parameters of semen. The mean volume of semen in dogs receiving salmon oil exhibited a more than threefold greater percentage increase in volume compared to the control group. A 31.6% increase was observed in the volume of experimental group semen, while the control group demonstrated a 9.8% increase from the beginning of the study ($p < 0.05$). The sperm concentration in semen of the control group has decreased, whereas that of the experimental group has increased almost two times from 37.33 (standard deviation (SD) = 7.37) $x10^6$ /mL to 70.67 (SD = 29.94) x106 /mL. The mean of sperm motility increased by 26.0% in the experimental group after the study, and decreased by 6.7% in the control group ($p > 0.05$). The mean viability of the experimental group's semen increased by 36.3%, while that of the control group increased only 18.3%. The morphological changes in spermatozoa were not significantly affected by the salmon oil in the study ($p > 0.05$), potentially due to the relatively short duration of the study, which did not cover the full cycle of canine spermatogenesis.

Discussion and conclusions

The research findings indicate that salmon oil has the potential to be a valuable addition to the field of veterinary science and animal breeding. It can be used as a natural and accessible dietary supplement to positively influence various aspects of canine reproduction.

Keywords: dog, semen quality, fertility, salmon oil, omega – 3.

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Effect of mitotempo on short term semen storability in rams

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Introduction and aim

The fact that ram sperm membrane is rich in unsaturated fatty acids (PUFA) increases sensitivity to oxidative stress. Increased oxidative stress peroxidizes both lipids and proteins in the cell (1). Therefore, increased lipid peroxidation (LPO) has been associated with decreased motility, increased morphological disorders, increased acrosomal damage, disruption of membrane integrity, increased rate of dead sperm, decreased mitochondrial activity, and increased Deoxyribonucleic acid (DNA) damage (2). Mitotempo is a mitochondria-targeted antioxidant substance with superoxide and alkyl radical scavenging properties (3). While the electron transport chain in mitotempo cells acts as an antioxidant to maintain the stability of the phospholipid layer bilayer membrane, it prevents ROS formation and transport thanks to its hydroxylamine-like structure (4). In this study, it was aimed to determine the effect of adding mitotempon to the extender in vitro at different doses in rams on the short-term storage of semen by determining the changes in motility, morphology, plasma membrane integrity, dead spermatozoon and oxidative stress parameters and to reveal how effective it is on the storage of semen.

Methods

In the study, 6 Akkaraman breed rams, 2 years old, were used as animal material. 6 repetitions were performed for the study. Tris + egg yolk extender was prepared and used as a diluent for short-term storage of semen. In the study, mitotempo semen extender groups were set as 0 (Control), 0.1, 0.5, 1, 5, 10, 20, 50, 100 and 200 µM. After the initial motility of diluted semen was determined by CASA at 5 °C, it was analyzed at the 24th, 48th, 72nd and 96th hours (2). Eosin-nigrosin staining was used to determine the ratio of dead spermatozoa (5), Diff Quick staining set was used to determine the ratio of abnormal spermatozoa (5), Hypoosmotic swelling test was used to determine the integrity of plasma membrane (6) and antioxidant parameters were measured at 0 and 96 hours. Before the analyses, sperm samples were centrifuged, and the supernatant was removed. After weighing the cellular pellet, it was diluted and homogenized with Tissue Lyser homogenization device. The homogenates obtained were centrifuged in a cooled centrifuge

for MDA (7), GSH (8), CAT (9), GSH-Px (10) and GST (11) analyses and the supernatants were used in the analyses.

Results

When the changes in total motility findings of control and diluted ram semen containing different doses of mitotempo were examined over time and compared with the other groups, the total motility rate was higher in the group containing 5 µM mitotempo at the 96th hour ($p<0.01$). Dead sperm values of control and diluted ram semen containing different doses of mitotempo were lower in 1 µM, 5 µM, 10 µM, 20 µM, 50 µM and 200 µM groups compared to control and 0.1 µM groups at 96th hour ($p<0.01$). The average spermatozoon anomaly values of control and diluted ram semen containing different doses of mitotempo were lower in the 1 µM, 5 µM, 10 µM, 50 µM and 200 µM groups compared to the control group at the 96th hour ($p<0.01$). While MDA levels at 0 and 96 hours, GSH-Px and GST levels at 96 hours showed a statistically significant difference in 1 µM, 5 µM and 20 µM doses compared to the control group ($p<0.01$).

Discussion and conclusions

As a result, it was determined that 1 and 5 µM mitotempo doses increased total motility, protected plasma membrane integrity, increased viability, decreased spermatozoon anomaly rate and prevented oxidative stress when spermatozoa were stored for a short time by adding mitotempo.

Keywords: Mitotempo, ram, semen, short-term, storability.

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A study of bacterial species present in the semen of breeding dogs

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Introduction and aim

The microbiota of the male reproductive tract in dogs has been described in the scientific literature on a limited basis. The presence of specific bacteria in the semen of other animal species has been demonstrated to have an adverse effect on sperm quality, with the potential to result in reduced fertility (1). A higher number of bacteria in dog semen (bacteriospermia)

has been associated with a reduction in sperm viability and the presence of morphologically abnormal spermatozoa (2). However, there is a data deficiency on the microbial composition of semen in breeding dogs. As bacterial testing of semen prior to breeding is not mandatory, the prevalence of different bacteria in breeding dogs remains unclear. The identification of bacterial species and an understanding of their prevalence can assist in the development of guidelines for breeders, thereby ensuring better breeding outcomes. The aim of this study was to determine the presence and prevalence of bacteria in the semen of breeding dogs, providing insights that could contribute to better management practices in canine breeding.

Methods

A total of 30 semen samples were collected from clinically healthy breeding dogs of various ages and breeds. Semen samples were collected either in the presence of a female dog in estrus or using swabs containing vaginal secretions from a female dog in estrus. The sperm-rich fractions were collected into sterile plastic bags via manual manipulation (3). Collected samples were then transported to the microbiology and animal reproduction laboratories. In order to identify the present bacterial species, 10 µL of each sample were inoculated onto Columbia Agar plates with 5% sheep blood using serial dilutions. The inoculated plates were incubated at a temperature of 37 $\pm$ 0.5 °C under aerobic conditions for a period of 24 to 48 hours. Subsequently, bacterial colonies were selected for further analysis. The bacterial genera and families were identified based on their biochemical properties. Bacteria that could not be identified under laboratory conditions were biotyped using matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS). Progressive and non-progressive sperm motility as a key indicator of sperm quality was evaluated using software (Sperm Class Analyzer, Microptic, Spain). The dogs were divided into two age categories: group A included dogs younger than six years of age, while group B included dogs that were six years and older. The statistical data analysis was performed using "IBM SPSS Statistics 29.0.0.0".

Results

The bacterial isolates included both Gram-positive and Gram-negative bacteria in canine semen. The most frequently isolated bacteria were gram-positive. *Staphylococcus* spp. was identified in 63.3% of semen samples, *Corynebacterium* spp. in 43.3%, *Streptococcus* spp. in 40.0%, *Enterococcus* spp. in 16.7%, *Micrococcus* spp. in 10.0% and *Canibacter* spp. in 3.3%. Gram-negative bacteria were also present, although in lower proportions. *Moraxella* spp. was identified in 16.7% of samples, *Neisseria* spp. in 13.3%, and *Acinetobacter* spp. in 10.0%. *Escherichia* spp., *Haemophilus* spp., *Frederiksenia* spp., *Pasteurella* spp. and *Limosilactobacillus* spp. were each detected in less than 10.0% of the ejaculates tested. The *Enterococcus* spp. bacteria were identified with greater frequency in older dogs ($p < 0.001$). In group A this bacterium was detected in 4.8% of the semen samples. In contrast, in group B, the prevalence was more than nine times higher (44.4%) ($p < 0.001$). The presence of bacteria such as beta-hemolytic *E. coli* and *Enterococcus* spp. in semen samples was found to be associated with higher non-progressive sperm motility ($p < 0.05$). In samples containing either one of these bacteria, non-progressive motility was 28.4% (standard deviation (SD) = 9.2), in comparison to 12.6% (SD = 8.1) in samples lacking these bacteria ($p < 0.05$).

Discussion and conclusions

This investigation provides an overview of the bacterial species present in the semen of breeding dogs. The coexistence of both Gram-positive and Gram-negative bacteria within canine semen demonstrates the complexity of the microbial environment and its potential implications for reproductive health. The genera *Enterococcus* spp. and *E. coli* were identified as potentially harmful to dog spermatozoa, as evidenced by a higher incidence of non-progressive motility in samples containing these bacteria compared to those where they were absent. It has been demonstrated that contamination of semen with these bacteria is also associated with a reduction in semen quality in other mammals (4, 5). The presence of pathogens in the male reproductive tract increases the risk of infection in both the stud dog and the bitch. It is possible for bacteria from the dog's reproductive tract to be transferred to the oral microbiota, with the subsequent contamination of the owner via closer contact with contaminated dog saliva. These insights

highlight the importance of monitoring the microbiota of canine semen in breeding dogs to prevent the spread of pathogens that could affect both dogs and their handlers.

Keywords: Breeding dogs, microbiota, seminal bacteria, pathogens, sperm motility.

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Effect of apilarnil addition on ram semen quality after freezing

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Introduction and aim

Apilarnil is a natural bee product with a higher protein content. According to several studies this compoound has antioxidant properties and is able to increase testosterone levels in blood serum (1-3). In addition, Apilarnil seems to have anti-inflammatory and anti-apoptotic properties (4). In traditional medicine Apilarnil is commonly used for the treatment of reproductive issues and enhance males libido (5,6). The objective of this study was to examine the impact of incorporating Apilarnil, into ram semen at varying concentrations on its storage stability.

Methods

The study was conducted during the sheep breeding season (september-december) six clinically healthy aged two years Akkaraman rams, with a live weight of 55-60 kg were used. The rams were first accustomed to ejaculate the artificial vagina using ewes as phantom. Ejaculates with at least 70% motility and with a concentration higher than 2 billion/ml were selected and pooled. The ejaculates were collected twice a week for a period of three weeks with a total of six repetitions.

A solution of tris and egg yolk was prepared (with a without glicerol) and used as a diluent for the semen (7). The final glycerol concentration was set at. After pooling ejaculates were into five aliquotes experimental groups: Control (no apilarnil), 0.5%, 1%, 1%, 1.5% and 2% Apilarnil. The sperms were pre-diluted at 38°C with Tris + egg yolk diluent at a ratio of 1/1. The reconstituted spermatozoa were then combined with the experimental groups, resulting in a final concentration 0.5%, 1%, 1.5% and 2% Apilarnil. Samples were then placed in abeker glass and chilled at 4°C, within two hours in a refrigerator. Following the addition of the glycerolated extender, the samples were equilibrated for three hours. Placed in 0.25ml straws, frozen on liquid nitrogen vapour at -140°C and the sperm's motility and kinematic parameters were afterward evaluated, after thawing at 38°C for 25 seconds, with the aid of a computer-assisted sperm analyzer (CASA). Moreover, the malondialdehyde (MDA) levels, the sperms plasma membrane integrity and viability, the mitochondrial activity and the acrosomal integrity were analysed through flow cytometry (8-10).

Statistical analyses were conducted using SPSS (Version 22.0, Inc., Chicago, USA). Data are presented as mean ± standard error (SEM). Non-parametric Kruskal-Wallis ANOVA determined group differences, followed by Mann-Whitney-U tests for pairwise comparisons. Statistical significance was set at $p < 0.05$

Results

A comparison of the total motility values revealed a statistically significant increase in the 0.5%, 1% and 1.5% apilarnil groups. The highest increase was observed in the 1% apilarnil group ($p < 0.05$). it was observed that there was a statistically significant increase in VCL and VAP values in the 0.5% apilarnil group and in ALH values in the 1% and 1.5% apilarnil groups at the $p < 0.05$ level.

A statistically significant increase was observed in the high mitochondrial membrane potential (HMMP) value in the 2% apilarnil group, and in the low mitochondrial membrane potential (LMMP) value in all groups except the 1% apilarnil group ($P < 0.05$). A reduction in acromial damage values was observed in all apilarnil-containing groups. The greatest reduction was observed in the 1.5% apilarnil group ($P < 0.05$). A statistically significant increase in plasma membrane integrity was observed in the 0.5%, 1%, and 1.5% apilarnil groups ($P < 0.05$). Furthermore, a statistically significant ($P < 0.05$) decrease in malondialdehyde (MDA) levels was observed in all groups.

Discussion and conclusions

The results indicated that the inclusion of 1% apilarnil in semen diluents was more effective in the cryopreservation of ram semen. The beneficial impact of Apilarnil on the freezing of ram semen can be attributed to its ability to act as an osmoprotectant, whereby the proline present in its structure plays a crucial role in stabilising cellular structures and enzymes, clearing ROS and maintaining redox balance in unfavourable circumstances (11).

Keywords: Apilarnil, semen, diluent, cryopreservation, ram.

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Correlation of the amplitude of the lateral head with the kinetic of the bulls and boars spermatozoa

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Introduction and aim

Motility is one of the most important properties associated with the ability to fertilise oocytes and is related to the structural integrity of the sperm cell membrane. The parameters of sperm velocity largely determine fertility and are of great importance for the process of sperm capaCitation and its ability to fertilise an egg. The sperm head is the carrier of genetic information during the fertilisation process, so its size and shape can play an important role in this process. Some authors believe that head shape is related to chromatin structure and integrity and thus to male fertility (1). The spermatozoa of highly fertile boars have smaller and shorter heads compared to the sperm of boars that are less effective in terms of fertilisation performance (2). Some authors point to differences in the intensity and movement style of sperm depending on head shape (3). The aim of our study was to investigate whether the amplitude of the lateral head (ALH) of the bulls and boars spermatozoa has an influence on the parameters of sperm

kinetics (VSL straight line velocity, VCL curved line velocity, BCF beat cross frequency).

Methods

The ejaculates of thirty-four boars and forty frozen bull spermatozoa were analysed. Various motility and kinetic parameters were analysed using the CASA system. After heating, 2.7 µl of the sample was added to the Leja chambers. 10 fields of view were analysed using a phase contrast microscope with a built-in heating plate, and the mobility parameters were analysed automatically using AndroVision software. Statistical data processing was performed with the statistical software GraphPad Prism 8.00 (GraphPad Software Inc., San Diego, CA, USA). The degree of correlation of the analysed parameters was determined using Pearson's correlation coefficient and by checking the statistical significance of the correlation coefficient ($P < 0.05$).

Results

ALH showed a strong positive correlation with VCL of bull ($r = 0.9545$) and boar spermatozoa ($r = 0.9668$) at the level of statistical significance $P < 0.001$.A strong positive correlation ($P < 0.001$) was also found between ALH and VSL of bull ($r = 0.7799$) and boar spermatozoa ($r = 0.7262$). There was no correlation between BCF and ALH.

Discussion and Conclusions

Previous studies have shown that in addition to the various locomotion traits investigated using the CASA method, various speed parameters such as VCL, VSL and VAP can be used as predictors of fertility in bulls (4) and boars (5, 6). VSL indicates sperm forward movement and multiplication, VAP reflects capaCitation and VCL expresses average velocity (7). The authors (8) found that Duroc spermatozoa have slightly larger heads compared to Pietrain spermatozoa, Landrace (9) and Jorksire (10), which are significantly longer, have a larger circumference and shorter tails, while the head size of the Holstein-Friesian bull (11) is larger than that of the Simmetal bull (12), while there is no literature data on head size for the other breeds. A larger number of ALH during sperm movement has a direct effect on their velocity, as evidenced by a very strong positive correlation between ALH with VSL

and VCS of the bulls and boars spermatozoa. Higher ALH values could be a consequence of larger head dimensions in the mentioned breeds of boars and bulls, but further research is needed.

Keywords: CASA, ALH, VSL, VCL, bull, boars, spermatozoa.

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Graphical evaluation of the agreement of flowcytometric analyses with different sperm concentrations

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Introduction and aim

Achieving successful fertilization is directly related to the quality of semen. With the developing reproductive biotechnology, semen quality can be easily determined using different methods. Flow cytometry, one of the fluorescence-based methods, stands out as a method that analyzes objectively and facilitates the evaluation of semen in terms of quality. This method provides information about the sperm quality parameters such as sperm viability, lipid peroxidation and capaCitation by scanning spermatozoa

stained with different dyes. However, to our best knowledge, there is no information on the suitable cell concentration to obtain exact results from the flow cytometrical semen analysis. In this study, the aim was to evaluate the compatibility between two different spermatozoa groups (1,000 vs 10,000 spermatozoa) with a graphical approach, using lipid peroxidation and capaCitation levels measured by the flow cytometry method.

Methods

The study material consisted of 100 samples of frozen semen collected at different times from Simmental bulls (n=10). The study groups were determined as 1,000 spermatozoa and 10,000 spermatozoa, which is frequently preferred in the literature. In each study group, lipid peroxidation level was analyzed by BODIPY C11/PI double staining, capaCitation level by Fluo-4, AM, cell permanent method using CytExpert 2.2 software (Beckman Coulter, Fullerton, CA, USA) on the flow cytometry device (Cytoflex, Beckman Coulter, Fullertone, CA, USA). The agreement between the two groups of spermatozoa was determined by a Bland–Altman plot with limits of agreement of bias$\pm$1.96 standard deviations (SD) of the difference. In addition, for agreements between the two groups, one-sample t test was used to assess whether the mean differences were different from 0. $P<0.05$ was considered as the significance level. All statistical analyzes were carried out using IBM SPSS 23.0 and GraphPad 8.

Results

Negative and positive BODIPY (RED and GREEN) results for lipid peroxidation levels, and negative and positive FLUO (RED and GREEN) results for capaCitation levels were evaluated. Accordingly, the mean differences of BODIPY (RED) and (GREEN) were found to be -5.408 and 4.781, respectively. The mean differences of FLUO (RED) and (GREEN) were determined as 3.952 and -3.777, respectively. For agreement between the two spermatozoa groups, all variables showed statistically significant differences (all p's <0.05) according to the one sample t test result.

Discussion and Conclusions

In terms of semen quality, it is desired for negative lipid peroxidation (BODIPY (RED)) and capaCitation (FLUO (RED)) levels to be high and positive levels to be low. According to the results of the study, the 10,000 spermatozoa group was higher for BODIPY (RED) than the 1,000 spermatozoa group, and lower for BODIPY (GREEN). For FLUO (RED) and (GREEN), the 1,000 spermatozoa group was found to be higher and lower, respectively, compared to the 10,000 spermatozoa group. Moreover, these two spermatozoa groups were not compatible in terms of the lipid peroxidation and capaCitation levels. Consequently, it has been determined that 10,000 spermatozoa should be counted when evaluating the lipid peroxidation level in the flow cytometry device, and 1,000 spermatozoa are sufficient for evaluating the capaCitation level. It was thought that the analysis time and workload could be decreased by reducing the number of spermatozoon counted for the capaCitation level.

Keywords: Bland-Altman, one sample t test, lipid peroxidation, flow cytometry, capaCitation, spermatozoon.

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Cholesterol's impact on post-thaw motility and viability of bull semen: A meta-analysis study

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Introduction and aim

Today, artificial insemination is the most preferred assisted reproductive technology in cattle breeding. Additionally, more than 95% of artificial insemination practices are performed with frozen bull semen. However, spermatozoa are highly damaged by oxidative stress caused by high amounts of ROS formation produced by cold shock and atmospheric oxygen during semen freezing. For this reason, adding antioxidants to the extender in bull

semen freezing is an issue that has been studied for decades. Among the different types of antioxidants, cholesterol, a non-enzymatic antioxidant, stands out as a frequently preferred additive component in bull semen freezing. In this study, it was aimed to investigate the effect of cholesterol on post-thaw motility, progressive motility, and viability and to obtain a single effect size for its effect in bull semen freezing.

Methods

Search Strategy: A comprehensive literature search was performed on Scopus and Web of Science databases between January 2000 to January 2024. The search strategy used combinations of words and keywords in English, including "cholesterol", "motility", "progressive motility", "viability", "sperm", and their synonyms. In addition, the origin of cholesterol in the studies was cholesterol-loaded cyclodextrin.

Inclusion and exclusion criteria: The criteria for inclusion in the study are listed as follows: (1) Having an original research article published in a peer-reviewed journal, (2) The study was published between 01/01/2000 and 01/01/2024, (3) The language of the article is English, (4) The study conducted on cryopreserved bull semen (5) Having numerical results (Average $\pm$ Standard Error or Deviation) obtained from post-thawed semen analysis. Exclusion criteria from the study are listed as follows: (1) The study is not an original research article, (2) Irrelevance of the study, (3) The study was conducted on fresh or cooled semen analysis, (4) The study has graphical results without numerical results.

Meta-analysis: The meta-analysis was performed using Stata v.18 software (StataCorp LP, College Station, TX), with the level of significance set at $p<0.05$. Absolute effect/raw difference was calculated and presented since all the variables were presented in the same units across all studies. A random effect meta-analysis using Hunter–Schmidt estimator was implemented. Cochran's Q test and I2 test statistic were used to assess heterogeneity. A random effects meta-regression analysis was performed to assess the heterogeneity of effect between studies using breed and dose as moderators.

The forest plot was used in the visual presentation of the findings. Begg test and Funnel plots were used to assess potential publication bias.

Results

A total of 20, 14, and 19 comparisons met the study criteria for motility, progressive motility, and viability, respectively. The study revealed a mean difference of 8.32 [95% CI: 6.52-10.12], 4.73 [95% CI: 1.25-8.21], 8.9 [7.06-10.73] in favor of the treatment for motility, progressive motility, and viability, respectively. The 95% confidence interval for the overall estimate, along with the z-test statistic of -9.08, 2.66, 9.49 and the p-values of <0.001, 0.008, <0.001 suggest that was statistically significantly different from 0 for motility, progressive motility, and live sperm count, respectively. No small study effect was observed for all three outcomes. The results of the I2 statistic indicated a high degree of heterogeneity for all three outcomes, with values of 97%, 94%, and 96% for motility, progressive motility, and viability, respectively. While the inclusion of moderators such as breed and dose in the meta-regression did result in a reduction in heterogeneity, the level of heterogeneity remained relatively high for all three outcomes.

Discussion and Conclusions

Cholesterol supplementation significantly boosts post-thawed motility, progressive motility, and viability of bull semen according to the findings of this meta-analysis. All three of the parameters were statistically improved which indicates that cholesterol acting as an additive can ameliorate damage caused by oxidative stress induced during freezing of semen. This meta-analysis provides compelling evidence that cholesterol supplementation significantly improves post-thawed motility, progressive motility, and viability of bull semen. These results underscore the potential of cholesterol as a beneficial supplement in semen extenders to enhance the efficiency of artificial insemination within bovine breeding programs. However, the high heterogeneity observed suggests that further research is needed to identify and control other variables influencing the outcomes. Future studies should focus on standardizing protocols and exploring additional moderators to optimize the use of cholesterol in semen cryopreservation.

Keywords: Meta-analysis, cholesterol, post-thawed, bull semen, motility, viability.

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Development of sperm DNA fragmentation kit based on artificial intelligence (A.I.)

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Citation
Arı, U.Ç., Toprak, T., Arı, A., Karatepe, İ., Sonalcan, İ.H. Development of sperm DNA fragmentation kit based on artificial intelligence (A.I.).

Introduction and aim

The aim of the current preliminary study was to determine the requirements of Turkey's first commercial sperm DNA fragmentation kit based on artificial intelligent and the differences between species, including humans, as "one health" concept. This kit contains a special slide (sps. slide), a solution used for binding sperm cells to the spc.slide, and other solution(s) used for determine DNA fragmentation of sperm cells in bulls, rams and also humans. Microscopic images were recorded with camera attached to microscope with x40 object. Since the sperm cells of each species have their own membrane and ultrastructural structure, it was determined that

this may cause different patterns and appearance in microscopic imaging (1-6). While these differences were seen in the human sperm cell, the loss of the head and tail structure and the resulting dot and circle forms; It was observed that bull and ram spermatozoa partially retain their unique head structure, while the tail disappeared. The tail can be protected and visible with different modifications of solution(s) and process, especially duration of solutions. In this case, it has been revealed that different solutions or solution modifications may be needed for each species. In addition, pattern differences require the testing and development of different approaches to artificial intelligence. Research and development (R&D) studies are ongoing for special artificial intelligence strategies that detect, segment, and deep-learning on this intended object for DNA fragmentation.

Methods

ESAS SDF kit contains a special slide, a solution used for binding sperm cells to the special slide, and other solution(s) used for determine DNA fragmentation of sperm cells in bulls, rams and also humans. Imagine results from different species semen samples were obtained with microscopy and camera records.

Results

Sperm cells of each species have their own membrane and ultrastructural structure, it was determined that this may cause different patterns and appearance in microscopic imaging in DNA fragmentation analyses. While these differences were observed in the human sperm cell, the loss of the head and tail structure and the resulting dot and circle forms; It was observed that bull and ram spermatozoa partially retain their unique head structure, while the tail disappeared.

Discussion and conclusions

The tail may be protected and visible with different modifications of solution(s) and process. In this case, it was revealed that different solutions or solution modifications may be needed for different species. In addition, pattern differences require the testing and development of different approaches in artificial intelligence. Research and development (R&D) studies

are ongoing for special artificial intelligence strategies that detect, segment, and deep-learning on this intended object (1-6).

Keywords: DNA Fragmentation, Sperm Cells, Ruminants, Human, Artificial Intelligence, Research and Development.

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Antioxidant effects of the addition of olive extracts in buffalo frozen semen

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Esposito, R., Mora, M.P.B., Prete, C.D., Aiudi, G., Piscopo, F., Calabria, A., Masiello, L., Carbonari, A., Gasparrini, B. Antioxidant effects of the addition of olive extracts in buffalo frozen semen.

Introduction and aim

The damage to spermatozoa during cryopreservation is attributed to the increase in oxidative stress, due to the thermal and osmotic insults. Therefore, the enrichment of semen extender with antioxidants before freezing has become a common strategy to mitigate cryopreservation-

induced oxidative stress in various domestic species. The interest in the use of herbal natural antioxidants has recently increased, attributed to their reduced toxicity, minimal residue, and high content of beneficial compounds such as polyphenols, flavonoids, carotenoids, gallic acid, tannins, and essential oils. The olive fruit extract (OFE) is a blend of natural antioxidants that can synergistically enhance cellular function. This product can be readily obtained from discarded olives, typically wasted during commercialization processes. The OFE contains several phenolic compounds in particular the hydroxytyrosol (HT), that is known for its antioxidant properties, acting as a natural ROS scavenger, reduces oxidation of low-density lipoproteins, protects from hydrogen peroxide-induced cytotoxicity and minimizes the lactate dehydrogenase activity [1]. The composition of buffalo spermatozoa renders them highly susceptible to oxidative damage induced by cryopreservation. In a previous work, we demonstrated that the enrichment of the buffalo extender with the addition of D50 OFE, containing 50 µM of hydroxytyrosol (HT), improved post-thaw sperm membrane integrity, the percentages of total live and acrosome-intact live sperm, as well as total and progressive sperm motility [2]. The aim of this study was to investigate whether the observed positive effect on post-thaw semen quality is attributed to the antioxidant properties of OFE. Specifically, we aimed to evaluate the impact of OFE supplementation on oxidative stress markers, such as the levels of biological antioxidant potential (BAP) and Reactive Oxygen Metabolites (ROMs).

Methods

The OFE was obtained by hot extraction with acidified water and the HT content of 10.8% was obtained by high-pressure liquid chromatography analysis with a calibration line. The experiment was carried out on a farm located in Paraguay (Cordillera Department, Arroyos y Esteros), two ejaculates from 16 Murrah buffalo bulls aged 3-10 years were collected by electroejaculation with equipment 0.5 volt upward discharging to a maximum of 20 volts, as used in cattle. To contain the buffaloes, a partially hydraulic and iron operating stall (cattle treatment cage) was used for the safety and welfare of the operators and animals. Before inserting the rectal probe, the rectum was emptied of fecal matter and the accessory glands

were massaged. The probe was inserted in the rectum and placed above the prostate gland. The ejaculation was stimulated using the type 2 curve of the device. Each ejaculate was split into two aliquots and diluted at 37°C with Tryladil® extender (Minitube®, Tiefenbach, Germany) without (control) and with the addition of OFE (treated), to a final concentration of 30 × 106 spermatozoa per mL., the concentrations of OFE refers to the levels of the most represented polyphenol, hydroxytyrosol (HT), determined by high-pressure liquid chromatography (HPLC). Specifically, the extender was supplemented with 72 mL/L of OFE, corresponding to 50 µM HT. The semen was then frozen in two steps: 1- diluted samples were cooled from 37°C to 5°C (cooling rate 2°C/3min), equilibrated at 5°C for 6 h, exposed to the liquid by nitrogen vapor for 10 min and then plunged into liquid nitrogen. At thawing, BAP and ROMs were evaluated in control and treated semen. Differences between groups were analyzed by Student's t-test.

Results

The enrichment of the extender with D50 OFE increased the post-thaw biological antioxidant potential (1616 $\pm$132 vs. 2705 $\pm$ 218 µmol/L HClO, in control and treated groups, respectively; $P<0.01$) and reduced the ROMs levels in sperm seminal plasma (88 $\pm$18.1 vs. 34 $\pm$ 6.2 UCARR, in control and treated groups, respectively; $P<0.05$).

Discussion and Conclusions

This is the first work reporting the antioxidant effects of OFE in frozen buffalo semen. The significantly increased BAP and the reduced ROS levels found in semen treated with D50 OFE compared to the control confirm that the beneficial effects of OFE on buffalo post-thaw semen quality are due to its antioxidant action. The reduction of ROS levels and the preservation of antioxidants can be attributed to the synergistic interaction among HT, and various bioactive phenolic compounds, with known antioxidant activities [3,4,5,6]. In conclusion, enriching the extender with a natural plant-derived antioxidant additive such as OFE was effective in counteracting excess ROS formation during cryopreservation, which is known to be a major cause of sperm damage.

Keywords: sperm cryopreservation, spermatozoa, reactive oxygen species, oxidative damage.

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A true prolapsed vagina in a bitch resulting from forceful separation in mating

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Citation
Uçar, E.H., Peker, C., Nalbant, S., Erdoğan, G. A true prolapsed vagina in a bitch resulting from forceful separation in mating.

Introduction and aim

Even though vaginal hyperplasia cases related to high oestrogen levels are commonly seen in the bitch, true vaginal prolapse is a very rare condition. Extreme tenesmus during dystocia (1,2), constipation (3), oestrogenic influences (4), forced separation during coitus, and size discrepancy between breeding animals play a role as etiologic factors for this pathology (5,6). In some cases, urinary bladder retroflexion (7), bladder incarceration, or irreducible rectal prolapse (3) can be seen concomitantly, worsening the prognosis. In this report, the clinical findings of a case of a complete vaginal prolapse caused by the bitch being forcibly separated from the male during unwanted mating are presented.

Methods

A 3-year-old female Cane Corso dog was brought to our clinic with the complaint of a mass protruding from the vagina in the last 3 hours. It was stated that the bitch, known to be in oestrus according to anamnesis, was seen mating with another male that was not of its breed and that an attempt

was made to move the bitch to prevent mating so that she would not become pregnant. It was reported that approximately 3 hours ago, while they were in the copulatory tie, she was forcibly separated from the male, and then this mass came out. On clinical examination, the animal showed lethargy, loss of appetite, and the behaviour of constantly turning and licking the oedematous and long-shaped mass protruding from the vulva. The size of the mass and the presence of the cervical ostium at the caudal end helped to identify the case of a complete vaginal prolapse. There was no evidence of urinary straining or dysuria, according to the owners. Moreover, the ostium urethra externa was clearly visible, and no necrotic mucosal lesion was observed. On ultrasonographic examination, neither the urinary bladder nor intestinal loops were found in the mass, but the invagination area was observed under the urinary bladder in the abdominal scans. No significant pathology was seen following the patient's hemogram and blood biochemistry analysis, and all values were within the normal reference limits. In the first step of the treatment, the mass was washed with warm antiseptic solutions, and no wounds, tears, or necrotic areas were found on the surface. The catheterization procedure throughout the orificium urethra externa was completed easily. To reduce the oedema of the mass caused by venous insufficiency, a tight bandage was applied using sterile gauze, starting from the caudal end and ending at the vulva level, and a gentle massage was made from the far end to the cranial edge of the mass for approximately 15 minutes. After the shrinking of the mass, the midline celiotomy was performed for the rejection of the protruded vagina and the evaluation of the uterine condition. The uterus was pulled back from the area where intussusception took place with gentle movements, and the vaginal position was normalized. Due to intense venous insufficiency in the uterus, both the cornu uteri and ovaries were removed surgically. To prevent the recurrence of the vaginal prolapse, the cervix was fixed to the abdominal wall (cervicopexy) at the level of ligation. Two doses of postoperative analgesia (0.2 mg/kg, Meloxicam, Maxicam, Sanovel, Turkey) were also administered every other day. In addition, a parenteral antibiotic (10 mg/kg, Amoxicillin+Clavulanic acid, Synulox, Zoetis, Italy) was administered subcutaneously for seven consecutive days.

Results
The patient well-tolerated cervicopexy and recovered uneventfully. After surgery and additional medical treatments, the bitch successfully recovered within 1 week postoperatively without vaginal relapse or discharge, and the vulvar oedema disappeared.

Discussion and Conclusions
Owners and canine breeders should take into consideration that the traumatic interference in coitus for preventing pregnancy carries a very high risk for vaginal prolapse in their pets. After such manipulations, their pets can lose their fertility even if they do not have any other organ complications or are referred to the clinics within a few of hours. In addition to the sterility risk, concomitant complications such as uraemia and irreducible malformations would worsen the prognosis and cause a high mortality risk.

Keywords: prolapsus vagina, vaginal invagination, cervicopexy, bitch.

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Disseminated transmissible venereal tumour in the deep portion of the cranial vagina in a bitch

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Citation
Peker, C., Uçar, E.H., Nalbant, S., Erdoğan, G. Disseminated transmissible venereal tumour in the deep portion of the cranial vagina in a bitch.

Introduction and aim

Transmissible venereal tumour (TVT) is a round-cell neoplasm seen in immunodeficient individuals, especially in uncontrolled populations with unknown history. Tumoral cells are often transmitted by coitus; the mass localizes in the external genital organs (1). Additionally, due to licking behaviours in animal socialization, the tumours may be seen in different parts of the head, such as the eyes, mouth, and nasal cavity (2-4). Affected dogs often live mainly with access to the street because tumour implantation would happen during coitus or by intimate social interaction with another sick canid. On inspection, the typical morphology of the tumour, the serosanguineous, haemorrhagic genital discharge, and the bad odour suggest the TVT. Through cytology and histopathology, TVT is easily diagnosed in the samples by detecting round TVT cells. The classification of TVT cells is based on their cytomorphological types in three classes: lymphocytoid type, plasmacytoid type, and mixed type (5,6). Chemotherapy with vincristine sulphate is preferred over surgical approaches due to the practical and easy procedure, high success rate, and low side effects. After

1-3 treatments, the animals respond immediately with an improvement in clinical signs (3). This case report presents the clinical findings of a case of TVT that could have gone undiagnosed due to its rare position.

Methods

A twelve-year-old, 35 kg, spayed Mongrel stray bitch was referred to our clinic, with a history of intermittent vaginal haemorrhage for 3 months, according to referring people. Although the patient had been treated with parenteral antibacterial therapies in another veterinary clinic, similar vaginal bleeding recurred after a few days. Inspection of the perineum and vaginal palpation revealed no protruding tissue mass from the vulva, and no suspicious mass in the vaginal lumen. When vaginal smear samples were collected and examined microscopically, round cells with little cytoplasm and vacuoles were found, which were referred to as TVT cells. To detect the exact localization of the tissue, pelvic ultrasonography was performed. The examination revealed a solid mass with no internal blood flow and no cystic areas at the level of the cranial vagina. During scans, a couple of amorphous suspicious masses were identified. Therefore, it was decided to remove the masses with an operation and then start chemotherapy. During the midline celiotomy, multiple multilobular dissaminated masses were observed in the deep portion of the cranial vagina, close to the cervix. All masses were completely and easily removed from the mucosa. Macroscopic examination showed typical cauliflower-shaped masses with bleeding but a lighter colour. A parenteral antibiotic (10 mg/kg, Amoxicillin+Clavulanic acid, Synulox, Zoetis, Italy) was administered subcutaneously for seven consecutive days.

Results

After completing the operation without any problems, the patient was given antibacterial treatment for a week. However, to eliminate the tumour cells from the lumen, intravenous chemotherapy (0.025 mg/kg/week Vincristine Sulphate, Kocak Farma, Turkey) was injected in sufficient doses for 3 cycles. At the end of chemotherapy, the elimination of TVT cells was confirmed by a smear test. It was reported that the vaginal bleeding stopped after the first week, and her general condition improved in the following days.

Discussion and conclusion: In this report, this bitch was infected with TVT due to social interactions, despite having been spayed previously. But the fact that the mass cannot be detected despite inspection and vaginal palpation can be confusing for the clinicians. Even if the tumoral mass is not detected on palpation, firstly, vaginal cytology should be performed to verify the presence of TVT cells by taking smear samples. Secondly, pelvic ultrasonography would reveal more accurate information about the location of the mass. The easy removal of these tumours in our surgical approach and the lack of additional bleeding were remarkable; moreover, their surfaces were pale yellow-white, unlike the classic TVT appearance. It should be noted that this disease, which is easily contagious and zoonotic, especially in uncontrolled populations, can occur in such rare manifestations. It should be considered that monitoring and control of these stray dogs with routine sterilization operations are crucial for public health.

Keywords: Canine transmissible venereal tumour, surgery, chemotherapy, dog

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In vitro fertilization developments in dogs

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Introduction and aim

The success rate in in vitro embryo production studies in dogs is lower than in other animal species. To achieve the best success with these techniques, it is helpful to know the effects on the maturation ability of the individual animal and the oocytes. Studies on the retrieval of oocytes, their maturation, fertilization, maturation and transfer of the resulting embryos are ongoing in dogs. In this review, information about obtaining oocytes from dogs, their maturation and fertilization is given.

In vitro fertilization (IVF) is the process of bringing together a mature oocyte and spermatozoa capable of fertilization under optimal conditions in the laboratory (1). In vitro fertilization consists of the stages of obtaining ova, maturation of oocytes preparation of spermatozoa, in vitro fertilization of oocytes and in vitro culture of fertilized oocytes (2). Optimizing the culture medium to produce an in vitro environment similar to that obtained in the oviduct and uterus is an important step to achieve this goal (3).

Methods:

Obtaining oocytes

Following ovariectomy in dogs, the ovaries are sliced in the laboratory and washed with proper solutions. The washout fluid is collected in a petri dish and examined under a stereomicroscope (15x magnification) to search for oocytes (4,5).

In vitro oocyte maturation

Oocytes with darkly pigmented cytoplasm and surrounded by at least one layer of cumulus cells are classified and selected as Class I. Selected oocytes are transferred to four-well petri dishes containing 500 µL of TCM-199 maturation medium supplemented with 10% fetal calf serumand 50 mg/mL gentamicin sulfate under mineral oil for 96 hours at 38°C (6,7)

In vitro preparation of spermatozoons

In the percoll method, which is one of the methods used for motile spermatozoon selection; The semen deposited on two different percoll layers (90-45%) is centrifuged at 1500 rpm for 15 minutes and then the supernatant is discarded. The remaining pellet is diluted 1:20 with Hepes buffered synthetic oviduct fluid (HSOF) and centrifuged again at 600 rpm for 6 minutes. After the supernatant is discarded, the density of the rediluted spermatozoa is determined (8).

In vitro fertilization of oocytes

Following in vitro maturation, oocytes with expanded cumulus are removed from the maturation medium and transferred to a special petri dish containing fertilization solution, where cumulus and corona cells are removed by gently pipetting for 3-5 minutes. Then 10 µL in vitro capaCitation spermatozoon suspension ($0.8x10^6$ spermatozoon/mL) is added to each compartment containing approximately 50 µL medium and in vitro maturated ova containing 5 to 10 oocytes. In order for in vitro fertilization to occur, oocytes and spermatozoa are incubated at 38 °C for 20 hours (9).

In vitro culture of fertilized oocytes

Following incubation with spermatozoa, oocytes that are considered to be fertilized are transferred from the fertilization medium to the culture medium and cultured for 7 days. Culture media are kept at 38 °Ċ in an environment where low oxygen levels are provided (5% CO_2 and 5% O_2). Embryos are formed at the end of the 7-day period. After the oocyte maturation period, the pH level of the environment containing the oocyte is adjusted to 7.4 - 7.8. After the spermatozoa matures, 3 drops of spermatozoa are added to 10 mL of medium. The oocyte is then incubated for 30 hours at 38.5 °C, 5% CO_2 and 90% humidity (8).

Following incubation with spermatozoa, oocytes that are considered to be fertilized are transferred from the fertilization medium to the culture medium and cultured for 7 days. Embryos are formed at the end of the 7-day period. Culture media are kept at 38 °Ċ in an environment where low oxygen levels are provided (5% CO_2 and 5% O_2).

Discussion and Conclusion

Although in vitro fertilization studies in dogs may seem like routine methods at first glance, studies have shown that there are many more obstacles in the maturation and fertilization stages. Only further studies and scientific collaboration can find a solution for IVM and IVF in animals and improve the survival of animal embryos after transfer.

Keywords: Dog, in vitro fertilization, maturation, medium, spermatozoon.

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The relationship between endometrial cytology and echotextural parameters in cows

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Introduction and aim

Reproduction is one of the indispensable elements of animal husbandry enterprises (1,2). Ultrasound and computer-aided image analysis (CAIA) are important routine tools in veterinary medicine for the assessment of physiological and pathological changes in the reproductive system (3). The mean grey level (MGL), homogeneity (HOM), and contrast (CON) are major variables of the digital images to make inferences about tissue echotexture (4). Endometrial cytology is thus preferred for the diagnosis, although it is an invasive procedure (5). The aim of this study was to investigate the relationship between echotextural parameters (MGL, HOM, CON and GRD) determined using a computer-assisted image analysis programme in cows whose uterine health was determined by cellular infiltration density in endometrial cytobrush samples.

Methods

The study was conducted in 88 Simmental cows. Cows among the cows which were between 45-50 days postpartum and 2-5th parity, had no systemic infection findings or metabolic problems, clinically genital tract infection was not detected. Digital images were taken B Mode ultrasonography device. Images were taken cross-sectionally from the greater curvature of the uterine cornu uterus and saved in JPEG format. At least 3 images were recorded from the uterus in each application. Image analyses were performed with specially developed computer software. Cytological samples were taken according to the method described by Kasimanickam et al (6). Giemsa staining was performed for cell counting in cytological samples. Microscopic evaluation of the smears was performed at 40X magnification. Classification was performed according to Chapwanya et al. scoring system (7). Each imaging region HOM, CON, MGL, and GRD parameters were determined (8).

Results

According to the results of the cytopathological evaluation, the groups were classified as follows: S0 (n = 20), S1 (n = 32), S2 (n = 21) and S3 (n = 15). No difference was found between the mean grey level in all groups (P>0.05). Homogeneity between S3 (0.0889 ± 0.003) and other groups was found to

be statistically different (P<0.0016). There was no difference (P>0.05) in the contrast values between the S1 and S2 groups., but a significant difference was found between the other groups (P <0.0001). A linear increase between S1 (6.741 ± 0.10001) and S3 (7.401 ± 0.12089) groups was observed when analysing the gradient (P <0.0003)

Discussion and Conclusions

Endometrial biopsy and histopathology are the most reliable methods for evaluating the uterus (7,9). However, endometrial biopsy was avoided in this study because it is an invasive approach.

It is difficult to detect uterine inflammation in cows by ultrasound. Some studies have focused on quantitative echotextural changes in the endometrium in recent years (4, 10). Researchers have identified ecotextual changes with quantitative values using the CAIA method (5). Therefore, the relationship between cell infiltration and echotextural parameters was investigated in our study. In the present study, it was found that HOM values increased with decreasing cell infiltration density, there were differences in CON values and there was no change in MGL. Similar results have been found in our study and in other studies (4, 8, 10). In this study, it was concluded that HOM may be a more definitive parameter than other parameters in determining inflammation in the uterus.

Keywords: Computer-assisted image analysis, Cytobrush, Cow, Homogeneity, Mean Gray Level, Contrast, Gradient

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Exceptional recovery of 5 embryos from the uterine flushing of a non-superovulated donor mare

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Introduction and aim

Embryo transfer (ET) is a useful technique to permit the reproduction of valuable broodmares, including either the ones still in sport activity or too old or young to carry the pregnancy to term. The mare generally ovulates one follicle per cycle, although double or triple ovulations are possible, mostly in Warmblood and mature mares [1]. In the equine species, twin pregnancies are undesirable and need to be reduced to a singleton as soon as possible. However, in an ET program, the occurrence of donors' multiple ovulations

increases embryo recovery rates and the ratio of pregnant recipient mare per cycle [2]. Differently from bovines, superovulation treatments are not available for horses. A clinical program of ET has been running at the Veterinary Teaching Hospital of the University of Pisa since 1998. Over the years, this program has become a cornerstone of the hospital's reproductive services, owning and managing more than 70 recipient mares per year. Moreover, this program provides invaluable hands-on learning opportunities for veterinary students, allowing them to acquire practical skills in both equine internal medicine and reproduction. During 23 breeding seasons (2002-2024), 2433 ovulations from donor mares, of different breeds and ages, were recorded out of 1703 cycles (mean: 74 cycles/year): occurrence of single (1OV), double (2OV), triple (3OV), and quadruple (4OV) ovulation was 72.6% (N=1236), 24.9% (N=424), 2.4% (N=41), 0.1% (N=2), respectively. Uterine flushing for embryo recovery was performed usually 8 days after ovulation and resulted in the recovery of 859 embryos (50.4% embryos/cycle and 35.3% embryos/ovulation). Embryo recovery rates per cycle were higher when more ovulations were detected (1OV: 43.8% vs 2OV: 66.5% vs 3OV: 85.4%, respectively: $P<0.001$). Differently, embryo recovery rates per ovulation were significantly lower in 3OV and 2OV compared to 1OV (28.4% and 33.2% vs 43.8%, respectively: $P<0.001$). This paper aims to report an extraordinary case of simultaneous recovery of 5 embryos from a single cycle of a donor mare, considering the embryo recovery rates observed in the previous 1703 cycles at the Veterinary Teaching Hospital of the University of Pisa.

Methods

In June 2024, a 9-year-old Belgian Warmblood donor mare was referred to the Veterinary Teaching Hospital of the University of Pisa for embryo recovery and transfer. The estrous cycle of the mare was monitored via ultrasound examination (US) by a private veterinarian. At the detection of follicles of diameter greater than 35 mm, ovulation was induced with hCG (Corulon®, 2500 IU, IV). The day after the induction, artificial insemination (AI) was performed with cooled fresh semen of a stallion of proven fertility shipped overnight from Belgium. The day after AI, 4 ovulations were detected.

Results
Eight days after ovulations, uterine flushing was performed, and 5 blastocysts were recovered. The embryos were evaluated, using a stereo microscope, in terms of developmental stage, quality, and size: all the embryos were blastocysts of excellent quality, while the diameter was relatively different (550.0±279.3 μm), suggesting some level of asynchrony in the ovulations' timing. The embryos were non-surgically transferred into 5 recipient Standardbred mares, aged between 3-7 years old, mostly pluriparous, from 5 to 7 days post-ovulation, previously checked via transrectal palpation and US to assess the quality of the tone of the uterus and cervix, and of structure and vascularization of the corpus luteum. Three recipients became pregnant and the pregnancies are all still ongoing.

Discussion and conclusions
To the best of the Authors' knowledge, this is the first report about the recovery of 5 embryos from a single uterine flushing of a not-superovulated donor mare. However, although the collection of more than one embryo represents a desirable event, this could be a problem in breeds in which the Studbook does not allow multiple pregnancies. Furthermore, this case underlines how fundamental is to have many recipient mares available when undergoing an equine ET program, highlighting the importance of a close collaboration between private practitioners and Veterinary Teaching Hospitals

Keywords: embryo, superovulation, uterine flushing, multiple ovulations, embryo transfer, mare

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Different reproductive rhythms and housing systems for improving welfare in rabbit does

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Introduction and aim

In wildlife conditions, the rabbit (*Oryctolagus cuniculi L.*) is a gregarious animal with social units that include, on average, 2–9 females, 2–3 bucks, and their progeny (1). In commercial farm conditions, breeding does are individually housed in standard wire cages with an external nest (2).The most widespread reproductive rhythm practiced in rabbit farming is based on artificial insemination (AI)(3) performed at around 11 days postpartum. This approach results in high production rates but requires high yearly replacement of animals that cannot sustain the enormous energy demand. Rabbit does that are in energy deficit have relatively short reproductive careers. Moreover, current housing systems do not allow animals to express the typical behavioural pattern of the species (4), with presence of abnormal behaviours that contribute to reduce welfare. Thus, the present study aimed to evaluate the best combination of housing systems and reproductive rhythms through multiple indicators (performance (5), behaviour, corticosterone (6) assessments) to improve does' welfare.

Methods

A total of 110 nulliparous rabbit does of the Grimaud hybrid maternal line were randomly allocated to 2 different housing systems, an innovative type of cage, Combi cage (C),vs. the conventional cage currently used in intensive farming, Standard cage (S), within which 3 reproductive rhythms: Intensive (I), with AI 11 days postpartum; Alternating (A), with AI alternated between 11 and 30 days postpartum; or Extensive (E), with AI 30 days postpartum, were applied. All rabbits underwent 6 consecutive reproductive cycles, according to a multi-factorial balanced experimental plan (2 × 3), as follows: CI (20 does) vs. CA (20 does) vs. CE (20 does) vs.SI (5 does) vs. SA (5 does) vs. SE (5 does). At the main critical phases (AI, kindling, and peak lactation), we conducted reproductive, behavioural, and salivary corticosterone (6) assessments. All animals were handled in accordance with the Turin University Bioethics Committee recommendations (Prot. N 256053 of 4/07/2017).

Results

CE group showed higher motor activity, lower feeding rates, and increases in number of live kits and weaned kits, which resulted in better reproductive performance in the C cages. The SA group displayed the highest number of live-born kits/litter ($P < 0.02$), the highest total weight of the weaned litter ($P < 0.01$), and the lowest pre-weaning mortality ($P < 0.04$) among S cages. The housing system also influenced behaviour: S does displayed the highest frequencies of self-grooming ($P < 0.01$), feeding ($P < 0.001$), and stereotypical behaviours (sniffing and biting bars, $P < 0.01$), which indicated frustration from a lack of stimuli and consequent boredom. Statistical analyses were performed with the SPSS 16.0 software package, using the two-way variance analysis (ANOVA) and setting $P<0.05$.

Discussion and Conclusion

The lack of space could have let to excessive increases in weight and BCS and a consequent lower conception rate, as the reproductive career progressed (7). Our study supported this hypothesis among does in CE group, that showed higher motor activity, lower feeding rates and increases in number of live and weaned kits, which resulted in better reproductive

performances in the C cages. Based on our results, we concluded that AI after kit weaning (E) was the best reproductive rhythm for does in C cages, and the A rhythm was best for does in S cages. However, from an ethological point of view, in the S cages, stereotypes related to the small size of the housing system remain.

Keywords: Rabbit does, housing system, reproductive rhythm, welfare, performance.

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INOS and TSHR localization in follicles and corpus luteum during development in Bruna cow ovaries

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Introduction and aim

Numerous studies on both animal and human ovaries have demonstrated that thyroid-stimulating hormone receptor (TSHR) and inducible nitric oxide synthase (iNOS) play a crucial role in regulating ovarian functions such as folliculogenesis, oogenesis, ovulation, puberty onset and the lifespan of the corpus luteum (CL) (1-6). This study aimed to examine the immunolocalization of TSHR and iNOS in the ovarian follicles and in the corpus luteum (CL) to determine if their expression is influenced by their different developmental stages in the cow's ovary.

Methods
Ten ovaries were collected from 5 Bruna cows. The estrus cycle phases have been determined post-mortem (7). Three of them were cyclic (follicular, early luteal and late luteal phases), aged between 12-24 months, two were acyclic (prepubertal and in anestrus, 10 months and 8 years old, respectively) and the ovaries have been classified based on the presence or absence of the CL, in CL+ and CL- ovaries. The immunohistochemical (IHC) processing for iNOS and TSHR has been performed. The evaluation of the peroxidase reaction has been done by quantifying the intensity of staining and classifying it by gradation (from negative to strongly positive).

Results
Our results confirmed the presence of TSHR and iNOS in various structures of the bovine ovary in their different developmental phases. Also, the primordial, primary, secondary, terziary follicles within the same ovary showed up a different mmunolocalization. The immunostaining was present or absent in both the germinal and somatic follicular components with different combinations. Specifically, follicles in the same developmental stage within the same ovary either expressed or did not express TSHR and iNOS, indicating phases where expression was silenced. The iNOS reaction was strongly present also in the wall of corpora atretica, suggesting a role during the follicle atresia processes by a crosstalk with the inflammatory infiltrate. Furthermore, the research revealed the immunolocalizaton of TSHR and iNOS in the CL, in which the expression changed based on the different developmental phase: the protein immunolocalization was strongly present in the large luteal cells during the late diestrus with a decrease in the subsequent estrus phases until a total silencing for the TSHR in the corpus albicans.

Discussion and Conclusions
Our results may indicate that these proteins play a critical role in the exit from dormancy and, consequently, in the development or atresia of follicles and oocytes. In addition, their different expression in the different CL stages may imply for these proteins a putative involvement in the growth, maintenance, and regression of bovine luteal tissue. The findings validated the role of the

iNOS-nitric oxide (NO) and TSHR pathway in regulating the dynamic vascular processes associated with the morpho-functional changes in the bovine ovary.

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Mini Review
7-station OSCE model in animal reproduction education

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Citation
Tekin, K., Adaca, A.Ü. 7-station OSCE model in animal reproduction education.

Abstract
Objective Structured Clinical Examinations (OSCEs) are a hands-on, practical form of testing that provides a standardized and objective method to assess students' clinical competence (1). They consist of a series of stations where candidates are observed and assessed as they go through a series of clinical tasks (2). Despite its widespread adaptation in advanced veterinary schools, there is limited scientific documentation of OSCE implementation in Türkiye. This mini-review examines the establishment of a 7-Station OSCE for evaluating veterinary students' skills in animal reproduction at Ankara University. This initiative addresses a significant gap in the Turkish veterinary curriculum, promoting a fair and comprehensive assessment methodology (3).

The OSCE blueprint is critical for structuring the examination, encompassing seven distinct stations that assess various practical skills in animal reproduction. Input from several experts was incorporated to ensure comprehensive coverage and relevance (4). Each blueprint detailed the station's content, the rater's and students' characteristics, the exam

environment, and the materials used (5). The development phase included extensive consultations with faculty members, experts in veterinary reproduction, and educational technologists to ensure that the examination would be both comprehensive and practically feasible (6).

The 7- Station OSCE Model included tasks such as veterinarian-client communication, history taking in cryptorchidism, breeding soundness examination, monitoring techniques (smear, USG, blood), desicion making "spay or not" (surgical or nonsurgical), timing of insemination or natural mating, and pregnancy diagnosis. Each station was designed with specific objectives and skill sets in mind. These stations were designed to assess a range of skills from basic communication to advanced clinical techniques (7). The veterinarian-client communication station, for instance, involved interactions with standardized clients to simulate real-world scenarios, thereby testing students' interpersonal and diagnostic skills (8).

The implementation of the OSCE involved a variety of materials, including standardized clients, 3D-printed organ models, hybrid simulators, and real patient images (9). Each station's duration ranged from 5-10 minutes, allowing a thorough yet efficient assessment of student skills (10). The use of 3D-printed models and hybrid simulators represented a significant advancement in veterinary education, providing students with realistic and repeatable practice opportunities (11). Standardized clients were trained to provide consistent responses, ensuring that each student faced comparable challenges (12). The 3D-printed organ models allowed for realistic practice of clinical procedures, enhancing the practical learning experience (13). Hybrid simulators, which combined physical models with digital feedback, provided real-time guidance and assessment, improving skill acquisition and confidence (14).

The evaluation process focused on both knowledge application and practical execution, providing a balanced assessment of student capabilities. Each station required a minimum of seven skills and a maximum of ten skills to be demonstrated by the students (15). The assessment criteria were meticulously designed to capture various aspects of clinical competence. Checklists

and global rating scales were employed to evaluate students' performance objectively (16). The checklists ensured that all critical steps were assessed, while the global rating scales provided an overall judgment of competence (17). This combination of assessment tools allowed for a comprehensive evaluation of both technical skills and professional behaviors (18). The results indicated that students found the OSCE to be a fair and comprehensive assessment method, with positive feedback regarding the realism and relevance of the stations (19).

The impact of OSCE extends beyond individual student assessment. It promotes a culture of continuous improvement and reflective practice among educators and students alike (20). By providing detailed feedback, OSCE encourages students to identify their strengths and areas for improvement, fostering lifelong learning and professional development (21). Additionally, the structured nature of OSCE facilitates benchmarking and standardization of assessment practices across institutions, contributing to the overall advancement of veterinary education (22).

The introduction of OSCE in the veterinary curriculum at Ankara University marks a significant advancement in educational assessments. It highlights the importance of incorporating current methods and technology in veterinary education to ensure comprehensive skill development (23). This initiative sets a precedent for other institutions in Türkiye and potentially worldwide (24). The use of standardized clients and advanced simulation technologies can bridge the gap between theoretical knowledge and practical application, enhancing the overall educational experience (25). By providing a rigorous and objective assessment framework, OSCE can help ensure that veterinary graduates are well-prepared to meet the challenges of clinical practice (26). Moreover, the success of this initiative could inspire other veterinary schools to adopt similar approaches, leading to a more standardized and comprehensive evaluation system across the field (27). The success of this OSCE model suggests that similar approaches could be beneficial in other areas of veterinary education, such as surgery, internal medicine, and emergency care (28). Another potential area of investigation is the adaptation of OSCE to assess other competencies, such as ethical decision-making, informed consent, and veterinarian-client communication (29).

While the implementation of OSCE in veterinary education has shown promise, it also presents several challenges. The development and maintenance of high-quality simulation materials, the training of standardized clients, and the logistical aspects of administering the OSCE require significant resources (30). Future research should focus on evaluating the long-term impact of OSCE on student performance and professional competence. Additionally, exploring the integration of newer technologies, such as virtual reality and artificial intelligence, could further enhance the assessment process (31).

The 7-station OSCE model in animal reproduction education at Ankara University represents a pioneering effort in Turkish veterinary education. This structured and objective assessment method not only evaluates theoretical knowledge but also practical skills, ensuring a holistic evaluation of students. Future research should focus on the long-term impact of OSCE on veterinary education and explore the integration of additional technological advancements.

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Impact of selenium on male animal reproduction: A comparison of organic and inorganic forms

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Citation
Selçuk, M., Nizam, M.Y. Impact of selenium on male animal reproduction: A comparison of organic and inorganic forms.

Abstract
Selenium (Se) is a trace mineral that plays a crucial role in many physiological functions. It is involved in antioxidant defense, reproductive health, thyroid metabolism, and immune response. Se exists in both inorganic and organic forms in nature and is absorbed by plants from the soil. When soil Se levels are insufficient, Se supplementation in animal feed is necessary. The most common inorganic Se source is sodium selenite. The USA and EU allow specific Se levels in animal feeds. Se has significant effects on male reproductive health. Dietary Se reaches high concentrations in the testes, increasing tissue Se levels. Organic Se is more effective than inorganic Se in this regard. Se positively impacts testosterone levels, spermatogenesis, and overall semen quality. Ensuring optimal Se levels, particularly in regions with low soil Se, is critical for animal health and productivity.

Introduction

In nature, Se exists in both inorganic and organic forms. Inorganic Se appears in three oxidation states: selenite (Se^{4+}), selenate (Se^{6+}), and selenide (Se^{2-}). Plants absorb selenite or selenate from soil to produce Se-containing amino acids. Soils with insufficient Se or Se forms inaccessible to plants, or the presence of substances in plants that bind Se, can reduce Se availability (1). Thus, supplementing animal feed with Se is necessary in regions with low soil Se levels. Se supplements in animal feed can be either inorganic or organic. Sodium selenite is the most cost-effective inorganic source and is widely used in animal diets. The United States FDA allows up to 0.3 ppm of inorganic Se in feeds for chickens, turkeys, ducks, swine, sheep, and cattle (2). Recently, 0.3 ppm of organic Se as selenized yeast has been approved for use in feeds for chickens, swine, turkeys, and cattle (3). In the EU, the permitted Se supplementation level in animal feeds is 0.5 mg/kg, while Polish swine feeding standards allow 0.1–0.3 mg/kg Se (4). Inorganic Se is absorbed and metabolized similarly to other trace minerals, whereas organic Se is utilized like many amino acids. Organic Se absorption is slower than inorganic forms (5) and is non-toxic at higher doses.

Effect of Selenium on Male Reproductive Performance

In male Baladi goats, feed supplemented with 0.15 ppm organic Se significantly increased testosterone secretion compared to a control group on a basal diet (6). Se deficiency in rats was associated with significant reductions in testicular weight, impairing reproductive performance (7). Bulls injected with 50 mg Se, followed by 30 mg after three weeks, exhibited elevated Se concentrations in the testes and seminal vesicles, but not in the epididymis (8). Generally, vitamin E has synergistic effects with Se. Ali et al. (2009) observed that vitamin E plus Se improved the reproductive performance of rams more than vitamin E alone (9) . In male rats, excessive dietary Se (6.0 and 8.0 ppm of sodium selenite) led to reduced body weight, testicular and cauda-epididymal weight, and spermatogenic cell numbers, as well as decreased seminiferous tubule diameter, lumen diameter, seminiferous epithelial height, and cauda-epididymal tubule diameter (10).

Spermatogenesis

Adequate Se levels in the male reproductive tract are essential for normal spermatogenesis, with Se playing a fundamental role in sperm maturation. Both deficient and excessive Se levels disrupt sperm maturation. As semen quality and fertility depend on sperm maturation, any abnormal process can lead to poor semen quality and reduced fertility in males. Se has striking effects on the enzyme system of the male reproductive tract (11). Precise tissue Se concentrations in male reproductive organs are not well-known, but the testes are suggested to have the highest Se concentration. Dietary Se at various supplementation rates significantly increases tissue Se concentrations (12, 13). However, 0.3 ppm dietary sodium selenite does not increase Se tissue concentrations as much as 0.3 ppm organic Se, since a larger proportion of inorganic Se is incorporated into selenoproteins (14). Se functions in the reproductive tract independently of other physiological processes in the body (15) . Selenium concentration was significantly higher in the seminal plasma of rams fed a diet containing 0.5 ppm organic Se compared to those receiving 0.2 ppm organic Se (16).

Semen Characteristics

Marai et al. (2009) reported that 0.1 ppm sodium selenate improved semen quality in rams by increasing ejaculate volume, sperm motility and concentration, and reducing the percentage of dead spermatozoa, sperm abnormalities, and acrosome damage (17). Edens and Sefton (2009) noted that broiler breeder roosters lacking dietary Se had a high percentage of abnormal spermatozoa at 26 weeks, unlike those given feed with 0.2 mg/kg sodium selenite or organic Se (Sel-Plex), which had 30% and 40% more normal spermatozoa, respectively, than controls. Broiler breeder roosters fed 0.2 mg/kg organic Se had the lowest sperm midpiece and head abnormalities. At 32 and 42 weeks, roosters on 0.3 mg/kg organic Se (Sel-Plex) showed higher percentages of normal sperm, better semen quality indices (SQI), and lower percentages of dead spermatozoa and sperm midpiece and head abnormalities compared to those fed 0.3 mg/kg sodium selenite (18). Baiomy et al. (2009) found that Ossimi rams receiving 0.5 mg organic Se per kg of feed had higher semen concentration, semen volume

per ejaculate, sperm motility, motile spermatozoa per ejaculate, and total sperm count than those on 0.2 ppm organic Se (16).

Conclusion

Selenium (Se) is indispensable for numerous physiological processes, notably in antioxidant defense, reproductive health, and various metabolic functions. Its role in male reproductive health is particularly significant, influencing testosterone levels, spermatogenesis, and overall semen quality. The evidence suggests that both organic and inorganic forms of Se are beneficial, although organic Se generally demonstrates greater efficacy in increasing tissue Se concentrations and improving reproductive outcomes. Moreover, Se supplementation, especially when combined with vitamin E, can enhance reproductive performance by improving sperm motility, morphology, and reducing abnormalities. Therefore, ensuring adequate Se levels through dietary supplementation is crucial, particularly in regions with low soil Se levels, to maintain optimal animal health and fertility. Given the reproductive efficiency improvements observed in cows supplemented with organic selenium in the form of Se-yeast, it is appropriate to research its potential positive effects on fertility parameters in male animals. Further research may focus on refining supplementation strategies to maximize the benefits of Se in animal husbandry and potentially in human health contexts.

Keywords: male animal, reproductive health, semen, selenium

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Role of microRNAs in bull spermatogenesis, fertilization and early embryonic development: A mini-review

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Yüksel, M., Akyüz, B., Arslan, K. Role of microRNAs in bull spermatogenesis, fertilization and early embryonic development: A mini-review.

Abstract
Estimation of bull fertility is one of the most important factors determining optimum productivity in cattle breeding. Numerous studies have been promising on the potential effects of bull microRNAs (miRNAs) on predicting bull fertility. Based on the existing evidence, bull germ cell differentiation occurs through the well-coordinated miRNA profiles of spermatogenesis. This compilation of studies has also shown promise in using miRNAs as markers for sire conception rate and non-return rate. The fact that sperm-borne miRNAs continue to have effects throughout preimplantation and embryonic development highlights the importance of individual bull fertility.

Introduction

As artificial insemination becomes more widely used, the fertility of a single bull may have a significant impact on the reproductive success of thousands of female cattle (1). Molecular biomarkers provide a promising solution to current challenges in bull fertility prediction by directly linking to the etiology behind fertility failure (2). MiRNAs that can produce phenotypic effects by regulating gene expressions are endogenous and non-protein-coding RNAs that are approximately 22 nucleotides in length (3). Spermatozoal miRNAs have been considered to be associated with bull fertility due to their functionality in sperm development, oocyte activation, fertilization and subsequent embryonic development (4). Several studies have reported miRNAs expression related to sperm quality or the profile of fertility in bulls. This mini-review considers the field of bull miRNAs as the focus of studies in the processes of bovine spermatogenesis, fertilization and early embryonic development.

MicroRNAs in Spermatogenesis

Spermatogenesis is the process of producing sperm from mature haploid spermatozoa or diploid spermatogonial stem cells. The development process is complexly managed and executed with precision and timeliness. In mammals, the process of spermatogenesis is regulated by transcriptional and post-transcriptional mechanisms (5, 6). Expression profiling studies of miRNAs that play a role during the mitotic, meiotic and post-meiotic stages of spermatogenesis have identified different miRNAs in mammal testicles, including those expressed in male germ cells (7). Pre-fertilization miRNAs are found in both testicular spermatogonia, Sertoli cells, Leydig cells, and mature spermatozoa. This demonstrates their role in normal testicular development, function and spermatogenesis (8).

Xu and colleagues have shown that the down-regulation of bta-miR-449a in bulls prevents male reproductive cells from transitioning from the mitotic phase to the majonic phase (9). High expression of bta-miR-146b in bull testicles has been found to be associated with proliferation and apoptosis of bull germline stem cells (1). Sun et al. have demonstrated that miR-34c has a crucial role in regulating bull spermatogenesis. MiR-34c directly

regulates the AXL gene by targeting a sequence in the 3'-UTR region. This situation has a role in controlling proliferation, programmed cell death, and relative transcripts abundance gene in bovine Sertoli cells (10). Bta-miR-6531 overexpression in Leydig cells of bovine testicles has affected the intracellular calcium concentration by restraining ATP2A2 gene expression. These results have demonstrated that bta-miR-6531 could play a role in regulating sperm mobility (11). Sahlu and colleagues have presented detailed findings that provide important information to enhance the reproductive capacity of male mammals by studying the specific roles of miRNA and mRNA in spermatogenesis during testicular development in young and mature Holstein bulls (12).

MicroRNAs in Fertilization and Early Embryo Development

Fertilization is a complex series of events that irreversibly change male and female gametes, resulting in the fusion of the paternal and maternal genomes in the zygote. During fertilization, the sperm undergoes the loss of its acrosome and plasma membrane components, so it transmits chromosomes, centrioles, perinuclear theca proteins, and regulatory RNAs, including miRNAs, to the zygote (13).

A study on the Holstein breed examined the expression profiles of 178 miRNAs in spermatozoa from bulls with high and medium non-return rates. Additionally, the authors determined seven sperm miRNAs (miR-502-5p, miR–1249-3p, miR–320a, miR–34c-3p, miR–19b-3p, miR–27a-5p, miR–148b-3p) that had lower expression in the high-fertility bulls (14). Sperm-borne miRNAs are thought to be essential for the development of preimplantation embryos. For example, miR-365-2 acts as a negative regulator of the BCL2 gene and consequently allows a fertilized oocyte to progress to the dividing stage (15). Sperm-borne miRNAs were investigated in two-cell embryos produced by bull sperm, which were divided into two groups as high and low-fertile according to pregnancy rates. MiR-33b, miR–126-5p, miR–205, miR–500, miR–505, miR–532 and miR–542-5p were found to have lower expression in spermatozoa from high-fertility bulls. Especially miR-216b has been expressed at lower levels in high-fertility sperm cells and zygotes. Research has shown that this modulation may effectively

control early development by disrupting the first division and improving the blastocyst quality (16). Menezes et al. have demonstrated that low-fertility bulls expressed higher levels of miR-15a and miR-29 in their sperm compared to high-fertility bulls (17). Keles et al. have conducted a study on high and low fertility groups, measuring sperm motility parameters and the non-return rate. They investigated the miRNA profiles of both groups and found that miR-10a-5p and miR-9-5p were expressed differently in low and high fertility bulls (18). Donnellan et al. investigated high and low fertility in Holstein-Friesian bulls based on conception rate. Six sperm miRNAs were found to be up-regulated; seven sperm miRNAs were found to be down-regulated in bulls with high fertility (19). Kasimanickam et al. compared Holstein bulls with high and low fertility based on sire conception rates in their article. Consequentially, 33 sperm miRNAs were up-regulated, while 23 sperm miRNAs were down-regulated in high-fertility bulls (20).

Conclusion

This mini-review has emphasized that the miRNA expression profiles of bulls are important in the processes of spermatogenesis, fertilization and early embryonic development. However, studies for identifying miRNA expression and its mechanisms during the stages of spermatogenesis remain uncertain. Furthermore, few studies have investigated the effects of bull spermatozoal miRNAs on embryonic development. This data suggests that we still have a long way to go with bull fertility-related miRNAs. MiRNAs play an important role in both spermatogenesis and the fertilization process of the oocyte and they can even affect the phenotype of the offspring.

Keywords: bull, miRNA, spermatogenesis, fertilization, early embryo development

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Hormonal influence on vaginal epithelial changes in Italian Mediterranean Buffaloes

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Felice, D.D., Spada, S., Aires, L.N., Kosior, M., Matera, R., Russo, M. Hormonal influence on vaginal epithelial changes in Italian Mediterranean Buffaloes.

Vaginal cytology is an important examination method for diagnosing gynecological disorders and staging the oestrus cycle in female dogs (1). It provides insights into oestrogen's effects on epithelial changes and the overall health status of the vagina. Sampling cells, preparing and staining a smear can be performed quickly and inexpensively in routine clinical practice (2,3). Detecting ovulation in buffaloes may be challenging due to the lack of specific clinical signs, making laboratory tests and ultrasound necessary to assess cycle stage. While vaginal cytology revealed a useful method to detect

hormonal changes in goat (4) and bovine (5), it is not effective in sheep (6). To our knowledge, no data are available in the literature regarding its use in buffaloes. This study aims to explore the potential of vaginal cytology for predicting hormonal changes in buffaloes.

For the present study, ten healthy buffalo heifers were synchronized using a modified OV-Synch method, organized as follows: animals were treated with 200 UI Pregnant Mare Serum Gonadotropin (PMSG) for 5 days prior to the classic OvSynch protocol, in which Day 1 corresponded to the first administration of PMSG and Day 6 the first administration of Gonadotropin-Releasing Hormone (GnRH).

The heifers were evaluated at day 1, 3, 5, 12 and 15 using a standardized protocol that included clinical examination, vaginal cytology and blood collection for hormonal assessment. For vaginal cytology, a 15-cm-long cotton-tipped sterile swab was inserted into the vagina to a depth of about 5 cm, ensuring cells were sampled from the vaginal roof. The swab was then removed, rolled onto a clean glass slide, and stained using a modified Wright- Giemsa method.

The smears were then scanned to perform measurements and define the cell types present following the flowchart published by Reckers et al. (2022) (1). Cellular length, nuclear area size, presence of cornification lines, presence or absence and grade of degradation of the nucleous were used to distinguish the following cell types; parabasal cells (P), intermediate cells (I), superficial cells (S) and anuclear cells (A). Presence of neutrophiles, erythrocytes and clusters was recorded.

Ten ml of blood were collected from the jugular vein into vacutainer tubes (lithium heparin anticoagulant). Samples were centrifuged at 1500 × g for 15min and the plasma was aliquoted into Eppendorf tubes (1mL) and stored at –20°C. Oestradiol and progesterone levels were assessed for all specimens.

Statistical analyses, including the Shapiro-Wilk test to assess data distribution, were conducted. Non-parametric tests were used, specifically the Friedman test to detect the effect of time on vaginal epithelial changes and the Wilcoxon signed-rank test to find any difference among the days. Spearman correlation tests were performed between hormonal levels and number of S and A cells.

For all animals, it was possible to identify all four cell types, predominantly represented by I cells. Even with low progesterone and estradiol levels, P cells were extremely low in numbers. Neutrophiles were occasionally found, especially after the placement of the progesterone releasing intravaginal device (PRID). Cellularity was consistently high and interestingly no erythrocytes were found in any smear. Effect of time was detected when looking at superficial and anuclear cells, which tended to decrease from day12 (PRID removal) to day15, characterized by a reduction in progesterone levels. A strong positive correlation was found on day5 between oestradiol concentration and the number of S cells ($r=0.7$; $p=0.04$), and on day1 between oestradiol and A cells ($r=0.87$; $p=0.001$). Additionally, high numbers of superficial cells were always associated with high levels of oestradiol. However, no correlation was observed between concentration of any cell type and progesterone levels.

The present study showed promising results concerning the use of vaginal citology to detect hormonal changes. The increased number of superficial cells related to the high concentration of oestradiol is in accordance with other studies (5,7), and may favour its use for a potential detection of ovulation. Vaginal epithelial changes were similar to goats and bovines, as low quantity of anuclear cells and erythrocytes were detected (5,6). The use of objective measurements enabled to characterize more precisely the percentage of cell type present. Further studies should be performed to determine whether these changes occur even with non-synchronized females and whether the method may be useful to detect the day of ovulation in this species.

Keywords: Vaginal citology, buffaloes, cells, hormones, oestrus cycle.

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Isolation, characterization and freezing of mesenchymal stem cells from bovine fetal bone marrow

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Bucak, M.N., Akçali, K.C., Zeytin, I.C., Yilmaz, H., Özer, Z.B., Dastouri, M., Ataman, M.B. Isolation, characterization and freezing of mesenchymal stem cells from bovine fetal bone marrow.

Induced Pluripotent Stem Cells (iPSC), developed by reprogramming somatic cells, have potential for applications in many areas of human and animal health. IPSCs are a promising cell type for animal health with clinical applications such as administration of mesenchymal cells to tissue areas with impaired functional structure, spermatozoon and oocyte production. The first step in producing iPSCs is the isolation of isomatic cells, such as mesenchymal cells, which are reliable, uncontaminated, and well-characterized. In our study, bovine mesenchymal stem cells were isolated from fetal bone marrows. The fluid aspirated from bone marrows of femur

or humerus bones was centrifuged for 5 minutes at 1500 rpm in DMEM low glucose media containing antibiotics and amfoterisin B. The pellet was dissolved in 10% FBS, 2% penicillin-streptomycin, 7µL/mL amfoterisin B and 2% L-lutamine, and seeded in flasks. The cells were cultured in an incubator at 37°C and 5% CO_2. The cells that reached confluence were passaged with 0.5% trypsin. The PCR method was used to characterize the cultured cells. Firstly, RNA isolation was performed from the cells. Then, cDNA was synthesized after measuring the amount of RNA. In general, 5 different markers are used in mesenchymal stem cell characterization. Cells that are positive for CD29, CD73 and CD90, and negative for CD34 and CD45 are defined as mesenchymal stem cells. After the PCR reaction performed with these markers, the products obtained were run on the agarose gel and visualized. When the bands seen in the CD29, CD73 and CD90 wells, and not in the CD34 and CD45 wells, the cells were shown to be mesenchymal stem cells. In order to obtain induced pluripotent stem cells through reprogramming in the future, the isolated and characterized mesenchymal stem cells were frozen. Freezing media containing 10% FBS, 10% DMSO and 80% DMEM low glucose media was used to freeze these cells. Freezing media was added to the cell pellet in the cryovial. The cryovials were kept at -80°C for 24 hours in a box, then placed in a liquid nitrogen tank. Cells stored in this way have the potential for using for different experiments after thawing process.

Keywords: Bovine fetal bone marrow, Cell freezing, Mesenchymal stem cell

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Plasma metabolic parameter level changes in superovulated cattle on different FSH treatment days

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Okuroğlu, A., Tirpan, M.B., Özen, D., Sevgi, R., Yilmaz, M.A., Ünal, I., Coşkun, M.I., Ünay, E., Kaya, U., Satilmiş, M. Plasma metabolic parameter level changes in superovulated cattle on different FSH treatment days.

Introduction and aim

This study aims to determine the difference of some metabolic parameter levels in superovulated Holstein cattle with more than 5 embryos and less than 5 embryos. Moreover, the alteration in the parameters on the 1st and 4th days of FSH application were examined in this study.

Methods

In this study, 14 Holstein donors with similar milk yields and feding requime were treated with a superovulation protocol, artificial insemination, and flushing. Then, the donors were classified into two equal groups based on produced more than five (5>) (n=7) or less than five (<5) (n=7) embryos. Blood samples were collected from the tail vein on the 1st and 4th days of FSH administration. Plasma Glucose (GLU), Non-esterified Fatty Acid (NEFA), Cholesterol (CHO), Blood Urea Nitrogen (BUN), and Total Protein (TP) levels were determined by ELISA commercial kits. The mixed model analysis of variance was used for statistical analysis. The plasma levels of the parameters were compared according to the 1st and 4th days of FSH applications within the groups and between the groups.

Results

In results, a statistical difference was determined only in plasma cholesterol levels, while there was no difference in plasma NEFA, GLU, BHB, BUN, TP levels. Plasma cholesterol level was higher in (>5) group than the (<5) group. On the other hand, no difference was determined between plasma NEFA, GLU, CHO, BUN, TP levels, while there was a statistical difference between plasma BHB levels. In both groups, plasma BHB levels were higher on the 4th day of FSH treatment than the 1st day of FSH treatment.

Conclusion

It can be concluded that FSH may not have a significant impact on altering plasma GLU, NEFA, CHO, BUN, and TP levels. However, plasma CHO levels may change according to the number of embryos obtained.

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Containment of hybrid wolf through surgical infertilization in Life Wolfalps EU project

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Citation
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Introduction and aim

Anthropogenic hybridization is widely considered a threat to biodiversity conservation. Regarding wild wolves (Canis lupus), hybridization with domestic dogs is quite common in Europe and central Italy, while in the Alps, the phenomenon is potentially manageable due to the low number of cases, although timely intervention is necessary to control the presence of hybrid individuals. Traditional control procedures include culling or gonadectomy, but more recently, the interruption of genital pathways (infertilization) has been proposed. The opposition to the elimination of hybrids, due to public disapproval, is a clear issue that has been addressed by the scientific community. The Life Wolfalps EU project, approved by ISPRA, has also tackled this issue with action C5, providing guidelines for fertility control as proposed by CANC.

Methods

The infertility procedure was carried out by CANC and involves the interruption of the reproductive tract (vasectomy or salpingectomy).

Four hybrid wolves, two males and two females, were captured during three sessions (October 2022, April 2023, and October 2023). All subjects were premedicated with a mixture of tiletamine and zolazepam (Zoletil 100®, 50/50 mg/ml, Virbac S.r.l, Milan) at 8.7 mg/kg i.m., and induction was performed with intravenous administration of Propofol (Proposure®, 10 mg/ml, Boehringer Ingelheim Animal Health Italy S.p.a) at 2.5 mg/kg. Maintenance was performed with isoflurane via a tube in pure oxygen, and during the entire procedure, the animal remained connected to a multiparametric monitor (Infinity Delta®, Dräger Italia S.p.A., Corsico, Italy) for intraoperative monitoring of vital signs.

In male subjects, a laparotomic vasectomy was performed due to its minimal invasiveness and very short execution time with intradermal suturing at very close intervals. In the two females, the minimally invasive method of salpingectomy was opted for, involving the opening of three 3-5 mm surgical breaches, which then healed by second intention without the application of sutures. The intervention time was longer compared to the laparotomic technique, but the reduced invasiveness allowed for quicker post-surgical recovery and faster release.

At the end of the intervention, 8 mg/kg cefovecin (Convenia®, Zoetis Italia S.r.l., Rome) were administered to ensure two weeks of antibiotic coverage; analgesia was provided subcutaneously with Carprofen (Rimadyl®, Zoetis Italia S.r.l., Rome). The males, equipped with a GPS collar positioned during anesthesia, were released in the center of the pack's territory within three hours of awakening and reunited with the other members without being attacked or expelled. The two females are housed in captivity at the "Uomini e Lupi" center in Entracque (CN – Italy) for monitoring of sexual hormone patterns following the salpingectomy.

Discussion and conclusions

Surgical fertility control as an alternative to euthanasia is considered an acceptable method by the public for managing wild animal populations. Since wolves exhibit significant social and hierarchical characteristics induced

by hormones, the depletion of hormonal stimuli following gonadectomy would cause a profound alteration in pack behavior. Therefore, orchiectomy and ovariectomy are not considered appropriate methods. On the other hand, the interruption of the reproductive tract, preserving the gonads, allows for the control of the fertility of captured subjects without altering their behavioral characteristics, as confirmed by their return to the packs. Complications, especially in females such as paraovarian cysts reported in older domestic dogs, need to be verified. Since the results of this initial phase of the project were encouraging, further capture sessions will be planned for the management of hybrids through infertility.

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Calcium's impact on calving, first AI, parity and BCS in cows with subclinical hypocalcemia

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Arslan, S., Yenilmez, K. Calcium's impact on calving, first AI, parity and BCS in cows with subclinical hypocalcemia

Introduction and aim
This study aimed to evaluate the relationship between calcium (Ca) levels and the interval between calving and first insemination, parity, and Body Condition Score (BCS) in Holstein cows diagnosed with Subclinical Hypocalcemia (SCH).

Methods
The study was conducted on 60 clinically healthy cows, which had recently calved and did not suffer from puerperal diseases (dystocia, retained placenta, endometritis, laminitis, displaced abomasum), with parities ranging from 1 to 5. All animals were kept under the same care, feeding, and reproductive management conditions and were fed ad libitum twice a day with a Total Mixed Ration (TMR) specifically designed for the transition period. Clean drinking water was continuously available. Blood samples were collected from these cows on the 10th day postpartum. The blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which

was then stored at -80°C until analysis. Calcium measurements from the samples were performed using an autoanalyzer (Randox, RX imola. Crumlin, United Kingdom). Cows with a serum total Ca level of <8.6 mg/dL were classified as SCH (n=30), and those with a serum total Ca level of >8.6 mg/dL were classified as normal (n=30).

Results

The incidence of SCH was calculated to be 50%. SCH cows were divided into 5 groups according to their lactation numbers. It was found that SCH occurred at a rate of 16.6% in the 1st lactation, 26.6% in the 2nd lactation, 30% in the 3rd lactation, 16.6% in the 4th lactation, and 10% in the 5th lactation. The interval from calving to first insemination was calculated in days using farm records. No significant difference was found between the groups in terms of Ca and BCS values ($p>0.05$). No correlation was found between Ca levels and BCS. It was determined that the incidence of SCH was widespread and that parity and BCS did not affect the risk of SCH. Additionally, the interval from calving to first insemination was significantly ($p<0.05$) higher in SCH cows (122.00 $\pm$ 9.71 days) compared to normal cows (96.55 $\pm$ 19.17 days).

Discussion and conclusions

In conclusion, SCH diagnosed on the 10th day postpartum may adversely affect reproduction.

Keywords

Subclinical hypocalcemia, Body Condition Score, Parity, calving to first insemination interval

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