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A retrospective case-control study of pregnancy failure in Thoroughbred horses in Australia

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ABSTRACT

Pregnancy failure is a serious economic and welfare concern in the Thoroughbred horse industry, yet its incidence and risk factors in Australia remain unclear. This retrospective, nested, case-control study investigated pregnancy failure in resident mares on studs in the Hunter Valley, Australia, in 2021—2022, in early (46–150 days), mid (151–270 days), and late (>270 days) gestation. We found an annual incidence risk of 5.4 pregnancy failure cases/100 45-day pregnancies (0.05 cases/mare; 95 % CI 0.04—0.07), with full-term (perinatal) losses (≥320 days) accounting for 24 % of cases (95 % CI 14—37 %). There was no significant difference in loss according to mare age or reproductive history. Control mares had nearly six times higher odds of being vaccinated against Salmonellosis (*Salmonella enterica* subsp *enterica* serovar *Typhimurium*) compared to case mares (OR 5.92, 95 % CI 1.2—29.7). Environmental factors like paddocks with native trees were also associated with increased losses. The study provides evidence that *Salmonella* sp. might be an important cause of pregnancy failures in Australia. In addition, the findings contribute valuable baseline data for developing targeted surveillance strategies for pregnancy failure in Australian Thoroughbreds. Further investigation of factors such as *Salmonella* sp. is warranted to enhance breeding success and ensure welfare of Thoroughbred mares in Australia.

1. Introduction

The Australian Thoroughbred breeding industry is a significant contributor to the national economy, generating $1.16 billion in gross value-added impact (direct and indirect) annually and employing nearly 8000 full-time equivalent people in directly and indirectly associated work (Hardy and Limoli, 2019). The percentage of live foals/mares bred has increased from 59.83 % in 2010/11 to 66.49% in 2019/20 (Racing Australia Ltd., 2021). The Hunter Valley in New South Wales (NSW) is a major center of Thoroughbred breeding in Australia, producing approximately one third of foals in Australia annually (Hardy and Limoli, 2019).

Variations in definitions for pregnancy failure and gestational periods make comparing incidence and burden between studies difficult, but it is likely that losses and their causes vary between global regions (Macleay et al., 2022). Population level information is also often lacking in studies. For example, several studies solely analysed submissions to diagnostic laboratories (Hong et al., 1993; Tengelsen et al., 1997; Smith et al., 2003), and therefore, do not reflect the case proportions at a population level, or allow for investigation of risk factors (Roach et al., 2021). Globally, it has been reported that pregnancy failure among Thoroughbred mares occurs in 3.7—15.2 % of mares depending on region and gestational period (Morris and Allen, 2002; Allen et al., 2007; Bosh et al., 2009; Miyakoshi et al., 2012; Lane et al., 2016; Rose et al., 2018; Roach et al., 2021; Roach et al., 2022). Roach et al. (2022) reported pregnancy failures of 3.7 % between day 70 and 300 gestation in United Kingdom (UK) and Irish-based Thoroughbreds, while Miyakoshi et al. (2012) observed higher losses of 8.7 % between day 35 gestation and foaling in Japanese Thoroughbreds. In Ireland, Lane et al. (2016) documented a pregnancy failure of 15.2 %, though the specific gestational period was not specified, and in Kentucky, USA, Bosh et al. (2009) reported pregnancy failures of 12.9 % between day 40 gestation and foaling. These losses are not only a concern for animal welfare, but also impact the economic sustainability of the industry.

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Research conducted worldwide has identified various risk factors associated with pregnancy failure in Thoroughbred mares. These include mare-specific risk factors such as age, reproductive history, body condition score (BCS) and health status, as well as environmental risk factors such as paddock conditions, infectious diseases, and management practices (Carrigan et al., 1991; Morehead et al., 2002; Bosh et al., 2009; Miyakoshi et al., 2012; Lane et al., 2016; Roach et al., 2021; Roach et al., 2022). Additionally, vaccination against infectious agents such as equine herpes virus 1 and 4 (EHV) has reduced losses in some regions (Smith et al., 2003; Loynachan, 2020), but lapses in vaccination can result in outbreaks (Stasiak et al., 2020). Despite extensive research worldwide, there remains a significant gap in understanding the specific causes, incidence, distribution, and risk factors associated with pregnancy failures in Australian Thoroughbreds.

A greater number of pregnancy failures have been reported to occur between 40 days gestation and foaling relative to embryonic losses at < 40 days gestation (12.9 % and 8.9 %, respectively; Bosh et al., 2009). Several studies have reported more frequent losses at > 6 months gestation (Hong et al., 1993; Smith et al., 2003; Laugier et al., 2011), suggesting that risk of loss might increase during gestation. Additionally, foal mortality is highest in the first 7 days after birth and is strongly associated with adverse gestational or parturient events (Smith et al., 2003). Regionally, risks also vary. For example, Australian Thoroughbreds have causes of pregnancy failure, specifically equine amnionitis and fetal loss (EAFL) syndrome, that have not been reported globally (Todhunter et al., 2009; Cawdell-Smith et al., 2012). In addition, Australia is the only country to have reported a large outbreak of pregnancy failure due to infection with *Chlamydia psittaci* (Chan et al., 2017; Jenkins et al., 2018; Begg et al., 2022). Overall, region-specific causes will influence the incidence and distribution of pregnancy failure.

The objective of the current study was to describe the incidence of pregnancy failure (≥ 45 days gestation) in Thoroughbred mares, investigate associated risk factors, and explore potential causes in a major Thoroughbred breeding region in Australia. A retrospective, nested, case-control study was conducted during 2020—2021 in the Hunter Valley, New South Wales (NSW), Australia. This study provides a baseline for surveillance of pregnancy failure in the region and provides insights into potential risk factors that could be mitigated to improve reproductive outcomes in Thoroughbreds in Australia.

2. Methods

2.1. Study design

A retrospective, nested, case-control study was conducted on resident mares from studs in the Hunter Valley, NSW on which Thoroughbred mares were bred to produce foals for the Thoroughbred racing industry. Studs were selected by convenience in that stud managers responded to invitation and gave consent to participate. The study was conducted in accordance with the protocol approved by the Human Research Ethics Committee at Charles Sturt University (Protocol Number H21456).

On participating studs, all resident mares that were ≥ 45-days pregnant from the 2020 breeding season were counted and eligible as cases or controls for the study. Residency was defined as living on the stud at detection of pregnancy at 45 days' gestation in the 2020 breeding season until at least the end of that pregnancy, either due to pregnancy failure or parturition at full-term gestation.

Current industry practice in Australia is to confirm pregnancy beyond the embryonic stage from 45 days of gestation with ultrasound examination. Case mares were all resident mares which lost their pregnancy > 45 days' gestation. Pregnancy failure was defined as early, mid, and late loss at 46—150 days, 151—270 days, > 270 days gestation, respectively. Perinatal losses (>320 days) were included as pregnancy failure. Foals that were born alive, including apparent full-term, but did not stand within one hour or drink from the mare within two hours were classified as cases because the death was considered due to prenatal complications. For each case, two control mares in which pregnancy failure > 45 days gestation did not occur were randomly selected from the rest of the cohort of resident mares on the same stud. A decision tree was provided to participating studs to aid identification of case mares (Figure S1).

2.2. Data collection

Participating studs were given a spreadsheet comprising three pages for stud, case mare, and control mare information (one page each). Telephone support from the research team was provided to assist with data entry.

Stud data included the geographic size of the stud, the number of mares – including resident, seasonal (lived on the stud for the 2020 breeding season only), and walk-on (mares visiting for breeding with stallions only) – and stallions, and whether other farming operations were conducted on the stud. Stud-level practices to prevent pregnancy failure were also recorded, including the level of foaling supervision, control of pest species (caterpillars, birds, vermin, insects), feeding practices, biosecurity, and quarantine practices. A copy of the spreadsheet used for stud data collection is available in the Supplementary Material.

Mare data included birth year, years resident on the stud, age at which first bred, total number of 45-day pregnancies (prior to 2020 season), total number of pregnancy failures (excluding 2020 breeding season onwards), last date of service, and number of serves to achieve the 45-day pregnancy in the 2020 breeding season. General mare health questions for 2020—2021 included information about vaccination status, nutrition, antibiotic and progestin treatments to prevent pregnancy failure and other health conditions, and illnesses that occurred during gestation. Vaccinations (when administered) are given at manufacturers' specified times: EHV at 5, 7, and 9 months of gestation; tetanus, strangles, rotavirus and *Salmonella* at 2 months before expected foaling date; Hendra virus vaccine is given to maintain the annual requirement and will depend on the month that the initial vaccination course was administered. Administration dates of vaccination were not requested for vaccines except *Salmonella* (following preliminary analysis). Information about each mare's environment was also collected regarding paddocks in which each mare was kept (for example, proximity to native trees, types of water source, presence of bat roosts, and proximity to a mare that experienced pregnancy failure >45 days gestation in 2020—2021). Note that in Australia, mares are not routinely stabled on studs. Due to the large number of possible stallions, data about the paternity of each pregnancy was not collected. For case mares, date of pregnancy failure detection, method of detection, examinations and diagnoses, and details of the foal if available (size and sex) were recorded. For control mares, date of parturition, foal size and sex, and signs of prematurity or dysmaturity were recorded. A copy of the spreadsheet used for mare data collection is available in the Supplementary Material.

2.3. Analysis

All statistical analyses were conducted in R (R Core Team, 2022). Descriptive analyses of farm-level and mare-level data were conducted, including plots and summary statistics of incidence risks (where appropriate), proportions with 95 % confidence intervals (CI), odds ratios and chi-squared tests (alpha = 0.5).

For investigation of the association of environmental variables with pregnancy failure, generalized linear models (binomial link) were used with stud included as a random effect if data from more than one stud were included in the analysis. Although the temporality of events was not ascertained (movement before or after pregnancy failure and the duration of occupation in each paddock), all estimates were adjusted for whether the mare was monitored or on hormonal treatment to prevent pregnancy failure, because mares who received these interventions would have been in paddocks that were easier to access and were also considered to be at a higher risk of pregnancy failure (i.e. the interventions had a common causal association with the exposure and outcome).

3. Results

3.1. Participating studs

Seven studs participated in the study, ranging in size from 40 to > 4000 ha and minimum 17 to maximum 460 mares (resident and seasonal) during the 2020 breeding season (August – December). This gave a total of approximately 1680 mares (one stud estimated 150 seasonal mares) during the 2020 breeding season, of which at least 962 remained as residents on the studs (some studs only recorded how many mares foaled, but not whether they were resident or seasonal mares, or how long they remained on the stud) to the 2021 season and were included in the study.

Four of the seven studs had stallions (range 7—9) during the 2020 breeding season, and three of these also had seasonally-resident stallions from the northern hemisphere ('shuttle stallions'; range 1—7). Four studs had walk-on mares ranging from 571 to > 1000 mares during the 2020 season.

3.2. Stud management

Management factors with respect to pregnancy failure prevention were similar between participating studs. All studs had staff who monitored foaling mares overnight (known as 'night watch'), and none used cameras to monitor foaling. Four studs used foaling monitors (for example, collars), three of these studs specified that monitors were used when only a few mares were left to foal down. No studs fed silage or haylage to mares. All participating studs conducted caterpillar (*Ochrogaster lunifer*) control to reduce the probability of pregnancy failure due to equine amnionitis and fetal loss syndrome (EAFL). Caterpillar control methods included active searching and passive spotting with removal of nests, offering monetary rewards to staff who found nests, avoiding putting pregnant mares in paddocks in which caterpillars are frequently found, and fencing native trees. All studs conducted vermin control using a variety of methods (baits, dogs, and cats), one conducted active bird control (shooting), several covered stored feed to prevent bird access, and most conducted fly control (fly veils, fly spray on mares when undergoing procedures such as farriery, use of insecticides and parasiticides). Mosquito control was not conducted on any of the participating studs.

All studs had a dedicated quarantine area which was separated by distance or double fencing from the resident and seasonal mares. On all studs, newly arrived resident mares were kept in quarantine for at least two weeks and staff implemented biosecurity protocols to prevent disease transmission from mares in quarantine to horses on the rest of the stud; for example, one stud described a separate foaling kit for quarantined mares.

All studs implemented biosecurity procedures in the event of an observed pregnancy failure, with all studs describing the use of personal protective equipment (PPE; such as gloves, face masks, goggles, overalls, boot covers). Many studs described the availability of specific kits including PPE, plastic sheeting, hand sanitizer and disinfectant (VirkonTM) for footwear, vehicles, and equipment at gateways into affected paddocks, as well as boot washing facilities. Affected mares or paddocks had separate equipment including headcollars and lead ropes. On some participating studs, the affected mare was transferred to an isolated area of the stud, and most studs kept all the mares in the affected paddock under quarantine conditions. Three studs described having a separate vehicle that remained solely with the affected paddock and two studs described disinfection of vehicles that had been in the affected paddock. One stud described how staff would go home, shower and change clothes before returning to work with other horses on the stud.

3.3. Incidence risk of pregnancy failure > 45 days gestation

The incidence risk of pregnancy failure > 45 days gestation was calculated using data from studs that supplied accurate rather than estimated total numbers of resident mares (N = 6 studs; total 44 incident cases). On these studs, 810 resident mares were pregnant at 45 days gestation in the 2020 breeding season and 44 mares subsequently experienced pregnancy failure, giving an overall annual incidence risk of 5.4 cases of pregnancy failure/100 45-day pregnancies (0.05 cases/mare; 95 % CI 0.04—0.07; Fig. 1).

On individual studs, the incidence risk ranged from 4 cases of pregnancy failure/100 45-day pregnancies (N = 25 mares; 0.04 cases/mare, 95 % CI 0—0.2) to 18 cases of pregnancy failure/100 45-day pregnancies (N = 17 mares; 0.18 cases/mare, 95 % CI 0.04—0.43). There was no significant difference between the 95 % CI for all included studs (Fig. 1).

3.4. Comparison of case and control mare characteristics

Data for a total of 59 case and 120 control mares were provided by participating studs. Due to the lack of an accurate denominator of resident mares for all studs (one stud estimated the number of resident mares), odds ratios were calculated instead of relative risks which would otherwise have been feasible in this nested case-control (case-cohort) study design.

Fig. 2 showed mares' mean age in 2021, age at first breeding, and duration of residency on the respective studs. The mean age of case and control mares in 2021 was 10.7 years (95 % CI 5—19.1 years) and 9.7 years (95 % CI 5—16.1 years; Figure S2), respectively. This age difference was not statistically significant either by mean age (t = -1.8, df = 177, P = 0.08, 95 % CI −2.1—0.1 years), or when categorized to age groups (young mares ≤ 6 years, middle-aged mares 7–11 years, old mares ≥ 12 years; X^2 4.2, df = 2, P = 0.12).

Mean age at first breeding was 4.8 years (95 % CI 3—7 years) and 4.9 years (95 % CI 3—7 years) in case and control mares, respectively (Stud 4 was excluded due to lack of data). Case and control mares had been resident on the stud for mean 4.8 years (95 % CI 1—13.7 years) and 4.4 years (95 % CI 1—12 years), respectively. A density plot of the last day of service for control or case mares demonstrated similar distributions, indicating equivalent rates of pregnancy for each group throughout the season (Fig. 3).

In case mares, the mean number of previous 45-day pregnancies/mare was 3.9 (95 % CI 0–11) and the mean number of previous pregnancy failures was 0.4 (95 % CI 0—2). In control mares, the mean number of previous 45-day pregnancies/mare was 3.2 (95 % CI 0—9), and the mean number of previous pregnancy failures was 0.3 (95 % CI 0—2) which were not significantly lower. In the subset of mares having experienced ≥ 1 45-day pregnancy prior to 2020 (maiden mares excluded to leave 52 case mares and 100 control mares), the odds of pregnancy failure in 2020–21 following one or more previous reported pregnancy failures in previous years was no greater in case than control mares (1.7, 95 % CI 0.84—3.4).

The mean number of times bred to achieve pregnancy at 45 days gestation in the 2020 season was very similar in case and control mares (1.47 (95 % CI 1—3.0) and 1.39 (95 % CI 1—3.0), respectively, and there was no significant difference between the proportions of case and control mares served on their resident stud or by a stallion on another stud (X^2 = 1.08, df =1, P = 0.30).

Most mares did not leave their stud other than for breeding with the stallion (no significant difference between case and control mares, X^2 = 0.42, df = 1, P = 0.51). Six mares experienced illness (2 colic requiring surgery, 3 laminitis, 1 foot abscess) in the period between the 2020 and 2021 breeding seasons (no significant difference between case and control mares, X^2 = 0.80, df = 1, P = 0.37). Only two control mares

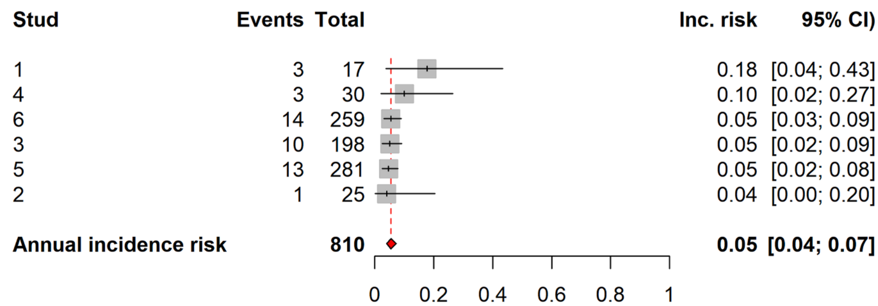

Fig. 1. Annual incidence risk of pregnancy failure > 45 days gestation on individual studs and combined, in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

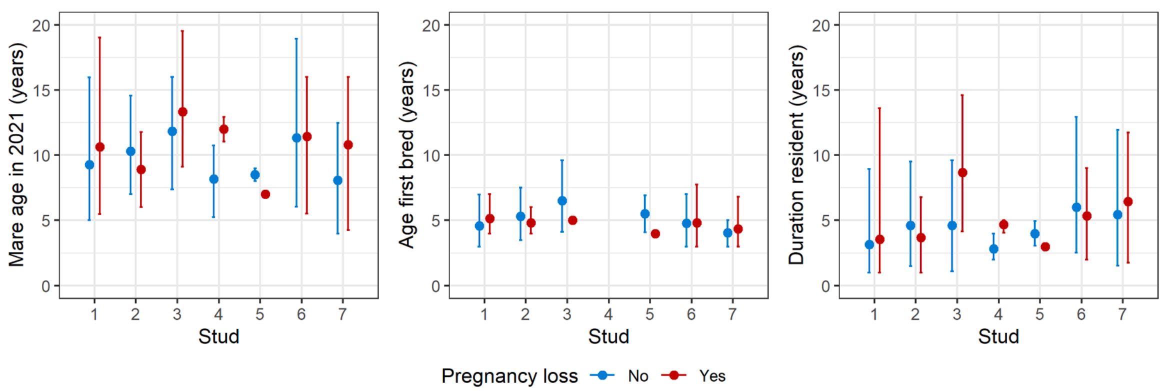

Fig. 2. Mare age in 2021, age at which first bred, and duration of residency on the stud, of case (experienced pregnancy failure) and control mares in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

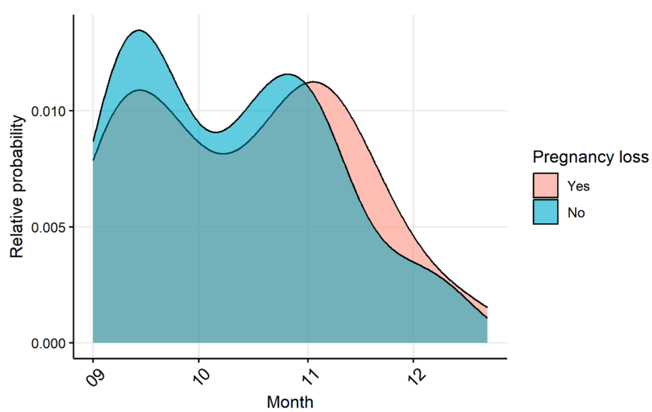

Fig. 3. Density plots of the day of breeding (service) of mares in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

had dental treatment (other than standard preventive dental care), and only three case mares were reported to receive specific diets or supplements (biotin for feet, L-arginine to prevent pregnancy failure, and a specific diet to manage laminitis).

3.5. Comparison of interventions to case and control mares

Greater proportions of case mares (some statistically significant; Table 1) were monitored, had undergone Caslick procedures, and received treatments with the aim of preventing pregnancy failure (antibiotics, anti-inflammatories, and hormones).

All mares were vaccinated against EHV-1 except two case mares (both foals were born at term with congenital abnormalities). One of these mares was also not vaccinated against Hendra virus disease, strangles or tetanus. All other mares were vaccinated against Hendra virus disease, strangles and tetanus. Vaccination against rotavirus disease was conducted in nearly all mares on three studs (except for 2 of 62 mares on one of these studs), and no mares on the other four studs. As a combined group, the proportion vaccinated against rotavirus disease was 66 % (95 % CI % 53—78 %) and 70 % (95 % CI 61—78 %) in case and control mares, respectively ($X^2 = 0.3$, df = 1, P = 0.60).

Table 1
Comparisons of interventions in case and control mares in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

Intervention	Mares (%, 95 % CI)		Statistical testing
	Cases	Controls	Chi-squared test, degrees freedom, P value
Caslick procedure	51 % (37—64 %)	42 % (31—51 %)	$X^2 = 1.3$, df = 1, P = 0.25
Antibiotics	19 % (10—31 %)	8.3 % (4—15 %)	$X^2 = 3.1$, df = 1, P = 0.08
Anti-inflammatories	15 % (8—27 %)	6.7 % (3—13 %)	$X^2 = 3.4$, df = 1, P = 0.07
Hormonal treatments	39 % (26—53 %)	32 % (24—41 %)	$X^2 = 0.6$, df = 1, P = 0.42
Monitoring	37 % (25—51 %)	18 % (12—26 %)	$X^2 = 7.7$, df = 1, P < 0.01

Vaccination against salmonellosis was only conducted on two studs. One of these studs vaccinated all mares and was therefore excluded from the analysis of vaccination due to no variation in the exposure status of mares. On the stud on which vaccination against salmonellosis was variable, a vaccination schedule using Zoetis Equivac® EST (*Salmonella enterica* subsp *enterica* serovar Typhimurium) commenced on 22 June 2021, prior to the expected foaling period. Vaccine supply was limited and was allocated based on the potential value of the foal (mares with expected higher value foals received vaccination). Therefore, the vaccine was randomly allocated with respect to the potential outcome (pregnancy failure). Mares only received one dose and not the recommended two doses for a primary course of the vaccine due to the limited supply. Subsequent to this, mares who experienced pregnancy failure were significantly less likely to have been vaccinated. The proportion of case and control mares vaccinated against salmonellosis was 56 % (5/9; 95 % CI % 22—85 %) and 88 % (37/42; 95 % CI 74—96 %), respectively, with an odds of vaccination nearly six times greater in control mares than mares that experienced pregnancy failure (OR 5.92, 95 % CI 1.2—29.7). If the first pregnancy failure is excluded (7 July 2021; pregnancy failure in an unvaccinated mare due to hydrops which is an externally visible condition), the odds of vaccination was over four times greater in mares that foaled at full term than mares that experienced pregnancy failure (OR 4.44, 95 % CI 0.8—24.5). The fetus and fetal membranes from these pregnancy failures were not examined by an equine medicine specialist or pathologist. Diagnoses were reported by an equine field veterinarian and included unspecified congenital defects (n = 2), seizure, lack of oxygen, placentitis (including red bag foaling; n = 2), prematurity, and unknown.

3.6. Pregnancy failure events

The dates of pregnancy failure and foaling for all case and control mares are shown in Figure S3. The relative probability of observed losses compared to the dates and gestation length of the control group are shown in Fig. 4.

Of the 59 cases, the largest group was late-term losses (≥270 days gestation, n = 26), with loss of foals at full-term (≥320 days gestation) accounting for a quarter of all pregnancy failures (n = 14; 24 %, 95 % CI 14—37 %). These were either stillbirths (defined as delivery of a dead foal at full term, n = 8 [57 %]), full-term live foals with a condition incompatible with life (n = 2 [14 %]), or full-term live foals that did not stand within 1 hour or suck from the mare within 2 hours and subsequently died or underwent euthanasia (n = 4 [29 %]).

Of foals born live at full-term (n = 6), three had congenital abnormalities, one was dysmature, one had an umbilical torsion, and one had a seizure. Of stillbirths (n = 8), two followed dystocia, one had a suspected umbilical torsion, one was diagnosed with *C. psittaci* infection, three had placentitis, and one had no cause detected (no postmortem examination conducted). The fetus and fetal membranes from 11 of these cases were examined (one examined off farm) and three had samples sent for analysis. The overall relative probabilities that the fetus and fetal membranes would be examined dependent on the stage of pregnancy failure are shown in Fig. 5.

For mares with late-term losses, mean age was 11.5 years (95 % CI 7—19.4 years). Last service dates ranged from 5 September to 10 November 2020, the mean number of serves in 2020 was 1.4 (95 % CI 1—2.7 serves), the mean number of previous > 45-day pregnancy failures/45-day pregnancy was 0.08 (95 % CI 0—0.3), and gestation length was up to 382 days.

Approximately one quarter of all pregnancy failures (n = 14; 24 %, 95 % CI 14—37 %) were unobserved and instead, detected by routine ultrasound examination (conducted on studs in April to June, prior to the next breeding season to coincide with barren mares being placed under lights to promote cyclicity). Mean age for this group was 8.7 years, (95 % CI 5.3—14.4 years), last service dates ranged from 7 September–24 November 2020, the mean number of serves in 2020 was 1.4 (95 % CI 1—2.7), and the mean number of previous > 45-day pregnancy failures /45-day pregnancy was 0.09 (95 % CI 0—0.4).

Of the 19 cases of observed pregnancy failures < 270 days gestation, most were mid-term (n = 17). Mean mare age for all 19 losses < 270 days gestation was 11.2 years, (95 % CI 4.8—17 years), last service dates ranged from 1 September–22 December 2020, the mean number of serves was 1.5 in 2020 (95 % CI 1—3.5), and the mean number of previous > 45-day pregnancy failures /45-day pregnancy was 0.3 (95 % CI 0—1.6). The probability that the fetus and fetal membranes from these would be examined off-farm by an equine medicine specialist and samples sent for pathology examination was much higher than late-pregnancy failures (Fig. 5). Diagnoses included umbilical cord occlusion/torsion (n = 8), unknown (n = 5, including one mummified fetus), other placentitis (n = 5, including focal mucoid [1] and fungal [1]), poor perfusion (4), *C. psittaci* placentitis (n = 3), congenital deformities (n = 3), EAFL placentitis (n = 2), and EHV-1 (n = 1).

3.7. Comparison of environments of case and control mares

Table S1 shows univariable odds ratios (OR) of the odds of an environmental exposure given full-term normal delivery (control mare). If studs had two or fewer mares in one exposure status for an environmental feature, their data were excluded. Therefore, different studs were used to estimate different effects, and some estimates were not identifiable because exposure status did not vary within any of the studs (for example, in the case of feeding mares under trees, studs either fed all mares under trees [one stud], or away from trees all together [three studs]).

Some environmental features were significantly associated with being a case or control mare; control mares were likely to have been kept in paddocks that were next to main roads and highways, had fenced or

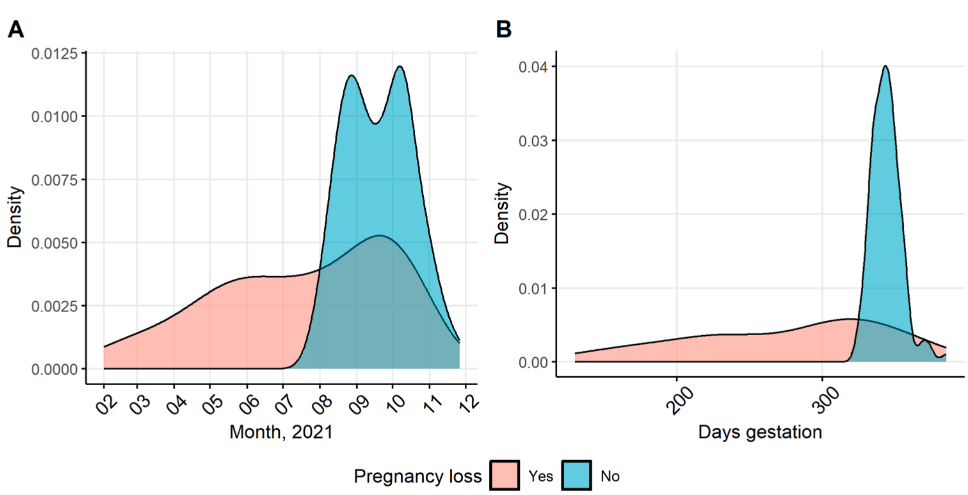

Fig. 4. Density plots of pregnancy failure and foaling by date (A) and days gestation (B), in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

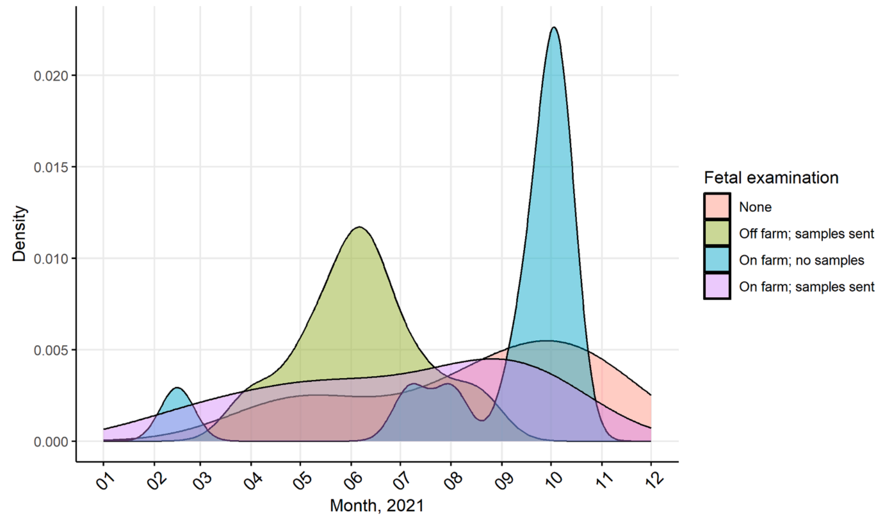

Fig. 5. Relative probabilities of examination of the fetus and fetal membranes in observed cases of pregnancy failure in a case-control study of pregnancy failure in Thoroughbreds in the Hunter Valley, New South Wales, Australia, from 2020—2021.

non-native trees, or were near bushland (i.e. these features appeared to be protective against pregnancy loss). Specifically, in all studs with sufficient data (Studs 1, 2, 6, and 7), the odds of a mare having been kept in a paddock next to a main road or highway were eight times greater in mares that did not experience pregnancy failure. Also, in mares that did not experience pregnancy failure, the odds of having been kept in a paddock with fenced trees (native or non-native trees) or non-native tree lines were high (Studs 2, 6, and/or 7; OR >3). The odds of having been kept in a paddock next to bush land was also higher (nearly four times) in mares that did not experience pregnancy failure (Studs 2 and 7).

4. Discussion

Our finding of a 5.4 % incidence risk for pregnancy failure > 45 days gestation is lower than the 12.9 % loss reported by (Bosh et al., 2009) in the USA, and 15.2 % observed by Lane et al. (2016) in Ireland, but higher than 3.0 % reported by Hanlon et al. (2012) in New Zealand. Comparatively, studies like Rose et al. (2018), Roach et al. (2021) and Allen et al. (2007) in the UK reported similar risks of 7.0 %, 7.3 % and 6.2 %, respectively. This variation highlights the complexity of comparing pregnancy failure rates globally, with both regional disease differences and methodological variations (including variation in durations for gestational periods) likely influencing estimates.

In our study, we found that mare age did not significantly influence the likelihood of pregnancy failure > 45 days gestation. This is similar to other studies. Lane et al. (2016) in Ireland and Roach et al. (2022) in the UK did not establish a clear association between mare age and pregnancy failure beyond the early gestational period. Likewise, Bosh et al. (2009) in the USA found no difference in pregnancy failure rates between age groups > 40 days gestation, and Miyakoshi et al. (2012) in Japan also reported no significant age-related risk > 35 days gestation. Interestingly, the pregnancy failures that were unobserved and identified through ultrasound examination occurred in a relatively younger group of mares compared to observed pregnancy failures. This suggests that these younger mares may not have exhibited obvious signs of pregnancy failure and were possibly not expected to experience complications, leading to less intensive monitoring compared to other groups. In contrast, late-term pregnancies in older mares, tend to be monitored more closely by stud staff, possibly because older mares are perceived to be at higher risk for pregnancy failure. This pattern suggests that age plays a role in monitoring practices, with younger mares potentially receiving less scrutiny due to the lower perceived risk of loss. Pregnancy monitoring, Caslick procedures and treatments such as antibiotics and hormones were more common among mares that experienced pregnancy failure (case mares) compared to those that did not (control mares). This suggests that case mares were already perceived to be at higher risk and were therefore subjected to closer scrutiny. Understanding the reasons for instigating monitoring and treatments and the effectiveness of these interventions on pregnancy failure requires further investment in research. This could lead to the development of more targeted and effective management practices, thus reducing required resources, including time.

A history of pregnancy failure was not a significant predictor of future pregnancy failure in our study which is consistent with the finding that age was not a predictor. Our findings contrast with those of Roach et al. (2022) in which UK Thoroughbred mares with two or more previous losses were at a higher risk of subsequent pregnancy failure. This suggests there may be different causes of pregnancy failure in Australia because specific reasons for past losses – whether infectious, anatomical, environmental, or genetic– likely influence future losses. For example, latent infections such as equine herpesvirus (EHV) have been suggested as potential causes of recurrent losses (Lunn et al., 2009; Roach et al., 2022) but could be of lower prevalence in Australian mares. If age-associated changes that compromise pregnancy are not a large contributor to pregnancy failure risk in Australia, then cumulative pregnancy failures would also be less likely a characteristic of mares who experience pregnancy failure. We did not collect detailed information on prior pregnancy failures – future research could focus on lifetime cohorts of mares to identify cumulative risk factors in regions in which age or previous losses are associated with increased risk of pregnancy failures.

Vaccination for *Salmonella enterica* subsp. *enterica* serovar Typhimurium emerged in this study as possibly protective against equine pregnancy failure. Interestingly, pregnancy failures in sheep due to *Salmonella enterica* subsp. *enterica serovar* Abortusovis are reduced by vaccination with attenuated live *Salmonella* Typhimurium (Linde et al., 1992). *Salmonella* sp. are a known cause of equine pregnancy failure, however *Salmonella* Typhimurium is uncommonly reported (Giles et al., 1993; Hong et al., 1993; Ricard et al., 2022; Ruby and Janes, 2023). The most frequently reported serovar is *Salmonella enterica* subspecies *enterica* serovar Abortusequi (*S.* Abortusequi) which can cause abortion in mares and donkeys (Madić et al., 1997; Wang et al., 2019). Mares appeared to be susceptible to pregnancy failure from *Salmonella* Typhimurium infection up to about 50–60 days of gestation but less susceptible after that time (Daels et al., 1987). While the pathogenesis of *Salmonella* Typhimurium pregnancy failure requires further investigation with a larger number of mares, the evidence suggests a potentially significant benefit of vaccination. *Salmonella* infection can result in severe enteritis, sepsis and a systemic inflammatory response syndrome which may compromise a pregnancy and may infect the placenta (Spier, 1993; Mansfield et al., 2017). If temporality can be confirmed and given the random allocation of vaccination relative to pregnancy outcomes in this study, it is plausible to consider that *Salmonella* sp. may be a significant but poorly recognized cause of equine pregnancy failure in Australia. With the increasing detection of *Salmonella* sp. in mares and foals (Madić et al., 1997; Butler et al., 2011; Marenzoni et al., 2012) and the growing interest in vaccinating mares, further epidemiological studies, such as a difference-in-difference approach, could provide more robust insights into the effectiveness of this intervention across multiple studs.

Environmental factors also played a role in pregnancy failure prevention, evidenced by the association between certain paddock features and pregnancy outcomes. Paddocks adjacent to public roads, containing fenced trees, or with non-native tree lines are often indicative of highly maintained environments. While these features themselves are unlikely to directly influence pregnancy, they can be associated with reduced exposure to risk factors such as processionary caterpillars or roosting birds, which have been linked to equine amnionitis and fetal loss syndrome (EAFL) and potentially, placentitis due to *Chlamydia psittaci* (Todhunter et al., 2009; Cawdell-Smith et al., 2012; Jenkins et al., 2018). Paddocks next to uncultivated land ('bush') are likely to be inspected more vigilantly for caterpillar control and elimination, especially if pregnant mares are kept in them. Additionally, paddocks close to main buildings or roadside areas likely benefit from higher visibility by staff, leading to increased monitoring and caterpillar or bird removal. Although the results are not definitive, they underscore the need for more comprehensive studies that assess the impact of environmental features on pregnancy outcomes.

Limited traceability (recording of individual animal movements) was apparent in this study in which estimates of numbers of mares limited the epidemiological effect measures that could be calculated. Critically, lack of traceability limits identification of horses at risk in the event of a disease outbreak, slowing response times and potentially exacerbating impacts. Disease transmission pathways in breeding operations are numerous due to extensive movements of horses, including stallions (both local and 'shuttle' stallions that travel between northern and southern hemispheres each year), walk-on mares, sales, and movements between studs. Investigation of barriers to recording horse movements both on and between farms could provide valuable information to improve traceability and the efficacy of an infectious disease outbreak response.

Biosecurity on studs in this study indicated a focus on observed pregnancy losses and prevention of introduction of infectious disease onto studs because biosecurity procedures in the event of pregnancy failures were conducted thoroughly by studs in this study and all studs had quarantine protocols. However, one biosecurity gap was the lack of mosquito control measures. Horses are susceptible to various flaviviral infections, with outbreaks of neurological diseases in Australia linked to Murray Valley encephalitis virus and West Nile virus (Kunjin strain) (Prow et al., 2013). More recently, Japanese encephalitis virus exposure has been detected in the region (Mackenzie et al., 2022). Although pregnancy failure due to these mosquito-borne flaviviruses has not been documented in horses, these viruses are known to cause pregnancy failure and stillbirth in other species (Mansfield et al., 2017). We also found that the probability of fetal material being examined off-stud by a specialist was higher for losses occurring before 270 days of gestation compared to those lost in late pregnancy. This suggests a more proactive approach in investigating earlier pregnancy failures, possibly due to the perceived greater uncertainty and variability of causes in earlier stages. Examining late losses more thoroughly could provide valuable insights and identify underlying causes to prevent late-term losses.

A limitation of this study was the exclusion of stallion factors such as health, fertility, and breeding practices, which can significantly influence pregnancy outcomes (Allen et al., 2007; Lane et al., 2016). For instance, Roach et al. (2022) found that older stallions might reduce the odds of pregnancy failure in mares with a history of prior losses. Additionally, we did not account for the status of mares before pregnancy (e.g., maiden, foaling, barren), which has shown variable impacts on pregnancy failure in other studies (Miyakoshi et al., 2012; Lane et al., 2016). Furthermore, we did not evaluate factors like Body Condition Score (BCS) or nutrition, both critical to mare health and pregnancy success (Miyakoshi et al., 2012). Lastly, the observational nature of the study limits causal inference regarding risk factors and this is most. In addition, due to the differences identified between this study and other regions, our findings are not transportable to other Thoroughbred breeding populations, possibly even in other areas of Australia. Future studies incorporating multi-regional comparisons would be of benefit to identify region-specific measures to improve pregnancy outcomes.

5. Conclusion

This study provides a baseline for the incidence and characteristics of pregnancy failure in Thoroughbred mares $\geq$ 45-days gestation in a breeding center in Australia, and highlights several factors associated with pregnancy failure. These include the continued influence of environmental features (areas on studs that were not curated paddocks or inspected for processionary caterpillars appeared to be higher risk), the potential protective effect of *Salmonella* Typhimurium vaccination, and the extensive practices and interventions to reduce pregnancy failure. Additionally, it demonstrates need for improved traceability of mare movements both within and between studs to enhance biosecurity and disease outbreak responses. Attributable causes of pregnancy loss cannot be globally generalized (increasing mare age and previous losses do not appear to predict future losses in the current study), and therefore, region-specific research is required to understand causes of losses.

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CRediT authorship contribution statement

Cara S. Wilson: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jane Heller:** Writing – review & editing, Investigation, Conceptualization. **Victoria Brookes:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Joan Carrick:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Patrick Shearer:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106424.

References

Allen, W., Brown, L., Wright, M., Wilsher, S., 2007. Reproductive efficiency of flatrace and national hunt Thoroughbred mares and stallions in England. Equine Vet. J. 39, 438–445.

Begg, A.P., Carrick, J., Chicken, C., Blishen, A., Todhunter, K., Eamens, K., Jenkins, C., 2022. Fetoplacental pathology of equine abortion, premature birth, and neonatal loss due to Chlamydia psittaci. Vet. Pathol. 59, 983–996.

Bosh, K.A., Powell, D., Shelton, B., Zent, W., 2009. Reproductive performance measures among Thoroughbred mares in central Kentucky, during the 2004 mating season. Equine Vet. J. 41, 883–888.

Butler, C., Werners, A., Newton, R., 2011. Surveillance of infectious and non-infectious causes of equine abortion in the UK: 2006–2011. DEFRA/AHT/BEVA Equine Q. Dis. Surveill. Rep.

Carrigan, M., Cosgrove, P., Kirkland, P., Sabine, M., 1991. An outbreak of Equid herpesvirus abortion in New South Wales. Equine Vet. J. 23, 108–110.

Cawdell-Smith, A., Todhunter, K., Anderson, S., Perkins, N., Bryden, W., 2012. Equine amnionitis and fetal loss: mare abortion following experimental exposure to Processionary caterpillars (Ochrogaster lunifer). Equine Vet. J. 44, 282–288.

Chan, J., Doyle, B., Branley, J., Sheppeard, V., Gabor, M., Viney, K., Quinn, H., Janover, O., McCready, M., Heller, J., 2017. An outbreak of psittacosis at a veterinary school demonstrating a novel source of infection. One Health 3, 29–33.

Daels, P., Starr, M., Kindahl, H., Fredriksson, G., Hughes, J., Stabenfeldt, G., 1987. Effect of Salmonella typhimurium endotoxin on PGF-2 alpha release and fetal death in the mare. J. Reprod. Fertil. Suppl. 35, 485–492.

Giles, R., Donahue, J., Hong, C., Tuttle, P., Petrites-Murphy, M., Poonacha, K., Roberts, A., Tramontin, R., Smith, B., Swerczek, T., 1993. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). J. Am. Vet. Med Assoc. 203, 1170–1175.

Hanlon, D.W., Stevenson, M., Evans, M.J., Firth, E.C., 2012. Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses. N. Z. Vet. J. 60, 329–334.

Hardy, G., Limoli, P., 2019. Measurement of Economic Impact of the Australian Thoroughbred Breeding Industry. AgriFutures Australia, Wagga Wagga, NSW.

Hong, C., Donahue, J., Giles Jr, R., Petrites-Murphy, M., Poonacha, K., Roberts, A., Smith, B., Tramontin, R., Tuttle, P., Swerczek, T., 1993. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. J. Vet. Diagn. Investig. 5, 560–566.

Jenkins, C., Jelocnik, M., Micallef, M.L., Galea, F., Taylor-Brown, A., Bogema, D.R., Liu, M., O'Rourke, B., Chicken, C., Carrick, J., Polkinghorne, A., 2018. An epizootic of Chlamydia psittaci equine reproductive loss associated with suspected spillover from native Australian parrots. Emerg. Microbes Infect. 7, 88.

Lane, E., Bijnen, M., Osborne, M., More, S., Henderson, I., Duffy, P., Crowe, M., 2016. Key factors affecting reproductive success of Thoroughbred mares and stallions on a commercial stud farm. Reprod. Domest. Anim. 51, 181–187.

Laugier, C., Foucher, N., Sevin, C., Leon, A., Tapprest, J., 2011. A 24-year retrospective study of equine abortion in Normandy (France). J. Equine Vet. Sci. 31, 116–123.

Linde, K., Bondarenko, V., Sviridenko, V., 1992. Prophylaxis of Salmonella abortus ovis-induced abortion of sheep by a Salmonella typhimurium live vaccine. Vaccine 10, 337–340.

Loynachan, A., 2020. Equine abortion: the 2016-2019 breeding seasons in central Kentucky. Equine Dis. Q.

Lunn, D.P., Davis-Poynter, N., Flaminio, M.J.B.F., Horohov, D.W., Osterrieder, K., Pusterla, N., Townsend, H.G.G., 2009. Equine herpesvirus-1 consensus statement. J. Vet. Intern. Med. 23, 450–461.

Mackenzie, J.S., Williams, D.T., van den Hurk, A.F., Smith, D.W., Currie, B.J., 2022. Japanese encephalitis virus: the emergence of genotype IV in Australia and its potential endemicity. Viruses 14, 2480.

Macleay, C.M., Carrick, J., Shearer, P., Begg, A., Stewart, M., Heller, J., Chicken, C., Brookes, V.J., 2022. A scoping review of the global distribution of causes and syndromes associated with mid- to late-term pregnancy loss in horses between 1960 and 2020. Vet. Sci. 9, 186.

Madić, J., Hajsig, D., Sostari, B., Ćurić, S., Seol, B., Naglić, T., Cvetnić, Z., 1997. An outbreak of abortion in mares associated with Salmonella abortusequi infection. Equine Vet. J. 29, 230–233.

Mansfield, K.L., Hernández-Triana, L.M., Banyard, A.C., Fooks, A.R., Johnson, N., 2017. Japanese encephalitis virus infection, diagnosis and control in domestic animals. Vet. Microbiol. 201, 85–92.

Marenzoni, M.L., Lepri, E., Proietti, P.C., Bietta, A., Coletti, M., Timoney, P.J., Passamonti, F., 2012. Causes of equine abortion, stillbirth and neonatal death in central Italy. Vet. Rec. 170, 262.

Miyakoshi, D., Shikichi, M., Ito, K., Iwata, K., Okai, K., Sato, F., Nambo, Y., 2012. Factors influencing the frequency of pregnancy loss among Thoroughbred mares in Hidaka, Japan. J. Equine Vet. Sci. 32, 552–557.

Morehead, J.P., Blanchard, T.L., Thompson, J.A., Brinsko, S.P., 2002. Evaluation of early fetal losses on four equine farms in central Kentucky: 73 cases (2001). J. Am. Vet. Med Assoc. 220, 1828–1830.

Morris, L.H., Allen, W., 2002. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Vet. J. 34, 51–60.

Prow, N.A., Tan, C.S., Wang, W., Hobson-Peters, J., Kidd, L., Barton, A., Wright, J., Hall, R.A., Bielefeldt-Ohmann, H., 2013. Natural exposure of horses to mosquito-borne flaviviruses in south-east Queensland, Australia. Int J. Environ. Res. Public Health 10, 4432–4443.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Racing Australia Ltd, 2021. Fact Book 2021: Racing Season Ended 31 July 2021. Racing Australia Ltd, pp. 1–100.

Ricard, R.M., St-Jean, G., Duizer, G., Atwal, H., Wobeser, B.K., 2022. A 13-year retrospective study of equine abortions in Canada. Can. Vet. J. 63, 715–721.

Roach, J.M., Arango-Sabogal, J.C., Smith, K.C., Foote, A.K., Verheyen, K.L., de Mestre, A. M., 2022. Multivariable analysis to determine risk factors associated with abortion in mares. Reprod. Fertil. 3, 301–312.

Roach, J.M., Foote, A.K., Smith, K.C., Verheyen, K.L., de Mestre, A.M., 2021. Incidence and causes of pregnancy loss after day 70 of gestation in Thoroughbreds. Equine Vet. J. 53, 996–1003.

Rose, B., Firth, M., Morris, B., Roach, J., Wathes, D., Verheyen, K., De Mestre, A., 2018. Descriptive study of current therapeutic practices, clinical reproductive findings and incidence of pregnancy loss in intensively managed thoroughbred mares. Anim. Reprod. Sci. 188, 74–84.

Ruby, R.E., Janes, J.G., 2023. Infectious causes of equine placentitis and abortion. Vet. Clin. North Am.: Equine Pract. 39, 73–88.

Smith, K.C., Blunden, A.S., Whitwell, K.E., Dunn, K.A., Wales, A.D., 2003. A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. Equine Vet. J. 35, 496–501.

Spier, S.J., 1993. Salmonellosis. Vet. Clin. North Am.: Equine Pract. 9, 385–397.

Stasiak, K., Dunowska, M., Rola, J., 2020. Outbreak of equid herpesvirus 1 abortions at the Arabian stud in Poland. BMC Vet. Res. 16, 1–8.

Tengelsen, L.A., Yamini, B., Mullaney, T.P., Bell, T.G., Render, J.A., Patterson, J.S., Steficek, B.A., Fitzgerald, S.D., Kennedy, F.A., Slanker, M.R., 1997. A 12-year retrospective study of equine abortion in Michigan. J. Vet. Diagn. Investig. 9, 303–306.

Todhunter, K.H., Perkins, N.R., Wylie, R.M., Chicken, C., Blishen, A.J., Racklyeft, D.J., Muscatello, G., Wilson, M.C., Adams, P.L., Gilkerson, J.R., Bryden, W.L., Begg, A.P., 2009. Equine amnionitis and fetal loss: the case definition for an unrecognised cause of abortion in mares. Aust. Vet. J. 87, 35–38.

Wang, H., Liu, K.J., Sun, Y.H., Cui, L.Y., Meng, X., Jiang, G.M., Zhao, F.W., Li, J.J., 2019. Abortion in donkeys associated with Salmonella abortus equi infection. Equine Vet. J. 51, 756–759.