CYTOGENETICS

– Introduction and applications in animal breeding

Cytogenetics

- Examination of chromosomes under the microscope
  - Necessary to induce cells to undergo mitosis in order to see individual chromosomes
  - Molecular cytogenetic techniques (e.g., FISH)

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Reasons to do a cytogenetic study

- Diagnose constitutional disorders
  - i.e., disorders present at birth
  - Typically involve more than one cell line

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Specimen requirements -- Room Temp

- Blood
  - Volume: 2-5 ml in a SODIUM heparin tube

Tissue culture

- Unlike many other lab tests, cytogenetics requires cells to be cultured so that they can undergo mitosis
  - Lymphocytes are differentiated cells that normally do not undergo spontaneous cell division
  - In culture process, a mitogen is added (e.g., phytohemagglutinin / PHA) to stimulate cells to replicate DNA and enter mitosis
  - Lymphocytes remain in culture (in tissue culture medium) for 48 – 72 hours

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Tissue culture

- Cultures can be synchronized by addition of methotrexate
  - Blocks cells in S phase; this allows more of the cells in culture to enter and remain at S phase
  - Cells then proceed synchronously to mitosis
- Colcemid -> prevents synthesis of spindle fibers – thus, mitosis is stopped in metaphase (no separation of sister chromatids)
  - Metaphase is the best time of cell cycle to see and examine chromosomes

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Tissue culture

- Ethidium bromide (DNA intercalating agent) can be added to slow or prevent chromosome condensation --> longer chromosomes
- Hypotonic solution is added to cells to get them to swell and lyse – thus releasing the chromosomes
- Fixed specimen is “dropped” onto glass slides and stained with Wrights-Giemsa

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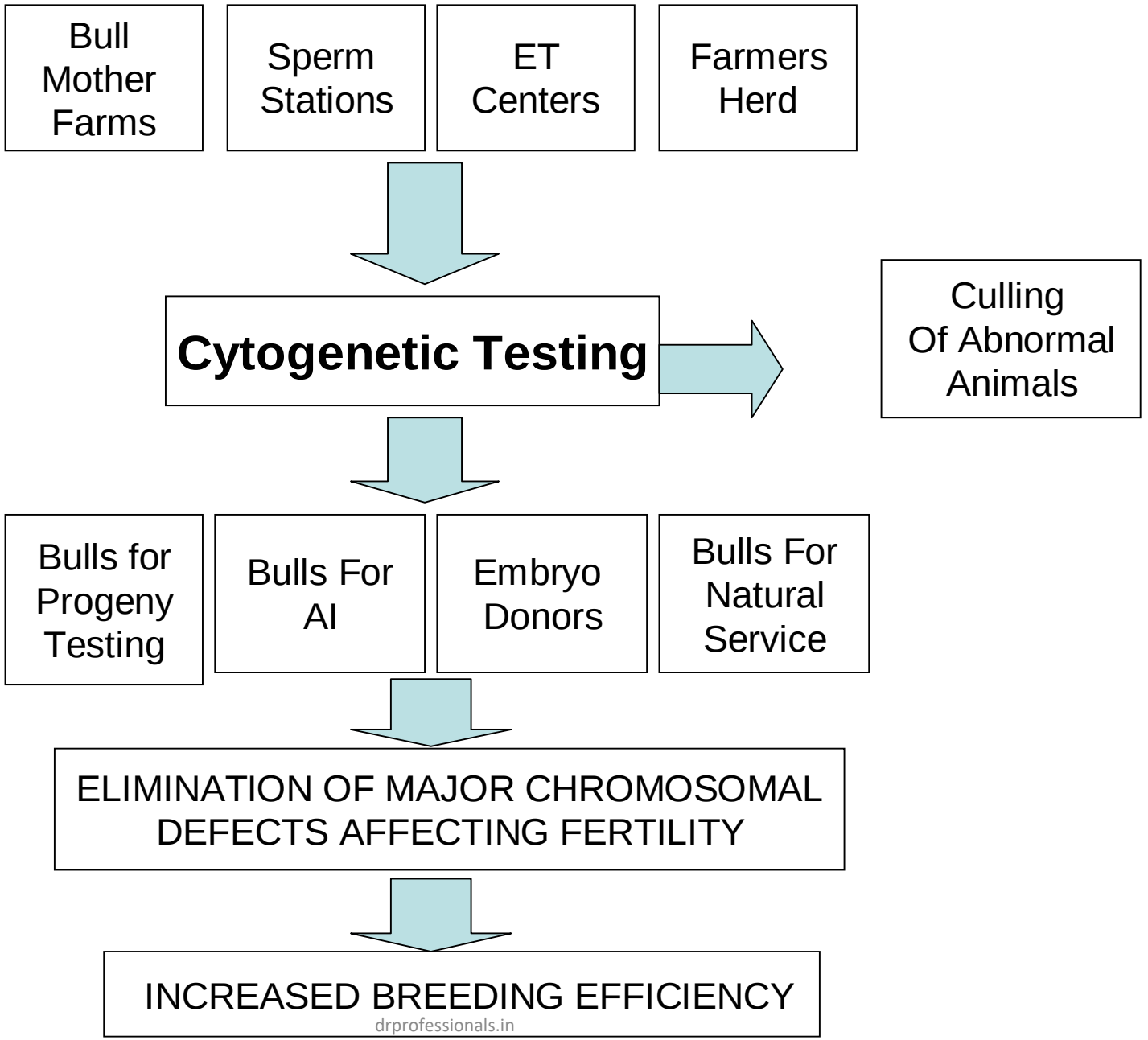
Analysis

- 20 metaphase cells are analyzed by G-banding

Identification of these abnormalities is important for:

- Differential diagnosis
- Determining therapy
- Monitoring therapy
- Providing information about prognosis


Bull Mother Farms
Sperm Stations
ET Centers
Farmers Herd
Cytogenetic Testing
Culling Of Abnormal Animals
Bulls for Progeny Testing
Bulls For AI
Embryo Donors
Bulls For Natural Service
ELIMINATION OF MAJOR CHROMOSOMAL DEFECTS AFFECTING FERTILITY
INCREASED BREEDING EFFICIENCY
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- Chromosomal abnormalities can be diagnosed **before birth** using **prenatal tests** [amniocentesis or chorionic villus sampling (CVS)] or after birth using a blood test.
- Cells obtained from these tests are grown in the laboratory, and then their chromosomes are examined under a microscope.
- The lab makes a **picture (karyotype)** of all the person's chromosomes, arranged in order from largest to smallest.
- The karyotype shows the **number, size and shape** of the chromosomes and helps experts identify any abnormalities.
- The chromosomal screening can reduce embryonic and foetal mortality up **to 20-30%** (Roberts, 1971).

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Way of Expression of the effects on fertility

Pigs	-	Litter Size
Sheep	-	Lambing Percentage
Goat	-	Kidding Percentage
Bulls	-	Non return rates(Number of cows failing to return to oestrus after service irrespective of whether natural service or AI practiced)
Cows	-	No of service/Conception

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Freemartinism:

- It is form of intersexuality
- Mostly in cattle bearing **heterosexual twins** External genitalia: female
- Less in goat, sheep, horse and pig
- **60,XX/XY**

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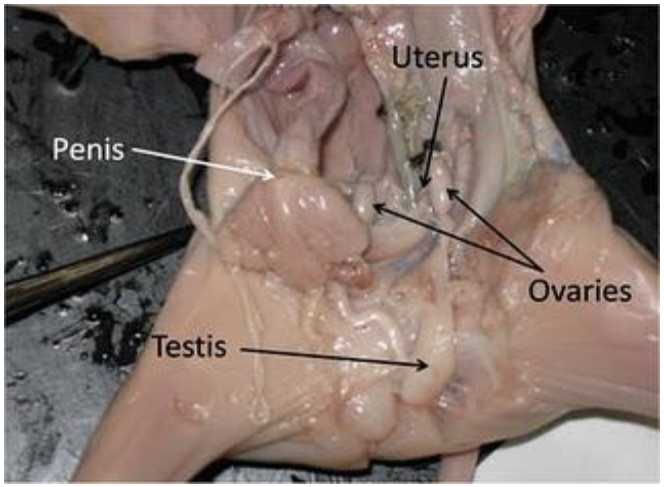
Hermaphroditism/Intersex:

- True Hermaphrodite:-Pig
  - **Both gonad tissues in function**
  - XX/XY
  - Common in **cattle ,Pig and goat**
- Male Pseudohermaphrodite:
  - **Testes as the gonads**
  - XY or XX
    - **Chimera, sexchromosome aneuploidy, translocation** of Y chromosome and gene mutation

Figure 5. Hermaphrodite pig with large clitoris.



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External genitalia of Intersex goat-an enlarged clitoris

Occurrences and Effects in Livestock

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Reproductive Failure	Species	Chromosome Aberration	Karyotype
Testicular hypoplasia	Cattle	Disomy-X	61,XXY
	Sheep	Chimera	60,XX/60,XY
	Pig	Disomy-X	55-XXY
		Disomy-X	39,XXY
Ovarian hypoplasia	Horse	Monosomy-X	63,XO
		Trisomy-X	65,XXX
		Mosaic	63,XO/64,XX
	Cattle	Trisomy-X	61,XXX
Repeat breeding	Cattle	Mosaic	60,XX/60,XY
		Mosaic	59,XO/60,XX
		Mixoploid	59,XO/60,XX/61,XXX
Infertility	Cattle	Robertsonian translocation	59,XY rob (1.29)
Embryonic mortality	Pig	Reciprocal translocation	38,XY,t(7q-;11q+)
			38,XY,t(13q-

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1. Cattle

SN	REPRODUCTIVE FAILURE	CHRTIONOMOSOMAL ABBERATION	KARYOTYPE	
1.	Reduced fertility in bull	Chimerism	60XY/60XX	
2.	Repeat breeding associated with still birth	Robertsonian translocation	59,XX	
3.	Repeat breeding	-do-	59,XY	
4.	Low fertility	Chromatid gaps and breaks	60,XY	
5.	Testicular hypoplasia	Chromatid gaps	60,XY	

6.	Infertile heifer	Unusual chimerism	60,XX/XY	
7.	Repeat breeder in jersey crossbred cattle	Translocation	60,XX	
8.	Azoospermic bull	Reciprocal trnslocation	60,XY	

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SN	Breed	Reproductive Failure	Chromosomal Abberation	Karyotype	
1.	**Jersey cross**	History of **anoestrus**	Rob(1;29) along with chromatid gaps and breaks	59,XX	
2.	HF cross bred bull (bull centre of **Mumbai**.)	Low fertility henotypically normal with abnormal sperms	**Rob(7;16)**	59XY	
3.	HF- Gir crossbred bull	Reduced fertility	Chromatid gaps and breaks	60,XY	
4.	Jersey x Gir crossbred bull	Delayed puberty	1;29 CFT	59,XY	
5.	**Deoni (Bos indicus)**	History of **anoestrus**	16,20 CFT	59,XY	

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2. Buffalo

SN	Breed	Reproductive Failure	Chromosomal Abberation	Karyotype	
1.	River buffalo bull	Reduced fertility	Alteration involving chromosome 3	50,XY with 3 p+	
2.	Water buffalo	Delayed puberty	Alteration involving chromosome 3	48,XX with 16 p+	
3.	Murrah buffalo bull	Phenotypically normal with abnormal sperms	Aneuploidy	48,XY/49,XY/51,XY	
4.	Murrah buffalo bull	Low fertility	Fragile sites on different chromosome	50,XY	
5.	Mehsana buffalo male calf	_____	Translocation	XXY karyotype with X;X translocation.	

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3. Sheep and Goat

S N	Breed	Reproductive Failure	Chromosomal Abberation	Karyotype	
1.	Saanen	Azoospermia ,disturbed fertility	Translocation	60,XY	
2.	Saanen	Delayed puberty	6,17 rob translocation	59,XY	
3.	Sannen	--do---	6,17rob translocation	59,XY	
4.	Polled Saanen goat	Disturbed fertility	Mixoploidy	XO/XX/XXX	
5.	Polled Saanen goat	Sterility	Chimera	60XX/XY.	
6.	**Malga**	**Disturbed fertility**	**Chimera**	**60XX/XY**	

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4.Pig

SN	Reproductive Failure/	Chromosomal Abberation	Karyotype	
1.	Reduced fertility	13,17 Robertsonian translocation	37,XY	
2.	Subfertility in boars	Mosaicism	37,X/38,XY/39,XXY	
3.	Decreased litter size	16-17 reciprocal translocation	38,XY	
4.	Infertility	16,17 Robertsonian translocation	37,XY	
5.	Decreased litter size	1-9/11-13 reciprocal translocation	38,XY	

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5.Horses

SN	Reproductive Failure/	Chromosomal Abberation	Karyotype	
1.	Reduced fertility	Mosaicism	63X, /64,XX	
2.	Sterile	Klienfilter syndrome	65,XXY FIG.	
3.	Infertility	Monosomy	63,XO	
4.	Infertility	Mosaicism	63,X0/64,XX	
5.	Infertility	Mosaicism	63,X/64,XX/65,XXX	

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- **Animal cytogenetics** has several applications in animal improvement
- Detection of chromosomal abnormalities.

- Most of the numerical chromosomal abnormality causes **sterility /infertility** or **death** in domestic animals.

- Structural chromosomal abnormalities causes altered functions or reduced fertility (sub-fertility).

- For the improvement in reproduction status of farm animals, **proper record keeping** with pedigree sheet, thorough **cytogenetic screening** of breeding stock, strictly culling of affected animal must be followed.

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