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Assessment of ovulation rate in breeding bitches based on vaginal Exfoliative cytology and progesterone analysis patterns in and around Chennai

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Abstract

The study was conducted to assess the ovulation rate in breeding bitches based on vaginal exfoliative cytology and serum progesterone profiling in and around Chennai. A total of 30 healthy adult female dogs presented for mating were selected and randomly divided into 3 groups (10 animals/group). Vaginal smears were collected at regular intervals, stained with Giemsa stain, and evaluated for cell types to determine the stage of the estrous cycle. Simultaneously, serum progesterone levels were measured using chemiluminescence immunoassay to confirm the optimal breeding window. Bitches were bred based on the concurrence of cytological estrus (predominance of superficial and cornified cells) and progesterone levels between 4-10 ng/mL. Conception was confirmed by ultrasonography 25-30 days post-mating. The study revealed a higher conception rate when breeding was synchronized with both cytological and hormonal indicators, compared to either method alone. These findings highlight the importance of combining exfoliative cytology and progesterone analysis for accurate estrus detection and improved reproductive success in breeding bitches.

Keywords: Bitches, Estrous cycle, progesterone analysis, vaginal cytology, breeding, Chennai

1. Introduction

The reproductive cycle of the bitch comprises of four phases: proestrus, estrus, diestrus, and anestrus. Ovulation typically occurs 1-2 days after the luteinizing hormone (LH) surge, during the estrus phase, but its timing is variable and difficult to predict based on external signs alone. Compounding this issue is the fact that ova are ovulated in an immature state and require 2-3 days for maturation before they are capable of fertilization (England & Concannon, 2002). Current ovulation detection methods include vaginal cytology, which assesses epithelial cell changes but lacks precision; progesterone assays, which are reliable yet require repeated blood sampling and laboratory analysis; LH assays, which detect the pre-ovulatory surge but are costly and not routinely used; and ultrasonography, which allows direct follicular monitoring but depends on skilled operators.

Each of these has limitations in terms of accessibility, cost, or accuracy. Therefore, there is a pressing need for simpler, more precise diagnostic markers—ideally measurable in a single sample—that correlate strongly with ovulation. The Exfoliative cytology is the primary test which can be employed to identify the breeding stage of the dog. The haematological parameters indicate the health of the breeding dogs which also play a major role in the fertility and conception rate of the bitches. Hence correlating the haematological values and vaginal cytology values will help in understanding the reproductive behaviour and conception in the bitches.

Materials and Methods

Selection of Animals

Thirty apparently healthy bitches, aged between 2 to 6 years, atleast once whelped which were presented to Small Animal Gynaecology Unit, Madras Veterinary College Teaching Hospital, Chennai were utilized for the study.

Study Design

The animals which were presented for breeding advice were selected and were allowed to mate based on the inference of the vaginal Exfoliative cytology (Group I), Based on progesterone values (Group II) and combination of both tests (Group III). Those animals were ultrasonographically evaluated thirty days' post mating to confirm pregnancy.

Veterinary medical history and clinical signs

Information regarding breed, age and parity, history of last whelping was recorded. General appearance, behaviour, feed and water intake, deworming and vaccination schedule, body temperature, conjunctival mucus membrane, pulse rate, respiratory rate was registered. Thorough clinical examination of external genitalia like vulval edema, vulval mucus membrane and vaginal discharge if any was taken in consideration.

Blood Collection

Five ml blood sample was collected via cephalic or saphenous vein puncture from bitches in all groups during anestrus, proestrus, estrus, and at the time of pregnancy diagnosis. Of the total sample, 2 ml was placed in an EDTA (Ethylene diamine tetra acetic acid) vial, while the remaining 3 ml was equally distributed between two clot activator vials. A blood smear was also prepared for differential leukocyte count. The whole blood from the EDTA vial was used for hematological analysis. Whereas, serum from one clot activator vial was used for biochemical evaluation. The serum from the second clot activator vial was used for s progesterone estimation.

Preparation of vaginal smear

Stage of cycle	Epithelial cell	Other cells
Proestrus	Shift from parabasal and intermediate to superficial cells	RBCs
Estrus	Predominance of superficial and cornified cells	Sometimes in early stages RBCs can be visualised
Diestrus	Predominance of intermediate cells, few parabasal cells, Foam cells can be seen sometimes.	Neutrophils
Anestrus	Predominance of parabasal cells, some intermediate cells. Foam cells can be seen sometimes.	-

Pregnancy Verification

For confirmation of pregnancy, the bitches bred were subjected to trans-abdominal ultrasound scanning 30 days after the last date of mating, for detection of gestational sac and foetus using B-mode ultrasound using multi frequency (2 to 8MHz) convex sector probe (Trivitron healthcare).

Conception Rate

Fertility rate was calculated by dividing number of bitches that had shown fertile estrus resulting in conception with number of animals mated following fertile estrus multiplied

Group	Total Dogs Mated (N)	Pregnant	Conception Rate (%)
Group I	9	6	66.67%
Group II	10	7	70.00%
Group III	8	7	87.50%

These findings strongly suggest that the integration of both cytological and hormonal parameters improves the precision of estrus detection and breeding timing, thereby enhancing successful conception. Similar observations were reported by Moxon *et al.* (2023) ^[2], who concluded that combining cytological analysis with hormonal profiling increases reproductive efficiency in bitches. Arlt and Heuwieser (2005) ^[3] also emphasized that progesterone-guided breeding results in optimal fertility outcomes when complemented with cytological confirmation of estrus.

By gently everting the vulval lips, a moistened cotton swab was carefully inserted into the craniodorsal aspect of the vagina. The swab was gently rotated in at least three directions to ensure contact with the mucosal surface and adequate collection of epithelial cells. The collected sample was then transferred onto a grease-free glass slide by gently rolling the swab from one end of the slide to the other, creating an even smear while avoiding overlapping of cell layers. The slide was then air-dried and subsequently stained for examination.

Staining of smear

Field stain was used for rapid processing of vaginal smear. Principle of field stain involve eosin and methylene blue dye that stain basophilic and acidophilic cellular elements. The basic component of white cells (cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic, the acidic component (nucleus with nuclei acid) takes blue to purple shades of the basic dye and is called basophilic. The staining procedure involved fixing air-dried vaginal smears in methanol for one minute or by dipping them 5-7 times in a coupling jar. The fixed smears were then sequentially dipped 5-6 times in Field Stain B (red stain) followed by Field Stain A (blue stain). After staining, the slides were air-dried and examined under a microscope for cellular interpretation.

Interpretation of vaginal smear

Depending on presence of predominant cell type, the slide can be interpreted for stage of oestrous cycle. Interpretation is given as described below:

with hundred and expressed in percentage.

Results and Discussion

The conception rates observed across the three groups showed variation based on the timing and method of estrus detection. Group I, relying solely on vaginal cytology, recorded a conception rate of 66.67%. Group II, which used progesterone assay alone, had a slightly higher conception rate of 70.00%. Notably, Group III, where both vaginal exfoliative cytology and serum progesterone analysis were combined for estrus detection, achieved the highest conception rate of 87.50%.

The enhanced conception in Group III may be attributed to accurate identification of the fertile window, characterized by a predominance of superficial and cornified epithelial cells and serum progesterone levels between 4-10 ng/mL, which is consistent with the ovulation window (Concannon, 2011) ^[4]. These results highlight the importance of a multimodal approach for estrus detection in breeding bitches, especially in field conditions where accurate timing significantly influences reproductive outcomes. The peak of keratinization occurs with the beginning of the increase in progesterone levels, but at the beginning of estrus, no characteristic features are

observed that indicate the beginning of the fertile period (Simpson *et al.*, 2005) [5].

Vaginal cytology

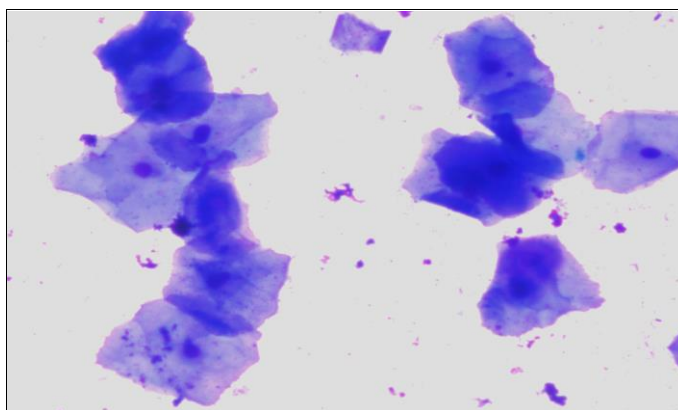


Fig 1: Pro - Estrus stage

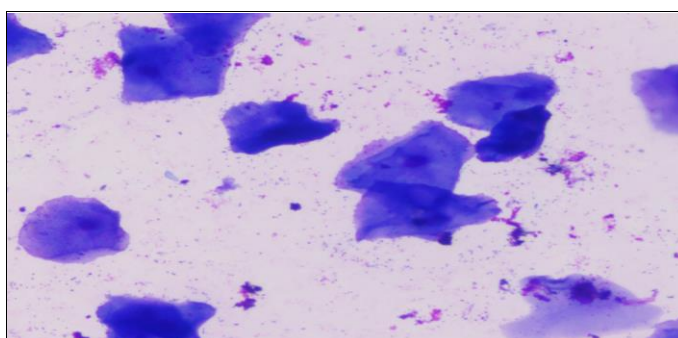


Fig 2: Estrus stage



Fig 3: Pregnancy verification at Day 30



Fig 4: Pregnancy verification at Day 30

Vaginal cytology of the samples

Animal	Parabasal	Intermediate	Superficial Intermediate	Superficial	Cornified	Total_Cells	% Superficial	% Cornified
1	10	6	10	37	32	95	38.94	33.68
2	3	4	14	34	33	88	38.63	37.50
3	4	4	14	31	39	92	33.69	42.39
4	6	5	11	35	40	97	36.08	41.23
5	6	9	11	30	41	97	30.92	42.26
6	7	8	18	19	47	99	19.19	47.47
7	8	8	15	35	30	96	36.45	31.25
8	1	1	12	19	66	99	19.19	66.66
9	9	5	14	27	41	96	28.12	42.70
10	4	6	16	22	49	97	22.68	50.51
11	3	4	17	31	43	98	31.63	43.87
12	2	4	13	47	33	99	47.47	33.33
13	2	7	15	32	42	98	32.65	42.85
14	6	7	18	16	50	97	16.49	51.54
15	6	9	16	26	41	98	26.53	41.83
16	8	2	19	25	45	99	25.25	45.45
17	1	3	19	36	40	99	36.36	40.40
18	6	6	12	24	48	96	25	50
19	2	4	11	35	46	98	35.71	46.93
20	4	8	25	12	49	98	12.24	50
21	4	8	17	31	35	95	32.63	36.84
22	5	9	10	37	38	99	37.37	38.38
23	3	1	18	40	32	94	42.55	34.04
24	9	2	18	31	36	96	32.29	37.5
25	1	6	13	30	46	96	31.25	47.91
26	2	2	11	40	39	94	42.55	41.48
27	7	5	16	28	41	97	28.86	42.26
28	6	4	19	21	43	93	22.58	46.23
29	4	9	12	28	44	97	28.86	45.36
30	2	6	12	31	45	96	32.29	46.87

Progesterone values of the samples

Animal	Proestrus (ng/ml)	Estrus (ng/ml)
Group - II		
11	0.35	4.01
12	1.2	4.7
13	2.1	5.2
14	2.1	4.9
15	2.9	5.6
16	1.6	5.5
17	1.7	4.4
18	1.4	5.3
19	1.8	4.6
20	1.2	4.5
Mean	1.64	4.87
Group - III		
21	2.3	4.2
22	2.5	4.6
23	2.6	4.8
24	2.4	4.5
25	2.7	4.2
26	3.1	4.9
27	3.2	5.6
28	2.4	5.4
29	2.9	5.5
30	2.8	5.4
Mean	2.69	4.91

Conflict of Interest

Not available

Financial Support

Not available

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