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Transvaginal insemination in a 3-year-old Labrador retriever: A case report

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Abstract
This case report represents the conception of a 3-year-old female Labrador Retriever by transvaginal insemination at College of Veterinary Science and Animal Husbandry, Anjora, Durg, India. The dog exhibited a history of vaginal bleeding for six to ten days and showed reluctance to engage in natural mating. Vaginal cytology confirmed the presence of 80 to 90% cornified cells, indicating the estrous cycle. As natural mating was not feasible, artificial insemination (AI) was selected as an alternative approach. Semen collection was done by digital pressure and massage technique and transvaginal insemination was conducted with the assistance of an elevated hindquarters position and a sterile AI sheath. Pregnancy confirmation was carried out via ultrasonography 35 days post insemination. The case highlights the effectiveness of transvaginal insemination as a viable solution for female dogs experiencing difficulties with natural mating, offering breeders and veterinarians a valuable reproductive tool.

Keywords: Transvaginal insemination, artificial insemination, vaginal cytology, canine reproduction

1. Introduction
Semen from the male is taken during artificial insemination (AI), and it is then put inside the female reproductive system. The Italian natural philosopher Spallanzani conducted the earliest known investigations of AI on dogs. Despite having such a stellar lineage, interest for AI in dogs has been reluctant to grow since many breed associations have historically shown strong opposition to it. Since this viewpoint has evolved (UK Kennel Club 2017), there has been a noticeable rise in the application of AI in canines. Parallel to this, techniques for breeding non-domestic Canidae for commercial or conservation objectives have been developed.

In a dog's natural mating practice, the male's penis is inserted into the female's vagina, with the cingulum locking the penis bulb into the vagina. However, not all mating attempts are successful, and factors like slip matings or conformational issues may hinder the achievement of natural mating. This is where artificial insemination plays a crucial role, offering an alternative method to facilitate the breeding process (Mason, 2018) [7].

Various forms of insemination in female dogs have been described, which includes: Vaginal insemination, Endoscope-assisted transcervical insemination (EIU), Non-EIU (Norwegian catheter), Laparotomy insemination, Laparoscopic insemination (Mason, 2018) [7]. In this article, we presented a case report on trans-vaginal insemination in a Labrador dog, highlighting the significance of AI in addressing breeding challenges and ensuring successful reproduction in canines.

2. Anamnesis and Observations
A three years old female Labrador dog was presented to TVCC, Durg, College of Veterinary Science and Animal Husbandry, Anjora, DSVCKV, Durg (C.G.) with the history of vaginal bleeding for the past six to ten days and unwillingness to mate naturally. Further, history revealed that female was reluctant to natural mating. On clinical examination, all physiological parameters were found to be normal. It was observed that the female was too aggressive and inexperienced. The details are given in Table 1.

3. Diagnosis and Procedure
3.1 Predicting the stage of estrus in Bitch
Upon examination, external signs of estrus were noted, and vaginal cytology was performed to confirm the phase of the estrous cycle. Confirmation of oestrous cycle was done by vaginal cytology revealing 80 to 90% of cornified cells (Antonov, 2017) [1]. Vaginal Cytology was performed using cotton swab technique as recommended by Aydin *et al.,* (2011) [2]. The smears were stained using Giemsa staining and observed under 100x. Based on Exfoliative vaginal Cytology, it was predicted that bitch was in estrus. The details of vaginal cytology were given in Table 2.

3.2 Semen collection and Evaluation
The use of an artificial vagina, manual collection, and electrical stimulation are the most frequently used methods for artificial insemination (AI) in dogs (Payan-Carreira *et al.*, 2011) [9]. Semen collection by AV is not advised because to latex rubber's negative effects on canine sperm. Most dogs' sperm may be easily extracted by digitally stimulating the penis. The process is made easier by the presence of a teaser bitch, preferably in oestrus. The manual collection method was used in this case study to collect semen. The male dog was put in this situation in a calm, stress-free setting with enough room and a non-slip surface for pawing. Semen was collected from the male using the digital pressure and massage technique, as recommended by Jahangirbasha *et al.,* (2018) [5].

Digital manipulation was used for collecting semen when certain male dogs did not show signs of sex interest. In order to encourage the male dog to mount the female, the female dog in estrus should be in front of the male dog. The preputial skin was gently moved caudally to reveal the glans penis once the male dog indicated sex desire. A gloved hand applied hard and continuous pressure through the prepuce to the base of the penis behind the bulbus glandis. Up until erection was attained, this technique was repeated with backward and front motions (Fig. 1). For additional analysis, the ejaculated semen was collected in a sterile test tube. The evaluation of collected semen was performed as per the procedures described by Roberts *et al*., (2016) [11]. Macroscopic and microscopic tests were carried out to evaluate the effectiveness of semen for AI. The details of semen evaluation were given in Table 3.

3.2 Procedure for Trans- vaginal Artificial Insemination
The female dog was then placed in a serene, stress-free setting with lots of room and a non-slip surface. With the aid of a second person, the female dog's hindquarters were raised to a height of 45 to 60 degrees above the surface of the examination table (Fig. 2). Semen was placed as near as possible to the exterior os of the cervix during transvaginal insemination (Burke 1986). The bitch's hindquarters were raised during and for 10 minutes after the semen was implanted with a shorter bovine insemination catheter, allowing the semen to run cranially into the cervix and minimising backflow. Semen is frequently flushed into the vagina from the catheter. After that, during insemination, the dorsal roof of the vagina was stimulated with a gloved finger to cause uterine contractions to increase and aid in the transfer of the deposited semen. Fresh semen can result in pregnancy at a rate of about 80% (England & Miller 2008) [4].

3.3 Confirmation of Pregnancy
After 35 days post-insemination, pregnancy was confirmed through ultrasonography (USG). Fig no.3.

Table 1: Physical examination - General parameters

General parameters	Observations
General appearance	No abnormalities detected
Behavioural habit	Active and alert
Feeding habit	Normal
(Cmm)	Pale pink
(Vmm)	Pink and edematous
Rectal temperaturevg)	101.5° F

Table 2: Vaginal Exfoliative Cytology

General parameters	Observations
Anucleated Keratinized Cells	92%
Superficial Nucleated Cells	08%
Intermediate Cells (small & large)	0%
Parabasal Cells	0%
RBC	Present

Table 3: Evaluation of male semen sample

Parameters	Method of Evaluation	Results
Volume	Graduated Test tube	7 ml
Colour	Visual Technique	opalescent
Progressive Forward Motility	Phase Contrast Microscope (Roberts *et al.,* 2016) [11].	85%
Sperm Concentration	Haemocytometer (Payan-Carreira *et al.,* 2011) [9].	170 millions/ml
Live Spermatozoa	Eosin - Nigrosin	85%

Fig 1: Semen collection by Digital manipulation

Fig 2: Transvaginal insemination

Fig 3: Confirmation of pregnancy by USG

Fig 4: Pups delivered successful after fulterm

4. Discussion

In this case report, artificial insemination (AI) was performed on a Labrador dog using fresh semen collected from the male counterpart through manual manipulation technique. The semen collection involved constant and pulsating pressure applied behind the bulbous glandis to ensure an adequate quantity of semen. These findings were consistent with previous studies by Shukla (2011) [10] and Jahangirbasha *et al.,* (2018) [5]. The volume of semen collected was 7 ml, which aligns with the observations made by Payan-Carreira *et al.* (2011) [9]. Semen volume can vary based on factors such as age, breed, frequency of semen collection, prostate gland size, and the size of the dog. However, semen volume is not a factor for semen quality assessment in dogs, as opined by Payan-Carreira *et al.* (2011) [9]. They noted that a decrease in ejaculate volume could be attributed to various factors such as prostatic cysts, benign prostatic hyperplasia, and inflammatory lesions.

The progressive motility of spermatozoa in this case report was 85%, which meets the criteria set by Roberts *et al.,* (2016) [11] who recommended at least 70% progressive motility. Factors such as water or urine contamination, blood presence, prolonged sexual rest, and systemic or infectious diseases can contribute to a decline in the percentage of motile spermatozoa (Payan-Carreira *et al.*, 2011) [9]. The percentage of live spermatozoa in this study was 85%, similar to the recommendation by Malati *et al.* (2022) [6] for a good semen sample to have a minimum of 80% morphologically viable spermatozoa. Eosin-nigrosin staining technique was used to differentiate dead spermatozoa with disintegrated plasma membranes (appearing pink) from live spermatozoa (appearing transparent) (Payan-Carreira *et al.*, 2011) [9].

Regarding sperm concentration, this case report recorded 170 million sperm/ml, while Roberts *et al.,* (2016) [11] reported a normal sperm concentration range of 300-2000 million/ml. An inverse correlation exists between sperm concentration and semen volume. Upon transabdominal ultrasonography, pregnancy was confirmed in the cases with the presence of fetal parts in fetal sacs. Normal whelping occurred at 62 days of gestation. The study demonstrated that digital manipulation is a cost-effective technique for semen collection in dogs. Semen evaluation and artificial insemination prove to be valuable tools for successful breeding (Payan-Carreira *et al.,* 2011) [9].

5. Conclusions

Transvaginal artificial insemination is a valuable and cost-effective method for facilitating successful breeding in female dogs experiencing challenges with natural mating. This case highlights the importance of understanding canine reproductive techniques and considering AI as a suitable option when addressing certain breeding difficulties. Further research and case studies will contribute to improving AI success rates and reproductive outcomes in canine breeding practices.

6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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