

**Prolonged Proestrus Bleeding and Medical Treatment in an English Pointer Dog**Abdulkadir Kaya<sup>1,a</sup>, Ömer Varışlı<sup>1,b\*</sup><sup>1</sup> Kırıkkale University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Kırıkkale, Türkiye<sup>a</sup>ORCID: 0000-0001-7903-4358; <sup>b</sup>ORCID: 0000-0002-2777-3586

\*Corresponding Author

E-mail: omervarisli@kku.edu.tr

Received: November 13, 2021

Accepted: January 23, 2022

**Abstract**

Prolonged proestrus bleeding is a rare pathology observed in bitch. This case is on a British Pointer breed bitch, which came to our clinic for artificial insemination. Artificial insemination was applied to the dog because of vaginal cytology findings, anamnesis, and vaginal discharge. It was thought that estrus is accompanied by proestrus bleeding. Proestrus bleeding was persisting after four days post artificial insemination, therefore GnRH treatment has been applied. After the treatment, bleeding and estrus were simultaneously over. A total of 10 puppies was born.

**Keywords:** Artificial insemination, GnRH treatment, vaginal cytology.

**INTRODUCTION**

Bitches display one or two estruses per year. The average gestation period is 63 days (57-68). The estrus cycle consists of proestrus (9 days), estrus (9 days), diestrus (2 mounts), and anestrus (120 days) phases. Reproductive times in bitches are highly variable, with cycles ranging from 5-12 months, anestrus 1-8 months, proestrus, and estrus can prolong 3-21 days. The time from preovulatory LH surge to estrus ranges from 2 to 5 days, and the time from fertile mating to birth varies between 57-68 days. In addition, it takes 2 days from LH surge to ovulation, about 4 days to oocyte maturation after ovulation, and about 18 days until implantation (Concannon, 1989; Johnston, 2001; Alaçam, 2005).

In the proestrus phase of the sexual cycle, vulva swelling and edema, and vaginal discharge are seen externally. In proestrus male dogs are attracted to the bitch but are not permitted to mount. But in estrus bitch flags her tail and stands for the male dogs. In the female in the proestrus, behavioral interest in the male but refusal to mating, a lick of the vulva, frequent urination, restlessness, increase in drinking is observed. Interpretation of vaginal cytology (VC) result; restrained intermediate and parabasal cells, an increase in the number of erythrocytes, and a small number of keratinized, superficial cells are observed. The proestrus period is an average of 9 days in dogs. Symptoms of the estrous period are edema in the vulva, yellowish vulva discharge, and acceptance of the male. In addition, increased intensive keratinized superficial cells are observed in vaginal cytology (Kırışan et al., 2000; Baştan et al., 2003; Alaçam, 2005).

It is important to determine the time of artificial insemination (AI) because dogs have estrus once or twice a year and the observed estrus duration vary. Therefore, it is essential for dog owners not to miss the time of estrus. If proestrus bleeding in dogs is considered day 1, ovulation

occurs on the 12th day and the appropriate mating time is the 14th day. This schedule can be used in dogs where proestrus bleeding is calculated as 9 days (Freshman, 1991). If the dog's proestrus and duration of estrus are unknown, it is critical to detect ovulation. In general, the first artificial insemination is done on the day of ovulation with fresh semen and the second is done at the following 48th hour. Because canine spermatozoa survive up to seven days in the bitch's tract and ova are fertile for only 12 to 24 hours. If frozen semen is used, the first insemination is made two days after ovulation and the second insemination 48 hours after that. Vaginal cytology, hormone analysis and, ultrasound methods are used to determine the time of appropriate artificial insemination in dogs. Superficial and keratinized cell frequency is sought in vaginal cytology. Progesterone value in the range of 4-10 ng/ml indicates ovulation (Payan-Carreira et al., 2011). In artificial insemination practice, it is sufficient for semen to have high motility (70% and above motility) and 150-200 million spermatozoa per milliliter (Tsutsui et al., 1988; Linde and Forsberg, 1991; Wilson, 1993).

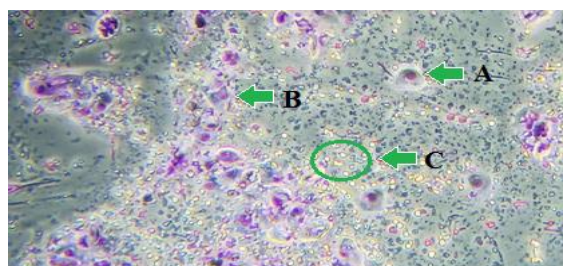
The phenomenon of prolonged proestrus/estrus bleeding in dogs is a rare reproductive disorder among reproductive disorders. Prolonged proestrus bleeding is caused by estrogen or LH hormone and may last for three weeks or longer. In dogs with prolonged proestrus bleeding, estrus and ovulation do not occur due to the inadequate level of estrogen hormone, and as a result of prolonged bloody vaginal discharge, cornified cells higher than 50-90% in vaginal cytology indication and serum progesterone level down 2 ng/ml is observed. Medical treatment or ovariohysterectomy operation is performed to reorganize the sexual cycle in cases of proestrus discharge. In medical treatment, primarily progesterone, GnRH (Gonadotropin Releasing Hormone), hCG (Human Chorionic Gonadotropin) hormones and antibiotics,

b-carotene, D3, and, E vitamins can be used to support the

treatment (Flatscher, 1999; Kırşan et al., 2000; Güvenç et al., 2006; Arlt et al., 2011).

### CASE HISTORY

Artificial insemination was requested for the British Pointer breed dog brought to Kırıkkale University Faculty of Veterinary Medicine Research and Application Animal Hospital, Reproduction and artificial insemination Clinic on 12.09.2019. According to the anamnesis information, vaginal discharge was started on 31.08.2019 and the bleeding was continuing. However, the male dog was unable to mate despite the bitch's desire to mate (Impotentia Coeundi). Vaginal cytology was inspected on the dog before routine artificial insemination. A high rate of intermediate and superficial cells and dense erythrocytes was observed in vaginal cytology (Figure 1). On the same day, semen was collected from a 1.5-year-old British Pointer breed male dog by digital manipulation for artificial insemination. The collected semen (4 ml) was examined in terms of motility under a phase-contrast microscope with a heating table and it was determined that semen had 80% motility. The spermatozoon concentration was determined as 300 million spermatozoa per milliliter. These data showed that the obtained semen is quite suitable for artificial insemination. Afterward, the bitch was artificial inseminated deeply vaginally using the entire spermatozoa-rich fraction. On the 4th day (16.09.2019) following artificial insemination, a single dose of 1ml GnRH (Ovarelin®, Ceva) was injected due to the continuing proestrus bleeding. Two days after GnRH injection (18.09.2019), it was reported that according to the cytological examination findings, estrus was over, proestrus bleeding was still continuing, but the female did not accept the male. The second GnRH application was made on 18.09.2019 and it was learned by the owner of the animal that the proestrus bleeding was over two days.



**Figure 1.** An Image of a Vaginal Smear (A: Intermediate Cell, B: Superficial Cell, C: Erythrocyte)

### RESULTS

A positive pregnancy was detected in the ultrasonographic examination performed in our hospital on the 32nd day after artificial insemination. The birth took place on the 64th day after artificial insemination and a total of 10 healthy puppies were born (Figure 2).

### DISCUSSION AND CONCLUSION

Estrus cycle pathology in dogs can be classified under main headings as prolongation and shortening of interestrus time and prolongation of estrus. Proestrus prolongation is a rare pathology in reproductive physiology, and few reports have been made on this subject (Çiftçier and Uysal, 2014). This situation is thought to be caused by the failure in follicle development due to insufficient LH and estrogen secretion (Kırşan et al., 2000; Risvanli et al., 2016). Prolonged proestrus bleeding is a condition that needs to be treated. Among the

treatment options, ovariectomy and medical treatment may be preferred. If the dog is desired to continue a fertile life, medical treatment is recommended instead of ovariectomy. In the presented study, it has been shown that medical treatment can be effective and that a large number of puppies can be obtained simultaneously with the treatment protocol.



**Figure 2.** Bitch and Puppies.

In a study by Çiftçier and Uysal (2014), 1 mL GnRH (Gonadotropin Releasing Hormone) and 400 IU hCG (Human Chorionic Gonadotropin) hormones were administered intramuscularly for treatment in a Rottweiler breed dog that was three years old and had proestrus bleeding for 18 days. Pregnancy was obtained by artificial insemination. In this case, proestrus bleeding lasted for 20 days and, bleeding ended four days after GnRH injection. In the mentioned study, it was stated that artificial insemination was performed in estrus after the medical treatment for proestrus bleeding was completed, but artificial insemination was performed in this study although the presence of vaginal bleeding (during the proestrus period). Waiting for the end of bleeding causes missed ovulation time and delayed artificial insemination. Thus, in cases of proestrus bleeding, both artificial insemination can be applied with estrus detection and stimulation of ovulation with hormonal therapy can be done in the same time period.

Proestrus occurs under the activity of the estrogen hormone secreted from the developing follicles. Estrogen also increases vascularity in the vaginal tissue and causes blood cells to infiltrate into the lumen, causing a bloody vaginal discharge. Estrogen thickens the vaginal epithelial layer and causes edema in tissues such as the vulva. The vaginal cytology of early and middle proestrus consists of dense intermediate and less superficial cells from parabasal cell. In late proestrus, on the other hand, superficial cells with pyknotic nuclei are found instead of intermediate cells. Superficial and keratinized cells in estrus constitute 80% or even 100% of total vaginal cells. In vaginal cytology, the distinction between late proestrus and estrus is the presence or absence of erythrocyte cells. Prolonged proestrus is defined as the failure of ovulation due to insufficient estrogen and LH hormone secretion (Rişvanlı et al., 2016). In this case, hormonal data could not be compared since there were only anamnesis, clinical findings, and vaginal cytology data. According to the vaginal cytological examination, it was diagnosed that the animal was in proestrus (Figure 1). However, in the

previous breeding experience of the animal that estrus and proestrus lasted less than 9 days. According to clinical findings, anamnesis and also the literature (the proestrus bleeding in dogs is considered the first day and ovulation on the 12th day and the 14th day as the appropriate mating time (Freshman, 1991)), the bitch was decided to do artificial insemination. In the following days, medical treatment was applied due to the persistence of bloody vaginal discharge. As a result of this patient's medical treatment, not only vaginal discharge but also estrus was over. After clinical follow-up, it was determined that the reported non-ovulation status would not always occur for prolonged proestrus and proestrus bleeding, and insemination around the 14th day after the onset of proestrus was appropriate in bitches with an average of 9 days of proestrus and estrus.

In conclusion, although vaginal cytology findings provide important information about the correct insemination time, it has been observed that the most important symptom of estrus is to accept the male. Furthermore, in cases of proestrus or proestrus bleeding, a successful pregnancy can be achieved by insemination with GnRH treatment.

#### Financial support

This research received no grant from any funding agency/sector.

#### Conflict of Interest

The authors declared that there is no conflict of interest.

#### REFERENCES

- Alaçam E. 2005. Evcil Hayvanlarda Doğum ve İnfertilite, 5. Baskı, Medisan Yayınevi, Ankara, Türkiye.
- Arlt SP, Spankowsky S, Heuwieser W. 2011. Follicular cysts and prolonged estrus in a female dog after administration of a deslorelin implant. *New Zealand Veterinary Journal*, 59(2): 87-91.
- Baştan A, Çetin Y, Güngör Ö, Başaran DA. 2003. Endometrial Cytology Findings at Different Stages of Sexual Cycle, Pregnancy and Pyometra in Bitches *Turkish Journal of Veterinary & Animal Sciences*, 27(4): 893-897.
- Concannon PW, Mc Cann JP, Temple M. 1989. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *Journal of Reproduction and Fertility Supplement*, 39: 3-25.
- Çiftçier B, Uysal O. 2014. Fertility in bitch with prolonged proestrus. *Livestock Studies*, 54(2): 71-76.
- Flatscher C. 1999. Programm der Postersitzungen-Freitag, 19. Februar 1999-ab 09.30-Postersession IV-Gynagologie 2 (PV/52-66)-Effects of b-carotene on the ovarian and uterine cycle in the bitch. *Reproduction in Domestic Animals*, 34(1): 38.
- Freshman JL. 1991. Clinical approach to infertility in the cycling bitch. *Veterinary Clinics North American Small Animal Practice*, 21: 427-431.
- Güvenç K, Toydemir F, Sontaş H. 2006. A Cocker Spaniel bitch with uterus unicornis (unilateral corneal agenesis). *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, 32(3): 69-73.
- Johnston SD. 2001. The Canine Estrous Cycle In: *Canine and Feline, Theriogenology*, Ed: SD Johnston, MVR Kustritz, PNS Olson.
- Kırşan İ, Şenünver A, Kaşıkçı G, Güvenç K, Gürbulak K. 2000. Dişi köpeklerde uzayan proöstrüsün medikal tedavisi. *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, 26(1): 223-234.
- Linde C, Forsberg C. 1991. Achieving canine pregnancy by using frozen or chilled extended semen. *Veterinary Clinics of North America: Small Animal Practice*, 21: 467-485.
- Payan-Carreira R, Miranda S, Nizanski W. 2011. Artificial Insemination in Dogs. *Artificial Insemination in Farm Animals*. InTech, Rijeka 51-78.
- Risvanli A, Ocal H, Kalkan C. 2016. Abnormalities in the sexual cycle of bitches. *Canine Medicine: Recent Topics and Advanced Research*, Hussein Abdelhay Elsayed Kaoud, IntechOpen, 127-138.
- Tsutsui T, Tezuka T, Shimizu T, Murao I, Kawakami E, Ogasa A. 1988. Artificial insemination with fresh semen in beagle bitches. *Japan Journal Veterinary Science*, 50(1): 193-198.
- Wilson M.1993. Non-surgical intra uterine artificial insemination in bitches using frozen semen. *Journal of Reproduction and Fertility Supplement*, 47: 307-311.