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Optimal Breeding Time Determination in Bitch Using Vaginal Cytology: Case Report

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ABSTRACT

Two rotweilers and one local mongrel bitches were presented to a veterinary practice for optimal breeding time (OBT) determination at different time. This was done using vaginal cytology. The swab technique was used to collect the vaginal epithelial cells from the posterior vagina. The clinical examinations of the first two bitches were similar with a soft and bulging vulva; and faint red bloody discharge. The bitches also exhibited Amantea reaction (relaxed when hands were put on their back). The vaginal cytology revealed the presence of mostly superficial as seen in estrus and bitches were recommended for breeding (3times consecutively) from that day upward. Whereas, the third bitch being on late pro-estrus was crossed 5 days later when the cytology revealed estrous. At the end of 61-67 days gestations, the three bitches delivered 8, 10 and 5 puppies respectively. This result once again emphasized the accuracy of vaginal cytology as a useful tool to determine an optimal breeding time in bitch. Hence, vaginal cytology, though can not detect ovulation day, will continue to be patronized by small animal practitioners because it is simple, fast, cheap, can detect the stage of the estrous cycle and to some extent accurate in determining optimal breeding time in a bitch particularly when combine with good clinical examinations.

Key words: Bitch, estrus, optimal breeding time, ovulation and vaginal cytology

INTRODUCTION

According to Durrant *et al.* (2003), the diagnostic value of stained vaginal smear for estrous characterization was first recognized by Stockard and Papanicolau in 1917 with the keratinization of the guinea pigs vaginal epithelium at the time of estrus. Thereafter, vaginal cytology has been used in many animals including dogs (Schutte, 1988). The bitch has a unique reproductive cycle quite distinct from other domestic animals. The peculiarities in the bitch cycle include physiological anaestrus, progesterone not being diagnostic of pregnancy and the length of diestrus that is almost the same in pregnant and non-pregnant bitch (Morrow, 1986). The vaginal cytology is a simple technique that can be employed by small animal practitioner to characterize different stages of reproductive cycle in the bitch. It can be useful for estimating the optimal breeding time for the bitch (Holst, 1986) and also for diagnosing pregnancy and reproductive disorders (Johnson, 1991) as well as in predicting the time of whelping (Wright, 2005).

The predominant cells at pro-estrus are intermediate cells with many blood cells. Very few superficial cells are encountered at late pro-estrus. The estrus is indicated by 100% superficial cells; while the first day of diestrus is a more reliable stage that can be identified, as the cells in the swab abruptly change from 100% cornified cells (at estrus) to about 50% non-cornified epithelial cells and Polymorphonuclear (PMN) cells are present (Baker and Lunsden, 2001) However, vaginal cytology does not identify ovulation day.

Optimal breeding time is that time at which a bitch on estrus can be bred to give maximum litter size. As dog's breeding especially the exotic breeds is becoming a potential business all around the globe, the small animal practitioners have to be conversant with various techniques of detecting optimal breeding time in bitch of which vaginal cytology is one of the simplest methods. In view of inadequacy of sophisticated facilities require for other methods such as hormonal assay and ultrasound in developing countries, this case is reported to consolidate on the

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use of vagina cytology to determine optimal breeding time in a bitch on 'heat'.

Case history

Three adult bitches (2 rotweilers and I local mongrel) presented to a veterinary practice in Ibadan at different time were used for the study. The owners wanted to know the right time to breed the bitches, having noticed the bloody discharge from vulva few days prior to presentation and were not sure of the day the heat actually commenced. The bitches were 2, 3 and 2½ years respectively. The first and the second had never whelped despite been crossed two times in the past, the third bitch had whelped once.

Management

The rectal temperatures were taken with digital rectal clinical thermometer, while the heart rates were measured using the stethoscope. The vulvae were critically examined for texture, size and colouration of their discharges. Vaginal cytology was indicated to be able to arrive at the right time of breeding the bitch (Holst, 1986).

Swab collection

This involved collecting the vagina swab using a sterile cotton swab. The bitch allowed to stand on the table and properly restrained using a muzzle. The vulva was properly cleansed with a sterile cotton swab. The left hand was used to press the major and the minor vagina cantus so that the vulva was fully opened. A cotton swab was then inserted into the vagina via cranio-dorsal angulation to avoid the clitoral fossa. When it has touched the vagina wall, it was rotated slightly in a clockwise direction to exfoliate and collect the cells of the vaginal wall.

Slide preparation

The slides were prepared using the cranial vagina as described by (Marti, 2005). The cells were quickly transferred from the swab on to a clean slide by gently rolling the swab on the slide once. Three slides were made in this way using three different swabs for each observation. The slides were air-dried and then fixed in alcohol for 30 minutes before Giemsa stain was applied. The slides were observed under a light microscope (× 400).

Vaginal cytology was done alternate days in the first two bitches and once in three days in the third bitch until when it indicated diestrus stage (Fig. 4). This is necessary because diestrus information is more reliable and used retrospectively to evaluate if the breeding (at estrus) had been done in the appropriate window of fertility. The slides were interpreted as described by Reskin and Meyer (2001)

RESULTS

The rectal temperatures of the bitches ranged between 38.7ºC and 39.3ºC.

Bitch 1 and 11

The clinical examinations of the first two bitches were similar with a soft and bulging vulva; and faint red bloody discharge. The bitches exhibited Amantea reaction (relaxed when hands were put on their back). The vaginal cytology revealed the presence of mostly superfiscial cells, which are sharp, flat angular cytoplasmic border with a small, pyknotic nuclei (Fig. 1) or anuclear squeamated cells (Fig. 2) typical of late estrus. Hence the bitches were recommended for breeding from that day upward. The bitches were asked to be bred from that day for three consecutive days.

Bitch 111

The third bitch did not exhibit Amantea, her vulva was soft and quite swollen while the blood discharge was thick red in colouration. The vaginal cytology revealed scattered and few superfiscials cells but with presence of red blood cells (Fig. 3) as seen in late proestrus. This bitch was recommended for breeding (at least thrice two days apart) after 5 days when cytological estrus was observed. At the end of 61 to 67 gestations, the three bitches delivered 8, 10 and 5 puppies, respectively.

DISCUSSION AND CONCLUSION

There are two schools of thought as regards the point of cells collection for cytology. The first one felt it is necessary to collect from the anterior vagina (Schutte, 1988), the other believed it is better at the posterior portion (Marti, 2005). The later was followed in this study based on the simplicity, the possible contamination along the posterior-anterior route and the sufficiency of using the vulva labie. Among the stains used for vagina cytology include Romanowsky stains (Wright, Giemsa, Leisman, Diff-Quik^{R}), new methylene blue, toluidine blue and haemotoxylin and eosin (H & E) (Baker and Lumsden, 2000). The preference for Giemsa stain in this study was founded on its low cost, availability and production of consistent staining adequate for cytological study (Manothaiudom,and Johnson, 1991)

The normal vagina is lined with squamous epithelium of few cell layers thick that is very prone to injury even

by slightest touch. For instance, petechial haemorrhages are produced by vaginoscopy at any time other than proestrus or estrus. 17β estradiol and estrone are the estrogens secreted during the follicular phase of the cycle by mature follicles (Macdonald and Pineda, 1989) and these affect the vaginal epitheliumin in three ways; by proliferation, maturation and exfoliation (Montes and Lugue, 1988).

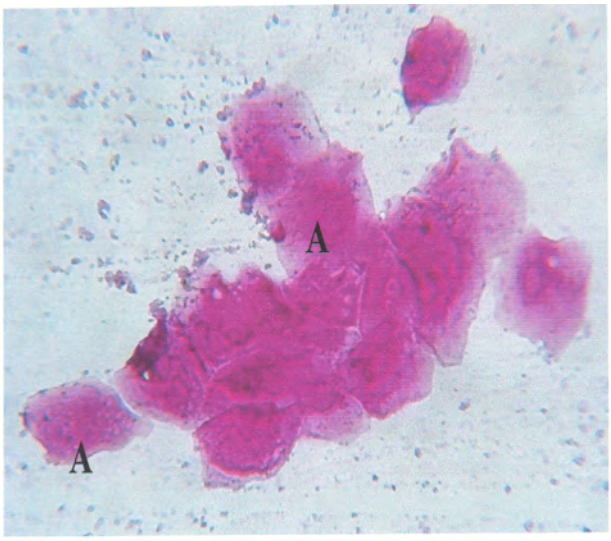

Fig. 1. Photomicrograph showing superficial cells that are keratinized, largely anucleated and have angular folded cell margins indicating estrus. × 400

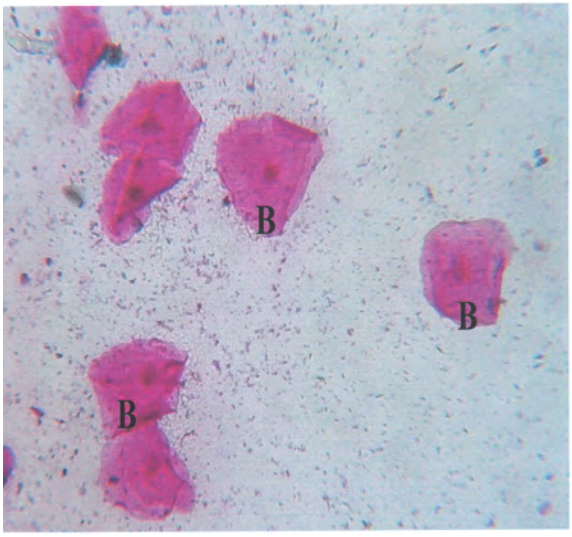

Fig. 2. Photomicrograph showing many superficial cells with pyknotic nucleus (B) × 400

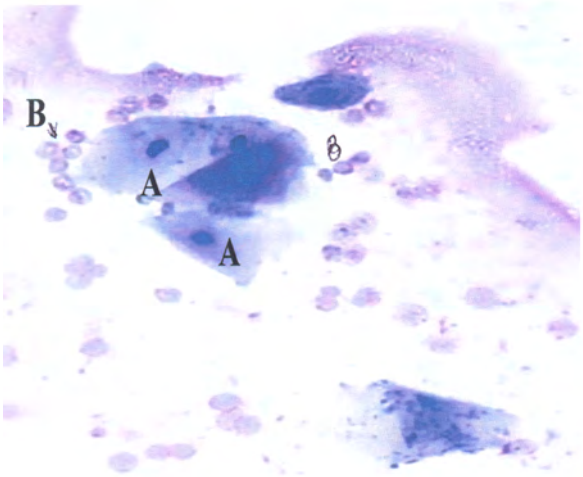

Fig. 3. Photomicrograph showing presence of scattered superficial cells (A) and erythrocytes (B) typical of late pre-estrus. × 400

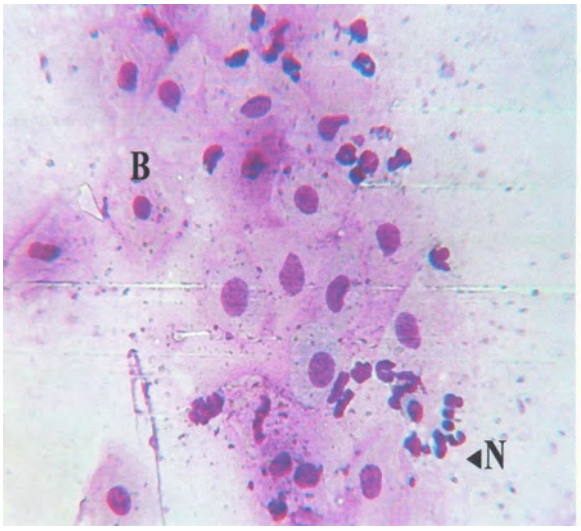

Fig. 4. Photomicrograph showing presence of non-cornified intermediate cells (B) and degenerate neutrophils (N) of diestrus. × 400

Therefore, the vaginal epithelium becomes thickened 20 to 40 cells layers to protect the vagina during copulation, while exfoliative cytology reveals changes in the epithelial lining from noncornified to cornified epithelial cells or superfiscial cells. Superficial cells have been defined as the largest cells in vaginal cytology with a sharp , flat angular cytoplasmic borders and a small pyknotic, fading nuclei (Fig. 2) or no nuclei (Fig. 1)and their dominance corresponds with the estrus (Raskin and Meyer, 2001). Thus, vaginal cytology is a spontaneous effect of estrogen activities which is at peak one to two days prior to estrus. At proestrus, vaginal cytology shows many erthrocytes cells and scattered superficial cells (Fig. 3) while vulvar edema, hyperaemia and serosanguineous discharges are observed. Presence of non-cornified intermediate cells and degenerate neutrophils of the cytology 2 days post crossing indicate diestrus (Fig. 4) is a retrospective way of adjudging that the crossing was done at the right time (Wright, 1990). Most often, the bitch allows mating only during the estrus around which period, the release of the ova from a graffian follicle (ovulation) occurs. Ovulation timing in the bitch is not always an easy

procedure and variation in behavioural changes exist between bitches and breed at this period (Hewitt and England, 2000). It takes place with the liberation of immature oocytes two days after the peak of LH. These oocytes take at least 48 hours before maturity and being ready to be fertilized. Reynaud *et al.*, (2005) demonstrated that the canine oocytes cannot be penetrated by spermatozoa immediately after they are released because they are still immature. Therefore, even if it is essential for a veterinarian to be as accurate as possible to detect the day of ovulation, this day is reached not less than 48 hours before the day of fertilization. Since an ovum is viable for 2-3 days, while spermatozoa can last 6 to 9 days in an estrogen-primed reproductive tract, optimal breeding time occurs between 2 days before ovulation to 4 days after ovulation, when the oocytes are fully matured and have not underwent degeneration. However, a fertile period can be as open as 5 day before and 5 days after ovulation (Levy and Fontbonne, 2007)

None of the clinical assessments, like the vulval oedema, the quantity and colouration of the vulval discharge, vaginoscopy, the Amantea sign or even the acceptance of the bitch to be mounted by the male is precise enough to detect the occurrence and day of ovulation in a particular bitch (Levy and Footborne, 2007). Ultrasonography can determine the time of ovulation accurately and its combination with progesterone assay produced 10% increase in the accuracy (Levy and Fontbonne, 2007). All the same, it is well known that there is no reliability on a predetermined ovulation day, and consequently, a predetermined mating date. Some bitches may ovulate as early as day 5 of the heat period, and others as late as day 30. In the same bitch, it has been shown that significant variations of the day of ovulation may occur among successive heat periods in around 44% of the cases (Badinand and Fontbonne, 1993). The later hypothesis further strengthens our reliance on the estimation of the optimal breeding time derived from the vaginal cytology with disregard for ovulation day. Vaginoscopy is another simple method that can be used along with vaginal cytology. It reveals the status of the vagina that becomes flattened and fold with moist red mucosa when under the influence of estogens at proestrus or estrus. In some conditions, it is highly recommended to use clinical examinations mentioned earlier in conjunction with the vaginal cytology. The conception in all the three bitches as well as high number of puppies is a pointer once again to the effectiveness of vaginal cytology to characterize estrous stages and in determining optimal breeding time in bitch. We conclude that despite limitation of vaginal cytology as regards detecting ovulation day, it will continue to be patronized by small animal practitioners because it is fast, cheap, simple; can detect the stage of estrous cycle; and to some extent accurate in determine optimal breeding time especially when combine with good clinical examinations.

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