Original article

Advocacy for quantitative progesterone assay in the breeding management of bitches

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ABSTRACT

Peripheral progesterone titers characterizing the reproductive cycle of the bitch are fairly well-documented in literature. Progesterone titers obtained via fluorescence-immunoassay technique at different points to the appearance-of-bloody-vulva-discharge (ABVD) in apparently healthy, exotic, multi-breed bitches (n = 76) that were subsequently inseminated were summarized using descriptive statistics. The recommended/predictable progesterone titer-ranges- PPTRs (ng/mL) and modality for insemination(s) were: 7.6-8.5 (inseminate-after-24-hours), 8.6-9.9 (inseminate-after-12-hours) and 10.0-20.0 (inseminate-immediately). The length of days from ABVD to the point of obtaining a PPTR (Mean ± SD) was 12.43 ± 3.38 days (range 8-17 days). The frequencies of progesterone assay per bitch were: one-fold (39.47%), two-fold (39.47%). three-fold (18.43%) and four-fold (2.63%). Observations on pregnancy statuses of bitches based on frequency of progesterone assay(s) were: 57.90% (pregnant, multiple-assay), 5.26% (pregnant, one-fold-assay), 26.32% (not-pregnant, multiple-assay) and 10.52% (not-pregnant, single-assay). The litter sizes at the PPTRs (Mean ± SD) i.e., 7.83 ± 1.86 (7.6-8.5 ng/mL), 7.86 ± 1.73 (8.6-9.9 ng/mL) and 8.83 ± 1.86 (10.0-20.0 ng/mL) were comparable with that for the entire study i.e., 8.16 ± 1.87. Similarly, the range of litter sizes at the three PPTRs as well as that for the entire study were same i.e., 5-10 puppies. It is concluded as follows: multiple progesterone assays resulted in higher pregnancy outcome and, litter sizes were not affected as long as insemination(s) occurred within the recommended progesterone titer-ranges. We recommend a comprehensive investigation of all pregnancy determinants, even for apparently healthy dogs, by dog-lovers and, regarding progesterone, multiple-assays, rather than one-fold assay, will upscale their breeding business.

Keywords: bitches, counsel, insemination timing, pregnancy, progesterone assays

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INTRODUCTION

The determination of optimal breeding time, just as many other aspects of canine reproduction, has often posed challenges to dog breeders and dog lovers. The clinician in many parts of the world, especially where relevant facilities are lacking, is not exempted from the seeming embarrassment often associated with this feat. The intricacies surrounding this aspect of canine reproduction is also well established in literature (Arlt, 2016; Obafemi *et al.,* 2021; Pavlo *et al.,* 2022). Although, various techniques aiding the seamless determination of the right time to inseminate the bitch have been described (Hollinshead and Hanlon, 2018; Pavlo *et al.,* 2022), many of these have few clinical applications. Of late, the most widely used technique of vaginal cytology, perhaps in many parts of sub-Saharan Africa, has been reported to be associated with some inadequacies (Antonov, 2016; Reckers *et al.,* 2022). In an on-going work investigating the status of vaginal exfoliated cells with that of peripheral progesterone during oestrus, findings with the cytological picture of a substantial number of bitches have shown non-characteristic patterns, mostly during oestrus. Interestingly, those for some other bitches do conform to known patterns (Leigh *et al.,* 2013). Some literatures as well as practitioners have underscored the relevance of peripheral progesterone as an indicator of the reproduction status of the bitch. The progesterone concentration in the bitch begins to rise just before or at the start of LH surge, which has been associated with pre-ovulatory follicular luteinization (Hollinshead and Hanlon 2018; Marinelli *et al.,* 2009). In many developing countries however, the technique has not become popular compared to vaginal cytology. In view of growing complaints of infertility associated with breeding at periods determined via vaginal cytology, and its increasing frequency of non-clarity/diagnostic outcomes, this study investigated peripheral progesterone titers in adult, multi-breed, exotic bitches from points of appearance of bloody vulva discharge, in order to determine their right breeding time. This paper discussed observations and findings which appeared to justify an advocacy for progesterone monitoring and modality, for a more effective determination of the right breeding time in bitches.

MATERIALS AND METHODS

Experimental animals

Adult (≥ one parity), apparently healthy, exotic, multi-breed (Boerboel, Caucassian, and German Shepherd) bitches, (n= 76) showing bloody vulva discharge that signifies heat were used. The day of appearance of bloody vulva discharge was obtained from history.

Sample collection and serum preparation

Intravenous blood (1.5-2.0 mLs) collected at different points from the appearance of bloody vaginal discharge (ABVD) in the bitches were allowed to stand for 30 - 60 minutes at 22-25 °C in order to clot. Serum was thereafter centrifuged at 4000 rpm for 15 minutes.

Assay procedure

A portable veterinary progesterone analyzer (Model MSL-YT02, Medsinglong Co. Ltd. China), based on fluorescence immunoassay detection method, was used. Eighty microliter of serum was pipetted into the round hole of the reagent card. Once the serum diffused into the strip detection area, the charged end is inserted into the instrument card slot and the machine is operated to enter a fifteen-minute detection mode, leading to display of the progesterone titer.

Predictable progesterone titer-ranges and insemination procedure

Three predictable progesterone titer-ranges (ng/mL) for insemination, according to manufacturer's recommendations were adopted as follows: 7.6-8.5 (inseminate-after-24-hours), 8.6-9.9 (inseminate-after-12-hours) and 10.0-20.0 (inseminate-immediately). Repeat insemination(s), as recommended, was allowed, at most once or twice, depending on result of the assay. The artificial expandable penile shaft kit was used for the insemination, following standard procedures.

Parameters investigated

The difference (days) between the appearance of bloody vulva discharge (ABVD)

and when a progesterone titer within the predictable progesterone titer-ranges (PPTRs) was obtained in each bitch. The frequencies of progesterone assays carried out per bitch till a titer within the PPTRs was obtained. Pregnancy diagnosis of bitches was carried out between days 30-35 post insemination using the WITNESS® Relaxin Rapid Test kit (Zoetis, US). The litter size was recorded at the points of whelping in each bitch.

Data analysis

Data obtained were summarized with descriptive statistics (Mean ± SD, and proportions) using SPSS 21.0 and presented in tables.

RESULTS

The length of days between ABVD and the point at which a progesterone titer within the PPTRs for insemination was 12.43 ± 3.38 (8-17) days, while the frequencies of progesterone assays per bitch and proportion of bitches involved were: one-fold (39.47%), two-fold (39.47%). three-fold (18.43%) and four-fold (2.63%). Observations on pregnancy statuses based on frequency of progesterone assay(s) revealed the proportion of the four categories i.e., pregnant, multiple assays; pregnant, single assay; non-pregnant, multiple assays and non-pregnant, single assay (Table 1). Based on PPTRs and subsequent recommended period of insemination of the total 76 bitches, data on litter size of each PPTRs was obtained (Table 2).

Table 1 Observations on pregnancy statuses of bitches based on frequency of progesterone assay(s)

pregnancy status of bitches (n = 76)	assay frequency of bitches: multiple/yes- > one-fold; single/no- one-fold	proportion (%)
pregnant	yes	57.90
pregnant	no	5.26
not pregnant	yes	26.32
not pregnant	no	10.52

Table 2 Litter sizes (Mean ± SD) in the study with respect to predictable progesterone titer-ranges as basis for insemination time(s)

no. of bitches in the group/ no. that has littered (n= 76)	predictable progesterone titer-range (ng/mL)	period of insemination as recommended	number of puppies/Mean ± SD	total no. of puppies/Mean ± SD	range (no. of puppies)
24/6	7.6 - 8.5	after 24 hours	47/7.83 ± 1.86	155/8.16 ± 1.87	5 – 10
28/7	8.6 - 9.9	after 12 hours	55/7.86 ± 1.73		
24/6	10.0 - 20.0	immediately	53/8.83 ± 1.86		

DISCUSSION

The present study shows that days 9 to 15 appear to be the commonest window when bitches attained the PPTRs at which successful insemination(s) may be carried out. This window incorporates the usual mating/insemination period that is popular among dog breeders. The caveat however is the common knowledge that events in consecutive oestrous cycles often differ in some respects in the same bitch (Badinand and

Fontbone 1993). The implication of this is that some elements of probability, and therefore infertility, may surround sticking to a predetermined insemination period in bitches that is not scientifically supported. Progesterone monitoring that is associated with multiple assays (two-to four-fold) per bitch, successfully predicted the time of insemination with high pregnancy rate in this study. This is similar to previous studies (Marinelli *et al.*, 2009) on multiple progesterone evaluation in cycling

bitches. In normal estrous cycles of bitches, progesterone titer gradually increases from early proestrus to about 6 – 7 ng/mL (mid-proestrus) when ovulation occurs. Other cycle events i.e. oocyte maturation, mating time, diestrus, implantation and pregnancy, are well described with reference to changes in progesterone values (Concannon and Lein 1989). However, a slightly greater than a tenth of the bitch population that were non-pregnant in this study may easily be explained away as having occurred due to low frequency of progesterone monitoring. Worrisome is the higher proportion of bitches which, in spite of multiple assays were not pregnant. These observations along with others may simply re-emphasize the fact that pregnancy establishment is subject to numerous factors other than the single female factor considered (i.e. progesterone) in this study. Optimization of other pregnancy determinants such as health and fertility of sex partners as well as inseminator efficiency will therefore be complementary to the usefulness of progesterone monitoring for higher pregnancy rates. The total proportion of pregnant bitches in the study was slightly above 60%. This appears to be proportionate to the total frequency of bitches that were assayed more than one-fold and predicates the need to counsel breeders and dog-lovers to embrace progesterone assay for the determination of the best-fit time for insemination at every oestrus cycle. The comparability of the mean values of litter sizes under the three PPTRs as well as that of the entire study suggests that none of the PPTRs is superior to another, at least, with respect to litter size outcomes. For this opinion to hold and within the limits of the current study, there should be close similarities in parity, age, breed as well as other determinants of litter size in the bitches included in the study.

CONCLUSION

Although, multiple progesterone assays were associated with high pregnancy outcome, about 40% of bitches were presented for monitoring just once (i.e. one-fold). Litter sizes were comparable and were not affected by the points at which insemination(s) occurred, as referenced by the predictable progesterone titer-ranges.

RECOMMENDATION

Dog-lovers are encouraged to routinely carry out a comprehensive investigation of all pregnancy determinants, even in apparently healthy dogs and, regarding progesterone, multiple-assays, rather than one-fold assay, should be embraced in breeding management, in order to upscale their business.

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