



Prediction of the optimal time for insemination using frozen-thawed semen in a multi-sire insemination trial in bitches



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ABSTRACT

The aims of the study were to determine which of Days 5, 6 or 7 after the blood plasma progesterone concentration (PPC) of bitches first reached 6–9 nmol/L (Day 0) yield the highest fertility and whether day of insemination affects the gender ratio of conceptuses. Six bitches were inseminated on Days 5 and 6 and 6 on Days 6 and 7. Ten million progressively motile frozen-thawed sperm from each of 5 dogs were pooled for the first insemination. The same number of sperm from 5 other dogs were pooled for the second insemination. Only one batch of semen from each dog was used on all bitches, which largely prevented any effect of male and semen. Twenty-three autosomal microsatellites and the amelogenin gene were used to determine the paternity and gender of the conceptuses. Pregnancy rate was 100%. Out of 103 ovulations 66 conceptuses were conceived (conception rate: 64%). The proportion of available oocytes fertilised was 0.11, 0.56, and 0.27 for Days 5, 6, and 7, respectively. The odds of fertilisation was 16.7 and 4.2 times higher from insemination on Day 6 compared to Day 5 ($P < 0.001$) and Day 7 ($P = 0.013$), respectively. The numbers of male- and female conceptuses were equal (33 each) and gender was independent of insemination day ($P = 0.18$). This study suggests that intrauterine insemination of bitches should best be done 6 days after PPC first reaches a value between 6 and 9 nmol/L with a second insemination one day later.

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1. Introduction

Insemination with frozen-thawed spermatozoa has become a well-established procedure in bitches in many parts of the globe. Yet, there is a need to improve the methodology in order to obtain better efficiency and fertility.

Based on a large retrospective study, Thomassen et al. (2006) concluded that bitches should optimally be inseminated with frozen-thawed spermatozoa 2 and 3 days

after the estimated time of ovulation. Only two studies (Badinand et al., 1993; Tsumagari et al., 2003) on bitches were, however, of a design suitable to compare the fertility of inseminations with frozen-thawed spermatozoa performed on different days during the same oestrous period of a bitch. Being able to compare the fertility of different days during the same oestrous period is essential to determine the day of the oestrous period on which frozen-thawed spermatozoa will yield the highest fertility. This is a prerequisite for determining the minimum effective number of inseminations and the minimum effective inseminating dose.

In the bitch, the LH surge induces ovulation, which precedes oocyte maturation and fertilisation. The

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concentration of progesterone in the blood plasma or -serum (PC) follows a similar pattern among bitches. PC starts rising due to pre-ovulatory follicular luteinisation, becoming progressively more pronounced during the period leading up to ovulation (Concannon et al., 1977). PC usually starts rising at approximately the same time as the onset of the LH surge (Concannon et al., 1977; Wildt et al., 1979; Badinand et al., 1993; Bysted et al., 2001; de Gier et al., 2006; Bergeron et al., 2013). Occasionally, PC only starts rising at the time of the LH peak or as much as 24 h thereafter (de Gier et al., 2006). Mean PC values at the time of the LH peak varies among studies: 8.1 (SD 2.3) nmol/L (Concannon et al., 1977), 14.0 (SD 14.3) nmol/L (Wildt et al., 1979), 3.8 (SD 0.7) nmol/L (Jeffcoate and England, 1997), 4 nmol/L (Bysted et al., 2001) and 9.8 (SD 3.4, minimum 4.8, maximum 14.6) nmol/L (Bergeron et al., 2013). The large standard deviations relative to their means reported in most of these studies as well as the minimum and maximum shown suggest considerable variation in PC at the time of the LH peak among bitches. These studies also show that PC subsequently continues to rise throughout the period during which the LH peak, ovulation, maturation of the oocytes and fertilisation occur, and that the variability in PC among bitches increases with PC and time after the onset of the LH surge.

In spite of the variation among bitches the above pattern of change in PC in relation to reproductive events during oestrus renders it useful to determine the time of insemination with frozen-thawed spermatozoa. So, for example, Thomassen et al. (2006) concluded that bitches should best be inseminated with frozen-thawed spermatozoa 2–3 days after the concentration of progesterone in serum has increased to 15–20 nmol/L. Badinand et al. (1993) phenotypically determined the sire of the offspring of bitches inseminated with frozen-thawed spermatozoa from a different male on each day starting when PC first increased until the onset of cytological dioestrus. In line with the conclusion by Thomassen et al. (2006), Badinand et al. showed that conception resulted from inseminations done 1.5–4.5 days after the plasma progesterone concentration (PPC) first exceeded 16 nmol/L.

Nöthling and Volkmann (1993) and Tsumagari et al. (2003) used the time when pre-ovulatory follicular luteinisation has progressed sufficiently to yield PPC values of 6 nmol/L as an indicator from which to determine when to inseminate the bitches. Nöthling et al. (2003) found that each of 13 bitches inseminated into the uterus at 5 and 6 days ($n=3$), 6 days ($n=1$), 6 and 7 days ($n=8$) or 7 and 8 days ($n=1$) after PPC first exceeded 6 nmol/L conceived and on average produced 6.0 (SD 2.7) pups. Deriving the time of insemination in a similar way than Nöthling et al. (2003), Tsumagari et al. (2003) inseminated Beagle bitches with frozen-thawed sperm from two different dogs 5 and 7 days after PPC first exceeded 6 nmol/L. They determined the paternity of each pup—and, hence, the day of insemination resulting in conception. Their bitches yielded mean litter sizes of 4 ± 2.4 (SD), 6.6 ± 2.5 and 6.5 ± 2.5 from the inseminations done 5, 7 and 5 and 7 days after PPC exceeded 6 nmol/L, respectively. Their results suggest that good fertility is possible from inseminations done 5 days after PPC first exceeded 6 nmol/L. Unfortunately, the study

by Tsumagari et al. (2003) does not permit a comparison between the fertility of inseminations done on either Day 5 or Day 7 with those done on Day 6.

Tsutsui (1975) and Bysted et al. (2001) showed that embryonic development is synchronised among embryos within a bitch, suggesting that fertilisation occurs over a short period of time within a bitch. Furthermore, there is a strong, positive, linear correlation between the interval since the LH peak and the stage of embryonic development, with the first potentially fertilised oocytes and zygote identified 7 d after the LH peak (Bysted et al., 2001). In the light of the findings by Nöthling et al. (2003) and Tsumagari et al. (2003), this synchrony suggests a need to more closely compare the fertility of Days 5, 6 and 7 after PPC first reaches a value between 6 and 9 nmol/L.

Some dog breeders believe that the time of mating or the time of artificial insemination has an effect on the gender ratio of the resulting litters. If such a belief is true, it holds large economic and breeding value to dog breeders and is worthy of proper characterisation. Insemination with frozen-thawed spermatozoa 5 and 7 days after PPC first exceeded 6 nmol/L yielded no difference in the proportions of male and female pups born (Tsumagari et al., 2003). Ennis and Gallagher (1994) established a method of sex-determination using the amelogenin locus in cattle, which is also being used in horses and pigs. No published research is available at present about sex-determination in canine conceptuses using the amelogenin locus but should the method be accurate in dogs it would enable a researcher to determine the gender of conceptuses before their gender would be phenotypically evident.

Using frozen-thawed spermatozoa to inseminate bitches between 5 and 7 days after PPC first reaches a value between 6 and 9 nmol/L (Day 0), the first aim of this study was to compare the fertility achieved on Day 5 with that on Day 6 (Group A), on Day 6 with that on Day 7 (Group B), in bitches that were each inseminated on these 2 days only during a single oestrous period. The second aim of this study was to determine whether insemination 5, 6 or 7 days after PPC first reaches a value between 6 and 9 nmol/L affects the gender of offspring in bitches.

2. Materials and methods

2.1. Experimental animals

The current study was approved by the Animal Use and Care Committee of the University of Pretoria (Project number V020/05).

Twelve nulliparous bitches (8 German Shepherd dogs, 3 Rhodesian Ridgebacks and one Belgian Shepherd dog between 1 and 2.5 years of age) and 20 male dogs (all German Shepherd dogs between 1 and 6 years of age) used in the study belonged to the South African National Defence Force (SANDF), Potchefstroom, South Africa. All animals were vaccinated annually against distemper, parvovirus, parainfluenza virus, adenovirus and rabies. All animals were dewormed once every three months and

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