USE OF LH AND PROGESTERONE ASSAYS FOR OVULATION TIMING AND OPTIMIZING CONCEPTION IN BITCHES

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Received : 14.09.2010 Accepted : 23.01.2012

ABSTRACT

The most appropriate method for estimating the time of ovulation and fertile breeding time in bitches and its relationship with plasma progesterone and LH concentrations were studied in 20 sub fertile bitches. The LH surge occurred on an average 9.95 ± 0.67 days from day 1 of proestrual bleeding and the calculated time of ovulation (LH surge + 48.00 hrs.) was 11.95 ± 0.67 days from the onset of proestrual bleeding and ranged from 9 to 20 of the cycle. Quantitative estimation of progesterone showed that the levels tend to increase just prior to LH surge upto the time if ovulation and the respective levels were 1 and 5 ng/ml. Progesterone levels of > 10ng/ml marked the onset of fertile period. The qualitative progesterone assay kit was able to detect progesterone levels of <3,4 to 10 and > 10 ng/ml in 100, 80 and 100 per cent of the samples accurately and hence could be used for detecting the fertile period.

KEY WORDS: Ovulation, LH surge, Progesterone Assay, Bitches

INTRODUCTION

An important aspect to be considered when attempting to achieve a successful mating is the variation in the estrous cycle which occurs both between bitches and between cycles in the same bitch. Despite this many breeders continue to time matings using a standard number of days from the start of vulval bleeding (onset of proestrus) with the result, bitches are often mated at the inappropriate time, leading to an apparent and usually avoidable infertility (Hewitt and England, 2000). It is very important to note that most of these apparently infertile bitches might be normal healthy fertile bitches whose "apparently infertile" problems might be related to a misunderstanding of proper breeding management.

The preovulatory luteinizing hormone (LH) surge is considered as the central event of the cycle because of its role in stimulating ovulation and transition to the progesterone dominated luteal phase, diestrus. LH surge in bitches occurs about 48 hours before ovulation (Holst and Phemister, 1975) and mature oocytes are fertilized during the 3-8 days following LH surge (Feldman and Nelson, 1996). Therefore, the detection of LH surge by RIA is a highly reliable and non-invasive method to predict time of ovulation. The bitch is unique in that there is a preovulatory rise in plasma progesterone concentration which is brought about by luteinisation of the granulosa cells of the follicle (Concannon et al., 1977). Knowing precisely and rapidly the concentration of progesterone in plasma would help to determine the time of ovulation in dogs and to decide when to breed (Wright, 1990). The present study was taken up with the objective of evaluating the usefulness of the LH, quantitative and qualitative progesterone assays in predicting ovulation and to assess the efficacy of optimum breeding on conception rate and litter size in bitches.

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MATERIALS AND METHODS

Twenty bitches of different breeds presented to the small animal obstetrics and gynaecology ward of Madras Veterinary College on the first day of proestrual bleeding formed the experimental animals for the study.

Almost all the bitches were referred for investigation because they had failed to conceive after two or more matings on different occasions.

Daily blood samples were collected from the cephalic vein starting from day 7 of proestrus until the day of breeding (as determined by the qualitative progesterone assay) and plasma was obtained by centrifugation. Part of the plasma was used for the quantitative progesterone assay while the remaining plasma was stored at -80°C for quantitative progesterone and LH estimation.

Plasma LH concentrations were determined using a double antibody radioimmunoassay as described by Miller and Aehnelt (1977) using ovine LH antiserum (NIDDK - anti - OLH - 1) and purified ovine LH (NIDDK - OLH - 1 -4) both for iodination and for use as reference standard (Provided by NIDDK, Bethesda, MD, USA).

Plasma concentrations of progesterone were determined by a solid phase radioimmunoassay (RIA) kit using 125 I (Prog-CTK-4, Diasorin, Italy)

The progesterone in the sample was determined using a commercially available "In- hospital' ELISA kit "Ovucheck-Premate" (Vetoquinol, UK). The premate kit was a semi quantitative plasma progesterone kit that consisted of low progesterone standard (A), high progesterone standard (B), conjugate and substrate. The test was performed as per the instructions of the manufacturer and the results were interpreted by comparing the colour change produced by the sample with that of the standards. If the test sample was the same colour or lighter than B, it indicated that ovulation had occurred and owners were advised to breed the bitches immediately and rebreed after 48 hrs. Bitches were subjected to pregnancy diagnosis using ultrasonography at four weeks after breeding. Details on whelping and number of pups delivered were obtained and the conception rate and litter size were studied.

RESULTS AND DISCUSSION

In the present study, LH levels rose from below detectable levels to around 10.4 ± 5.02 ng/ml one day before the surge to reach peak levels of 166 ± 12.06 ng/ml (80 to 260 ng/ml) during the period of surge and thereafter fell to non-detectable levels 2 days later. Peak levels of 166 ± 12.06 ng/ml obtained in the present study were within the range reported by Olson et al. (1982) (80 to 749 ng/ml, mean 402 ng/ml). The basal and mean concentrations of LH reflected the magnitude and frequency of pituitary LH release in response to pulsatile release of hypothalamic GnRH into the portal vessels, the extent of negative feedback of ovarian hormones in pituitary LH secretion and the amount of LH that would be released from pituitary cells. LH surges of much shorter duration have been reported in ewes (Goding et al., 1969) and cows (Hansel and Echternkamp,1972)

The LH surge occurred on an average 9.95 ± 0.67 (7 to 18) days from day 1 of proestrual bleeding with a range of 7 to 14 days which was in accordance with the findings of England (1992). In 25 per cent of bitches, LH surge occurred 24 to 72 h prior to onset of estrus, in another 25 per cent on the day of estrus and in the remaining 50 per cent 48 to 72 h later. The mean duration of LH surge was prolonged and occurred over 1.95 ± 0.17 days. The LH surge was observed to be elevated for 2 to 3 days in 70 per cent of the bitches and for a single day in 30 per cent of the bitches.

The calculated time of ovulation in relation to LH surge (LH surge + 48 h) in the present study was 11.95 ± 0.67 days from onset of proestrual bleeding and ranged from 9 to 20 which was in accordance with the findings of Johnston (1980). Following ovulation 3 days were required for the secondary oocytes to mature so that fertilization occurred and was completed approximately 6 days after LH peak. Wright (1991) reported that ovulations generally occurred 48 h after the surge but in few bitches 24 or 72 h after the surge and thus for most bitches the time of fertilization was 4 to 5 days after the LH surge.

The mean progesterone concentrations as determined by RIA in the present study, were non-detectable during early proestrus, gradually started increasing 4 days before the LH surge to reach levels of 0.73 ± 0.16 ng/ml 1 to 2 days before the LH surge. The mean progesterone level was 1.67 ± 0.14 ng/ml

around the time of LH surge with a range of 1 to 3 ng/ml. Progesterone levels continued to increase to reach a mean level of 5.8 ± 0.35 ng/ml at the time of ovulation and was within the range of 3.5 to 8.5 ng/ml. At the onset of fertile period the mean circulating progesterone level was 13.00 ± 0.73 ng/ml and ranged from 10 to 21.8 ng/ml. The rate at which the progesterone levels increased from 0.73 ng/ml prior to the LH surge to around 1 to 3 ng/ml at the time of LH surge was highly variable in the 20 bitches studied. In some bitches the increase occurred over several days while in others it took place within 24 to 48 hrs. The difference between these two rates of hormone rise probably reflected the pattern of LH surge which was caused by the interaction of oestradiol 17 â from follicles and GnRH. This was in turn related to the number of follicles and their state of maturity and therefore oestrogen production (Renton et al., 1992).

Since, plasma LH surge is more closely associated with ovulation, the calculated time of ovulation based on LH assay was taken as the standard in the present study and the time of ovulation based on progesterone assay was compared with it. A high coefficient of correlation ($P < 0.01$) of 0.97 was observed between LH and quantitative progesterone assay.

The overall agreement of Ovucheck ELISA kit and RIA at low (<3 ng/ml), intermediate (4 to 10 ng/ml) and high (>10 ng/ml) range was 100, 80 and 100 per cent respectively. The rise of progesterone > 10 ng/ml (range 10 to 21.8 ng/ml) as determined by RIA was correctly identified by the qualitative ELISA kit and was a diagnostic mark for detecting the fertile period and thus the mating time in bitches.

In the present study, although LH assay was considered to be the most accurate method for estimating the time of ovulation in the bitch, the need for daily sampling and specialized laboratories to carry out the assay makes this technique limited only to research purpose. However, by monitoring the peripheral plasma progesterone concentrations in a sequential manner, it is possible to predict the time of LH surge, ovulation and fertilization period. Unlike the daily sampling that would be required for LH assay, peripheral progesterone concentrations could be measured every second or third day because the initial rise was gradual. Two types of kits were used for this purpose, the quantitative RIA and qualitative (semi quantitative) ELISA kits. Quantitative RIA kits enabled the accurate assessment of the progesterone concentrations in canine plasma and this was corroborated by Jeffcoate and Lindsay (1989), Renton et al. (1992), Feldman and Nelson (1996) and Root and Johnston (2000). However, the quantitative estimation required the use of equipment which were usually available only in laboratories. In contrast, the qualitative type of plasma progesterone ELISA kit (Ovucheck Premate) required no special skills or equipment to run the assay and the results were produced in as little as 30 minutes. There was 100 per cent agreement between the ELISA and RIA kit in determining the fertile period and the use of this kit in the present study had resulted in significant increase in pregnancy rate.

In the present study, the conception rate following breedings during the fertile period based on the qualitative progesterone assay was 80 per cent with a whelping rate of 75 per cent. A mean litter size of 7.6 ± 0.64 was observed with a range of 2 to 12. Considering that all bitches chosen for the study were sub fertile with conception failure following more than half of the breeding attempts, the conception rate of 80 per cent obtained in the present study could be considered as high which was equivalent to the conception rates reported in normal bitches (Root and Johnston, 2000). The hormone assays performed in the present study to assess the time of ovulation proved that all the bitches were apparently normal healthy fertile dogs whose apparent infertility might be due to improper time of breeding or an infertile male.

Six out of the sixteen bitches that conceived had breeding dates ranging from day 16 to 24 from the onset of proestrus, of which one bitch had a proestrus length of 14 days and 2 had a proestrus length of 15 days. These three bitches might have been forcefully mated during the period of previous proestrus without

considering whether or not the bitch had demonstrated true signs of estrus.

Hence to conclude, although LH assays were more precise in determining the time of ovulation, both quantitative and qualitative progesterone assays proved to be more useful due to their practicality. The cost factor of the qualitative progesterone assay kits have been overcome by the indigenous development of a solid phase residual binding progesterone enzyme immunoassay(EIA) by Suryanarayanan (2007) using polyclonal antibodies against progesterone-BSA and progesterone-3-CMO-HRPO conjugate.

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