ABSTRACTS

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Time of establishment of the number of follicles to be ovulated in dogs

Tsutsui, T1; Hori, T2; Takahashi, F2 and Concannon, PW3

1International Institute of Small Animal Medicine (Bio Plus), 2-7-2, Sumiyoshi, Koutou-ku, Tokyo, 135-0002, Japan; 2Department of Reproduction, Nippon Veterinary and Life Science University, 1-7-1, Kyonan-cho, Musashino-shi, Tokyo, 180-8602, Japan and 3Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850, USA

tsutsui-t@bioplus.jp

INTRODUCTION AND AIM: In dogs, ovulation occurs two days after the LH surge and on average two days after the onset of estrus. A recent study of 390 estrus cycles in 102 bitches (1) examined the interval from the onset of proestrus (OPE), i.e., first day of observed vaginal serosanguinous discharge, to the day of ovulation (DO), i.e., when plasma progesterone increased over 2 ng/ml. The OPE-DO interval ranged from 3 to 31 days and averaged 11.1±0.2 (SEM) days. This interval was in general consistent within most animals, typically varying by only a few days, and did not vary with length or number. The extents to which the variation in the observed length of the OPE-DO interval among bitches represents variation in the length of the associated wave(s) of follicle growth, the character of the plasma estrogen profile, the external versus internal presence of serosanguinous fluid, or other factors, are not known. The time when the number of follicles that will ovulate becomes established in individual dogs is not known. Such information would further characterize the follicular phase in dogs and potentially have clinical implications. We therefore conducted a study, in which the OPE-OD interval was determined in two successive cycles, and at the second cycle we removed one ovary at selected times post OPE and examined the residual ovary during the subsequent luteal phase. OPE-OD intervals were compared between cycles, and the numbers of proestrus follicles and numbers of ovulations per ovary in the residual ovary were compared, in the hopes of elucidating the mechanism that establishes the timing of follicle dominance and of the number of follicles that will ovulate.

MATERIALS AND METHODS: Twenty-one female beagles at 2.4-7.6 years of age were used and selected for the study based on having one or more prior cycles with an observed OPE-DO between 8 and 14 days. The female dogs were observed daily for pudendal enlargement and the presence or absence of “vulval bleeding”, and the first day of proestrus (regarded Day 0) was determined based on onset of serosanguinous vaginal discharge. The ovary on the side with a greater number of follicles was excised on Days 2 (n=5), 5 (n=4), or 8 (n=6) after OPE. Hemiovariectomy (hemi-ovx) was performed by midline incision under general anesthesia to minimize tissue damage. The suspensory ligament of the ovary to be excised was fixed with forceps for ovarian fixation (Fujihira, Japan). The ovarian bursa was then incised, and the ovary was fixed with forceps for ovarian excision (Fujihira, Japan) and excised using an electric scalpel. The left ovary was excised when follicles could not be counted, and the ovary on the side with a greater number of prominent follicles was excised when they could be counted. In the control dogs (n=6), a laparotomy was performed on Day 8 post OPE. The size and number of follicles were recorded. Ovulated follicles in the residual ovary were counted by laparotomy 72 days after ovulation in the late luteal phase, and the number of corpora lutea was regarded as the number of ovulated follicles. Results for each bitch were compared to those in its preceding estrus cycle. The number of ovulated follicles in the estrus following hemi-ovx was determined by counting corpora lutea in the ovary excised for sterilization after ovulation. Means are reported ± SEM. Blood was collected daily from day 6 after the onset of vulval bleeding, and the ovulation day was estimated from the blood P4 level [2].

RESULTS: In control bitches, ovulation occurred 9-12 days (mean: 10.5±0.5 days) after OPE in the control group, similar to that before the experiment (8-12 days, mean: 10.5±0.7 days). The total number of follicles ovulated by the bilateral ovaries was 6-9 (mean: 8.0±0.5); 2-5 (mean: 3.8±0.4) from the left and 3-5 (mean: 4.2±0.3) from the right ovary, showing no significant difference between the bilateral ovaries. In the Day 2 hemi-ovx group, there were many small follicles with a diameter of 1.5-2.0 mm in the bilateral ovaries, and accurate counting was difficult. The ovulation day was 10-14 days (mean: 13.0±0.9 days) after OPE and that before the experiment was 10.8±1.1 days in these animals. The ovulation time appeared to be slightly delayed, but the difference was not significant. The number of ovulated follicles in the residual ovary was 6-10 (mean: 8.2±0.9), and significantly greater than the 4.0 ± 0.2 in individual ovaries of the control bitches (P<0.01) and similar to the 8.0±0.5 ovulated by both control ovaries combined. In the Day 5 hemi-ovx group, the follicular size at Day 5 post OPE was 3.0-5.0 mm and follicles could be readily counted. Ovulation occurred 10-14 days (mean: 12.0±1.1 days) post OPE, which was similar to that before the experiment (mean: 12.0±1.0 days). The number of follicles in the residual ovary at Day 5 was 3-5 (mean: 4.0±0.5); the number of subsequently ovulated follicles in that ovary, 2-6 (mean: 3.8±1), was similar and also similar to the 4.0 average number of ovulating follicles in single control ovaries.
In the day 8 hemi-ovx group, the follicular size was 4.0-5.0 mm. Ovulation ranged from 12-16 days and averaged 14.5±1.0 days post OPE, compared to 12.5±1.0 days in the cycle before experiment. Any delay was slight and the difference was not significant. The number of follicles ova in the residual ovary was 2-4 (mean: 3.2±0.3) at the time of laparotomy was the same as the number subsequently ovulated follicles 2-4 (mean: 3.2±0.3).

DISCUSSION. These results can be reviewed in relation to the time of the expected or subsequent preovulatory LH surge as well as the time after OPE. The estimated day of ovulation in each group can be assumed to have occurred 2 days after the LH surge. The predicted day of the LH surge based on the events of the previous cycle might be relevant in examining the results for the Day 2 hemi-ovx group as the effects of ovary removal in that group may have lengthened, albeit not significantly, the interval from OPE to DO from what it would have been in the absence of surgery. The results suggest that the time when the number of follicles that will ovulate following the ensuing preovulatory LH surge in bitches with 8-14 days OPE-DO intervals is determined in mid proestrus at approximately 5 days prior to the LH surge. Further, at 7-9 days before the LH surge, shortly after the onset of proestrus, the ovaries have both a cohort of growing follicles that includes follicles that will eventually be ovulated and an equal number or more of follicles that can be rescued from impending atresia and progress towards peak maturation given a sufficient stimulus. Such a stimulus was provided by the hemi-ovx procedure. The mechanism presumably involves a transient gonadotropin increase in response to acute declines in circulating estrogen and inhibin predicted to occur after hemi-ovx in proestrus. From early proestrus onward in dogs, LH and FSH concentrations are near their lowest concentrations of the entire cycle. That is presumably due to an ever-increasing negative feedback suppression by increasing estrogen and inhibin concentrations that does not abate until the preovulatory LH surge is triggered. Monitoring of plasma inhibin, E2, FSH and LH concentrations in similarly treated bitches would help explain the observed increase in the number of ovulations per ovary. There was a total or near total failure of a similar response to the same surgery and presumably same endocrine sequellae performed at 2-4 days later at 5 days before the impending preovulatory LH surge in the Day 5 hemi-ovx group. That observation demonstrates that in the short interval between day 8 and day 5 before the LH surge rapidly growing dominant follicles destined to ovulate suppress a cohort of an equal or greater number of only slightly less mature follicles that must undergo atresia and not enter the pool of follicles that will ovulate and form corpora lutea.

Our interpretation assumes the follicles that ovulate after Day 2 hemi-ovx include an average of 4 follicle per ovary that were originally destined to become the dominant ovulating follicles and an additional group averaging 4 per ovary that are recruited from follicles that would not have otherwise ovulated. It is not impossible however that the Day 2 hemi-ovx results in endocrine changes that damage or destroy the most or all of the most dominant follicles and recruits an entirely new set of dominant follicles from the available maturing proestrus follicles. In either case, the results suggest that making available a physiological increment if circulating gonadotropin in early proestrus at about day 8 before the impending LH surge can result in a doubling of the number of follicles that will ovulate from an ovary. Whether the important moiety is LH, FSH or a combination of the two would be informative in looking at scenarios for potential clinical management of bitches with a history of producing small litters.

In conclusion, the follicles that will be ovulated in response to an LH surge, in dogs with 10-14 day OPE-DO intervals, established in mid- proestrus at 5-6 days post OPE and 5-6 days before the LH surge. The extent to which the current results would apply to bitches with observed OPE-DO intervals that are short (3-6 days) or long (16-27 days) is not known, and the mechanisms determining the length of the follicular phase in bitches are not known. Likewise, whether the timing for the selection and determination of the follicles that will undergo terminal maturation and ovulate is a function of time after OPE, time prior to the LH surge or something else is not known. It could potentially be studied comparing bitches with routinely short versus long OPE-OD intervals.