ABSTRACTS

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PROLONGED DURATION OF FERTILITY OF DOG OVA

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Objectives - The fertile period for natural mating in dogs extends from approximately 7 days before ovulation until day 5 after ovulation. This relates to intra-uterine survival of canine sperm for up to 7 days or more, the delay in maturation of oocytes until 2 to 3 days after ovulation in the lower oviduct, and viability of secondary oocytes for an additional 48-60 h or more [1,2]. We have also observed in dogs with uterine fistulas that spermatozoa did not enter the uterus after vaginal insemination in the last stage of estrus, even if mating occurred [3]. Verstegen et al. [4] confirmed late-estrus “cervical closure” occurring on average 5 days after ovulation, but also observed that, even 72 h after the cervical, one of five intrauterine inseminations resulted in conception, indicating that canine ova can be fertile perhaps up to 8 days after ovulation and 6 days after oocyte maturation. Therefore, in the present study conducted to further clarify the duration of fertility of canine ova, we examined pregnancies after intrauterine artificial insemination (IUAI) between 6 and 9 days after ovulation, with the time of ovulation initially estimated from plasma progesterone and later confirmed by LH levels.

Materials and methods - Beagles used were 1.9 to 6.7 years old, including 27 females at 4.3 ± 0.3 (mean ± sem) yr and 5 males at 6.9 ± 0.8 yr. Plasma was collected and assayed for progesterone (EIA) daily from day 8 after onset of vulval bleeding, and IUAI was performed at known days post-ovulation, based on the day of ovulation being estimated as the first day that progesterone exceeded 2 ng/ml [5]. At a later date, plasma LH levels measured by RIA allowed a re-estimation of ovulation time as having occurred 2 days after the preovulatory LH peak. The IUAI was performed surgically in 5, 7, 8, and 7 dogs on days 6, 7, 8, and 9 post-ovulation, respectively. Each IUAI deposited 2 x 10^7 sperm in 100µl into the upper portion of the uterine horn ipsilateral to the ovary with the greatest number of ovulations. The vulva was examined for the appearance of sperm at 0.5, 1, and 2 h post-IUAI by microscopic exam of smears obtained by saline-moistened swabs from the vulva and vestibule. Pregnancy was diagnosed by ultrasound (7.5-MHz probe) exam and fetal development in pregnant dogs was examined at 5-day intervals beginning at 20 days post ovulation. The number of pups was counted on the day of birth.

Results - The day of ovulation estimated from plasma progesterone was corrected using LH levels in 3 (11.1%) of the 27 dogs and thus changed to a date 1 day earlier or 1 day later in 1 and 2 dogs, respectively. The rates of conception following IUAI at 6, 7, 8, and 9 days after ovulation were 100% (5/5), 71.4% (5/7), 37.5% (3/8), and 0% (0/7), respectively. Of the 13 conceptions, complete resorption of the litter occurred in 20% (1/5), 40% (2/5), and 33.3% (1/3) of bitches inseminated at 6, 7, and 8 days after ovulation, respectively, yielding an average litter resorption rate 30.8% (4/13). In the remaining 9 pregnancies, partial-litter fetal resorption occurred in 50% (2/4) and 33.3% (1/3) of dogs artificially inseminated at 6 and 7 days after ovulation dogs. The mean litter size and time from ovulation to delivery were 4.3 ± 1.6 pups and 64.0 ± 0.0 days (n=4), 4.0 ± 1.4 pups and 66.3 ± 0.4 days (n=3), 1.4 pups and 67.69 days (n=2) for IUAI at 6, 7, and 8 days after ovulation, respectively. The litter size was larger than the estimated number of ova ovulated on the inseminated side in only 2 (2/5, 40%) and 1 dog (1/5, 20%) after IUAI at 6 and 7 days after ovulation, respectively. Spermatozoa were observed in the vestibule within 2 h in all dogs except one day-8 (complete
litter resorption) dog and one day-9 (non-conception) dog. The timing of the appearance of sperm in the vestibule varied among dogs and was unrelated to time of IUAI after ovulation.

**Discussion** - The high pregnancy rates with IUAI at 6 and 7 days after ovulation, confirms that many canine oocytes are fertile at 4-5 days after maturation. The high rate of post-implantation partial or full litter resorption was presumably due to the aging of ova and/or asynchrony between embryonic development and the intrauterine environment. That asynchrony becomes an increasingly important factor as day of insemination post-ovulation increases agrees with the prolonged (67-69 d) gestation lengths relative to the day of ovulation seen in most of the pregnancies resulting from insemination 7 or more days after ovulation. The 2 pregnancies resulting from IUAI at 8 days post ovulation confirm that, in the extreme, some oocytes remain viable until that time and thus 6 d after oocyte maturation. The low percentage of fertilization of ova from the contralateral ovary in this study was presumably due to the decreased motility and fertility of spermatozoa in the intrauterine environment at 6 days after ovulation or later. The appearance of spermatozoa in the vaginal vestibule at by 2h post IUAI suggests that failure of spermatozoa to enter the uterus from the vagina (or remain viable after doing so) at 6 days or more after ovulation is not due to the physical closure of the cervical canal, but because of other and possibly biochemical changes in the cervical or intrauterine environment.

**References**