WEEKLY CYCLICAL OVULATION COULD BE MADE BY $PGF_{2\alpha}$ INJECTION IN RUMINANTS

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Abstract: Follicular growth of ruminant species occurs more than once during an estrous cycle either resulting in ovulation (ovulatory follicular wave) or not resulting in ovulation (latent follicular wave). Using Shiba-goats, we examined if ovulation could be induced from every latent follicular wave and if the resulting ova were fertile. Ovarian follicular development was monitored daily by transrectal ultrasonographic examination in 20 female goats for 2 consecutive estrous cycles. After the second spontaneous ovulation (day 0 = ovulation), all animals were each treated with a luteolytic dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on days 3 and 4, which resulted in ovulation from the first latent wave. Fertile males goats (n=2) were immediately introduced to the first group of animals (n=5), resulting in 4 pregnancies. For the second group of goats (n=9), 4 consecutive short estrous cycles were induced by the $PGF_{2\alpha}$ treatment at a mean interval of 6.5 ± 1.2 days with a range of 4-9 days. Five of 9 goats were allowed to mate following 4 consecutive short ovulatory cycles; normal behavioral estrus was observed at each estrus, and four of five animals became pregnant. The remaining goats (n=6), in addition to $PGF_{2\alpha}$ treatment, received exogenous progesterone supplement which allowed latent follicular development to occur, but prevented ovulation until the effect of progesterone supplement subsided. These results suggest that female Shiba-goats possess physiological mechanisms for final steps of follicular maturation and subsequent ovulation at short intervals when progesterone production from the newly developed corpus luteum is terminated.

Key words: Follicular wave, Ovulation, Progesterone, Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), Goat

Introduction

Introduction of ultrasonography allows day-to-day monitoring of changes in individual follicular growth in the ovary of farm animal species such as cattle [1], goat [2] and ewe [3]. With this technique, waves or distinct groups of follicular development have been observed twice or more times during the early or mid luteal phase of the estrous cycle. Only dominant follicles of the final wave following the luteal phase ovulate, whereas those of previous waves undergo atresia and are therefore termed as latent waves. In heifers the dominant follicle of the first latent wave is capable of ovulation when luteolysis is induced by prostaglandin (PG) $F_{2\alpha}$ 5-8 days after spontaneous ovulation [4, 5]. These observations suggest that final steps of follicular maturation in the latent waves were prevented by a negative feedback effect of progesterone on gonadotropin secretions. The miniature "Shiba-goat", a breed indigenous to Japan, exists in a closed colony maintained for research purposes, particularly as a model for domestic ruminants. They are non-seasonal breeders under natural daylight and other aspects of their reproductive characteristics have been reported elsewhere [6, 7]. However, induction of ovulation or repeated ovulations at short intervals has not been investigated in Shiba-goats. The objectives of the present study were to test if the dominant follicles from the first latent wave would ovulate following luteolysis induced by $PGF_{2\alpha}$, to determine if goats were fertile after the first-induced short estrous cycle or four consecutive-short estrous cycles, and to examine the effect of progesterone replacement on follicular growth and ovulation following the $PGF_{2\alpha}$ induced luteolysis.

Materials and Methods

Animals, ultrasonographic examination and $PGF_{2\alpha}$ treatment

Female Shiba-goats, about 1.5-year old at the beginning of the investigation and weighing 24-30 kg, were used. All experiments described here were completed within a year. An ultrasonograph (SSD-550, Aloka Co. Ltd., Tokyo, Japan) equipped with a Transrectum Electronic Linear Probe (UST-660-7.5, 7.5 MHz, Aloka Co. Ltd.) was used to monitor follicular and corpus luteum (CL) development. The ultrasonographic examination was done once daily at around 15:00 h, but 2-3 times per day when the dominant follicles came close to ovulate. Spontaneous ovulation occurred was judged from the disappearance of a large follicle(s) that had been present on the previous examination [1,8]. Twenty goats whose estrous cycles were regular for two consecutive cycles were selected for the experiments. After confirming the second spontaneous ovulation, these animals were treated with $PGF_{2\alpha}$ (Fuji Chemical Industries Ltd., Takaoka, Japan) when functional CLs were present and the diameter of a dominant follicle(s) exceeded 5.5 mm, usually 3 days after the ovulation. The $PGF_{2\alpha}$ treatment regimen employed in the present investigation consisted of two successive intramuscular injections, 4 mg $PGF_{2\alpha}$ on the first day (3 days after ovulation) and 2 mg on the following day. Goats (n=20) were randomly assigned to one of three treatments: **a**) 5 animals were mated immediately following the $PGF_{2\alpha}$ induced ovulation from the first latent wave (day 0 = ovulation), **b**) four consecutive short estrous cycles were induced in 9 animals, and 5 of these animals were mated after the fourth short ovulatory cycle, and **c**) in the remaining animals, progesterone replacement therapy was administered on the day of $PGF_{2\alpha}$ treatment. Goats (n=5) from group **a**) were left alone until normal delivery. Animals (n=9) of group **b**) were received the $PGF_{2\alpha}$ treatment repeatedly for 4 more times when the largest follicles grew more than 5.5 mm in diameter. In addition, five of nine goats were randomly chosen and mated with a male of proven fertility following the fourth $PGF_{2\alpha}$-induced ovulation. Goats (n=6) from group **c**) were treated intramuscularly with 60 mg progesterone (Luteogen-L; Long lasting progesterone, 200 mg/5 ml, Sankyo Pharmaceutical Co., Ltd., Tokyo, Japan) on the day of first $PGF_{2\alpha}$ treatment and 40 mg on the following day. Ultrasonographic examinations were continued until two days after induced ovulation or the subsequent estrus.

Blood sampling and hormone assay

During the entire experimental period except once pregnancy was recognized, blood samples (5 ml) were drawn daily at around 15:00 h by jugular venipuncture into heparinized tubes (Terumo Venoject Ⅱ, Tokyo, Japan) and immediately centrifuged. Plasma was collected and stored frozen at -20 °C until assayed. Plasma progesterone and estradiol concentrations were determined in duplicate by an enzyme immunoassay (Cayman Chemical Company, Ann Arbor, MI, USA) with an assay protocol supplied by the manufacturer. Intra- and inter-assay coefficients of variation for progesterone were 7.0% and 10.5%, respectively, and the sensitivity of this assay was 18.2 pg/ml. Intra- and inter-assay coefficients of variation for estradiol were 9.4% and 13.5%, respectively, and the detection limit of this assay was 2.2 pg/ml. Plasma (50 μl) was extracted with 2 ml diethyl ether and the ether extract was assayed for progesterone without chromatography. For estradiol, 2 ml plasma were extracted with 6 ml diethyl ether and the ether extract was re-dissolved in water and methanol (50:50, v/v), and washed with hexane. The water/methanol layer was evaporated and used for the assay as recommended by the manufacturer. Recovery rates for the extracted steroids were averaged at ≧91%.

Statistical analysis

Data were primarily analyzed by ANOVA followed by Duncan's multiple range test, and if excessive variations are realized, χ^2 test was applied. Differences were considered significant when p values were less than 0.05. Results are means ± SEM.

Results

Follicular waves and steroid hormone concentrations during the estrous cycle

The mean length of 40 estrous cycles studied in 20 animals was 20.8 ± 1.1 days with a range of 17-22 days. Representative patterns of follicular development are shown in Fig. 1, in which changes in diameter of individual follicles larger than 3.0 mm are plotted. Among the 40 cycles observed, one latent follicular wave during the luteal phase was identified in 9 cycles (23 %, Figure 1A) and two latent follicular waves occurred in the remaining 31 cycles (77 %, Figure 1B). Thus, latent follicular waves occurred after each estrus. The number of follicles ovulated in each cycle was 2.3 ± 0.9 (n=40) and mean diameter of the follicles one day before ovulation was 6.9 ± 1.1 mm (n=92). Plasma progesterone and estradiol levels throughout the estrous cycle in 6 different goats with two latent follicular waves are shown in Figure 2. Plasma progesterone levels began to decrease 4 days before ovulation, accompanied by an elevation in plasma estra l peaking one day before ovulation. In Shiba-goats with two latent follicular waves, the maximum diameter of follicles from the first latent follicular wave was 8.6 ± 1.2 mm, 6.6 ± 0.9 mm in the second one and 7.2 ± 0.9 mm in the third wave, which resulted in ovulation. An increase in plasma estradiol levels was detected on day 5 of the first latent wave, no elevation was found with the second wave, and a large increase in estradiol levels occurred during the third ovulatory wave immediately before ovulation (Figure 2).

Induction of ovulation from the first latent follicular wave by $PGF_{2\alpha}$

In 20 goats whose dynamics of follicular growth had been monitored ultrasonically, luteolysis was induced from the first latent follicular wave by the $PGF_{2\alpha}$ injections for 2 successive days. As shown in Figure 3, the first $PGF_{2\alpha}$ injection resulted in an immediate depletion of plasma progesterone concentrations and a concomitant elevation in plasma estradiol. Dominant follicles of the first wave in all goats thus treated were ovulated 2 or 3 days after two successive $PGF_{2\alpha}$ injections, followed by the growth of another cohort of follicles. Relatively large SEM associated with plasma estradiol levels (Figure 3) reflected a time of ovulation, which differed as much as 24 hours. The mean number and diameter of ovulated follicles were 2.2 ± 0.5 and 7.0 ± 0.2 mm, respectively, which were not different from the animals with regular estrous cycles. Immediately after the second $PGF_{2\alpha}$ treatment, five of 20 goats (group **a**) were randomly chosen and placed in a pen with two fertile males. Four of these goats became pregnant and delivered kids in 148-152 days.

Induction of 4 consecutive short ovulatory cycles with $PGF_{2\alpha}$

Another 9 goats (group **b**) in which two successive regular cycles had been observed were subjected to the $PGF_{2\alpha}$ treatment for the induction of four consecutive short ovulatory cycles. Ovulation occurred within 2 or 3 days after the $PGF_{2\alpha}$ treatment (Figure 4A, data from one representative animal is shown). Among 36 ovulations (4 x 9 goats) observed, one ovulation occurred spontaneously without the $PGF_{2\alpha}$ treatment in an animal whose progesterone level was not elevated after the second $PGF_{2\alpha}$-induced ovulation (Figure 4B, the 3rd ovulation). Although two successive injections of $PGF_{2\alpha}$ were applied, the first $PGF_{2\alpha}$ treatment caused a precipitous decrease in progesterone levels. Then, rapid follicular growth was observed between days 5 and 7, and 1 to 5 follicles were recognized as dominant follicles destined to ovulate. One day after the depletion of plasma progesterone, estradiol concentrations increased dramatically, which then returned to basal levels after ovulation. The mean ovulation interval in 36 short cycles thus observed was 6.5 ± 1.2 days with a range of 4-9 days. The mean diameter of ovulated follicles was 7.0 ± 1.1 mm (n=83) and number of follicles ovulated per ovulation was 2.3 ± 0.6 (n=36), which were similar to those of intact animals mentioned above. In addition, all of these animals exhibited normal estrous behavior at each eatrus. Five of 9 animals chosen randomly were allowed to mate after 4 consecutive short ovulatory cycles and four of 5 animals were confirmed pregnant with one or two fetuses by ultrasonic examination. The resulting kids, delivered after 147-151 days of pregnancy, had normal birth weight and length.

Effect of progesterone treatment on follicular growth growth and ovulation

Goats (n=6, group **c**) were treated with a combination of $PGF_{2\alpha}$ and progesterone for two successive days. Not as in the case of $PGF_{2\alpha}$ alone, however, ovulation was not immediately induced in these goats treated with the exogenous progesterone; ovulation was detected 18.5 ± 1.5 days later. Dynamics of follicular growth of one representative animal and plasma progesterone levels of these 6 animals are illustrated in Figure 5.

Discussion

The present study demonstrated that estrous cycles in Shiba-goats were mostly characterized by three waves of follicular development, two of which were latent waves and follicles from the third wave resulted in ovulation. Saanen goats display up to four waves of follicular development in each estrous cycle [2]. Despite this minor difference in the number of follicular waves, it is evident that like other ruminant species Shiba-goats have non-ovulatory or latent follicular waves during the luteal phase. In the present study it was demonstrated that ovulation can be induced repeatedly at 6 to 7-day intervals by the successive $PGF_{2\alpha}$ treatments. Subsequent induction of luteolysis permitted growth of follicles fr the next latent follicular wave and resulted in another ovulation. These sequential events could be repeated up to four times. The number and diameter of follicles ovulated after $PGF_{2\alpha}$ induced luteolysis were not different from those of control females.

Similar to observations made in cattle [9], peak level of plasma estradiol during the first latent wave was smaller than that of the third wave immediately before ovulation,. The frequency of pulsatile GnRH release from the hypothalamus in Shiba-goats is reduced by progesterone in combination with estrogen [10,11]. Significant increase in estradiol secretion does not occur from follicular development at the first latent wave because the frequency of gonadotropin pulses may not be sufficient due to high progesterone levels. Injections of $PGF_{2\alpha}$ 3 and 4 days after ovulation resulted in immediate depletion of plasma progesterone levels and concomitant elevation of plasma estradiol levels; the latter observation was resulted from follicular growth. This plasma estradiol level appeared to be high enough to induce the gonadotropin surge and all goats thus treated with two successive $PGF_{2\alpha}$ injections ovulated 2 or 3 days later. This observation is consistent with the data from previous reports in cattle [4,5]. The ovulation induced by $PGF_{2\alpha}$ appeared to result from a depletion of peripheral progesterone levels, since exogenous progesterone supplement along with $PGF_{2\alpha}$ treatment completely blocked $PGF_{2\alpha}$ induced ovulation. Thus, the present study supports the notion that follicular development occurs during the luteal phase, but high levels of progesterone prevent the final step of follicular maturation and thus ovulation [12,13]. These results are further supported by an observation in which goats actively immunized against progesterone display shortened ovulatory interval [14]. It should be noted that the short ovulatory cycles were consecutively induced for 4 times. Hormonal profiles and behavioral estrous activity following the second through fourth induced short cycles were similar, if not the same, to those of the first one. In addition, the pregnancy rates between goats mated at the first and fourth short cycles were the same, suggesting that the quality of ova even after four short ovulatory cycles are the same as those of the first one. On an average 75% of goats with normal estrous cycle become pregnant at the University farm. Thus, the pregnancy rates after $PGF_{2\alpha}$-induced ovulation from the first and fourth latent follicular wave were similar to that of normal cycling goats without $PGF_{2\alpha}$ treatment. These results indicate that ova shed from the first latent wave were as fertile as that after 4 consecutive 6 to 7-day ovulatory cycles. These results suggest that as long as progesterone concentrations are controlled at low levels, the female goats have ability to maintain short and fertile estrous cycles.

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