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OVARIAN FOLLICULAR DYNAMICS AND CONCENTRATIONS OF OVARIAN AND PITUITARY HORMONES DURING THE PERIOVULATORY PHASE OF THE POSTPARTUM GOATS.

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A B S T R A C T

To declare the ovarian and hormonal changes during the transition from the anestrus to the cyclic state, five postpartum goats were daily examined by ultrasound. Two waves of follicular growth were followed and emerged 5.20±0.37days before and 2.2±0.37days after LH peak. The corpus luteum attained its widest diameter on Day4.71±0.36. The total inhibin secreted during the luteal phase was higher than that of the periovulatory period. Plasma LH levels showed an increase above basal levels on Day 0 and Day 8. Prolactin levels showed a noticeable declined (-40%) in most of the ovulated goats on the day of LH peak. Conclusion: the decreased prolactin and an increased of LH levels might induced the ovulatory event in the anestrus goats.

KEY WORDS: Goat, Ovarian activity, Postpartum

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1. I N T R O D U C T I O N

Prolonged postpartum anestrus is one of the reasons for low reproductive efficiency and economic loss for animal breeders [34]. The lack of information regarding the ovarian activity during postpartum in goats had detrimental effects on the success of animal productivity and synchronization regimens. Follicular waves have been fully described during lactation in sheep [5] and cattle [25], but not in goats. In goats, follicular activity resumes 13 day postpartum [1] and following the first luteal function; estrus precedes the onset of ovarian activity by four days [19]. Alternatively, the progesterone remains at basal levels during the postpartum anestrous period and increases with the resumption of postpartum cyclicity [18]. Conversely, low estradiol levels persist for two weeks and fluctuated during the next three weeks postpartum [1].

The objectives of the current study were to characterize the ovarian follicular and luteal activities and the accompanying hormonal changes during the shift from

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the anestrus to the cyclic conditions in postpartum goats.

2. MATERIAL AND METHODS

2.1. Animals and experimental design

Five parturient *Shiba* goats (2 to 6 years old) with an average body weight 36.9±3.8kg delivered normally in summer, 2007 were used. Each goat was kept in an isolated box with their kid (s) under natural daylight and was fed hay cubes (600g/goat daily). The goats' ovaries were investigated using a real time B-mode scanner (ECHOPAL ultrasound scanner, Hitachi Medical Corporation, Tokyo, Japan) equipped with a 7.5 MHz transducer from Day 5 to Day 70 after kidding as previously described [22]. Follicles that were first detected at 4 mm in diameter were assumed to have been 3mm in diameter the previous day [11]. The term "follicular wave" referred to one or more antral follicles growing from <3 to ≥5mm in diameter before regression or ovulation [24, 26]. Individual follicles emerging at different times within a maximum of 48h were regarded as a single follicular wave [21]. The following characteristics of the follicular waves were determined per animal: i) The day of wave emergence (the day on which the follicle was 2 or 3mm in diameter) and ii) The maximal diameter attained by each wave dominant follicle. The day of ovulation was judged when the largest follicles being monitored could not be detected at the next examination [26].

2.2. Blood sampling and hormonal assay

Blood samples were collected daily between 0700 and 0900 hours through jugular vein and the separated plasma were assayed for estradiol, progesterone, FSH, LH, immunoreactive (ir-) inhibin and prolactin by a double antibody radioimmunoassay (RIA) system using ^{125}I-labeled radioligands as described previously [13, 15, 23, 30, 31].

2.3. Data analysis

To analyze the effect of ovulation on the total secretion of each hormone by using Student's t test, the area under the curve (AUC) was calculated [10] using "GraphPad Prism" software. The basal hormonal levels were calculated in each animal as the average of the five lowest measurements in all samplings [2]. A sustained rise above 0.5 ng/ml of progesterone was taken as a clear-cut value for luteal activity [20]. The obtained data were normalized to day of LH peak (Day 0). A value of $P<0.05$ was considered to be significant.

3. RESULTS

The changes in the follicle growth, corpora lutea development and the concomitant hormonal levels around the first postpartum ovulation in goats are presented in Fig 1. The diameter of the ovulatory follicle was 3.21±0.06mm when it was first detected 5.20±0.37 days before LH peak, and reached its maximal diameter (7.3±0.3mm) before ovulation which occurred 1-2 days after LH peak. The postovulatory wave emerged 2.20±0.37days post-LH peak and reached their maximal monitored diameter (6.5±0.3mm) on Day 7-8. The following luteal phase was short (5.0±0.3 days) and the corpus luteum (s) was detected as a roughly circumscribed hypoechoic area (Fig 2), and attained its widest diameter (8.37±0.35mm) 4.71±0.36 days later before its regression.
There was variability in FSH levels before or after ovulation among the examined goats, but showed a peaking (>50%) on day of LH surge (Fig 1B). The total luteal phase secreted ir-inhibin was significantly higher ($P<0.5$) than that of the periovulatory period and was negatively correlated with plasma FSH (Fig 1C). Estradiol showed a tiny elevation above basal levels coincident with LH and FSH peaks, before re-increase significantly on Day 8 (4.5±0.8 pg/ml) and coincident with

the decline of progesterone concentrations (Fig 1D). Plasma LH levels did not show a dramatic change in basal levels except for the LH peak and the re-increase on Day 8 (Fig 1E). Plasma progesterone levels increased from Day 2, but still below the cut value of luteal activity until Day 5 (0.8±0.2ng/ml).The total hormone secretion during the luteal phase was significantly higher (P<0.01) than that of the periovulatory period (Fig 1G). Prolactin levels showed a noticeable declined (-40%) in most of ovulated goats (80%) on the day of LH peak (Fig 1H).

4. DISCUSSION

The current study for the first time described fully the changes in the ovarian function and hormonal levels around the first ovulation in postpartum anestrus goats. The present results showed the emergence of the ovulatory follicle 5.20±0.37 days before LH peak (range 4-6 days), and ovulated 1-2 days post-LH peak, indicating the long growth phase of the follicle and its retarded response to LH surge, suggesting the low maturity of the granulosa cells that is necessary for optimal ovulation and/or luteinization in

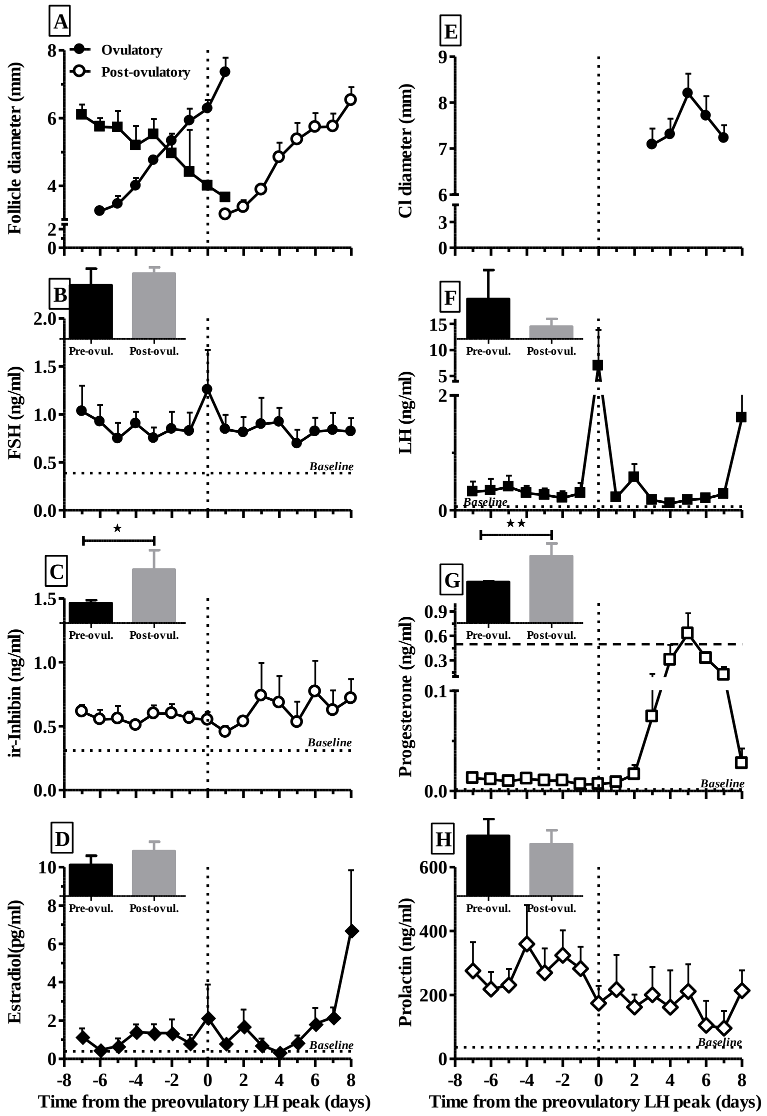

Fig 1 Pattern of follicular and luteal activity and the associated hormonal changes around the first postpartum ovulation in goats. (A) follicular and luteal dynamics, (B) FSH (●), ir-inhibin (○), (C) LH (■), progesterone (□), (D) estradiol (◆) and prolactin (◇). Data shown were pooled from all animals (n=5), presented as mean (±SEM) and were normalized to day of LH surge (vertical dashed line). Basal hormonal levels were represented by the horizontal dotted line. The small upper graphs represented the total hormonal secretion during preovulatory (■) and postovulatory (■) periods. Values with superscript were significantly different (★ P<0.05 and ★★ P<0.01).

response to the LH surge [4]. Failure of postpartum dominant follicle to undergo terminal maturation may be due to depleted pituitary circulating LH stores [29] or low IGF-1 and insulin “metabolic signals” [17]. The first postpartum ovulation was followed by a short luteal phase (4 to 6 d) and was associated with low progesterone production. In cows, the first postpartum ovulation was followed by an increase in progesterone lasting only 5 to 9 days [33]. Premature regression of CL may be in consequence of early release of prostaglandin from the uterine endometrium [9], as prostaglandin biosynthesis is more active in subnormal CL [16]. It is now clear that a subtle dialogue exists among the hypothalamic-pituitary axis, ovary and uterus and probably responsible for the appearance and constant duration of short cycles [31].

The monitored FSH among the examined goats was fluctuated before or after ovulation, but showed a peaking (>50%) concomitant with the day of LH surge; as described previously [7]; and one day before the postovulatory wave emergence, signifying the role of FSH in pre-ovulation follicle development and as a trigger for follicle recruitment [21, 22]. In cows, variable plasma FSH profiles was observed during the postpartum period with no changes associated with the onset of ovarian activity or the first estrus [27].The current evaluation of the luteal phase ir-inhibin showed its higher levels than that of the periovulatory period and was negatively correlated with plasma FSH. Correspondingly, plasma inhibin A levels increased concomitant with emergence of follicular waves and were inversely related with FSH concentrations during each follicular wave [16]. The increased secreted inhibin during postovulatory period, indicated the improved folliculogenesis and indicating the role of subluteal progesterone priming in the resumption of normal ovarian activity during the postpartum period [24, 32] through its effect on hypothalamic-pituitary function and rise in LH release that increases follicular turnover and prolongs the lifespan and dominance of the largest follicle postovulatory wave [6].

The plasma estradiol showed a slight increase on the day of LH peak and on Day 8, concomitant with maximal diameter of the dominant follicle, but

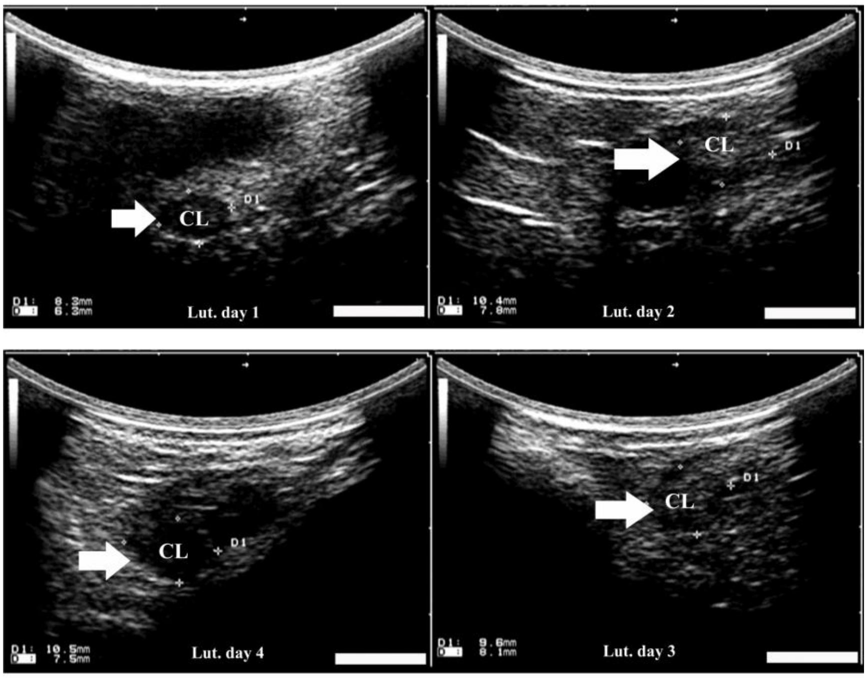

Fig 2 Representative ultrasound pictures of the first postpartum developed corpora lutea (CL) in the goats. The CL appeared as a hypoechoic structures in a hyerechoic ovarian stroma. Luteal regression was indicated by the lack of CL echogenicity (Day 4). Scale bar=10mm.

reflects low follicular maturation or inadequate aromatization of androstenedione [29] or body weight loss that influences the secretion of ovarian steroids and eventually induces ovarian quiescence [28]. Accordingly, earlier reports verified low plasma estradiol-17α at the first postpartum estrus in cows [31]. The plasma LH increased above basal levels in all goats two days before ovulation with a further increase 8 days later. Earlier reports showed that, the close the time of ovulation, the higher LH concentrations released [12] and verified that LH is an important factor in follicular maturation and ovulation [36]. The plasma progesterone levels showed an increase above the cut value of luteal activity on Day 5 post-LH peak (0.8±0.2ng/ml) that might be due to lower proportion of large to small luteal cells as compared with cyclic CL [8].

Analysis of prolactin in this study showed a noticeable declined prior to LH peak in four of the ovulated animals (80%), indicating the overcrowding role of prolactin on LH and consequently ovulation in postpartum goats. Likewise, bromocriptine treatment (prolactin antagonist) increased the LH response to LH-RH stimulation [35].

In conclusion, the increased basal levels of LH levels coincident with drop of circulatory prolactin might responsible for the induction of first postpartum ovulation in goats. The subluteal progesterone secretion during the postpartum period played a significant role in enhancement of follicular turnover and the resumption of ovarian cyclicity in postpartum anestrus goats.

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