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The effect of the absence or presence of a corpus luteum on the ovarian follicular population and serum oestradiol concentrations during the estrous cycle in Sanjabi ewes

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ABSTRACT

The aim of this study was to determine if the presence or absence of a corpus luteum (CL) during estrous synchronization in ewes can affect the ovarian follicular population and the serum oestradiol concentrations. The estrous cycles of 197 Sanjabi ewes were synchronized using a 12-day treatment with intravaginal progestagen sponges (Chronogest®). Estrus was detected in 144 ewes, 27–39 h after sponge removal. Blood samples were taken daily from day 2 and continued for 19 days and analyzed for serum oestradiol concentration. Nine ewes were slaughtered on each experimental day (days 1–16 after estrus) for ovary collection. The ovaries per ewe were classified as those without, or with one or two CL's, for each slaughter day. Visible follicles on the surface of the ovaries were classified, based on their diameter, into (i) very small (<2 mm), (ii) small (2–3.4 mm), (iii) medium (3.5–5 mm) and (iv) large (>5 mm) categories, and the respective numbers recorded. Results indicated, the number of ovarian follicles to decrease ($P < 0.01$) from days 1 to 5 of the cycle and showed a significant increase on day 7. Numbers were high again on day 11 and decreased ($P < 0.01$) on day 16 of the estrous cycle. The serum oestradiol concentrations were significantly higher ($P < 0.001$) in the double than in the single ovulating animals (one or two CL's, respectively) on days 2–0. However serum levels were also significantly higher ($P < 0.001$) in single, than twin ovulating animals on days 4–5 and 12–16 of the estrous cycle. There were no significant differences in the total number of very small follicles between animals without and those with two CL's. The number of small, medium and large follicles in ewes, with or without a CL on the ovary was significantly higher ($P < 0.01$) than ewes with two ovulations at certain stages of the estrous cycle. The present study provides evidence of differences in the follicular ovarian population in ovaries without CL's and double ovulations. The existence of an intraovarian effect of the CL numbers on follicular population is demonstrated.

1. Introduction

Folliculogenesis is a continuous process and a group of ovarian follicles enter the growth phase daily. In sheep this phenomenon occurs from puberty, throughout adult life. During her lifetime, only a few follicles from a pool of several million will grow to an ovulatory size, and even fewer will ovulate (Driancourt, 2001; Gupta et al., 2007). One of the most intriguing phenomena of folliculogenesis in domestic ruminants is follicular dominance, the process whereby the selected follicle(s) continue to develop, while others undergo atresia (Webb and Armstrong, 1998). Evi-

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dence concerning follicular dominance in small ruminants (sheep and goats), and their relationship with hormone concentrations and follicular development during the estrous cycle, is still unclear. Several researchers have postulated the existence of follicular dominance in cyclic ewes and goats (Campbell et al., 1999; De Castro et al., 1999; Evans et al., 2000; Gonzalez-Bulnes et al., 2001; Evans, 2003). Others however question this fact (Duggavathi et al., 2004; Gonzalez-Bulnes et al., 2005). Medan et al. (2005) found several follicles to grow simultaneously during each follicular wave, without affecting each other's growth. This could then not confirm a clear powerful follicular dominance in goats. Gonzalez-Bulnes et al. (2004) reported a greater decrease in the number of small follicles developing to large sizes on the ovary, ipsilateral to the dominant follicle. Hunter et al. (2004) again reported that the processes of recruitment and selection of follicles lead to the development of a number of ovulatory follicles that is specific for the particular specie and breed.

A high correlation has been recorded between the serum progesterone (P4) concentration and oestradiol (E2) concentration and the development of ovarian follicles (Menchaca and Rubianes, 2002; Yu et al., 2005). Previous studies have demonstrated that the circulating progesterone concentration may affect the follicular size (Ginther and Kot, 1994; Tenorio Filho et al., 2007). The luteal progesterone concentration, via the suppression of the LH pulse frequency, may contribute to the follicular turnover (Bodensteiner et al., 1996), based on previous observations in ewes (Vinoles et al., 1999; Bartlewski et al., 2001). It may be concluded that the effect of progesterone on the growth and fate of ovulatory-sized antral follicles in cyclic ewes (prolonged follicle lifespan during exposure to submaximal levels of progesterone), is mainly systemic/endocrine. Studies on the relationships between the incidence of single and double ovulations and circulating hormones in cattle and mares have been reported (Mann et al., 2007; Ginther et al., 2008). Mann et al. (2007) reported plasma oestradiol concentrations around ovulation to reveal no differences between single and double ovulating ewes. To date, on the basis of current knowledge, reports of comparing the effect of different ovulation rates on serum oestradiol concentrations and follicular population throughout the estrous cycle in ewes and goats are limited. The purpose of the present experiment was thus: (i) to determine the effect of the day of estrous cycle on ovarian follicular population and serum oestradiol concentration; (ii) determine the effect of the absence or presence of a CL on the ovarian follicular population between days of the estrous cycle; (iii) compare serum oestradiol concentrations related to single versus double ovulations in Sanjabi ewes.

2. Materials and methods

2.1. Experimental animals and ovary collection

The Sanjabi breed is native to central Iran, bordering on Iraq, and akin to the Kurdi sheep breed. The Sanjabi is one of the largest breeds found in Iran. The ewes weigh 60 kg and the rams 70–80 kg. The body color is white, the feet and face are brown and the woolly fleece is of carpet grade – the most sought after Iranian breed (staple length 10–15 cm; fleece weight 2.0–3.0 kg). Sanjabi ewes are generally good milkers and lambs fatten readily (Devendra and Mcleroy, 1982). Ewes are seasonally polyestrous, with the young being born during spring, the most favorable time of year.

The present study was conducted outside the breeding season, at the Razi University sheep farm, Kermanshah province, Iran (34°18′ N and 47°3′ E) from April to June (spring) 2007. With respect to maintaining the body condition of the ewes and health and also considering the seasonality of the breed, this time (spring) of the year was chosen to perform the trial. Rainfall mainly occurs during November–April (fall, winter and early spring), with the precipitation ranging between 400 and 800 mm per annum. The mean monthly maximum and minimum ambient temperatures, range from 8.7 to 35.1 °C and from 3.6 to 27.8 °C, respectively. The mean temperature during the synchronization period was 23 °C.

A total of 197 clinically healthy Sanjabi ewes, 2–3 years of age and weighing 54–61.5 kg, were used in the trial. Upon arrival, all ewes were managed by trained university personnel, using methods as described in the experimental protocol, approved by the Institutional Animal Care and Use Committee (IACUC) of the Razi University. All ewes received a daily maintenance diet of alfalfa pellets, while water, hay, and salt bars were available ad libitum.

Time of estrus was synchronized using intravaginal sponges, containing 20 mg Cronolone (Chronogest®, Intervet, Cambridge, UK) for 12 days. Ewes were checked for signs of overt estrus from 24 h after pessary removal, using 22 intact rams, fitted with aprons – this allowed mounting, but preventing intromission. The time of estrus was recorded as the time of onset of the estrous cycle (day 0 = day of estrus). Ewes showing estrus were separated from the rest of the flock. Ewes were then transported to a local abattoir, where 9 ewes were slaughtered on each experimental day (days 1–16 after estrus), for ovary collection. The ovaries of the ewes were excised immediately after slaughter and transported to the laboratory in 0.9% normal saline, supplemented with procaine penicillin G 2 MIU and dihydrostrepto-mycine 2 g, within 30 min of slaughter. At the laboratory, the ovaries were again washed in saline.

2.2. Blood samples and hormone assays

Each morning blood samples (10 ml) were collected by jugular venopuncture into vacutainers (Becton Dickinson, Rutherford, NJ, USA), starting on day 2 and continuing for 19 days. Blood samples were centrifuged at 1500 × *g* for 10 min, within 2 h after collection, and the serum harvested and stored at −20 °C, until assayed. Serum oestradiol concentrations were determined using commercially available ELISA kits (Calbiotech, Ultra-Sensitive Oestradiol lumELISA, Catalog No. ES561S). The validity of the assay for use in ovine was tested by setting up new standard curves of ewe oestradiol. Standard curves were recorded parallel to the standard curves of the kit, indicating that the ovine serum did not interfere with the binding of the assay. The sensitivity of the assay was 0.4 pg/ml and the intra- and inter-assay coefficients of variation 4.6% and 7.8%, respectively. Serum oestradiol per ewe was calculated for each day of slaughter.

2.3. Experimental design and data analysis

The ovaries per ewe were classified as without or with one or two CL's for each day of slaughter. Visible follicles on the surface of the ovaries were classified, based on their diameter into: (i) very small (<2 mm), (ii) small (2–3.4 mm), (iii) medium (3.5–5 mm) and (iv) large (>5 mm); the respective number of follicles was recorded (Duggavathi et al., 2003). Briefly, the number of ovarian follicles classified as without a CL, and the serum oestradiol concentrations of single and double ovulations were summarized for each ewe on each day of slaughter. The experiment was performed, using a split-plot time design (effects of the ovaries per ewe on follicular population and serum oestradiol concentration were evaluated with each sampling from days 1 to 16 of the estrous cycle, and data analyzed using the SAS GLM procedure (SAS Institute Inc., Cary, NC, USA). Means were compared using the Duncan's multiple range test. A Pearson correlation coefficient was calculated to determine the correlation between the serum oestradiol levels and the very small, small, medium, large follicles and the total follicular population.