Research in Veterinary Science 99 (2015) 37–40

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Functional histology of the ovarian follicles as determined by follicular fluid concentrations of steroids and IGF-1 in *Camelus dromedarius*

M. Kafi [a,*], M. Maleki [b], N. Davoodian [a]

[a] Department of Animal Reproduction, School of Veterinary Medicine, Shiraz University, 71345, Shiraz, Iran
[b] Department of Pathobiology, School of Veterinary Medicine, Mashad University, 48974 Mashad, Iran

ARTICLE INFO

Article history:
Received 22 July 2014
Accepted 6 January 2015

Keywords:
Ovarian follicles
Histology
Steroids
Dominant follicles
Camelus dromedarius

ABSTRACT

Ovaries were collected from sexually mature non-pregnant dromedary she-camels. Follicles 6 to 19 mm in diameter per pair of ovaries were randomly selected and classified into clear (n = 30), or opaque (n = 14) based on macroscopic examination of the follicle surface, and then were divided into four classes: clear follicles with 6– 9.9 and 10–19 mm diameter; opaque follicles with 6– 9.9 and 10–19 mm diameter. Follicular fluid (FF) was aspirated for measurement of estradiol-17β, progesterone and IGF-I concentrations, and then a section of tissue through the exposed surface of the follicle wall was removed and fixed in and processed for histological examination. Mean (±SE) number of clear follicles observed on the ovaries that contained a large dominant follicle was less than that on the ovaries which contained a large atretic follicle ($p < 0.05$; 2.6 ± 1 vs 8.6 ± 0.6). In conclusion, the estrogenic large follicles have suppressive effects on the growth of other follicles.

1. Introduction

The macroscopic appearance of excised follicles is highly correlated with histological signs of atresia in cattle (Kruip and Dieleman, 1985), sheep (Moor et al., 1978), pigs (Bjersing, 1967), and mice (Wilkinson et al., 1979). In addition, studies in cows (Hendriksen et al., 2003; Lavranos et al., 1999; Rodgers and Irving-Rodgers, 2010) and mares (Bridges et al., 2002; Watson et al., 2002) have shown that there is strong agreement between morphological findings and follicular fluid (FF) steroids concentration as the antral follicle is in a healthy or atretic (growing or atretic) status.

Many researchers have measured steroid hormones in the ovarian FF in addition to the different indicators of atresia or cell death to further explore the reliability of steroid concentrations as a good marker of follicle atresia in the bovine (Jolly et al., 1994; Rodgers and Irving-Rodgers, 2010). These researchers have shown that healthy follicles have higher levels of estradiol-17β than atretic follicles, and atretic follicles have higher levels of progesterone or thecal products such as testosterone or androstenedione. In the mare (Bridges et al., 2002) and in the cow (Sudo et al., 2007), it was shown that estradiol-17β and insulin-like growth factor-I (IGF-I) concentrations in FF were greater in large ovulatory than in medium size follicles. In dromedary camel, Salem et al. (1997) and Bukhari et al. (2008) reported that FF concentrations of estradiol-17β and progesterone increased with the growth of the follicles. IGF-I stimulates estradiol-17β synthesis which is important for final maturation of the preovulatory follicle and the oocyte (Spicer, 2004; Webb et al., 2004).

Ovarian follicular development occurs in a wave-like pattern in most domestic animals including cows, sheep, goats and horses (Evans, 2003). In dromedary (Manjunatha et al., 2012; Skidmore, 2011) and Bactrian (Niasari-Naslaji, 2008) female camels, waves of follicular development are also observed during either their breeding or non-breeding seasons. In the dromedary, the growth phase starts with a cohort of small follicles that grow at a rate of 0.5 to 1.0 mm per day until they reach 1.0 cm in diameter and then usually only one follicle continues to grow to become the dominant follicle, while the other follicles regress (Skidmore, 2011). No detailed information is available regarding the relationship between the morphology, histology and hormonal status of follicles that appear in a follicular wave in the dromedary camels. Therefore, the following study was designed to examine the relationship among surface opaqueness of follicles, histological signs of atresia and FF estradiol-17β, progesterone and IGF-I concentrations in *Camelus dromedarius*.

2. Materials and methods

Ovaries from sexually mature (between 5 and 10 years of age) non-pregnant dromedary camels were collected at a local abattoir and placed in a thermos containing ice and then transferred to the

* Corresponding author. Department of Animal Reproduction, School of Veterinary Medicine, Shiraz University, 71345, Shiraz, Iran. Tel.: +9871 36139803; fax: +9871 36940228.
E-mail address: kafi@shirazu.ac.ir (M. Kafi).

http://dx.doi.org/10.1016/j.rvsc.2015.01.001
0034-5288/

laboratory within 30 min of slaughter. The presence of a corpus luteum for each pair of ovaries was used to estimate pregnancy or taking an infertile mating (Al Eknah, 2000). Therefore, only the ovaries from 22 camels which contained no corpus luteum were used in the present study.

2.1. Processing of ovaries

Macroscopic examination of the surface of a follicle was used to determine whether it was grossly clear or opaque. A grossly clear follicle appeared as translucent, whereas an opaque follicle had a cloudy or milky appearance. A follicle was visibly classified as clear if more than 70% of its exposed surface was clear or as opaque if at least 70% of its exposed surface was cloudy (De los Reyes et al., 2006; Grimes et al., 1987). Remaining follicles including hemorrhagic and cystic follicles (Bukhari et al., 2008) were excluded from the study. Then diameters of the clear and opaque follicles were measured by placing a caliper across the interface of the external boundary of a follicle with the body of the ovary. Follicles of different diameters (6–19 mm) per pair of ovaries were classified as clear (n = 30), or opaque (n = 14). Follicles were further subdivided into four classes: 1) clear follicles with 6–9.9 mm diameter (n = 11), 2) clear follicles with 10–19 mm diameter (n = 19), 3) opaque follicles with 6–9.9 mm diameter (n = 6) and 4) opaque follicles with 10–19 mm diameter (n = 8). The wall of each large follicle (10–19 mm) was also grossly examined and the degree of vascularization in each follicle was recorded. Follicles were then categorized into two groups, with either poor or good level of vascularization (Kenney et al., 1979). Poor vascularization in follicles was defined by visibly pale follicular walls whereas follicles with high amount of vessels in their walls were defined as having a good level of vascularization. FF was then gently aspirated using sterilized 19-gauge needles and syringes, centrifuged at 3000 × g for 15 min to remove debris, and stored in small aliquots at –20 °C for hormone assay. A section of tissue through the exposed surface of the follicle wall (4–6 mm^2) on each ovary containing grossly clear or opaque follicles was removed and placed into Karnovsky fixative for histological evaluation.

2.2. Histological evaluation of follicular tissues

Follicular samples were routinely processed for histology. Two serial sections were obtained for each sample. One was stained with hematoxylin and eosin and one with Period acid-Schiff (PAS) for the evaluation of granulosa-theca cells and basal membrane respectively. The degree of atresia was assessed histologically using light microscopy. Based on the histological condition of each section of the follicular wall, follicles were divided into non-atretic (healthy), moderately atretic and strongly atretic follicles (Grimes et al., 1987).

2.3. Hormone assays

Concentrations of estradiol-17β, progesterone and IGF-I in the FF samples were measured using validated commercial radioimmunoassay kits (Immunotech kit, France). A parallelism test was designed to measure follicular fluid progesterone, estradiol and IGF-1 concentrations. Known concentrations of hormone standards were added into the samples and the recovery percentages were calculated. The sensitivity of the tests and the recovery rate for estradiol-17β progesterone and IGF-I were 0.5 pg/ml and 85.5 to 109%; 0.05 ng/ml and 85 to 110%; and 2 ng/ml and 91 to 103%, respectively. Macroscopically clear follicles (10–19 mm in diameter) with an estradiol-17β to progesterone (E:P) ratio greater than 1 were defined as large dominant follicles.

2.4. Statistical analyses

All proportions were subjected to chi-square or Fisher exact tests to compare follicular morphology, histology and hormonal status of the follicles where appropriate. Of 44 follicles, only 31 follicles with undetached granulosa layers were diagnosed as suitable for the histological examinations. The others had either luteinized or detached granulosa layers and therefore were excluded from the histology. As there were heterogeneous variances in the concentrations of the hormones measured, a natural log transformation was made to statistically compare the means. The data were then subjected to a t-test to statistically compare the differences. Pearson's correlation analyses were used to evaluate the relationships among the hormone concentration and follicle diameters. P values less than 0.05 were considered as significant difference.

3. Results

3.1. Follicular opaqueness and histological findings

Out of 31 follicles suitable for histological examination, 18 follicles (58%) were observed as atretic (atretic degrees combined) and the remaining 13 follicles (42%) were diagnosed as non-atretic. Atretic follicles were characterized by disorganized and degenerated granulosa cell layers, abundant pyknotic granulosa cells, basement membrane breakdown, leukocytes infiltration in theca and granulose layers and loosely arranged theca interna. A greater percentage ($p < 0.05$) of opaque (91%) follicles showed histologic signs of atresia than that of the clear (40%) follicles (10 out of 11 vs 8 out of 20). While 8 out of 11 (72%) opaque follicles were strongly atretic, only 1 out of 20 (5%) clear follicles was strongly atretic ($p < 0.05$). Data available from 16 clear large follicles (10–19 mm) showed that 93.7% (15 out of 16) of them had a good level of vascularization in their wall while only 6.3% (1 out of 16) of follicles were poorly vascularized. In addition, of 15 clear large follicles that had good level of vascularization, 6 (40%) showed moderate signs of atresia. Poor vascularization was observed in 6 out of 8 (75%) large opaque follicles (10–19 mm).

3.2. Follicular opaqueness, hormonal status and histological findings

The mean (±SE) FF concentration of estradiol-17β was greater ($p < 0.05$) in the clear follicles 10–19 mm in diameter than in the clear follicles 6–9.9 mm in diameter (156 ± 42.6 vs 29 ± 12.5 pg/ml, respectively). However, mean (±SE) concentrations of progesterone, IGF-I and E:P ratio in the FF were not significantly different between the clear and the opaque follicles in different diameter classes. Mean (±SE) FF concentrations of estradiol-17β were significantly greater for clear than opaque follicles (92.5 ± 27.5 vs 6.67 ± 0.65 pg/ml, $p < 0.05$).

Mean (±SE) FF concentrations of estradiol-17β, progesterone, IGF-I and E:P ratio for each of the histologically non-atretic and atretic follicles are shown in Table 1. Mean (±SE) FF concentrations of estradiol-17β were greater for non-atretic (n = 11) than for atretic (n = 13) follicles 10–19 mm in diameter ($p < 0.05$). No significant

Table 1
Mean (±SE) follicular fluid concentrations of E_2, P_4, IGF-I and E:P ratio of non-atretic and atretic follicles (10 to 19 mm).

	Non-atretic follicles	Atretic follicles
No. of follicles	11	13
Estradiol-17β (pg/ml)	231 ± 40[a]	1.91 ± 2.4[b]
Progesterone (ng/ml)	22.3 ± 4.9	18.8 ± 5.5
IGF-I (ng/ml)	159.3 ± 21.4	162.1 ± 17.1
E:P ratio	10.3 ± 8.16	0.10 ± 0.43

Values in the same row with different superscripts are different ($p < 0.05$).