

Ovarian Antral Follicular Dynamics and Follicular Fluid Composition in Non-descript Goats of Karnataka

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Abstract

A study was carried out to elucidate the ovarian antral follicular dynamics and the profile of certain biochemical components in ovarian antral follicular fluid of non-descript goats of Karnataka. Three hundred ovaries were collected from two to five year old healthy non-descript goats slaughtered at Civil Meat Processing and Production Centre, Bangalore during the months of September to December, 2012. The surface ovarian antral follicles were categorized into three groups based on the diameter as Group I (small, 1 to 2.9 mm), Group II (medium, 3 to 5 mm) and Group III (large, >5 mm) follicles. The follicular fluid was collected and pooled as per the different groups of follicles and it was analysed for the levels of glucose, total protein, albumin, total cholesterol, estradiol-17 β , alkaline phosphatase and acid phosphatase activities. The results of the present study revealed that the number of medium sized follicles were significantly ($P < 0.05$) higher compared to small and large sized follicles. The glucose content and acid phosphatase activities were significantly ($P < 0.05$) higher in small follicles compared to medium and large follicles. Total cholesterol and estradiol-17 β levels were significantly ($P < 0.05$) higher in large follicles compared to small and medium follicles. The total protein, albumin levels and alkaline phosphatase activities did not differ significantly ($P > 0.05$) between various groups of follicles. It was concluded that the findings of the present study on ovarian antral follicular dynamics and biochemical profile of follicular fluid could be useful for the scientists working on *in vitro* maturation and *in vitro* fertilization in goats.

Keywords: Ovarian antral follicles, follicular dynamics, Biochemical profile, follicular fluid, goats.

Introduction

The goat is the earliest ruminant animal domesticated at around 10 thousand years ago and it has been considered as the poor man's cow. The goat rearing generates employment and livelihood sources for small, marginal and landless farmers. Reproduction is most essential component in the life of any animal species as it is needed for the propagation and continuity of the species (Pineda, 2003). The ovarian folliculogenesis is most important physiological event that significantly affects the success of animal breeding. Understanding the pattern of follicular development in different species is most important for designing improved methods to manipulate reproduction in domestic animals (Evans, 2003). Ovarian follicular fluid is a complex mixture consisting of water and solutes derived from plasma and metabolites of follicular cells (Bagavandoss *et al.*, 1983). The composition of follicular fluid has also drawn much interest of the workers because of the remarkable success in *in vitro* maturation and *in vitro* fertilization of the oocytes. The limited knowledge of ovarian follicular dynamics and biochemical composition of follicular fluid in goats (Bordoloi *et al.*, 2001; Bordoloi *et al.*, 2003) necessitated the study of ovarian physiology in non-descript goats of Karnataka.

Materials and Methods

Collection of ovaries and classification of the follicles

Three hundred ovaries were collected from healthy adults of two to five year's old belonging to non-descript goats (*Capra hircus*) at Civil Meat Processing and Production Centre, Frazer Town, Shivajinagar, Bangalore from September 2012 to December, 2012. Ovaries were recovered immediately after the slaughter and placed in sterile plastic container containing 0.9 per cent sodium chloride solution and transported to the laboratory in ice cold condition within one hour. The extraneous and adipose tissues were removed for better examination of the ovaries. Each ovary was observed for the antral follicles visible on the ovarian surface. A Vernier calliper was used to measure the surface diameter of the follicles. Hand lens and scale were also used to measure the surface diameter up to one mm sensitivity. The ovarian antral follicles were categorized into three groups based on the surface diameter as Group I (small, 1 to 2.9 mm), Group II (medium, 3 to 5 mm) and Group III (large, >5 mm) follicles (Goswami *et al.*, 1998; Yu *et al.*, 2005).

Collection of ovarian antral follicular fluid

In the laboratory, the ovaries were washed thoroughly twice with sterile normal saline before collection of follicular fluid to avoid the contamination of the follicular fluid. Fluid from each follicle was drawn with the help of disposable sterilized insulin syringe fitted with 26 gauge needle. For each group of follicles, different needle and syringe was used for the collection of fluid as per the method described (Leroy *et al.*, 2004; Sowmya, 2010).

Processing and storage of follicular fluid

The follicular fluid recovered from each group of follicles was pooled. The pooled samples of follicular fluid were subjected to refrigerated centrifugation at 1500 rpm at 5 °C for 15 minutes in Remi C-23, cooling centrifuge to remove the blood cells, oocytes and granulosa cells. The cell free follicular fluid samples were stored at -20 °C for further analysis of biochemical parameters.

Analysis of Follicular Fluid for Biochemical Components

The glucose content in follicular fluid was assayed by using commercial reagent kits of Merck Specialities Pvt. Ltd., Mumbai based on glucose oxidase – peroxidase method as described by Burtis and Ashwood (1996). Reagent kits of ERBA diagnostics Mannheim GmbH Mallaustr, Germany were used for the estimation of total protein (Tietz, 1986), albumin (Doumas *et al.*, 1972; Tietz, 1986), total cholesterol (Allain *et al.*, 1974) and alkaline phosphatase activities (Tietz, 1976). The activities of acid phosphatase was estimated by reagent kits manufactured by Aspen Laboratories Pvt. Ltd., Delhi based on the method of Tietz (1976). The concentration of estradiol-17 β was determined by the reagent kits manufactured by Roche diagnostics, GmbH, Germany which is based on electrochemiluminescence immunoassay method by using competition principle.

Statistical analysis

The data was statistically analysed by one way analysis of variance (GraphPad Prism version 5.01, 2007) with Bonferroni post test. The significance was measured at $P < 0.05$.

Results and Discussion

Dynamics of ovarian antral follicles in non-descript goats

The mean \pm SE values of the number of different size ovarian antral follicles

recorded in the non-descript goats are presented in Table 1. The results of the present study revealed that there was significant difference ($P < 0.05$) between Group I and Group II and also between Group II and Group III follicles. Hence, in the present study there were more number of medium sized follicles compared to small and large sized follicles.

Table 1. Mean \pm SE values of number of follicles in different groups of ovarian antral follicles present in non-descript goat ovaries

Number of follicles in different groups	Group I (Small, 1 to 2.9 mm)	Group II (Medium, 3 to 5 mm)	Group III (Large, >5 mm)
		8.14 \pm 0.84 b	12.1 \pm 1.66 a

Values bearing different superscript differs significantly ($P < 0.05$).

Biochemical composition of the follicular fluid

The mean glucose concentration in the follicular fluid of large follicles showed a significant decrease ($P < 0.05$) compared to small and medium size follicles (Table 2). The difference in the total protein concentrations in different size follicles was non-significant ($P > 0.05$). The albumin concentrations in the follicular fluid did not differ significantly ($P > 0.05$) between various groups of follicles. The concentration of albumin in the follicular fluid decreased numerically as the size of the follicles increased (Table 2).

Table 2. The mean \pm SE values of biochemical parameters in different groups of ovarian antral follicles of non-descript goat ovaries

Biochemical parameters	Group I	Group II	Group III
Glucose (g/dl)	28.10 \pm 0.56 ^a	23.40 \pm 0.50 ^b	19.70 \pm 0.45 ^c
Total protein (g/dl)	7.39 \pm 0.29	6.82 \pm 0.28	6.55 \pm 0.25
Albumin (g/dl)	4.82 \pm 0.24	4.19 \pm 0.30	3.93 \pm 0.31
Total cholesterol (mg/dl)	32.60 \pm 2.43 ^a	43.00 \pm 2.17 ^b	51.80 \pm 2.52 ^c
Alkaline phosphatase (IU/L)	24.90 \pm 1.48	22.80 \pm 1.43	20.40 \pm 1.35
Acid phosphatase (IU/L)	7.41 \pm 0.82 ^a	5.15 \pm 0.62 ^b	3.34 \pm 0.37 ^c
Estradiol-17 β (pg/ml)	1450.60 \pm 108.16 ^a	2765.47 \pm 158.29 ^b	3942.02 \pm 175.41 ^c

Values in the same rows bearing different superscript differs significantly ($P < 0.05$).

The concentration of total cholesterol was significantly ($P<0.05$) higher in large follicles compared to small and medium size follicles. The concentration of total cholesterol increased significantly ($P<0.05$) as the size of the follicle increased (Table 2).

The activities of alkaline phosphatase were non-significant between small, medium and large follicles (Table 2). The activity of acid phosphatase differed significantly ($P<0.05$) between Group I, Group II and Group III. The activity in different groups of follicles decreased as the size of the follicle increased (Table 2).

The concentration of estradiol-17 β differed significantly ($P<0.05$) between small, medium and large follicles. The concentration increased significantly ($P<0.05$) as the size of the follicle increased (Table 2).

The significant ($P<0.05$) reduction in the large sized follicles compared to medium sized follicles could be due to higher incidence of apoptosis during transition from medium to large sized follicles as opined (Yu *et al.*, 2005). The significant ($P<0.05$) increase in the number of medium sized follicles compared to small sized follicles in non-descript goat ovaries collected from abattoir were in agreement with Goswami *et al.* (1998) who observed more number of medium sized follicles compared to small sized follicles .

The number of small follicles was numerically higher compared to large follicles. This was in accordance with Ravindra *et al.* (1994) who observed the increased number of follicles with more than two mm diameter on all the days of estrous cycle in ewes except for the days 1, 5, 15, 16 and 17 of the estrous cycle. Further, more number of Group I follicles were observed during mid-luteal phase (Ariyaratna and Gunawardana, 1997). There could be species specific multiple numbers of follicles which continue growing while the other follicles regress at variable intervals at the same time (Evans, 2003). It was inferred that non-descript goats of Karnataka have a higher population of medium size follicles compared to small and large follicles.

The decrease in the glucose concentration in the large follicles may possibly attributed to utilization of the glucose by the follicular oocytes during its maturation process as reported (Bordoloi *et al.*, 2001). Further, the significant ($P<0.05$) depletion of glucose in large follicles was in accordance with Alkalby *et al.* (2012) who opined that the decreased concentration of glucose in large follicles implies that very little glucose is synthesized locally by granulosa cells of follicles and main source of glucose in follicular fluid was plasma.

The observations of numerically decreased levels of total protein in the follicular

fluid as the follicle size increased in the present study was in accordance with Bordoloi *et al.* (2003) who opined that decreased levels of total protein in follicular fluid could be attributed to its utilization for metabolic activities of the follicular cells during steroidogenesis or due to change in permeability of the follicular wall for total protein. The high correlation between total protein content in follicular fluid and in serum suggests that a substantial part of the protein content in follicular fluid originates from serum (Wise, 1987).

The decrease in the albumin levels in the large follicles in the present study was in accordance with Wise (1987) who reported that the actively growing follicles need amino acids and the ovary is one of the most active tissues in catabolizing albumin. Further, the decreased level of albumin in the follicular fluid as the follicle size increased in the present study was in agreement with Albomohsen *et al.* (2011) who suggested that the higher levels of albumin in small follicles suggest an active inward transport of albumin compound from blood into follicles, which may be required for various physiological functions such as growth and maturation of follicles.

In the present study, the significant ($P < 0.05$) increase of total cholesterol levels in the large follicles is attributed to increased permeability of the follicular wall in large follicles leading to the entrance of high density lipoprotein fraction which is one of the cholesterol containing lipoprotein fraction that might lead to higher levels of total cholesterol in the large follicles as reported by Bagavandoss *et al.* (1983). Further, Thakur *et al.* (2003) opined that as cholesterol is the main precursor for steroidogenesis, it was one of the major components in the matured follicles.

The significantly ($P < 0.05$) higher levels of acid phosphatase activity in small follicles was attributed to limited ability of small follicles to respond to gonadotropins stimulation and few small follicles may undergo atresia. It could play a regulatory role in endocrine functions that control the growth and nutrition of the oocyte in developing follicles (Henderson and Cupps, 1990). The numerically higher level of alkaline phosphatase activity in small follicles was in agreement with Henderson and Cupps (1990) who opined that higher levels of alkaline phosphatase activity in the small follicles were attributed to its role in the regulation of follicular growth. The significantly higher level of estradiol-17 β as the follicle size increased was attributed to the change in the environment of follicular fluid from androgenic to estrogenic tissue. An increase in granulosa cell numbers and aromatase activity could account for the decreased concentrations of androgen and increased concentrations of estradiol-17 β in the follicular fluid with increased follicle size (Henderson and Cupps, 1990).

It was concluded that the findings of the present study with respect to ovarian antral follicular dynamics and composition of follicular fluid in non-descript goats of Karnataka will provide clues to the researchers who work on reproductive biology in goats and as well it also meets certain academic interests.

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