Study on Biochemical Constituents of Caprine Ovarian Follicular Fluid after Superovulation

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ABSTRACT : The experiment was designed on 42 non pregnant Black Bengal goat. Out of which 18 were subjected to a superovulatory treatment comprising of eCG and hCG for embryo transfer study. The remaining 24 goats received no treatment and served as control for parameter studied as well as recipient for embryo transfer studies. Important biochemical constituents such as acid and alkaline phosphatase, total protein and cholesterol and inorganic phosphorus were estimated in the follicular fluid of control and treated group and the values were separately recorded for small medium and large size follicle. The results indicated a significant effect on acid phosphotase activity due to size of follicle. The value increased progressively from small to medium and from medium to large follicles. Alkaline phosphotase activity showed reverse trend. Alkaline phosphotase decreased progressively as size increased. The concentration of inorganic phosphorus did not reveal any significant difference between the control and treatment groups and also between the different size follicles. The concentration of protein decreased significantly from small to medium and from medium to large, although no difference was observed between the control and treatment groups. The concentration of Cholesterol in the follicular fluid indicated a significant increase from small to medium and to large follicle. Here also no difference was observed due to treatment. Similar in the composition of follicular fluid in the respect of above mentioned constituents indicated no of super ovulatory treatment on follicular fluid composition. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1711-1715)***

Key Words : Goat, Superovulation and Follicular Fluid

INTRODUCTION

Follicular fluid consists of secretions of the follicular cells and transudate of plasma. The constituents of follicular fluid are considered as a regulating factor in follicular development and steroidogenesis. Mc Gauphey (1972) suggested that the follicular fluid contents vary before and after oocyte maturation and variations might be related to meiotic process. Follicular fluid, a serum transudate modified by follicular metabolic activities, contains specific constituents such as steroids and glyco proteins synthesized by the cells of the follicle wall. It plays a role in the physiological, biochemical and metabolic aspects of the nuclear and cytoplasmic maturation of the oocyte (Hafez and Hafez, 2000). Follicular fluid composition was under intensive investigation to know the ovulatory process (Luck et al., 2000), oocyte maturation (O' Callaghan et al., 2000), fertilization and embryonic development (Choi et al., 1998) and follicular atresia (Lebedeva et al., 1998). The objective of this study was to analyze a number of biochemical which traits like describe acid and alkaline phosphatase, cholesterol, protein and inorganic phosphorus and to obtain a better under standing of the biochemical events that takes place during the growth of follicle in normal and superovulated groups of goats.

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MATERIALS AND METHODS

Forty-two adult non-pregnant Black Bengal female goats between the age of 1.5 to 3.5 years were utilized for this study. The goats were maintained on a normal balanced ration having following composition, Maize -42.25 kg/quintal, Wheat bran -37.00 kg/quintal, Ground Nut Cake -18.50 kg/quintal, Mineral mixture -2.00 kg/quintal, Common salt -0.250 kg/quintal and Vitamin AD_3 was mixed at 20 mg/quintal. The goats were allowed to graze 3 to 4 h daily and the green fodder was made available *ad libitum* during the period of investigation. Diet contained 16% crude protein and 70% total digestible nutrient. All goats were synchronized by $PGF_2\alpha$ (Dinofertin, Alved Pharmaceutical Chennai, India) at 5 mg/goat intramuscularly 11 days apart. Goats under superovulation group received equine chorionic gonadotropin (eCG), (Folligon: Intervet Holland: at 750 IU/goat intramuscularly) on day 10th of the estrus i.e. 1 day prior to the last synchronization treatment (Ishwar and Memon, 1996) to promote follicular development. Prostaglandin $F_2\alpha$ (Dinofertin) 5 mg/goat IM was given on the next day of the eCG treatment to ensure luteolysis. Human chorionic gonadotropin (hCG) (Chorulon Intervet Holland: 5,000 IU/goat intravenous) was given on the day of return of

Table 1. Showing acid phosphatase activity (KAU/100 ml) in the follicular fluid of different size follicle

Groups	Size of follicles			
	Small (<3mm)	Medium (3 to 5 mm)	Large (<5mm)	Overall
Control	17.68±2.09 (4)	23.99±1.61 (5)	28.09±0.82 (5)	23.65±1.40 (14)
Treatment	18.74±1.10 (4)	24.16±1.81 (5)	27.35±0.84 (6)	23.99±1.15 (15)
Overall	18.21±1.10[a] (8)	24.08±1.13[b] (10)	27.69±0.57[c] (11)	

Figures in parenthesis indicate number of observations. Mean with different superscripts differ significantly ($p<0.01$).

Table 2. Showing alkaline phosphatase activity (KAU/100 ml) in the follicular fluid of different size follicle

Groups	Size of follicles			
	Small (<3mm)	Medium (3 to 5 mm)	Large (<5mm)	Overall
Control	37.47±5.8 (4)	31.42±2.92 (5)	24.78±1.12 (5)	30.07±2.54 (14)
Treatment	38.47±5.8 (4)	33.08±3.79 (5)	25.10±1.15 (6)	31.32±2.37 (15)
Overall	38.97±4.30[a] (8)	32.25±2.28[b] (10)	24.95±0.77[c] (11)	

Figures in parenthesis indicate number of observations. Mean with different superscripts differ significantly ($p<0.05$).

estrus following the above treatment to induce multiple ovulation and luteal development (Wani and Golderman, 1987). All the goats were mated twice at an interval of 12 h during estrus with a fertile buck available. Laparotomy was performed in the animals of both groups on third day after the mating as per the method of Dziuk (1971). The ovary was exposed. The follicular surface of each ovary was cleaned with the help of a sterile and dry muslin cloth to remove blood clot and other watery fluid present on the surface. The follicular fluid was aspirated from the base of follicle with the help of 1 ml sterilized tuberculin syringe fitted with 18 gauges disposable needle Separate tuberculin syringe and needles were used for collection of follicular fluid from different sizes of follicle (small, medium and large). The tip of fine needle was inserted into the base of follicle to avoid oozing out of fluid and at the same time suction pressure was made in the syringe by pulling the piston of syringe. The ovarian follicles were classified into three groups as small, medium and large (Ishwar, 1989). The follicle less than 3 mm in diameter were considered as small follicle, 3 to 5 mm diameter as medium follicle, while the follicle which were more than 5 mm considered as large follicles. The follicles from superovulated and normal groups were pooled separately. Total protein, total cholesterol, inorganic phosphorus, alkaline phosphatase and acid phosphates were analysed as per the method of Lowry et al., 1951, Zak et al., 1954, Fisk and Subbaraw 1925, respectively. Analysis of variance and critical difference was calculated according to the method suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

There was non significant effect of treatment but significant ($p<0.01$) effect of follicular size on acid phosphatase activity in follicular fluid. Further critical difference test showed significantly highest acid phosphatase activity in the follicular fluid of large size follicle followed by medium and small (Table 1). Non-significant effect of treatment and significant effect of follicular size for alkaline phosphatase activity was observed in the follicular fluid but average values indicated progressive decrease of alkaline phosphatase activity with increase of follicular size. Further critical difference showed significantly lowest alkaline phosphatase activity in large size follicle than those of medium followed by small size. However, difference between small and medium size follicle was significant ($p<0.05$) statistically (Table 2). The alkaline phosphatase activity is an important enzyme which catalyses several reactions in the body and is involved in the active transport of phosphates across the cell membrane synthesis of proteins and DNA turnover in nucleus (Moog, 1946 and Denielli, 1953). It is also involved in the hydrolysis of many esters of phosphoric acid and in phosphate fixation in the soft tissue and bones during the developmental stages. The activity of alkaline phosphatase in the different tissue is partially controlled by the ovarian hormones (Sykes et al., 1955). Steroid binding receptors are phosphorylated proteins (Auricchio et al., 1981; Dougherty et al., 1982) and binding activity can be altered by the action of acid and alkaline phosphatase upon receptors. In our studies, acid phosphatase activity increased with

Table 3. Showing inorganic phosphorus (mg/100 ml) in the follicular fluid of different size follicle

Groups	Size of follicles			Overall
	Small (<3mm)	Medium (3 to 5 mm)	Large (<5mm)	
Control	9.8±1.30 (3)	10.00±5.21 (3)	10.66±0.66 (3)	10.16±1.56 (9)
Treatment	10.25±0.94 (3)	09.00±1.5 (3)	9.83±3.77 (3)	9.69±1.21 (9)
Overall	10.02±0.72 (6)	09.50±2.41 (6)	10.24±1.72 (6)	

Figures in parenthesis indicate number of observations.

Table 4. Showing total protein concentration (gm/100 ml) in the follicular fluid of different size of follicle

Groups	Size of follicles			Overall
	Small (<3mm)	Medium (3 to 5 mm)	Large (<5mm)	
Control	5.80±0.36 (6)	3.97±0.12 (6)	2.96±0.14 (6)	4.24±0.31 (18)
Treatment	5.84±0.26 (6)	3.87±0.19 (6)	2.72±0.25 (6)	4.14±0.33 (18)
Overall	5.82±0.21[a] (12)	3.92±0.01[b] (12)	2.80±0.14[c] (12)	

Figures in parenthesis indicate number of observations. Mean with different superscripts differ significantly ($p<0.01$).

Table 5. Showing total cholesterol (mg/100 ml) in the follicular fluid of different size follicle

Groups	Size of follicles			Overall
	Small (<3mm)	Medium (3 to 5 mm)	Large (<5mm)	
Control	43.67±3.42 (6)	70.15±15.35 (6)	172.28±14.34 (6)	95.37±15.00 (18)
Treatment	51.71±3.34 (6)	74.99±5.97 (6)	171.20±13.45 (6)	99.30±13.40 (18)
Overall	47.69±2.57[a] (12)	72.57±7.86[b] (12)	171.70±9.35[c] (12)	

Figures in parenthesis indicate number of observations. Mean with different superscripts differ significantly ($p<0.01$).

follicular development, which is in agreement with the finding of Janakiraman and Mehta (1990) in goats. However, Wise (1987) and Chang et al. (1976) observed decrease in alkaline and acid phosphatase activity increase in follicular size in bovine. It is thought that follicular acid and alkaline phosphatase activity might be an excellent indicator of atrophy due to lysosomal enzyme effect upon phosphorylated receptor, which would lead to atresia.

Neither treatment nor size had significant effect on inorganic phosphorus concentration. Average value is presented in Table 3. These values was higher than those reported by Lutwak Mann (1954), Olds and Van Demark (1957), Burgogyne et al. (1979) and lower than those reported by Krishna Murty et al. (1986). However, our value was in agreement with Chang et al. (1976) and Thakur (1990).

There was non-significant effect of treatment but significant effect ($p<0.01$) of follicle size on total protein concentration. Average value presented in Table 4 showed significant highest protein level in small size follicle followed by medium and large size follicle. The present values are similar to those reported by Caravaglios and Cilotti (1957), Wise (1987), Thakur (1990) and Thakur et al. (2003). Since volume of antral fluid increased the protein concentration decreased. Perkins and Goods (1966) did not get any difference in protein content of follicular fluid of natural and synchronized estrus cycle. O'callaghan et al. (2000) also reported that concentration of the 34.22, and 20 K Da IGF binding proteins were lower in follicle from superovulated ewes compared with unstimulated ewes ($p<0.05$). Non-significant effect of treatment but significant effect of size ($p<0.01$) was found on total cholesterol level of follicular fluid. Average value presented in Table 5 showed significantly highest concentration in large size follicle than that of medium followed by small follicle. The values for total cholesterol were higher than the values reported by Janikiraman and Mehta (1990) and Thakur (1990) for goats. However, in our study level of total cholesterol increased with increase in follicular size which is in agreement with the findings of Brantmeier et al. (1987), for cows, Janakiraman and Mehta (1990), Thakur (1990) and Thakur et al. (2003) for goats. Cholesterol in ovary can

be derived from two sources cellular denovo synthesis from acetate or uptake from plasma lipoprotein. Cholesterol present in the follicular fluid is in the form of a constituent of high-density lipoprotein (Chang et al., 1986; Brantmeier et al., 1987). Grummer and Carrol (1988) suggested that cholesterol is the precursor for steroid synthesis and the follicular fluid contains only high-density lipoprotein so the avascular granulosa cells of the follicles totally depend on the cholesterol from high-density lipoprotein. Jonas (1975) observed that the high-density lipo protein, present in the follicular fluid is derived from the blood plasma by crossing the basement membrane of granulosa cells. From above discussion, it can be easily derived that since production of steroid increase, as the follicle develops (Wise et al., 1986) the level of cholesterol also increases.

Since similar in the composition of follicular fluid in the respect of above mentioned constituents is control and as well as treated group and the trend of increase or decrease in level is also similar, therefore it can be concluded that superovulation does not cause any adverse effect on the growth and development of oocytes within the follicle.

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