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SHORT COMMUNICATION

Total protein, albumin and electrophoretic pattern of follicular fluid of goat (*Capra hircus*)

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ABSTRACT

To investigate various biochemical constituents in follicular fluid of small (2-3 mm), medium (>3-5 mm) and large (>5 mm in diameter) follicles, goat ovaries were obtained from abbatoir immediately after slaughter. For analysis of total protein, albumin and electrophoretic pattern of different protein fractions, pooled follicular fluid from identical follicles (based on diameter) was used. Both total protein and albumin levels in follicular fluid demonstrated declining trend along with increase in follicular size. Electrophoresis of follicular fluid showed five bands in contrast to six bands control serum samples. The protein bands of γ-globulin was not visible in the follicular fluid.

Key words : Total protein, albumin, electrophoretic pattern, follicular fluid, goat

Follicular fluid has drawn the interest of the workers because of the remarkable success in *in vitro* fertilization and *in vitro* maturation of the oocyte, where follicular fluid is incorporated in the medium. To understand the interaction of the constituents of follicular fluid with follicular cells and oocytes for their development and maturation, examination of total protein and albumin concentration with electrophoretic pattern in different phases of follicular development is essential. The present study was designed to monitor the concentration of total protein and albumin in follicular fluid according to the follicular size in goat.

A total of 372 ovaries of Assam local goat were collected from the abbatoir immediately after slaughter and carried to the laboratory in a prewarmed (37°C) thermosflask. Ovaries were then cleaned thoroughly and each ovary was subjected to follicle dissection. Isolated follicles following dissection was classified according to its diameter as small (2-3 mm), medium (>3-5 mm) and large (>5 mm). Follicles belonging to same class were placed in a sterile cavity glass slide and ruptured under stereo zoom microscope. The follicular fluid was then drawn from the cavity glass slide with the help of 1 ml. sterile syringe and transferred to a centrifuge tube. The pooled follicular fluid of each class of follicle was centrifuged at 2000 rpm for 15 minutes and the supernatant was drown out with the help of a pasteur pipette. Total protein and albumin in supernatant follicular fluid were estimated following the methods of Lowry *et al.* (1951) and Amino (1976) respectively.

Polyacrylamide gel electrophoresis of goat serum (control) and follicular fluid was carried out according to Davis (1964). Relative proportion (Rp%) of different protein bands in serum and follicular fluid was calculated from the optical density value of eluted dye from Coomassie brilliant blue stained polyacrylamide gels. Elution of dye was performed by cutting out stained zones, extracting the dye with 4 ml. of 25% pyridine for six hours at room temperature (Fenner *et al.*, 1975). Eluted dye was collected by passing through a small syringe stuffed with glass wool and optical densities were recorded at 580nm using spectrophotometer. Relative mobility (Rm, Av.) of different protein bands in serum and follicular fluid was calculated as per Hedrick and Smith (1968).

The mean concentration (g%) of total protein and albumin in follicular fluid of small, medium and large follicle was 7.98±0.48 and 4.63±0.28, 7.73±0.60 and 4.16±0.11, and 6.57±0.39 and 3.81±0.23 respectively. Although statistically not significant, total protein and albumin concentrations in follicular fluid decreased as the size of the follicle increased. Slightly lower total protein concentration in follicular fluid with increased size of the follicles was probably due to utilization of protein for metabolic activities of the follicular cells during steroidogenesis or due to change in permeability of the follicular wall for protein.

Electrophoretic patterns of total protein in serum (control) and follicular fluid are depicted in Fig.1. In serum, six bands representing albumin, α_1-globulin, α_2-globulin, β_1-globulin, β_2-globulin and γ-globulin were observed. The

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Table 1. Relative proportion (rp%) and relative mobility (rm, av.) of different bands in serum and follicular fluid of different classes of follicle

Protein Bands	Serum		Small follicle		Medium follicle		Large follicle	
	Rp (%)	Rm (Av.)	Rp (%)	Rm (Av.)	Rp (%)	Rm (Av.)	Rp (%)	Rm (Av.)
Albumin	32.55	0.70	37.00	0.70	36.50	0.70	36.70	0.70
α_1-globulin	6.38	0.51	8.38	0.51	8.38	0.51	8.37	0.51
α_2-globulin	7.25	0.39	9.42	0.39	9.42	0.39	9.43	0.39
β_1-globulin	4.89	0.27	6.10	0.27	6.10	0.27	6.40	0.27
β_2-globulin	36.17	0.12	39.10	0.12	39.00	0.12	39.40	0.12
γ-globulin	12.70	0.03	-	-	-	-	-	-

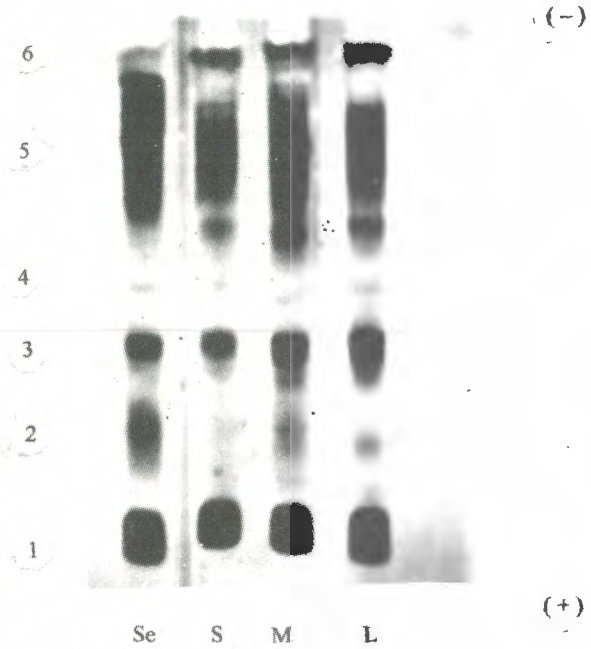

Fig. 1. PAGE of serum and follicular fluid of goat
1. Albumin 2. α_1-globulin 3. α_2-globulin
4. β_1-globulin 5. β_2-globulin 6. γ-globulin
Se – Serum, S – Small follicle,
M – Medium follicle and L – Large follicle

protein band in the region of γ-globulin (Fig.1, '6') was not visible in follicular fluid of all classes of follicle. Lack of γ-globulin in follicular fluid of different classes of follicle in the present study (Table 1) may be due to nontransportation of it from the blood to the follicular fluid during transudation process. The electrophoretic pattern of proteins present in follicular fluid of different classes of follicle was almost similar when they were compared among them. There was no difference in relative proportion (Rp%, Av.) and relative mobility (Rm, Av.) of different protein bands of follicular fluids, but some differences were observed when values were compared with serum (Table 1).

As defensive mechanism is maintained by γ-globulin, the lack of γ-globulin in follicular fluid may be responsible for low defensive mechanism, which was related with vitality and viability of the oocyte during in vitro maturation process. On the other hand, γ-globulin was reported to be present in follicular fluid of buffalo by Murty *et al.* (1987) and Parmar and Mehta (1991). However Murty *et al.* (1987) reported that γ-globulin was not present in the follicular fluid of buffalo during oestrus. Absence of γ-globulin fraction in caprine follicular fluid reflects species difference in follicular fluid constituents. However, it was felt that thorough knowledge on follicular fluid was essential prior to its incorporation in the in vitro maturation medium.

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