BIOCHEMICAL COMPOSITION OF CAPRINE FOLLICULAR FLUID AND OOCYTES

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Abstract: The aim of this study was to biochemically characterize caprine follicular fluid and oocytes after maturation. Ovaries were collected from adult and cycling non-pregnant slaughtered goats (*Capra hircus*). A total of 212 pairs of ovaries were investigated and these data were then compared. Follicular fluid was aspirated from small (< 2 mm), medium (2–5 mm) and large (> 5 mm) non-atretic ovarian follicles. The follicular fluid was centrifuged at 4°C and 2000 rpm for 15 min to remove any cells and stored at –80°C prior to assay. Follicular fluid samples were analyzed for total protein, urea, creatinine, triglycerides, calcium, glucose, cholesterol, phosphorus, sodium, chloride, magnesium and potassium. Data were analyzed by the linear regression model. As follicles became larger, creatinine, triglycerides, calcium, glucose, cholesterol, phosphorus and sodium significantly ($P < 0.05$) increased while those of total protein, urea, chloride, magnesium and potassium significantly ($P < 0.05$) decreased. As the oocytes get matured, there was uptake of protein, calcium and phosphorus. The total number of protein bands for sera and follicular fluid were 19 and 18 respectively as evidenced by SDS PAGE- coomassie blue staining.

Keywords: biochemical composition, follicular fluid, oocyte, goat

Introduction: The follicular fluid plays an important role in physiological, biochemical and metabolic aspects of nuclear and cytoplasmic maturation of the oocyte and release of egg from the ruptured follicle and fertilization [1]. In recent years, follicular fluid received due interest because of the remarkable success made in *in vitro* fertilization and *in vitro* maturation of the oocyte [2]. Existing reports OR chemical composition of follicular fluid, particularly in goat are limited. Within the ovarian follicle, developing oocyte is surrounded by the follicular fluid (FF). Besides meeting nutritional requirement of the growing oocyte, FF also maintains proper environment for growth and maturation of the oocyte. Follicular fluid is an avascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a 'blood-follicle barrier' [3]. Besides a transudate of serum, FF is partially composed of locally produced substances, which are related to the metabolic activity of follicular cells [4]. This metabolic activity, together with the 'barrier' properties of the follicular wall, is changing significantly during the growth phase of the follicle [3, 5, 6]. Therefore, a different biochemical composition of the FF in different sized follicles can be expected. Changes in biochemical constituents of blood are important indicators of physiological state of an animal [7]. Hence, the present study was undertaken to biochemically characterize caprine follicular fluid and oocytes after maturation.

Materials And Methods: Ovaries of goats (n = 424) were collected from local slaughter house, Blood was collected before slaughter of animal. Samples were transported to laboratory in ice pack. After measuring the diameter of surface follicles, they were then classified as small (< 2 mm diameter), medium (2-5 mm diameter) and large (>5 mm diameter). Follicular fluid was aspirated using disposable syringe, centrifuged at 2000 r.p.m. for 15 minutes and the supernatant taken out using a Pasteur pipette, stored at - 20° C until further analysis [8]. Biochemical constituents of follicular fluid and serum were analysed by span diagnostics kit. For in vitro maturation of Goat oocytes, ovaries of unknown reproductive state were collected from local slaughter house and placed in normal saline containing gentamicin (50µg/ ml), transported to laboratory within 2 hrs of slaughter [9]. Protein, calcium and phosphorus of in vitro matured oocytes with different growth factors were analysed by span diagnostics kit. SDS PAGE of serum and pooled follicular fluid from same animal and in vitro matured oocytes were run with 4% stacking and 12.5% with separating gel. Data were analysed by Software gel doc Gene-tools syngene U.S.A. Viable oocytes of goats were cultured at 38.50C with 5% CO_2 in air in the presence of growth factors: (GDF-9) [0,10,20,30 ng/ml] or bFGF [0,10,20,30 ng/ml] in medium for 24 hours. The viability and maturation rates were examined. The matured oocytes were *in vitro* inseminated and the fertilization and cleavage rates were examined. The conditioned medium/used medium after one day was screened for the protein profile by gel electrophoresis. This experiment consists of two groups as follows: a) Culture of oocytes without Growth factors, b) Culture of oocytes with GDF-9 (20 ng/ml) and bFGF (20 ng/ml). The statistical software "Graph Pad Prism" San Diego, USA was used for analyzing the data. Data were analyzed by the linear regression model. Maturation rate with different growth factors were analysed by ANOVA followed by Tukey"s multiple comparison test (the percentage values were transformed to arcsine values before

analysis). Maturation rates were analysed by unpaired 't' test (the percentage values were transformed to arcsine values before analysis).

Results: As caprine ovarian follicles became larger, creatinine, triglycerides, calcium, glucose, cholesterol, phosphorus and sodium significantly ($P < 0.05$) increased while those of total protein, urea, chloride, magnesium and potassium significantly ($P < 0.05$) decreased (Table 1). As the oocytes get matured, there was uptake of protein, calcium and phosphorus. The protein, calcium and phosphorus contents were significantly increased as the oocytes got matured in medium containing GDF-9, FGF or the combination of GDF and FGF (Table 2). The total number of protein bands for sera and follicular fluid were 19 and 18 respectively (Table 3).

Table 1: Concentration of different biochemical components of follicular fluid of different size categories of ovarian follicles of goat

Biochemical Constituents	Small Follicle	Medium Follicle	Large Follicle
Total Protein (mg/dl)	5.387±0.08^{ab}	5.107±0.06^{b}	4.817±0.05^{b}
Urea (Mm)	4.2900.02^{a}	4.200±0.00^{b}	4.080±0.00^{c}
Creatinine (mg/dl)	1.520±0.01^{a}	1.620±0.01^{b}	1.787±0.02^{c}
Triglycerides (mg/dl)	20.67±0.49^{a}	16.37±0.11^{b}	14.53±0.22^{c}
Calcium (mg/dl)	9.940±0.02^{a}	10.34±0.06^{b}	11.01±0.02^{c}
Glucose (mg/dl)	20.30±0.20^{a}	27.20±0.86^{b}	29.19±0.35^{bc}
Cholesterol (mg/dl)	54.84±0.89^{a}	67.45±1.00^{b}	97.70±0.63^{c}
Phosphorus (mg/dl)	11.34±0.06^{a}	11.78±0.11^{b}	11.95±0.01^{bc}
Sodium (mEq/l)	107.6±2.02^{a}	115.8±0.68^{b}	121.4±0.62^{c}
Chloride (mEq/l)	115.0±0.40^{a}	109.9±0.36^{b}	103.1±0.59^{c}
Magnesium (mEq/l)	2.590±0.15^{a}	2.267±0.02$^{a\,b}$	1.987±0.01$^{b\,c}$
Potassium (mEq/l)	14.42±0.13^{a}	10.78±0.23$^{a\,b}$	9.870±0.12^{c}

Superscripts bearing different letters in the same row differ significantly (P<0.05).

Table 2: Effect of incorporation of growth factors on biochemical components of caprine oocytes\

Biochem-ical Constitu-ents	GDF		FGF		GDF+FGF	
	Immature	Mature	Immature	Mature	Immature	Mature
Protein (mg/dl)	63.3±3.04^{a}	148.3±2.70^{b}	64.7±1.85^{a}	149.5±1.47^{b}	63.61 ± 0.65^{a}	144.00± 2.65^{b}
Calcium(mg/dl)	0.68±0.03^{a}	2.15±0.08^{b}	0.65±0.03^{a}	1.85±0.06^{b}	0.64 ± 0.04^{a}	1.68± 0.03^{b}
Phosphorus (mg/dl)	0.59±0.05^{a}	1.63±0.04^{b}	0.61±0.03^{a}	1.57±0.05^{b}	0.60± 0.01^{a}	1.51± 0.04^{b}

Superscripts bearing different letters in the same row differ significantly (P<0.05).

Table 3. Protein profile of caprine serum and follicular fluid

Serum protein molecular weight (kDa)	Follicular fluid protein molecular weight (kDa)
217.4±0.48	215.23±3.23
208.2±0.23	139.89±4.23
206.1±3.23	141.30±3.23

148.7±3.27	86.05±2.13
126.4±2.34	66.6±1.11
99.3±2.38	64.2±2.22
75.39±2.78	56.7±2.14
67.17±2.11	44.7±2.17
39.96±1.78	39.0±1.29
35.51±1.67	30.1±1.98
29.99±1.86	28.45±1.24
26.83±1.11	26.87±1.25
24.9±1.92	25.86±2.10
23.0±1.28	24.66±1.29
22.1±0.79	22.42±1.28
21.0±1.10	21.48±0.98
13.8±1.27	16.38±1.23
5.6±0.56	3.06±1.01
4.4±0.28	-

Discussion: As follicles become larger, the concentrations of glucose, cholesterol, creatinine, calcium, phosphorus and sodium increased in a linear manner, while those of total protein, urea, triglycerides, chloride, potassium, magnesium decreased. The glucose concentration in small follicles was significantly lower than that measured in medium and large follicles [10]. Leese et al. (1994) [11] has stated that glucose concentrations in FF in women is a result of both glycolysis taking place in mural granulosa cells and influx of same molecules from the plasma into the fluid. Leroy et al. (2004) [12] observed that the concentration of glucose in FF of small follicles was less than half of the level found in serum but only 21% lower in large follicles. Leroy et al. (2004) [12] also reported that the FF glucose concentration was closely correlated with the serum levels and that it was consistently higher than in serum, possibly due to an active inward transport. This finding strongly suggest that post partum changes in glycemia are well reflected in the FF of dominant follicles but that the oocyte is more or less protected from low glucose concentrations. Nandi et al. (2013) [13] reported that the cholesterol concentration increased with an increase in follicular size, which was in agreement with the findings of [14], [15] and [16] in goat and [17] in cattle. Cholesterol in follicularfluid derived from two sources, cellular de novo synthesis from acetate and uptake from plasma lipoprotein. Cholesterol in the follicular fluid was in the form of a constituent of high-density lipoprotein [17]. Cholesterol was the precursor for steroid synthesis and the follicular fluid contained only high-density lipoprotein, therefore, the avascular granulosa cells of the follicles totally depended on the cholesterol from high-density lipoprotein, which was derived from the blood plasma by crossing the basement membrane of granulosa cells [16]. As the production of steroids increased, the follicle's level of cholesterol also increased [5]. However, the result of the present study differed from that reported in pigs [18] and in buffalo [19]. The decreased cholesterol level in the large follicles in those studies might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis. Leroy et al. reported that the total cholesterol in FF was about 42% of the concentration found in blood serum and there was a significant increase of the total cholesterol content from small to large follicles. Cholesterol, present in FF, is bound to the high-density lipoprotein fraction (HDL) because the only other cholesterol-containing lipoprotein fraction, the low-density lipoprotein fraction (LDL), is too large to pass the blood–follicle barrier. The higher total cholesterol concentration in large follicles can be explained by the increased permeability of the follicular wall in that follicle class, permitting the entrance of the larger HDL fraction. The triglycerides concentration was higher in small follicles because might be the alternate sources of energy for the cells in follicles. Another reason for the high concentrations of triglycerides in small follicles was that triglycerides did not pass through the follicular membrane and follicular triglyceride levels were mainly a result of local metabolic processes. Triglyceride levels in small follicles were significantly higher than in serum and significantly lower in large follicles. The total protein content of the follicular fluid in the present study was comparable with earlier reports in goats [20] and cattle [12]. However, our

results differed from those of in sheep [21], cattle [17] and [5] and buffalo [19], who reported a decrease in the total protein concentration as the follicle size increased. It was reported that as the follicular fluid volume increased with an increase in follicle size, the protein concentration decreased [16]. Continuous protein equilibrium existed between plasma and follicular fluid and the protein concentration was reported to be similar in small and large follicles in cattle [22]. The difference in concentration of urea in various sizes of follicles was expected as lower urea concentration favors oocyte development [12], and oocytes retrieved from large follicles were found to have higher developmental competence [23]. The sodium, potassium, chloride, calcium and magnesium concentrations in overall follicular fluid in the present study were comparable with an earlier report in sheep [6]. Trends in the present ion profiles compared favorably with the values reported in cattle by [24], except for the potassium concentration. The linear increase in concentration of calcium and sodium in large follicles observed in the present study was in accordance with those in cattle [5], buffalo [25] and goat [26]. Increased calcium concentration with follicular development had a role in steroidogenic capability of growing follicle and calcium played an important role in gonadotrophin regulation of ovarian steroidogenesis and ovulation. Increased follicular fluid sodium concentrations were related to follicle viability and were linked to the active follicular synthesis of estrogen. A compound with potent sodium retention action, 19-hydroxyandrostenedione, was found in large concentrations in the large follicles [5]. Enlargement of the follicle dimension with follicular growth was largely due to the movement of water from blood to antrum, a process that requires an osmotic gradient across the follicular wall. Thus, increased sodium concentration in large follicles could create an osmotic gradient across the follicular wall to facilitate osmosis [27]. The phosphorus level increased in a linear manner with an increase in the size of the follicle in the present study, which was in contrast to an earlier report in goat [16]. The higher concentrations of potassium and magnesium in small follicles compared to those in large ones in this study were in accordance with the earlier reports in cattle [5], buffalo [25] and goat [26]. The decreased concentration of follicular fluid potassium with follicular development could be due to the increased use of glucose by developing follicles, a process that leads to transfer of potassium ions from extracellular sites to intracellular sites [28]. Magnesium could substitute for calcium in thrombin formation under low Ca/Mg ratio conditions that exist in small follicles. As magnesium was antagonistic to calcium, the decreased magnesium with follicular development facilitated the calcium action in large follicles [29]. Information on biochemical changes during oocyte maturation was limited. The ion content in oocytes after in vitro maturation was limited to a study wherein it was found that phosphorus, calcium and protein uptakes were increased in the oocytes after culture [23]. It was reported that maturation of sheep oocytes was totally dependent on the synthesis of new proteins. There were also changes in the relative rate of synthesis of specific proteins during oocyte maturation that was independent of follicle size from which the oocytes were collected. In conclusion, as follicles became larger, creatinine, triglycerides, calcium, glucose, cholesterol, phosphorus and sodium significantly increased while those of total protein, urea, chloride, magnesium and potassium significantly decreased. As the oocytes get matured, there was uptake of protein, calcium and phosphorus. The total number of protein bands for caprine sera and follicular fluid were 19 and 18 respectively.

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