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Seasonal Changes in Concentrations of Proteins and Lipids in Growing Goat Oocytes

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ABSTRACT : Proteins and lipids not only provide a source of energy to the cell, but also play vital roles in modifying the physical properties and function of the biological membranes. In the present study, we investigated the biochemical constituents, viz. proteins and lipids, in growing oocytes of goat antral follicles during summer and winter seasons. Goat genitalia in phosphate buffered saline (pH 7.4) were brought to the laboratory within one hour of slaughter under aseptic conditions at 37°C. Oocytes were aspirated from normal small (<3 mm in diameter) and large (>3 mm) follicles and pooled for biochemical estimations. A significant increase in the amount of protein and lipid was observed with the growth of the oocyte. The amount of protein varied non-significantly with the season, while the amount of lipid varied significantly. The amounts of phospholipid, cholesterol, free fatty acid, and triglyceride increased with the growth of the oocyte, but no significant effect of season in these constituents was observed. Lysolecithin, sphingomyelin, and sterols were the polar lipids identified in both oocytes prepared from small follicles (small oocytes) as well as large follicles (large oocytes). In addition, the small oocytes also contained phosphatidyl serine, while large oocytes contained phosphatidyl glycerol phosphate and phosphatidyl inositol. Among non-polar lipids, triglycerides and long chain alcohols appear only in small oocytes and not in large oocytes. Monoglycerides, 1,2-diglycerides, 1,3-diglycerides and o-dialkyl glycerol ethers, fatty acids, fatty acid methyl esters, and wax esters were identified in both small and large oocytes. Information on biochemical composition of growing oocytes is relevant to oocyte and embryo competence, culture and cryopreservation. (**Key Words :** Goat, Lipid, Oocyte, Protein)

INTRODUCTION

As the oocyte grows, its size and volume increase by accumulating RNAs, proteins, lipids and organelles (Guraya, 2000). The extensive reserves of informational molecules, organelles and lipid droplets support the synthetic, nutritional and regulatory requirements of early embryos (Guraya, 2000). The proteins are important for the success of post maturation development, in addition to having a role in the final phases of maturation itself. The changes in protein synthesis patterns occurring during oocyte maturation are correlated with the acquisition of competency to complete maturation *in vitro* and also for normal fertilization and embryogenesis (Sharma and Chowdhary, 1998; Guraya, 2000; Sharma et al., 2000; Malakar and Majumdar, 2002). Lipids not only provide a source of nutrient to the cell but also play a vital role in modifying the physical properties and functions of biological membranes and have potential effects on cell-cell interactions, cell proliferation and transport (Stubbs and Smith, 1984; McEvoy et al., 2000).

Biochemical analysis has demonstrated the qualitative and quantitative aspects of synthesis and accumulation of stage specific proteins during oogenesis in pig, sheep, cattle and mouse (Guraya, 2000). Detailed studies on lipids in the oocytes or embryos in farm animals have been done for cattle, pig and sheep (Homa et al., 1986; McEvoy et al., 2000). Sheep oocytes were found to contain abundant stores of lipids for membrane synthesis (phospholipids: PL), energy provision (triglycerides: TG) and other roles in post fertilization (Coull et al., 1998). Fatty acid composition relates to the quality of oocytes and chilling resistance in sheep (Zeron et al., 2002). Arav et al. (2000) suggested a close relationship among cold susceptibility, lipid phase transition and lipid profiles in animal gametes.

Despite the significant roles of the proteins and lipid reserve in cell structure and function very few studies have provided detailed description of its nature and composition in mammal oocytes. Biochemical studies depicting protein and lipid changes in growing oocytes of goat are yet to be made. Information concerning lipid and protein reserves in

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Table 1. Amounts of proteins and lipids in small and large oocytes in different seasons

Biochemical components	Small oocyte		Large oocyte	
Mean+±SE (μg/oocyte)	Winter	Summer	Winter	Summer
Proteins	0.367+0.080	0.235+0.056	0.747+0.130*	0.568+0.089*
Lipids	0.058±0.005	0.027+0.005*	0.110+0.001*	0.055+0.005*
Phospholipids	0.015±0.003	0.008+0.001	0.026+0.045*	0.013+0.004*
	(26.86)	(29.63)	(23.63)	(23.64)
Cholesterol	0.006+0.001	0.004+0.001	0.013+0.004*	0.008+0.004*
	(9.63)	(13.33)	(11.82)	(13.64)
Free fatty acids	0.019+0.004	0.011+0.004	0.03+0.001*	0.020+0.003*
	(32.76)	(40.74)	(27.27)	(36.36)
Triglycerides	0.017+0.010	0.005+0.003	0.04+0.016	0.013+0.006*
	(29.31)	(18.52)	(36.36)	(23.64)

* Values are significantly different from the corresponding large oocyte value ($p<0.01$).
Figures in parenthesis indicate the percent composition of various classes of lipids in small and large oocytes in different seasons.

growing oocytes may prove crucial for developmental programming and culture *in vitro* and for improvement in cryopreservation programmes.

MATERIALS AND METHODS

Ovaries from mature and nonpregnant goats (mainly Beetal) were collected during winter (Dec-Feb) and summer (May-June) from the local abattoir and transported to the laboratory in phosphate buffered saline (PBS, pH 7.4), supplemented with 50 μg/ml gentamycin at 37°C within 1-2 h of slaughtering and blot dried.

On arrival at the laboratory, the ovaries were rinsed in PBS at 37°C. The visible follicles on the surface of the ovaries were measured using a vernier caliper. Based on their sizes, follicles were divided into two groups: small (<3 mm in diameter) and large follicles (>3 mm). Cumulus oocyte complexes (COCs) were retrieved by aspiration of small and large follicles separately.

COCs with good cumulus investment were collected, pooled and transferred to approximately 1.0 ml PBS and were then subjected to pipetting to remove the adherent cumulus cells. After pipetting, the fluid was placed in a clean pre-warmed glass petridish and small and large oocytes with intact zona pellucida that had been fully divested of all the adherent cells and had uniform cytoplasm were re-selected during winter and summer for biochemical estimations. The diameters of oocyte (including the zona pellucida) were also determined during summer and winter season.

A known number of oocytes were taken in 1 ml of PBS and vortexed at high speed to break zona pellucida and suspend the cytoplasmic contents in PBS. Total soluble proteins present in oocytes were estimated by the method of Lowry et al. (1951). A known number of oocytes were homogenized in 20 ml chloroform: methanol (2:1 v/v) and total lipids were extracted by Folch et al. (1959) method. The total lipids were dissolved in 1.5 ml of chloroform and used for quantitative analysis of phospholipids (PL) (Ames, 1966), total cholesterols (free and esterified) (Chiamori and Henry, 1959) and free fatty acids (FFA) (Lowry and Tinsley, 1976). The total triglycerides (TG) content was measured by subtracting the sum of the amounts of phospholipids, cholesterols and free fatty acids from the amount of total lipids. Lipid extracts were also subjected to thin layer chromatography (TLC) on silica gel-G using a solvent system of petroleum ether: ethyl ether: acetic acid (80:20:1 v/v/v) for non-polar lipids and chloroform: methanol: 7 N ammonia (65:25:4 v/v/v) for polar lipid separation (Randhawa , 1986).

All results were subjected to statistical analysis by two way ANOVA (Steel et al., 1986) and level of significance was checked at 1%. Results were expressed as mean±SE μg/oocyte.

RESULTS AND DISCUSSION

In the present study, the mean diameter of small follicle's oocytes with zona pellucida during winter was 138.95±4.76 μm (range: 112.5-160.0 μm), while it decreased during summer to 111.51±2.70 μm (range: 103.96-126.51 μm). In large follicle's oocytes also the mean diameter decreased during summer from 157.17±5.69 μm (range: 145.0±186.0 μm) in winters to 122.01±2.70 μm (range: 132.20-168.50 μm) in summers. Wolfenson et al. (2000) have also reported that summer heat stress reduces the number of follicles and impairs oocyte quality in cattle. The amount of proteins in small oocytes during winter was 0.367±0.080 μg/oocyte. It increased significantly ($p<0.01$) in large oocytes and was found to be 0.747±0.130 μg/oocyte. In summer, the amount of proteins decreased as compared to winter (Table 1). The increase in the amount of proteins with the growth of the oocyte may be due to the fact that during oocyte growth and maturation, proteins are synthesized and stored that help in fertilization and embryo development (Schultz and Wassarman, 1977; Canipari et al., 1979; Gall et al., 1993; Guraya, 2000; Sharma et al., 2000;

Table 2. Identification of polar lipids by thin layer chromatography

Category	Rf value	Identification
Small oocytes	0.08	Lysolecithin
	0.11	Phosphatidyl inositol
	0.22	Sphingomyelin
	0.50	Phosphatidyl serine
	0.83	Sterols
Large oocytes	0.08	Lysolecithin
	0.11	Phosphatidyl inositol
	0.22	Sphingomyelin
	0.40	Phosphatidyl ethanolamine
	0.67	Phosphatidyl glycerol phosphate
	0.86	Sterols

Table 3. Identification of non-polar lipids by thin layer chromatography

Category	Rf value	Identification
Small oocytes	0.03	Monoglycerides
	0.15	1,2-Diglycerides
	0.21	1,3-Diglycerides
	0.30	o-Diakyl glycerol ethers
	0.32	Long chain alcohols
	0.39	Fatty acids
	0.60	Triglycerides
	0.77	Fatty acid methyl esters
	0.88	Wax esters
Large oocytes	0.03	Monoglycerides
	0.15	1,2-Diglycerides
	0.21	1,3-Diglycerides
	0.30	o -Diakyl glycerol ethers
	0.39	Fatty acids
	0.77	Fatty acid methyl esters
	0.88	Wax esters

Malakar and Majumdar, 2002). In our study, the increase in the amount of proteins with the increase in the size of the oocyte was two-fold, which is in accordance to the studies of Schultz and Wassarman (1977) who have demonstrated that with each doubling of the volume of the oocyte, there was doubling in the amount of proteins in mice. The decrease in protein content during summer may be attributed to decrease in the diameters of small and large oocytes during summer as compared to winter (Zeron et al., 2001) as the protein content is a measure of the surface area of oocytes (Grealy et al., 1996).

The amount of total lipids increased with the growth of oocyte significantly in winters as well as summer ($p<0.01$) (Table 1). In small and large oocytes lipids decreased significantly ($p<0.01$) in summer season as compared to winter season. Lipids may act as a reservoir of latent energy (McEvoy et al., 2000). Our results on the total lipid content are consistent to the findings of Coull et al. (1998) on sheep oocytes that have determined the amount of total lipids to be 0.096 μg/oocyte. The increase in the amount of total lipids with the growth of the oocyte may be due to increased transportation via synthesis or through *de novo* synthesis (Sharma et al., 2000). Fair et al. (1995) have also demonstrated that in bovine oocytes lipid content increases with increasing size.

The amount of phospholipids and cholesterols increases significantly from small to large oocytes with the growth of the goat oocytes. Their amount decreased during summer ($p<0.01$) as compared to winter season (Table 1). Increase in the phospholipid content with the growth of the oocytes is attributable to increased intracellular membranes as phospholipids and cholesterol are prerequisites for the formation of membranes that are crucial requirement during growth phase (Coull et al., 1998; McEvoy et al., 2000). The marked increase in cholesterol level with the growth of the oocyte may also be due to the increased transportation of cholesterol from follicular cells (Buccione et al., 1990). Cholesterol constituted about 9 to 13% of lipid proportion in goat oocytes (Table 1). In sheep, Coull et al. (1998) have detected that cholesterol esters constitute 16-17% of total oocyte lipid. The decrease in phospholipids and cholesterol during summer may be due to degenerative changes in various organelles including smooth endoplasmic reticulum and mitochondria and may also be due to non-exihibition of estrous by goats due to heat stress and consequently, lesser transportation (Wilson et al., 1998, 2000; Guraya, 2000).

During winters, the amount of free fatty acids (FFA) in small oocytes was 0.019±0.004 μg/oocyte and in large oocytes was 0.03±0.001 μg/oocyte ($p<0.01$). During summer, the FFA decreased significantly ($p<0.01$) in both small and large oocytes (Table 1). Our findings on FFA are in accordance to the reports of Mc Evoy et al. (2000) who detected FFA amounting to 0.167±0.018 μg/oocyte (pig), 0.063±0.006 μg/oocyte (cattle) and 0.089±0.007 μg/oocyte (sheep). The decrease in FFA proportion in large oocytes (Table 1) may be attributed to increased transportation of FFA from the oocyte to compensate the increased utilization of acyl CoA in the formation of cholesterol and in steroid hormone production as compared to small follicles (Yao et al., 1980; Sangha and Guraya, 1989). The increase in FFA proportion in large and small oocytes during summer as compared to winter reflects the non-utilization of FFA for cholesterol synthesis during summer (Zeron et al., 2001). Alteration of fatty acid composition in bovine oocytes might improve maturation and cryopreservation (Kim et al., 2001).

The amount of triglycerides increased with the growth of the oocyte from small oocytes to large oocytes in winters. Triglycerides decreased during summer ($p<0.01$) both in small and large oocytes (Table 1). The increase in triglycerides content with the growth of the oocytes may be related to the increased synthesis and accumulation of lipids in the growing follicles (Guraya, 2000). Triglycerides are also the energy stores in the mammalian oocytes (Coull et al., 1998; Guraya, 2000) and also impede conventional cryopreservation protocols for different animal species as

this form of lipid influences sensitivity to chilling (Mc Evoy et al., 2000).

By TLC, the polar lipids identified in small oocytes were lysolecithin, phosphatidyl inositol, sphingomyelin, phosphatidyl serine and sterols and those identified in large oocytes were lysolecithin, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl glycerol phosphate and sterols (Table 2). Among non-polar lipids, monoglycerides, 1,2-diglycerides, o-dialkylalcohol, ethers, fatty acids, fatty acid methyl esters and wax esters were identified in both small as well as large oocytes (Table 3). Triglycerides and long chain alcohols appear only in small oocytes but not in large oocytes. Yao et al. (1980) have also purified all methylated samples of porcine ovarian follicular fluid and serum. Presence of lysolecithin revealed qualitatively, accounts for increased production of cholesterylesters (McEvoy et al., 2000). Phosphatidyl serine that accounts for 10% of PL in pig oocytes influences second messenger functions and plays a vital role in purine synthesis (Homa et al., 1986). Hydrolysis of phosphatidy-4,5-bishosphate (PIP2) yields two second messengers, inositol-1,4,5-triphosphate (IP3), which stimulates kinase C (Cran and Moor, 1990; Nishizuka, 1992; Mc Evoy, 2000), and diacylglycerol. Phosphatidyl inositol influences calcium centered cascade of events at fertilization indirectly (Cran and Moor, 1990).

In conclusion, the present study quantified the total soluble proteins, total lipids and lipid fractions in goat oocytes with intact zona pellucida during summer and winter and indicated a decrease in the amount of biochemical constituents during summer due to non–exhibition of estrous during acute heat stress. Information on these components are relevant to oocyte and embryo competence, culture and cryopreservation and also play a vital role in development during and after fertilization. Detailed qualitative analysis of proteins and lipids, fatty acids, in particular, are still to be made.

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