

Biochemical Parameters of Follicular Fluid in Cyclic and Acyclic Sheep

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The study was conducted to estimate biochemical profiles in follicular fluid in cyclic and acyclic sheep. Reproductive tracts of sheep were collected from a local Municipal abattoir located in Jammu city. The genitalia were classified into two groups, cyclic and acyclic, each group having 10 genitalia. A non-significant difference was recorded in the means of total protein, cholesterol, albumin and globulin in follicular fluid of cyclic and acyclic sheep. However, the glucose level was significantly higher ($P < 0.05$) in the follicular fluid of cyclic sheep than that of acyclic sheep.

KEY WORDS

Follicular fluid, biochemical parameters, cyclic and acyclic sheep.

INTRODUCTION

Follicular fluid (FF) is a mixed secretion of follicular cells and transudate of plasma. The constituents of follicular fluid are considered as a regulating factor in follicular development and steroidogenesis (Gosden *et al.*, 1988; Thakur *et al.*, 2003). The composition of follicular fluid varies greatly depending on the stage of follicular development and exerts variable effects on oocyte development. This necessitates the characterization of follicular fluid at different stages of follicular development so that fluid from follicle at appropriate stage of development can be used to establish optimal environment for maturation of viable oocyte, which could improve the efficiency of in-vitro fertilization (Kalmath and Ravindra, 2007).

Blood glucose appears to be one of the key

nutrients affecting ovarian activity in farm animals. Concentration of glucose in blood of animals may influence the rate of steroidogenesis and gonadotropin synthesis and secretions (Lynn *et al.* 1965).

Proteins are found in all living cells where they are intimately associated with various phases of activity that constitute the life of a cell. Fergusson and Chalapa (1989) postulated that imbalances in proteins and energy supply may affect metabolism and energy status of an animal. Similarly, the deficiency of nitrogen by products or energy utilization may alter the functions of hypothalamus pituitary-ovarian axis. Cholesterol is the precursor for steroid synthesis and follicular fluid contains only high density lipoproteins. Thus, increased levels of follicular fluid cholesterol results in increased steroid production (Wise, 1987).

MATERIALS AND METHODS

The present study was conducted at Division of Animal Reproduction, Gynaecology and Obstetrics. Faculty of Veterinary sciences and Animal Husbandry R.S. Pura, Jammu during the period between March to October, 2011.

Reproductive tracts of sheep were collected from a local Municipal abattoir located in Jammu city. The genitalia were classified into two groups, cyclic and acyclic, each group having 10 genitalia.

Group I: Genitalia having ovaries with a corpus hemorrhagicum (CH), a large CL and >5mm follicle(s) in diameter or a regressing CL with follicle(s) >6mm in diameter were classified as active and the animals as cycling.

Group II: Genitalia having ovaries without a CL or CH or the presence of a regressed CL without <5mm in diameter follicle(s), such ovaries were

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classified as inactive and the animals as non-cycling (Azawi *et al.*, 2008).

Collection of follicular fluid and reproductive tissues

Reproductive tracts were collected daily from March 2011 to October 2011 from municipal slaughter house. No information regarding identity and history was included in this study. Immediately after slaughter, complete female genitalia of sheep with no apparent clinical abnormalities was collected and transported to the laboratory within 30 minutes in Phosphate Buffered saline in an ice box. Follicular fluid was aspirated by using a separate hypodermic tuberculin syringe for each ovary and was pooled irrespective of their size. It was collected by applying slight pressure to avoid additional traumatisation of follicles. Aspirated fluid was centrifuged at 3000rpm for 15minutes to remove cellular debris. The sample was kept at -20°C till further use.

Estimation of Follicular Fluid Biochemical Metabolites

Follicular fluid samples were assayed for glucose, total protein, albumin and cholesterol with the help of a double beam UV/VIS spectrophotometer (Labtronics, Laboratory Instruments) using commercial diagnostic kits (ERBA diagnostics Mannheim GmbH, Transasia Bio-Medicals Ltd) adopting standard procedures.

Concentration of biochemical constituents =

$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Estimation of glucose

Glucose was estimated by using Trinder's method as described by Trinder (1969). Using standards kit procured from Erba diagnostics Mannheim GmbH. Development of pink color determined the end point and was measured spectrophotometrically between 500-540nm. The standard concentration of glucose was 100mg/dl.

Estimation of total protein

Total protein was estimated by using Biuret method as described by Tietz (1986). Using standard kit from Erba diagnostics Mannheim GmbH. Development of blue-violet color

indicated the end point and was measured spectrophotometrically at 546nm. The standard concentration of total protein was 6.0g/dl.

Estimation of albumin

Albumin was estimated by using Bromocresol green (BCG) method as described by Doumas *et al.*, (1972). Using standard kit from Erba diagnostics Mannheim GmbH. Development of blue-green color indicated the end point and was measured spectrophotometrically at 625nm. The standard concentration of albumin was 3.6g/dl.

Estimation of cholesterol

Cholesterol was estimated by using Modified Roeschlau's method as described by Roeschlau *et al.*, (1974). Using standard kit from Erba diagnostics Mannheim GmbH. Cholesterol was measured spectrophotometrically at 505nm. The standard concentration of cholesterol was 200mg/dl.

Estimation of globulin

Globulin was calculated by subtracting albumin from total protein.

RESULTS

The average glucose concentration (mg/dl) of follicular fluid was significantly higher ($P < 0.05$) in cyclic sheep (70.60 ± 10.85) as compared to acyclic sheep (36.41 ± 4.76). While as the average cholesterol concentration (mg/dl) in follicular fluid was higher (56.25 ± 8.99) in cyclic sheep as compared to (47.87 ± 5.42) in acyclic sheep. However, the difference was non-significant ($P > 0.05$). Similarly the average total protein concentration (gm/dl) in follicular fluid was non-significantly higher ($P < 0.05$) in cyclic sheep (6.79 ± 0.80) as compared to acyclic sheep (4.89 ± 1.02) and the average albumin concentration (gm/dl) in follicular fluid was also non-significantly ($P > 0.05$) higher in cyclic sheep (2.41 ± 0.24) as compared to in acyclic sheep (2.33 ± 0.35). The average globulin concentration (gm/dl) in follicular fluid was higher (4.38 ± 0.79) in cyclic sheep as compared to (2.55 ± 0.83) in acyclic sheep. However, the difference was non-significant ($P > 0.05$).

DISCUSSIONS

Glucose (mg/dl)

Glucose concentration in follicular fluid in cyclic sheep was significantly higher ($P < 0.05$)

than that of acyclic sheep. The finding was in accordance with the reports of Landau *et al.*, (2000); Leroy *et al.*, (2004); Nandi *et al.*, (2008) and Razzaque *et al.*, (2012) who observed that as the follicle size increases glucose levels in the fluid also increase in cows and buffalos. This suggests that glucose metabolism is less intense in large follicles of cyclic sheep as compared to small follicles of acyclic sheep. An increase in the volume of follicular fluid and increased permeability of the blood follicle barrier during follicular growth (Bagavandoss *et al.*, 1983) could be attributed to higher glucose levels in large size follicles (Gosden *et al.*, 1988). Hypoglycaemia leads to depression in hypothalamic functions causing loss of ovarian activity which is due to failure of release of gonadotropin hormone.

Total protein (g/dl)

Total protein concentration in follicular fluid in cyclic sheep (6.79 ± 0.80) was non-significantly higher ($P > 0.05$) than in acyclic sheep (4.89 ± 1.02) indicating that the total protein concentration may not have any specific bearing on the process of follicular development and is not affected by the reproductive state of the animal. The results are consistent with the previous reports of Arshad *et al.*, (2005) and Abd Allah *et al.*, (2010) wherein a non-significant change in the concentration of follicular fluid total protein either with the stage of the oestrus cycle or during development of the follicle was observed.

Cholesterol (mg/dl)

Cholesterol levels in follicular fluid was found to be non-significantly ($P > 0.05$) higher in cyclic sheep (56.25 ± 8.99) than in acyclic sheep (47.87 ± 5.42). Increased levels of cholesterol with increase in follicle size were reported in goat Bordoloi *et al.*, (2000); Thakur *et al.*, (2003) and Mishra, (2003) and Cattler Brantmeier *et al.*, (1987). Cholesterol is the precursor for steroid synthesis and follicular fluid contains only high density lipoproteins. Thus increase in steroid production leads to increased levels of follicular cholesterol (Wise, 1987).

Albumin (g/dl)

Albumin concentration in follicular fluid of cyclic sheep (2.41 ± 0.24) was observed to be

non-significantly higher ($P > 0.05$) than in acyclic sheep (2.33 ± 0.35). Andersen *et al.*, (1976) indicated that albumin content of follicular fluid was inversely related to follicular size. Also the levels of albumin in follicular fluid tend to be higher in atretic follicles, particularly towards the later portion of oestrus cycle. This may indicate that the likely estrogen and water uptake relationship in growing follicles may dilute follicular albumin concentrations (Schuetz and Anisowicz, 1974). In contrast a significant increase in albumin in follicular fluid was studied by Abd Allah *et al.*, (2010) in buffaloes. Also albumin may be required for binding some chemicals as well as minerals inside the follicular fluid for various physiological functions including growth and maturation of follicles (Arshad *et al.*, 2005).

Globulin (g/dl)

Globulin has a significant importance in the body due to its immunity producing activity. Globulin concentration in follicular fluid was recorded to be 4.38 ± 0.79 in cyclic sheep which was non-significantly higher ($P > 0.05$) than that of acyclic sheep (2.55 ± 0.83). The globulin in follicular fluid might be necessary for protecting the follicle from external environments. Andersen *et al.*, (1976) reported that the albumin content of follicular fluid was inversely related to follicular size.

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TABLES

Table 1: Biochemical profiles in follicular fluid of cyclic and acyclic sheep (Mean \pm S.E)

Variable	Cyclic (n=10)	Acyclic(n=10)	t- value
Glucose (mg/dl)	70.60 \pm 10.85	36.41 \pm 4.76	2.98*
Cholesterol (mg/dl)	56.25 \pm 8.99	47.87 \pm 5.42	0.78 ^{NS}
Total protein (gm/dl)	6.79 \pm 0.80	4.89 \pm 1.02	1.57 ^{NS}
Albumin (gm/dl)	2.41 \pm 0.24	2.33 \pm 0.35	0.21 ^{NS}
Globulin (gm/dl)	4.38 \pm 0.79	2.55 \pm 0.83	1.68 ^{NS}

FIGURES

Fig. 1: Average concentration of glucose and cholesterol in follicular fluid (mg/dl)

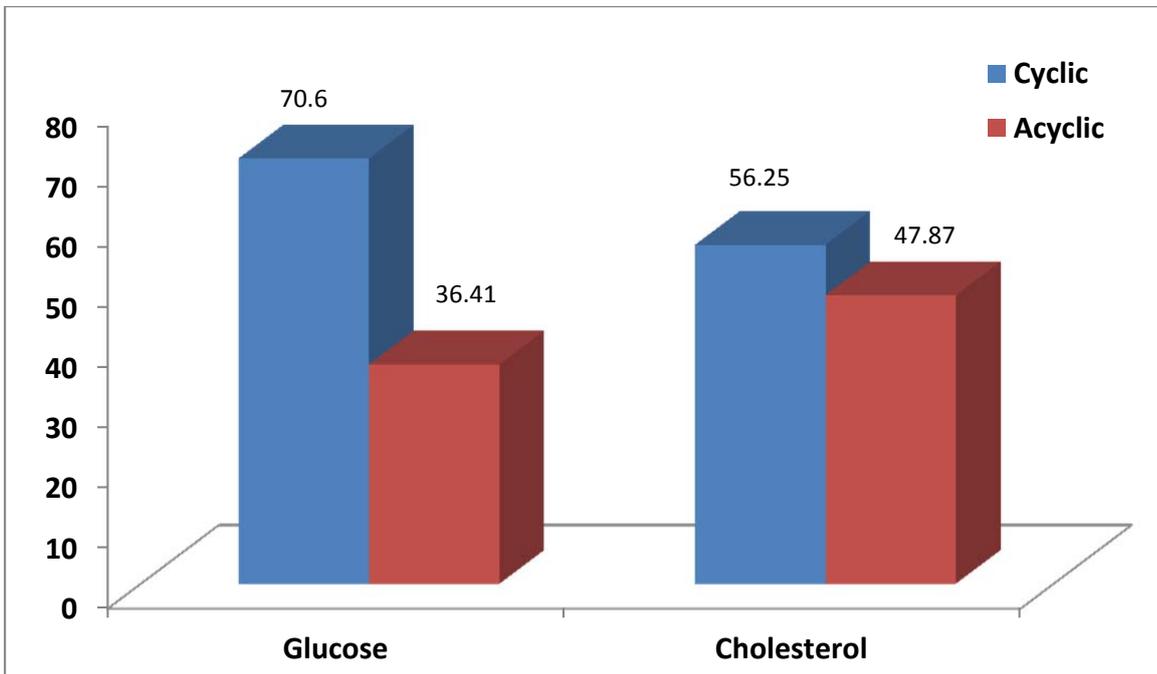


Fig. 2: Average concentration of total protein, albumin and globulin in follicular fluid (gm/dl)

