BIO-CHEMICAL MILIEU OF BUFFALOES OVARIAN FOLLICLES DURING THEIR DEVELOPMENT*

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ABSTRACT

Biochemical constituents of ovarian follicular fluid were studied in relation to size of follicle in buffaloes. In the present study, ovaries were collected immediately after slaughter and the follicular fluid was aspirated and stored at -20°C. Sampling was done and were analysed for various biochemical constituents. Small follicle had significantly lower (P<0.05) concentrations of glucose, cholesterol, oestradiol, progesterone and sodium, while as the concentration of alkaline and acid phosphatase and potassium was significantly (P<0.05) higher.

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Key words: Buffalo, Ovarian follicular fluid, Biochemical milieu

INTRODUCTION

The ultimate goal of *in-vitro* embryo culture is to perfectly mimic the conditions of oocyte maturation, fertilization and embryo development. Follicular fluid composition reflects changes in the secretory processes of the granulosa cells and theca interna and alterations in the components of plasma due to physiological or pathological processes. It has been suggested that the decline in fertility in high yielding dairy cattle is mainly a problem of inferior oocyte and embryo quality, rather than being the result of a disruption in gonadotropin secretion (O'Callaghan and Boland, 1999). It is not unlikely that metabolites which are present in the follicular fluid can influence oocyte quality. Several *in-vitro* studies showed that metabolites, such as glucose, proteins, phosphatases, sodium and potassium might influence the competence of bovine oocytes to mature and after fertilization, to develop to the blastocyst stage (Edwards, 1974). Therefore, a variation in biochemical composition of the follicular fluid in different sized follicles could be expected. Before focusing on the possible effects of metabolic changes on follicle and oocyte quality, it is necessary to determine the physiological concentrations of the most common metabolites in follicular fluid of different sized follicles.

MATERIALS AND METHODS

Ovaries (n = 250) were collected from a local slaughterhouse in normal saline containing antibiotics (Penicillin 400IU and streptomycin 400µg/ml) maintained at 4°C. Extra tissues of ovaries were removed and then ovaries were washed two to three times with normal saline at 4°C. After proper washing the ovarian surface follicles were counted and measured by using vernier calliper and were grouped into three categories viz. small follicle (1-4 mm), medium sized follicle (6-8mm) and large sized follicle (more than 10mm)

Follicular fluid from each category was aspirated using a sterile disposable syringe fitted with a 20 G needle. The follicular fluid from different sized follicles was aspirated and pooled separately. The pooled samples were centrifuged at 600g for 30 minute at 4°C and the supernatant was stored at -20°C without addition of any preservative for biochemical components,

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hormones, enzymes and macro elements using commercial kits and flame photometer. The estimation of glucose was done as per Kaplan, (1984), cholesterol (Warnick *et al.,* 1985) and protein, albumin and globulin (Gendler, 1984). Sodium and potassium estimation was done by flame photometry (Hawk *et al.,* 1954). Both progesterone and oestradiol estimation were done by ELISA based kits as per Erickson (1995). Acid phosphatase estimation was done as per Hillman (1971) and Alkaline phosphatase (Moss and Henderson, 1994). The data generated were statistically analysed including one way ANOVA (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The appearance of follicular fluid aspirated from different sized antral follicles ranged from clear to pale yellow with some turbidity.

Glucose mean concentration was significantly lower in small than large sized follicle. The concentrations in the small, medium and large follicle were 31.81±1.60, 35.56±3.48 and 38.04±3.22 mg/dl, respectively. The present findings are consistent with the results of Landau *et al.* (2000) and Leroy *et al.* (2004) in dairy cattle.

The possible reasons for the present finding might be that the glucose metabolism (per unit follicular fluid volume) was less intensive in large follicles compared with small ones, resulting in a lower consumption of glucose from follicular fluid and in a reduced secretion of lactate into the follicular fluid (Leroy *et al.*, 2004). In a large follicle, a relatively smaller number of granulosa cells consumed glucose from follicular fluid and secreted lactate into a relatively lesser amount in the follicular fluid (Gosden *et al.*, 1988), thus causing an increase in glucose and a decrease in lactate concentrations in large follicles.

In the present study the follicular total protein concentration in relation to size of follicle varied insignificantly from small to large follicle. The concentrations in small, medium and large follicle were 6.37±0.15, 5.50±0.36 and 6.27±0.26 g/dl, respectively. Our results were in consistency with the reports of Abd Ellah *et al.* (2010) in buffalo; Leroy *et al.* (2004) in cattle. The possible reason might be that there is a continuous equilibrium between follicular fluid and plasma for proteins. Hence, the protein concentration was reported to be similar in various sized follicle (Andersen *et al.,* 1976).

Cholesterol concentration in relation to size of follicle increased significantly ($P<0.05$) as follicle diameter increased. Cholesterol in follicular fluid of small follicle was 35.86±3.83 mg/dl, increased in the follicular fluid of medium follicle (41.97±2.90 mg/dl), and reached the highest value in follicular fluid obtained from large follicle (51.13±2.72 mg/dl).The finding is in accordance with findings in cattle (Brantmeier *et al.*, 1987; Leroy *et al.*, 2004).

The cholesterol in the follicular fluid is derived from two sources, cellular *de novo* synthesis from acetate and uptake from plasma lipoprotein. It is in the form of a constituent of high density lipoprotein (Brantmeier *et al.,* 1987). Cholesterol is the precursor for steroid biosynthesis and follicular fluid contains only high density lipoprotein, therefore, the avascular granulosa cells of the follicles totally depend on cholesterol from high density lipoprotein, which might be derived from blood plasma by crossing the basement membrane of granulosa cells. As the production of steroids increase the follicle level of cholesterol increase (Wise, 1987).

The albumin concentrations in relation to size of follicle varied insignificantly. The albumin concentrations in the small, medium and large follicle were 3.51±0.12, 2.94±0.15 and 3.40±0.22 g/dl, respectively. The finding was in synchrony with the finding of Abd Ellah *et al.* (2010).The mean concentration of globulin in relation to size of follicle varied insignificantly as size increases. The concentrations in small, medium and large follicle were 2.85±0.20, 2.56±0.20 and 2.87±0.31 g/dl, respectively. This indicated that follicular growth did not seem to have any effect on its globulin contents.

The mean concentration of sodium in the follicular fluid in different sized follicles increased significantly and was the lowest (105.29±1.60 mEq/L) in the small

follicle and the highest in the large follicle (118.59±3.22 mEq/L) and intermediate concentration in follicular fluid of medium follicle (113.44±3.48 mEq/L).The concentration of potassium in the follicular fluid of buffaloes decreased significantly from small to large follicle and was found significantly ($p<0.05$) higher in small follicle (7.34±0.51 mEq/L) than medium (6.28±0.22 mEq/L) and large follicle (5.40±0.14 mEq/L). These observations in the present study were in accordance with the finding of Abd Ellah *et al.* (2010).

The increase in follicular sodium concentration could be linked to the active follicular synthesis of oestrogen. Increased follicular fluid sodium in the large follicles could create an osmotic gradient across the follicular wall to facilitate osmosis (Sharma *et al.*, 2003). The decrease in concentration of potassium with the follicular development could be due to increased use of glucose by developing follicle, a process that leads to transfer of potassium ions from extracellular sites to intracellular sites (Sharma *et al.*, 2003).

The mean concentration of progesterone in relation to size of follicle varied significantly ($P<0.05$) such as lower concentration (8.80±0.58 ng/ml) in small follicle than medium follicle (10.45±1.01 ng/ml), reaching to its maximum value in the follicular fluid of large follicle (18.58±2.22 ng/ml). Our results were in agreement with the findings of Eissa (1996) and McNatty (1978), who have found the significant positive correlation between follicular progesterone and estrogen and demonstrated that the follicular progesterone serves as the precursor to androgens, and subsequently estrogen production by the follicle of buffalo cows. Our results were inconsistent with the findings of Henderson *et al.* (1982) and Wise (1987), who reported no relationship between follicular progesterone and oestrogen concentrations. The variations in the findings can be attributed to breed variations. The possible reason for the increase in the concentration of progesterone in the large follicular might be due to increased activity of granulosa and thecal cells (McNatty, 1978).

The mean oestradiol concentration in relation to size of follicle decreased significantly ($P<0.05$) in small (815.45±22.25 pg/ml) than medium (872.55±21.80 pg/ml) and large follicle (918.50±9.38 pg/ml). The increase in the follicular oestradiol in the large sized follicle can be justified by higher number of granulosa cells with the advancement of follicular development, as the granulosa cells are the principle site of androgen aromatization into oestradiol (Henderson *et al.*, 1982). Our results are consistent with results of Wise (1987) and Mekkawy *et al.* (1988) in buffaloes and Henderson *et al.* (1982) and Kruip and Dieleman (1985) in cattle, who found the oestrogen concentration to increase as follicle size increased.

With respect to different sizes, the acid phosphatase activity was significantly ($p<0.05$) higher in the small (19.15±0.96U/L) and medium follicle (16.89±0.66U/L) as compared to large follicle (13.02±0.61U/L).The results in the present study coincided with the findings of Wise (1987) in bovines. The possible reason for decreased acid phosphatase activity in the large follicle might be due to change in hormonal milieu along the development of the follicle.

Follicular fluid alkaline phosphatase activity changes between sizes of the follicle with significantly ($P<0.05$) higher activity in small follicle (371.48±6.64 U/L) than medium (310.79±20.41U/L) and large follicle (286.41±13.89U/L). The present finding is in accordance to the finding of Madan *et al.* (1998). The higher alkaline phosphatase activity in the initial stages of follicular development might be due to progesterone and androgen dominant environment that exists in the small follicle and thus a higher concentration of progesterone and androgen could be conducive to phosphatase activity (Kalmath, 2000). The decreased follicular fluid alkaline phosphatase activity with the development of the follicle in the present study could be due to increase in the oestrogen level in the follicle.

Finally, it might be concluded that the follicular glucose, cholesterol, oestradiol, progesterone, electrolytes, alkaline and acid phosphatases have significant effect on follicular development. Therefore, these parameters might be considered as a guiding milieu for selection of follicle for oocyte production or

modification of *in vitro* maturation media for improving *in vitro* embryo production.

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