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Minerals, enzymes and electrolytes profiles of follicular fluid of surti buffaloes in relation to follicles size

P. A. PARMAR[1], A. M. PANDE[2] AND R. G. SHAH[3]

Department of Veterinary Biochemistry and Biotechnology
College of Veterinary Science and Animal Husbandry,
Anand Agricultural University, Anand - 388 001 (Gujarat)

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ABSTRACT

A total of 210 ovaries were collected, of which 91 were having corpus luteum and 119 were without corpus luteum. The follicles of ovaries were classified in to three categories on the basis of its diameter and the presence or absence of corpus luteum. Follicular fluid samples were assayed using commercial kits for minerals and enzymes and electrolytes were estimated by Flame photometer. The number of all the three size of follicles of all the three categories was significantly different ($P < 0.05$) on the ovaries with and without corpus luteum. The vales of calcium, inorganic phosphorus and sodium in follicular fluid were non significantly increased from small to large follicles where as the values of magnesium, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and potassium in follicular fluid were significantly ($P < 0.05$) decreased in the ovaries with and without corpus luteum.

Key words: Buffalo, ovary, follicular fluid, minerals, enzymes, electrolytes.

The follicular fluid forms the minerals, enzymes and electrolytes environment of the oocyte before ovulation (Gosden *et al.,* 1988 and Józwik *et al.,* 2001) and is a vascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a 'blood-follicle barrier' (Okuda *et al.,* 1982 and Bagavandoss *et al.,* 1983). Follicular fluid, is, in part, exudates of serum and is in addition partially composed of locally produced substances, which are related to the metabolic activity of follicular cells (Gérard *et al.,* 2002). This metabolic activity, together with the "barrier" properties of the follicular wall, is changing significantly during the growth phase of the follicle (Wise, 1987 and Gosden *et al.,* 1988). Follicular fluid composition has been under intensive investigation in recent times in a bid to increase knowledge of follicular development, oocyte maturation and follicular atresia (Mishra *et al.,* 2003). Metabolic activity and blood–follicle barrier properties change during the growth phase of the follicle and hence a different biochemical composition of the follicular fluid in different size follicles could be expected.

MATERIALS AND METHODS

The present study was carried out on 210 Surti buffalo ovaries collected from abattoir. Ovaries were transported to the laboratory in 0.9 per cent chilled (4°C) saline supplemented with gentamicin (50 mg/ml). The ovaries were washed twice with chilled saline then cleaned with spirit or 70 per cent alcohol. The ovaries were further classified as per the presence or absence of corpus luteum. The number of ovaries were 119 and 91, respectively. The follicles present on ovaries were classified on the basis of diameter: (a) small (1 to 4 mm), (b) medium (5 to 8 mm) and (c) large (9 to 12 mm) (Leroy et al., 2004, Parmar and Mehta 1990). The ovaries were

[1]Assistant Research Scientist, Amul Research & Development Association, Amul Dairy, Anand [2]Professor & Head, [3]Associate Research Scientist, Department of Gynaecology & Obstetrics

also grouped as per the presence and absence of corpus luteum. The follicle size in both categories was measured by digital Vernier Caliper. The follicular fluid was aspirated from all three size of follicles of ovaries with and without corpus luteum by using a 22-gauge needle attached to a 1 ml plastic syringe and was pooled accordingly on a weekly basis and collected in separate eppendorf tubes. The total follicular fluid collected from ovaries with corpus luteum was 18, 18 and 33 ml, respectively for small, medium and large size follicles and the corresponding values for ovaries without corpus luteum were 9, 6 and 9 ml. The follicular fluid collected was made cell-free by centrifuging at 5°C at 1500 r.p.m. for 30 min. The aliquots of follicular fluid were stored in a deep freezer (−20°C) till minerals, enzymes and electrolytes analysis. Follicular fluid samples were assayed for minerals (calcium, inorganic phosphorus and magnesium) and enzymes (acid phosphatase, alkaline phosphatase and lactate dehydrogenase) estimated using BS-120 Chemistry Analyzer where as the Electrolytes (sodium and potassium) was estimated by Flame photometer (Hawk et al., 1954).

RESULTS AND DISCUSSION

Follicular Development

A total of 210 ovaries were collected, of which 91 were having corpus luteum and 119 were without corpus luteum. The total numbers of follicles present on the above ovaries were 1158 and they were distributed as per their size. The overall numbers of follicles of different size irrespective of presence or absence of corpus luteum on the ovaries. The mean follicle numbers of three different size present on the ovaries with and without corpus luteum was significantly different. The mean of number of small follicles (4.94 ± 0.17) on ovaries with corpus luteum was significantly higher than of their medium (0.69 ± 0.08) and large (0.41 ± 0.06) size follicles. The mean of number of small follicles (3.98 ± 0.17) on the ovaries without corpus luteum was significantly higher than there of medium (0.77 ± 0.09) and large (0.35 ± 0.05) follicles.

The overall mean numbers of follicles per ovary was significantly different among different size follicles significantly higher number of small follicles (4.40 ± 0.12) were recored as compared to medium (0.73 ± 0.06) and large (0.38 ± 0.04) size follicles. The mean of number of the follicles per ovary with or without corpus luteum in present study was found to be higher than that reported by Parmar and Mehta (1992). They reported that the mean of number of small, medium and large size follicles per ovary was 1.34, 0.25, and 0.18 per ovary, respectively. The overall mean of number of the follicles per ovary irrespective of with or without corpus luteum in the present study was also found to be higher than that reported by Makwana and Shah (2009) but lower than that reported by Parmar and Mehta (1990), Amer *et al.* (2008) and Singh and Shah (2010). Amer *et al.* (2008) reported 6.8 follicles in type III (without corpus luteum), followed by 5.2 in type II (regressed corpus luteum) and 4.4 in type I (functional corpus luteum) ovaries in buffalo.

The results showed that the buffaloes have less number of follicles available on the ovaries. The reason for the less number of primordial follicles in buffaloes compared to cattle is not known, but it could be due to a lower number of the original germ cells, a lower rate of multiplication during the mitotic divisions or an increased frequency of atresia, especially during the fetal life (Samad and Nasseri, 1979).

Follicular fluid Minerals

Calcium

The mean calcium concentration in follicular fluid of small, medium and large size follicles with and without corpus luteum on the ovary was 7.59 ± 0.25, 8.13 ± 0.21 and 8.37 ± 0.09 mg/dl and 7.57 ± 0.23, 8.12 ± 0.10 and 8.39 ± 0.23 mg/dl, respectively. The calcium concentration of follicular fluid of all the three size of follicles with and without corpus luteum was not significantly different and increased with the increase the size of follicle. The overall mean values of calcium in follicular fluid of small, medium and large size follicles was 7.57 ± 0.17, 8.12 ± 0.09 and 8.38 ± 0.16 mg/dl, respectively. The calcium concentration in follicular fluid of all the three size of follicles was not significantly different and it increased with the increase in the size follicle. The overall mean calcium concentration in follicular fluid was 8.02 ± 0.13 mg/dl and 8.11 ± 0.13 mg/dl for ovaries in which corpus luteum was present and absent, the difference being non significant among the group. The overall calcium concentration was 8.08 ± 0.1 mg/dl.

The calcium levels non significantly found to be increased with the increase in the diameter of follicle such finding correlated well with the study of Wise (1987) and Kaur *et al.* (1997). 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. Calcium ions are involved in the regulation of oocyte and meiosis restoration at the beginning of oocyte maturation. The calcium deposits were quickly depleted after oocyte isolation from the follicle (Marketa *et al.*, 2002).

Inorganic Phosphorus

The mean inorganic phosphorus concentration in follicular fluid of small, medium and large size follicles was 4.93 ± 0.26, 5.70 ± 0.34 and 6.31 ± 0.39 mg/dl and 4.63 ± 0.23, 5.94 ± 0.27 and 6.03 ± 0.19 mg/dl, respectively for ovaries with and without corpus luteum. The inorganic phosphorus concentration in follicular fluid of all the three size of follicle with and without corpus luteum was not significantly different and it increased as the follicular diameter increased. The overall mean values of inorganic phosphorus in follicular fluid of small, medium and large size follicles was 4.75 ± 0.17, 5.86 ± 0.21 and 6.10 ± 0.17 mg/dl, respectively. The overall mean of phosphorus concentration in follicular fluid of all the three size of follicles was not significantly different and had showed increasing trend with the increase in the size of follicle. The overall mean inorganic phosphorus concentration in follicular fluid of follicles was 5.64 ± 0.23 mg/dl and 5.64 ± 0.15 mg/dl, respectively, for ovaries with and without corpus luteum and the difference being non significant. The overall inorganic phosphorus was 5.64 ± 0.13 mg/dl.

The phosphorus concentration increased non significantly as the follicular diameter increased in

Table 1 Distribution of different size of follicles on ovaries with and without corpus luteum of buffaloes.

Follicles	Ovaries having corpus luteum (n = 91)			Ovaries without corpus luteum (n = 119)			Overall distribution of different size of follicles irrespective of with or without CL on the ovaries (n = 210)		
	Number of follicles	Mean number of follicles/ ovary	Per cent distribution	Number of Follicles	Mean number of follicles/ ovary	Per cent distribution	Overall Number of follicles	Overall Mean of number of follicles/ ovary	Overall Per cent distribution
Small	450	4.94 ± 0.17^{a}	81.82	474	3.98 ± 0.17^{a}	77.96	924	4.40 ± 0.12^{a}	79.79
Medium	63	0.69 ± 0.082^{b}	11.45	92	0.77 ± 0.93^{b}	15.13	155	0.73 ± 0.64^{b}	13.38
Large	37	0.41 ± 0.06^{c}	6.73	42	0.35 ± 0.53^{c}	6.91	79	0.38 ± 0.04^{c}	6.83
Total	550		100	608		100	1158		100

Table 2 Minerals profiles of follicular fluid in buffaloes in relation to different follicle size of follicles on the ovaries the presence and absence corpus luteum.

Ovaries	Calcium (mg/dl)				Inorganic phosphorus (mg/dl)				Magnesium (mEq/l)			
	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E
With corpus luteum	7.59 ± 0.25^{b}	8.13 ± 0.21^{a}	8.37 ± 0.09^{a}	8.02 ±0.13	4.93 ± 0.26^{b}	5.70 ± 0.34^{a}	6.31 ± 0.39^{a}	5.64 ± 0.23	2.61 ± 0.05^{a}	2.42 ± 0.04^{b}	2.21 ± 0.01^{c}	2.42 ± 0.04
Without corpus luteum	7.57 ± 0.23^{a}	8.12 ± 0.10^{a}	8.39 ± 0.23^{a}	8.11 ± 0.13	4.63 ± 0.23^{b}	5.94 ± 0.27^{a}	6.03 ± 0.19^{a}	5.64 ± 0.15	2.60 ± 0.07^{a}	2.40 ±0.02^{b}	2.22 ± 0.01^{c}	2.37 ± 0.03
Overall Mean ± S.E.	7.57± 0.17^{a}	8.12± 0.09^{a}	8.38± 0.16^{a}	8.08± 0.10	4.75± 0.17^{b}	5.86± 0.21^{a}	6.10± 0.17^{a}	5.64± 0.13	2.60± 0.04^{a}	2.41± 0.02^{b}	2.22± 0.01^{c}	2.38± 0.24

Table 3 Enzymes profiles of follicular fluid in buffaloes in relation to different follicle size of follicles on the ovaries the presence and absence corpus luteum.

Ovaries	Acid phosphatase (U/L)				Alkaline phosphatase (U/L)				Lactate dehydrogenase (U/L)			
	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E.	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E
With corpus luteum	12.75 ± 0.16^{a}	11.65 ± 0.41^{b}	10.31 ± 0.44^{c}	11.56 ± 0.30	293.24 ± 3.44^{a}	290.51 ±3.59^{b}	225.02 ± 3.65^{c}	267.44 ± 7.99	319.04 ± 7.12^{a}	281.45 ± 7.16^{b}	227.51 ± 7.31^{c}	275.83 ± 9.51
Without corpus luteum	12.64 ± 0.28^{a}	11.56 ± 0.29^{b}	10.14 ± 0.26^{c}	11.16 ± 0.22	307.47 ± 8.41^{a}	250.39 ± 4.48^{b}	239.22 ± 9.77^{c}	259.94 ± 6.73	320.64 ± 0.82^{a}	268.45 ± 6.56^{b}	235.43 ± 7.63^{c}	266.28 ± 7.10
Overall Mean ± S.E.	12.68± 0.18^{a}	11.59± 0.23^{b}	10.19± 0.22^{c}	11.29± 0.18	300.68 ± 5.49^{a}	264.67± 7.1^{b}	235.43 ± 7.28^{c}	262.37± 5.22	320.36 ± 6.94^{a}	272.79 ± 5.08^{b}	233.32 ± 5.9^{c}	269.37 ± 5.69

Table 4 Electrolytes (Sodium (mM/L) and Potassium (mM/L)) in follicular fluid of buffaloes in relation to different follicle size of ovaries with and without corpus luteum during peak breeding season.

Ovaries	Sodium (mM/L)				Potassium (mM/L)			
	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E.	Small Follicles	Medium Follicles	Large Follicles	Overall Mean ± S.E
With corpus luteum	126.25 ± 2.06^{a}	130.15 ± 2.07^{b}	132.65 ± 1.28^{b}	129.64 ± 1.16	9.62 ± 0.27^{a}	8.75 ± 0.40^{b}	6.47 ± 0.23^{c}	8.24 ± 0.34
Without corpus luteum	126.46 ± 1.7^{a}	127.85 ± 1.54^{b}	132.62 ± 0.93^{b}	129.77 ± 0.84	9.7 ± 0.27^{a}	8.65 ± 0.23^{b}	6.71 ± 0.17^{c}	8.00 ± 0.23
Overall Mean ± S.E.	126.38 ± 1.29^{a}	128.62 ± 1.23^{b}	132.62 ± 0.75^{b}	129.73 ± 0.67	9.69 ± 0.19^{a}	8.68 ± 0.20^{b}	6.65 ± 0.14^{c}	8.08 ± 0.19

present study was in agreement with the results of Abd Ellah *et al.* (2010); Nandi *et al.* (2007) and Mishra *et al.* (2003). The higher concentration of phosphorus in small follicles compared to those in large ones irrespective of with or without corpus luteum of ovaries was in accordance with the earlier reports by Wise, (1987), Kaur *et al.*, (1997) and Bordoloi *et al.*, (2001). Phosphorus is known to be involved in high energy bonds that link oxides of phosphate to carbon or to carbon nitrogen compounds (such as adenosine triphosphate, ATP). Phosphorus is also intimately involved in acid-base buffer systems of blood and other body fluids (Zilva *et al.*, 1991).

Magnesium

The mean magnesium concentration in follicular fluid of small, medium and large size follicles was 2.61 ± 0.05, 2.42 ± 0.04 and 2.21 ± 0.01 mEq/l and 2.60 ± 0.07, 2.40 ± 0.02 and 2.22 ± 0.01 mEq/l, respectively for ovaries having presence or absence of corpus luteum. The magnesium concentration of follicular fluid of all the three size of follicles with and without corpus luteum was significantly different and it decreased with the increase diameter of follicle.The overall mean values of magnesium in follicular fluids of small, medium and large size follicles was 2.60 ± 0.04, 2.41 ± 0.02 and 2.22 ± 0.01 mEq/l, respectively. The overall mean magnesium concentration in follicular fluid of all the three size of follicles was significantly different which decreased with increase in follicular size. The overall mean of magnesium concentration in follicular fluid of follicles with corpus luteum (2.42 ± 0.04 mEq/l) and without corpus luteum (2.37 ± 0.03 mEq/l) did not differ significantly. The overall magnesium concentration for both groups was 2.38 ± 0.24 mEq/l.

In present study the magnesium level significantly decreased as the follicular diameter of the increased which these finding corroborate with study of Arshad *et al.* (2005) and Abd Ellah *et al.* (2010) in buffaloes. Magnesium acts as a co-factor in enzymatic reactions and induces meiotic maturation of *Xenopus* oocytes (Belle *et. al.* 1986). Further, low concentrations of divalent cations (Mg and Ca) inhibit the resumption of meiosis, and the inhibitory effect of low Mg concentration is greater than that of low Ca (Jagiello *et al.*, 1982). Low concentration of Mg and Ca in the IVM medium impaired developmental competence, but did not affect the progression of nuclear maturation. On the other hand, a high Mg concentration (2 mM) and high Mg/Ca ratio (2:1) improved early embryo development because Mg regulates Ca homeostasis in zygotes (Lane *et al.* 1998). However, increasing Mg to 2 mM from 0.5 mM improved developmental competence and hastened the progression of nuclear maturation, irrespective of the Ca concentration.

The higher concentration of magnesium in the small follicles could help the mitosis of the follicular cells through the formation of thrombin, a potent mitogen. Magnesium could substitute calcium in thrombin formation under the condition of low Ca/Mg ratio that exists in small follicles. As magnesium was antagonistic to calcium, the decreased magnesium with follicular development facilitated the calcium action in large follicles (Kalmath, 2000).

Follicular fluid Enzymes

Acid phosphatase

The mean acid phosphatase concentration in follicular fluid of small, medium and large size follicles was 12.75 ± 0.16, 11.65 ± 0.41 and 10.31 ± 0.44 U/L and 12.64 ± 0.28, 11.56 ± 0.29 and 10.14 ± 0.26 U/L, respectively for ovaries having presence and absence of corpus luteum. The acid phosphatase concentration in follicular fluid on all the three size of follicles in both groups was significantly different and was decreased as the follicular diameter increased. The overall mean values of acid phosphatase in follicular fluids of small, medium and large size follicles was 12.68 ± 0.18, 11.59 ± 0.23 and 10.19 ± 0.22 U/L, respectively, the difference being significant among the different size of follicles. The acid phosphatase concentration decreased with the increase in the follicular diameter. The overall mean acid phosphatase levels in follicular fluid was 11.56 ± 0.30 U/L and 11.16 ± 0.22 U/L respectively for both group, the difference being non significantly. The overall acid phosphatase levels for both group was 11.29 ± 0.18 U/L.

The present finding of the acid phosphatase concentration which significantly decreased with the increase in the follicular diameter was in agreement with study of Wise (1987) and Kalmath and Ravindra (2007) in buffaloes. Acid phosphatases are lysosomal enzymes which catalyze various reactions and involved in the active transport of phosphates across the cell membrane, synthesis of protein and DNA turnover in nucleus (Mishra *et al.*, 2003).

Alkaline phosphatase

The mean alkaline phosphatase concentration (U/L) in follicular fluid of small, medium and large size follicles was 293.24 ± 3.44, 290.51 ± 3.59 and 225.02 ± 3.65 and 307.47 ± 8.41, 250.39 ± 4.48 and 239.22 ± 9.77 for both the groups of ovaries respectively. The alkaline phosphatase concentration in follicular fluid of all the three size of follicles of ovaries with and without corpus luteum was significantly different and it decreased as the follicular diameter increased. The overall mean values of alkaline phosphatase in follicular fluid of small, medium and large size follicles was 300.68 ± 5.49, 264.67 ± 7.1 and 235.43 ± 7.28 U/L, respectively which decreased as the follicular size increased. It differences was significantly among the diameter of follicles. The overall mean alkaline phosphatase concentration in follicular fluid was 267.44 ± 7.99 U/L and 259.94 ± 6.73 U/L, respectively for presence and absence of corpus luteum on the ovaries, the difference being non significant. The overall alkaline phosphatase for both the group of ovaries was 262.37 ± 5.22 U/L. The finding of alkaline phosphatase level for both the group of ovaries was significantly with the decreased in follicular diameter increased which was in close proximity with the results of Wise, (1987). Alkaline phosphatases are lysosomal enzymes which catalyze various reactions and involved in the active transport of phosphates across the cell membrane, synthesis of protein and DNA turnover in nucleus (Mishra *et al.* 2003).

The higher alkaline phosphatase activity in the initial stages of follicular development might be due to progesterone and androgen dominant environment that exits in the small follicle, in that a higher concentration of progesterone and androgen could be conducive to phosphatase activity (Kalmath 2000). The activity of alkaline phosphatase in follicular fluid with the development of the follicle decreased due to the shift in the follicular hormonal milieu from androgen to estrogen dominant follicle.

Lactate dehydrogenase

The mean lactate dehydrogenase concentration in follicular fluid of small, medium and large size follicles with and without corpus luteum on the ovary was 319.04 ± 7.12, 281.45 ± 7.16 and 227.51 ± 7.31 U/L and 320.64 ± 0.82, 268.45 ± 6.56 and 235.43 ± 7.63 U/L, respectively. The lactate dehydrogenase concentration of follicular fluid of all the three size of follicles with and without corpus luteum was significantly different and decreased with the increase in the size of follicle. The overall mean values of lactate dehydrogenase in follicular fluid of small, medium and large size follicles was 320.36 ± 6.94, 272.79 ± 5.08 and 233.32 ± 5.9 U/L, respectively. The lactate dehydrogenase concentration in follicular fluid of all the three size of follicles was significantly different and it decreased with the increase in the size of follicle. The overall mean of lactate dehydrogenase concentration in follicular fluid was 275.83 ± 9.51 U/L and 266.28 ± 7.10 U/L for ovaries in which corpus luteum was presence and absence, the difference being non significant among the group. The lactate dehydrogenase concentration was 269 ± 5.69 U/L.

In present study the lactate dehydrogenase concentrations significantly found to be decreased with the increase in the diameter of follicle such finding correlated well with the study of Wise (1987) and Abd Ellah *et al.* (2010) in buffaloes. The higher serum lactate dehydrogenase activity indicated that there was an inward movement of the lactate dehydrogenase from serum to the follicular fluid as reported by Abd Ellah *et al.* (2010). Increased activity of lactate dehydrogenase (LDH) in follicular fluid in small follicles indicated early follicular degeneration (Wise, 1987). This suggested that as the follicle degenerated, there were distinct biochemical changes that accompanied the degenerative processes. Isoenzyme analysis of lactate dehydrogenase activity in follicular fluid might increase the accuracy and sensitivity of lactate dehydrogenase for a biological atresia marker in ovine follicles (Nandi *et al.*, 2007).

Follicular fluid Electrolyte

Sodium

The mean sodium concentration in follicular fluid of small, medium and large size follicles was 126.25 ± 2.06, 130.15 ± 2.07 and 132.65 ± 1.28 mM/L and 126.46 ± 1.7, 127.85 ± 1.54 and 132.62 ± 0.93 mM/L, respectively for ovaries with and without corpus luteum. The sodium concentration in follicular fluid of all the three size of follicles was not significantly different and it increased as the follicular diameter increased. The overall mean values of sodium in follicular fluid of small, medium and large size follicles was 126.38 ± 1.29, 128.62 ± 1.23 and 132.62 ± 0.75 mM/L, respectively. The overall mean of sodium concentration in follicular fluid of all the three size of follicles was not significantly different and had showed increasing trend with the increase in the size of follicle. The overall mean of sodium concentration in follicular fluid of follicles was 129.64 ± 1.16 mM/L and 129.77 ± 0.84 mM/L respectively for ovaries with and without CL, the difference being non significant. The overall sodium concentration was 129.73 ± 0.67 mM/L.

The sodium levels non significantly increased as the follicular size increased in present study corroborate well with the study of Arshad *et al.* (2005), Wise, (1987) and Kaur *et al.* (1997). 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 (Wise, 1987). 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 (Sharma *et al.*, 1995).

Potassium

The mean potassium concentration in follicular fluid of small, medium and large size follicles was 9.62 ± 0.27, 8.75 ± 0.40 and 6.47 ± 0.23 mM/L and 9.7 ± 0.27, 8.65 ± 0.23 and 6.71 ± 0.17 mM/L, respectively for ovaries having corpus luteum and without corpus luteum. The potassium concentration in all the three size of follicles for both the group was significantly different and it decreased with the increased follicular diameter. The overall mean values of potassium in follicular fluid of small, medium and large size follicles was 9.69 ± 0.19, 8.68 ± 0.20 and 6.65 ± 0.14 mM/L, respectively. The difference being significant and it decreased with the increase in the follicular size. The overall mean of potassium concentration of follicular fluid was 8.24 ± 0.34 mM/L and 8.00 ± 0.23 mM/L for ovaries bearing corpus luteum and without corpus luteum, the difference being non significant. The overall potassium value for both the group was 8.08 ± 0.19 mM/L.

In present study the potassium concentration significantly decreased as the follicular size increased. Similar finding was results of Iwata *et al.* (2004); Leroy *et al* (2004); and Abd Ellah *et al.* (2010). 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 to intracellular (Chang *et al.*, 1976). The concentration gradient of potassium between serum and follicular fluid suggests an active inward transport of the cation and is involved in local metabolism (Gosden *et al.*, 1988).

CONCLUSION

Calcium, inorganic phosphorus and sodium non significantly increased in the follicular fluid of small to large follicles in both with and without corpus luteum of ovaries. The concentration of magnesium, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and potassium significantly ($P < 0.05$) decreased in the follicular fluid of small to large follicles in both with and without corpus luteum of ovaries. The result of present study suggested that the oocyte and the granulosa cells of ovarian follicles of buffalo grow and mature in a changing minerals, enzymes and electrolytes from small to large follicles.

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REFERENCES

Abd Ellah, M. R., Hussen H. A., and Derar, D. R. 2010. Ovarian follicular fluid constituents in relation to stage of estrus cycle and size of the follicle in buffalo. *Veterinary World.* **3** (6):263-267.

Amer, H. A., Hegab, A. O. and Zaabal, S. M. 2008. Effects of ovarian morphology on oocyte quantity and quality, granulose cells, *in vitro* maturation, and steroid hormone production in buffaloes. *Animal Reproduction,* **5**: 55-56.

Arshad, H. M., Ahmad, N., Zia-ur-Rahman, Samad, H. A., Akhtar, N. and Ali S. 2005. Studies on some biochemical constitutes of ovarian follicular fluid and peripheral blood in buffaloes. *Pakistan Veterinary Journal* **25**: 189.

Bagavandoss, P., Midgley, A. R. and Wicha, M., 1983. Developmental changes in the ovarian follicular basal lamina detected by immunofluorescence and electron microscopy. *Journal of Histochemistry Cytochemistry* 633-640.

Belle, R. Mulner-Lorillon, O., Marot, J. and Ozon, R. 1986. A possible role for Mg^{2+} ions in the induction of meiotic maturation of *Xenopus* oocyte. *Cell Differentiation.* **19:** 253–261.

Bordoloi, P. K., Sarmah, B. C., Dutta, D. J. and Deka, B. C. 2001. Macro and micro minerals in caprine follicular fluid. *Indian Journal of Animal Reproduction* **22**: 23–25.

Chang, S. C. S., Jones, J. D., Ellefson, R. D. and Ryan, R. J. 1976. The porcine ovarian follicle: I. selected chemical analysis of follicular fluid at different developmental stages. *Biology of Reproduction* **15**: 321-328.

Gérard, N., Loiseau, S., Duchamp, G. and Seguin, F. 2002. Analysis of the variations of follicular fluid composition during follicular growth and maturation in the mare using proton nuclear magnetic resonance (1H NMR). *Reproduction.* **124**: 241-248.

Gosden, R. G., Hunter, R. H. F., Telfer, E., Torrance, C. and Brown, N. 1988. Physiological factors underlying the formation of ovarian follicular fluid. *Journal of Reproduction Fertility,* **82**: 813 825.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954 Practical Physiological Chemistry, 13th edn., p. 878, McGraw-Hill, New York, Toronto and London.

Iwata, H., Hashimoto, S., Ohota, M., Kimura, K., Shibano, K. and Miyake, M. 2004. Effects of follicle size and electrolytes and glucose in maturation medium on nuclear maturation and developmental competence of bovine oocytes. *Reproduction* **127**: 159–164.

Jagiello, G., Ducayen, M. B., Downey, R. and Jonassen, A. 1982. Alterations of mammalian oocyte meiosis I with divalent cations and calmodulin. *Cell Calcium* **3**: 153–162.

Józwik, M., Józwik, M., Wolczynski, S., Józwik, M. and Szamatowicz, M. 2001. Ammonia concentration in human preovulatoy ovarian follicles. *Europian Journal of Obstetetric Gynecology and Reproductive Biology,* **94**: 256-260.

Kalmath, G. P. 2000. Biochemical analysis of ovarian antral follicular fluid in buffaloes (M.V.Sc. Thesis). University of Agricultural Sciences, Bangalore, India.

Kalmath, G. P. and Ravindra, J. P. 2007. Enzymatic profiles of acid and alkaline phosphatases in ovarian antral follicular fluid of buffaloes. *Indian Journal Animal Research* **41** (2): 106-110.

Kaur, J., Takkar, O. P. and Khera, K. S. 1997. Mineral elements in follicular fluid of buffalo ovary. *Indian Journal of Animal Reproduction* **18**: 36–38.

Lane, M., Boatman, D. E., Albrecht, R. M. and Bavister, B. D. 1998. Intracellular divalent cation homeostasis and developmental competence in the hamster preimplantation embryo. *Molecular Reproduction and Development.* **50**: 443–450.

Leroy, J. L. M. R., Vanholder, J. R., Dalanghe, G., Opsomer, A., Van Soom, P. E. J. Bols and de Kruif, A. 2004. Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. *Animal Reproduction Science* **80**: 201-211.

Makwana P. M. and Shah R. G. 2009. Influence of different categories of follicle and presence of CL on recovery rate, quality and quantity of buffalo oocytes. XXV Annual convention of the ISSAR and international symposium, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India, pp 286-287 (Abstr.).

Marketa, S., Rozineck, J. and Petr, J. 2002. Labeling of bound calcium in pig egg ultrastructure. 2nd Annual meeting of CSMS Vranoska Ves. *Tema Microscopy.* **59**: 307-322.

Mishra, O. P., Pandey, J. N. and Gawande, P. G. (2003). Study on biochemical constituents of caprine ovarian follicular fluid after superovulation. *Asian Australasian Journal of Animal Science* **16**: 1711–1715.

Nandi., S., Girish Kumar., V., Manjunatha., B. M. and Gupta., P. S. P. 2007. Biochemical composition of ovine follicular fluid in relation to follicle size. *Development Growth Differentiation* **49**: 61–66.

Okuda, Y., Okamura, H., Kanzaki, H., Takenaka, A., Morimoto, K. and Nishimura, T. (1982). An ultrastructural study of capillary permeability of rabbit ovarian follicles using horseradish peroxidase as a tracer. *Nippon Sanka Fujinka Gakkai Zasshi*. **34**: 181-186.

Parmar, A. P. and Mehta, V. M. 1990. Seasonal influences on endocrine and biochemical changes in developing ovarian follicles of surati buffaloes. M.V.Sc. Thesis. GAU. Anand Campus.

Parmar, A. P. and Mehta, V. M. 1992. Study of biometry of surti buffalo ovaries and development of ovarian follicles in relation to different season. *Indian Journal of Animal Reproduction* **13**(2): 157-160.

Samad, H. A. and Nasseri, A. A. 1979. A quantitative study of primodial follicles in buffalo heifer ovaries. I*compendium 13th FAO/SIDA International Course on Animal Reproduction, Uppsala, Sweden.*

Sharma, R. K., Vats, R. and Sawhney, A. (1995). Changes in electrolytes of antral follicles in goat. *Indian Journal of Animal Reproduction* **16**: 18–21.

Singh, R. and Shah, R. G. 2010. Influence of different categories of follicles on quantity and quality of oocytes with respect to their *in vitro* maturation in surti buffaloes. M.V.Sc. Thesis. AAU. Anand.

Wise, T., 1987. Biochemical analysis of bovine follicular fluid: albumine, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. *Journal of Animal Science* **64**: 1153-1169.

Zilva, J. F., Ponnall P. R. and Mayne P. D. 1991. Plasma enzymes diagnosis. Clinical chemistry in diagnosis and treatment. 5th Ed. ELBS. Edward Arnold, UK. Pp:307-322.