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CHANGES IN CONCENTRATION OF MINERALS IN FOLLICULAR FLUID OF GROWING FOLLICLES

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ABSTRACT: The present study was undertaken in goat ovaries to study the changes in mineral content of follicular fluid during growth and maturation of follicles. Goat ovaries were procured from local slaughterhouse. The diameter of surface follicles was measured and grouped into small (< 5 mm) and large (≥ 5 mm). From these follicles the fluid was aspirated and after centrifugation and dilution was estimated for Na, K, Ca, Mg, Fe, Cu, Mn, and Zn using atomic absorption spectorphotometer (AAS). It was found that the concentrations of Na and Ca were significantly higher in large follicle (199.41 ± 6.13 mEq/L and 6.44 ± 0.57 mEq/L respectively) as compared to small follicles (155.43 ± 4.2 mEq/L and 6.07 ± 0.07 % respectively). The concentrations of K, Zn and Fe were significantly higher in small follicles (21.36 ± 0.98 mEq/L, 2.79 ± 0.03 % and 5.99 ± 0.07 % respectively) as compared to large follicles (15.06 ± 1.3 mEq/L, 1.98 ± 0.03 % and 2.25 ± 0.01 % respectively). The levels of Mg, Mn and Cu did not differ significantly between small and large follicles. This study emphasizes the changes of minerals in folliculogenesis of goats.

Key words: Goat, Ovary, Follicle, Maturation, Minerals.

INTRODUCTION

If the genetic potential from *in-vitro* fertilization is to be maximized, a thorough understanding of the basic changes ongoing during follicular development is required so that the optimal environment can be established for the maturation of viable oocytes (Wise 1987). The details of biochemical and physiological interrelation will provide an index to examine deviation in situation wherein normal growth and development of antral follicle is threatened in *in-vitro* system. The follicular fluid seems to provide normal osmotic pressure, steroid binding proteins, and enzyme necessary for nuclear and cytoplasmic maturation of oocytes, release of eggs from ruptured follicle and fertilization (Hafez 1987). The presence of minerals in follicular fluid is considered as regulatory factor in follicular development and steroidogenesis. Minerals are

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not only participating as co-factor, and activation of various enzyme systems for oocyte development and maturation but also affect ovarian function and thus fertility. Keeping the above facts in view, the present study of estimation of mineral contents in follicular fluid in goat ovaries was undertaken.

MATERIALS AND METHODS

Ovaries of goats (n = 100) were collected from local slaughterhouses and placed in normal saline containing gentamicin (50 mg/ml) and then transported to the laboratory in a thermoflask. After measuring the diameter of surface follicles they were then classified as - small (< 0.5 mm diameter) and large (≥ 0.5 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. Fifty samples of follicular fluid were collected from small and large follicles and stored at - 20° C until further analysis.

Before analysis the follicular fluid was diluted 200 times for the estimation of Na and K and 10 times for Fe, Cu, Mn, Zn, Mg and Ca. The concentration of these minerals was then estimated in Atomic Absorption Spectrophotometer (Perkin Eimer) using stantdard procedures. The AAs was standardized using standard solutions of different mineral elements after fitting of lamp specific for each mineral element separately. After proper standardization of AAS, the diluted samples were fed to atomizer for 30 seconds separately in order to estimate the concentration of mineral of interest. The data of AAS were multiplied by dilution factors for calculating the final concentration of minerals in the follicular fluid.

The difference in concentration of minerals between small and large follicles was analyzed by t-test.

RESULTS AND DISCUSSION

The concentrations of Na and Ca were significantly higher in large follicles as compared to small follicles (Table 1) which was consistent with the findings of Thakur *et al.* (2003). Increased follicular Na concentration in large follicles observed in present study might be related to viability of follicles and most probably linked to active estrogen synthesis (Wise 1987). Higher concentration of Ca in large types of follicle might be associated with proliferation of granulose and theca cells and thus indirectly reflected the steriodogenic capabilities of the growing follicles as Ca plays an important role in gonadotropic regulation of ovarian steroidogenesis (Bordoloi *et al.* 2001).The increase in the Ca concentration in follicular fluid along with increase in size of follicles observed in the present study was in agreement with the observation of Bordoloi *et al.* (2001).

The concentrations of K, Zn and Fe were significantly higher in small follicle as compared to large follicles (Table 1). Sharma *et al.* (1995) and Thakur *et al.* (2003) reported higher concentration of potassium/follicle as the size decreased. Follicular fluid potassium concentration reflects the physiological status and metabolic activity of the follicular cells. Anoxia and intracellular acidosis resulting from ischemia could lead to degeneration of granulose cells resulting in high potassium concentration in follicular cells (Kundson *et al.*1979). These values for Fe and Zn were in agreement with earlier finding of Sharma and Vats (1998) and Bordoloi *et al.* (2001). These

Table 1: Concentration of macro and micro minerals in caprine follicular fluid in two class of follicle.

Class of follicle	Na (mEq/L)	K (mEq/L)	Ca (mg%)	Mg (mg%)	Zn (mg%)	Fe (mg%)	Cu (mg%)	Mn (mg%)
Small (< 5 mm in diameter)	155.93 ± 4.20^{a}	21.36 ± 0.98^{b}	6.07 ± 0.07^{a}	4.02 ± 0.41	2.79 ± 0.03^{b}	5.99 ± 0.07^{b}	0.58 ± 0.003	0.11 ± 0.03
Large (> 5 mm in diameter)	199.41 ± 6.13^{b}	15.06 ± 1.30^{a}	6.44 ± 0.17^{b}	4.46 ± 0.07	1.98 ± 0.03^{a}	2.25 ± 0.01^{a}	0.55 ± 0.001	0.13 ± 0.01

* Values are Mean ± S.E. M
* Two different superscripts within the same column differ significantly.
* One different superscripts within the same column differ significantly.

variations are attributed to increased levels of steroid hormones (estrogen and progesterone) that induce increased hemodynamic pulses in the vascular shunt of the developing follicles (Sangha*et al.*1999). In ovulatory follicles, the declined iron level might be due to ischemia leading to rupture of follicle wall at stigma (Sharma and Vats 1998).

The levels of Mg, Mn and Cu did not differ significantly between small and large follicles. Wise (1987) reported that the Mg is a key nutrient in proliferative cells for attainment of steriodogenic capabilities of the growing follicle and thus may be important in follicular fluid of rapidly growing follicle which support our findings wherein greater concentration of Mg was found in large follicles that are actually growing. These biochemical findings further endorse the earlier findings of Thakur *et al.* (2003). The fluid from small follicles contained non-significant higher level of Cu as compared to large follicle. The variation of two category of follicles might be due to synergistic effect of progesterone and estrogen (Sharma and Vats 1998).Thus, the higher Cu concentration in small follicle corresponds to estrogen secreting stage of the follicle. The increase in Mn concentration with advancement of follicular size though not significant was similar to those reported by Sharma and Vats (1998) and Bordoloi *et al.* (2001). Sikka (1992) suggested that high concentration of Mn in large follicles might be due to involvement of this ion in major energy producing reactions.

CONCLUSION

The concentrations of Na, Ca, Mg, and Mn were found to be greater in large follicles than in small follicles while the concentrations of K, Zn and Cu decreased with the increase in size of follicles. Therefore, it provides information that deficiency of a single and combined trace element can induce reproductive failure.

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