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Quantitative estimation of some macro- and micro-minerals in follicular fluid of indigenous pigs

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There are great differences in the developmental competence of oocytes collected from individual swine ovaries. Oocytes grow and mature in the follicular fluid (FF). Hence, FF 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). Macro- and micro-minerals play very important role in different body functions and present appreciably amounts in all types of body fluids. Unfortunately, the information of mineral profiles of follicular fluid was scanty in follicular fluid of indigenous breed of porcine in India. Therefore, the present study was planned to determine the concentration of macro-minerals in FF of different size of follicles.

Ovaries (200), free from gross pathological lesions were collected from slaughter house in Kolkata between March and May 2011, from clinically healthy sows. Average body weight of the animals varied between 95 and 110 kg. After collection, the ovaries were taken to the laboratory within 3 h after slaughter. The diameter of various follicles (1-12 mm) present on each ovary was measured using geometrical divider and scale. According to the follicular diameter, the follicles were divided into 3 groups i.e. small (1–2 mm), medium (3–5mm) and large (6–12 mm). FF was aspirated by sterile insulin syringes from all the 3 categories of follicles individually, by holding them in forceps and giving negative pressure on the follicles. Separate pools were made for each size of follicles, and then kept into properly coded sterilized graduated vials. A total number of 10 pooled samples of 2 ml each were aspirated from 170, 65 and 20 numbers of follicles of small, medium, and large sized, respectively.

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Biochemical estimations: Sodium (Na) and Potassium (K) concentrations were estimated as per Tietz (1976) with certain modifications. Determination of the quantity of Ca (Schwartzenbach *et al*. 1956), magnesium (Whitmore and Evans 1964), and phosphorus (Amador and Urban 1977) was made with certain modifications. Estimation of iron was as per Siedel *et al*. (1984), copper and zinc concentration in FF as per Agarwal and Henkin (1985) and of lithium concentration as per Vonknoring *et al*. (1976) with certain modifications.

Statistical analysis: The mean values (n±SE) for concentrations of various macro- and micro- minerals of FF from different sized follicles were computed. Differences between the biochemical constituents among the different sized follicles were analyzed, using SPSS package following standard procedures as described by Snedecor and Cochran (1994) for ANNOVA and Duncan's multiple range tests.

Mean concentrations of various macro- and micro-mineral constituents in fluid from small, medium and large follicles are represented in Table 1. Although concentration of all the

Table 1. Concentration of macro-minerals (sodium, potassium, calcium, phosphorus and magnesium) and micro-minerals (zinc, copper, iron, lithium) in porcine FF with relation to follicular size

Parameter	Small	Medium	Large
Na (mmol/ litre)	131±1.48	133.35±0.92	135.03±0.76
K (mmol/ litre)	11.16 ±0.34	10.3 ±0.225	9.26±0.28
Ca (mg/dl)	8.4±0.054^{b}	8.565±.0.046^{b}	8.85±0.058^{a}
P (mg/dl)	8.28 ±0.211^{b}	8.478±0.09^{b}	9.225±0.18^{a}
Mg (mg/dl)	3.365±0.14	3.198±0.207	2.971 ±0.188
Zn (µg/dl)	1.708±0.01	1.6±0.024	1.521±0.09
Cu (µg/dl)	1.842±.01^{c}	2.462±.0136^{b}	2.624±.078^{a}
Fe (µg/dl)	244.9±1.048^{b}	259.5±1.046^{b}	282.5±2.86^{a}
Li (mEq/ litre)	2.03±.041^{a}	1.138±.071^{b}	0.5310±.022^{c}

Values were expressed as mean ± SE, values with different superscripts in a row differ significantly (P<0.05).

macro- and micro-minerals either increased or decreased in concentration with increase in follicular size but only Ca, P, Cu and Fe increased significantly, and only Li decreased significantly in follicular fluid.

Na concentration in the present study was congruent with the findings of Chang *et al*. (1976) in pig. Though actual cause is unknown but a potent Na retention action compound 19 hydroxyandrostenedione, was found in greater concentration in the large follicles (Wise *et al*. 1987). Regression of follicular fluid K concentration with follicular development as found in this study was supported by the study of Knudsen *et al*. (1979) in pig.

Ca concentration as found in the present study was supported by the findings of Chang *et al.* (1976) in pig and Wise (1987) in cow. Ca is one of the macro-minerals which play a major role in reproduction; its deficiency can affect fertility (Boland 2003). Marketa *et al*. (2002) stated that free Ca ions seem to be involved in the regulation of oocyte growth and meiosis restoration at the beginning of oocyte maturation. Abd Ellah *et al*. (2010) analysed P concentration in buffalo follicular fluid, the findings of which was in support of the present study. The higher concentrations of Mg in the small follicles could help the mitosis of the follicular cells through the formation of thrombin, a potent mitogen. Magnesium could substitute for calcium in thrombin formation under low Ca:Mg ratio conditions, that exist in small follicles. As magnesium was antagonistic to calcium, the decreased magnesium with follicular development facilitated the calcium action in large follicles (Kalmath 2000). Magnesium acts as a cofactor in enzymatic reaction, and induces meiotic maturation of xenopus oocyte (Belle *et al*. 1986).

The present study is supported by the findings of Arshad *et al.* (2005), where Zn concentration significantly increased with follicular size. Leonhard (2000) opined in a review, that zinc ions are involved in the process of cell division, development and differentiation and in the control of gene expression. But the exact function of Zn in follicular fluid is still to be discovered.

Increased Cu concentration in large follicles could be correlated with the increased estrogen levels that were related to increases in ceruloplasmin synthesis (Sharma and Sharma 1997).

Fe concentration in follicular fluid was significantly higher ($P<0.01$) in large follicles than small and medium follicles. Increased Fe concentration in the developed follicles was due to the increased level of steroid hormones, which induce increased hemodynamic pulses and vascular shunt of the developing follicles (Sangha *et al*. 1993). Guraya *et al*. (1991) suggested that the variations in Fe content were in accordance with the hemodynamic changes observed in follicular wall during follicular development and maturation and these changes were regulated by intrafollicular factors.

Bagger *et al.* (1993) in their study on mice found that Li enhanced the capability of mouse oocyte for continuation of meiosis by activation of cyclic AMP second massager path in the cumulus cells.

Therefore, change of concentration of Li in different sized follicular fluid might be related to folliculogenesis, and particularly maturation of follicles. Cumulus cells induce follicular cells to synthesize a meiosis inducing substance which stimulates the oocyte to resume meiosis.

SUMMARY

In the present study, characteristics of porcine follicular fluid (FF) harvested from different sized ovarian follicles and developmental competences of enclosed oocytes in relation to their sizes were investigated. It has been observed that some mineral components of FF like Ca, P, Cu and Fe significantly increased as the follicles increased in size, while only Li, which is first reported here in porcine FF decreased significantly with increase in follicular size. Therefore, it may enhance more success in reproduction in this species, which may improve the certain macro and micro mineral level in follicular fluid of oocytes.

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