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Review Paper

Role of Biochemical factors and Mineral Supplementation in Livestock ration for Maintenance of their Fertility and Healthy Reproductive Status: A Review

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Abstract

The biochemical components and factors like protein and enzyme components of follicular fluid like lipase, amylase and creatinine phosphokinase concentration show an increasing trend as the follicles mature, while some other components like Alpha-fetoprotein, Acid Phosphatase and Alkaline Phosphatase concentration decreases with increase in follicular size. Moreover, presence of amylase, lipase and CPK and their respective concentrations in the in the follicular fluid can also been detected. The micro-minerals play an important role in increasing pregnancy rates in apparently barren and reproductively abnormal mares. Provision of micro-mineral supplemented ration containing copper, zinc, iron and manganese during the vernal period of breeding cycle proves to be useful. The pregnancy rate increase significantly in micro-mineral supplemented livestock. Some mineral components of follicular fluid like Ca, P, Cu and Fe significantly increases as the follicles increase in size, while only Li, concentration significantly decreases with increase in follicular size. Therefore, it may enhance more success in reproduction, which may improve the certain macro- and micro-mineral level in follicular fluid of oocytes.

Keywords: Biochemical factors, fertility, livestock, reproduction.

Introduction

Follicular fluid where oocytes grow and mature is a mixture of serum exudates and locally produced metabolites of follicular cells[1] and is found in an avascular compartment within the ovarian follicle. Incorporation of follicular fluid into culture medium showed encouraging results in *in-vitro* maturation and fertilization of oocytes[2,3]. Alpha-fetoprotein, amylase, lipase and creatinine kinase remains present in FF. The present article is constructed to highlight the importance of quantitative biochemical estimation of different proteins like total protein, albumin, globulin and Alpha-fetoprotein and enzymes like alkaline phosphatase, acid phosphatase, lipase, amylase, creatinine kinase concentrations of FF harvested from ovarian follicles.The accurate diagnosis of mineral deficiency is revealed by clinical and production response[4]. Old mares have lower pregnancy rates per cycle and increased pregnancy loss rates[5]. Deficiency of minerals causes various reproductive failures such as infertility, repeat breeding, embryonic loss, poor conception rate, anestrous condition etc[6]. The influence of micro-minerals on reproductive efficiency, ovulatory mechanism and maintenance of pregnancies and regulation of endocrine functions in the animals is important[7].

Investigation reports and research findings

Proteins in the living cells are intimately associated with various phases of activity that constitute the life of the cells[8]. The gradual decrease in the concentration of total protein, albumin and globulin in the different sized follicles is related with estrogen and water uptake in growing follicles and due to this water uptake there is dilution of follicular protein concentration. It was observed that total protein concentrations in small, medium, and large sized goat ovarian follicular fluid were 84.24%, 69.16%, and 71.02% of total protein of serum, respectively[9,10]. A number of proteins reside in follicular fluid and surrounding granulosa cells, therefore, a small change in hydration could produce large effects on protein concentrations as follicles were growing[11].

Developing follicles need amino acids for their activity. Ovary is one of the most active tissue in catabolizing albumin[12]. There is active inward transport of albumin from blood into follicles which 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[13].

AFP is present in high level in fetal fluids, certain neoplasm and in regenerating liver. In human AFP, Carcino Embryonic Antigen (CEA), and CA-125 have been found in FF after ovarian stimulation[14]. Presence of AFP in serum, bile, CSF and feces suggests that AFP plays a similar role in body homeostasis. Presence of AFP in the developing follicles of the ovary, in human ovarian follicle, and in the developing and newborn brain, suggests may somehow be involved in the

maturation and programming of the positive feedback exerted by ovary-derived estrogen on brain LH and FSH levels.[15] Physiological studies on AFP-estrogen interaction revealed rat Alpha-feto protein was able to inhibit the formation of water-soluble metabolites of E_2 and E_1, when microsomes from rat liver were incubated in the presence of NADPH, and to regulate the activity of 17β-hydroxy steroid dehydrogenase *in vitro*[16]. AFP has also been associated with the delay in the onset of puberty in postnatal rat pups[17]. The injection of AFP during the prepubertal period in rats has resulted in a decreased number of primordial and primary follicles in the ovary[18].

The changes in enzymatic activity of ACP have an important role in ovulation, and it seems that there is relationship between its presence and oocyte fertilization by spermatozoon[19]. However, ACP may be important for steroidogenesis. Phosphatase enzymes were implicated in both growth and atresia of follicles. A progressive decrease in the ACP and ALP activity with follicular maturation was noted.

ALP and ACP are lysosomal enzymes which catalyze various reactions in the body and are involved in the active transport of protein and DNA turnover in nucleus.[20] The higher alkaline phosphatase activity in the initial stages of follicular development might be due to a 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[21]. The decreased follicular fluid alkaline phosphatase activity with the development of the follicle could be due to the shift in the follicular hormonal milieu from androgen to estrogen dominance, with the development of the follicle. Thus, the changes in the phosphatase activity could reflect changes in metabolic activity on tissue synthesis mechanisms induced by the different hormones.

The role of the endothelial-bound lipases in ovarian physiology is largely unknown. The ovary is metabolically very active organ that needs fatty acids as energy substrate for synthesis of lipid mediators and cholesterol for hormone synthesis. The rat ovary has been shown to be highly dependent on oxidation of fatty acids for the generation of adenosine 5'-triphosphate for energy metabolism, growth, and steroidogenesis[22,23]. The lipase would facilitate the supply of fatty acids from lipoproteins to cells of several parts of the ovary, including the corpus luteum and the growing follicle with its enclosed oocyte and the avascular granulosa layer.

Lipases bound to the luminal side of the vascular endothelium may thus be the first in line among several mechanisms facilitating the uptake of fatty acids and cholesterol by ovarian cells[24].

Alpha- amylase activity in ovarian tissue was more during proestrus when follicular size was large and low in diestrus when follicular size was small, because follicular fluid is partially derived from ovarian epithelium.[25] Kasperczyk et al.[25] also suggested that change of amylase concentration might be due to action of sex hormones on ovarian tissues.

According to Malnick et al.[26], brain-type isozyme of creatinine kinase (CKBB) responds to estrogen in female reproductive tract. Chang *et al.*[27] reported that estrogen concentration increased as follicular size increased in pig.

Higher serum copper level in normal mares than in apparently barren animals was observed by Joy and Nair[28] and Prasad and Rao[29]. Prasad and Rao[29] and Kalita *et al.*[30] found significantly low serum zinc level in repeat breeding animals. Increased serum iron level in apparently barren animals was reported by Eltohamy *et al.*[31] and Singh and Pant[32]. The higher concentration of serum manganese level was observed by Eltohamy *et al.*[31] and Prasad and Rao[29] respectively.

Islam et al.[7] carried out a study to determine the role of micro-minerals in increasing pregnancy rates in apparently barren and reproductively normal mares. For this study, normal healthy mares and apparently barren (infertile) mares were selected randomly and were subjected to micro-mineral supplemented ration containing copper, zinc, iron and manganese for 20 days during their vernal period of breeding cycle. After the investigation, it was found that the pregnancy rate in micro-mineral supplemented group was significantly higher ($P<0.05$) than in non-supplemented group. It was evident that pregnancy rate in supplemented group of apparently barren mares was 55.56%, while in non-supplemented group was 33.33%. The pregnancy rate in supplemented group of normal healthy mares was 60% while in non-supplemented group of normal mares was 50%.[7]

Chakraborty et al.[8] in his study reported on the 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 protein and enzyme components of FF like lipase, amylase and CPK increased in concentration as the follicles increased in size, while some other components like AFP, ACP and ALP were found to be decreasing order with increase in follicular size. A few unexplored components like AFP and Li have been studied here in the present study. Moreover, presence of amylase, lipase and CPK in FF and their concentrations was also detected in the porcine FF for the first time in this study. The albumin content had no significant difference between small and medium follicles but differed significantly ($p\leq0.05$) in large follicles. This indicated that follicular growth does not seem to have any effect on albumin content in small and medium follicle. Globulin plays a significant role in the body due to its immunity producing activity. In the present study, globulin was found non-significant in follicular fluid. The small quantity of globulin might be necessary for protecting the follicles from external environments.

The injected rat alpha-fetoprotein was localized to the zona pellucida of follicles undergoing atresia. Although, physiological role of AFP remains an enigma, its presence was found in FF and this study demonstrated that AFP was not only present in porcine FF but its concentration decreased significantly ($p<0.01$) with the development of follicles. However, the functions of AFP in FF remain to be investigated.

Lipase activity in this study found to be highest in large follicular fluid and lowest in small follicular fluid. Amylase activity in this study found to be highest in large follicular fluid and lowest in small follicular fluid. In the present study, CK concentration was found significantly higher in large follicles than in the medium follicles[8].

Ca is one of the macro-minerals which play a major role in reproduction; its deficiency can affect fertility[33]. Marketa *et al*.[34] have 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*.[35] 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[36]. Magnesium acts as a cofactor in enzymatic reaction, and induces meiotic maturation of xenopus oocyte[37].

Fe concentration in follicular fluid was significantly higher ($p\leq0.01$) 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[38]. Guraya *et al*.[39] 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.*[40] 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.

The concentration of all the macro- and micro-minerals in the porcine follicular fluid either increased or decreased with increase in follicular size, but only Ca, P, Cu and Fe concentrations significantly increased and only Li significantly decreased in follicular fluid. The 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[41].

Conclusion

Mineral supplementation at appropriate concentrations can improve the pregnancy rates by reducing the incidences of repeat breeding in breeding livestock. So, optimum formulation of livestock ration mixed with adequate quantity of macro- and micro-minerals proves a stimulating factor in maintenance of pre- and post-pubertal stages in livestock. It also proves to be helpful in remediation of cases of silent heat, infertility, sterility and other reproductive disorders.

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