ENZYME AND ANTIOXIDANT PROFILE OF FOLLICULAR FLUID IN SHEEP

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ABSTRACT

The follicular fluid consists of locally produced substances that act as regulatory factors in follicular development and steroidogenesis. Among the various constituents, alkaline phosphatase, lactate dehydrogenase and antioxidants in the follicular fluid provide information about the metabolic activities and degenerative changes that take place in the cells during follicular growth. Hence, this study was conducted to evaluate the enzyme and antioxidant concentrations of follicular fluid in relation to follicular size. About two hundred and fifty pairs of ovaries of Madras Red sheep in apparently good health were randomly collected after slaughter from Corporation Abattoir, Chennai. Follicular fluid was aspirated from small (less than 2 mm), medium (2-4 mm) and large (greater than 4 mm) ovarian follicles and centrifuged in a cooling centrifuge at 4°C at 3000 rpm for 15 minutes. The supernatant fluid from the three categories of follicles was collected and analyzed for alkaline phosphatase, lactate dehydrogenase, antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxide as per the standard protocol. The data were analyzed statistically by ANOVA. It was observed that the alkaline phosphatase and lactate dehydrogenase activity significantly decreased ($P < 0.05$) as the follicle size increased. The activity of superoxide dismutase and catalase significantly decreased ($P < 0.05$) with an increase in follicle size whereas, glutathione peroxidase activity significantly increased ($P < 0.05$) with increased follicle size.

Keywords: Alkaline phosphatase, lactate dehydrogenase, antioxidants, follicular fluid, sheep.

INTRODUCTION

The ovarian follicle is surrounded by follicular fluid that provides an important microenvironment for the development of oocyte. It contains numerous biochemical components that are essential for steroidogenesis, follicle growth and maturation of oocytes, ovulation and oviductal transport of the oocyte. It also consists of locally produced substances that are related to metabolic activity of the follicular cells (Gerard et al., 2002). This metabolic activity changes during different growth phase of the follicles. The metabolite, ion and enzymatic characteristics of follicular fluid and follicle or oocytes development are highly correlated (Iwata et al., 2006).

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Reactive oxygen species (ROS) are powerful oxidants and physiological by-products of metabolically active gametes and embryos (Agarwal and Allamaneni, 2004). At low concentrations, ROS tend to play important physiological roles *in vitro* such as promoting the developmental competence of oocytes and regulating the rate of preimplantation embryo development. In the follicular fluid of developing oocytes, enzymatic antioxidants, such as superoxide dismutase, catalase and glutathione peroxidase may have an important role in reproductive processes such as folliculogenesis. The reports on the antioxidant levels in the follices are scanty in the ovines. Hence, this study was conducted to evaluate the antioxidant status and enzyme concentration in ovine follicles and to determine their relationship with follicle size.

MATERIALS AND METHODS

Five hundred ovaries of Madras Red sheep in apparently good health were randomly collected after slaughter from Corporation Abattoir, Chennai and transported to the laboratory in 0.9% chilled (4 ^{o}C) normal saline within one hour of slaughter (Thangavel, 1994). The ovaries were washed twice in chilled normal saline. The follicles on the ovarian surface were categorized by measuring the diameter (Shailaja and Kumari, 1984) into small (less than 2 mm), medium (2 - 4 mm) and large (greater than 4 mm). The follicular fluid was aspirated from small, medium and large follicles individually by using 2 mL graduated disposable syringe with 26 gauge needle and pooled separately.

The cellular debris in the aspirated follicular fluid of each group was removed by centrifuging the samples in a cooling centrifuge at 4 ^{o}C at 3000 rpm for 15 minutes. The supernatant fluid of three categories of follicles was collected and enzymes were estimated in the freshly collected follicular fluid. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activity in the follicular fluid were determined spectrophotometrically using standard kits purchased from Agappe Diagnostics, Kerala. Superoxide dismutase was estimated as per Marklund and Marklund (1974), Catalase activity as per Caliborne (1985) and Glutathione peroxidase as per Rotruk et al. (1973).

Statistical Analysis

The data were subjected to one – way analysis of variance (ANOVA) and post hoc analysis were carried out using Duncan's test for multiple comparisons using SPSS software version 20 for windows.

RESULTS AND DISCUSSION

ALKALINE PHOSPHATASE (ALP)

The concentration of alkaline phosphatase in the small, medium and large follicles are presented in Table 1. The present findings indicate a significant decrease ($P< 0.05$) in the alkaline phosphatase activity as the follicle size increased. This was supported by Nandi et al. (2007) who observed a similar significant decrease in alkaline phosphatase activity of the follicular fluid of ovine as the follicular size increased. Henderson and Cupps (1990) and Wise (1987) reported a significant increase in alkaline phosphatase activity in the smallest follicle when compared to medium and large follicles of bovine due to increase in response of follicles to gonadotropin stimulation. This was in accordance with the findings of Kalmath (2000) that the higher alkaline phosphatase activity in the initial stages of follicular development may be due to progesterone and androgen dominant environment that exists in the small follicle, in that a higher concentration of progesterone and androgen could be conducive to phosphatase activity. The decreased activity of alkaline phosphatase activity in follicular fluid with the development of the follicle could be due to the shift in the follicular hormonal milieu from androgen to estrogen dominant phase. The higher alkaline phosphatase concentration in actively

growing small follicles in this study may be due to the influence of Gonadotropin Releasing Hormone (GnRH) and Follicle Stimulating Hormone (FSH) as reported by Henderson and Cupps (1990).

LACTATE DEHYDROGENASE (LDH)

The concentration of lactate dehydrogenase in the small, medium and large follicles are presented in Table 1. The LDH activity showed a significant decrease ($P < 0.05$) as the follicle size increased. The trend observed in this study was similar to the findings of Nandi et al. (2007) in sheep and Wise (1987) in bovines. This may be due to early follicular degeneration and biochemical changes that accompanied the degenerative process (Wise, 1987). The follicles which lacks the support of FSH when a crop of follicles are developing in the active ovary, they tend to go into a physiological process of degeneration process called atresia. Atretic follicular physiology and biochemistry varies. This could have been reasoned for change in LDH level which is seen in our study.

SUPEROXIDE DISMUTASE (SOD)

The concentration of superoxide dismutase in the small, medium and large follicles are presented in Table 1. The SOD activity was significantly increased ($P < 0.05$) in the small follicles when compared to medium and large sized follicles. Similar findings were observed by Singh et al. (1998) in goats and sheep, Louis Paolella and Catherine Combelles (2007) in bovine and Lasota et al. (2009) in pig. Sabatini et al. (1999) reported that a high SOD activity could inhibit the rupture of follicles, a process which is the mechanism based on ROS. Laloraya et al. (1989) observed that SOD activity was localized in developing follicles and in postovulatory follicles. The studies on rat and human ovaries suggested that anion superoxide and SOD may play a role in the ovulation process and in the development of oocytes (Shiotani et al., 1991, Sato et al., 1992) by increasing the level of SOD in the small follicles. In large follicles, ROS may need to be above a certain threshold, and keeping SODs at reduced concentrations within the follicular fluid milieu might provide the appropriate balance of superoxide anion and hydrogen peroxide for normal cell function (Combelles et al., 2010). Similarly, as the follicles grow the demand for oxygen availability (Fischer et al., 1992) and metabolic requirement (Harris & Picton, 2007) increases and it is likely that mechanism exists to manage oxidative stress in the antral follicles, thereby altering the SOD levels.

CATALASE

The concentration of superoxide dismutase in the small, medium and large follicles are presented in Table 1. The catalase activity in the follicular fluid significantly decreased ($P< 0.05$) as the follicle size increased. The value was significantly higher in the small follicles when compared to the medium and large follicles. Sajal Gupta et al. (2011) reported highest levels of catalase in the small follicles of bovine and suggested that catalase may represent a dominant antioxidant of defence in the initial stages of folliculogenesis.

GLUTATHIONE PEROXIDASE

The concentration of glutathione peroxidase in the small, medium and large follicles are presented in Table 1. The concentration of glutathione peroxidase significantly increased ($P< 0.05$) as the follicle size increased. Similar findings were observed by Sesh et al. (2001) in sheep and goat follicular fluid. In contrast, Basini et al. (2008) observed a decrease in glutathione peroxidase activity with an increase in follicle size in swine, whereas, Koli et al. (2007) found no significant difference in glutathione peroxidase with follicle size in antral follicles of bovine.

Glutathione peroxidase is an antioxidant enzyme that catalyzes the destruction of a

variety of organic hydrogen peroxides. Hydrogen peroxides have an inhibitory effect on aromatase activity and thereby decrease the production of estrogen. As glutathione peroxidase scavenge the free radicals, it helps in the sustenance of estradiol level, thus playing an indirect role in the process of steroidogenesis (Sesh et al., 2001).

CONCLUSION

In the present study it has been found that follicular enzymes (ALP and LDH) play an essential role in the development of follicles as they contribute to physical changes and the follicular dynamics. It is obvious that ovaries undergo a phase of stress during active follicular wave pattern due to the influence of gonadotropic hormones as there is a race of follicular dominance. This clearly indicates that the protection from antioxidant enzymes is most essential to combat this physiological phenomenon. This study proves by way of analyzing antioxidant status that there is a protection favouring ovarian activity.

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TABLE 1

ENZYMES AND ANTIOXIDANTS CONCENTRATION IN FOLLICULAR FLUID OF SHEEP (Mean ± SE)

Composition	Small Follicle	Medium Follicle	Large Follicle
Alkaline Phosphatase (IU/L)	331.08 ± 10.12 [a]	312.86 ± 10.23 [b]	248.79 ± 10.97 [c]
Lactate Dehydrogenase (IU/L)	134.36 ± 4.0 [a]	115.16 ± 4.43 [b]	93.34 ± 3.73 [c]
Superoxide Dismutase (mg /L)	371.05 ± 8.36 [a]	329.22 ± 11.70 [b]	237.69 ± 9.10 [c]
Catalase (mMol/L)	923.56 ± 22.7 [a]	832.86 ± 26.33 [b]	745.09 ± 18.49 [c]
Glutathione Peroxidase (µg / ml)	256.16 ± 7.37 [a]	282.86 ± 9.17 [b]	310.19 ± 8.1 [c]

Values with different superscript in the same row differ significantly ($P < 0.05$).