The Indian Journal of Animal Reproduction; 23(I): 38-41; June 2002,

Enzymatic changes in granulosa cells of developing goat follicles

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Received : August 16, 2000
Accepted : March 26, 2002

ABSTRACT

Enzyme activities were measured in granulosa cells from small (<2mm), medium (2-5mm) and large sized (>5mm) follicles of goat ovaries. Acid and alkaline phosphatases and lactate dehydrogenase increased in the granulosa cells from small to large follicles. Levels of enzyme 3β HSDH was very low in small and medium sized follicles and it increased dramatically in the granulosa cells of large follicles. The changes in enzyme systems have been correlated with the metabolism and the steroid biosynthesis in the granulosa cells of the maturing follicles.

Key words: Granulosa cells, ovary, goat, ACP, AKP, LDH, 3βHSDH

INTRODUCTION

The thecal and granulosa cells of the follicle are responsible for maintaining the microenvironment in which the oocyte develops and these cells differentiate in response to the cyclical pattern of gonadotrophin secretion characteristic of the estrous cycle. Information about the control of granulosa cell differentiation has burgeoned over the last few years due to the establishment of culture conditions under which cells would remain viable and acquire differentiated functions in response to hormonal stimuli (Hsueh *et al.* 1984, Guraya.1998). The ultrastructural transfiguration from a protein type profile to that of a steroid producing cell as the follicle changes from secondary to pre-ovulatory stage has been analyzed both qualitatively and using morphometric techniques (Amsterdam *et al.* 1991; Zoller 1991; Guraya 1998,2000). Histochemically lipids, carbohydrates and dehydrogenase showed varied reactivity in different layers of granulosa cells in various mammalian species (Miyamoto *et al.* 1981; Guraya 1985, 1998,2000; Sharma and Guraya 1990; Guraya *et al.* 1998). The present study discusses the enzymatic changes viz. Acid and alkaline phosphatase, lactate dehydrogenase and Δ5-3β hydroxy steroid dehydrogenase in grnulosa cells as they differentiate in the developing follicles, which will help in understanding the mechanisms governing mammalian oocyte maturation.

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MATERIALS AND METHODS

The goat (*Capra hircus*) ovaries were obtained from the mature, non-pregnant goats from the abattoirs of Ludhiana. They were transported immediately to the laboratory in polythene bags packed with freezing mixtures. Upon arrival at the laboratory, the ovaries were dissected free from the uterus and most of the fallopian tube. The ovaries were rinsed with Phosphate Buffered Saline (PBS) and thereafter the granulosa cells were harvested by puncturing the visible normal follicles. Three types of follicles were used as a source of granulosa cells i.e. Small follicles (<2mm), medium follicles (2-5mm) and large follicles (>5mm). The granulosa cells from these three different sized follicles were aspirated by inserting 5ml syringe with a 20-23gauge needle. The cells were separated from follicular fluid by centrifugation at 3000-4000 rpm for 15minutes in cold. The follicular fluid was decanted and 1ml of PBS added and recentrifuged at 4000 rpm for 15 min. to remove the traces of follicular fluid and debris. Supernatant discarded and pellet of granulosa cells was suspended in 1ml of PBS. Cell counting was done by using haemocytometer. Only cells that have clearly defined nuclei and cytoplasm were counted.

The granulosa cells after their isolation from different sized follicles were homogenised in phosphate buffer (0.1 M pH 7.4). The homogenate was centrifuged at 3000-4000 rpm for 10-15 minutes in cold. The supernatant was collected and used for the estimation of enzymes.

Acid and Alkaline phosphatase activity was estimated by the method of Bessy *et.al* (1946) using 0.05M citrate buffer, 5.5x10-3M p-nitrophenyle phosphate, pH 10.5 and 0.05M glycine buffer, 5.5x10-3M p-nitrophenyl phosphate pH 4.8 as buffer-substrate solutions respectively. P-nitrophenol served as control. Activity of Lactate dehydrogenase was measured by the method of King (1965) using sodium pyruvate as substrate. 3-β-Hydroxy Steroid dehydrogenase was estimated according to the method of Agular *et al.* (1992) using pregnenolone as substrate.

Data was evaluated by one way ANOVA and the level of significance was taken at 5%.

RESULTS AND DISCUSSION

Acid phosphatase activity increased significantly in granulosa cells from small to medium follicle and then to large follicle (Table 1). Alkaline phosphatase activity was found to be 2.68 + 0.05 in granulosa cells from small follicles and it increased to 3.67 + 0.30 in the granulosa cells of large follicle. LDH activity showed an increasing trend from small follicle's granulosa cells to large follicle through medium follicle granulosa cells (Table 2).

The enzymatic activity of 3β HSDH is very low in granulosa cells from small and medium sized follicle (Table 2). It increased dramatically in large follicle as compared to small follicle and medium follicle (Table 2). No significant change was observed in the enzyme activity of 3β HSDH in granulosa cells of small and medium follicle.

Phosphatases are involved in many different processes that require mobilization of phosphate ions or dephosphorylation as part of anabolic, catabolic or transfer processes (Henderson and Cupps 1990). Acid phosphatase is a lysosomal enzyme and related to intracellular transport system during synthetic and secretory processes (Bansal and Roy 1997). A concomitant increase in Acid phosphatase activity in the granulosa cells of large follicle is attributed to synthesis

Table 1. Variation (Mean + SE) in activity of acid phosphatases (ACP) and alkaline phosphatase (AKP) in granulosa cells of three different sized follicles

GCs of growing follicles	Enzymes	
	Acid phosphatase (ACP)	Alkaline phosphatase (AKP)
SFg	9.39±0.07^{c}	2.68±0.05^{c}
MFg	13.60±0.14^{b}	3.67±0.30^{b}
LFg	33.70±0/42^{a}	5.06±0.23^{a}

Key to abbreviations:
SFg = Small follicle's granulosa cells.
MFg = Medium follicle's granulosa cells.
LFg = Large follilcle's granulosa cells.

Note: Any two means in a column having same lower case superscript were not significantly different at 5 per cent level of significance.

Table 2.Variation (Mean + SE) in activity of lactate dehydrogenase (LDH) and -3β-hydroxy steroid dehydrogenase (units/mg protein) in granulosa cells of three different sized follicles.

GCs of growing follicles	Enzymes	
	Lactate dehydrogenase (LDH)	-3ß-hydroxy steroid dehydrogenase (-3β-HSDH)
SFg	0.2±0.05^{c}	3.92±0.99^{b}
MFg	0.3±0.07^{b}	4.21±0.87^{b}
LFg	0.5±0.09^{a}	14.55±1.64^{a}

Key to abbreviations:
SFg = Small follicle's granulosa cells.
MFg = Medium follicle's granulosa cells.
LFg = Large follicle's granulosa cells.

Note: Any two means in a column having same lower case superscript were not significantly different at 5 per cent level of significance.

and accumulation of lysosomal enzymes involved in the development/maturation of the follicle (Elfont *et al.*1977; Brietenecker *et al* 1978; Narimoto *et al* 1985; Sangha and Guraya 1989).

Alkaline phosphatase plays a role in ionic movement across the cell membrane and is also associated with secretory and absorptive processes of the cell (Bansal and Roy 1997). Alkaline phosphatase insreased in the granulosa cells with the follicular growth. The Alkaline phosphatase activity in the granulosa cells suggest that it may play its role in active solute transfer. Henderson and Cupps (1990) similarly found high concentration of alkaline phosphatase in the largest follicle group. The pre-ovulatory phase of the estrous cycle is characterized by high concentration of estradiol and gonadotrophins in blood which may increase follicular alkaline phosphatase (Kesner and Comey 1982). Goody *et al* (1982) cited direct and indirect evidence for a role of alkaline phosphatase in steroid receptor inactivation. The change in the enzyme systems have been correlated with the steroid biosynthesis in the granulosa cells of maturing follicles of mammalian ovary (Gore-Langton and Armstrong, 1994; Guraya 1998, 2000).

Lactate dehydrogenase activity increased significantly in the granulosa cells of growing follicles.The high metabolic activity of granulosa cells is correlated with the lactate dehydrogenase activity which increases with the growth of the follicle (Guraya ,1985, 1998). The feasible importance of LDH in the energy supply of the egg cell and in the resumption of meiosis has been reported (Greenwald and Roy, 1994). Earlier histochemical studies of Brietenecker *et al.*(1978) have also shown strong activity of LDH in the granulosa cells of graffian follicles.

The presence of 3β HSDH, an enzyme related to steroid biosynthesis, may simply indicate that the granulosa cells of maturing follicles develop the potential of steroid hormone synthesis which possibly occurs in them during the pre and post-ovulatory periods (Guraya,1985;1997;1998). It may be proposed that theca interna produces the androgens which are transported to the granulosa cells where they are aromatized into 17α–Oestradiol (Guraya 1985,1998); 3βHSDH is involved in the production of progesterone from pregnenolone. However, complete activation of granulosa cell progesterone biosynthesis awaits the LH surge and breakdown of the blood-follicle barrier associated with follicular rupture (Hillier,1994). This latent potential for progesterone secretion is reflected in relatively high levels of steroid which accumulates in antral fluid even before LH surge onset (Hillier 1994). Extensive *in vitro* biochemical studies have revealed the steroidogenic nature of granulosa cells and their ability to convert pregnenolone to progesterone and androgens to oestrogens(Hsueh et al. 1984; Guraya 1998). Hoyer and Anderson (1977) have also observed histochemically that 3β HSDH activity is strongly expressed in rat granulosa cells of pre-ovulatory follicle but is only weakly expressed in earlier antral stages.

REFERENCES

Agular, BM., Vinggaard, AM and Vind, C (1992). Regulation by dexamethasone of the 3ß-hydroxy steroid dehydrogenase activity in adult rat Leydig cells. J. Steroid. Biochem. Mol. Biol., **43:** 565-571.

Amsterdam, A., Suh, BS., Himmelhoc, S., Baum, G and Ben-Zetey, A.(1991). Modulation on granulosa ultrastructure during differentiation; The role of cytoskeleton. In: Familiar, G., Makabe, S., and Motto, PM. (ed) Ultrastructure of the ovary. Kluwer Academic Publishers, pp. 101-112.

Bansal. N. and Roy, KS. (1997) Histoenzymic effects of chlorphrifos toxicity on rabbit genitalia. Indian J. Anim. Sci., **67:** 125-127.

Bessey, OA., Lowry, OH., and Brock, MJ.(1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem., **164:** 321-329.

Brietenecker, G., Friedrich, F and Kemeter, P. (1978). Further investigation on the maturation and degeneration of human ovarian follicles and their oocytes. Fertil Steril., **29:** 336-341.

Elfont, EA., Roszka, JP and Dimino, DT. (1977). Cytochemical studies of acid phosphatase in ovarian follicles: A suggested role for lysosomes in steroidogenesis(rat). Biol. Reprod., **17:** 787-795.

Gore-Langton, RE and Armstrong, DT. (1994). Follicular steroidogenesis and its control. In : Knobil, E and Neill, JD (ed) The Physiology of Reproduction, Raven Press, New York, Vol I, 2nd ed. pp 571-628.

Greenwald, GS and Roy, SK. (1994). Follicular development and its control. In: Knobil, E and Neill, JD (ed) The Physiology of Reproduction, Raven Press, New York, Vol I, 2nd ed., pp. 629-724.

Goody, WW., Schrader, WT and Malley, BWO. (1982). Activation, transformation and sub-unit structure of steroid hormone receptors. Endocrinol. Rev., **3**: 141.

Guraya, SS. (1985). Biology of Ovarian Follicles in Mammals. Springer-Verlag, Berlin, Heidelberg, New York.

Guraya, SS. (1997). Ovarian Biology in Buffaloes and Cattle. ICAR, New Delhi.

Guraya, SS. (1998). The comparative cell biology of accessory somatic (Follicleor Granulosa) cells in the animal ovary. Proc. Indian Nat. Sci. Acad., **64**: 161-196.

Guraya,SS. (2000). Comparative cellular and molecular biology of ovary in mammals-Fundamental and applications. Science Publishers, USA.

Guraya, SS., Monga, S., Kaur, P and Sangha, GK. (1998). Comparative morphological and histochemical studies of ovarian follicles in the goat and sheep. Indian J. Anim. Sci., **68**: 619-628.

Henderson, KA and Cupps, PT.(1990). Acid and alkaline phosphatase in bovine antral follicles. J. Anim. Sci., **68**: 1363-1369.

Hillier, Sg. (1994). Hormonal control of folliculogenesis and luteinization. In: *Molecular Biology of the Female Reproductive System*. Findly, JK (ed) Academic press, San Diego, USA. Pp 1-37.

Hoyer, PE and Anderson, H.(1977). Histochemistry of -3ß-hydroxy steroid dehydrogenase in the rat ovary. Histochemistry, **51**: 167-193.

Hsueh, AJW., Adashi, EY., Jones, PBC., Welsh, TH.(1984). Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocrinol. Rev., **5**: 76-126.

Kesner, JA and Comey, EM.(1982). Interaction of estradiol and luteinizing hormone releasing hormone on follicle stimulating hormone release in cattle. J. Anim. Sci., **54**: 817.

King, J.(1965). *Practical Clinical Enzymology*. D Van Nostrand Co., London.

Miyamoto, H., Ishibashi, T. and Utsumi, K.(1981). The histochemical distribution of succinate dehydrogenase in the goat ovary. Japan J. Zootech. Sci., **52**: 58-62.

Narimoto, K., Okamura, H., Mori, T., Sakai, M., Espey, LL., and Ogawa, K. (1985). Cytochemical localization of acid phosphatase in rabbit ovarian follicle. Acta. Histochem. Cytochem., **18**: 525-538.

Sangha, GK., and Guraya, SS.(1989). Histochemical changes in acid and alkaline phosphatase activities in the growing follicles and corpora lutea of the rat ovary. Acta. Morphol. Neerl. Scand., **26**: 43-50.

Sharma, RK and Guraya, SS.(1990). Granulosa heterogenity: A histochemical lectin staining and scanning electron microscopic study on *Rattus rattus* ovary. Acta. Embriol. Morph. Exp., **11**: 107-129.

Zoller, LC.(1991). Quantitative analysis of the membrana granulosa in developing and ovulatory follicles. In: Familiar, G. Makabe, S. and Motto, PM.(ed). Ultrastructure of the Ovary. Kluwer Acdemic Publisher, pp.73-90.