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Activity of glutathione related enzymes and ovarian steroid hormones in different sizes of follicles from goat and sheep ovary of different reproductive stages

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The investigations on enzymes related to glutathione like glutathione-S-transferase (GST) and glutathione peroxidase (GSH-Px) have been carried out mostly in human and rat ovaries, however the studies on these enzymes in ruminants are relatively absent. In the present study the changes in the activity of these enzymes, in different sizes of follicles from goat and sheep ovaries of different reproductive stages, were investigated. The results demonstrated that the activity of the enzyme GST increased with the increase in size of the follicles from small to large follicles of follicular phase ovary and from small to medium follicles of luteal phase ovary in both the species, thereafter it decreased in large follicles of luteal phase ovary. There was increasing pattern in the activity of GSH-Px in the follicular phase follicles and a decreasing pattern in the luteal phase follicles from both the species. Thus the changes in the activity of glutathione related enzymes namely GST and GSH-Px in different size follicles from both the species during different reproductive phases are evident from the results. It is reasonable, therefore, to assume that these enzymes may have functional role in the steroid hormone metabolism in ruminant ovary as reported in human ovary.

The glutathione (GSH) is an ubiquitous tripeptide that contributes extensively to intermediary metabolism and to toxification and detoxification processes[1,2]. Despite the intense investigation on glutathione regulation in hepatic and renal tissues, there have been few studies on this important intracellular thiol compound in ovarian tissue. Mettison and coworkers have reported the presence of glutathione in the rat ovary[3]. The enzymes related to GSH like glutathione S-transferase (GST)[4] and glutathione peroxidase (GSH-Px)[5] were also found in the mammalian ovary. The glutathione S-transferases are multifunctional proteins present in the many organs[6] and show tissue specific expression[7,8]. They have capacity to detoxify electrophilic xenobiotics including cytotoxic drugs and carcinogens by catalyzing their conjugation and with reduced GSH[6,7]. The biological function and endogenous substrate of GSTs are not very much clear but they are known to bind corticosteroids and steroid hormone metabolites and play an important role in the intracellular transport of these substances[9,10]. Recently, we have demonstrated the subcellular distribution of GST, change in its activity during estrous cycle and increase in its activity by estradiol-17ß in rat ovary which indicates the functional role of this enzyme in the ovary under endocrine regulation[11]. The activity of GSH-Px involved in the hydroperoxide and GSH metabolism has been found to change during estrous cycle[12]. The age related changes in the activity of GSH-Px have also been documented in human ovary[5]. The biochemical function performed by GSH-Px in this organ have been poorly elucidated. Most of the investigations on these enzymes have been carried out in human and rat ovary while studies on glutathione related enzymes namely GSTs and GST-Px in ruminants in relation to follicular development are very few. The present study was, therefore, undertaken to evaluate the changes in the activity of GST and GSH-Px in different sizes of follicles from goat and sheep ovary of different reproductive stages.

Collection of samples and tissue preparation — Goat and sheep ovaries at random stages of the estrous cycle were obtained within 10-20 min after slaughter of animals from local slaughter house at Karnal. The ovaries were placed in cold saline and transported to the laboratory. These ovaries were further washed in normal saline gently 2-3 times. The stromal tissue surrounding the ovaries were clipped off using scissors. These were kept at 4°C until further processing. The follicles from ovaries of both the species were isolated mechanically with the help of scissors, forceps and 24G needle. These

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follicles were grouped into six categories and pooled according to their size as per the modified method of Moor *et al*[13]. According to size, the follicles were grouped as follicular small and luteal small (>3 mm), follicular medium and luteal medium (3-6 mm), and follicular large and luteal large (>6 mm) follicles. The pooled follicles in each size group were taken for further processing.

Preparation of follicular homogenate—The number of follicles taken in the follicular small, medium and large groups were in the range of 80-85, 10-12 and 4-6 respectively, while those in the luteal small, medium and large groups were in the range of 18-20, 3-5 and 2-4 respectively for sheep. Likewise for goat, the number of follicles taken in the follicular small, medium and large groups were 22-25, 5-6 and 4-5 respectively, whereas those in the luteal small, medium and large groups were 10-21, 3-4 and 1-2, respectively. These follicles in each group were weighed and homogenized separately using tissue homogenizer in 3 ml of 0.25 *M* sucrose solution, at the speed of 10,000 cycles, twice, each for 10 sec at 4°C. The homogenate was centrifuged at 600*g* for 10 min at 4°C. The supernatant collected from each group were stored separately in sample vials at 4°C for the estimation of proteins and enzyme assays and about 0.5 ml of the supernatant from each group was stored separately below -20°C for the hormone assay.

Enzyme assay—The enzyme glutathione-S-transferase was assayed by the method of Habig *et al.*[14] using 1-chloro-2,4 dinitrobenzene (CDNB) as substrate. The rate of reaction was followed by measuring the increase in absorbance at 340 nm against the control cuvette (without enzyme solution). The activity was calculated using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. The specific activity of enzyme was expressed as nmol of 1-chloro-2,4- dinitrobenzene (CDNB) conjugated/mg protein/minute.

Glutathione peroxidase was assayed by the method of Mohands *et al.*[15] using NADPH as co-factor. The disappearance of NADPH was shown by the decrease in absorbance at 340 nm with 15 secs of intervals for 2 min against control cuvette (without co-enzyme solution). The specific activity was calculated using an extinction coefficient of 6.22 mM^{-1} cm^{-1} and expressed as nmol of NADPH oxidised/min/mg protein.

Hormone assay—Steroid hormones were assayed in follicular homogenate by modified method of Arora and Panday[16]. The sensitivity of the assay calculated on the basis of different standard curves were 2.5 and 7.5 pg for estradiol-17ß and progesterone, respectively. The inter-assay and intra-assay variation in hormone was 12.60% and 11.09% and 11.20% and 11.09% for estradiol-17ß and progesterone, respectively.

Activity of GST

Sheep—The GST activity increased progressively from follicular small (248.74 ± 10.40) to follicular large (346 ± 10.21) and then from luteal small (229.67 ± 9.2) to the luteal medium (328.50 ± 11.34) follicles. After luteal medium, the activity dropped down to 142.26 ± 6.75 in the luteal large follicles (Fig. 1).

Goat—The GST activity determined in the different sizes of goat follicles showed a similar pattern as that of sheep. The specific activity showed a progressive increase from follicular small (227.59 ± 7.22) to follicular large (330.95 ± 0.14) follicles and from luteal small (189.66 ±4.06) to luteal medium (245.25 ± 7.26) follicles and then decreased to 155.26 ± 5.31 in the luteal large follicles (Fig. 1).

Activity of GSH-Px

Sheep—The highest specific activity of GSH-Px was in the follicular large (42.41 ± 1.22) and lowest was in luteal large 18.36 ± 0.16 (Fig.2).Thus specific activity showed an increasing pattern in the follicular phase and a decreasing pattern in the luteal phase.

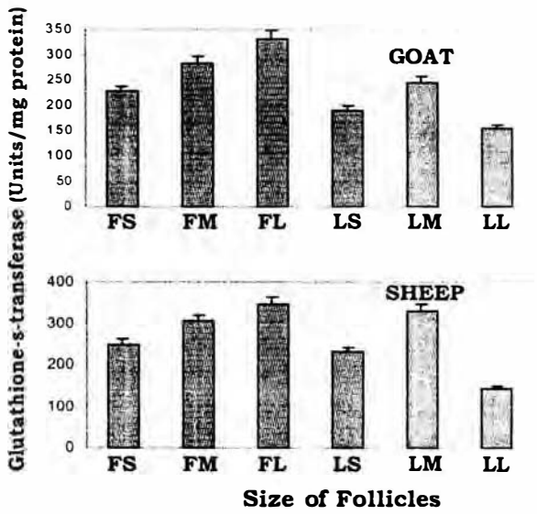

Fig. 1—Activity of glutathione-s-transferase in different sizes of follicles from sheep and goat ovaries. Value represents mean±SE of three experiments with pooled follicles in each group. FS=follicular small, FM=follicular medium, FL=follicular large, LS=luteal small, LM=luteal medium, LL=luteal large.

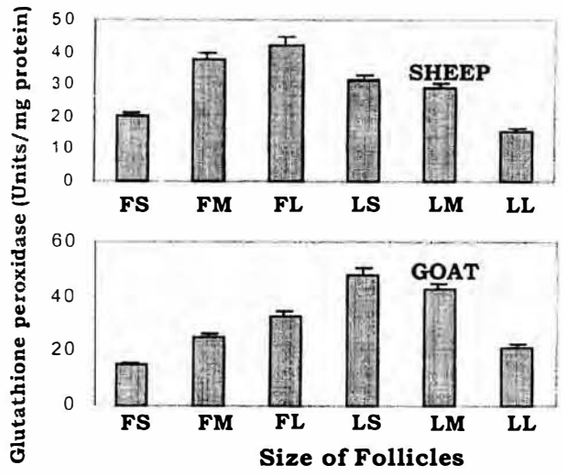

Fig.2 — Activity of glutathione peroxidase in different sizes of follicles from sheep and goat ovaries. Value represents mean±SE of three experiments with pooled follicles in each group. FS=follicular small, FM=follicular medium, FL=follicular large, LS=luteal small, LM=luteal medium, LL=luteal large.

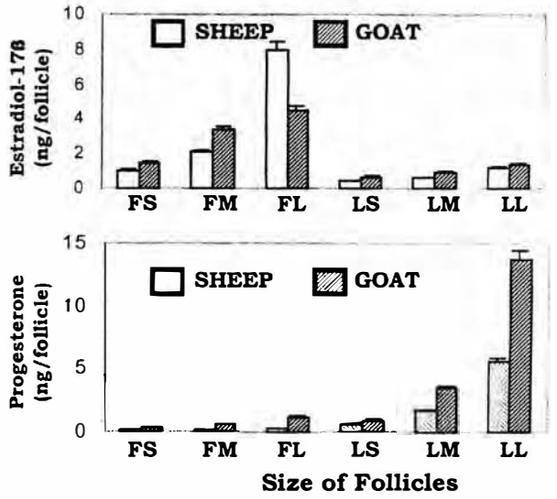

Fig. 3—Content of estradiol-17ß and progesterone in different sizes of follicles from sheep and goat ovaries. Value represents mean±SE of three experiments with pooled follicles in each group. FS=follicular small, FM=follicular medium, FL=follicular large, LS=luteal small, LM=luteal medium, LL=luteal large.

Goat—The highest specific activity (48.23 ± 0.48) of GSH-Px was present in luteal small follicles and the lowest in follicular small (15.23 ± 0.48) follicles, in contrast to that of sheep. However, the pattern of GSH-Px activity in both follicular phase and luteal phase follicles was same in case of sheep. The range of specific activity of the enzyme was 15.23 ± 0.20 to 32.82 ± 0.42 from follicular small to large and 48.23 ±0.48 to 21.77 ± 0.26 from luteal small to large follicles (Fig 2).

Steroid hormones—The estradiol-17ß expressed as ng/follicle(Fig 3) was highest in follicular large (8.0 ± 0.46 in sheep and 4.5 ± 0.73 in goat) and lowest in luteal small (0.43 ± 0.09 and 0.66 ± 0.08 in sheep and goat respectively) follicles. The content of estrodiol-17ß increased progressively, as the size increased ranging from 1.02 ± 0.14 and 1.50 ± 0.18 to 8.00 ± 0.46 and 4.5 ± 0.73 in case of sheep and goat follicular phase follicles, respectively. The content of progesterone(Fig 3) was highest in the luteal large follicles (5.68 ± 0.23 and 13.70 ± 0.37 in sheep and goat ovary, respectively) followed by luteal medium, luteal small, follicular large, follicular medium and follicular small follicles (0.16 ± 0.04 and 0.22 ± 0.05 in case of sheep and goat respectively).

The results of this study demonstrated that the activity of the enzyme GST increased with the size of the follicles from follicular small to follicular large and from luteal small to luteal medium in both the sheep and goat follicles. Thereafter it decreased in large luteal. It has also been reported that the maximum activity of GST was seen at oestrus stage of oestrous cycle in rats, and estradiol-17ß was able to induce GST[11]. It is well recognised that GSTs are inducible by compound known to alter the expression of drug metabolizing enzymes[8]. A recent report indicated that the activated oxygen species may activate GST which supports the concept of enzyme action. Glutathione itself is clearly an antioxidant and it has been suggested, that essentially all of the glutathione linked enzymes can be considered to be, directly or indirectly, involved in the metabolism of reactive products of oxidative metabolism[8]. Rahilly *et al.*[17], based on their studies on distribution of GST isozymes in human ovary, concluded that the GST is closely associated with the glutathione dependent enzyme -5, 3-ketosteroid isomerase which catalyses the conversion of pregnenolone to progesterone along with 3ß-HSD and dehydroepiandrosterone to androstenedione. GST binds a range of steroid hormone exhibiting high affinity for progesterone[10]. In general GST may transfer cytoplasmic bound steroid to high affinity nuclear receptors[10]. As the result of this study shows an increasing pattern of GST in the follicular phase and luteal phase with size of the follicles, it becomes evident that GST definitely has a functional role in the process of folliculogenesis and steroidogenesis.

Glutathione peroxidase is antioxidant enzyme that catalyzes the destruction of a variety of organic hydrogen peroxides. Pinto and Bartley[12] reported that

the changes in the GSH-Px activity was associated with the changes in estrogen having a prominent effect. Raymond *et al.*[18] on their studies on rat ovary reported that the activity of GSH-Px increased in follicular phase and decreased in luteal phase, and the enzyme is under hormonal control. Pinto and Bartley[19] have also reported that, the activity of GSH-Px was maximum at oestrous and minimum at dioestrus in the rat ovaries. Okatani *et al.*[5] showed the inhibitory effect of peroxide on aromatase activity, which was attenuated by GSH-Px. They also concluded that there is a remarkable decline in the activity of antioxidative scavenger system (glutathione peroxidase) in human ovary with age, in contrast to the smaller decrease in free radical production. These free radical will decrease the estrogen production through their effects on aromatase activity. As the GSH-Px scavenge the free radicals, it helps in the sustenance of estradiol level, thus playing an indirect role in the processes of steroidogenesis. The results of the present study showed an increasing pattern of GSH-Px activity in the follicular phase and a decreasing pattern in the luteal phase follicles, thus in close agreement with the results of Pinto & Bartley[19] and Raymonds *et al*[18]. It is clear that there seems to be a requirement of GSH-Px in follicular phase for the estrogen production (indirectly) while in the luteal phase it is not required due to the progestational dominance. These observations agree completely with those of estradiol-17ß and progesterone content in different groups of follicles of both the species in this study (Fig. 3). Thus the change in the activity of glutathione related enzymes namely GST and GSH-Px in different size follicle from both the species of ruminant during different reproductive phases is evident. It is reasonable, therefore, to assume that these enzymes may have functional role in the ruminant ovary as in human ovary. However, further study is still required to establish its functional role in the follicular development and differentiation.

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