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Research Article

HISTOENZYMIC LOCALIZATION OF PHOSPHATASES AND OXIDOREDUCTASES IN ATRETIC FOLLICLES OF INDIAN BUFFALO OVARY

ADHIRAAJ SINGH, DEVENDRA PATHAK*, OPINDER SINGH AND NEELAM BANSAL
Department of Veterinary Anatomy, College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

ABSTRACT

An increased rate of follicular atresia has been implicated as one of the major factors for reproductive failure in buffalo. The present study was aimed to histolocalize the phosphatases and oxidoreductases in ovary of buffalo to correlate its localization with type and stage of atresia. Cryostat sections of 6-7 µ thickness of ovary at -20°C were obtained on glass slides with cryostat microtome and incubated with different substrates for the demonstration of phosphatases and oxidoreductases. Histoenzymic activities of phosphatases and oxidoreductases were not much distinguishable in healthy primordial and primary follicles and the atretic ones. Granulosa cells of tertiary follicles undergoing atresia showed reduced activity of these enzymes. Reduction in the activity of the enzymes in the granulosa cells was a gradual process and the activity reduced from the first degree to third degree of atresia. The theca cells of cystic atretic follicles and proliferating cells of obliterative atretic follicles showed strong to intense reactions for phosphatases and oxidoreductases. The higher enzyme activity of these enzymes might correspond to the higher secretory activity of these cells. The obliterative atretic follicles with high enzyme activity might contain epitheloid type of cells which might be responsible for steroidogenesis.

Key words: Atretic follicles**,** buffalo, histoenzyme, ovary, oxidoreductases, phosphatases

Reproductive efficiency is the primary determinant affecting productivity in female buffalo, but is greatly hampered by late attainment of puberty, seasonality of calving, long postpartum anestrus and subsequent calving interval (Barile, 2005). Ovary is the primary dynamic reproductive organ having dual function of gametogenesis and steroidogenesis. Folliculogenesis is a dynamic event which finally leads to ovulation or atresia.An increased rate of follicular atresia has been implicated as one of the major factors for reproductive failure in buffalo (Rajesha *et al.,* 2001). Atresia of ovarian follicles could be associated with various morphological, biochemical and histological and histoenzymic changes. Follicular atresia may have a significant impact on the number of follicles available for maturation and fertilization in adult. Availability of different enzymes is required for the follicular growth and atresia. The present study was aimed to localize the phosphatases and oxidoreductases in ovary to correlate its localization with type and stage of atresia.

MATERIALS AND METHODS

Fresh unfixed tissues from ovary of Indian buffaloes were collected from slaughter houses immediately after the slaughter of the animals and placed in tissue freezing medium (Leica) and frozen in liquid nitrogen. Cryostat sections of 6-7 µ thickness at -20°C were obtained on glass slides with cryostat microtome and incubated with different substrates for the demonstration of phosphatases and oxidoreductases. The phosphatases included alkaline phosphatase (AKPase) and glucose-6-phosphatase (G-6-Pase) whose activity was tested as suggested by Barka and Anderson (1963). The oxidoreductases included succinic dehydrogenase (SDH), lactate dehydrogenase (LDH), glutamic dehydrogenase (GLD), glucose-6-phosphate dehydrogenase (G-6-PD), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase), and reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase) whose activity was tested using the protocol of Pearse (1972). The positive and negative controls were used wherever possible.

*Corresponding author: drdevendra@gmail.com

RESULTS AND DISCUSSION

Histoenzymic activities of phosphatases and oxidoreductases were not much distinguishable in healthy primordial and primary follicles and the atretic ones. Variable activity of enzymes was present in the primordial follicles and primary follicles but was not much distinguishable as healthy or atretic ones. Histoenzymic distribution of these enzymes was differentially noted in antral follicles undergone different types of atresia.

PHOSPHATASES

Alkaline Phosphatase: The AKPase activity was moderate to strong in primordial and primary follicles

(Fig. 1). Atretic follicles showed weak to moderate reactions of AKPase. A decreasing trend of AKPase activity in the atretic follicles of buffalo ovary was reported by Bhardwaj and Roy (2001). Intense reaction was observed in the membrane granulosa and theca cells in a healthy tertiary follicle (Fig. 2). The reaction was moderate to strong in the granulosa cells detached from the tertiary follicles during the follicular atresia while it was intense in theca cells similar to the healthy follicles (Fig. 3). In the cystic atresia the theca cells showed strong reaction for AKPase while there was no reaction in the lumen (Fig. 3). In the obliterative atresia the proliferating cells which were destined to fill the lumen and form scar tissue showed strong to intense reaction for AKPase. Thus it could be postulated that the theca cells in cystic atretic follicles and proliferating cells are active and hence atresia is an active degenerative process as this enzyme might be involved in the proliferative activity. A1KPase is known to play a role in the metabolism of phosphate esters (Sangha and Guraya, 1989). Alkaline phosphatase and acid phosphatase are lysosomal enzymes which catalyze various reactions in the body and are involved in the active transport of protein and DNA turnover in nucleus (Mishra *et al*., 2003). Similar observations in ovaries have been made by Henderson and Cupps (1990) in bovine ovaries and Khera *et al.* (1994) in buffalo and Bordoloi *et al.* (1999) in goat ovary.

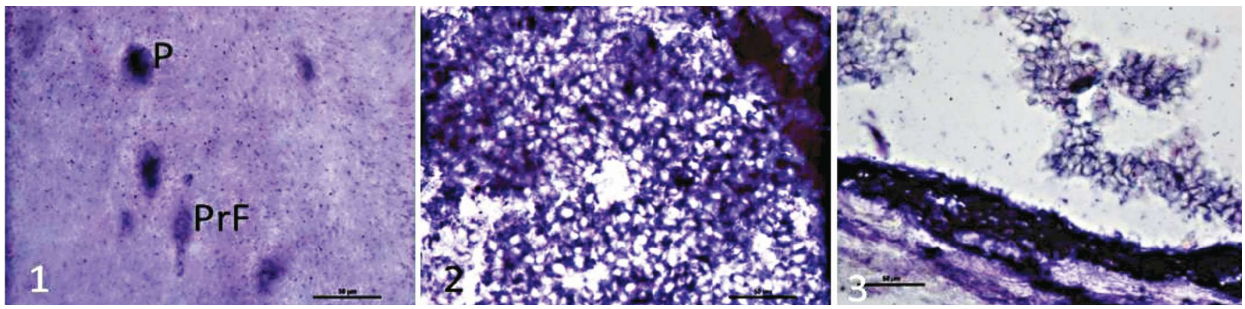
Glucose-6-phosphatase (G-6-Pase): The reaction of G-6-Pase was strong in the membrane granulosa and theca cells of healthy tertiary follicles (Fig. 4). The reaction was weak to moderate in membrana granulosa of the early atresia tertiary follicles while it was weak in the late atretic tertiary follicles. Lumen of cystic atretic follicles was devoid of any enzyme activity (Fig. 5). The activity was moderate in the proliferating cells of obliterative type of atretic follicles.G-6-Pase enzyme is responsible for glucose metabolism and may be associated with steroid synthesis of the cells and thus its low availability indicates that there may be reduced metabolic activity in atretic cells. Similar observations have been recorded by Bhardwaj (1996) in buffalo ovary.

OXIDOREDUCTASES

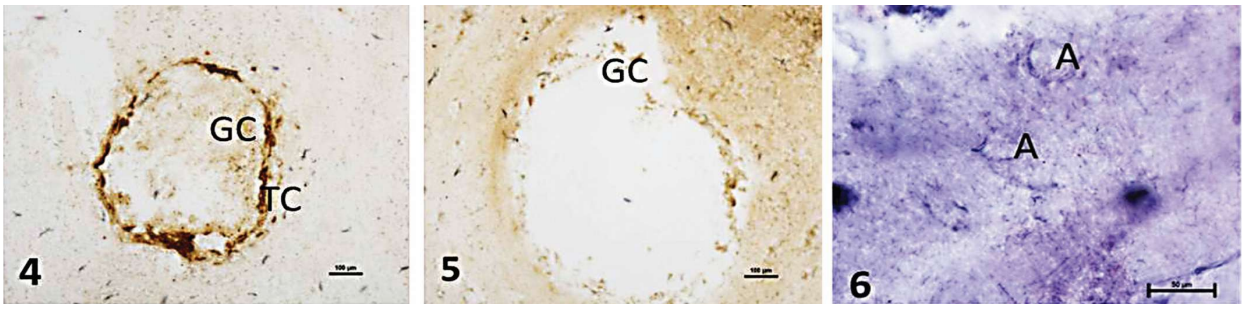
Glucose-6-Phosphatase Dehydrogenase (G-6-PD): The reaction of G-6-PD was strong and intense in primordial and primary follicles however, the atretic follicles had reduced activity of this enzyme (Fig.6). Its reactivity was strong in the membrana granulosa and theca cells of healthy tertiary follicles. The reaction was weak to moderate in membrana granulosa of the early atresia tertiary follicles (Fig. 7) while it was weak in the late atretic tertiary follicles. Lumen of cystic atretic follicles was devoid of any enzyme activity while the theca cell boundary of the cyst showed strong reaction for the enzyme. It was moderate in the proliferating cells of obliterative atretic follicles (Fig. 8) and weak to moderate in scar tissue formed at completion of atresia. Alterations in G-6-PD have been reported toalter the supply of energy to the cells (Zhang *et al.,* 2000).

Succinic Dehydrogenase (SDH): The reactivity of SDH enzyme was strong to intense in membrane granulosa and was moderate in the theca layer of healthy tertiary follicles (Fig. 9). The cystic atretic follicles showed strong reaction in the theca cells while the granulosa cells were lost due to degenerative changes (Fig. 10). The obliterative atretic follicles showed strong reaction during proliferative stage of atresia (Fig. 11) while it was moderate during the later stage.SDH is known to play an important role in steroidogenesis as it is closely linked to cytochrome system (Motta and Hafez, 1980).The higher enzyme activity might correspond to the higher secretory activity of membrana granulosa cells and in proliferating atretic cells.

Lactate Dehydrogenase (LDH): The activity of LDH was intense in the healthy as well as atretic primordial and primary follicles (Fig. 12). The follicular cells of growing follicles showed intense reaction (Fig. 12 inset) while strong and moderate reactions were observed in the membrana granulosa cells of the normal tertiary follicles and theca cells, respectively (Fig. 13 inset). The membrane granulosa cells of early atretic follicles showed moderate reactions while theca cells exhibited strong reaction of LDH (Fig. 13).Weak reaction of LDH was observed in the membrana granulosa cells and strong reactions were seen in the theca cells of late atretic follicles and at the last stage of atresia, only theca layer with strong reaction were observed with no granulosa cells. In the early stage of obliterative type of atretic follicles, proliferating cells showed moderate reactions for LDH which became stronger during the later stage of proliferation and intense at later stage of atresia(Fig.14). The LDH enzyme is responsible for conversion of



Cryostat sections of buffalo ovary showing Fig. 1. Intense reaction of alkaline phosphatase in the healthy primary and primordial follicles and weak in atretic follicle; Fig. 2. Intense reaction of enzyme alkaline phosphatase in the healthy follicle both in the theca layer an in granulosa cells in atretic follicle; Fig. 3. Intense reaction of enzyme alkaline phosphatase in the theca layer and weak in granulose cells in atretic follicle. Inset showing intense reaction in the collapsed theca layer of atretic follicle. Azo dye method. Original magnification x 400.



Cryostat sections of buffalo ovary showing Glucose-6- phosphatase; Fig. 4. Strong reaction of G-6-Pase in granulosa cells (GC) and theca cells (TC) of the healthy tertiary follicles. Original magnification x 100, Fig. 5. Moderate reaction in the theca layer and weak in granulosa cells (GC) in late atretic follicle. Lead nitrate method. Original magnification x 100. Cryostat sections of buffalo ovary showing G-6-PD activity Fig. 6. Weak to moderate reaction in atretic preantral follicles; Nitro BT method, Original magnification x 400.

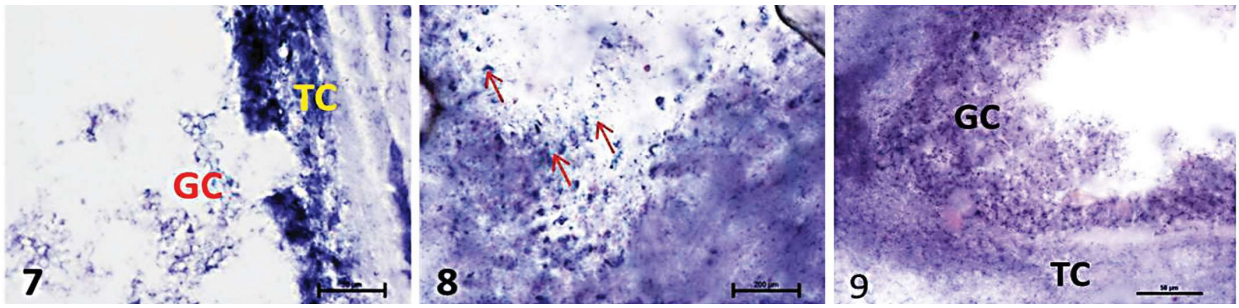


Fig. 7. Moderate weak reaction in granulosa cells (GC) and intense in theca the theca layer (TC) of tertiary atretic follicle; Nitro BT method, Original magnification x 400. Fig. 8. Moderate reaction in the proliferating cells (arrows) of obliterative type of atretic follicles. Nitro BT method, Original magnification x 100. Cryostat sections of buffalo ovary showing Fig. 9. Strong reaction of SDH in the granulosa cells (Gc) of healthy tertiary follicles. Original magnification x 400.

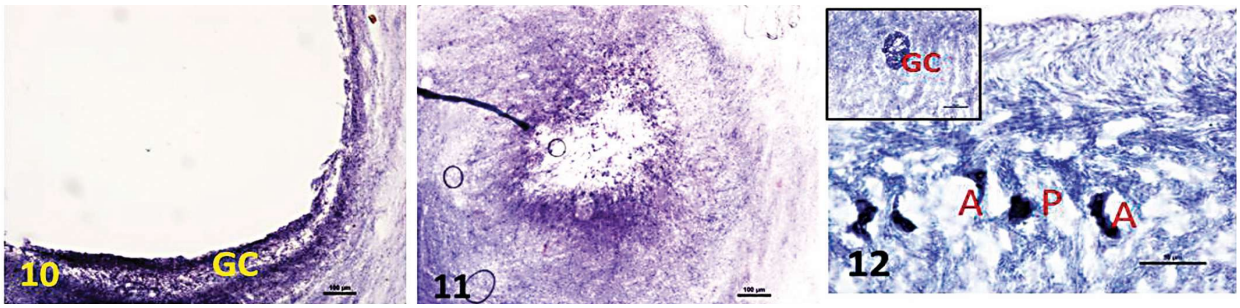


Fig. 10. Strong reaction theca cells (Tc) of cystic atretic follicles, Original magnification x 100; Fig. 11. Strong reaction in proliferating cells of obliterative type of atretic follicles. Nitro BT method. Original magnification x 100. Cryostat sections of buffalo ovary showing Fig. 12.Intense reaction of LDH in the healthy Primordial (PrF) and primary follicles (P). Inset showing intense reaction in granulosa cells of healthy growing follicle.

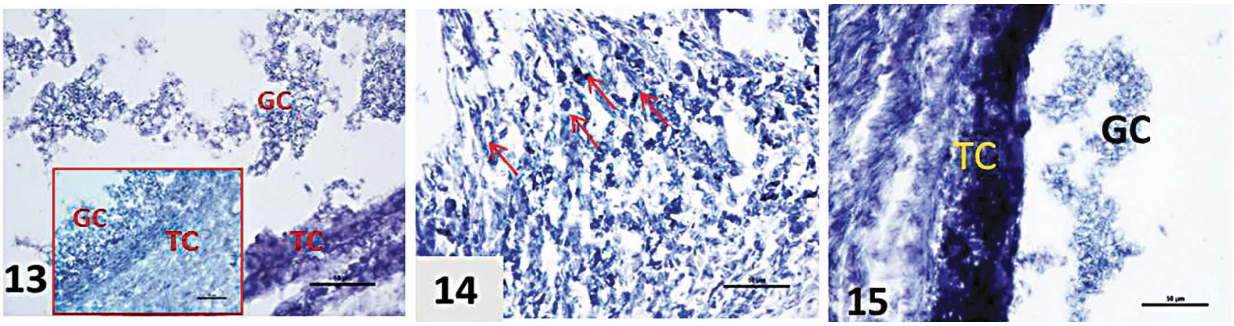


Fig. 13. Moderate to strong reaction in granulosa cells (Gc) and strong in theca layer (Tc)of early tertiary atretic follicle; Inset showing strong reaction in granulosa cells (Gc) and Moderate in theca the theca layer (Tc) of healthy tertiary atretic follicle; Fig.14. Strong reaction in the proliferating cells (arrows) in the late obliterative type of atresia. Nitro BT method. Original magnification x 400. Cryostat sections of buffalo ovary showing Fig. 15. Weak reaction of NADHD in granulosa cells and intense reaction in theca layer of atretic follicles.

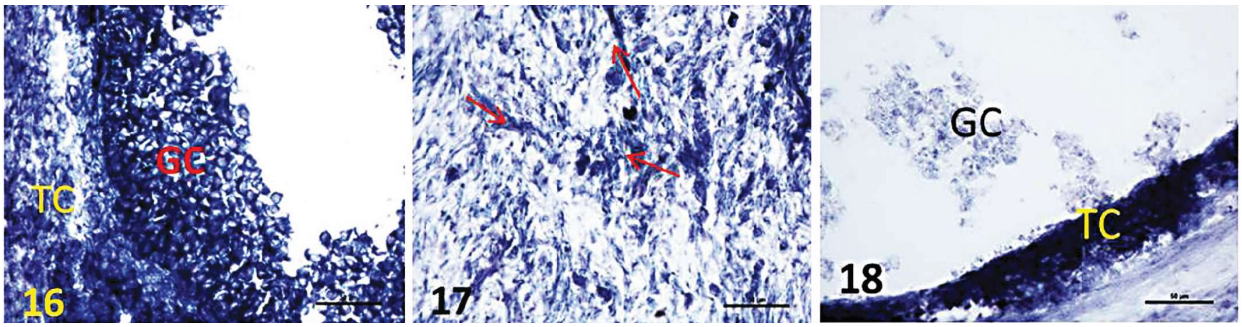


Fig. 16. Intense reaction in granulosa cells and strong reaction in of healthy tertiary follicle; Fig. 17. Strong reaction in the proliferating cells (arrows) in the obliterative type of atresia. Nitro BT method. Original magnification x 400. Cryostat sections of buffalo ovary showing Fig. 18. Weak reaction of NADPHD in granulosa cells and intense reaction in theca layer of atretic follicles.

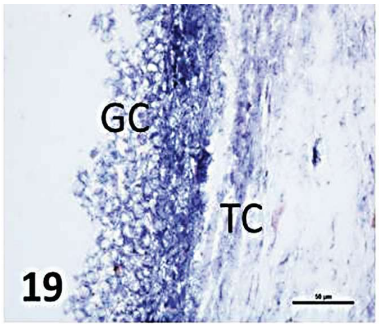


Fig. 19. Intense reaction in granulosa cells and strong reaction in of healthy tertiary follicle.

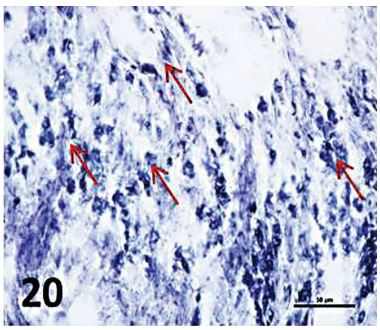


Fig. 20. Strong reaction in the proliferating cells (arrows) in the obliterative type of atresia. Nitro BT method; Original magnification x 400.

glucose to acetyl coenzyme-A, which is utilized for the active synthesis of fatty acids and steroids. Nandi *et al*. (2007) observed the trend of higher LDH activity in small follicles where atresia was more common. Our findings are in accordance with an earlier study in cattle (Wise *et al*., 1987) in which these authors suggested that as the follicle degenerated, there were distinct biochemical changes that accompanied the degenerative process. Isoenzyme analysis of LDH activity in follicular fluid might increase the accuracy and sensitivity of LDH for a biological atresia marker in follicles.

NADH Diaphorases: The activity of NADH diaphorases was moderate in the atretic granulosa cells of tertiary follicles while theca layer showed an intense reaction (Fig.15). The healthy tertiary follicle exhibited strong to intense reaction both in granulosa cells and theca cells (Fig.16). In the cystic type of atretic follicles, the lining theca cells showed moderate reaction while no granulosa cells were observed in antrum. In the early stage of obliterative type atresia, the proliferating cells invaginating towards the centre of the follicle showed strong reaction while degenerating cells showed weak

reaction. In the later stage of the obliterative type of atresia, the proliferating cells exhibited strong to intense reaction (Fig.17). The scar tissue was formed at the last stage of obliterative type of atresia. Only few proliferating cells showed moderate to strong reaction, while majority of cells exhibited weak reaction. Thus it can be concluded that in the obliterative type of atresia, the proliferative cells were enzymatically active. Similar observations have been recorded by Bhardwaj (1996) in buffalo ovaries.

NADPH Diaphorases: In the cystic type of atretic follicles weak reaction of NADPHd was noticed (Fig.18). In the healthy tertiary follicles, moderate granular reaction of enzyme was observed in the granulosa cells as well as slightly intense reaction in the theca cells (Fig.19). Granular reaction was observed in the cytoplasm of granulosa cells. In the obliterative type of atresia the proliferating cells invaginating towards the antrum showed strong NADPHd reaction while others showed very weak reaction. A typically strong NADPHd reaction was observed in obliterative atretic follicles whereas strong reaction in theca cells and moderate reaction was observed in the corpus atreticum (Fig. 20). Similar observations were made by Bhardwaj (1996) in buffalo ovary.The enzyme is responsible in steroidognesis by converting cholesterol to progesterone (Sorenssen and Singh, 1973) and fatty acid synthesis (Hoyer 1980).Thus we can conclude that only proliferating cells invaginating in obliterative atresia and theca cells in normal healthy follicle were enzymatically active. Granulosa cells were active only in healthy follicles.

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