Haryana Vet. (Dec., 2018) **57 (2)**, 140-142

Research Article

ENZYME HISTOCHEMISTRY OF OVARIAN RETE SYSTEM IN BUFFALO *(BUBALUS BUBALIS)*

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Received: 20.09.2017; Accepted: 21.09.2018

ABSTRACT

The present study was conducted on the fresh ovaries of six buffaloes collected from the abattoir immediately after sacrifice of the animal. Depending upon the age, the animals were divided into three groups with two animals in each; prenatal, prepubertal and pubertal. The cryostat sections of 10-12 µ thickness were obtained at -23°C and were incubated in different substrates for demonstration of phosphatases, oxidoreductases and esterases. The study revealed a variable activity of phosphatases (alkaline phosphatase, glucose-6-phosphatase), oxidoreductases (succinic dehydrogenase, lactate dehyrodenase, glucose-6-phosphate dehydrogenase**,** reduced nicotinamide adenine dinucleotide phosphate diaphorase, reduced nicotinamide adenine dinucleotide diaphorase) and esterases (non-specific esterases) in rete system of buffalo during different age groups. The enzymatic activity has been correlated with the physiological function of the extraovarian rete, connecting rete, intraovarian rete at different stages of reproduction.

Key words: Buffalo, Prenatal, Prepubertal, Pubertal, Ovarian rete system.

Ovary is responsible for both exocrine and endocrine functions. The structure of ovary varies with the species, age and phase of reproductive cycle. Ovaries are differentiated into an outer cortex and inner medulla (Dellmann and Eurell, 2006). The medulla consisted of loose connective tissue strands of smooth muscles, numerous blood vessels, lymph vessels, nerves and network of irregular channels called rete ovarii. According to Byskov (1978), rete system is divided into three parts as extraovarian rete (ER), connecting rete (CR) and intraovarian rete (IR). Rete is a dynamic system of cells and tubules which plays a role in ovarian development prenatally and in ovarian function postnatally (Smith, 2011). The present study is planned with the aim to observe the distribution pattern of different enzyme in the rete system of buffalo during prenatal, prepubertal and pubertal life. Some studies have been conducted on the enzyme histochemistry of rete ovarii (Bhardwaj and Roy, 2003) and ovarian medulla in buffalo (Singh, 2014).

MATERIALS AND METHODS

The fresh ovarian tissue samples from six animals were collected immediately after slaughter, and were grouped as prenatal, prepubertal and pubertal groups based on their age. The age of prenatal animals was estimated by measuring the crown rump length and was converted into days by using prenatal formula (Soliman 1975).

$$Y = 73.544+2.256X$$

Where Y is the age in days and X is the crown vertebral rump length (CVRL) in cm (>20).

The age of prepubertal and pubertal was determined from dentition (Saini *et al.,* 1993). The tissues were subjected to cryostat sectioning at -23°C. The sections of 10-12 µ thickness were cut and incubated for demonstration of various enzymes viz; alkaline phosphatase (AKP) and G-6-Phosphatase (G-6-P) by coupling azodye method (Barka and Anderson, 1963), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) and glucose-6-diaphorase (G-6-PD) by nitro BT method (Pearse, 1972) and non-specific esterase (NSE) by Naphthol acetate method (Barka and Anderson, 1963). The observations were recorded and necessary photomicrographs were taken with digital microscope.

RESULTS AND DISCUSSION

The histoenzymic activity of phosphatases, oxidoreductases and esterases in various components of ovarian rete system of buffalo at different age groups is described as under .

1. Phosphatases:

a. Alkaline phosphatase (AKP): A fine granular moderate AKP activity was observed in the luminal part of rete tubules which was strong in the rete cords in all the age groups studied (Fig. 1). A moderate to strong AKP was found in connecting rete tubules during prenatal and prepubertal groups (Fig. 2), which became weak to moderate in the pubertal animals. The reaction of AKP in extraovarian rete tubules was reported to be weak to moderate in prenatal and prepubertal groups, which increased in pubertal animals (Fig. 3). The higher concentration of AKP activity in the rete system of buffalo may be related with steroidogenic activity particularly androgen. (Harrison and Wier, 1977). Similar finding have been reported in rete ovarii of buffalo ovary at different age groups (Bhardwaj, 1996), and ovarian medulla in prenatal buffalo (Singh, 2014). However, Goswami (1985) reported weak activity in the rete ovarii of adult buffalo. Gropp and Ohno (1966) also reported the AKP activity in the follicular cell cords of developing ovary in cattle.

b. Glucose-6-phosphatase (G-6-P): The activity of G-6-P was found to be weak in intraovarian rete tubules and cords during prenatal (Fig. 4) and prepubertal groups, which became weak to moderate in the pubertal animals. A moderate reaction of G-6-P was present in the connecting

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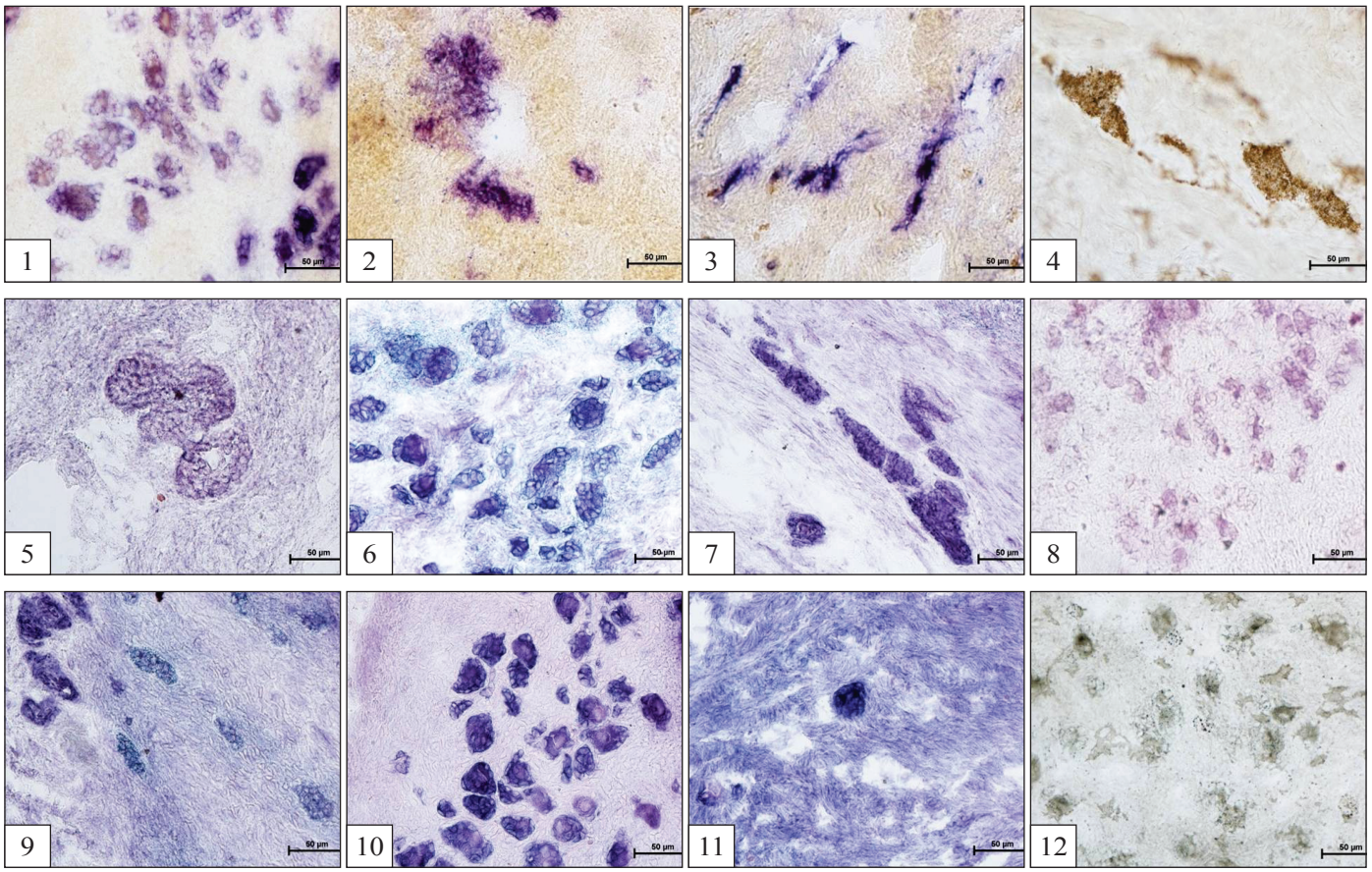

Fig. 1: Prenatal buffalo ovary showing moderate to strong AKP activity in the intraovarian rete cords and tubules. ×400; 2: prepubertal buffalo ovary showing moderate to strong AKP activity in the connecting rete. ×400; 3: Cryostat section of pubertal buffalo ovary showing moderate to strong AKP activity in the extraovarian rete. ×400; 4: Cryostat section of prenatal buffalo ovary showing moderate G-6-P activity in the connecting rete. ×400; 5: Cryostat section of prepubertal buffalo ovary showing weak to moderate SDH activity in the connecting rete. ×400; 6: Cryostat section of prenatal buffalo ovary showing strong to intense LDH activity in the intraovarian rete cords and tubules. ×400; 7: Cryostat section of prenatal buffalo ovary showing strong to intense LDH activity in the connecting rete. ×400; 8: Prenatal buffalo ovary showing weak to moderate G-6- PD activity in the intraovarian rete cords and tubules. ×400; 9: prenatal buffalo ovary showing moderate to strong NADH activity in the intraovarian rete cords and tubules. ×400; 10: prenatal buffalo ovary showing moderate to strong NADPH activity in the intraovarian rete cords and tubules. ×400; 11: pubertal buffalo ovary showing moderate to strong NADPH activity in the intraovarian rete cords and tubules. ×400; 12: Prenatal buffalo ovary showing weak to moderate NSE activity in the intraovarian rete cords and tubules. ×400.

rete tubules during prenatal, moderate to strong in prepubertal which became weak in the pubertal group. The reaction of G-6-P in extraovarian rete was weak in all three groups studied. The weak to moderate activity of G-6-P in the rete system is indicative of low glucose metabolism during steroidogenesis. Similar findings have been reported in rete ovarii of buffalo by Bhardwaj (1996), prenatal buffalo ovaries by Singh (2014).

2. Oxidoreductases

I Dehydrogenases

a. Succinic dehydrogenase (SDH): A weak to moderate activity of SDH was observed in the intraovarian rete tubules and cords in prenatal group, while negligible to weak activity was found in prepubertal and pubertal groups. A negligible to weak reaction of SDH was found in connecting rete tubules and cords of prenatal animals but it became weak to moderate in the prepubertal (Fig. 5) and pubertal animals. The reaction of SDH in extraovarian rete was weak in all three age groups. The variation in SDH activity in different components of rete system at different age groups in buffalo can be related with the steroidogenesis (Boos, 1988), cellular metabolism and proliferation (Boos *et al.,* 1989) of ovarian tissue. Similar findings have been reported in rete ovarii of buffalo by Bhardwaj and Roy (2003) and in ovarian medulla by Singh (2014).

b. Lactate Dehydrogenase (LDH): The activity of LDH was strong in lumen of intraovarian rete tubules and strong to intense in rete cords of prenatal animals (Fig. 6). A moderate to strong LDH activity was observed in the rete cords and tubules of prepubertal, which decreased to moderate in the pubertal animals. A strong to intense LDH was found in connecting rete during prenatal animals (Fig. 7), which beacme moderate to strong in the prepubertal and pubertal groups. In extraovarian rete, a weak to moderate activity of LDH was observed in prenatal and prepubertal groups which became moderate to strong in pubertal animals. The present findings showed that the activity of LDH decreased in the intraovarian and connecting rete tubules with the increase in age of animal, but reverse pattern was observed in the extra ovarian rete. Presence of LDH indicates its role in the steroidogenesis as it breaks glucose to produce acetyl coenzyme A which is utilized for the synthesis of fatty acids

and steroids. Similar findings have been reported in rete ovarii of buffalo ovary by Bhardwaj and Roy (2003) and in marmosets by Gudes and Miraglia (1977).

c. Glucose-6-phosphate Dehydrogenase (G-6-PD): The activity of G-6-PD varied from weak to moderate in lumen of intraovarian rete tubules in prenatal animals (Fig. 8), which became negligible to weak in prepubertal and pubertal groups. A moderate activity of G-6-PD was found in connecting rete tubules and negligible to weak in extraovarian rete of prenatal, prepubertal and pubertal groups. The activity decreased with the increase in the age of animal. As the presence of G-6-PD indicates its role in the steroidogenesis and glucose metabolism, so less activity in present study denotes reduced functional activity of rete system in prepubertal and pubertal animals. Similar findings have been reported in rete ovarii of buffalo ovary by Bhardwaj (1996) and Singh (2014).

II Diaphorases:

a. Reduced nicotinamide adenine dinucleotide diaphorase (NADH): A moderate to strong activity of NADH was observed in lumen of intraovarian rete in prenatal animals (Fig. 9), which reduced to moderate in prepubertal and pubertal groups. A moderate activity of NADH was found in connecting rete of all the age groups studied. In extraovarian rete, moderate activity of NADH was observed in prenatal and prepubertal groups, which increased from moderate to strong in pubertal animals. The variation in enzymatic activity of different components related with the steroidogenic activity of NADH. Similar findings have been reported in the developing ovaries of human foetus by Pryse-Davies (1970), in marmosets by Gudes and Miraglia (1977) and in buffalo ovaries by Bhardwaj (1996).

b. Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH): The activity of NADPH was reported strong to intense in lumen of intraovarian rete in prenatal and pubertal groups (Fig. 10 and 11), a moderate to strong in the prepubertal animals. A moderate to strong NADPH was found in connecting rete and a weak to moderate activity in extraovarian rete of all the age groups studied. Activity of this enzyme is also involved with steroidogenic activity of the ovary. The present findings corroborates well with the observations of Bhardwaj (1996) in buffalo ovary and in marmosets by Gudes and Miraglia (1977).

3. Esterases:

a. Non specific esterases: A weak to moderate activity of non specific esterase was found in the intraovarian rete, negligible in connecting rete and extraovarian rete of all the age groups studied (Fig. 12). This enzyme plays important role in the lipid metabolism as it breaks the lipid membrane of secretory granules and activates the secretion (Leonieni and Rechardt, 1972). Similar findings have been reported by Singh (2014) in prenatal buffalo ovaries, however Bhardwaj (1996) observed moderate to strong NSE activity in rete ovaries of buffalo during neonatal, prepubertal and adult ovary.

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