Journal of Animal Research: v.9 n.2, p. 253-256. April 2019

DOI: 10.30954/2277-940X.02.2019.6

Enzyme Histochemistry of Thyroid Gland in Prenatal Indian Buffalo (*Bubalus bubalis*)

Jatinderveer Kaur Billing, Opinder Singh* and Devendra Pathak

Department of Veterinary Anatomy, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

**Corresponding author: O Singh; E-mail: singhopinder68@gmail.com*

Received: 24 Nov, 2018 **Revised:** 06 March, 2019 **Accepted:** 18 March, 2019

ABSTRACT

The present study was conducted on buffalo foetuses (n=19) ranging from 7.4 (62 days) to 108 cm (317 days) curved crown rump length (CVRL) to study the distribution of phosphatases and oxidoreductases in thyroid gland during prenatal development. A progressive increase in phosphatase activity from 12.50 cm CVRL (85 days) to 41 cm CVRL (166 days) was observed around the blood vessels in the developing thyroid. Mild activity of Succinate dehydrogenase (SDH) was observed at 13.50 cm CVRL (89 days) whereas Lactate Dehydrogenase (LDH) activity was absent at this stage. SDH activity was correlated with mitochondrial localization in developing thyroid. Weak to moderate LDH activity was observed at 56 cm CVRL (200 days) suggesting the presence of glycolytic pathway in developing thyroid. Mild to moderate Reduced Nicotinamide Adenine Dinucleotide diaphorase (NADH-diaphorase) and Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-diaphorase) was noticed in the follicular cells and interfollicular spaces of the thyroid gland at 12.50 cm CVRL (85 days) which progressively increased with gestational age and became intense at 56 cm CVRL (200 days) indicating increase in metabolic activity.

Keywords: Histoenzyme, Thyroid, Prenatal, Buffalo

Thyroid gland is the first endocrine gland, to develop as a ventral- midline- endodermal diverticulum from the floor of foregut. It is primarily concerned with heat production and regulation of the body temperature as well as the adaptation to the surrounding environment. Rapid advances have been made in knowledge and understanding of thyroid in human (Fagmanand Nilsson, 2010) due to its functional importance and various endocrine disorders such as congenital hypothyroidism and thyroid dysgenesis. The elucidation of various histoenzymic factors responsible for growth and development of thyroid gland in buffalo will be critical for an improved understanding of development and functions of the gland. So, the present study was conducted to elucidate distribution of various enzymes in different cell types in thyroid gland of buffalo during prenatal development.

MATERIALS AND METHODS

The present study was conducted on thyroid glands of buffalo fetuses (n=19). The fetuses of different gestational age were obtained from pregnant non-descript buffaloes slaughtered at Gazipur Slaughter House, New Delhi and Veterinary Clinical complex, GADVASU, Ludhiana. After the collection, the foetal body length was measured as curved line in centimeter with the help of inelastic thread along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and designated as crown rump length (Edward, 1965).

The age of the foetuses was calculated by using the formula given by Soliman (1975).

$Y = 28.66 + 4.496 \times (CVRL < 20\ cm)$

$Y = 73.544 + 2.256 \times (CVRL > 20\ cm)$

Where, Y is age in days and X is CVRL in centimeters

Based on CVRL the foetuses were divided into three groups. Group I comprised of foetuses of CVRL between 0-20 cm, Group II above 20 to 40 cm and Group III

above 40 cm. The fresh thyroid gland from foetuses of different age groups were immediately collected and stored in liquid nitrogen. These tissues were subjected to cryostat sectioning at -20°C. The sections of 10-12 µm thickness were cut and incubated in different substrate for demonstration of various enzymes viz; alkaline phosphatase by simultaneous coupling azo dye method (Barka and Anderson,1963), Glucose-6-Phosphatase by Lead nitrate method (Barka and Anderson,1963), Succinate dehydrogenase **(**SDH), Lactate Dehydrogenase (LDH), Reduced Nicotinamide Adenine Dinucleotide diaphorase (NADH-diaphorase) and Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-diaphorase) by Nitro BT Method (Pearse, 1972). The positive and negative controls were carried out wherever possible.

RESULTS AND DISCUSSION

Phosphatases

Alkaline phosphatase

During prenatal development at 12.5 cm CVRL (85 days) in Group I weak to moderate alkaline phosphatase activity was observed around the blood vessels in the developing thyroid gland (Fig.1A). Sinha *et al*., (2016) reported AKPase activity in lining cells of thyroid gland in ducks. At 31 cm CVRL (143 days) in Group II, AKPase activity was moderate around blood vessels in the capsule and in the interfollicular space. In Group III, at 41 cm CVRL (166 days) moderate to strong AKPase activity was observed around the blood vessels in the parenchyma and capsule. At 56 cm CVRL (200 days) strong activity of AKPase was observed around blood vessels in the parenchyma (Fig.1B).

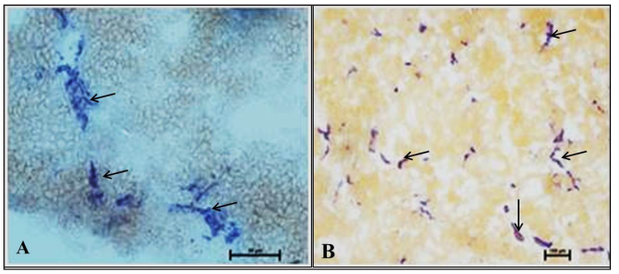

Fig. 1: Thyroid gland of buffalo foetus, **(A)** at 12.5 cm CVRL (85 days) showing mild to moderate activity of AKPase around the blood vessels in the parenchyma (arrows), Azo Dye Method x400; **(B)** at 56 cm CVRL (200 days) showing strong AKPase activity around the blood vessels in the parenchyma (arrows), Azo Dye Method x100.

Uppal and Bansal (2008) also observed localization of AKPase around blood vessels in thyroid gland of buffalo calves below one month of age and correlated it with transport of electrolytes across the wall indicating high activity in thyroid gland during foetal life.

Glucose-6-Phosphatase

Weak G-6-Pase activity was observed around blood vessels and follicular cells in parenchyma at 13.5 cm CVRL (89 days). At 31 cm CVRL (143 days) in Group II, weak to moderate G-6-Pase activity was observed in the interfollicular spaces and in the capsule. In Group III, at 41 cm CVRL (166 days) G-6-Pase activity continued to be weak to moderate in the follicular cells however at 56 cm CVRL (200 days), moderate activity of G-6-Pase was observed in the thyroid follicular cells in Group III (Fig. 2). Glucose-6-phosphatase consisted of amino acids, anchored to the endoplasmic reticulum (ER) and is involved in the release of glucose into the circulation.

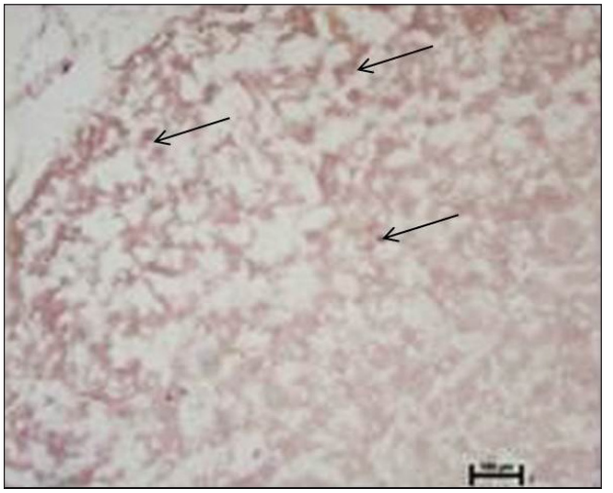

Fig. 2: Thyroid gland of buffalo foetus at 56 cm CVRL (200 days) showing moderate Glucose-6-phosphatase activity in the thyroid follicular cells (arrows), Lead nitrate method x100

Oxidoreductases

Succinate dehydrogenase (SDH)

At 13.5 cm CVRL (89 days), weak SDH activity was observed in between the follicles of the thyroid gland. Weak activity of this enzyme indicated low metabolic activity of the gland at this stage. At 31 cm CVRL (143 days), weak to moderate activity of SDH was observed in the parenchyma of the thyroid gland. At 56 cm CVRL (200 days), in the follicular cells moderate activity of SDH was observed in the parenchyma (Fig.3A). SDH is a mitochondrial enzyme that is involved in generation of energy by oxidation reduction reaction in the cell as opined by Uppal and Bansal (2008) in the thyroid glands of buffalo calves below one month of age.

Lactate dehydrogenase (LDH)

LDH activity was absent in the thyroid gland at 13.5 cm CVRL (89 days). At 31 cm CVRL (143 days), weak activity of LDH was observed in the parenchyma of the gland (Fig.3B). At 56 cm CVRL (200 days), weak to moderate activity of LDH was observed. The presence of LDH activity in thyroid gland suggested the presence of glycolytic pathway in the cellular elements of thyroid gland. Sarma *et al*., (2017) also reported weak activity of LDH during early postnatal life in Assam goat.

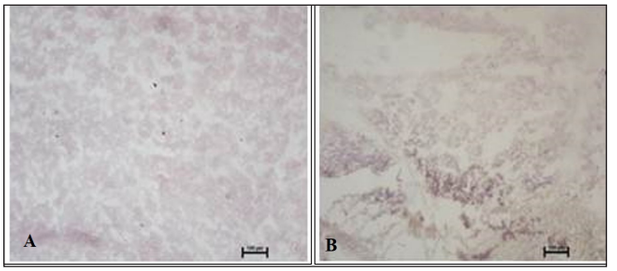

Fig. 3: Thyroid gland of buffalo foetus, **(A)** at 56 cm CVRL (200 days) showing moderate SDH activity in the follicular cells; **(B)** at 31 cm CVRL (143 days) showing weak LDH activity in the follicular cells. Nitro BT Method x100

Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-diaphorase)

Moderate activity of NADPH-diaphorase was observed in the follicular cells and interfollicular spaces of the thyroid primordia at 12.5 cm CVRL (85 days). At 31 cm CVRL (143 days), activity was moderate to strong in the follicular cells and in the capsule of the thyroid gland. At 56 cm CVRL (200 days), intense activity of NADPH-diaphorase was observed in follicular cells (Fig.4A). NADPH-diaphorase is a coenzyme dehydrogenase and is an indicator of metabolic activity of the cell.Uppal and Bansal (2008) also observed strong to intense activity of NADPH-diaphorase in the follicular and parafollicular cells of thyroid glands of buffalo calves below one month of age and correlated it with metabolic activity of cell as it is a coenzyme dehydrogenase.

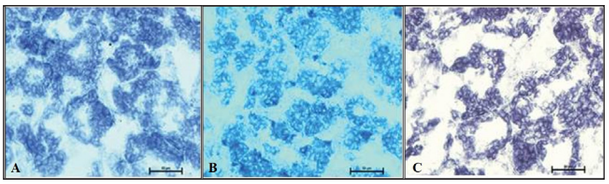

Fig. 4: Thyroid gland of buffalo foetus, **(A)** at 56 cm CVRL (200 days) showing intense NADPH-diaphorase activity in the follicular cells; **(B)** at 41 cm CVRL (166 days) showing moderate to strong NADH-diaphorase activity in the follicular cells; **(C)** at 56 cm CVRL (200 days) showing intense NADH-diaphorase activity in the follicular cells. Nitro BT Method x100

Nicotinamide Adenine Dinucleotide diaphorase (NADH-diaphorase)

At 12.5 cm CVRL (85 days), moderate activity of NADH-diaphorase was observed in the follicular cells and interfollicular spaces of the thyroid primordia. At 31 cm CVRL (143 days), activity was moderate to strong in the follicular cells and in the capsule of the gland. At 41 cm CVRL (166 days) NADH-diaphorase activity was moderate to strong in the follicular cells and in the capsule (Fig.4B). At 56 cm CVRL (200 days), intense activity was observed in the follicular cells (Fig.4C). NADH-diaphorase is a coenzyme dehydrogenase and acts in the cell as a part of hydrogen transport chain. Uppal and Bansal (2008) also observed strong to intense activity of NADH diaphorase in the buffalo calves below one month of age. The increased activity of the enzyme suggested maturation and differentiation of cells in thyroid gland with the advancement of gestational age.

CONCLUSION

The distribution of various enzymes viz: phosphatases and oxidoreductases were observed in the developing thyroid. The phosphatase activity reflected the electrolyte transport potential across cell membranes and release

of glucose into circulation indicating high activity in thyroid gland during foetal life. Succinate dehydrogenase activity reflected the mitochondrial density, whereas Lactic dehydrogenase activity suggested the presence of glycolytic pathway in cells of thyroid gland. The presence of Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase and Nicotinamide Adenine Dinucleotide indicated high metabolic activity in thyroid gland during prenatal development,

REFERENCES

Barka, T. and Anderson, P.J. 1963. Histochemistry: Theory, Practice and Bibliography. 1st Ed., Harper and Row Publishers Inc. New York. pp. 237-316.

Edward, M.J. 1965. Observations on the anatomy of the reproductive organs of cow. *New Zealand Vet. J.,* **13**: 25.

Fagman, H. and Nilsson, M. 2010. Morphogenesis of thyroid gland. *Mol. Cell. Endocrinol*., **323**(1): 35-54.

Pearse, A.G.E. 1972. Histochemistry; Theoretical and applied. 3rd Ed., Vol. II. Churchill Livingstone, London.

Sarma, K., Kalita, S.N. and Devi, J. 2017. Postnatal Gross Morphological and Histoenzymic Studies on the thyroidgland of Assam goat. *J. Anim. Res*., **7**(3): 571-574.

Sinha, S., Sarma. M., Goswami. S., and Devchoudhoury, K.B. 2016. Histochemical and ultrastructural studies on the thyroid gland of Pati Ducks (*Anasplatyrhynchos domesticus*) of Assam. *J. Anim. Res.,* **6**(2): 101-104.

Soliman, M.K. 1975. Studies on the physiological chemistry of the allantoic and amniotic fluids of buffalo at various periods of pregnancy. *Indian Vet. J*., **52**: 106-11.

Uppal, V. and Bansal, N. 2008. Histoenzymic studies on thyroid gland of neonatal buffalo calves. *Indian J. Anim. Sci.,* **78**(2): 154-155.