Histogenesis of Testicular Parenchyma During Prenatal Life in Buffalo

Histogénesis del Parénquima Testicular Durante la Vida Intrauterina en el Búfalo

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SUMMARY: The study was conducted on the testes of 18 buffalo foetii to reveal histogenesis and differentiation of different cells of testicular parenchyma. At 8.0 cm CVR (65 days) the seminiferous tubules were present at gonadal periphery and a network of polygonal mesenchymal cells was seen in the centre of testis. These tubules were surrounded by a distinct basement membrane and a single layer of peritubular cells at 10 cm CVR (74 days), which became double layered at 88.0 cm CVR (272 days). The testicular parenchyma at 12.0 cm CVR had two zones; outer zone having longitudinal tubules and inner zone having rounded tubules. But a reverse pattern of their arrangement was observed at 14.0 cm CVR (92 days). The pre-Sertoli cells were first observed in buffalo foetii of 8.0 cm CVR (65 days) in the periphery of seminiferous tubular epithelium whereas the gonocytes were demonstrable in the centre of tubules at 10.6 cm CVR (76 days). The fetal Leydig cells were also reported at 8.0 cm CVR (65 days) but at 14.0 cm CVR (92 days), the interstitium had considerably expanded due to the differentiation of mesenchymal cells into the Leydig cells.

KEY WORDS: Histogenesis; Testicular parenchyma; Prenatal life; Buffalo.

INTRODUCTION

The differentiation of testes is a complex process and involves a number of mechanisms at various stages of development. Any disturbance in the development of the organ during critical period of organization can lead to low reproductive efficiency during the adult life. In literature reports are available on the histogenesis and morphogenesis of testes in sheep (Hochereau de Riviers *et al.*, 1995), goat (Tekade, 2001), cattle (Wrobel, 2000; Abd-Elmaksoud, 2005) but scanty information is available in the buffalo. So the present study was conducted to understand the normal developmental process of testes and to evaluate the unrecognized defects in the histogenesis of the organ which can hamper the reproductive efficiency during adult life and data obtained can be used to improve the production and reproduction in buffalo.

MATERIAL AND METHOD

The present study was conducted on testes of 18 Indian buffalo foetii of different ages obtained from abattoir and Veterinary clinics. The foetal body length was measured

as a curved line in centimeters with a calibrated inelastic thread along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and designated as curved crown rump length (CVR) (Edward, 1965). The approximate age of the foetii was calculated by using the following formula given by Soliman (1975).

$$Y = 28.66 + 4.496 X \text{ (CVR} < 20 \text{ cm)}$$

$$Y = 73.544 + 2.256 X \text{ (CVR} \geq 20 \text{ cm)}$$

Where Y is the age in days and X is the CVR in centimeters. Based on CVR the samples were divided into three groups viz:

Group I: Foetal samples of CVR between 0 cm to 20 cm

Group II: Foetal samples of CVR between 20 cm to 40 cm

Group III: Foetal samples of CVR > 40 cm

Both the right and left testes were collected and fixed in Bouin's and 10% neutral buffered formalin (NBF) fixatives. The tissues were processed for paraffin blocks preparation by acetone-benzene schedule (Luna, 1968) and

Table. I Micrometrical data on testes of buffalo during prenatal life.

Age Groups	Longitudinal seminiferous tubular diameter		Rounded seminiferous tubular diameter		Diameter of pre Sertoli Cell		Diameter of gonocyte		Diameter of nucleus of Gonocyte		Diameter of Leydig cells	
	RT	LT	RT	LT	RT	LT	RT	LT	RT	LT	RT	LT
I	102.36	103.89	38.73	39.39	10.72	10.83	13.24	13.27	7.78	7.83	8.57	8.57
	± 3.95	± 3.57	± 1.47	± 1.44	± 0.35	± 0.34	± 0.41	± 0.42	± 0.26	± 0.25	± 0.24	± 0.23
II	112.56	113.62	41.59	42.00	12.98	13.11	13.79	13.21	7.16	7.24	9.83	9.83
	± 5.07	± 4.61	± 1.32	± 1.17	± 0.46	± 0.42	± 1.42	± 1.21	± 0.63	± 0.67	± 0.49	± 0.30
III	112.56	113.62	41.59	42.00	12.98	13.11	13.79	13.21	7.16	7.24	9.83	9.83
	± 5.07	± 4.61	± 1.32	± 1.17	± 0.46	± 0.42	± 1.42	± 1.21	± 0.63	± 0.67	± 0.49	± 0.30

sections of 5-7mm thickness were cut with rotary microtome. These paraffin sections were stained with hematoxylin and eosin and Masson's trichrome stains to study the histomorphological details. The micrometrical observations on diameters of seminiferous tubules, Sertoli cells, gonocytes, Leydig cells and their nuclei were recorded on hematoxylin and eosin stained sections with the help of filar and ocular micrometer duly calibrated with stage micrometer.

RESULTS AND DISCUSSION

The micrometrical data on the longitudinal and rounded seminiferous tubules, sertoli cells, Leydig cells and gonocytes of right and left testes in group I, II and III have been summarized in Table I and the values have been statistically analyzed in Table II.

Seminiferous tubules. The testicular parenchyma comprised of seminiferous tubules and intertubular tissue. The seminiferous tubules were observed first in buffalo foetii of 8.0 cm CVR (65 days) at the gonadal periphery whereas the inner part of parenchyma consisted of network of polygonal mesenchymal cells and thin walled blood vessels. At 12.0 cm CVR (83 days), the testicular parenchyma was further subdivided into two zones. The outer zone contained the longitudinal/elongated/oval tubules whereas the inner zone had rounded tubules. The number of longitudinal tubules was more than the rounded tubules at this age group but the reverse pattern was observed at 14.0 cm CVR (92 days) (Fig. 1). With the advancing age of the foetus the central zone disappeared and was replaced by mediastinum testes at 18.2 cm CVR (110 days) whereas the outer zone was responsible for the formation of permanent seminiferous tubules. These seminiferous tubules developed from pre existing clusters of epitheloid cells. The present findings are in agreement with the earlier reports on the testicular parenchyma of dog (Kawakami *et al.*, 1993) and bovine fetuses (Wrobel, 2000). At 39.5 cm CVR (163 days) most of the seminiferous tubules were rounded in shape and only a few were longitudinal whereas in group III the number of longitudinal tubules were more than the rounded ones as observed earlier by Wrobel (2000).

The seminiferous tubules were surrounded by a distinct basement membrane and a layer of peritubular cells at 8.0 cm CVR (65 days) buffalo foetii. These peritubular cells were suggested to be originating from the tunica albuginea and then settled around the seminiferous tubules. These cells are responsible for maintaining the structural integrity of seminiferous tubules. It has been suggested that the basement membrane underlying the seminiferous tubular epithelium in post natal life is a shared product of Sertoli and peritubular cells (Skinner *et al.*, 1985). These peritubular cells were elongated in shape with a rounded nucleus. The single layer of these cells became double layer at 88.0 cm CVR (272 days) (Fig.5).

The number of seminiferous tubules increased with the advancing age of the foetii. From the data it is inferred that there was no significant difference between the diameter of longitudinal seminiferous tubules of right and left testes. A progressive increase in the diameter of seminiferous tubules was noticed with the increase in age of buffalo foetii. The present findings

Fig. 1. 14.0 cm CVR buffalo foetus showing blood vessels (BV) and seminiferous tubules (S) in outer zone. Hematoxylin and Eosin stain x 175.

Fig. 2. 12.0 cm CVR buffalo foetus showing the formation of fetal Leydig cells (LC) and fibroblasts (F) in the interstitium. Hematoxylin and Eosin stain x 875.

Fig. 3. Differentiation of pre-Sertoli cells (Ps), gonocytes (G), fibroblasts (F), peritubular cells (PC) and Leydig cells (LC) in the buffalo foetus of 14.0 cm CVR. Hematoxylin and Eosin stain x 875.

Fig. 4. Testicular parenchyma of left testis in 75.0 cm CVR buffalo foetus showing well defined basement membrane (B), peritubular cells (PC), pre-Sertoli cells (Ps), gonocytes (G) and Leydig cells (LC) could be seen. Hematoxylin and Eosin stain x 875.

Fig. 5. 88.0 cm CVR buffalo foetus showing more differentiation of pre-Sertoli cells (Ps), gonocytes (G), Leydig cells (LC) and peritubular cells (PC). Masson's Trichrome stain x 350.

Table II. Correlation of coefficient between CVR and micrometrical parameters of right and left testes and in between the groups of buffalo foetii.

Parameter	Right testis	Left testis	Group I RT vs LT	Group II RT vs LT	Group III RT vs LT
Diameters of seminiferous tubules					
Longitudinal tubules	0.212 ^{NS}	0.174 ^{NS}	0.29	0.15	1.96 ^{**}
Rounded tubules	0.528 ^{**}	0.527 ^{**}	0.32	0.23	0.52
Diameter of Sertoli Cell	0.429 ^{***}	0.394 ^{***}	0.23	0.21	0.14
Diameter of Sertoli Cell Nucleus	0.418 ^{***}	0.369 ^{***}	0.34	0.34	0.25
Diameter of Gonocyte	0.071 ^{NS}	0.098 ^{NS}	0.05	0.31	0.23
Diameter of Gonocyte Nucleus	0.149 ^{NS}	0.155 ^{NS}	0.14	0.09	0.27
Diameter of Leydig Cells	0.482 ^{***}	0.482 ^{***}	0.00	0.00	0.00
Diameter of Leydig Cells Nucleus	0.081 ^{NS}	0.115 ^{NS}	0.41	0.02	0.39

*** Significant at 1% (P=0.01) ** Significant at 5% (P=0.05) RT- Right testes LT- Left testes NS – Non significant

are in agreement with the findings of Singh *et al.* in goat fetuses.

The correlation in the diameters of the seminiferous tubules in right and left testes with respect to the age of buffalo foetii was statistically significant for rounded tubules ($p = 0.05$) and non significant for longitudinal tubules. No significant difference was observed regarding various parameters between right and left testes within the individual age groups except the diameter of longitudinal seminiferous tubules.

Pre Sertoli cell. The pre Sertoli cells were first observed in buffalo foetii of 8.0 cm CVR (65 days) at the periphery of the seminiferous tubular. Similar organization of pre Sertoli cells was also observed by Pelliniemi (1975) and Dang & Fouquet (1979) in pig and *Macaca fascicularis* fetuses, respectively.

The pre Sertoli cells were large in number and polygonal or conical in shape with irregular dark nuclei and pale cytoplasm (Fig.3). The shape of these cells changed to columnar with an elongated nuclei in the testis of buffalo foetii at 88.0 cm CVR and they were lying perpendicular to the basement membrane at this age. Similar observations were made in guinea pig and human fetal testes by Black & Christensen (1969) and Rath & Anand (1992), respectively. These cells were found to be closely arranged adjacent to the basement membrane of seminiferous tubule. The basement membrane act as a uniting media between the pre Sertoli cells and peritubular cells which are responsible for the formation of blood-testicular barrier (Byskov & Hoyer, 1994).

The maximum number of pre Sertoli cells was found

to be 16-20 per cross-sectional area and their number considerably increased with the progression of fetal life (Fig.4). Similar observations were also made by Abd-Elmaksoud in the bovine fetal testis. It was interpreted that there was no significant difference in the diameter of pre-Sertoli cells in left and right testes but it increased progressively with the advancing age of the buffalo foetii. Similar pattern was also observed with the nuclear diameter of these cells. The statistical analysis revealed a positive correlation between the size of pre Sertoli cells with respect to age of buffalo foetii, which was found to be significant at 1% level ($p=0.01$) in both the testes. The comparison of pre-Sertoli cell and its nuclear diameter in right and left testes showed that it was more in group I as compared to group III.

Gonocytes. The gonocytes were demonstrable in the centre of seminiferous tubules surrounded by pre Sertoli cells at 10.0 cm CVR (74 days) of buffalo foetii. These cells were rounded in shape with a prominent spherical nucleus at 14.0 cm CVR (92 days). The gonocytes were also located at the periphery and centre of seminiferous tubules (Fig.4) but these cells rarely come in contact with the basement membrane of seminiferous tubules. It is clear from the data that the size of gonocyte and their nucleus did not vary significantly with respect to age and testis of the buffalo foetii. This might be due to the fact that these cells continue to undergo mitotic replication and do not enter meiosis in the male fetuses (Mc-Carrey, 1993). The above findings are in agreement with the observations made on the fetal testes of pig (Black & Christensen; Black, 1971), goat (Singh *et al.*, 1979) and dog (Kawakami *et al.*). The correlation of coefficient between the diameters of gonocytes and their nuclear with respect to CVR of buffalo foetii was found to be non significant in both the testes.

Interstitial tissue. The interstitial tissue contained numerous blood vessels, lymph vessels, Leydig cells, loose network of mesenchymal cells, connective tissue cells and 1-2 layers of peritubular cells surrounding testicular tubules. These cells were present in close proximity to the blood vessels indicating their endocrine nature. At 8.0 cm CVR (65 days) the interstitium was comparatively less developed and showed the presence of darkly stained fetal Leydig cells. These cells were found to be polygonal in shape with rounded nuclei which is darker than the pre-Sertoli cells, the gonocytes and their cytoplasm was acidophilic. In addition to fetal Leydig cells, few fibroblasts were also observed at various stages of differentiation at 12.0 cm CVR (83 days) (Fig. 2) which may form the adult Leydig cells. Our findings are in agreement with the findings of Wrobel (2000). At 14.0 cm CVR (92 days), the interstitium significantly expanded due to the differentiation of mesenchymal cells into fetal Leydig cells and constituted the larger cell population of all the other components of interstitium which was well presented at 36.5 cm CVR (156 days). This concept is supported by the observation of Orth (1993) who found that the number of

mesenchymal cells decreased with the appearance of Leydig cells in pig testes. The number of fetal Leydig cells was found to be more as compared to the gonocytes and pre-Sertoli cells but a decrease in the number of Leydig cells with the advancement of fetal age could also be demonstrated. This could be due to degeneration of earlier Leydig cells or due to the proliferation of intertubular tissue during the prenatal development of testes (Wrobel, 1990). Our findings are also supported by Huhtaniemi & Pelliniemi (1992) and Singh (1996) who also reported a decrease in number of Leydig cells with increase in the age of the foetii. The diameter of fetal Leydig cells remained same in both the right and left testes but there was an increase in the diameter of fetal Leydig cells from group I to group III. Their nuclear diameter did not vary significantly with respect to age of the foetii and left and right testes. Our results correspond well with the findings of Singh *et al.* (1979) and Pachpande *et al.* (2006). There was a positive correlation between the diameters of Leydig cells and their nuclei with respect to age of foetus, which was found to be significant at 1% level ($p=0.01$) for Leydig cells and non significant for their nuclei.

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RESUMEN: El estudio fue realizado en los testículos de 18 fetos de búfalos, para revelar la histogénesis y diferenciación de las diferentes células de parénquima testicular. A los 8,0 cm de longitud corona-rabadilla (LCR) (65 días) los túbulos seminíferos estuvieron presentes en la periferia de la gónada y una red poligonal de células mesenquimales se observó en el centro del testículo. Estos túbulos estaban rodeados por una membrana basal y una sola capa de células peritubular a los 10 cm LCR (74 días), la cual se convirtió en una doble capa a los 88,0 cm LCR (272 días). El parénquima testicular a 12,0 cm LCR tenía dos zonas, zona exterior con túbulos longitudinales y zona interior con los túbulos redondeados transversalmente. Sin embargo, un patrón inverso en su disposición se observó a los 14,0 cm LCR (92 días). Las células pre-Sertoli se observaron primero en fetos de búfalos de 8,0 cm LCR (65 días) en la periferia del epitelio seminífero tubular, mientras que los gonocitos fueron visibles en el centro de los túbulos a 10,6 cm LCR (76 días). Las células de Leydig fetales también se observaron a los 8,0 cm LCR (65 días), pero a los 14,0 cm LCR (92 días), el intersticio tuvo una considerable expansión debido a la diferenciación de células mesenquimales en células de Leydig.

PALABRAS CLAVE: Histogénesis; Parénquima testicular; Vida prenatal; Buffalo.

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