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Histogenesis of testis in human foetuses at different weeks of gestation in eastern India

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Abstract
The male Gonads or Testes are embryologically derived from "Mesoderm" except for Primordial germ cells giving rise to spermatogonia which are derived from epiblast. The true sources are the mesothelium lining the posterior abdominal wall, underlying mesenchyme & Primordial germ cells. The gross changes occurring during development of male gonads are studied in much more detail as compared to histological changes therefore very few studies available on the same. One of the defining characteristic features of the living beings is their ability to continue the progeny which is emphatically the function of the testis with an additional course of endocrine function in human species. The present prenatal study of histogenesis of human foetal testis is carried out to know the occurrence of various cell populations like germ cells, Leydig cells, Sertoli cells and peritubular cells at different gestational periods. 10 aborted male human foetuses between 8- 26 weeks of gestational age with no obvious congenital anomalies were obtained for study.

Keywords: epiblast, leydig cells, sertoli cells, germ cells, foetuses

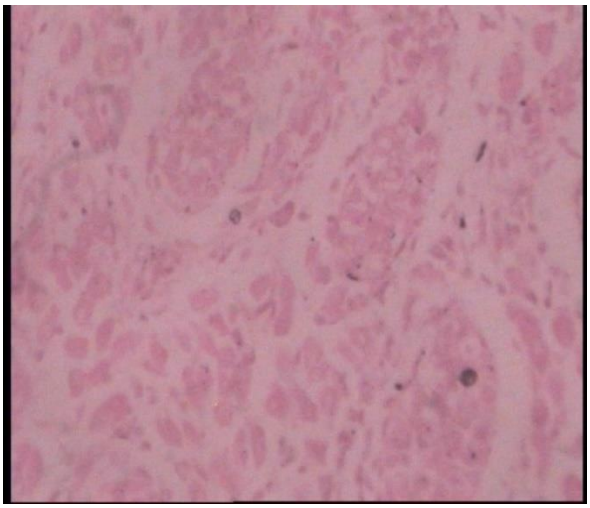
Introduction
The gonads are derived from three different components: the primordial germ cells, the coelomic epithelium and subjacent mesenchyme of the mesonephric ridge (Hamilton and Mossman, 1972) [1]. Gruenwald (1942) [2] noted that the gonads first appeared as a uniform gonadal blastema formed by the coelomic wall with contribution from mesothelium and mesenchyme and the first indication of sex differences appeared as a slightly better limitation and more parallel arrangement of cords in the testis in embryos of 15-17mm (early 7 weeks). According to Wyndham (1943) [3], the testes were represented by a thickening of the germinal epithelium in an embryo of 14mm (6 weeks) and Mc Kay *et al*. (1953) [4] observed that the male gonad was differentiated into testis in 23 mm (late 7 weeks) embryo. Elias (1974) [5] observed that cells in the testis were initially plates rather than cords. Mancini *et al*. (1960) [6] observed that foetal testis contained two cellular types: (a) primitive spermatogonium having a rounded nucleus and well-defined cytoplasm and (b) supporting or indifferent cell with a scarcity of cytoplasm, poorly defined limits and an elongated nucleus generally oriented with the longer axis perpendicular to the basement membrane. According to Vilar (1970) [7], the seminiferous epithelium of the newborn human test is comprised of solid cords about 50 µ in diameter where two types of cells were easily differentiated. Testicular dysgenesis and maldevelopment have been known to cause undescended testis which is present in approximately 4.5% of males at birth and is associated with various complications most significantly infertility and malignancy (Khatwa and Menon, 2000) [8].

AIMS and objectives
This study is done to know the chronological pattern of histogenesis of human foetuses in this eastern geographical region of India.

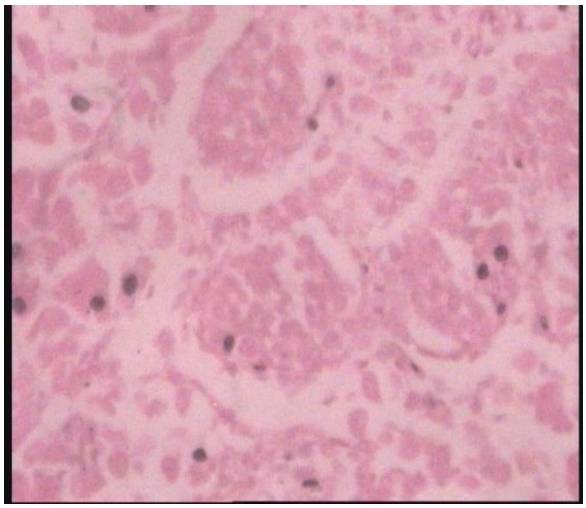
Materials and methods
This study was done to correlate the chronological pattern of testis development in this geographical eastern region of India, Odisha & compare the results from other researchers nationwide & worldwide. This is a hospital based, observational, cross sectional study conducted at Hi- Tech Medical Colleges & Hospital, Bhubaneswar, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013 on twenty aborted human foetuses without obvious congenital anomaly of gestational age between 8 weeks and 26 weeks collected within 6 hours of delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians. Foetuses were immediately fixed in 10% Formalin for 1-2 hrs. Testis was dissected by Dissecting Microscope, fixed in 10% Formalin for 48-72 hrs. After fixation by formalin, the tissues were transferred to 30%, 50%, 70%, 90% and Absolute alcohol each for 30 minutes. This ascending grading of the dehydrating fluid was done because when alcohol mixes with water, it produces diffusing current which can damage the tissues. Then the tissues were put in xylol for 24 hours to clear the residual

alcohol. These tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots containing only fresh melted paraffin at 90 minutes interval. Then the tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome. 5 mu sections were cut in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put in the warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the tissue was put in decreasing grades of alcohol (Absolute alcohol, 90%, 70%, 50% and 30%) then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water.2-3 drops of 1% acid alcohol (1cc HCl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish). Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 45X magnification. Thereafter photomicrographs were taken by camera using microscope adapter.

Observations



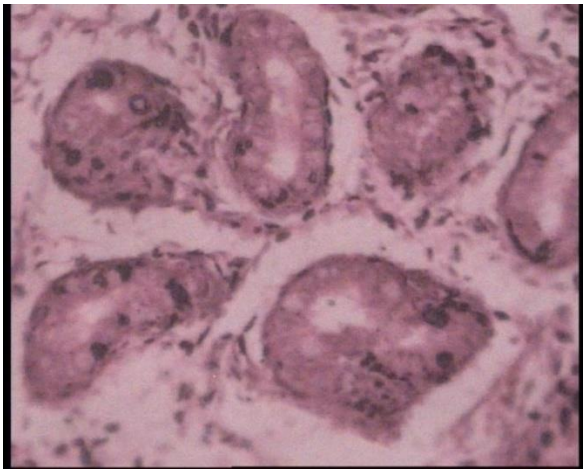
8 Weeks Gestation



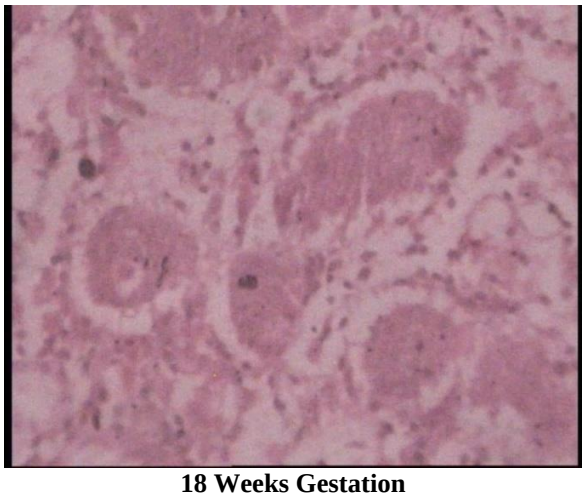
10 Weeks Gestation

12 Weeks Gestation

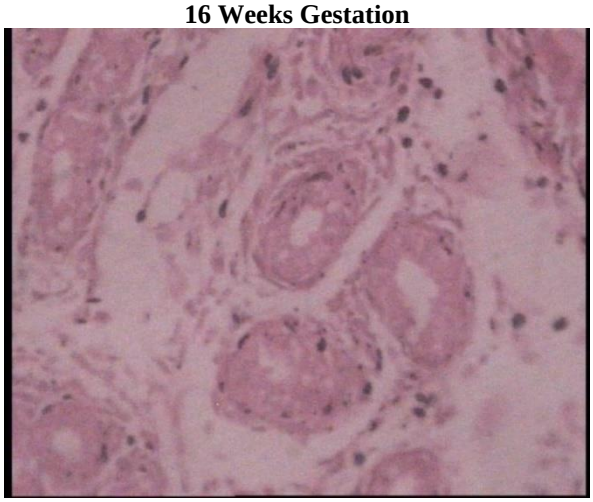
14 Weeks Gestation



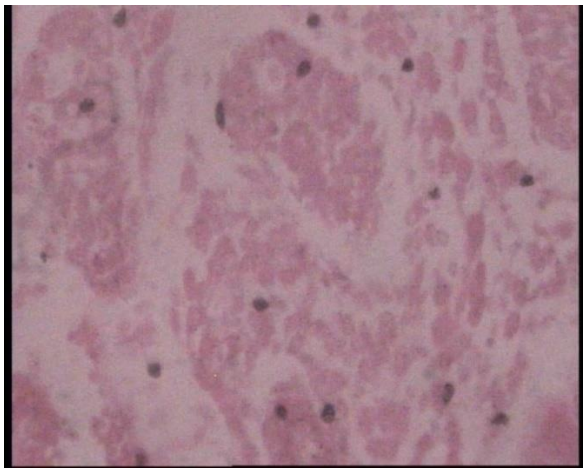
16 Weeks Gestation



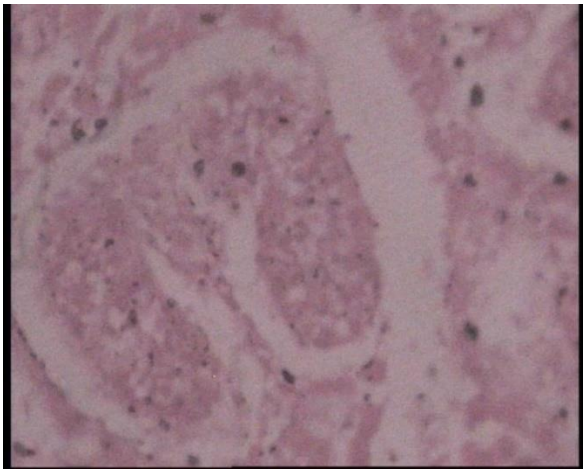
18 Weeks Gestation



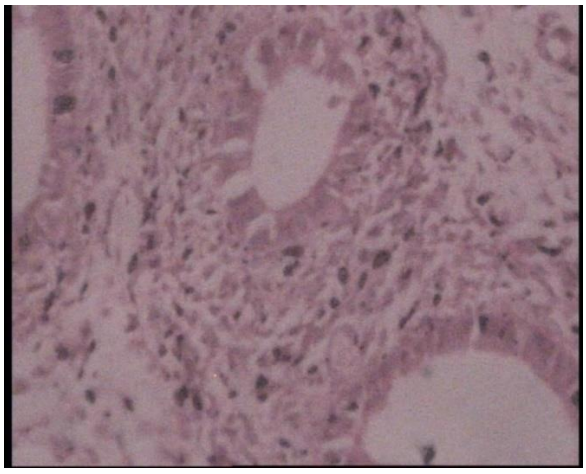
20 Weeks Gestation



22 Weeks Gestation



24 Weeks Gestation



26 Weeks Gestation

8 Weeks Foetus: Germinal epithelium lining was seen with supporting cell which are undifferentiated. Black dots were Sertoli cells.

10 Week Foetus: The findings were similar as 8th Week.

12 Week Foetus: Well developed seminiferous tubules with Sertoli cells.

14 Week Foetus: Rete testis was seen. Mediastinum testis with fibrous tissue were seen. Leydig cells were visible. Seminiferous tubules were seen to have 2-3 different layers.

16 Week Foetus: Rete testis and seminiferous tubules were increased. Well defined tunica albuginea and tunica vasculosa were seen.

18 Week Foetus: In low power efferent ductules are visible. In high power cells of Sertoli and differentiating different types of germ cells are visible.

20 Week Foetus: Seminiferous tubules were clearly visible. Rete testis and Mediastinum testis were well defined. Efferent ductules were visible. In high power efferent ductules were visible.

22 Week Foetus: The findings were similar as 22nd Week.

24 Week Foetus: Septa is developing but not yet completed. Tunica albuginea and

26 Week Foetus: Well defined seminiferous tubules divided by septae and well defined efferent and rete testis were seen. Well-developed germinal cells are visible. In high power seminiferous tubules with differentiated germ cell.

Discussion

Slightly different time of human testicular differentiation had been observed by different 3 workers in this field i.e. at 6 weeks (Wyndham, 1943), 2 early 7 weeks (Gruenwald, 1942) or at late 7 weeks 4 (Mc Kay *et al*, 1953). In the earliest specimen of the present study, Germinal epithelium lining was seen with supporting cell which are undifferentiated [9]. Opinions regarding the origin of the cells of the gonads are divergent. Many authors had stated that the sex cords were derived from the proliferating 2 coelomic epithelium (Gruenwald, 1942, Hamilton 1 9 and Mossman, 1972, Sadler, 1995 and Standring *et al*., 2005) [2, 10] whereas Satoh (1991) [9] stated that they originated from the mesonephros10. The present study agrees with the former authors. By the 4th month, the elongated mass of tissue comprising the embryonic testis had become sufficiently condensed and rounded to assume a form 12 suggestive of its adult shape (Ham, 1969) [11]. The present authors agree with this finding also.

Conclusion

This prenatal developmental morphology and histology of human testis is expected to be help full for the management of premature infants [12]. The knowledge will also helpful for the testicular tumour surgeries [13].

Acknowledgement

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Conflicts of interests: None

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