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ABP: androgen binding proteins

AJs: adherent junctions

ALC: adult Leydig cells

AMH gene: anti-Müllerian hormone gene

AR: androgen receptor

BL: basal lamina

BTB: blood testis barrier

CB: chromatoid body

DHT: dihydrotestosterone

DPP: days post-partum

ES: ectoplasmic specializations

FGF2: fibroblast growth factor

FSH: follicle stimulating hormone

GDNF: glial cell line-derived neurotrophic factor

GnRH: gonadotropin releasing hormones

HPG: hypothalamo-pituitary-gonadal

IL-1: interleukin-1

LH: luteinizing hormone

MIF: Müllerian -inhibiting factor

P450scc: cholesterol-side-chain cleavage enzyme

PCNA: proliferating cell nuclear antigen

PDGF: platelet-derived growth factor

PGCs: primordial germ cells

PLZF: Promyelocytic leukemia zinc factor

PND: postnatal day

RER: rough endoplasmic reticulum

ROS: reactive oxygen species

SER: smooth endoplasmic reticulum

SF-1 gene: steroidogenic factor-1 gene

SNT: seminiferous tubules

SSC: spermatogonial stem cell

StAR protein: steroidogenic acute regulatory protein

TDF: testis -determining factor

TGFβ: transforming growth factor beta

TJs: tight junctions

TNF: tumor necrosis factor

Abstract

Background and aim: Infertility is a major human health problem which can begin at any age. Testicular growth is important for future male fertility which shows acceleration during puberty. The current study was designed to evaluate the histological changes that occur in albino rat testis during pre-pubertal, pubertal and post pubertal periods. This would be of help in understanding the testicular behavior and their correlations to infertility problems.

Design: The study was conducted on 50 male albino rats that were divided into three groups. Group I (pre pubertal group) was classified into two weeks and one month old rats. Group II (pubertal group) consisted of rats of two months old. Group III (post pubertal group) was classified into three months and twelve months old rats. Examination of testes was performed by microscopic, immunohistochemical and morphometric studies.

Results: The current study showed that at early pre pubertal group (two weeks old rats) thin tunica albuginea capsule and small sized seminiferous tubules (SNT) were noticed. Spermatogenesis was arrested at the stage of primary spermatocytes with no detectable spermatids. Significant decreased immunological reaction for vimentin and AR compared to group II was

noticed. At late pre pubertal group (one month old rats) significant increase in the diameter and germinal epithelial thickness of SNT compared to the previous subgroup was seen. Progression of spermatogenesis was noticed with the appearance of spermatids in many tubules with no observable spermatozoa. Significant increase in the immunological reaction for vimentin and AR compared to the previous subgroup was found.

At puberty (two months old rats), the diameter of SNT and thickness of germinal epithelium were significantly increased. The number of different types of spermatogenic cells was significantly increased. In addition to an increase in the immunological reaction for vimentin and AR compared to group I. Rats of three months old showed the same results as group II.

Signs of early aging appeared at late post pubertal group (twelve months old rats). Tunica albuginea capsule and interstitial collagen fibers were significantly increased compared to group II. The diameter and germinal epithelial thickness of SNT were significantly decreased compared to group II. The SNT showed irregular outlines with exfoliation of spermatogenic cells and loss of their basal epithelial lining. The number of different types of spermatogenic cells was significantly decreased with significant decrease of the immunological reaction for vimentin and AR compared to group II.

The interstitium of the testis showed multiple thickened congested blood vessels, few mature Leydig and degenerated cells. The most important result of this subgroup was the presence of testicular niche consisted of embryonic like stem cells having large euchromatic nuclei to cytoplasmic ratio together with other dark stained nuclei most probably progenitor cells, macrophages, blood vessels and Leydig cells.

Conclusion: Testes of rats showed accelerated growth during puberty in which there were significant increase in the number of germ cells and immunological reaction for vimentin and AR. At late post pubertal periods, signs of aging started to appear including testicular fibrosis with multiple thickened blood vessels, significant decrease in the number of germ cells and in the immunological reaction for vimentin and AR.

Keywords: testis, leydig cells, sertoli cells, aging.

Introduction

Infertility is a major human health problem which can begin at any age. Approximately 15 % of couples are infertile, and among these couples, male factor infertility accounts for approximately 50 percent of causes. Testicular growth is important for future male fertility which shows acceleration during puberty **(Dygalo et al., 2014).**

Post-natal development of the testis depends on the action of the gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are secreted by the pituitary gland. FSH acts directly on the Sertoli cells through the FSH-receptor while LH acts to stimulate androgen secretion by the Leydig cells. This androgen then acts on all cells expressing the androgen receptor (AR) in the testis; primarily the Sertoli cells, peritubular myoid cells and the Leydig cells **(O'Shaughnessy et al., 2012).**

Leydig cells are the major source of androgen in the male mammal. Androgens are the keystone in fertility and intact sexual functions in males. It exerts its actions via androgen receptors present extensively in testicular cells. The alteration of androgen receptors in different testicular cells is usually accompanied by sexual disorders **(Kilcoyne et al., 2014).**

Spermatogenesis which is a highly ordered process includes mitotic and meiotic divisions that

results in generation of haploid germ cells (spermatozoa). This process is initiated at puberty and involves continuous and serial cellular proliferation and differentiation events **(Murta et al., 2013).**

The development and differentiation of cells involved in spermatogenesis requires highly regulated and coordinated interactions between cells. Intercellular communication plays a critical role in the development of germ cells during fetal development and during spermatogenesis in the adult**.** It represents a critical aspect of male reproductive function and fertility **(Kidder and Cyr, 2016).**

The Sertoli cells, which are the main regulators of spermatogenesis in the adult testis, regulate also the differentiation of testis during development. It forms the blood-testis barrier which is one of the tightest blood-tissue barriers in mammals **(De Freitas et al., 2016).**

Many studies performed on Spermatogenesis process have focused either on the hormonal events of the hypothalamus-pituitary-testicular axis or morphological events that take place in the seminiferous epithelium. Recent advances in biochemistry, cell biology, and molecular biology have shifted attention to understanding some of the key events that regulate spermatogenesis, such as Sertoli-germ cell communication, junction dynamics, germ cell apoptosis and cell cycle regulation **(Cheng et al., 2010).**

Aim of this work:

To evaluate the histological changes that occur in albino rat testis during pre-pubertal, pubertal and post pubertal periods. Furthermore, to make a comparable histological and morphometric study on testicular post natal development as it was not performed previously. Hopefully, this would be of help in understanding the testicular behavior and their correlations to infertility problems.

I- Gender determination and prenatal development of the testis

Gender differentiation is accomplished through a cascade of gene activations. Genetic sex is determined at fertilization by the presence or absence of the Y chromosome. The testes, however, do not form until the seventh week of development. **Gonadal sex** is determined by the presence of the **SRY gene** located in the **sex-determining region** of the short arm of the Y chromosome. The SRY gene triggers the sexual differentiation of the gonads into testis; thus, it is responsible for **gender determination.** It operates as a *master switch* that activates several genes on autosomal chromosomes 9, 11, 17, 19 and the X chromosome **(Makiyan, 2016)**.

A transcription factor called the **testis - determining factor (TDF),** encoded by the SRY gene causes the expression of other genes that initiate formation of not only the testes but also other male sex organs. Several other genes are expressed about the same time as the SRY gene, including the following:

• **WT-1 gene** (Wilms tumor 1 gene), which is required for the development of the urogenital system and for regulation of the SRY transcription.

• **SOX -9 gene** (SRY [sex determining region Y]-box 9 gene) found in the genital ridges activates the anti-

Müllerian hormone gene **(AMH gene)** that is responsible for Müllerian-inhibiting factor synthesis.

• **SF-1 gene** (steroidogenic factor-1 gene) that regulates the expression of a number of steroidogenic genes **(Ross and Pawlina, 2016).**

The **testes** develop in close association with the urinary system retroperitoneal on the posterior wall of the abdominal cavity. Testes (like ovaries) are derived from three sources:

• **Intermediate mesoderm** forms the urogenital ridges on the posterior abdominal wall, giving rise to Leydig cells (interstitial cells) and myoid cells (peritubular contractile cells).

• **Mesodermal epithelium (coelomic mesothelium)**

Lines the urogenital ridges and gives rise to finger-like epithelial cords called primary sex cords. The primary sex cords give rise to Sertoli cells.

• **Primordial germ cells** migrate from the yolk sac into developing gonads, where they are incorporated into the primary sex cords. Here they divide and differentiate into spermatogonia. At this stage, these primary sex cords are composed of primordial germ cells, pre-Sertoli cells, and a surrounding layer of myoid cells. Later, these cords differentiate into the seminiferous cords, which give rise to the seminiferous tubules, straight tubules, and rete testis (diagram 1) **(Bloom and Fawcett, 1996)**.