The Effect of Hormonal Male Contraception on Sertoli-germ Cell Interaction in Albino-mice Testes: Morphological Study

Abd Al-Jabber Falih Hussein
MBChB, MSc

Abstract:

Background: The Sertoli cells have special adhesive junction called ectoplasmic specialization make the adhesion between the Sertoli and germ cells ,there are also adhesive proteins which are produced under Follicle stimulating hormone and testosterone hormone control ,these junctions are important in translocation of germ cells through the epithelium toward the lumen .

Objective: To study the effect of hormonal male contraception on Sertoli-germ cell interaction.

Materials & Methods: 20 adult male albino mice *mus musculus* were used and divided into two groups :treated and control groups ; the treated group include 10 mice receive weekly intramuscular injection of 10 mg/kg of medroxyprogesterone acetate (as hormonal male contraception) and testosterone enanthate to keep testosterone level within physiological level ,while control group receive 0.1 ml of normal saline .

Results: The histological examination of treated testes reveal disorganization of the seminiferous epithelium with loss of adhesion between cells and some cells are sloughed into the lumen with formation of gap around the germ cells due to shrinkage of Sertoli cells.

Conclusions: Hormonal male contraception affects the production of adhesive proteins between sertoli and germ cells with disorganization of Sertoli cell cytoskeleton which leads to spermiation failure and germ cell sloughing and detachment.

Key words: Spermatid sloughing, spermiation failure, ectoplasmic specialization, hormonal male contraception

Introduction:

Morphological studies of the testis have identified that there are three types of junctions found in the cells of the seminiferous tubules are occluding junction , anchoring junction and Communicating gap junction [1].

The seminiferous epithelium contains an architecturally unique type of cell –cell junction termed as Ectoplasmic specialization (ES) [2] as shown in the (Figure 1).

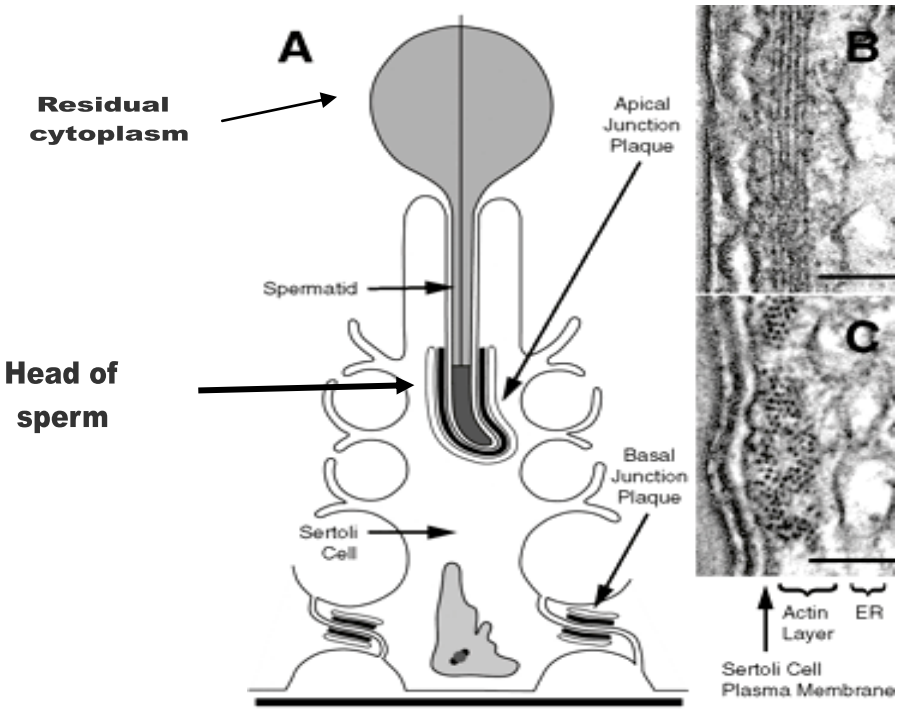

Figure (1): Position of ectoplasmic specializations in the seminiferous epithelium of the testis. (A) Ectoplasmic specializations are present only in Sertoli cells and occur apically at sites of adhesion to spermatids and basally at sites of adhesion to neighboring Sertoli cells. (B, C) Typical appearance of ectoplasmic specializations in transmission electron micrographs. The structures consist of the plasma membrane of the Sertoli cell, a layer of actin filaments and a cistern of endoplasmic reticulum (E.R.). (2)

Ectoplasmic Specialization (E.S) is considered to be an adhesion junction .As developing spermatids mature and elongate, E.S. are formed in Sertoli cells adjacent to spermatid heads, this site of adhesion between germ cell and Sertoli cell is disassembled as part of the process by which spermatids are released in the tubular lumen [3], these junctions are also believed to be fundamental in the maintenance of adhesion between neighboring Sertoli cells near the base of the epithelium to forms the bases of blood – testis –barrier [4] .

One of the functions of the ectoplasmic specialization (E.S) may be due to facilitation of translocation of elongated spermatids through the epithelium. At the completion of elongation, E.S is removed during spermiation [5].

The molecules reported to be involved in E.S include actin [6], Vinculin [7], actinin [6], Fimbrin [8] and espin [9], and the only transmembrane adhesion molecule known to be present is integrin [10, 11].

FSH is responsible for the alignment of the actin and vinculin filaments of the Sertoli cell cytoskeleton [12], which are needed for testosterone dependant adhesion between Sertoli cell and spermatids [13].

The functional role of integrin in the seminiferous epithelium is based on the disappearance of integrin concurrent with turnover of E.S [14], when spermatocytes translocate from the basal to the adluminal compartment, integrin expression disappears and then subsequently reappears [11]. Also the disappearance of integrin has been shown to coincide with disassembly of the apical ES and spermatid release into the tubular lumen [10].

Materials & Methods

1. **The Animals:** Twenty adult male albino mice *Mus. musculus* were used in the project were divided into the following groups:

Control group: The number of the animals in this group was ten, this group received 0.1ml normal saline by intramuscular injection with syringe of 1ml **Treated group**: The number of the animals in this group was also ten .These animals received weekly intramuscular injections of 10 mg/kg Medroxyprogesterone acetate (MPA) and 10 mg/kg of Sustanon. Each animal received four doses and then scarified.

All animals in this study were dissected under anaesthesia using chloroform. When the animals were anaesthetized and while the heart was still beating, a T-shaped abdominal incision was done and the right testis was removed.

2. **The Hormones:** The following hormones were used during the treatment:

A-Medroxyprogesterone acetate (MPA) is synthetic progesterone structurally related to progesterone given by weekly intramuscular injection in a dose of 10mg/kg as a long acting contraceptive which will suppress both FSH and LH and subsequently testicular testosterone [15].

B-Sustanon 100 mg/ml contains: Testosterone propionate 20 mg, Testosterone phenyl propionate 40 mg, Testosterone isocaproate 40 mg.

The dose of Sustanon that was used in the experiment was weekly intramuscular injection of 10 mg/kg to keep the animals within physiological level of testosterone.

Results:

The lumen of most of the seminiferous tubules of control testes were filled with sperms (**Figure2**), while those of treated testes were devoid from sperms The histological examination of treated testes stained with haematoxylin & eosin show the seminiferous epithelium which was disorganized , disrupted with loss of cellular cohesions , and showed arrested ,degenerated spermatogenic cells, there was a wide rim or gap of extracellular spaces between the germs cells and the surrounding cytoplasmic extensions of Sertoli cells probably caused by shrinkage of both cells **.(figure 3,4)**

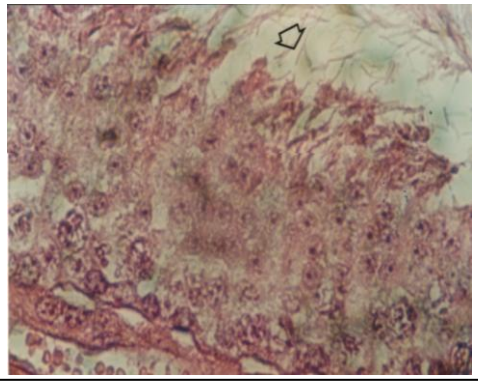

Figure (2): Transverse section of seminiferous tubules of animal of control group shows the normal cellular cohesions with the presence of sperms inside their lumen (open arrow). [stained with H&E. 600X]

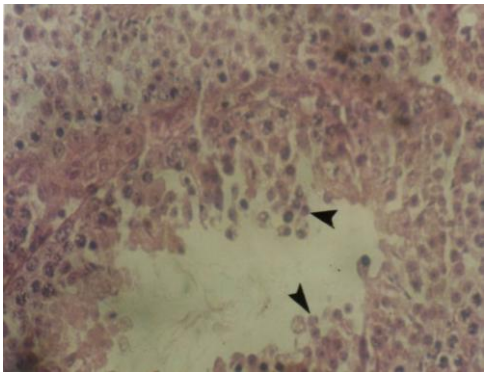

Figure (3): Seminiferous tubules of treated testis show the disruption of germ cell cohesions with the appearance of spermatids that are degenerated and sloughed into the lumen (arrow heads). [stained with H&E. 400X].

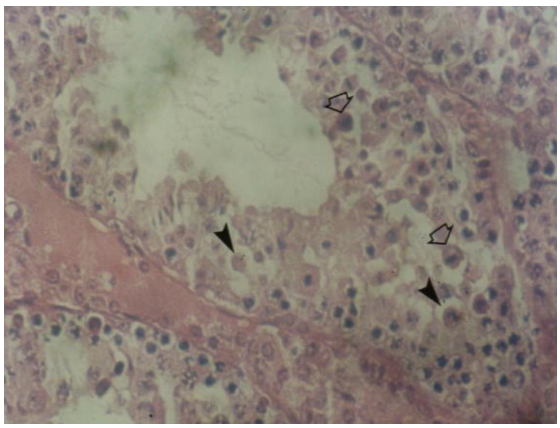

Figure (4): Seminiferous tubules of treated testis show the extracellular rim or gap around the germ cells (arrow heads) and the degenerated germ cells (open arrows). [stained with H&E. 400X].

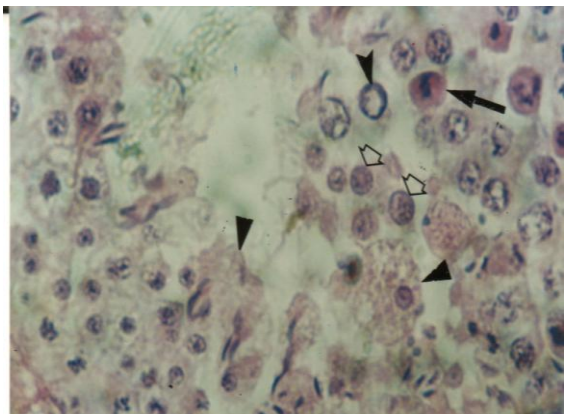

Figure (5): A lumen of seminiferous tubule of treated testis demonstrates the degenerated acidophilic germ cells (arrow), detached spermatids (open arrows), cytoplasmic debris (filled triangles), apoptotic bodies and intraepithelial vacuolation (arrow head). [stained with H&E. 600X].

But some of seminiferous tubules contained normal spermatids or immature germ cells due to their detachment or sloughing from the seminiferous epithelium, the lumen also contained large amount of spermatogenic debris. **(Figure 5, 6, 7)**

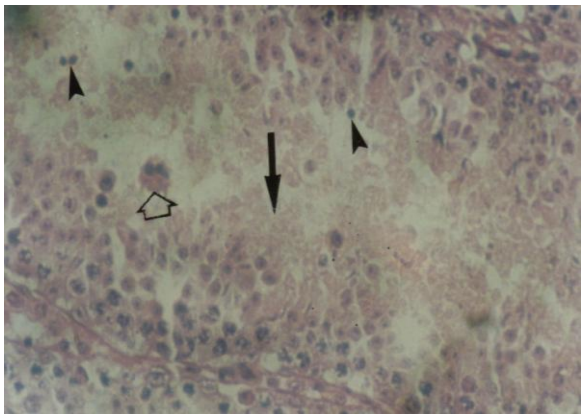

Figure (6): A lumen of seminiferous tubule of treated testis contains cytoplasmic debris, apoptotic bodies (arrow heads), degenerated inculcated cell (open arrow), degenerated spermatids and seminiferous fluid that is not reabsorbed (arrow). [stained with H&E. 400X]

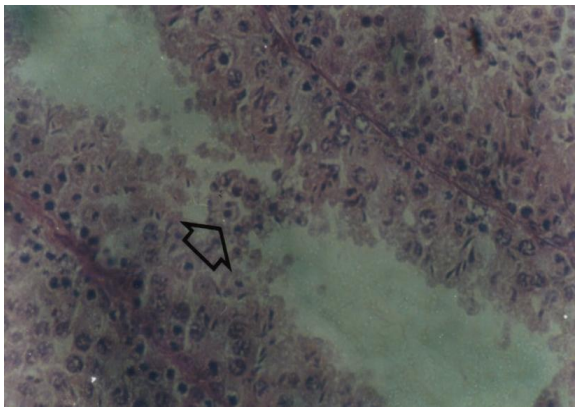

Figure (7): A lumen of seminiferous tubule of treated testis shows the sloughing of spermatids into the lumen (open arrow). [stained with H&E. 400X].

Discussion:

The suppression of either FSH or testosterone led to 10-16% of spermatids failing to spermatid, however the combined suppression of FSH and testosterone for the same period (1 week) led to a striking degree of spermiation failure, with half of the mature spermatids being retained [16].

The spermiation failure is an early event during spermatogenic suppression followed by spermatid degeneration and sloughing then degeneration of primary spermatocytes [16].

The retained spermatids have no ectoplasmic specialization attached to them as suggested by [17]. But the ectoplasmic Specialization (E.S.) were present in the seminiferous epithelium even when exposed to long time of gonadotropin suppression and the formation and recycling of these structure remain relatively normal , suggesting that the process of detachment is not due to the absence of E.S [18]. Adult rat hypophysectomy promotes disorganization of E.S, and of actin and vinculin distribution [19]. The finding that the E.S structures are present during hormonal suppression raised the question that why round spermatids detached from the Sertoli cell. There may be a deficiency in adhesive structure that normally formed between round spermatid and Sertoli cell E.S. [18]. The α6β1-Integrin could be one of the cell adhesion molecules [14], also N. Cadherin is actively involved in spermatid- Sertoli adhesion , the production of N-cadherin by Sertoli cell and the binding of spermatid to Sertoli cell is stimulated in synergistic manner by testicular testosterone and FSH [20], that are suppressed by MPA (medroxyprogesterone acetate).

The intermediate filaments of the Sertoli cells normally are centered on the nucleus, where they apparently terminate in the nuclear envelop and radiate to the plasma membrane, apically often a close association with microtubules is seen. Both in rat and human, changes in cell shape during spermatogenic cycle are associated with redistribution of intermediate filaments, these changes are hormone dependant (Vimentin phosphorylation subsequent to FSH stimulation), Vimentin filaments shortened at the stage of spermiation [21].

Suppression of testicular testosterone leads to degradation of vimentin filaments with collapsing of vimentin cytoskeleton around Sertoli cell nuclei at all stages of the spermatogenic cycle [22], retraction of the Sertoli cell processes, germ cell detachment [23].

Spermiation may provide positive signals to the Sertoli cell to continue with spermatogenesis, and when there is spermiation failure so these signals gradually decline and then round spermatids sloughing begin [24].

So it appears so clear that how Medroxyprogesterone acetate causes gonadotropin suppression FSH and LH with subsequent testicular testosterone which disrupt the adhesive junctions between the germ cells and Sertoli cells through the disorganization of ectoplasmic specializations and through the deficiency of FSH and testosterone dependant adhesive proteins that explain the sloughing or detachment of round spermatid from the Sertoli cells to be seen within the seminiferous tubules lumen and also explain the wide extracellular spaces between Sertoli cells and germ cells.

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Department of Anatomy College of Medicine Al-Mustansirya University