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Injection of Fresh versus Frozen testicular sperms in azoospermic males undergoing ICSI.

Thesis Submitted for partial fulfillment of the Master Degree in Dermatology, Venereology and Andrology

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Review of literature

Introduction

Infertility affects approximately 13-14% of reproductive-aged couples. It is defined as the inability to conceive after 1 year of properly timed, unprotected intercourse.
(**WHO, ICMART, 2009)**

Since the establishment of In Vitro Fertilization, it became evident that almost half of the couples failed to achieve fertilization and this phenomenon was attributed to a male gamete dysfunction. The adoption of assisted fertilization techniques particularly Intracytoplasmic Sperm Injection (ICSI) has been able to alleviate male factor infertility by granting the consistent ability of a viable spermatozoon to activate an oocyte. **(Queenie V. et al, 2014)**

Palermo et al achieved the first successful attempt of ICSI in 1992. In that case, sperms from oligoteratoasthenospermic males were used. **(Palermo et al, 1992)**

In 1993, researchers discovered the ability of testicular sperm to fertilize oocytes; this realization started a new era for the treatment of azoospermic patients. **(Craft et al, 1993; Schoysman et al, 1993 and Devroey et al, 1994)**

Hotchkiss and Engle have first reported testicular biopsy in 1933. Surgical techniques for testicular sperm retrieval began with open surgery. Other techniques included Fine Needle Aspiration (FNA) and Microscopic Testicular Sperm Extraction (mTESE). **(Sharif K, 2000)**

The introduction of an operating microscope has led to the microsurgical testicular sperm extraction (mTESE) technique, which shows higher sperm collection rates and fewer complications **(Dardashti et at, 2000)**. Testicular biopsy is now a prerequisite for treating infertile men **(Anderson et al, 2010)**.

Sperm cryopreservation is a technique that can keep sperms alive indefinitely. To date there is no agreement in the literature on whether or not cryopreservation affects sperm chromatin integrity. Testicular sperm cryopreservation was introduced as a logical step to reduce the number of procedures required to obtain testicular sperms **(Paoli et al, 2014)**.

There are conflicting reports of results of injection of fresh versus frozen thawed sperms regarding fertilization and pregnancy. While some concluded that the use of cryopreserved sperm yields inferior results as compared to fresh sperm **(Park et al, 2015 and Mustafa et al, 2008)**, others concluded that cryopreservation yields similar results **(Bruno et al, 2015 and Habermann et al, 2000)**

Some even stated that cryopreservation of testicular sperm and tissues is more suitable and of great benefit, it is particularly beneficial if carried out before ovulation induction of the female partner in preparation for ovum retrieval and not after. **(Tavukcuoglu et al, 2013)**

Aim of the work:

The aim is to assess whether cryopreservation of testicular sperms affects fertilization and pregnancy rates in azoospermic men undergoing ICSI.

Chapter I:

Testis Anatomy

Structure of the Testes

The human testes are two organs of the shape of rotation ellipsoids with diameters of 2.5 × 4 cm engulfed by a capsule (tunica albuginea) of strong connective tissue Thin septula testis divide the parenchyma of the testis to about 370 conical lobules. **(Middendorff et al, 2002)**.

The lobules consist of the seminiferous tubules and intertubular tissue, containing groups of endocrine Leydig cells and additional cellular elements. The seminiferous tubules are coiled loops. Both their ends open into the spaces of the rete testis. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered to the extra testicular portion of the excurrent ductal system of the epididymis.
(Holstein et al, 1996)
(Fig.1 and 2)

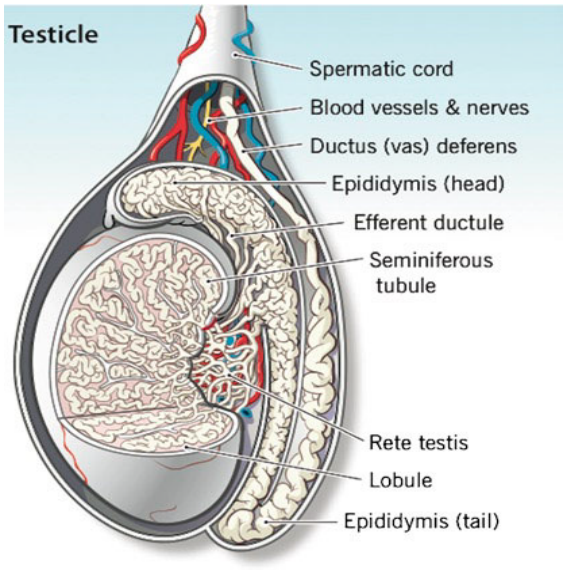

Fig 1. Anatomy of the testis. **(Zini Et al, 2011)**

Structure of the seminiferous tubule

The seminiferous tubule consists of the germinal epithelium and the peritubular tissue (lamina propria) **(Davidoff, 1990).**

The mean diameter of a seminiferous tubule is about 180 μm, the height of the germinal epithelium measures 80 μm and the thickness of the peritubular tissue is about 8 μm. The germinal epithelium consists of cells that include different developmental stages of germ cells, namely spermatogonia, primary and secondary spermatocytes and spermatids. These are located within invaginations of Sertoli cells.
(Holstein et al, 1999).

The prismatic Sertoli cells are connected by specialized zones of tight junctions of cellular membranes separating the germinal epithelium in a basal and an adluminal compartment.

These specialised zones, the so-called "tight junctions" form the blood-testis barrier of the testis. During maturation the germ cells pass this barrier entering the adluminal compartment where they find protection from diffusion of extraneous substances. Sertoli cells also exhibit increasing amounts of lipid droplets in correlation to advanced age being an indicator of the "biological clock" of the testis **(Holstein et al, 1999)**.

Further functions attributed to Sertoli cells include:

1. Sustentacular and nutritive functions for the germ cells.
2. Organization of the delivery of mature spermatids into the tubular lumen (spermiation).
3. Production of endocrine and paracrine substances for the regulation of spermatogenesis.
4. Secretion of androgen binding protein (ABP).
5. Interaction with the intertubular endocrine Leydig cells.

(Russell, et al, 1993)

The peritubular tissue (lamina propria of seminiferous tubules) consists of around five layers of myofibroblasts with intermingled connective tissue ground substance. The myofibroblasts cause peristaltic contractions of the seminiferous tubule giving rise to transport of the immotile spermatozoa to the rete testis The thickness of the peritubular tissue normally is about 8 μm. In cases of disturbed spermatogenesis the peritubular tissue may be thickened by connective tissue ground substance up to 12 μm.
(Zini et al, 2011).

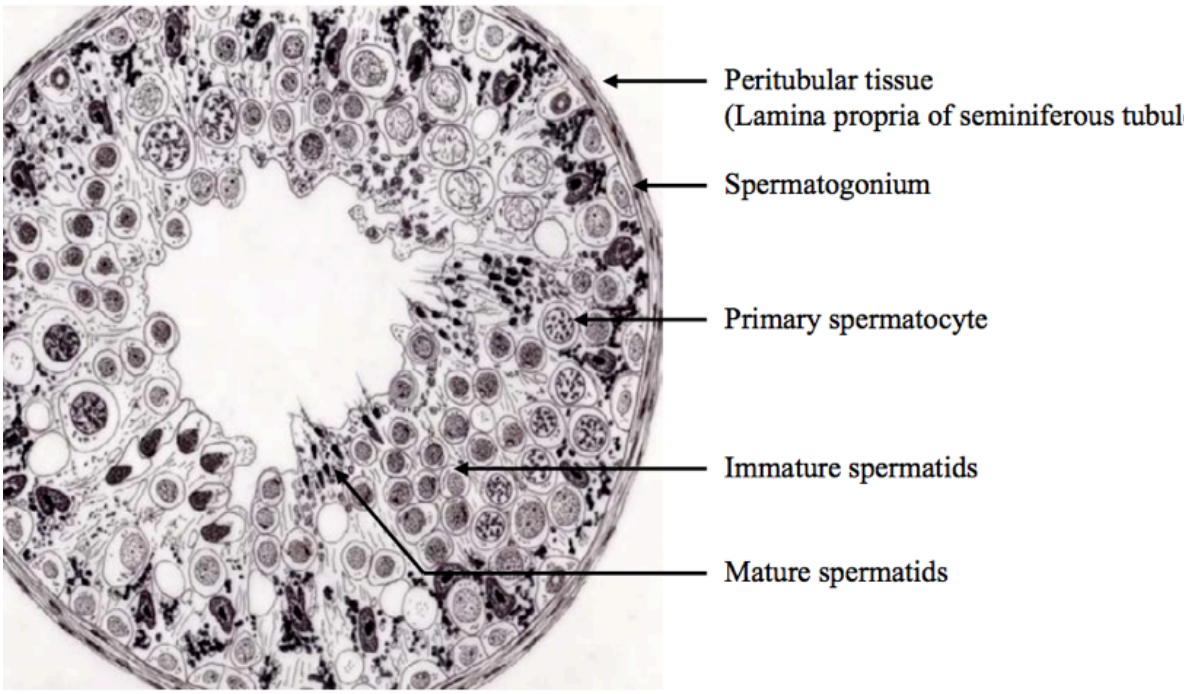

Fig 2. Microscopic stucture of Seminiferous tubules **(Schulze et al, 2003)**

Chapter II: Spermatogenesis

Introduction

Spermatogenesis begins at puberty after a long preparatory period of "prespermatogenesis" in the fetus and the infant. This process can be subdivided into three major steps:

(i) The multiplication of spermatogonia by the process of mitosis, known as spermatogoniogenesis;
(ii) Meiosis, which reduces the chromosome number from diploid to haploid. It commences with the entry of type B spermatogonia into the prophase of the first meiotic division. These cells, now called primary spermatocytes, divide to form secondary spermatocytes, and then divide again to form round spermatids;
(iii) Spermiogenesis; the successful transformation of the round spermatid into the complex structure of the spermatozoon.

Each of these steps represents a key element in the spermatogenic process. Defects in any of them can result in the failure of the entire process and lead to the production of defective spermatozoa and reduction or absence of sperm production. **(Russel el al, 1993)**

Spermatogoniogenesis:

Several types of spermatogonia are distinguished by their position in the basal part of the germinal epithelium, their morphology and stainability of nuclei. Fetal spermatogonia become transitional spermatogonia and later become spermatogonia type Ad (dark). Spermatogonial stem cells undergo proliferative events and produce a population of cells that have distinct nuclear appearance. **(Gunes et al, 2015)**

Dark type A spermatogonia are stem cells of the seminiferous tubules that have an intensely stained dark ovoid nucleus containing fine granular chromatin. These cells divide by mitosis to generate Dark and Pale Type A spermatogonia.

Pale Type A spermatogonia have pale staining and fine granular chromatin in the ovoid nucleus.

Type B spermatogonia are characterized by large clumps of condensed chromatin under the nuclear membrane of an ovoid nucleus.

(Gunes et al, 2015)

All type A spermatogonia are necessary for intact spermatogenesis. In reduced spermatogenesis, Type A dark spermatogonia are often absent. In cases of disturbed ability of spermatogonia to develop B-type spermatogonia the number of A pale type spermatogonia increases and bi- or multilayered groups of spermatogonia in the basal compartment are formed without further developed germ cell stages. This aspect represents an arrest of spermatogenesis at the stage of spermatogonia **(Holstein et al, 1998).**

Spermatogonia multiplicate continuously in successive mitoses. The daughter cells remain interconnected by cytoplasmic bridges so that a clone derived from one stem cell, forms a syncytium of cells. Syncytial connections are maintained through spermatogonial and spermatocytic stages and are dissolved only in advanced phases of spermatid development. It is thought that the formation of these clones is the basis for the synchronous development of germ cells. **(Zini et al, 2011)**

Type B spermatogonia divide mitotically to produce primary spermatocytes (preleptotene, leptotene, zygotene, and pachytene), secondary spermatocytes and spermatids (Sa,Sb,Sc,Sd1, and Sd2), **(Holstein et al, 2003).**