The Expression of Progesterone and Estrogen Receptors in Testicular Tissue among Obstructive and Non-obstructive Azoospermia

Thesis
Submitted for Partial Fulfillment of the Master Degree in
Dermatology, Venereology and Andrology

Presented by
Remon Mounir Shaker
M.B.B.Ch. Faculty of Medicine

Under Supervision of

Prof. Dr.Adel A. Halim Emam

Professor of Dermatology, Venereology and Andrology
Faculty of Medicine – Ain-Shams University

Dr. Khaled M. Abd El-Raouf EL-Zawahry

Lecturer of Dermatology, Venereology and Andrology
Faculty of Medicine –Ain-Shams University

Faculty of Medicine
Ain-Shams University
2013

*First, thanks are all due to **Allah** for Blessing this work until it has reached its end, as a part of his generous help throughout our life.*

*My profound thanks and deep appreciation to **Prof. Dr. Adel A. Halim Emam**, Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University for his great support and advice, his valuable remarks that gave me the confidence and encouragement to fulfill this work.*

*I would like also to express my deep and special thanks to**Dr. Khaled M. Abd El-Raouf El-Zawahry**, Lecturer f Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University for his generous help, guidance and patience through all the stages of this work. This work could not have reached its goal without his help.*

*I am also thankful to **Prof. Dr. Wafaa Helmy,** Professor of Pathology, Faculty of Medicine, Al-Azhar University for her valuable supervision, co-operation and direction that extended throughout this work.*

*I am deeply grateful to **my mother and father** who directed and encouraged me during the preparation of this work.*

*Lastly, Words fail to express my love, respect and appreciation to **my wife Dr. Gena Gamal** for her unlimited help, support and faith.*

Remon Mounir Shaker

List of Contents

List of Abbreviations

ABP	: Androgen binding protein
AF	: Activation function
AQP	: Aquaporins
AR	: Androgen receptor
ART	: Artificial reproductive techniques
ATG	:Adenosine-thimine-guanosine
ATPase	: Adenosine tri phosphatase
AZF	: Azoospermia factor
CAG	: Cytosine adenine guanosine
CBAVD	: Congenital bilateral absence of the vas deferens
CF	: Cystic fibrosis
CFTR	: Cystic fibrosis transmembrane regulator
DM	: Diabetes Mellitus
DBD	: DNA-binding domain
DBY	: DEAD box proteins
DES	: Diethylstilbestrol
DHT	: Dihydrotestosterone
DNA	: Deoxyribonucleic acid
E2	: Estradiol
ER	: Estrogen receptor
ERE	: Estrogen response element
FSH	:Follicle-stimulating hormone
FSH-R	: Follicle-stimulating hormone receptor
GnRH	: Gonadotropin-releasing hormone
GPER	: G protein-coupled estrogen receptor
H & E	: Hematoxylin and Eosin
hESR	: Human estrogen receptor
HGH	: Hypo-gonadotropic hypogonadism
HPG	: Hypothalamic-pituitary-gonadal
HS	: Highly significant
HSD	: Hydroxysteroid dehydrogenase
ICSI	: Intra cytoplasmic sperm injection
IF	: Inhibitory function

List of Abbreviations(Cont.)

IgG	: Immunoglobulin G
KAL gene	: Kallmann gene
kDa	: Kilodalton
KS	: Klinefelter Syndrome
LBD	: Ligand-binding domain
LC	: Leydig cells
LH	: Luteinizing hormone
LH-R	: Luteinizing hormone receptor
MRI	: Magnetic resonance imaging
mRNA	: Messenger ribonucleic acid
NOA	: Non-obstructive azoospermia
NS	: Non significant
PR	: Progesterone receptors
SCC	: Side chain cleavage
SCO	: Sertoli cell only
SD	: Standard deviation
SGP-2	: Sulphated glycoprotein 2
SP	: Synthetic peptide
SRY	: Sex-determining region Y protein
StAR	: Steroidogenic acute regulator
TB	: Tuberculosis
TESE	: Testicular sperm extraction
TGF	: Transforming growth factor
TRUS	: Transrectal ultrasound
TUR	: Transurethral resection
WHO	: World health organization

List of Tables

List of Figures

Introduction

Azoospermia

Azoospermia is a descriptive term referring to ejaculates that lack spermatozoa without implying a specific underlying cause. The condition is almost always an unforeseen finding when semen analysis is performed for any indication. Only in a few cases is azoospermia expected prior to semen analysis, such as in cystic fibrosis, Klinefelter's syndrome and previous vasectomy cases. Such azoospermic semen samples are found in up to 2% of the adult male population and 5-59% of infertile men.It is important for azoospermia to be distinguished from aspermia; specifically, the latter indicates the lack of semen formation or the lack of ejaculation, such as in the case of total retrograde ejaculation **(Aziz, 2013).**

The appropriateness of the term azoospermia and the reliability of diagnosing the absence of spermatozoa have been the focus of debate over the past decade. The traditional definition of azoospermia is ambiguous, which has ramifications on the diagnostic criteria **(Sharif, 2000).**

Thus, the accurate assessment of very low sperm counts is particularly important to avoid labeling severely oligospermic men as azoospermic **(Ezeh and Moore, 2001).**

The total number of sperm in an ejaculate is influenced by testicular sperm production, the integrity of the conducting system, the presence of retrograde ejaculation (partial or total), and the duration of abstinence before the analysis. The WHO laboratory manual for the examination and processing of human semen includes standards to enhance the accuracy and precision of the sperm number estimates to make them reproducible. Special attention is required to control patient-related factors, such as the optimal abstinence duration of 2-7 days and the complete collection of ejaculate. Frequent

ejaculation within a short period of time may deplete the epididymal stores, resulting in hardly any detectable sperm in the semen sample. Similarly, losing the first portion of the ejaculate, which is the sperm-rich portion, will significantly affect the accuracy of the assessment of spermnumber **(Bjorndahl and Kvist, 2003).**

Azoospermia is generally indicative of either complete bilateral obstruction of the male excurrent ductal system (obstructive azoospermia) or severely impaired sperm production (non-obstructive azoospermia). Fertility is only possible in affected men through medical or surgical intervention. The goals of the diagnostic evaluation are to identify underlying etiologies that are of medical or prognostic significance, to identify genetic abnormalities that may affect the patient's offspring, and to guide the selection of medical or surgical therapy **(Castilla et al., 2006).**

The initial evaluation of azoospermia should include a detailed medical history, a directed physical examination, measurement of serum testosterone, follicle stimulating hormone, This history is sufficient for differentiation of obstructive and non-obstructive azoospermia **(Chang et al., 2004).**

Sperm are often intermittently present in the ejaculate of patient with severely impaired sperm production. Rare sperm detected upon repeat semen analysis in patient who was previously azoospermic should be cryopreserved whenever possible **(Carlsen et al., 2004).**

Spermatogenesis :

Spermatogenesis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium.These hormonal messengers are

critical not only for regulation of male germ cell development, but also for the proliferation and function of the somatic cell types required for proper development of the testis **(McLachlan et al., 2002).**

These include the interstitial steroidogenic Leydig cells, whose primary function appears to be production of testosterone the myoid cells that surround the seminiferous tubules and provide physical support and contractile motion to these structures and the Sertoli cells, whose direct contact with proliferating and differentiating germ cells within the seminiferous tubules makes them essential for providing both physical and nutritional support for spermatogenesis. Each of these cell types is a direct target for one or more of the hormones whose actions are essential for unimpaired male fertility **(Holdcraft and Braun, 2004).**

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are glycoprotein hormones secreted by the anterior pituitary that act directly on the testis to stimulate somatic cell function in support of spermatogenesis. These hormones, part of the transforming growth factor (TGF) β superfamily of secreted growth factors, share a common α-subunit and are distinguished by their hormone-specific β-subunit. In males, FSH receptor expression (FSH-R) is limited to the testicular Sertoli cells, whileLH receptors (LH-R) are found primarily in the Leydig cells, although receptor staining is also observed in spermatogenic cells **(Lei et al., 2001).**

Genetic and pharmacological studies in rodents indicate that the primary role of FSH in spermatogenesis is stimulation of Sertoli cell proliferation during prepubertal development. Sertoli cell number largely determines the number of germ cells **(Heckert et al., 2002).**

These results are consistent with hormone depletion-replacement studies in human males, FSH can also rescue spermatogenesis in gonadotrophin-suppressed men independently of Testosterone **(Holdcraft and Braun, 2004).**

Testosterone and its metabolites, dihydrotestosterone (DHT) and estradiol (E2), are collectively referred to as the sex hormones. This is because of their primary role in the regulation of gonadal and germ cell development in both males and females as well as in the sexual differentiation of males **(Hess et al., 2000).**

Estrogen receptors in male :

Dorrington et al. have demonstrated that the testis wasable to produce estrogens **(Carreau et al., 2012).** In testes, as in other tissues, the aromatase is the enzyme which irreversibly transforms androgens into estrogens and in the adult mammalian testis it is well known that aromatase is localized in Leydig cells **(Carreau, 2007).**

Nevertheless, the last decade has provided a growing body of evidence that within seminiferous tubules, germ cells at least in the numerous studied species participate in the production of testicular estrogens **(Carreau et al., 2009).**

In order to exert a biological effect, estrogens should interact with estrogen receptors which in turn can modulate the transcription of target genes (genomic effect) and/or activate different signalization pathways located on the membrane (non-genomic effect) Two types of nuclear estrogen receptors have been described: Era (ESR1) and ERb (ESR2) **(Carreau and Hess, 2010).**

In a man, the presence of a biologically active aromatase and estrogen receptors (ER a and ERb) has been reported in Leydig cells, and also in immature germ cells and ejaculated

spermatozoa **(Carreau et al., 2010).** Moreover, human spermatozoa expressed the receptor coupled to a G-protein (GPER), and at the transcript level in the human biopsies from testisand our unpublished data. Concerning aromatase, the amounts of transcript and enzymatic activity were decreased in immotilecompared with motile sperm in humans **(Franco et al., 2011).**

Progesterone receptors in male :

In contrast to the established unequivocal roles of progesterone in female reproductive physiology, there are limited data on the role of progesterone in male reproductive events. The actions of progesterone are generally mediated via conventional intracellular progesterone receptors (PRs) that belong to the superfamily of transcription factors expressed in a variety of female foetal and adult tissues **(Conneely and Lydon, 2000).**

Capacitation is a multifaceted process occurring in the female genital tract by which spermatozoa acquires the ability to fertilize an oocyte. progesterone has been shown to activate several signalling pathways involved in the regulation of sperm functions. It was reported that progesterone induces hyperactive motility and acrosome reaction of mammalian spermatozoa during the transit along the female reproductive tract **(Gadkar et al., 2005)**.later, a non-genomic plasma membrane PRwas found in the acrosomal region. Specific progesterone sperm-binding sites are located on the plasma membrane of the spermatozoon. Binding studies also revealed the presence of two classes of PRs in the human spermatozoon: one class has an elevated affinity constant (nanomolar) and it is specific for progesterone, whereas the other class has an affinity constant in the micromolar range **(Wu et al., 2006).**

In humans, PR mRNA and protein are reduced in testis of azoospermic men with maturation arrest and hypo-spermatogenesis and also in spermatozoa of oligoastheno-teratozoospermic infertile men **(Gwen et al., 2011).**

These observations are definitive evidence to suggest that PR has a role in regulation of spermatogenesis and reduced PR expression is associated with defective spermatogenesis. However, the reason for the loss of PR expression in the testis of infertile men is yet not clear **(Ben-Yehoushua et al., 2007).**

Aim of the Work

The objective of this study is to determine the expression of progesteroneand estrogen receptors in the testicular tissue of the patients. In order to focus a light over the role of the PR and ERα in the men with infertility.

Chapter 1-1
Azoospermia

Definition and incidence:

Azoospermia is defined as the absence of sperm in the ejaculate based on centrifugation and examination of the pellet. Importantly, semen analysis has a detection limit of approximately 100 000/ml, meaning there may well be sperm present in an 'azoospermic' sample **(Cooper et al., 2006).**

This fact, combined with the intrinsic variability in sperm output, accounts for the well recognized phenomenon of 'intermittent azoospermia'. Increased sensitivity of sperm detection (e.g. fluorescent labelling) reduces the apparent incidence of azoospermia **(Mommers et al., 2008).**

In any event, at least two analyses are needed to confirm azoospermia; the finding of any sperm on any occasion substantially increases the likelihood that sperm will be available for ICSI in either semen or biopsy tissue by pointing to the presence of active spermatogenesis (**Tüttelmann et al., 2011).**

When the ejaculate volume is less than 1 ml, ejaculatory dysfunction, obstructive azoospermia from ejaculatory duct obstruction or endocrine dysfunction can be considered. The absence of sperm in the ejaculate and first void after ejaculation (post ejaculation urinalysis) rules out retrograde ejaculation **(John et al., 2011).**

Azoospermia and infertility :

Azoospermia, or the complete absence of sperm in the ejaculate, accounts for 10–20% of males presenting with infertility **(Sharlip et al., 2006).**

The goal of the serum hormonal evaluation is to differentiate between obstructive from Non-obstructive