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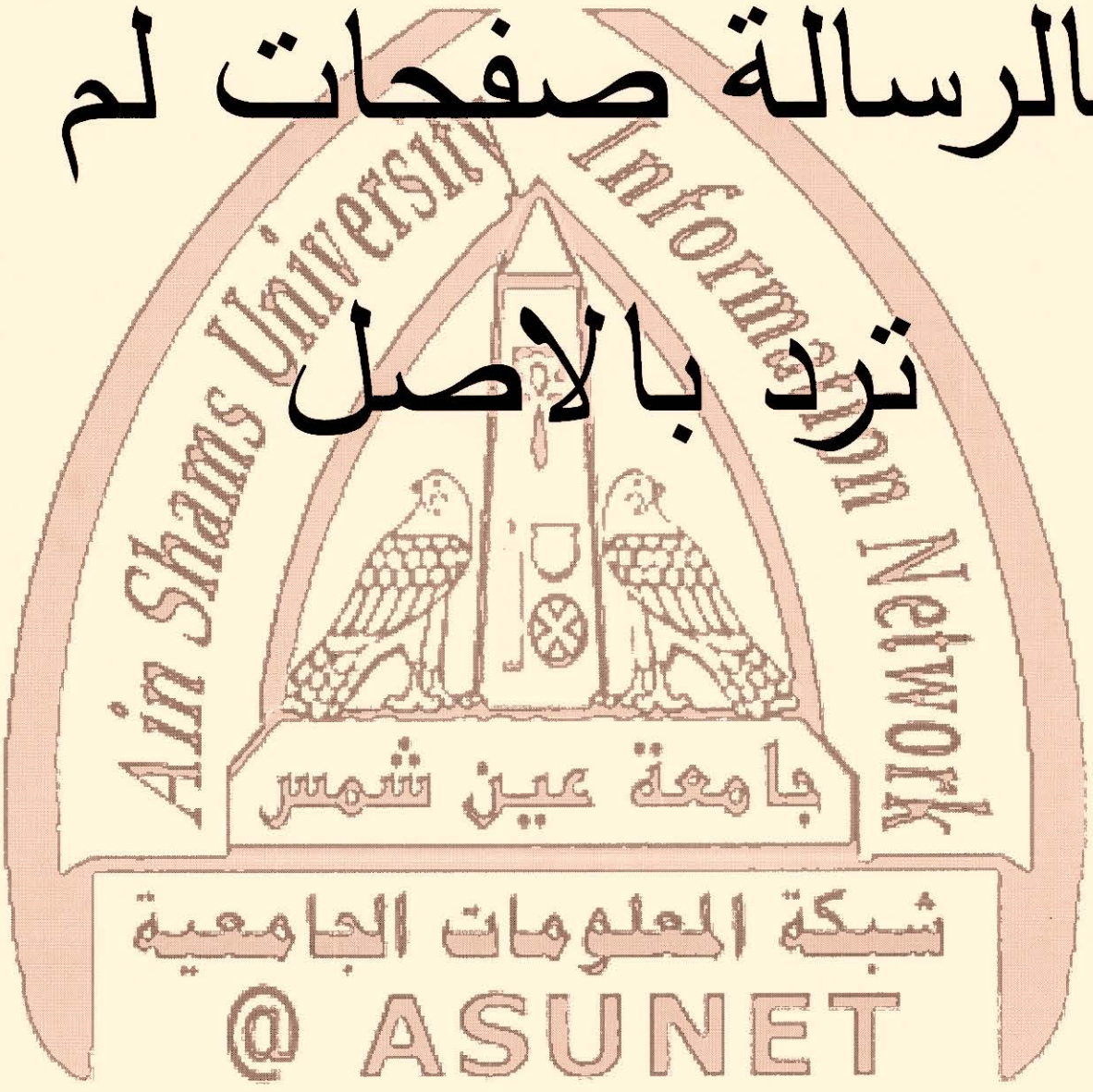

COMPARATIVE STUDY BETWEEN IN-VITRO FERTILIZATION (IVF) AND INTRACYTOPLASMIC SPERM INJECTION (ICSI) IN DIFFERENT INDICATIONS OF ASSISTED REPRODUCTIVE TECHNIQUES

Thesis submitted for partial fulfillment of the
MD degree in Obstetrics and Gynecology

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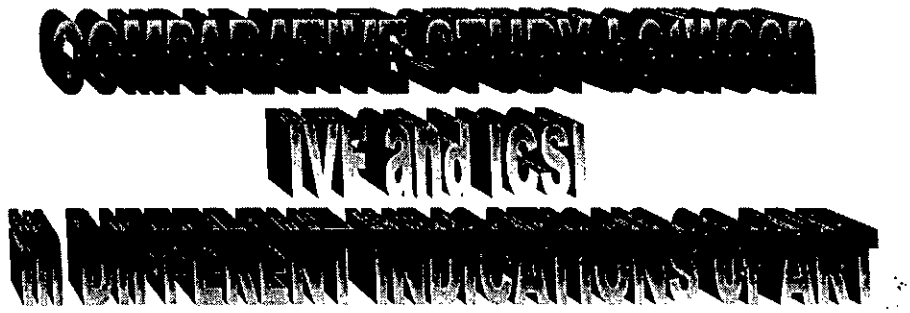
list of Abbreviations

AR	Acrosome reaction
ART	Assisted reproductive technology
CASA	Computer-assisted semen analysis
CBAVD	Congenital bilateral absence of vas deferens
CC	Clomiphene citrate
COH	Controlled ovarian hyperstimulation
Conv. IVF	Conventional in-vitro fertilization
D & C	Dilatation and curettage
DES	Diethylstilbesterol
DHEA-S	Dehydroepiandrosterone sulfate
DMSO	Dimethylsulfoxide
DRE	Digital rectal examination
EDD	Effective daily dose
ET	Embryo transfer
FSH	Follicle stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
GnRH-a	Gonadotropin releasing hormone agonist
GV	Germinal vesicle
h	Hour
hCG	Human chorionic gonadotropin
HEPES	Hydroxyethylpiperazine-N-2-ethanesulfonic acid
hMG	Human menopausal gonadotropin
HPF	High power field
HSG	Hysterosalpingography
HTF	Human tubal fluid
ICSI	Intracytoplasmic sperm injection
IGF-1	Insulin-like growth factor I
IU	International unit
IUI	Intrauterine insemination
IVF	In-vitro fertilization
IVF-ET	In-vitro fertilization – Embryo transfer
LH	Luteinizing hormone
LUF	Luteinized unruptured follicle
MAR	Mixed agglutination reaction
MCR	Metabolic clearance rate
MESA	Microepididymal sperm aspiration
min	Minute
OCC	Oocyte – cumulus complex
OHSS	Ovarian hyperstimulation syndrome
PCO	Polycystic ovary

PCOD	Polycystic ovarian disease
PCT	Post-coital test
PESA	Percutaneous epididymal sperm aspiration
PR	Percentage recovery of motility
PROH	1,2-propanediol
PVP	Polyvinylpyrolidone
PZD	Partial zona dissection
rhCG	Recombinant human chorionic gonadotropin
rLH	Recombinant luteinizing hormone
SUZI	Subzonal insemination
TESE	Testicular sperm extraction
TRUS	Trans-rectal ultrasound
TVS	Transvaginal sonography
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
ZP	Zona pellucida
β-hCG	Beta-human chorionic gonadotropin



The desire to reproduce is an intensely motivating human force. It is through children that we have continuity with the past and future; in this respect, children are our immortality. Couples may also experience strong religious, cultural and societal pressures to conceive. It is, therefore, understandable that when people have difficulty conceiving, most perceive their infertility as a major life crisis. Fertility is the ability of a man and a woman to reproduce. Similarly, infertility is an involuntary reduction in the ability to have children *(Hull et al., 1985)*.

Infertility was adressed in the Holy Quran: ***"To God belongs the dominion of the heavens and the earth; He creates what He wills; He bestows male or female, according to His will; or He bestows both males and females, and He leaves barren whom He will; for He is full of knowledge and power" (Sura 42: 49-50).*** However, treating infertility is not a defiance to God's will but to discover the absolute truth about the patient's capability to produce an own child *(ISESCO, 1996)*.

During the last two decades and with the advent of new technology, we have witnessed a great advancement in the medical sciences especially in the field concerned with the treatment of human infertility. Moreover, ever since the birth of the first "test tube" or in vitro fertilized baby (Louise Brown) in Britain in 1978, a real era was also born to offer a new hope for infertile couples to enjoy the benefit of their misfortune *(Eskandarani, 1996)*.

In vitro fertilization (IVF) has been successful for the alleviation of long-standing infertility due to tubal diseases, idiopathic and male factor infertility. It is well documented, however, that the results of IVF in male infertility are not as good as in those patients with normal semen parameters. In andrological infertility only 20-30% of the inseminated cumulus-oocyte complexes are normally fertilized; which is much lower than 60-70% fertilization in patients with normal semen parameters. Furthermore, a sizeable number of couples cannot be accepted for IVF when no spermatozoa are present in the ejaculate or when the number of morphologically normal and progressively motile spermatozoa is below a certain threshold number (conventionally 500.000) *(Tournaye et al., 1992)*.

Union of male and female gametes, either in vitro or in vivo, requires sperm penetration through the cumulus oophorus and zona pellucida. Failure of fertilization despite increased number of spermatozoa introduced into the oocyte's vicinity by IVF has been shown to be directly related to abnormalities in sperm cell morphology and motility *(Ng et al., 1989)*.

This has prompted the development of a new approach called **"GAMETE MICROMANIPULATION"** to circumvent the barriers that prevent sperm access to the ooplasm, namely the zona pellucida and the ooplasmic membrane. Various micromanipulation strategies have been suggested for prompting sperm-egg fusion. These fall into three categories: *(Cohen et al., 1994)*

- The first involves creation of an artificial gap in the zona to allow spermatozoa to interact with the oocyte directly following insemination, a procedure that has been broadly termed **"ZONA DRILLING".**
- The second category of micromanipulation techniques directed at facilitating sperm-egg interaction is the subzonal insertion of sperm **"SUZI".** More invasive than zona drilling, it completely bypasses the zona and involves direct placement of sperm into the perivitelline space.
- The third mode of microsurgical fertilization is the direct injection of sperm into the cytoplasm of the oocyte **"INTRACYTOPLASMIC SPERM INJECTION (ICSI)"**.

In 1992, the first pregnancy was established and in 1993 the first delivery occurred following transfer of embryos obtained after injection of a single spermatozoon into the ooplasm of a metaphase-II oocyte. It soon became evident that this novel procedure of assisted fertilization i.e. ICSI was much more efficient than PZD and SUZI *(Palermo et al., 1992 a; Van Steirteghem et al., 1993 a,b)*.

Initially ICSI was applied in couples with repeated fertilization failure after standard IVF. It soon became clear that ICSI could also be used in couples with a very limited number of spermatozoa in the ejaculate. ICSI proved soon also to be the preferred treatment for patients with congenital absence of vas deferens or failed reversal of vasectomy. ICSI with fresh and frozen-thawed epididymal spermatozoa was far superior to conventional IVF *(Tournaye et al., 1994)*.

In cases of obstructive azoospermia where motile spermatozoa cannot be obtained from the epididymis it is possible to carry out ICSI using spermatozoa isolated from a testicular biopsy specimen *(Silber et al., 1995)*.

Comparison between conventional in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in different indications of assisted reproductive techniques in terms of fertilizing capability, implantation potential, pregnancy outcomes and also a comparison of post-thaw results between cryopreserved embryos derived from both techniques.

EMBRYOLOGIC BACKGROUND

The development of a human being begins with fertilization, a process by which the **spermatozoon** from the male and the **oocyte** from the female unite to give rise to a new organism, the **zygot** *(Moore, 1985)*.

In preparation for fertilization, both male and female germ cells undergo a number of changes involving the chromosomes as well as the cytoplasm. The purpose of these changes is twofold: *(Salder, 1990)*

1- To reduce the number of chromosomes from the **diploid** (Greek diploos, double) number of 46 observed in somatic cells to the **haploid** number of 23 observed in the gametes. This is accomplished by the meiotic or maturation divisions and is necessary, since otherwise fusion of a male and a female germ cell would result in an individual with twice the number of chromosomes of the parent cells.

2- To alter the shape of the germ cells in preparation for fertilization. The male germ cell, initially large and round, loses practically all of its cytoplasm and develops a head, neck and tail. The female germ cell, on the contrary, gradually becomes larger as the result of an increase in the amount of cytoplasm.

Chromosomes during mitotic division

Before a normal somatic cell enters mitosis, each chromosome replicates its DNA and in fact becomes doubled. During the DNA replication phase the chromosomes are extremely long, diffusely spread through the nucleus, and cannot be recognized by the light microscope. With the onset of mitosis, the chromosomes begin to coil, contract, and condense, and these events mark the beginning of **prophase**. Each chromosome consists of two parallel subunits (chromatids) which are joined at a narrowed region common to both called the centromere (kinetochore). Throughout prophase the chromosomes will continue to condense and become shorter and thicker, but only at **prometaphase** will the chromatids become distinguishable. During **metaphase**, the chromosomes line up in the equatorial plane and their doubled structure is clearly visible. Each is attached by microtubules (mitotic spindle) extending from the kinetochore to the centriole. Soon the kinetochore of each chromosome divides, marking the beginning of **anaphase** followed by migration of the chromatids to opposite poles of the spindle. Finally, during **telophase**, chromosomes uncoil and lengthen, the nuclear envelope reforms and division of the cytoplasm occurs. Each daughter cell receives one-half of all the doubled chromosome material and thus maintains the same number of chromosomes as the mother cell*(Herbert et al., 1995)*.

First meiotic division

As in a mitotic division, the female as well as the male germ cells (primary oocyte and primary spermatocyte) replicate their DNA just before the first meiotic division begins. Hence, at the beginning of the maturation divisions, the germ cells contain double the normal amount of DNA and each of the 46 chromosomes is a double structure *(Moore, 1985)*.

The first characteristic feature of this meiotic division is the **pairing (synapsis) of the homologous chromosomes**, which are referred to as **bivalents**. The pairing is exact and point for point, except for the X-Y combination. The centromere regions of the homologous chromosomes do not pair. Since each individual chromosome is double-structured and contains two chromatids, the homologous pair consists of four chromatids. In a mitotic division the homologous chromosomes never pair.

The second characteristic feature of the first meiotic division is called **cross-over** and consists of the **interchange of chromatid segments** between the two paired homologous chromosomes (bivalents). When, subsequently, each double-structured member of the homologous pair splits longitudinally, one or more transverse breaks occur in the chromatids and an interchange of chromatid segments between two homologous chromosomes occurs. During the separation of the two homologous chromosomes, the points of interchange temporarily remain united and the chromosomal structure then has an X appearance, known as a **chiasma**. During the chiasma stage, blocks of genes are exchanged between homologous chromosomes. In the mean time, the separation continues and the two members of each pair become oriented on the spindle. In subsequent stages the members migrate to the opposite poles of the cell *(Lindner et al., 1983)*.

After the first meiotic division has been completed, each daughter cell contains one member of each chromosome pair and thus has 23 double-structured chromosomes. Since each chromosome is still double-structured except at the centromere, the amount of DNA in each daughter cell equals that of a normal somatic cell *(Byskov, 1982)*.

Second meiotic division

Shortly after the first meiotic division, the cell begins its second maturation division. In contrast to the first one, **no DNA synthesis occurs in advance of this division**. The 23 double-structured chromosomes divide at the centromere and each of the newly formed daughter cells receive 23 chromatids. The amount of DNA in the newly formed cells is now half that of the normal somatic cell *(Lindner et al., 1983)*.