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THE EFFECT OF SPERM PARAMETERS AND BOTH MATERNAL AND PATERNAL AGE ON OUTCOME OF INTRACYTOPLASMIC SPERM INJECTION

Milat Ismail Haje[1], Kameel M Naoom[2]
[1]Postgraduate student in College of Medicine, Hawler Medical University, Erbil/Iraq
[2]Professor in Embryology, College of medicine, Hawler Medical University, Erbil/Iraq
milathaji@yahoo.com

***Abstract*— The purpose of this study was to investigate any influence of maternal and/or paternal age, three sperm parameters (sperm count/ml, motility and morphology) on pregnancy outcomes in intracytoplasmic sperm injection (ICSI) cycles. In all, 785 ICSI cases were analyzed retrospectively. Pregnancy outcome were influenced by the age of the maternal, paternal partners and sperm count x10^6. The clinical pregnancy rate with respect to the age of female partner and male partner was revealed a significant inverse correlation between them with (P = <0.001) for each partner. The relationship between clinical pregnancy rate and sperm count x10^6/ml was revealed a significant difference between the groups (P= 0.046). On the other hand no basic semen parameters (motility and normal morphology) influence on ICSI pregnancy outcome was found in the subgroup of patients. We conclude that the influence on pregnancy outcome after ICSI is related mostly to maternal and paternal age.**

***Keywords*: Intracytoplasmic sperm injection, maternal age, paternal age, semen parameters, pregnancy outcome.**

I. INTRODUCTION

The outcome of assisted reproductive technology (ART) procedures is known to be influenced by multiple factors, including the etiology of infertility, patient age, semen parameter quality, the type of ovarian stimulation, and the level of follicular phase estradiol (E2). The subsequent number and quality of oocytes and the number of embryo transferred are affected by the different regimens of ovarian stimulation.

The negative impacts of advancing female age are well known. A classic study of the Hutterites observed a rise in sterility first noted at 35 years of age, with a sharp increase after the age of 39 years, reaching an almost complete inability after the age of 44 years (Tietze, 1957), the same trend has been noted in women undergoing ART. In women aged >35 years, success rates after ART start to decline. By the age of 40 years a marked decline is noted (Navot et al, 1991and Al-Shawaf et al 1992). On the other hand, male reproductive function does not cease abruptly as in women, but become fundamentally changed with age (Sartorelli et al, 2001). Some investigators have associated a decline in pregnancy rates after ART with advancing paternal age in couples in which the women is younger (Klonoff-Cohen and Natarajan, 2004), on the other hand, Spandorfer et al(1998) suggested that the pregnancy is not affected by male age.

Sperm morphology is consistently the most significant parameter that relates to fertilization. In this context Coetzee and colleagues (1998) have performed a metaanalysis of the data that confirmed the importance of sperm morphology for IVF success. Similar results were found by Liu et al 1994 and Nikolettos et al 1999. Other studies revealed little or no relationship between semen quality and fertilization with ICSI ((Nagy et al, 1995; Mercan et al, 1998). However, in extreme cases of defective spermatozoa fertilization and pregnancy rate reduced (Nagy et al, 1995). Chen et al (2009) conceded that the percentage of normal forms was no different in pregnant and non-pregnant groups. Mercan et al, 1998 and Karpuz et al, 2007 found that there was no significant difference for the ICSI outcome indicating pregnancy rate. In this study the main objectives were therefore to analyze the pregnancy rates retrospectively in 785 cycles, in relation to the age of both partners and to the three conventional sperm parameters (sperm count/ml, motility and morphology).

II. MATERIAL AND METHODS:

This study was performed retrospectively in Fertility and IVF center in Maternity Teaching Hospital in Erbil City-Iraq between (January 2011-December 2012). Out of 1055 infertile couples 785 underwent ICSI cycles. The average age of infertile men was (33.1±7.3) years. Seminal fluid analysis was done for each patient; analyses were done in the Andrology room according to WHO criteria (1992). Before sample collection patient were informed about the relevance of abstinence time (3-6) days and about importance of collecting the complete ejaculate and not using any soap during collection.

A. Ovarian stimulation, oocyte collection, and oocyte preparation for microinjection:

Controlled ovarian stimulation was done using down-regulation with gonadotropin-releasing hormone GnRH (Zoladex or decapeptyle 0.1mg)) agonist protocol with urinary FSH (Gonal F) or recombinant (Merional) or GnRH antagonist protocol with urinary or recombinant FSH. When at least two follicle reached a mean diameter of 18 mm, using transvaginal ultrasonography, 1000 IU HCG (Ovitrelle, pregnyl) was administered and oocyte retrieval was done by vaginal ultrasound- guided puncture of ovarian follicles, 36 hour after HCG administration. At the end of oocyte retrieval, the cumulus-corona cell complexes were removed enzymatically; immediately before micromanipulation; by incubating the oocyte in HEPES- buffered intracytoplasmic sperm injection (ICSI) medium(Hyalourindase IVC) for up to 2 min, enzymatic removal was enhanced mechanically by

aspirating the oocyte in and out of hand-drawn the pipettes. The denuded oocytes were examined to assess integrity and maturity. Inspected under the inverted microscope (olympus) at 20 x magnification and classified as mature metaphase 2 (MII), mature metaphase 1 (MI) and immature prophase (GV). Only MII oocytes with extruded first polar body were microinjected.

B. Semen preparation for ICSI:

For ICSI semen preparations were performed according to WHO (1992) using freshly ejaculated spermatozoa. The semen specimens were collected by masturbation, Semen was diluted 1-2(volume/volume) with (HTF, HEPES, IVC). After centrifugation at 250 xg for 10 min, and the pellets were resuspended and combined in 3 ml of the medium. The sperm suspension was centrifuged for 10 min; the pellet was resuspended in 0.7 ml of the medium and placed in incubator for 30-50 min. in this study, approximately 10 ul was used to determine the concentration and motility of the sperm. The remaining sample was used for ICSI.

C. Testicular sperm extraction (TESE):

TESE was performed under general anesthesia. The scrotal skin and tunica vaginalis were opened, a 0.5 cm incision was made in the tunica albuginea and one or two pieces of the extruding testicular tissue were excised using a pair of curved scissors. The specimen was then transferred into a Petri dish filled with ~ 2 ml modified HEPES-buffered Earle's medium and heparin 0.4% (HTF-HEPES-IVC).

D. Intracytoplasmic Sperm Injection (ICSI):

The 3-5 µl sperm- polyvinylpyrrolidone (PVP-IVC) droplet was placed in the center of a Petri dish (Falcon type 1006) and was surrounded by eight 5µl droplets of HEPES-buffered IVC medium. These droplets were covered by ~ 3.5 ml of lightweight paraffin oil. The ICSI procedures were carried out on the CRI-UK micromanipulation system attached to inverted microscope Olympus Ixs1 (Olympus company). Injection of oocytes was performed in microdroplets of HEPES-buffered ICSI medium covered with lightweight paraffin oil (IVC). A single spermatozoon with apparently normal morphology was immobilized by cutting across its tail with the injection pipette. After securing the oocyte onto the holding pipette, with the polar body at the 6 or 12 o'clock position, the injection pipette was pushed through the zona pellucida and the oolemma into the ooplasm at the 3 o'clock position. When penetration of the oolemma was verified by aspirating some cytoplasm, the spermatozoon was slowly ejected. The injection pipette was withdrawn gently and the oocyte released from the holding pipette. After injection oocytes were washed twice in HTF-IVC, then incubated at 37°c in a 50 µl drop of G1.2 medium under mineral oil in a CO^2 incubator (Galaxy CO^2).

E. Luteal phase support after Intra uterine insemination (IUI) and ICSI:

Luteal phase support was started with progesterone vaginal pessaries (cyclogest 400 mg bd) on the day after retrieval and was continued till 12wk of gestation if pregnancy positive.

F. Definition of pregnancy:

Serum β-human chorionic gonadotropin was measured after 12 days after embryo transfer. After 10 days clinical pregnancy was indicated by doing transvaginal sonography for detection of fetal sac.

G. Statistics:

All statistics were performed using the Statistical Package for the Social Science (SPSS- version 19). Difference in the pregnancy rate between the groups was tested using the chi square test.

III. RESULTS

Out of 1055 infertile couples, 785 underwent ICSI cycles revealed an overall clinical pregnancy rate of 34.8%.

Table 1 shows the evaluation of the clinical pregnancy rate with respect to the age of female partner. The results revealed a significant inverse correlation between them with (P = <0.001). The highest group was for women age <25 years, the clinical pregnancy rate (PR) was 46.6%, and lowest rate was the group +40 years which was 12.3%.

Evaluation of the clinical pregnancy rate with respect to the age of men partner revealed a significant inverse correlation between them with (P = <0.001), for men age <25 years the clinical PR was 41.2%, for the group 25-29 years were 56.6% (Table 2).

Semen parameters (count, motility and morphology) obtained on the day of the aspiration procedure was evaluated to determine if semen quality has an impact on ICSI outcome.

The relationship between clinical pregnancy rate and sperm count $x10^6$/ml is shown in Table 3. There was significant difference between the groups (P= 0.046), higher rate was with count $x10^6$/ml (1.9-9) $x10^6$ group which PR was 50%.

The relationship between clinical pregnancy rate and sperm total motility % is illustrated in Table 4. There was no significant difference between the groups (P= 0.107).

The relationship between clinical pregnancy rate and sperm normal morphology % is shown in Table 5. Also the results showed no significant difference between the groups (P= 0.185).

women age	Pregnancy negative	Pregnancy positive	Total
<25	39	34	73
	53.4%	46.6%	100.0%
25-29	93	71	164
	56.7%	43.3%	100.0%
30-34	122	72	194
	62.9%	37.1%	100.0%
35-39	102	50	152
	67.1%	32.9%	100.0%
+40	93	13	106
	87.7%	12.3%	100.0%
Total	449	240	689
	65.2%	34.8%	100.0%

Pearson Chi-Square P< 0.001

Table (1): The correlation of clinical pregnancy rates after ICSI with respect to the women age

Men age	Pregnancy negative	Pregnancy positive	total
< 25	10	7	17
	58.8%	41.2%	100.0%
25-29	36	47	83
	43.4%	56.6%	100.0%
30-34	86	54	140
	61.4%	38.6%	100.0%
35-39	108	59	167
	64.7%	35.3%	100.0%
40+	159	53	212
	75.0%	25.0%	100.0%
Total	399	220	619
	64.5%	35.5%	100%

Pearson Chi-Square P< 0.001

Table (2): The correlation of clinical pregnancy rates after ICSI with respect to the men age

Sperm countx10^6/ml	Pregnancy negative	Pregnancy positive	Total

<1	85	62	147
	57.8%	42.2%	100.0%
1.9-9	17	17	34
	50.0%	50.0%	100.0%
10-14.9	16	8	24
	66.7%	33.3%	100.0%
15-39.9	85	35	120
	70.8%	29.2%	100.0%
40-69.9	94	37	131
	71.8%	28.2%	100.0%
+70	108	55	163
	66.3%	33.7%	100.0%
Total	405	214	619
	65.4%	34.6%	100.0%

Pearson Chi-Square P=.0.46

Table (3): Sperm count x10^{6}/ml according to pregnancy outcome following ICSI

Total motility%	Pregnancy negative	Pregnancy positive	Total
< 1	100	66	166
	60.2%	39.8%	100.0%
1-9.9	3	5	8
	37.5%	62.5%	100.0%
10-19.9	11	9	20
	55.0%	45.0%	100.0%
20-39.9	43	21	64
	67.2%	32.8%	100.0%
40-99.9	251	114	365
	68.8%	31.2%	100.0%
Total	408	215	623
	65.5%	34.5%	100%

Pearson Chi-Square P=.01.7

Table (4): Sperm total motility percentage according to pregnancy outcome following ICSI

Normal morphology%	Pregnancy negative	Pregnancy positive	Total

< 1	130	87	217
	59.9%	40.1%	100.0%
2-3.9	29	14	43
	67.4%	32.6%	100.0%
4-9.9	52	23	75
	69.3%	30.7%	100.0%
10+	197	90	287
	68.6%	31.4%	100.0%
Total	408	214	622
	65.6%	34.4%	100%

Chi-Square P=.0185

Table (5): Sperm normal morphologic percentage according to pregnancy outcome following ICSI

IV. DISCUSSION

As the study of Hutterites has demonstrated normal fertility is highly dependent on female ageing (Tietze, 1957). It is also well established that the outcome of couples treated by IVF is significantly influenced by advancing female age. It is therefore, a logical assumption that the ICSI would be similarly affected by female age. In current study, the rate of pregnancy after ICSI inversely with women age , and this correlation was significant with (P<0.001) (Table 1). This result is in consistent with results of (Devroey et al, 1996 and Spandorfer et al 1998). Some investigators have associated a decline in pregnancy rates with advancing paternal age in couples in which the women is younger (Klonoff- Cohen and Natarajan, 2004), Some studies have suggested a negative trend in fertility with advanced male age (De La Rochebrochard et al, 2006; Ferreira et al 2010; Tsai et al, 2013).). On the other hand, Gallardo et al.,1996 and Spandorfer et al., 1998 suggested that the age of male partner didn't affect fertilization, embryo development or implantation. In this study there was a significant inverse correlation between pregnancy rate after ICSI and age of male partner with (P = <0.001). The combination of sperm morphology, progressive motility percent and sperm count has been demonstrated to be the best parameter to evaluate the fertility capacity of sperm in IVF (Lundin et al., 1997). In this study the effect of semen parameters on PR after ICSI were examined. The results showed that there was significant difference between the groups (P= 0.046), higher rate was with count $x10^6$/ml (1.9-9) $x10^6$ (Table 3). It means that pregnancy rate (PR) after ICSI was better when sperm count was low (Table 3).

The relationship between clinical pregnancy rate and sperm total motility % are shown in Table (4). The results showed no significant difference between the groups (P= 0.107), higher rate was with sperm total motility % (1-9.9%) group. Moreover, in the current study, the relationship between clinical pregnancy rate and sperm normal morphology % is shown in Table (5). There was no significant difference between the groups (P= 0.185), higher rate was with sperm normal morphology %(<1%) group. It means that PR after ICSI didn't depend on total sperm motility and normal morphology This result is in agreement with (Nagy, 1995; Karpuz et al., 2007) and Mansour et al, (1995) found no significant difference in the incidence of fertilization between patients with <5% normal forms and patient with >5% normal forms, . Chen et al (2009) conceded that the percentage of normal forms was no different in pregnant and non-pregnant groups. ICSI is also independent on sperm motility (Verheyen et al, 1999; Mercan et al, 1998) but fertilization rate was significantly higher in patients with more adequate sperm parameters. The only ultimate criterion for successful ICSI is the presence of at least one living spermatozoa per oocyte in the semen preparation used for microinjection. Nikolettos et al (1999) have concluded that the chance of a successful pregnancy is low with sever anomalies of the sperm head shape.

So according to previous reports and this study in ICSI, fertilization may be achieved, even in the presence of a few motile sperm, because natural selection steps are skipped in the presence of abnormal sperms (Van Steirteghem et al., 1993).

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