

## ORIGINAL ARTICLE

**Factors Affecting Pregnancy Rate in Infertile Women Underwent in Vitro Fertilization / Intracytoplasmic Sperm Injection Cycle**WAFAA MOHAMMED AL-JUBOURI<sup>1</sup>, MANAL TAHA AL-OBAIDI<sup>1</sup>, HUDA ALI HUSSAINI<sup>2</sup><sup>1</sup>High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University.<sup>2</sup>College of Medicine, Al-Nahrain UniversityCorrespondence to: Wafaa Mohammed Al-Jubouri, Email: [aljuborywafaa@yahoo.com](mailto:aljuborywafaa@yahoo.com)**ABSTRACT**

Intracytoplasmic sperm injection is more being used for non-male factor indications, although it was developed for overcoming infertility of males because pregnancy rate of in vitro fertilization is still < 40%. This work aims at evaluating the vitro sperm injecting cycle fertilization/ Intracytoplasmic efficiency in infertile women and the factors that predicting clinical pregnancy rate. Al-Nahrain University at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, we conducted a randomized clinical trial from 1<sup>st</sup> Sep. 2018 to 1<sup>st</sup> Sep. 2020. 40 women complaining from infertility underwent IVF / ICSI protocols were sampled for this work. Positive serum  $\beta$ hCG test were used to evaluate pregnancy r, two weeks following transferring embryo. In this study, 12 infertile women got pregnant with pregnancy rate of 30%. Duration of stimulation, number of mature eggs, fertilized eggs, and grade I transferred embryo, and changes in percentages in endometrial zone at day of pickup in comparison with that at day of hCG were significantly higher ( $P < 0.05$ ) in pregnant women than those who were not pregnant. Failure rate of pregnancy is still high and the most important factors that associated with failure of IVF/ICSI outcome were short duration of gonadotropin stimulation, few number of mature eggs and embryos, and suboptimal endometrial receptivity.

**Keywords:** IVF, ICSI, infertility, pregnancy rate, predictors, Iraq.**INTRODUCTION**

Infertility seems to be a big health problem universally. It is reproductive system disease due to failure for making a clinical pregnancy on year or longer than the regular unprotected sex<sup>(1)</sup>. Globally 10% of the people, suffer from infertility. It is considered as the fifth highest serious global disability<sup>(2)</sup>. About 186 million face infertility, most from the developing world<sup>(3)</sup>. In Iraq, infertility rose in the last 16 years from 2000 to 2016 for many reasons: war, stress, hereditary, lifestyle, occupation, smoking and eating habits<sup>(4)</sup>. Previous studies showed that female are 37% of the infertility causes (25% ovulatory disorders, 15% endometriosis, 12%, pelvic adhesions, 11% tubal blockage, 11% other tubal/uterine abnormalities and 7% hyperprolactinemia); 35% are caused by both male and female; and in 8%, the cause is male factor infertility<sup>(5)</sup>. Despite the fact that male infertility is crucial to any infertility review, this study discusses evaluating and treating female infertility. Infertility could be main and secondary. The first condition is woman diagnosed with a clinical pregnancy. The secondary is a woman unable to get pregnant but has previously had a clinical pregnancy<sup>(6)</sup>. The assisted reproductive technology (ART) emergence was hopeful for infertile couples. However, In Vitro Fertilization (IVF) success remains lower than 100% and not satisfactory. Therefore, important indicators of decision making stand for the success probability and cost effectiveness as crucial to patients with a low ratio<sup>(7)</sup>. So, how to increase the favorable outcome of ART has become a critical topic for both clinicians and infertile couples. Intracytoplasmic Sperm Injection (ICSI) is specialized IVF form utilized mainly to treat severe male-factor infertility in which a single spermatozoon is ejected into an oocyte mechanically in vitro for fertilization<sup>(8)</sup>. In contrast, ICSI increases the genetic defect transmission possibility of generation to the next through bypassing natural barriers to fertilization<sup>(9)</sup>. The high ICSI success rate increased using other to whom conventional IVF is an option, in particular non-male factor infertility<sup>(6)</sup>. ICSI can be expensive, time-consuming and causing unresolved concerns in damaging to gametes and the health newborn conditions<sup>(10)</sup>. The study evaluates the efficiency of IVF/ICSI cycle in infertile women and the factors that predicting clinical pregnancy rate.

**PATIENTS AND METHODS**

**Study design and setting:** In Al-Nahrain University, at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, the current article conducted randomized clinical trial from 1<sup>st</sup> Sep. 2018 to 1<sup>st</sup> Sep. 2020.

**Population and sampling:** From those been to the private clinic and High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, 40 women aged between 20 – 40 years are sampled. These women complained from infertility and underwent IVF / ICSI protocols. The study excluded those with intrauterine pathology (polyp, fibroid), hydrosalpinx, congenital malformation, moderate to severe endometriosis with an autoimmune disease history or thrombophilia. We obtained a signed informed consent for reviewing patients' medical archives for study uses and for keeping the patient medical record confidentiality and anonymity.

**Workup:** this work evaluated 40 patients having IVF/ICSI cycle according to the steps below:

- Full medical, surgical and obstetrical history assessing height & weight for getting Body Mass Index (BMI)
- Clinically and gynecologically examining for excluding abnormality.
- Testing Hormonal (FSH, LH, E2, Testosterone, Prolactin, TSH) for females two days after menstrual cycles
- For each patient, the study selects antagonist protocol as controlled ovarian hyperstimulation protocol.
- At trigger day, we sampled blood (VEGF, TGF-B1, E2, Progesterone) and assessed power Doppler US of subendometrial blood flow and US of endometrial patterns and thickness.
- Trans-vaginal ultrasound guided Oocyte retrieval conducted after causing ovulation with hCG approximately 35-36 hours.
- On Oocytes pick up day: Blood sampling for (VEGF, TGF-B1, E2, Progesterone).
- We conducted embryo transferring two-three days following Oocyte pickup based on the of embryo quantity and grading.

**IVF /ICSI Procedures:** gradual IVF procedures:

**Step 1:** We conducted Controlled Ovarian Hyperstimulation (COH) with FSH from the second day of a menstruation by an initial dose 150-225 IU daily according to patient's underlying medical conditions. Then, patients are administered a GnRH antagonist as a flexible technique according to the biggest follicles size if reached 13-14 mm consequently. We conducted serial vaginal U/S for number and size of follicles, endometrial thickness and serum level of E2.

**Step 2:** Oocyte Retrieval (OCR) was conducted 34-36 hours post hCG injection before follicular rupture. This takes about 20-30 minutes.

**Step 3:** Regarding Oocyte Grading and Quality, we classified early oocytes as immature (germinal vesicle (GV) or metaphase I (MI) stage), yet the mature is called MII.

**Step 4:** Fertilization and Embryo Culture: We collected semen collection prior to oocyte retrieval. We selected the mature eggs and held them by a specialized pipette, a very delicate, sharp and hollow needle for immobilizing and picking up a single sperm. Then, we inserted the needle carefully inserted through the shell of the egg into its cytoplasm and it remained in the CO2 incubator to give the result of cell division, with the aid of Nikon ICSI Microscope.

**Step 5:** Embryo Quality: After insemination, we assessed the embryos at the second day (43 - 45 hours following the insemination) and the third day (67 - 69 hours after insemination). We considered good quality embryos when homogeneous, with normal kinetics (the second, seventh, eighth and ninth day cells at the third day) and containing <10% of cytoplasmic parts.

**Step 6:** Embryo Transferring: (After two days of oocyte retrieval by a catheter of embryo transfer, we transferred 2-3 embryos). We decided on the selected transfer embryo number based on the embryonic quality, patient age, attempt rank and clinical history.

**Step 7:** Following up and Luteal Phase Support (LPS) and was conducted by vaginal progesterone (Cyclogest®400 mg two times: Cox Pharmaceuticals, Barnstaple, UK) or (Crinone, ® 8% progesterone gel, MERK). This was repeated every day for two weeks (until β-HCG done, if it was positive then she continued taking progesterone till 9-12 weeks when the placenta starts to function).

Positive serum βhCG test were used to evaluate pregnancy r, two weeks following transferring embryo. We also examined the gestational sac on transvaginal U/S (clinical pregnancy), 3 weeks following positive serum βhCG.

**Statistical analysis:** We used statistical Package for Social Sciences (SPSS) version 26. For data analysis: mean, ranges and standard deviation. Frequencies and percentages present the categorical data. By independent t-test (two tailed), we compared the continuous variables between pregnant and non-pregnant women. It is significant, if a P level– value is lower than 0.05.

**RESULTS**

The current work included 12 infertile women got pregnant with pregnancy rate of 30% as in figure (1).

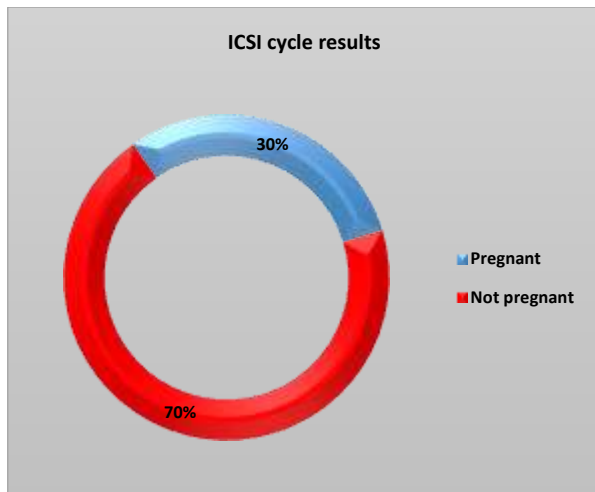


Figure 1: Distributing patients by ICSI cycle results

In comparison between women who got pregnant and those who didn't, we noticed that duration of stimulation, the nu mature egg quantity, quantity of fertilized eggs, and quantity of grade I transferred embryo seemed significantly higher (P < 0.05) in pregnant women than in those who were not. There were no

significant differences between women who got pregnant and those who didn't (P ≥ 0.05) in means of every parameter.

Table 1: Comparison in certain characteristics according ICSI cycle outcome

| Variable                          | ICSI cycle outcome     |                    | P - Value |
|-----------------------------------|------------------------|--------------------|-----------|
|                                   | Not pregnant Mean ± SD | Pregnant Mean ± SD |           |
| Age (Year)                        | 29.71 ± 4.6            | 29.25 ± 6.0        | 0.813     |
| BMI (Kg/m <sup>2</sup> )          | 24.85 ± 3.3            | 23.5 ± 2.6         | 0.175     |
| Duration of infertility           | 6.16 ± 3.8             | 6.12 ± 2.9         | 0.97      |
| Baseline hormonal parameter       |                        |                    |           |
| FSH (IU/L)                        | 7.25 ± 2.0             | 6.75 ± 1.9         | 0.464     |
| LH (IU/L)                         | 5.85 ± 2.4             | 4.54 ± 1.4         | 0.092     |
| TSH (mIU/L)                       | 2.35 ± 1.1             | 2.22 ± 0.9         | 0.701     |
| Prolactin (µg/L)                  | 19.74 ± 8.6            | 15.7 ± 5.3         | 0.08      |
| Estradiol day 2 (pg/mL)           | 43.09 ± 18.0           | 38.03 ± 12.3       | 0.313     |
| Clinical parameter                |                        |                    |           |
| Number of gonadotropin ampule     | 21.46 ± 8.5            | 24.75 ± 8.5        | 0.274     |
| Duration of stimulation (Day)     | 8.96 ± 1.4             | 9.91 ± 1.2         | 0.035     |
| No. of retrieved oocyte           | 9.25 ± 4.0             | 12.16 ± 5.0        | 0.058     |
| No. of mature eggs (MI)           | 5.67 ± 2.5             | 8.16 ± 3.9         | 0.019     |
| No. of fertilized eggs            | 4.53 ± 1.6             | 6.41 ± 2.7         | 0.042     |
| No. of transferred embryo         | 3.25 ± 0.8             | 3.41 ± 0.8         | 0.556     |
| No. of grade I transferred embryo | 1.75 ± 0.6             | 2.66 ± 1.0         | 0.01      |

Table (2) shows that mean of endometrium zone as significantly rising in the day of OPU (P = 0.031) in women becoming pregnant than in those who were not in comparison to that of hCG day. The women becoming pregnant and those who were not were not significantly different in the change percentage in any parameters at OPU day (P ≥ 0.05) in comparison to that at hCG day.

Table 2: Comparing hormone change and ultrasound results at Ovum Pick-Up day in comparison with that of the hCG day based on the ICSI cycle outcomes

| Change rate at day of Ovum Pick-Up in comparison with those of hCG day (%) | ICSI cycle outcome     |                    | P - Value |
|--|------------------------|--------------------|-----------|
|  | Not pregnant Mean ± SD | Pregnant Mean ± SD |           |
| Hormonal factor  |                        |                    |           |
| Estradiol  | - 10.68 ± 2.8          | - 11.79 ± 7.5      | 0.626     |
| Progesterone   | 633.1 ± 413.8          | 705.14 ± 234.6     | 0.491     |
| VEGF   | 13.74 ± 13.4           | 12.4 ± 6.3         | 0.667     |
| TGF  | 9.34 ± 5.3             | 10.72 ± 5.7        | 0.484     |
| Doppler U/S finding  |                        |                    |           |
| Endometrial thickness  | 8.72 ± 3.6             | 9.24 ± 5.9         | 0.78      |
| Resistance Index   | - 17.15 ± 6.6          | - 17.33 ± 11.7     | 0.96      |
| Pulsatile Index  | - 15.84 ± 9.4          | - 19.18 ± 9.7      | 0.326     |
| V1/V2  | - 19.73 ± 8.8          | - 8.95 ± 20.4      | 0.102     |
| Zone   | 19.04 ± 24.3           | 44.44 ± 33.6       | 0.031     |

**DISCUSSION**

An important role for implantation to be successful is the relationship between the developing embryo and maternal tissue (11). ICSI was used to help males with sperm problems, by putting one sperm directly into an egg, but now it suggests to be used for other causes of infertility as ICSI treatment cycles outnumber those of IVF by around three to one (12). Some think that ICSI is better, but the latest figures, which were for 2016, don't support that, although only a randomized clinical trial can find out for sure. This interventional study is designed to find pregnancy rate and examine factors showing pregnancy possibility for infertile women following conducted IVF/ICSI cycle.

In this study, pregnancy rate was 30% which is comparable to investigation performed by Smith ADAC et al in 2015 reporting live-birth rate of 29.5% (13) and slightly higher than result obtained by Shulman A et al study in 2002 (23.9%) (14). This study showed

that the most important predictors that determine successful outcome after IVF/ICSI were number of mature transferred embryo, prolonged duration of stimulation, number of fertilized and mature eggs in addition to endometrial receptivity. Agreements with this result were reported by Fauque P et al in 2007<sup>(15)</sup> and Cai Q et al in 2014<sup>(16)</sup> who made that transferred embryo quantity and quality are crucial indicators of IVF/ICSI results. Strong evidences support the good-quality embryo positive role to a better IVF result<sup>(17)</sup>. Determining the ideal embryo quantity and quality to be transferred, we proposed a cumulative embryo score (CES) and additional systems of scoring embryo according to the transferred improved-quality embryo numbers as embryos transferred in one cycle (multiple pregnancy) elevate the maternal and neonatal dangers in spite of the bigger live-birth likelihood than an embryo<sup>(18)</sup>. Regarding endometrial receptivity, agreements with our result were seen in studies conducted by Kasius A et al in 2019<sup>(19)</sup>, Zhang T et al in 2016<sup>(20)</sup> when confirmed that endometrial receptivity on the hCG administration day to be a possible prognostic tool for ART results. that quantity of embryos and suboptimal endometrial receptivity and changed embryo-endometrial dialogue are 1/3 failures of implantation and 2/3 respectively<sup>(21)</sup>. Duration of gonadotropin stimulation in this study is considered as important predictor confirming Yang YC et al (2019) who reported a shorter mean stimulation duration in weak pregnancy group responders in comparison to normal ones<sup>(22)</sup>. Oocyte maturity is important in the success of fertilization, developing embryo and pregnancy. Oocyte maturity and biosynthesize adequate estradiol requires sufficient LH and FSH action on theca cells. It also needs sufficient action on the granulosa cells<sup>(23)</sup>. In conclusion, failure rate of pregnancy is still high and the most important factors that associated with failure of IVF/ICSI outcome were short duration of gonadotropin stimulation, few number of mature eggs and embryos, and suboptimal endometrial receptivity.

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