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STUDY THE NUMBER OF TRANSFERRED EMBRYOS INCREASE THE PREGNANCY RATES IN FRESH ICSI-ET-CYCLES

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ABSTRACT
Low chances of implantation after the transfer of a single embryo have led the need to increase the number of embryos during embryo transfer for in vitro fertilization cycles, especially in countries that do not have legal limitations for the number of embryos to be transferred. However, because of the risk of multiple pregnancies, abortion, high cost of repeating IVF or vitrification and the demand of patients to increase the number of embryos in countries such as Iraq, there is still no consensus on the number of embryos to be transferred. This study was to investigate whether increasing the number of embryos during ET increases pregnancy rates. One hundred and twenty eight infertile Iraqi women (with normal baseline hormonal levels) were selected and subjected to an IVF stimulation program with an intra-cytoplasmic sperm injection. All of these women were considered eligible for embryo transfer (no visible indications for implantation failure), and only good quality embryos were transferred. Those women were divided randomly into two groups according to the number of transferred embryos; group A: sixty seven patients who accepted the transfer of (1-4) embryos and group B: sixty one patients who insisted on the transfer of 3 or 4 good quality embryos. In group A, thirty six patients became pregnant while in group B only thirty patients became pregnant. No significant difference was found in pregnancy rates after increasing the number of transferred embryos. The result showed that increasing the number of transferred embryos does not increase pregnancy rates in a fresh ICSI-ET cycles.

KEYWORDS: IVF, Pregnancy rates, embryo transfer, RIF, ICSI.

INTRODUCTION

Improvements in assisted reproductive technologies (ART) have helped many infertile couples and enabled them to have healthy children of their own [1, 3]. Although advances in ART and laboratory techniques and greater experience in these fields have led to increase pregnancy rates (PRs), limitations with implantation still remain challenging for in vitro fertilization (IVF) specialists. One of the strategies to increase implantation rates is increase the number of embryos during embryo transfer (ET), but the multiple pregnancies and abortion rates decline the goal of all IVF centers; a successful pregnancy after ET and the delivery of a single healthy baby in IVF or ICSI cycle [2, 4]. In many countries such as the United States, the transfer of a single embryo or small number of embryos to reduce the chances of multiple pregnancies is not encouraged by patients or IVF specialists due to possibility of low chances of pregnancy. However, in some countries, the number of embryos that can be transferred is restricted based on set guidelines, likely due to legal issues [5, 11, 12]. Nevertheless, older age of women is correlated with poorer ovarian response and a reduced lower number of viable embryos because of aneuploidy (which increases with age), gene mutations and reduction in mitochondrial activity of developing embryos. The number of poor quality embryos was found to be increased in old women (≥35 years) and in patients with recurrent implantation failure (RIF) [6], so increasing the number of transferred embryos was introduced to those women as a trial to increase chances of pregnancy which represents the term of demand high order multiple pregnancies (HOMP) such as in Middle East societies [7]. We thus conduct a randomized prospective study to compare between two groups of patients; group accept to lower the number of transferred embryos and a group of patients who did not accept (which is common in our society) to transfer less than 3 embryos during fresh ICSI cycle as a try to increase chances of positive pregnancy.

MATERIALS & METHODS

Patients

From patients who came to the Specialist Fertility Department/ Al-Bonook hospital, 128 infertile Iraqi women were selected and subjected to an IVF stimulation program (either agonist or antagonist) in a fresh ICSI-ET cycle. A full medical history was taken for each couple, hormonal baseline assays including (follicle stimulation hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol or E_2) with measuring the index of the body mass (BMI) and analyzing the seminal fluid (SFA) for each husband. All women who participated in this study (young < 35 years and old women ≥35 years) had a normal hormonal baseline and a normal uterus shape to be eligible for ET and decrease the reasons of failed pregnancy (maternal factors), they were divided randomly into two group; group A who accepted to lower the number of transferred embryos (1-4) embryos and group B who demanded to transfer at least 3 embryos, but no more than 4 embryos were transferred in both groups due to doctor's decision to decrease the chance of multiple

pregnancies and abortion. Group A consisted of 67 patients while group B consisted of 61 patients.

Stimulation Protocol

According to female age, antral follicle count (AFC), BMI, previous IVF stimulation program and other parameters, the patients of this study were enrolled either in agonist long or antagonist protocol. Agonist program started from day 21 of menstrual cycle by giving an injection of Decapeptyl 0.1 mg daily followed by injections of Gonadotropin (Gonal-f) which were started from day 2 of the exact ICSI cycle while the antagonist protocol (flexible antagonist protocol) started directly from day2 of ICSI cycle by giving gonadotropins (Gonal-f, Menogon Ferring and Merional IBSA). When leading follicles (at least 4) reached ≥ 16 mm under ultrasound (U/S), E_2 level ≥1500IU and progesterone (P4) levels must be under ≤1.3 ng/mL as well as the endometrium thickness ≥7 mm under U/S then ovulation was triggered by Ovitrelle injection (5000-10000) IU depending on BMI. If none of triggering parameters were observed, the patient was postponed or the embryos were vitrified and those patients were excluded from this study.

Intra cytoplasmic sperm injection and embryo transfer cycle

Thirty four to thirty five hours after ovulation trigger, the procedure of oocytes pick up (OPU) began under general anesthesia by using single lumen follicle aspiration 17g needle under transvaginal U/S guidance, aspirated follicular fluid was examined in IVF laboratory to detect and asses the quality of retrieved oocytes, then these oocytes were denuded and injected by husband's sperm, the last step at day of OPU was the incubation of injected oocytes in culture media. After 17-19 hours assessment of fertilization was made and the assessment of developmental stages was continued, quality of the developed embryos with changing dishes of culture media according to embryo's developmental stages was made by 2 embryologists and the day of ET with the number of transferred embryos was made as mentioned (demanding of patients, IVF medical team's decision and the day of ET) which ranged from (day2-day5). ET for all patients was made by using the same embryologist, same 2 gynecologists and same ET catheter (Cook medical company).

Follow up of the patients

Luteal support was started after OPU by given a 400 mg of Cyclogest and continued for up 14 days after ET. The pregnancy was detected by measuring the level of β-HCG. If this result was <2 mIU/mL negative pregnancy was confirmed, but when it was above, the test was repeated each weak until it was raised ≥1500 with sac detecting by using intravaginal U/S in order to follow up the pregnancy and the clinical pregnancy was confirmed after a positive embryonic cardiac activity (ECA).

Statistical analysis

Data were presented into two kinds; the continuous data presented as mean ± standard deviation (SD) and the comparison of means between two study groups was done using unpaired T test, while the categorical data were presented as frequency and the difference between frequencies of the two study groups was analyzed using fisher exact test and chi square test.

RESULTS

Table (1) illustrates the comparison of the characteristics data of all patients in this study between group A and group B. There was no significant difference in (age, type of infertility and duration of infertility, hormonal baseline assays) between both, but the number of RIF was significantly higher in group A than group B as the *P* value was 0.027.

TABLE 1: Characteristics data of all patients of this study

Parameter	Group A (67patients) Mean ±SD		Group B (61patients) Mean ±SD		P value
Age (year)	33.79+5.67		32.0+5.51		0.072
Duration of infertility (year)	7.87+4.27		7.38+3.62		0.485
RIF (trials)	1.01+1.8		0.41+1.22		0.027
FSH (mIU/mL)	5.66+1.71		5.56+1.61		0.737
LH (mIU/mL)	3.83+1.43		3.89+1.51		0.816
PRL (ng/mL)	17.92+7.39		19.27+6.73		0.282
E_2 (pg/mL)	46.0+16.7		46.97+17.17		0.749
Type of infertility	Primary	Secondary	Primary	Secondary	1.000
	62	5	56	5	

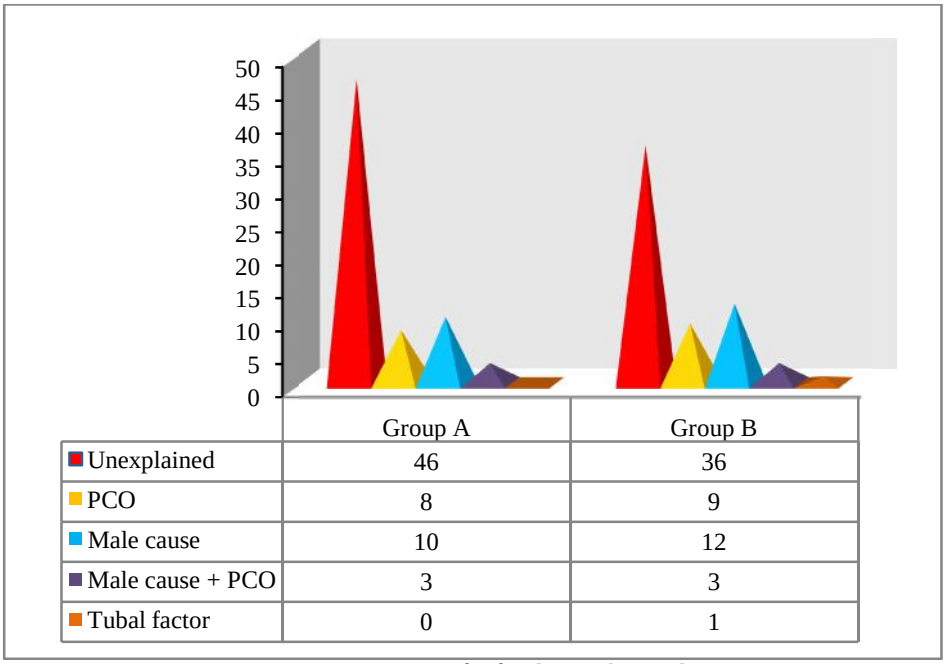
RIF: Repeated implantation failure, FSH: Follicle stimulation hormone, LH: Luteinizing hormone. PRL: Prolactin, E_2: Estradiol, SD: Standard deviation

Table (2) illustrated the parameters of stimulation protocols that were used in ICSI cycle of this study. A highly significant difference of stimulation data between both groups was noticed only in number of stimulated follicles with the size of (15-17) mm, the *P* value was 0.22. Figure 1 represented the causes of infertility in both groups and the *P* value was 0.702 and showed no statistical differences between both groups.

TABLE 2: data of stimulation cycles of both groups

Parameter		Group A (67patients) Mean±SD	Group B (61patients) Mean±SD	P value
Type of stimulation protocol	Agonist	40	39	0.716
	Antagonist	27	22	
Number of GnRH injections (ampule)		27.04+5.3	27.05+4.78	0.996
Duration of stimulation cycle (day)		9.24+1.48	9.25+1.5	0.979
Number of stimulated follicles under U/S		10.9+6.29	12.64+6.15	0.115
Number of follicle with size> 17.5 mm		1.82+1.06	2.15+1.01	0.077
Number of follicle with size (15-17) mm		3.69+2.34	4.62+2.21	**0.022**
Number of follicle with size < 15 mm		5.34+4.17	5.84+4.06	0.500
Endometrium thickness (mm) before HCG injection		8.2+0.85	8.28+0.76	0.550
Progesterone on day of HCG (ng/mL)		0.75+0.15	0.78+0.19	0.259

U/S: Ultrasound, mm: millimeter, HCG: Human chorionic gonadotropin, ng: Nanograms, mL: milliliter, SD: Standard deviation



	Group A	Group B
Unexplained	46	36
PCO	8	9
Male cause	10	12
Male cause + PCO	3	3
Tubal factor	0	1

FIGURE 1: causes of infertility in this study

Pregnancy outcomes

Figure 2 demonstrated that there were 36 pregnant women in group A while only 30 women became pregnant in group B, no statistical significant was observed as the *P* value was 0.724. Table 3 represented a comparison of pregnancy outcomes for all patients who were enrolled in this study to evaluate number of RIF and the pregnancy outcomes in both groups. In group A, 36 patients became pregnant, 15 of them had at least one failed ICSI trial (the range number of failed ICSI trials in this group was between no previous trial to 9 failed trials), while group B had 30 pregnant patients only 5 patients who had at least two failed ICSI trial became pregnant in this group (the range number of failed ICSI trials in group B was between no previous trial to 7 failed trials), this comparison showed that there was a highly significant increase in pregnancy outcomes in group A as the *P* value =0.038.

TABLE 3: Comparison of RIF between both groups by Mann Whitney U test

Parameter	Group A Median (Range)	Group B Median (Range)	P value
Positive pregnancy	0 (0-9) N=36	0 (0-7) N=30	0.038
Negative pregnancy	0 (0-4) N=31	0 (0-3) N=31	0.246
Total No.	67	61	

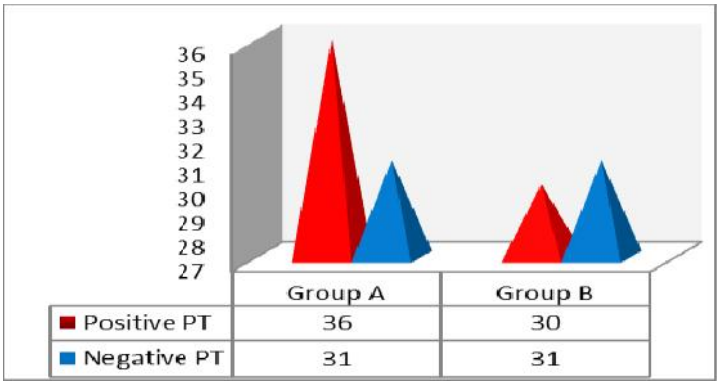


FIGURE 2: pregnancy outcomes in both groups

DISCUSSION

The black box in any ICSI cycle is the failure of good quality embryos to implant into normal uterus after a good ovarian stimulation respond, a good yield of oocytes in OPU, a high fertilization rate after ICSI, a good number of high quality embryos and a good ET procedure [16]. The dialogue of the endometrium and the embryo may be sometimes become irregular, unsynchronized and imperfect due to either the endometrium status (genetic, physiology or even molecular status) or due to the status of the embryo itself and sometimes both of them are responsible for this failure [16, 17], but some data showed that many transferred embryos failed to implant after ET so to increase the chance of pregnancy specialists tried to increase the number of transferred embryos in ET [8]. In the present study, characteristics data were compared in table 1 and the comparisons of these data showed no significant increase in PRs (figure2) even after increasing number of transferred embryos. Based on guidelines of 2008 which were approved by the Executive and Council of the Society of Obstetricians and Gynecologists of Canada and the Board of the Canadian Fertility and Andrology Society, any IVF center should evaluate the need of each patients and be able to predict chances of implantation according to patients' characteristics, the quality of embryos for each couple and the characteristics of their IVF or ICSI cycle in order to decrease the potential of abortion or risk of multiple pregnancy and maintain the overall acceptable PRs [12]. In most cases it is better to transfer fewer embryos when they are at blastocyst developmental stage especially in young female (1 is preferable) when she and her embryos have all the factors that increase the potential of a successful pregnancy such as good ovarian respond, good quantity and quality of good embryos [13]. In agreement with all studies, no increase in PRs after increasing number of transferred embryos [9, 10, 11], even in patients with RIF, actually when comparisons was made between those patients, the increase was found in patients who had a fewer number of transferred embryos (table 3). According to Giannini *et al*., PRs does not increase when number of transferred embryos is increased, but the implantation rates increased when the number of transferred embryos are two [14] . Many guidelines recommend to lower the number of transferred (1 or 2 and in rare cases 3) embryos in ICSI cycle depending on female age, her ovarian respond, the status and number of her embryos, if the couple are patients with RIF and most important the decision of IVF medical team unless the women is over forties then transfer 4-5 embryos at cleavage stage or 3 at blastocyst stage may be favorable [12, 13]. In our society (the Muslim societies) it is very difficult to conduct a law which restricts the number of embryos during ET especially with the high cost of these techniques or refusing most patients to pay for vitrification, but increasing experience in IVF treatments and increasing knowledge of the effect of abortion and multiple pregnancies with guidelines from different countries and following updates in this filed can guide many specialists to inform patients about these risks and insist to decrease low number. Many researchers have found PRs are increased by advances and improvements in IVF treatments, ICSI and ET procedures, culture and ET media, increasing number of ET procedure, but no one mentioned that increasing transferred embryos lead to increase PRs [15].

CONCLUSION

Increasing number of embryos during ET of a fresh ICSI cycle does not increase PRs even in patients with RIF, actually PRs increase in RIF when transferring low number on embryos even one good quality embryo can implant after good managed treatments and high quality techniques.

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