

## Number of Embryos Transferred and Pregnancy Outcome in *In Vitro* Fertilization

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### Abstract

**Objectives:** In developing countries, where blastocyst culture is not possible because of poor quality control in *in vitro* fertilization (IVF) laboratories, transferring of only one blastocyst is recommended. The aim of this study was to determine the relationship between the number of embryos transferred and pregnancy outcome in IVF cycles in developing countries.

**Materials and Methods:** In this retrospective study, that carried out on infertile women referred to infertility treatment center of Imam Khomeini Hospital in Sari, Iran, of 591 IVF cycles performed in infertile women during 5 years, from the beginning of 2011 to the end of 2015, 212 cases had positive pregnancies, of which 120 cases had inclusion criteria were included in the study.

**Results:** The mean age of women was  $34.21 \pm 6.55$  years. They had 53 (44.2%) Single, 52 (43.3%) twin, and 15 (12.5%) triplet pregnancy, which finally lead to 79 (65.8%) singleton, 18 (15%) twin and 2 (1.7%) triple births. Significant differences were shown between number of oocytes and twin pregnancy rates ( $P = 0.01$ ), but these differences were not seen between number of oocytes ( $P = 0.20$ ) and number of embryos ( $P = 0.18$ ) with twin birth. 63 (52.5%) participants lost their embryos during the pregnancy, so transferring one to three good quality embryos did not considerably increase the number of multiple births. Complications of pregnancy included preeclampsia (3.3%), preterm premature rupture of membrane (3.3%) and gestational diabetes mellitus (0.8%), hypertension (2.5%), hypothyroidism (5.8%). Neonate complication after IVF included Jaundice 7 (5.8%), Respiratory Distress Syndrome (RDS) 3 (2.5%), without complications 96 (80%). Data were analyzed using SPSS software, version 21. P values  $< 0.05$  were considered as statistically significant.

**Conclusion:** In developing countries, transfer of one or three embryos leads to multiple pregnancy but couldn't increase significantly multiple live birth rates in this condition due to wasting embryos in uterus during pregnancy. Therefore, transfer of one to three good quality embryos is a good option in *in vitro* fertilization.

**Keywords:** Multiple Birth; Fertilization In Vitro; Embryo Transfer; Sperm Injections; Intracytoplasmic

### Abbreviations

IVF: *In Vitro* Fertilization; SET: Single Embryo Transfer; DET: Double Embryo Transfer; ICSI: Intra Cytoplasmic Sperm Injection; PCO: Poly Cystic Ovary

### Introduction

Regardless of the risks related with multiple pregnancies, single-embryo transfer (SET) is accepted as the optional treatment in healthy patients, but most physicians transfer further embryos in order to increase pregnancy rate [1]. After transferring more than single embryo, maternal and neonatal complications increase. Among neonates born using in IVF, twins are vulnerable to premature birth and low birth weight, respiratory distress or hyperbilirubinemia [2]. Twins pregnancies are more likely to develop pre-eclampsia and also premature pre-term rupture of membranes (PPROM) and need to more Caesarean section. Although some clinicians and patients may think that having twins is cost effective, but the total cost of live birth (for mothers and babies) is the same for double transmission versus single embryo [3]. Ultimately, twins impose further cost, given the concomitant longstanding complications [4,5].

Gleicher and Barad reported twin pregnancies are pleasant for patients and there are more overestimation twin risks; therefore, deciding to plan single embryo transfer is not logical [6]. Baruffi., *et al.* concluded that double-embryo transfer (DET) had twin pregnancy and more live birth rates compared with single-embryo transfer (SET), but until recently, single embryo transfer success rates are increasing and twin complications are being reported all around the world [7]. Another study showed that live birth rates were not different between fresh and frozen DET versus SET [8]. But Lawlor., *et al.* and Nelson., *et al.* reported that in women above 40 years, transfer of three embryos could not increase the live birth rate [9].

During the recent years, the rate of multiple births decreased because of fewer transferring of three embryos [10], but it is still high in many developing countries. 76.4% intra-cytoplasmic sperm injection cycles (ICSI) comprise one or more embryos and 20.7% are related to transferring three or more embryos [11]. Another reason for transferring more embryos is embryologists, physicians, and patient preference. Patients are eager to have twin children because of the burden of IVF procedures and cultural points. They ask the physicians to transfer more embryos. Embryologists freeze three or more embryos in each cryopreservation because of financial issues. Then, they thaw all over the embryos and do not freeze them again due to low future success rates. They do not transfer a single blastocyst because of lack of media and increase vulnerability of these embryos for destruction when placed to grow up in traditional laboratory conditions instead of using time lapse microscopy and other advanced technologies in developed country, like European countries, in IVF laboratory settings [12].

According to the reasons mentioned, we evaluated multiple pregnancy and live birth rates and their complications in mothers and neonates as an important dilemma in IVF laboratory and clinics in developing countries.

### Materials and Methods

This retrospective study was conducted by investigating of the patients who referred to infertility treatment center of Imam Khomeini Hospital Sari, in Iran, during 5 years, from the beginning of 2011 to the end of 2015, and became pregnant using the IVF method. The study was accepted by Medical ethical committee of Mazandaran University of Medical Sciences with Code: IR.MAZUMS.IMAMHOSPITAL.REC.1397.027. Inclusion criteria include; cycles in which at least one embryo of grade A or B has been transferred to them, lack of donated eggs, and exclusion criteria include; endometriosis, Uncontrolled diabetes, polycystic ovary syndrome (PCO), women treated with gonadotropin releasing hormone (GnRH) and clomiphene citrate. Data were analyzed using SPSS software, version 21. Independent sample T test was run to compare the means between the two groups of normally distributed variables. Also, percentages of categorical variables were compared using the chi-square test ( $\chi^2$ ). P values < 0.05 were considered as statistically significant.

**Results and Discussion**

The total number of IVF carried out during 5 years were 591, including 212 positive IVF pregnancy tests (35.87%). Among women with positive IVF pregnancy, 120 patients that had inclusion criteria entered the study. Demographic and basic characteristics of all women (N = 120) undergoing IVF in table 1.

Variables	Mean ± SD
Age (years)	34.21 ± 6.55
BMI (kg/m <sup>2</sup> )	24.18 ± 1.348
Duration of infertility (years)	6.99 ± 3.800
Oocyte recovery (No.)	7.71 ± 2.766
Number of transferred embryos (No.)	2.93 ± 0.444
Maternal disease and complications	Number (percent%)
Hypothyroidism	7 (5.8%)
Hypertension	3 (2.5%)
Gestational Diabetes Mellitus (GDM)	1 (0.8%)
Preeclampsia	4 (3.3%)
Preterm Premature Rupture of Membrane (PPROM)	4 (3.3%)

**Table 1:** Demographic and baseline characteristics of all women undergoing IVF.

Result of pregnancies included 52 (43.3%) twin, 15 (12.5%) triplet, and 53 (44.2%) Single pregnancy, which finally lead to 18 (15%) twin and 2 (1.7%) triple and 79 (65.8%) singleton birth. Significant difference was shown between number of oocytes and twin pregnancy rates (P = 0.01) (Table 2). No significant statistical difference was observed between the number of oocytes (P = 0.20) and the number of embryos transferred (P = 0.18) with twin birth rate (Table 3). Therefore, transfer of one to three good quality embryos did not considerably increase the number of multiple births. Comparison of complication between single and twin and multiple pregnancy (Table 4). Neonate complication after IVF include Jaundice 7 (5.8%), Respiratory Distress Syndrome (RDS) 3 (2.5%), without complications 96 (80%).

Variable	Single pregnancy (mean ± SD)	Twin pregnancy (mean ± SD)	Mann-Whitney U	P Value
Mean oocyte recovery (N)	7.38 ± 2.88	8.34 ± 2.76	1357	0.01
Number of transferred embryos (N)	2.98 ± 0.5	2.85 ± 0.39	1573	0.17

**Table 2:** Comparison of number of oocytes and embryos transferred in single and twin pregnancy.

Variable	Single pregnancy (mean ± SD)	Twin pregnancy (mean ± SD)	Mann-Whitney U	P Value
Mean oocyte recovery (N)	7.84 ± 2.95	8.33 ± 2.19	759.5	0.20
Number of transferred embryos (N)	2.93 ± 0.45	2.78 ± 0.42	791	0.18

**Table 3:** Comparison of number of oocytes and embryos transferred in single and twin birth.

variables	Single pregnancies 53 (44.2%)	Twin pregnancies 52 (43.2%)	Multiple pregnancies 15 (12.5%)	Total 120 (100%)
Normal birth weight	93 (77.6%)	_____	14 (11.6%)	107 (89.2%)
LBW	5 (4.1%)	_____	6 (5%)	11 (9.1%)
Preterm birth	5 (4.2%)	_____	6 (5%)	11 (9.2%)
Term birth	109 (90.8%)	_____	_____	109 (90.8%)
Live birth	79 (65.8%)	18 (15%)	2 (1.7%)	
Miscarriage	_____	_____	_____	21 (17.5%)

**Table 4:** Comparison of complication between single and twin and multiple pregnancy.

The results of the present study showed that women who pregnant with IVF had 53 (44.2%) Single, 52 (43.3%) twin, and 15 (12.5%) triplet pregnancy, which finally lead to 79 (65.8%) singleton, 18 (15%) twin and 2 (1.7%) triple births. In the United States, 25 - 28% of the IVF births were twin and 1% were triplet [13], in Europe, in this group of population, twin birth rates were 18.6% and triplets were 0.6% [11].

Transmission of several embryos to increase the chance of pregnancy and live birth is a common practice, which results in a high proportion of multiple pregnancies with ART methods and studies shows that the frequency of multiple-birth and pregnancies depends on the number of transmitted embryos [14]. Currently in Iran, the infertility treatment policy is transfer three and more fetus, which probably increases the chances of multiple-pregnancy [15,16]. Causes transfer of more embryos are physicians, embryologists, and patient’s preference. Patients in Iran are eager to have twin children because of the burden of IVF and cultural issues, so they ask the physicians to transfer over three embryos. In the present study, no significant relationship was observed between the number of embryos transferred (excellent quality 8 - 12-cell embryos) and multiple-birth. In line with our study, another study showed that in patients who had extra embryos for cryopreservation, transferring more embryos increased multiple pregnancy with no increase in live birth. These authors showed that the age of the donor and embryo quality are prognostic factors [17]. Contrary to our results, Vahratian., *et al.* [18] showed that multiple-birth risk was significantly increased with a greater number of transferred embryos. These different results may be because of different population and treatment methods and better laboratory IVF conditions that lead to high-quality embryos who could remain alive longer in utero till the end of the pregnancy. Also, should be point that the poor quality embryo transfer is a risk factor for extra uterine implantation [19]. Whereas 63 (52.5%) participants in present study lost their embryos in uterus during the pregnancy. Eventually, we suggest in Iran and similar countries transfer of one to three good quality embryos can be a reasonable option.

In this study, we found significant differences between the number of oocytes retrieved and twin pregnancy rates. It means that more retrieved oocytes result in more twin pregnancy rates. Policy of retrieve less than 15 oocyte leads to fewer available embryos and increases live birth rates and reduce the multiple pregnancy rates [20]. The results of a study in 2016 showed more retrieved oocytes had led to more pregnancy rates [14] but didn’t survey the multiple pregnancy rates.

Over the decades, IVF has been associated with a high success rate in infertility treatment. however, this method may have complications for mother and neonate. Premature rupture of membranes, preterm delivery, miscarriage, hypertension, gestational diabetes, and multiple births are the complications of this technology [13]. In the present study, complications of pregnancy include preeclampsia (3.3%), preterm premature rupture of membrane (3.3%), and gestational diabetes mellitus (0.8%), hypertension (2.5%), hypothyroidism (5.8%). The results of a retrospective study by Zhu., *et al.* (2016) revealed that the increased risks of perinatal complications and adverse neonatal outcomes may be because of higher frequency of multiple pregnancy and the manipulation in ART processes. However, they

found more gestational diabetes and other prenatal complications, such as placenta previa, oligohydramnios, and preterm delivery, which may be because of different population and larger sample size [21]. Those did not report an increase of preterm labor in twin pregnant women after DET and concluded that this may be due to lower sample size and more dizygotic twin in DET, which has fewer complications compared with monozygotic twin.

In the present study, the frequency of neonate birth weight less than 2500 grams in multiple-birth (6%) was more than of single-birth, the frequency of neonates with normal weight (2500 - 4000 gr) was more in singleton rather than multiple-birth. Our results were consistent with Chowdhury, *et al* [22]. Low birth weight can be attributed to preterm delivery in multiple births.

Also, infertility duration was no statistically significant correlation with single and multiple pregnancies. This result was consistent with Berkkanoglu, *et al.* [23] and Vahid Roudsari, *et al* [24]. Despite this, in Matalliotakis, *et al.* study [25], showed that a significant correlation between the duration of infertility and the frequency of pregnancy with IVF, they stated that the shorter the infertility period, was higher the pregnancy rate with IVF. The reason for the difference in the result could be because of the diversity in demographic factors and the various therapeutic methods.

### Conclusion

Not all multiple pregnancies lead to live birth, several factors such as laboratory conditions, embryo quality, mother's age, early and late abortion and preterm delivery may decrease live birth rates. In developing countries, transfer of one or three embryos leads to multiple pregnancy but couldn't increase significantly multiple live birth rates in this condition due to wasting embryos in uterus during pregnancy. A point that should be considered is that the results obtained in the present study may not be generalizable to the society because of small sample size. In this regard, it is suggested that similar studies be conducted with a larger sample size.

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### Conflict of Interest

The authors declare that they have no financial conflict of interest.

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