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Impact of different embryo loading techniques on pregnancy rates in *in vitro* fertlization/embryo transfer cycles

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Abstract

BACKGROUND:

Embryo transfer (ET) technique is one of the important factors of *in vitro* fertlization success. Among the different steps in ET technique, less attention has been given to embryo loading (EL). The aim was to compare the impact of two different techniques of EL on pregnancy rate in IVF/ET cycles.

MATERIALS AND METHODS:

In this retrospective study, 144 and 170 patients were placed in groups A and B, respectively. In Group A, the embryos were drawn directly into the ET catheter from culture microdrop under the oil. In Group B, the embryos were transferred from culture microdrop into G2 medium in center-well dish. Then, the embryos were drawn into the catheter and finally transferred into the uterus. Both groups were adjusted for other parameters based on the EL technique. The main outcome measure was pregnancy rate.

RESULTS:

There were insignificant differences for etiology of infertility, source of sperm, type of stimulation protocol, percent of IVF or intracytoplasmic sperm injection type of ET catheter, cycles with good quality embryos and transferred embryos between two groups. The rate of both chemical and clinical pregnancy was higher in Group B compared to A, but the difference was insignificant ($P = 0.09$ and $P = 0.1$, respectively).

CONCLUSION:

It seems that there is no difference in the outcome by loading the embryo from microdrop or center-well dish.

KEY WORDS: Embryo loading technique, embryo transfer, pregnancy

INTRODUCTION

Assisted reproductive technology (ART) consists of sophisticated processes and the final and perhaps most critical procedure is embryo transfer (ET). Since the last decade, interest in studying the ET technique has increased, because it has been well established that ET can affect implantation and pregnancy rates. About

80% *in vitro* fertilization (IVF)/ET cycles reach the ET stage, but most of them do not achieve pregnancy.[1] Despite considerable progress in ART, it is estimated that the implantation rate (IR) is not above 20% following IVF.[2] Although, IR depends upon numerous factors, such as embryo quality and uterine receptivity, it is well recognized now that the ET itself is one of the important factors for IVF success.[3] In contrast to other aspects of ART procedures, ET technique has remained relatively unchanged for decades. Certainly, it is necessary to modify and optimize the standard protocol for ET technique in ART.

Regarding the laboratory aspects, some important factors have been previously mentioned such as the presence of blood on the catheter,[4] bacterial contamination of the catheter,[5] types of ET catheter,[6] air bubble in the catheter,[7] prolonged duration of procedure,[8] ET medium composition[9] and the volume of the transfer medium.[10] Among different steps in ET technique, less attention has been given to the embryo loading (EL) procedure. EL is the final step which is done by the embryologist. The embryo(s) is drawn from the culture medium into the catheter. Then, the catheter is handed to the gynecologist in order to enter the catheter into the uterus and eject the embryo(s). Although the EL technique looks simple and easy at first glance, it is considered a delicate step in ET. It may be necessary to optimize the EL technique in order to minimize the drawbacks. There are different methods for EL (e.g., air-fluid and fluid-only).[7] In this investigation, the main goal was to compare two different techniques for EL (in the air-fluid sub-categories). In one category, the embryos were loaded directly from the culture microdrop overlaid with mineral oil. In the second one, the embryos were moved to the transfer dish, then loaded into the ET catheter. To the best of our knowledge, no data have been reported yet for comparison between the effect of these EL techniques on ART outcomes.

MATERIALS AND METHODS

Patient selection

In total, 314 ETs were retrospectively reviewed during the period from March to October 2011. Couples were divided into male factor, female factor, and unexplained infertility. Semen samples were collected from ejaculated or non-ejaculated sources. An ET cycle was excluded from this study if details of the ET were incomplete or if patients were lost to follow-up without record of pregnancy outcome. Also, cycles with egg donation, surrogacy, frozen-thawed cycles, and *in vitro* maturation (IVM) were excluded from this study. All patients signed informed consents. This study was approved by our institute review board and ethics committee (Number: 822).

Controlled ovarian hyperstimulation

Controlled ovarian hyperstimulation was carried out with GnRH agonist (Diphereline S.R. 3.75 mg, Ferring, Germany) down-regulation in mid-luteal phase of spontaneous menstrual cycle. Human menopausal gonadotropine (hMG; Merioanal, IBSA, Lugano, Switzerland) (i.m., 150-225 IU) was used for gonadotropin stimulation from Day 2 of the cycle. Antagonist (Cetrotide, Merck-Serono, Switzerland) protocol was also used. Then, 10,000 IU of human chorionic gonadotrophin (hCG, i.m.; IBSA, Switzerland) was administered for ovulation trigger when at least 3 follicles >18 mm were seen. The ovarian response was controlled by transvaginal ultrasound and serum estradiol concentration. Oocyte retrieval was done approximately 36 h after hCG injection under transvaginal ultrasound-guidance.

ICSI and IVF procedures

Semen analysis was done according to World Health Organization (WHO) guidelines.[11] All sperm preparations were performed using the swim-up or density gradient centrifugation techniques as described elsewhere.[12,13] Conventional IVF and ICSI procedures were applied according to etiology of infertility as also described previously.[14,15]

Fertilization and embryo evaluation

After ICSI or denudation after conventional IVF, the oocytes were washed twice and placed in microdroplets of G1 (20 μl) (Vitrolife, Sweden) covered with mineral oil. The oocytes were checked under

microscope for presence of two pronuclear (2PN) 16-18 h later. The zygotes were kept in G1 until ET on Day 2. Good quality embryos were separated and 3 embryos were placed in each microdrop. About 48 h post injection or insemination, embryo evaluation was done according to Hill *et al*.,[16] [Figure 1]. Grades A and B were considered as high-quality embryos. Grade D embryos were not transferred.

Preparation of ET Dish

The day before ET, center-well organ culture dish (Falcon 3037) was filled with 500 µl G2 (Vitrolife, Sweden) in inner well and Ham's F10 medium in outer well, which was placed in the triple-gas incubator overnight (5% O2, 6% CO2).

Embryo catheter loading technique

After ID confirmation of the patient, the catheter was loaded by the embryologist and handed to the clinician for performing the ET. EL techniques were divided into two groups of A and B. In Group A, the embryos were drawn directly into the ET catheter from the G1 culture microdrop under the oil. Then, the catheter tip was thoroughly washed in equilibrated and warm Ham's F10 medium. Afterward, the G2 medium was drawn into catheter prior to ET. In Group B, the embryos were transferred from culture microdrop into G2 medium first. Then, the embryos were drawn into ET catheter, and finally transferred into the uterine cavity. The chemical compositions of G1 and G2 media are different.[17] The G2 medium has more glucose as well as essential amino acids which are necessary for further embryo development. In both groups, the catheters were first flushed using a 1-ml air-tight syringe before EL with Ham's F10 medium. The embryo-containing medium was bracketed by air bubbles. Total medium volume was 20-30 µl. Three types of catheter were used: Cook (Cook Medical, USA), Labotect (Labotect, Germany) and CCD (Laboratoire C.C.D., France).

Embryo transfer technique

Cleavage embryos were transferred in the lithotomy position without sedation. For luteal support in agonist protocol, the patients received progesterone (Progesterone, Aburaihan Co., Iran) 400 mg BID, suppository from the day of oocyte retrieval until the tenth week of gestation. Also, for luteal support in antagonist protocol, the patients received estradiol (Aburaihan Co., Iran), 2 mg BID, in addition to progesterone. A sterile speculum was inserted in the vagina for opening and exposing of the cervix. Aspiration of cervical mucus before ET was done by Mucat (Laboratoire C.C.D., France). Embryos were deposited at 1.5-2 cm below the fundus. The catheter was withdrawn gently, while maintaining the pressure on the syringe plunger. Following ET, immediately the catheter was returned to the laboratory for checking the retained embryos, mucus and blood by embryologist. Immediate reloading and transferring with the same or a new catheter was done, if embryos were retained. If there was no blood/mucus in the catheter or no reloading or alteration of catheter, the transfer was defined as easy. No assisted hatching was performed for embryos that were assigned for ET. Chemical pregnancy was determined by a positive βhCG level 14 days after ET. Fetal heartbeat was evaluated seven weeks after ET using ultrasound and indicated clinical pregnancy.

Statistical analysis

For statistical analysis, data was presented as mean ± S.E. The Chi-square and independent samples *t*-test were used wherever appropriate. All tests were two-tailed and significance level for *P* value was 0.05.

RESULTS

Maternal age was between 18-50 years and the mean ± SEM for groups A and B were 31.19 ± 0.4 and 30.19 ± 0.4, respectively. A total of 2517 MII oocytes were retrieved, of which 1653 were fertilized (fertilization rate = 65.6%). A total of 679 Day 2 embryos were transferred. The range of retrieved MII oocytes was between 1-30 oocytes. About half the couples were male factor for groups A and B (52% and 55.8%, respectively). Nearly 90% of the semen specimens were collected by masturbation in both groups.

There were insignificant differences for etiology of infertility, source of sperm, type of stimulation protocol, numbers of IVF or ICSI cycles, type of EL catheter, cycles with good quality embryos and

number of transferred embryos between the two groups [Tables 1 and 2]. The number of missing cycles to follow-up chemical and clinical pregnancy in Group A were 12 and 7 cycles, respectively. Also, in Group B, 7 patients were missed to follow-up for chemical pregnancy and we were unable to follow-up 3 cycles in order to confirm clinical pregnancy. A total of 86 and 45 chemical and clinical pregnancies were achieved, respectively. The clinical pregnancy rate was higher in Group B compared to A, but the difference was insignificant [Table 1].

DISCUSSION

IR has still remained low against huge progresses that have been made in the field of ART since the last three decades. This drawback has attracted many researchers around the world to conduct studies in order to improve the success rates. One of the important factors that can affect IR is certainly the ET technique. Although, the EL technique is considered the most simple and easy step in the ET procedure, it could have impacts on the ART outcome. The EL technique has not been changed much for years. However, more attention has been given to it in recent years in order to enhance the ART outcome.[18,19]

Some factors have been evaluated for the probable effect of EL on success rate in ART program. For example, the syringe used,[20] volume of transfer media,[10] concentration of protein,[21] catheter loading speed,[22] viscosity in transfer media,[23] and embryo placement in the catheter.[24] Most attentions have been paid for the volume of transferred media and presence of air bubbles. Ebner and co-workers (2001) demonstrated that the presence of air bubbles and the usage of a smaller transfer volume in EL (<10 μl) had a negative effect on the rates of IR and pregnancy.[10] Transfer of high volume of media (>60 μl) may increase the chance of embryo expulsion.[25] Montag *et al*., (2002) showed that using high fluid volume (40 μl) compared to low fluid volume (15-20 μl) increased the rates of IR and pregnancy.[26] The other controversial factor is the use of air bubbles in the catheter. Some clinicians believe that air bracket in the catheter makes the ET easy to identify culture medium with embryos during ultrasound-guided ET.[27] However, others believe that the air in the catheter is not involved in the physiologic process.[28] Also, some authors have suggested a new modification of the standard air-fluid EL which uses only one air bubble.[19] It seems that any effort to increase the ET outcome by modification or introducing a new EL technique is one of the inseparable components of ET investigation.

Our study was about the comparison of different EL techniques of loading directly from culture microdrop, and loading from transfer dish. The air-fluid and the fluid-only are two models for catheter EL techniques.[7] The different EL techniques, which were analyzed in this study, were placed in air-fluid sub-categories. For Method A, there are some advantages, e.g., the embryo(s) are directly drawn into the catheter from culture microdrop, which has growth factors that are released from the embryo(s). The preimplantation embryos may produce some beneficial factors for stimulating development of both self and adjacent embryos.[29] There are some established candidates for these autocrine/paracrine factors, e.g., platelet activating factor (Paf) and insulin-like growth factor I and II.[30] Paf, by creating autocrine loop can be useful for embryo survival and development.[30] The other autocrine embryo-trophic factors are leukemia inhibitory factor (LIF) and epidermal growth factor (EGF).[31] Also, it is well established that reducing the activity of these autocrine embryo-trophin factors may reduce the developmental potential of embryos.[32] Furthermore, these factors may be beneficial for further embryo development in the uterus as well as implantation success rates. In this technique, these growth factors are not diluted and remain with the embryo after injection into the uterine cavity. But, there are also some disadvantages of this method, e.g., the embryos were loaded directly from culture drop under the oil, so the catheter tip becomes oily. Though, the catheter tip was washed in the transfer media prior to ET. However, oil microdroplets may still remain on the catheter tip, which theoretically could be detrimental for embryo implantation. Shimada *et al*., (2002) assessed the role of mineral oil on nuclear maturation and developmental competence of pig oocytes in IVM media.[33] They showed that blastulation rate was higher in the group lacking mineral oil. They considered that the adverse effect of oil may be due to absorption of secreted steroid hormones by oil. It is well known that blastocyst can produce and secrete hCG[34] and because of detection of mRNA for hCG in 6- to 8-cell embryos,[35] it is accepted that human embryo starts to produce hCG before implantation. Therefore, if mineral oil remains on the tip of the catheter and is transferred to the uterine cavity, it has the potential for absorption of steroid hormones (e.g., hCG) which have both embryonic and

maternal sources. The other probable effect of transferred oil may be reduction of endometrial receptivity for hatched embryo. In our hypothesis, this is one of this technique's drawbacks and may be one of the probable causes of the trend towards a lower pregnancy rate in Group A. In some IVF centers, in order to overcome this problem, the tip of the loaded catheter is cleaned with sterile non-woven gauze before ET. Some prefer to cover the transfer medium using the mineral oil in Method B.[36] However, in our center, the risk of oil in Method B is fully omitted which is an advantage. But, the growth factors generated from cultured embryos are diluted in transfer media and the embryos are deprived of probable useful effect(s). Our data suggested a trend of higher pregnancy rates in Method B, but the difference was insignificant.

One of the important factors that affect the success of ET is the quality of the embryos.[3] In our study, the rates of good quality embryos were similar in both groups as well as other probable confounding factors such as causes of infertility, source of sperm, type of catheter, type of stimulation protocol, maternal age and number of transferred embryos in the groups [Tables 1 and 2]. We also tried to minimize other variables by matching different criteria between the two groups. To the best of our knowledge, this study is the first report to evaluate the efficacy of EL directly from under the oil versus other technique. Limiting factors may be the retrospective nature of the study, as well as lack of follow-up of the patients until delivery. Introducing a new EL technique with fewer drawbacks and more benefits will certainly improve the success rates in ET. This issue could be matter of more clinical investigations in the future.

In conclusion, our results did not show any significant differences for pregnancy rates between two techniques of EL; prospective studies and follow-up of patients are needed to draw final conclusion.

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Footnotes

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Conflict of Interest: None declared

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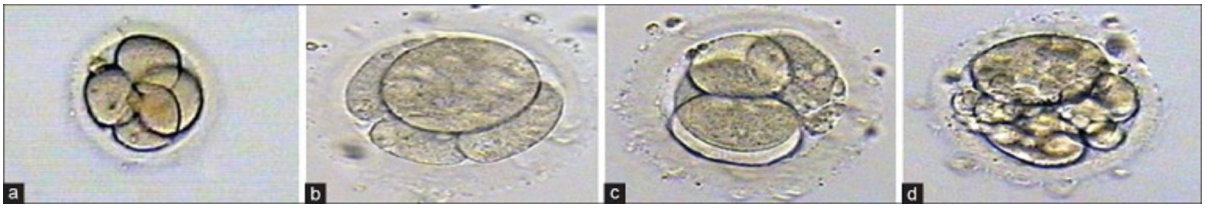
Figures and Tables

Figure 1



Embryo grading was done according to Hill *et al*.[16] Briefly, Grade a: Even blastomeres with no fragmentation, Grade b: A little inequality in blastomeres size, <10% cytoplasmic fragments. Grade c: Unequal sized blastomeres,<50% fragments. Grade d: Unequal blastomeres, severe fragmentation and large black granules

Table 1

Parameters	Group A (*n*=144)	Group B (*n*=170)	*P* value
Female age[a]	31.19±0.46	30.19±0.43	0.1
Causes of infertility			
Male factor	75	95	0.9
Female factor	45	49	
Unexplained	24	26	
Stimulation protocol			
Agonist	66	89	0.2
Antagonist	78	81	
Chemical pregnancy (%)	32/132 (24.2)	54/162 (35.1)	0.09
Clinical pregnancy (%)	15/25 (60)	30/51 (76.4)	0.1

[a]values are mean±SE

Comparison of different clinical characteristics between groups A (embryo drawn directly from microdrop) and B (embryo drawn from transfer medium)

Table 2

Parameters	Group A (*n*=144)	Group B (*n*=170)	*P* value
Source of sperm			
Ejaculated	128	154	0.9
Non-ejaculated	16	16	
Type of catheter			
Labotect	65	82	0.5
Cook	69	81	
CCD	10	7	
IVF/ICSI	29/115	31/139	0.6
Cycles with good quality embryos (%)	83.3	85.2	0.9
No. of transferred embryos[a]	2.24±0.06	2.1±0.05	0.1

[a]values are mean±SE

Comparison of different laboratory characteristics between groups A (embryo drawn directly from microdrop) and B (embryo drawn from transfer medium)