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EGG DONATION

The use of portable CO₂ incubator for cross-border shipping of embryos in an international egg donation program

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Abstract

Two groups of egg recipients were treated, one *in situ* (165 patients; 195 cycles) and one after cross-border embryo transportation (340 cycles; 340 cycles) using mobile CO₂ incubator. The positive pregnancy rate per cycle was 199/340 (58.6%) and 99/195 (50.7%) in the transportation and the traveling group, respectively (NS). The clinical pregnancy rate (fetal heart beat) was 48.1 and 43.1% per embryo transfer cycle, respectively (NS) and the delivery rate was 44.1 and 35.9% per embryo transfer cycle, respectively ($p = 0.01$). Long distance transportation of human pre-implantation embryos using portable CO₂ incubator is safe and do not jeopardize their developmental potential.

Keywords

Egg donation, embryo culture, embryo transportation, *in vitro* fertilization

History

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Introduction

As assisted reproductive therapy (ART) becomes more sophisticated, mobility of embryos rather than patients will become critical in order to provide good medical care for populations that are remote from the large metropolitan centers. Moreover, as a consequence of safety and liability issues, mobilization of human embryos between *in vitro* fertilization (IVF) laboratories, especially across international borders, is currently done after cryopreservation and therefore imposes a significant toll on success rates.

Transportation of fresh gametes and embryos is currently practiced mainly with or in between rural areas especially for agricultural or scientific purposes [1–3]. The devices that were developed for such purposes are, in principle, desiccators equipped with temperature control system [1,4,5]. These shipping devices can also maintain CO₂ levels; however, normal CO₂ levels can be maintained only for few hours and therefore they are not suitable for long trips.

Maintenance of controlled culture conditions is essential for normal embryo development. Today, CO₂ incubators are being designed specifically for IVF purposes and are equipped with high fidelity electronic systems and filtering devices that turn them into sophisticated environmental culture chambers. Many of these incubators are, in fact, relatively compact bench top systems that relay on a predetermined gas mixture supply. The ability to turn these systems into mobile incubators may have a significant impact on the way of IVF practice on both the national and global scales.

The legislation of egg donation in Israel, where only IVF patients could volunteer to donate oocytes, created a severe shortage of oocytes for donation. In Ukraine, however, donors

may undergo IVF and donate their oocyte for compensation. Under these circumstances, patients from Israel were seeking for donors in Ukraine either on a private base or through a binational medical enterprise.

In our IVF unit at the ISIDA Hospital in Ukraine, we are cooperating mainly with two countries, Israel and UK. According to the regulation, embryos transportation is allowed between Ukraine and Israel, but is not between Ukraine and UK. As a result, the common practice is that fresh human embryos are transported from Ukraine to Israel for transfer, while UK recipient are traveling to Ukraine for embryo transfer (ET).

Prompted by the aforementioned difference, we aimed to compare IVF–egg donation outcome using the two logistic practices: transport of embryos from Ukraine to Israel for fresh ET (transportation group) or the traveling of the recipients from UK to Ukraine (traveling group). This will aid both fertility specialists' counseling and their patients in deciding whether to transport the fresh embryos or to travel for fresh ET.

Materials and methods

The study was carried out after the approval of the local institutional review board of ISIDA Hospital in Ukraine as well as the institutional review board of Elisha hospital in Israel and the Bridge IVF Center in London, UK. All patients signed an informed consent before participating in the study.

The egg donors were stimulated according to a fixed protocol of mid-luteal administration of differelin followed by daily administration of 150 units of menotropins.

In the transport group, only frozen sperm samples were used for fertilization. In the traveling group, usually the first attempt was done with a fresh husband's sample. At the same time, cryopreservation was offered for future cycle cost saving.

Preparation of the endometrium was uniform in both groups and was done with administration of 2 mg of estradiol valerate three times a day followed by vaginal administration of micronized progesterone (Utrogestan) 300 mg three times a day

as off the day of oocyte pick-up. About 2–3 days prior to implementing the donation, all patients were screened for serum estradiol (E2) and progesterone (P4) levels, and endometrial thickness. Patients with increased P4 levels or with endometrium thickness below the cutoff of 6 mm were excluded from the current cycle.

In order to avoid unexpected fertilization failure and increase the cycle efficiency, intracytoplasmic sperm injection was carried out globally without respect to sperm quality unless the patient's preference was different.

Embryo transfer policy was practiced according to the guidelines from both ministries of health in Israel, UK and Ukraine. The decision about the number of embryos for replacement was done after consultation with the couple and granting their consent. As a rule no more than two good quality embryos were replaced in each patient during the first three treatment cycles. In cases with three or more recurrent implantation failures and at least two good quality embryos for ET, adding a third embryo was offered. In the UK, transfer of more than three embryos was unacceptable while in the Israeli patients with recurrent implantation failures and global poor embryo quality a maximum of four embryos was acceptable. According to our transfer policy, replacement of three or more good quality embryos was acceptable only if fetal reduction was an option in case of triplets.

For embryos shipping purpose, a LEC-960 portable incubator (LEC Instruments, Scoresby, Australia) was modified by sealing its culture chamber. A differential pressure transmitter (HK Instruments, Muurame, Finland) was set to open a flow valve (model VX21, Baccara Geva Ltd, Geva, Israel) when the chamber pressure dropped below 1000 mPa. The chamber was supplied with gas mixture of 6% CO₂ in air. The inlet pressure was reduced to <0.2 bars using a 3-l pin-index cylinder fitted with one-step gas flow regulator (Table 1).

Culture was carried out using Cook™ sequential culture media (Cook, Queensland, Australia) in 5 ml vials (Nunc™, Roskilde, Denmark). The maximal capacity of the heating block was 25 vial pits. During the “preparation” mode the battery was charged for at least 12 h. Embryos at Day 0–5 were transferred into the vials containing 0.5 ml of pre-gassed culture medium. The vials covered by their loosen cups were placed inside the heating block and the incubator's culture chamber was tight sealed. A Petri dish filled with sterile cotton pads soaked with distilled water was placed inside the chamber to increase humidity level and minimize media evaporation. Immediately after the culture chamber sealing, continuous air mixture flow was initiated until the chamber's air was exchanged with the gas mixture. This was verified by continuous CO₂ level measurements and took <5 min on average to accomplish. The incubator setting was switched to

“transportation mode” by activating the chamber pressure regulation and flow system. The incubator was then carried on board in the passenger cabin of regular commercial flights between Ukraine's and Israel's international airports. Airport security was cleared without exposing the incubator's contents to radiation.

Results

During January 2009 to August 2010 a total of 340 patients from Israel (340 cycles; 20 transportation cycles) were treated by our cross-border egg donation program using a portable incubator. The average duration of door to door transportation was 8.5 ± 2.5 h. During this period, there were three incidents of >1-h delay in departure. At the same period 165 patients (195 cycles) traveled from UK to have their egg donor IVF-ET *in situ* in Kiev, Ukraine.

The average egg donor age was 27.3 ± 3.1 years (range 21–31 years). The average cohort size was 14.1 ± 9.3 metaphase II oocytes. On average, two recipients shared oocytes with one donor.

The recipients were prepared and synchronized with the donors according to a fixed protocol of oral estradiol administration followed with vaginal administration of micronized progesterone. The average recipient age in the transportation and traveling group was 41.3 ± 7.2 years (range 23–54 years) and 43.4 ± 4.8 years (range 27–56 years), respectively ($p < 0.003$).

The average number of embryos transferred in the transportation and the traveling groups was 2.5 ± 0.5 (range 1–3) and 2.9 ± 0.6 (range 1–4), respectively ($p = 0.002$). The positive pregnancy rate per cycle was 199/340 (58.6%) and 99/195 (50.7%) in the transportation and the traveling group, respectively (NS).

The clinical pregnancy rate (fetal heart beat) was 48.1 and 43.1% per ET cycle, respectively (NS) and the delivery rate was 44.1 and 35.9% per ET cycle, respectively ($p = 0.01$).

In the transportation group, the overall implantation rate was 27.2% (239 fetal sacs out of 876 embryos replaced) with twinning rate of 23/163 (14.1%) and triplets rate of 8/163 (4.9%), of which seven underwent first trimester fetal reduction and one, second trimester fetal reduction.

In the traveling group, the overall implantation rate was 21.9% (124 fetal sacs out of 565 embryos replaced) with twinning rate of 15/84 (17.8%) and triplets rate of 8/84 (9.5%) of which seven underwent first trimester fetal reduction and one have had a spontaneous late abortion.

Discussion

Our present results demonstrate that long distance transportation of human pre-implantation embryos is safe and do not jeopardize their developmental potential.

By comparing the outcomes in the transported and the traveling groups of patients, it is evident that the outcome is comparable and the transportation is safe. Because the groups originated from different countries having different legislative, social, moral and cultural attitudes toward infertility issues, matching was done only according to the study period.

The traveling patients were significantly older probably because IVF treatment using donor eggs was less available, with more restrictions and more costs in the UK than in Israel. Additionally, there was no option for them to have their frozen embryos at home, therefore, on average these patients insisted more frequently to increase the number of embryos transferred.

The differences in the implantation rates and delivery rate between both groups probably results from the age factor and the fact that among the transportation group there were less patients

Table 1. Comparison of IVF outcome in the “transportation” and the “traveling” groups.

	Transportation group	Traveling group	<i>p</i> Value
Number of patients	340*	165	
Number of cycles	340	195	
Mean age (years)	41.3 ± 7.2	43.4 ± 4.8	0.003
Mean number of embryos replaced	2.5 ± 0.5	2.9 ± 0.6	0.002
Implantation rate	239/876 (27.2%)	124/565 (21.9%)	0.02
Clinical pregnancy rate	163/340 (48.1%)	84/195 (43.1%)	NS
Delivery rate	150/340 (44.1%)	70/195 (35.9%)	0.01
Twinning rate	23/163 (14.1%)	15/84 (17.8%)	NS
Triplet rate	8/163 (4.9%)	8/84 (9.5%)	NS

*Each patient had also embryos for cryopreservation.

with repeated implantation failures following egg donation. The traveling group have demonstrated relatively high incidence of uterine and endometrial pathologies as we have indicated in a recent study [6].

Although there are lately some reports [7] about comparable IVF outcome using fresh and frozen vitrified eggs and embryos, there is a wide consensus in the literature about the superiority of fresh cycles upon to the frozen ones. This was especially valid during the period this study was conducted.

There are several difficulties in long distance transportation of human pre-implantation embryos, especially when there is a need for airborne transportation.

There are three major concerns that deserve careful preparation and coordination before executing such a cross-border embryo transport project. First, coordination with the airport security authorities is of outmost critical importance before and during every trip. During the tight security arrangements in all airports worldwide, there is an extensive use of X-ray radiation. Since human pre-implantation embryos should not be exposed to any form of ionizing irradiation the security procedure should be carried out with the aid of trained dogs only.

Secondly, the incubator must be carried and stored on board in the passenger cabin due to the need to store it in an upright position and prevent it from being exposed to extreme temperature and depressurizing conditions.

The third concern is the legal issues of transporting human embryos between countries. These are sensitive issues and legal advice is strongly recommended before initiating such a project. Moreover, prior coordination with the custom authorities is crucial before attempting to clear customs in every trip.

The ability to ship pre-implantation embryos within the same country may change the availability of advanced reproductive technologies to remote and isolated places. The ever increasing sophistication and expertise needed in some areas of assisted reproductive technology limits its availability and significantly increase the costs of these services. Setting up centers of excellence for advanced ART techniques such as pre-implantation genetic diagnosis and fertility preservation may help reducing the costs and increasing the availability of these services within the country [7–11]. Oocytes and embryos may be shipped back and forth between peripheral clinics and a central laboratory where skilled embryologists and scientists may provide their services for the less equipped and skillful clinics [12,13].

As the IVF and its related techniques are becoming the central pivot of infertility therapy from the one hand and as the practice

of reproductive biology and science are becoming more intricate on the other hand, the need for shipping gametes and embryos will become more critical and important asset.

Declaration of interest

The authors report no conflicts of interests while conducting this research.

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