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A Novel Way of Enhancing Pregnancy Rates in ART Cycles – Cumulus Co-Culture and Cumulus Aided Embryo Transfer (COCAET)

Firuza R Parikh, *Suparna G* Nadkarni, *Nandkishor J* Naik, *Dattatray J* Naik
Shonali A Uttamchandani
Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, Mumbai 400 026.

OBJECTIVE – To evaluate pregnancy rates after the addition of expanded cumulus cells to the embryos at the time of embryo transfer. **METHOD** – A prospective study was undertaken involving 100 women undergoing ICSI. Of these 50 received expanded cumulus cells at the time of embryo transfer (Group A). Fifty women underwent embryo transfer without the addition of cumulus cells and acted as controls (Group B). Batches of women were alternatly allotted to one of the two groups. Inclusion criteria were at least three metaphase II oocytes for sperm injection or at least two embryos available for transfer. Embryos were co-cultured with patient's own cumulus cells in both groups and were transferred into the uterus with 100 picolitres of the expanded cumulus cells in Group A. In Group B, embryos were transferred without cumulus cells. **RESULTS** – There was a significant increase in the implantation rate (26.3% in Group A as compared to 15% in Group B, p=0.01). There was also a trend towards higher pregnancy rates. (50% in Group A as compared to 36% in the Group B, p=0.16). Although the incidence of multiple pregnancy was higher at 36% in Group A as compared to 22% in Group B, this was statistically not significant (p=0.3). **CONCLUSION** – Our study has demonstrated, for the first time, the use of autologous expanded cumulus cells at the time of embryo transfer and has evaluated its role in enhancing pregnancy and implantation rates.

Key words : cumulus co-culture, cumulus aided embryo transfer, growth factors, implantation factors, enhanced pregnancy rates.

Introduction

The hope of every ART practitioner is to offer better pregnancy rates to infertile couples undergoing ART. In spite of various advances in the field, including better stimulation protocols, use of sequential media, and blastocyst culture, the implantation process still remains elusive. One of the reasons for failure of implantation may be the paucity of embryotrophic factors that are essential for implantation and these factors may be absent in in vitro conditions, even when sequential media are used. We have used in 50 women undergoing ICSI, a co-culture system with expanded cumulus cells for embryonic growth and have added these cells to the embryos at the time of embryo transfer in order to provide a substrate for growth factors and to increase the adhesiveness of the embryos. A group of 50 women in whom the embryos were co-cultured but in whom the cumulus cells were not added at the time of embryo transfer, served as the control group. We observed better implantation and pregnancy rates in the first group as compared to the controls. This is the first report of improving implantation rates by adding expanded cumulus cells to the embryos at the time of embryo transfer.

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Correspondence :
Dr. Firuza R. Parikh
Department of Assisted Reproduction and Genetics.
Jaslok Hospital and Research Centre.
15, Dr. Deshmukh Marg, Mumbai 400 026, India.
Tel. 9122 56573275, 56573171 Email : parikh@bom3.vsnl.net.in

Material and Methods

Hundred women who were undergoing ICSI were selected for the study provided they met fixed criteria which included having at least three metaphase II oocytes for sperm injection or at least two embryos available for tansfer. Of the 100 women, 50 constituted the study group (Group A) and 50 constituted the control group (Group B). The two groups were matched for age and selection criteria. The indications for ICSI were similar in both the groups and included male factor infertility, pelvic factor infertility, premature ovarian failure, failed previous IUI, failed previous IVF, unexplained infertility, and polycystic ovarian disease (Table I). In the study group (Group A), cumulus co-culture was done and cumulus aided embryo transfer was also performed. In the control group (Group B), the embryos were cultured on expanded cumulus cells but were transferred without cumulus cells.

Ovarian stimulation, oocyte retrieval and ICSI

Luteal phase suppression by GnRHa was followed by controlled ovarian stimulation by HMG. At follicular maturity, 5000 to 10000IU hCG trigger was given,

Table I. Indications for ICSI

Parameters	Study Group (n=50)	Control Group (n=50)
Male factor infertility	21	22
Pelvic factor infertility	13	12
Premature ovarian failure (POF)	7	6
Previous failed IVF	1	1
Failed IUI and unexplained infertility	2	3
Polycystic ovarian disease (PCOD)	4	3
Advancing age	2	3

Table II. Results

Parameters	Study Group A	Control Group B
Number of cycles	50	50
Number of oocytes	284 (5.7 oocytes/cycle)	298 (5.9 oocytes/cycle)
Metaphase oocytes	235 (82.7;235/284)	230 (77.2%; 230/298)
Fertilization rate	74% (174/235)	74.3% (171/230)
Cleavage rate	100% (174/174)	100% (171/171)
Average number of embryos transfered	3.3	3.3
Pregnancy rate	50% (25/50)[a]	36% (18/50)[a]
Implantation rate	26.3% (44/167)[b]	15% (25/165)[b]
Abortion rate	20% (5/25)	22.2% (4/18)
Multiple gestaion	36% (9/25)[c]	22% (4/18)[c]

[a] p=0.16 [b] p=0.01 [c] p=0.3

followed by oocyte retrieval (Photograph 1). ICSI was performed using standard established protocols[1].

Cumulus Co-cultures

Fertilization check was carried out at 16 to 18 hours post ICSI. Forty-eight hours after ICSI the Day 2 embryos (two cell stage and above) were co-cultured on the patient's own cumulus cells, which had formed a monolayer.

Preparation of the expanded cumulus cells monolayer

After denudation of the oocytes with 80 IU hyaluronidase, the separated cumulus cells which tended to clump together were collected and washed in tissue culture medium, (Quinn's Advantage Cleavage Medium, Sage Biopharma, USA) diluted and then incubated at 37°C in 5% CO_2. After overnight incubation, expansion of these cells into fibroblast-like cells was seen (Photograph 2). The old medium was discarded and fresh

medium was added. At 36 hours, colonies were seen coalescing together. Some areas were plump whereas some were sparse. At this stage the cumulus cells were exposed to fresh medium and the Day 2 embryos were added. The next day the embryos were observed for further cleavage. Cumulus aided embro transfer was carried out on Day 3.

Embryo Transfer Procedure

In the study group 100 picolitres of cumulus cells were added to tissue culture medium containing 30 mg/ml of human serum albumin along with the embryos to be transferred (Photograph 3). The cleaving embryo and the expanded cumulus cells were loaded together in the embryo transfer catheter (Labotect, 320200; Germany) and embryo transfer was performed in the routine way. In the control group cumulus cells were not used, rest of the procedure remaining unchanged.

Results

In Group A (Study Group), the average age was 32.3 years and in Group B (Control Group), it was 32.0 years. In Group A, 284 oocytes were retrieved (5.7/cycle). Of these 235 (82.7%) were at Metaphase II stage. In Group B, 298 oocytes were retrieved (5.9/cycle). Of these 230 (77.2%) were at Metaphase II stage. Fertilization and cleavage rates in Group A were 74% and 100% and in Group B 74.3% and 100% respectively. Average number of embryos transferred were 3.3 in both the groups. The implantation rate of 26.3% in Group A was significantly higher than that of 15% in Group B (p=0.01). There was also a trend towards higher pregnancy rates, (50% in Group A as compared to 36% in the Group B, p = 0.16). Both the groups received the same luteal phase support in the form of 600 mg micronised progesterone vaginally daily alongwith 2000 IU of βhCG every 4th day. The abortion rate of 20% (5/25) in Group A was similar to 22.2% (4/18) in Group B. However although the incidence of multiple pregnancy was higher at 36% in Group A as compared to 22% in Group B, this difference was statistically not significant. (p=0.3) (Table II).

Discussion

To the best of our knowledge, this is the first report of the use of the combined technique of cumulus co-culture and cumulus aided embryo transfer, using autologous cumulus cells in patients being treated for infertility by ICSI.

Cumulus cells have been used as a feeder layer in a co-culture system[2]. These cumulus cells secrete embryotrophic factors such as insulin like growth factor I and II (IGF I and II)[3], epidermal growth factor (EGF)[4], leukemia inhibitory factor (LIF)[5], transforming growth factors (TGF α and β)[6], tumour necrosis factor (TNF α)[7], basic fibroblast growth factor (bFGF)[8] and interleukins (IL – 1 and IL – 6)[9]. A more recent report indicates that vascular endothelial growth factor (VEGF) is also secreted by cumulus cells[10]. These factors play an important role in enhancing uterine receptivity[11]. Blastocyst implantation takes place under the guidance of many of these factors[12,13].

In view of the above facts we postulate that the combined use of cumulus co-culture and cumulus aided transfer helps in regulating preimplantation embryonic development in vitro and play a role in aiding the implantation process in vivo. As shown in our study, the use of cumulus co-culture and cumulus aided transfer significantly increase the implantation rate. And although this results in higher pregnancy rate, the increase in pregnancy rate is not statistically significant.

We did observe a higher multiple gestation rate in our study group. This was not significant statistically. We are continuing to use this technique with great satisfaction. However, we now transfer less number of embryos in order to reduce multiple pregnancies.

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