

## DOES TRANSFER OF EMBRYOS AT THE BLASTOCYST STAGE INCREASE THE RISK OF MULTIPLE PREGNANCY?

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### Abstract

Prolonged *in vitro* cultivation allows us to obtain better quality embryos and provides better intrauterine conditions for transfer. The aim of this study was to compare the results of *in vitro* fertilisation (IVF) procedures in which 1, 2 or 3 blastocysts (group 1) were transferred with the results of IVF procedures in which 1, 2 or 3 embryos at the stage of 8 cells to morula, achieved under the identical cultivation conditions, were transferred (group 2). The study period was from September 1997 to September 1998.

Ovulation induction protocols including short or long GnRHa combined with gonadotrophins were used. Embryos were cultured in Medi-Cult IVF and M3 media. Maximally 3 embryos were transferred.

In group 1, from a total of 377 cultured embryos, 129 reached the blastocyst stage (34.2 %) and 88 transfers of embryos resulted in 29 clinical pregnancies (33.0 % pregnancy rate). In 5 cases (17.3 %) multiple pregnancy was detected.

In group 2, 274 embryo transfers were carried out and 56 pregnancies were achieved (20.4 % pregnancy rate), out of which 11 (19.6 %) were multiple pregnancies.

Transfer of embryos at the blastocyst stage improved the outcomes of the assisted reproduction method in our Department; it did not result in any increase in multiple pregnancies and no triplet pregnancy occurred.

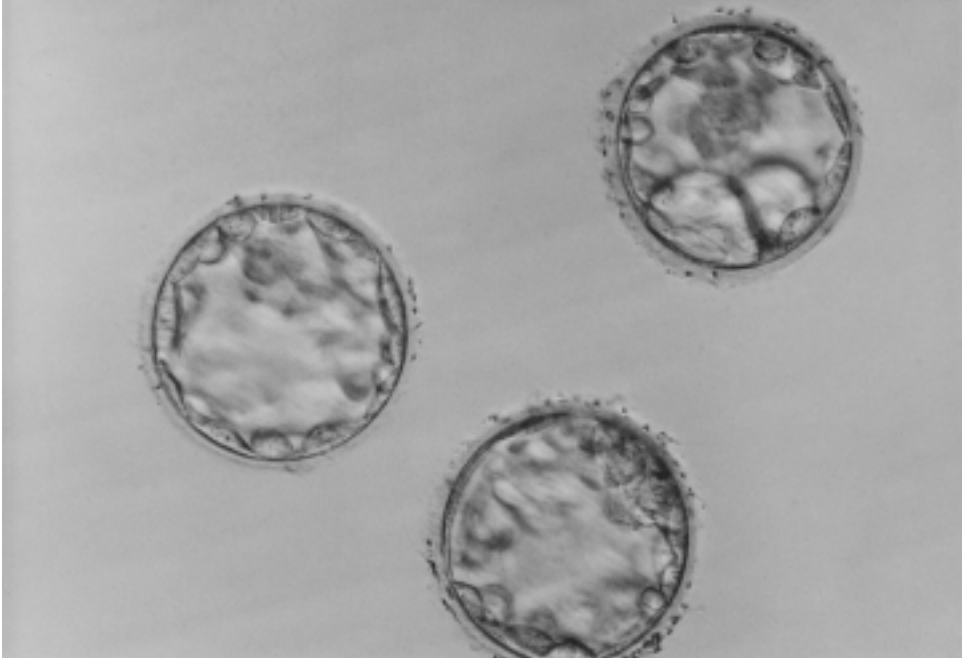
### Key words

embryo, transfer, blastocyst, multiple pregnancy

### INTRODUCTION

In a fertile woman, when an oocyte is fertilised, the resulting embryo undergoes cleavage to attain the compacted or cavitated stages (day 4 to 5) in the Fallopian tube before it descends into the uterus for further cleavage, dissolution of the zona pellucida and implantation (day 5 to 7).

In assisted reproduction, the transfer of human embryos at the blastocyst (BC) stage (*Fig. 1*) into the uterus is shown to have several advantages over the transfer of 2- or 3-day -old embryos. Firstly, there may be an improvement in success rates due to better synchronisation of the uterine endometrium and embryonic development. Secondly, it has been suggested that only “hardier” embryos achieve the blastocyst stage *in vitro* and, therefore, have an increased implantation



*Fig. 1*  
Blastocysts after 120 h of cultivation (M3 medium, Medi-Cult), light microscope.  
Magnification:  $\times 3000$ .

potential (1). This implies that embryos which may have some chromosomal or genetic abnormalities, though capable of early cleavage, fail to reach the blastocyst stage, which ensures self-selection (2).

In 1994, the first blastocyst stage embryos were prepared in our laboratory by their prolonged cultivation with human tubal epithelial cells (3, 4). Since the autumn 1995, the prolonged embryo cultivation has been carried out in synthetic media and the results have been reported (5,6).

The aim of this study was to compare the results of *in vitro* fertilisation (including multiple pregnancy rate) in relation to whether 1, 2 or 3 blastocysts or 1, 2 or 3 embryos without blastocyst stages were transferred.

#### MATERIALS AND METHODS

In the period between September 1997 and September 1998, embryo transfers were carried out in 362 women admitted to our Department during that period.

For ovulation induction, short or long GnRHa protocols in combination with gonadotrophins were used. Oocyte recovery was timed at 36 hours following an hCG injection and was carried out under ultrasound guidance.

Conventional IVF or intracytoplasmic sperm injection (ICSI) techniques were used for insemination. Freshly ejaculated semen was prepared by a swim-up procedure. Oocytes were cultivated,

inseminated and the resulting embryos were incubated in the IVF medium (Medi-Cult, Denmark) until they achieved the 4-cell stage. Subsequently, the cultivation was prolonged in the completely defined synthetic M3 medium (Medi-Cult, Denmark) till day 5 of embryo development. All procedures were carried out in a cultivation box (Heraeus) in the air atmosphere with 5% CO<sub>2</sub> at 37 °C.

The maximum of 3 embryos were transferred by a Trans Soft Embryo Replacement Catheter (COOK).

## RESULTS

In group 1, a total of 377 embryos underwent prolonged cultivation, which resulted in 129 embryos achieving the blastocyst stage (34.2%). These were used for 88 women and produced 29 pregnancies (33.0% pregnancy rate /PR/). Out of these, five (17.3 %) were multiple pregnancies (*Tables 1 and 2*).

*Table 1*

Comparison of clinical pregnancy and multiple pregnancy rates in transfers with embryos at (BC+) and before (BC-) the blastocyst stage

| 1 <sup>st</sup> Department of Obstetrics and Gynaecology in Brno | No. of embryos transferred at |             |
|--|-------------------------------|-------------|
|  | BC+ (1 - 3)                   | BC- (1 - 3) |
| Number of embryo transfers                                       | 88                            | 274         |
| Total number of pregnancies                                      | 29                            | 56          |
| Clinical pregnancy rate (%)                                      | 33.0                          | 20.4        |
| Number of multiple pregnancies                                   | 5                             | 11          |
| Multiple pregnancy rate (%)                                      | 17.3                          | 19.6        |

*Table 2*

Outcomes of transfers in relation to the number of blastocysts introduced

| 1 <sup>st</sup> Department of Obstetrics and Gynaecology in Brno | No. of blastocysts transferred |      |      |      |
|--|--------------------------------|------|------|------|
|  | 1                              | 2    | 3    | Σ    |
| Number of embryo transfers                                       | 56                             | 23   | 9    | 88   |
| Total number of pregnancies                                      | 16                             | 8    | 5    | 29   |
| Clinical pregnancy rate (%)                                      | 28.6                           | 34.8 | 55.6 | 33.0 |
| Number of multiple pregnancies                                   | 2                              | 2    | 1    | 5    |
| Multiple pregnancy rate (%)                                      | 12.5                           | 25.0 | 20.0 | 17.3 |

In 56 women who received one blastocyst, 16 clinical pregnancies were achieved (PR, 28.6 %). Twenty four transfers with 2 blastocysts resulted in eight pregnancies (PR, 34.8 %) and nine transfers with 3 blastocysts produced five pregnancies (PR, 55.6 %) (Table 2).

In group 2, a total of 274 women were fertilised with embryos before the blastocyst stage and 56 pregnancies resulted (PR, 20.4%); 11 of them were multiple pregnancies (19.6%). One-embryo transfers in 20 women resulted in two clinical pregnancies (PR, 10%), two-embryo transfers in 23 women produced six pregnancies (PR, 19.4%) and three-embryo transfers in 223 women resulted in 48 pregnancies (PR, 21.5%). (Tables 1 and 3).

Table 3  
Outcomes of transfers involving 1 to 3 embryos at the stage of 8-cells to morula

| 1 <sup>st</sup> Department of Obstetrics<br>Masaryk University, Brno, Czech Republic | No. of embryos transferred |      |      |      |
|--|----------------------------|------|------|------|
|  | 1                          | 2    | 3    | Σ    |
| Number of embryo transfers   | 20                         | 31   | 223  | 274  |
| Total number of pregnancies  | 2                          | 6    | 48   | 56   |
| Clinical pregnancy rate (%)  | 10.0                       | 19.4 | 21.5 | 20.4 |
| Number of multiple pregnancies   | 0                          | 1    | 10   | 11   |
| Multiple pregnancy rate (%)  | 0                          | 16.7 | 20.8 | 19.6 |

Transfer of embryos at the blastocyst stage resulted in a higher pregnancy rate and improved assisted reproduction results (33 % PR in group 1 vs. 20.4 % PR in group 2).

#### DISCUSSION

Success in production of most viable embryos for *in vitro* transfer lies in overcoming the block between day 2 and day 3 of the embryonic development and achieving the stage of blastocyst by prolonged cultivation. Some other advantages of this approach are: to allow genetically defective embryos to cease development, to enable the hardiest embryos to survive for longer periods *in vitro* and to introduce embryos into the uterine environment which is more physiological for the blastocyst stage than for the earlier cleavage stages.

The use of feeder cells such as human ampullary cells (7), monkey Vero cells (8), bovine uterine fibroblasts (9), etc., for this purpose is gradually abandoned. Recent studies have indicated that it is possible to obtain viable human blastocysts in defined culture systems in the absence of serum and somatic helper cells (10).

The development and use of sequential culture media, designed to cater for changing requirements of the embryo as it develops and differentiates, have been shown to produce blastocysts of high viability (11).

In our study, we did not find any difference in the number of multiple pregnancies between two groups of women, one of which received embryos at the blastocyst stage and the other at the early cleavage stage (17.3 % and 19.6%, respectively). We used transfers of up to three blastocysts in nine women and none had a triplet pregnancy.

The ultimate goal of IVF in humans is to optimise its outcome, particularly at the perinatal period. This may be achieved by insertion of a single, viable, good quality, 5-day embryo. Selection of such embryos for transfer may be through detailed morphological characteristics (inner cell mass size, cleavage speed and thin zona). At present, defined culture systems, in the absence of serum and somatic helper cells, seem to provide the best conditions for production of blastocysts with the desired characteristics.

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#### ZVYŠUJE TRANSFER EMBRYÍ VE STÁDIU BLASTOCYSTY RIZIKO VÍCEČETNÉHO TĚHOTENSTVÍ?

##### S o u h r n

Prodloužení kultivace in vitro nad 100 hodin dává možnost jednak získat embrya lepší kvality, jednak dosáhnout lépe připraveného děložního prostředí pro transfer. Cílem této práce bylo srovnat výsledky IVF po transferování 1, 2 nebo 3 blastocyst – skupina 1, s výsledky po transferu embryí (1–3) ve stádiu 8 buněk až moruly při stejných kultivačních podmínkách – skupina 2. Studované období zahrnovalo září 1997 - září 1998, kdy bylo provedeno 362 embryotransferů.

Ovulace byla u pacientek indukována podle krátkých a dlouhých stimulačních protokolů s GnRH analogy v kombinaci s gonadotropiny. Kultivace embryí probíhala v IVF médiu a M3 médiu (Medi-Cult). Transferována byla maximálně 3 embrya.

Ve skupině 1 z celkového počtu 377 kultivovaných embryí, dosáhlo 129 embryí stádia blastocysty (34,2 %). Z 88 embryotransferů s blastocystami vzniklo 29 klinických těhotenství (33,0 % těhotenský poměr). V pěti případech ze všech transferů s blastocystou bylo v 17,3 % detekováno vícečetné těhotenství.

Ve skupině 2 vzniklo z 274 transferů 56 těhotenství (20,4 %). Vícečetných těhotenství v této skupině bylo 11, což činí 19,6 %.

Transfer blastocyst zvýšil výsledky asistované reprodukce na našem pracovišti. Neprokázáli jsme vyšší výskyt vícečetného těhotenství po transferech s blastocystami, trojčetné těhotenství v této skupině nebylo zaznamenáno žádné.

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