

Original Article

# Selected single blastocyst transfers maintained pregnancy outcome and eliminated multiple pregnancies

TAKESHI KURAMOTO,<sup>1,2\*</sup> ARIEF BOEDIONO,<sup>2</sup> AKIYOSHI EGASHIRA,<sup>1,2</sup> MUTSURO MOTOISHI,<sup>1,2</sup> MICHIO SUGIOKA,<sup>1,2</sup> KIMIKO FUKUDA,<sup>1</sup> TOKI HIGASHIJIMA,<sup>1</sup> NAOMI YOSHIOKA<sup>1</sup> and RAJADURAI RAJAMAHENDRAN<sup>2</sup>

<sup>1</sup>Kuramoto Women's Clinic and <sup>2</sup>Fukuoka Reproductive Medicine Research Laboratory, Fukuoka, Japan

**Background:** Transfer of more than one embryo following *in vitro* fertilization/intracytoplasmic sperm injection cycles have increased pregnancy rate at the cost of increasing the incidence of triplets and twins. It has been proposed that prolonged culture to the blastocyst stage would automatically result in the selection of good quality embryos for transfer and minimize the incidence of triplets and twins.

**Methods and Results:** The objectives of the present retrospective analysis were to examine the pregnancy outcome, multiple pregnancy and related data following: (i) single blastocyst transfer (BT) and double BT; (ii) single BT in patients belonging to different age groups; and (iii) good, fair or poor quality of BT. A total of 260 BT were carried out between August 1998 and July 2002 and they are included in the current study. Sixty of the 260 BT patients received a single BT, and 41 of them

received selected single good quality BT (SSBT). The implantation rate has no significant difference between following single BT (53.3%) and double BT (42.8%). No multiple pregnancy occurred following single BT, while significantly higher ( $P < 0.05$ ) multiple pregnancy rate was observed following a double BT (45.8%). The clinical pregnancy and implantation rates following a single BT were similar ( $P > 0.05$ ) in patients belonging to <30 years (62.5%), 30–34 years (57.9%) and 35–39 years old (35.8%).

**Conclusion:** Selected single good quality BT maintained pregnancy and avoided multiple pregnancies. It is recommended for patients with a risk for high-order multiple pregnancy. (Reprod Med Biol 2004; 3: 13–18)

**Key words:** blastocyst transfer, cleavage transfer, multiple pregnancy, selected single blastocyst transfer.

## INTRODUCTION

TO OVERCOME LOW pregnancy and implantation rate from *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, fertility specialists have developed efficient ovarian stimulation protocols and laboratory techniques with the goal of obtaining multiple embryos for transfer. Pregnancy rate rose with transfer of multiple embryos; however, it also increased the incidence of multiple births, including twins, carrying extra risks of obstetric, and pre- and perinatal complications.<sup>1</sup> To minimize the risk of multiple pregnancy, many IVF clinics have reduced the number of embryos transferred from three to two embryos per transfer.<sup>2,3</sup> However, research reports show that transferring two or

three embryos yielded similar results, reducing only the incidence of triplets but not twins, resulting again in increased health-care cost.<sup>4–6</sup> In many clinics, more than three embryos are still transferred. This has led to the practice of embryo reduction in pregnancies with three or more fetuses. Embryo reduction still carries 10% risk of miscarriage even when carried out by experienced clinicians.<sup>7</sup> It is extremely mentally stressful for the couples and many regard it as ethically problematic. Embryo reduction can be completely avoided by transferring only one embryo per transfer.

Early reports showed a low pregnancy rate after a single embryo transfer.<sup>8,9</sup> These results originated from transfers with only one embryo available for transfer. Recently, the transfer of single selected day 2 or 3 embryos has been shown to result in acceptable pregnancy rate eliminating the risk of multiple pregnancy at birth.<sup>10,11</sup> Identification of day 2 or 3 embryos with a very high implantation potential is critical for single embryo transfer and morphological characteristics at

\*Correspondence: Takeshi Kuramoto MD PhD, Kuramoto Women's Clinic, 1-1-19 Hakataeki-higashi, Hakata-ku, Fukuoka 812-0013, Japan. Email: kwc@gol.com

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day 2 or 3 have been used to identify good quality embryos in approximately 75% of all IVF/ICSI cycles.<sup>12</sup>

It is proposed that culture of IVF/ICSI embryos to day 5, to the blastocyst stage, would automatically result in the selection of good quality single embryos for transfer and minimize the occurrence of multiple pregnancy. Although blastocyst culture has been attempted since 1990, the first blastocyst transfer (BT) was reported by Scholtes and Zeilmaker<sup>13</sup> and they concluded that the embryo transfer results after day 3 and 5 embryos were comparable. They also proposed that the replacement of one or two day 5 embryos would minimize the incidence of triplets. Recent reports on blastocyst stage transfer have shown that culturing embryos to the blastocyst stage would allow the reduction of embryos transferred from three to two.<sup>14–16</sup> Although reports on blastocyst stage transfer show better results than cleavage stage transfer, the pregnancy outcome and related data following single BT is not available.

Since 1998, we have offered the BT option to limit the number of embryos transferred for patients at risk for high-order multiple pregnancy. However, two BT have occurred where the risk twin pregnancy depends on the age of patients and/or the blastocyst qualities. The objectives of the present retrospective analysis were to examine the pregnancy outcome, the incidence of multiple pregnancy, and related data following: (i) single or double BT; (ii) single BT in patients belonging to different age groups; and (iii) good, fair or poor quality BT.

## MATERIALS AND METHODS

### Study group/population

FOUR HUNDRED AND sixty consecutive embryo transfers were carried out between August 1998 and July 2002 and are included in the present study. Embryos were obtained from patients <40 years using the conventional IVF and ICSI procedures. A total of 260 BT were carried out on day 5 after oocyte retrieval. Sixty of the 260 BT patients received single BT and 41 of them received a selected single good quality BT (SSBT).

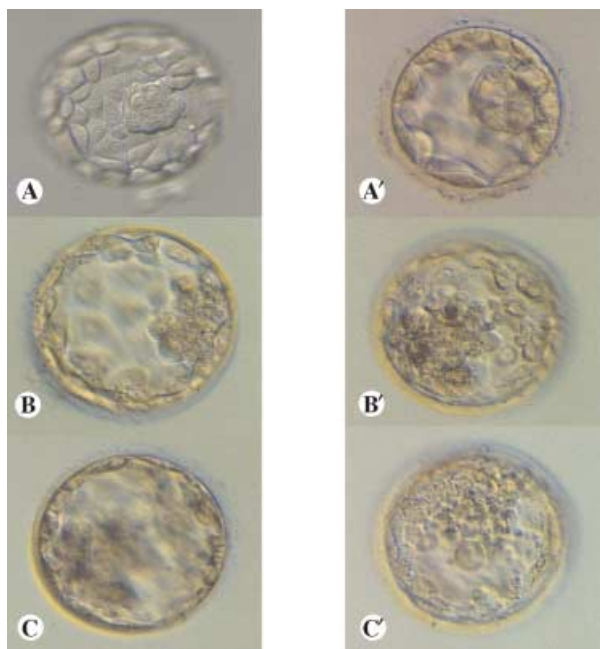
### Ovary stimulation, embryo production and transfer

The adopted ovarian stimulation, oocyte collection, IVF and ICSI procedures and embryo transfer method have been reported earlier.<sup>17</sup> Briefly, patients were down-regulated with gonadotropin-releasing hormone (GnRH) analog (Sprecur; Aventis Pharma, Tokyo, Japan)

beginning in the mid-luteal phase and ovarian stimulation was performed by administering pure follicle-stimulating hormone (FSH) (Fertinorm P; Serono, Tokyo, Japan) and human menopausal gonadotropin (hMG) (Humegon; Organon, Netherlands, Holland). Follicular growth was monitored by transvaginal sonography (TVS) and serum estradiol levels. When the leading follicle reached 17–18 mm in diameter and the endometrial thickness was over 8 mm, 5000–10 000 IU of human chorionic gonadotropin (hCG) (Profasi; Serono, Tokyo, Japan) was administered. Oocytes were harvested with an 18-gauge single lumen needle (Kitazato, Shizuoka, Japan) guided by TVS, 35–36 h after hCG administration. Oocytes were inseminated using IVF or ICSI procedure, 6–8 h after retrieval. Following assessment of fertilization, the zygotes were cultured in 20  $\mu$ L droplets of K-SICM medium (Cook, Brisbane, Australia) at 37°C in an incubator (Astec, Fukuoka, Japan) containing 5% CO<sub>2</sub>, 5% O<sub>2</sub> and 90% N<sub>2</sub> for 72 h. In order to obtain blastocysts; cleavage stage embryos were subsequently placed in 20  $\mu$ L droplets of K-SIBM medium (Cook) and cultured for additional 48 h. Blastocysts were graded into three categories on day 5; according to the morphology of the inner cell mass (ICM), the trophoblast, and the degree of fragmentation (Fig. 1). Embryo transfer was performed on day 5 after oocyte retrieval. Micronized vaginal progesterone (400 mg/day) was used from day 2 to day 20 after oocyte retrieval for luteal support. Urine pregnancy test was performed 14 days after embryo transfer.

### Data collection and analysis

Clinical pregnancy was identified by the presence of a gestational sac on TVS performed 7 weeks after transfer and the clinical pregnancy rate was calculated as the number of patients diagnosed as pregnant, divided by the number of transfers performed. The implantation rate was calculated as the number of a gestational sac, divided by the number of embryos transferred. The miscarriage and multiple pregnancy rates were determined by the incidence of miscarriage or multiple pregnancy, divided by the number of clinical pregnancies. Clinical pregnancy, implantation, miscarriage and multiple pregnancy rates were compared following: (i) single and double BT; (ii) single BT in patients belonging to different age groups (<30, 30–34, and 35–39 years); and (iii) grade A-A' (good), B-B' (fair), or C-C' (poor) BT. Statistical analysis was performed using the  $\chi^2$  test. A *P*-value of <0.05 was considered statistically significant.



**Figure 1** Blastocyst grading according to the morphology of the inner cell mass (ICM), the trophoctoderm, and the degree of fragmentation. Grade A–A' (good): blastocyst with ICM containing many tightly packed cells and trophoctoderm containing many cells forming a cohesive epithelium with no or few fragmented cells (<10%). Grade B–B' (fair): blastocyst with ICM containing several cells loosely grouped together and trophoctoderm containing few cells forming a loose epithelium with 10–30% fragmented cells. Grade C–C' (poor): blastocyst with ICM containing few cells loosely grouped together and trophoctoderm containing few but large cells forming a loose epithelium with >30% fragmented cell.

## RESULTS

**I**N THE CURRENT study, the age, the number of retrieved oocytes and the zygotes were not statistically different between IVF patients and ICSI patients (Table 1). The clinical pregnancy and implantation rates of IVF patients had a high tendency than those of ICSI patients, but there were not significantly different between those two groups (Table 1). In addition, the rate of ICSI patients was not statistically different between the single BT group and the double BT group (Table 2).

The clinical pregnancy was also not statistically different ( $P > 0.05$ ) following single (53.3%) and double (59.0%) BT (Table 2). The implantation rate was also not statistically different ( $P > 0.05$ ) following single (53.3%) and double (42.8%) BT (Table 2). No multiple pregnancy occurred following single BT, while significantly

higher ( $P < 0.05$ ) multiple pregnancy rate was observed following double BT (45.8%).

The clinical pregnancy, implantation and multiple pregnancy rates following single BT in patients with different age groups are shown in Table 3. The clinical pregnancy and implantation rates were similar ( $P > 0.05$ ) in patients belonging to the <30, 30–34 and 35–39 age groups. It is important to note that no multiple pregnancy was observed following single BT in patients belonging to the different age groups. Transfer of good (SSBT), and fair quality blastocysts resulted in similar ( $P > 0.05$ ) pregnancy and implantation rates (Table 4).

## DISCUSSION

**E**XTENDED CULTURE OF embryos to day 5 or blastocyst stage is being adopted in many IVF clinics as a method to prevent multiple pregnancy in young patients. This is based on observations that a high proportion of defective embryos cease to develop beyond four- or eight-cell stage and the best quality embryos with high implantation potential reach blastocyst stage and are available for transfer. However, from a randomized study standpoint blastocyst stage transfer is not necessarily to obtain higher pregnancy than that of cleavage stage transfer. However, it is the fact that the patients transferred two blastocysts increase the risk of twins considerably. Therefore, we evaluated whether single BT reduces or eliminate the incidence of multiple pregnancy without lowering the pregnancy rate.

In the current study, a high implantation rate was observed following single BT (53.3%) and no multiple pregnancy occurred following single BT. Recent reports by Strandell *et al.*<sup>10</sup> and Vilska *et al.*<sup>11</sup> have shown an acceptable pregnancy rate (approximately 29%) and elimination of multiple pregnancy following single day 2 or 3 embryo transfers. However, Gardner *et al.* reported a similar pregnancy rate after day 2 or 3 and a day 5 transfer, but the implantation rate from blastocyst stage transfer was significantly higher than the rate of cleavage stage transfer.<sup>16</sup> In addition, Cruz *et al.* also reported that higher pregnancy rate of 40 and 9.1% following day 5 compared to day 2 or 3 transfers, respectively, in patients with three or more unsuccessful cycles.<sup>14</sup> Regarding implantation rate after embryo transfer, the implantation viability of blastocyst might be considered higher than that of cleavage embryo. In fact, high implantation rate was achieved after single BT in our clinic. The higher pregnancy rate observed in the current study over day 2 or 3 single embryo

|  | IVF            | ICSI           |
|--|----------------|----------------|
| Number of patients                               | 144            | 116            |
| Mean age of patients (mean $\pm$ SEM)            | 33.3 $\pm$ 0.3 | 33.7 $\pm$ 0.3 |
| Age range (years)                                | 26–39          | 24–39          |
| Number of oocytes per retrieval (mean $\pm$ SEM) | 8.5 $\pm$ 0.3  | 8.8 $\pm$ 0.4  |
| Number of pronuclear embryos (mean $\pm$ SEM)    | 6.3 $\pm$ 0.3  | 5.4 $\pm$ 0.3  |
| Number of embryos transferred (mean $\pm$ SEM)   | 1.8 $\pm$ 0.04 | 1.8 $\pm$ 0.04 |
| Clinical pregnancy rate (%)                      | 60.4           | 54.3           |
| Implantation rate (%)                            | 48.0           | 39.4           |

**Table 1** Comparison of pregnancy outcome following *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI)

There were not significantly different between IVF and ICSI. ( $P > 0.05$ ). SEM, standard error of the mean.

**Table 2** Effects of the number of transferred blastocyst on pregnancy outcome

| Number of embryos | Number of transferred cycles | Number of ICSI patients | Clinical pregnancy (%) | Implantation (%) | Miscarriage (%) | Multiple pregnancy (%) |
|-------------------|------------------------------|-------------------------|------------------------|------------------|-----------------|------------------------|
| 1                 | 60                           | 24                      | 32 (53.3)              | 32 (53.3)        | 9 (28.1)        | 0 (0.0)*               |
| 2                 | 200                          | 92                      | 118 (59.0)             | 171 (42.8)       | 14 (11.9)       | 54 (45.8)*             |

\* $P < 0.05$ . ICSI, intracytoplasmic sperm injection.

**Table 3** Effects of the patient age on pregnancy outcome

| Patient age (years) | Number of transfers | Clinical pregnancy (%) | Implantation (%) | Miscarriage (%) | Multiple pregnancy (%) |
|---------------------|---------------------|------------------------|------------------|-----------------|------------------------|
| <30                 | 8                   | 5 (62.5)               | 5 (62.5)         | 2 (40.0)        | 0 (0.0)                |
| 30–34               | 38                  | 22 (57.9)              | 22 (57.9)        | 6 (42.9)        | 0 (0.0)                |
| 35–39               | 14                  | 5 (35.8)               | 5 (35.8)         | 1 (20.0)        | 0 (0.0)                |

There are no different rates among of three age groups in the same column ( $P > 0.05$ ).

**Table 4** Effect of the blastocyst quality and the number of blastocysts transferred on pregnancy outcome

| Embryo quality | Number of embryos | Number of transfers | Clinical pregnancy (%) | Number of embryos transferred | Implantation (%) | Miscarriage (%) | Multiple pregnancy (%) |
|----------------|-------------------|---------------------|------------------------|-------------------------------|------------------|-----------------|------------------------|
| Good           | 1                 | 41                  | 22 (53.7)              | 41                            | 22 (53.7)        | 3 (13.6)        | 0 (0.0)*               |
|                | 2                 | 35                  | 24 (68.9)              | 70                            | 40 (57.1)        | 1 (4.2)         | 15 (62.5)*             |
| Fair           | 1                 | 17                  | 9 (52.9)               | 17                            | 9 (52.9)         | 6 (66.7)        | 0 (0.0)**              |
|                | 2                 | 70                  | 41 (58.6)              | 140                           | 63 (45.0)        | 6 (14.6)        | 21 (51.2)**            |
| Poor           | 1                 | 2                   | 1 (50.0)               | 2                             | 1 (50.0)         | 0 (0.0)         | 0 (0.0)                |
|                | 2                 | 14                  | 7 (50.0)               | 28                            | 8 (28.6)         | 0 (0.0)         | 1 (14.3)               |

\*\*\* $P < 0.05$ .

transfers could be attributable to arrest in development of defective early stage embryos during long-term culture and the availability of embryos with high implantation potential.

It is well known that the outcome in assisted reproductive technology correlates closely to the patient age. In the present study the implantation rate of the young generation (under 34 years) was higher than the older



generation (over 35 years) in a single BT. Therefore, a single BT application should be considered for the patients. Many reports have also shown that the quality of the embryo is probably the most important factor, which predicts the outcome of transfers. Different procedures have been described to identify good quality pro-nuclear (PN) stage, cleavage stage, and the blastocyst stage embryos.<sup>9,18,19</sup> At the 2 PN stage, the polarity characteristics of the nuclei have been shown to correlate with a high implantation rate. The morphological characteristics of early stage embryos have been used to identify good quality embryos. In the present study, we graded the blastocysts into three categories (good, fair and poor) based on the development of the ICM, the trophoctoderm, and the degree of fragmentation. This finding is in agreement with previous reports using day 2 or 3 embryos.<sup>9,11,18,20</sup> Vilska *et al.*<sup>11</sup> reported a pregnancy rate of 34.0% with top grade day 2 or 3 single embryo transfers, in contrast to our pregnancy rate with good and fair quality single BT were 53.7 and 52.9%, respectively. This difference could be definitely attributable to selection of viable embryos during long-term culture and well after embryonic gene expression is turned on. Human gene expression has been shown to occur between the four- and eight-cell stages of embryo development.<sup>21</sup> It has been reported that the quality of early stage embryos can substantially influence blastocyst formation rate.<sup>22,23</sup> In our clinic, the development of embryos are monitored daily and it is our observation that good quality blastocysts always come from early cleavage stage embryos that develop progressively with minimal fragmentation.

Extended *in vitro* culture increases the degree of embryo selection, maximizing the choice of the best quality embryos for transfer and minimizing the need for transfer of greater numbers embryos. The extended culture to day 5 allowing the embryos to 'select' themselves by growth to the blastocyst stage. In the current retrospective study, we have shown for the first time that SSBT resulted in higher pregnancy rate (53.7%) and avoided multiple pregnancy. By this strategy, IVF/ICSI success could be optimized through increased pregnancy rate and would totally eliminate the incidence of twin pregnancies. The advantage of this option is that when multiple good blastocysts are present on day 5, one can transfer only the best quality of the blastocyst and freeze the remaining embryos. This avoids the risk of high-order multiple pregnancy; however, affords the patient an opportunity to undergo additional embryo transfer, with possibly a better technique or better uterine environment. To date, we have achieved a 57%

viable pregnancy rate with 44-embryo transfer of thawed blastocysts (data not presented).

It is clear from published reports that the incidences of multiple pregnancy including twins are high following IVF/ICSI cycles among young patients (<35 years). In the present study the clinical pregnancy and implantation rates were similar in patients belonging to <30 and 30–34 years, with no multiple pregnancy observed following single BT. Based on the results of the current study, SSBT is strongly recommended for patients at risk for high-order multiple pregnancy.

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