Running Title: Single embryo transfer

NUMBER OF EMBRYOS TRANSFERRED AND IMPLANTATION

Pierluigi Giannini[a], Claudio Piscitelli[a], Annalise Giallonardo[a], Marco Sbracia[b], Francesco Morgia[a], Monica Torti[a], Monica Montigiani[a], Mauro. Schimberni[a]

[a]Bioroma Centro di Riproduzione Assistita Casa di Cura "Paideia", Rome, Italy
[b]Center for endocrinology and Reproductive Medicine, Rome, Italy

Corresponding Author: Marco Sbracia,
Via Carlo Porta 10, 00153 Rome, Italy
Phone: +39-065812996;
Fax: +39-065880096;
E-mail: marcandrea@hotmail.com

Key words: embryo transfer, blastocyst, embryo cleavage stage, multiple pregnancy

Abstract

Multiple pregnancies are considered the most frequent and serious complication of assisted reproduction technology. In order to reduce the frequency of multiple pregnancies several centers have adopted the policy to reduce the number of embryos transferred in uterus, suggesting the single embryo transfer. Eventhough a relevant number of papers have been published on this issue no a general consensus exists about how many embryos replace in uterus and at which cleavage stage. We conducted a retrospective study on cycles performed along all the 2003 analyzing the relationship between number of embryo transferred and pregnancy and implantation rate, evaluating also the role of women age. No differences were found among the groups except for one embryo transferred women, which were mostly natural cycles, for estradiol levels, number of mature oocytes retrieved, number of top quality embryos and pregnancy rate. The implantation rate was significantly higher in the two embryos transfer versus three embryos transfer. We found higher pregnancy rate and implantation rate with similar multiple pregnancy rate in patients where only two embryos were transferred versus three embryos transfer when women were less than 35 years old. In women aged less than 35 years, which in turn have the higher expectancy of successful pregnancy and also the higher risk of multiple pregnancy, the single embryo transfer is a suitable choice for these patients.

Introduction

The outcome in IVF has been improved through the years, with the improvement of culture media, the improvement in identifying o of viable embryos, and the improvement of transfer procedure, other than selecting younger women undergoing IVF procedure. Recently it has been introduced the concept of birth emphasizing a successful singleton at term (BESST) in order to highlight the prevention of multiple pregnancies in IVF. Multiple pregnancies are considered the most frequent

and serious complication of assisted reproduction technology, as also showed from last SART registry release where the rate of multiple births is of 35.4 in all population and up to 38.6 in women younger than 35 years old[1]. Multiple pregnancies and multiple births are associated with increased maternal and baby morbidity and mortality. In the mother there is an increased risk of hypertension, polyhydramnios, premature labor, postpartum bleeding. In the babies there is an increased risk of pre-term delivery, low birth weight, higher perinatal mortality, congenital anomalies, cerebral palsy, intracranial hemorrhage and blindness. The frequency of multiple pregnancies is correlated with the number of high quality embryos transferred[2,3]. In order to reduce the frequency of multiple pregnancies several centers have adopted the policy to reduce the number of embryos transferred in uterus, suggesting the single embryo transfer. The strategy of a single embryo transfer clearly allows to avoid triplets pregnancies and reach the frequency of twin pregnancies close to 1%[4]. However the policy to reduce the number of embryos transferred has to be balanced with the needs for a high success rate. The reduction of embryos transferred in uterus should be performed in patients with higher chance of pregnancy and using embryos with higher chance of implantation, as showed by Martin et al[5].

The factors influencing embryo implantation are maternal age and the embryo quality as showed by several authors. It is well known that with the increase of maternal age the implantation rate decreases with also the chance of pregnancy[6].

To increase the implantation rate it has been suggested to select healthy embryos chosen on morphological characteristics or maintaining the in vitro culture through the blastocyst stage, in order to select embryos with higher chance to implant. Several reasons suggest to transfer blastocyst, such as other reasons suggest to transfer day 2/3 cleavage stage embryos. Blastocyst transfer allows to select embryos prolonging in vitro culture with the activation of embryo genoma. On the other side embryos transferred at day 2/3 cleavage allow to reduce the number of cancelled cycles, and to freeze more embryos.

Different studies have been conducted to evaluate if blastocyst transfer allows higher implantation rate and pregnancy rate with lower multiple pregnancies, with inconclusive results.

In a study conducted by Huisman et al[7] it has been shown that no significant differences in term of implantation rate were observed between 3 days or 5 days embryo transfer, except in case of top quality blastocyst (25.7%) versus top quality day 3 embryos (17.9%). Furthermore, in a recent meta-analysis conducted by Blake et al[8], no differences were found between blasocyst and day 2/3 cleavage stage embryo transfer in term of clinical pregnancy rate and multiple pregnacy rate, whereas day 2/3 cleavage stage embryo transfer performed significantly better than blastocyst transfer in term of embryo freezing and cancellation rate. However, Gardner et al[9] reported a high pregnancy rate with single blastocyst transfer (60.9%) with no twin pregnancy. On the other hand a high pregnancy rate (35.1%) and no multiple pregnancies has been reported by Gerris et al[4] with single top quality day 3 embryo transfer, data comparable with double embryo or three or more embryo transfer for pregnancy rate (36.2% and 25.2% respectively) but with higher multiple pregnancy rate (35.3% and 35.2% respectively).

Eventhough a relevant number of papers have been published on this issue no general consensus exists about how many embryos replace in uterus and at which cleavage stage.

We conducted a retrospective study on cycles performed along all the 2003 analyzing the relationship between number of embryo transferred, pregnancy and implantation rate, evaluating also the role of women age.

Material and Methods

A retrospective survey was conducted on all IVF cycles performed to Bioroma , from January 2003 trough December 2003. The data were extracted from our database sheets.

All patients undergoing IVF and participating in the study gave their informed consent. All patients underwent a standard infertility evaluation, and none of the patients eligible for the study showed FSH >10 IU/ml and Estradiol (E_2)>60ng/ml on cycle day 3.

A long protocol was used in all patients in which GnRHa was given as a pre-treatment and pFSH administration took place when pituitary desensitisation was established. At all patients Buserelin (Suprefact Hoecst, Milano, Italy) subcutaneously was administered, 0.4mg daily, on day 22 to 24 of their previous cycle until the HCG administration. Ovarian suppression was assessed by daily hormonal profiles of E_2, and ultrasound scan (US) of the ovaries every 3rd day. Suppression was confirmed when E_2, reached the level of <30pg/ml, and follicles with a dimension <15mm mean diameter were visible on US examination. When suppression was confirmed by estradiol and ultrasound examinations, FSH was commenced at 300 IU of pFSH, on the second day of the menstrual cycle.

From the 7th day of stimulation daily monitoring of follicle size by ultrasound was performed, and plasma levels of E_2, was measured. From this stage, the dose of FSH was adjusted, depending on the individual response of each patient. The criteria used for triggering ovulation with 10.000 IU of i.m. hCG (Profasi HP 5000, Serono, Italy) were: plasma E_2 between 1000 and 4500 pg/ml, at least four follicles > 16 mm diameter.

Oocyte retrieval was performed under ultrasound control by the transvaginal route on day 0, 36 h after the injection of hCG. Either local or general anaesthesia was used. ICSI was performed in all cases according to published procedures[10]. Oocytes were observed 18 hours after ICSI for their pronuclei and 44 hours after insemination for embryo development.

The embryos obtained were categorised on day 2 or 3 into three categories, depending on their morphological appearance. Grade A had equal and regular blastomeres without the presence of cytoplasm fragments respectively; Grade B had unequal blastomeres with or without cytoplasmatic fragments; Grade C were totally fragmented embryos which were not transferred[11].

Embryos were transferred 48-72h h after insemination using the Wallace embryo transfer catheter (H. G. Wallace Ltd, UK). All transfer procedures were performed by the same physician to avoid inter-operator variability. All pregnancies were confirmed by a rising titre of serum β-hCG from 12 days after embryo transfer and ultrasound demonstration of the gestation sac 4 weeks after the transfer. Biochemical pregnancies alone have not been included.

The same luteal phase support was used in both groups, 50mg daily of progesterone (Prontogest, AMSA, Italy) intramuscularly from the day of replacement.

Statistical Analysis

Mann Whitney U test, Students t'test, χ^2 and Fisher exact test were used when appropriate to evaluate the differences of the variables in the patient groups for number of embryos transferred (1, 2 , 3 and 4 embryos) and in three women's age subgroups (<35 years, >35-38< years, >39 years): clinical pregnancy rate for transfer were the primary outcomes, whereas secondary outcomes were E_2 at the day of hCG, number of oocytes collected, number of top quality embryos obtained, implantation rate and multiple pregnancy rate. All statistical analyses were performed using the SPSS statistical package.

Results

A total of 413 consecutive cycles were included in the study. In 79 cases only one embryo was transferred (19.1%), in 103 cases two embryos were transferred (24.9%), in 215 case three embryos were transferred (52.1%), and in 16 cases four embryos were transferred (3.9%). No differences were found among the groups except for one embryo transferred group, which were mostly natural cycles, for estradiol levels, number of mature oocytes retrieved, number of top quality embryos and pregnancy rate. The implantation rate was significantly higher in the two embryos transfer versus three embryos transfer ($P<0.01$) Data are reported in Table I.

When the patients were subdivided for women age groups (<35 years, ≥35-39≤, ≥40 years) we

found higher pregnancy rate and implantation rate with similar multiple pregnancy rate in patients where only two embryos were transferred versus three embryos transfer, when women were less than 35 years old ($P<0.05$). In women 35 years old or more no differences were observed for all parameters between two or three embryos transferred groups. The data are reported in Table II.

Discussion

Our data, even though they are retrospective, show that two embryos transfer is effective as three and more embryos transfer in term of pregnancy rate, with no differences also for multiple pregnancy rate. When we evaluated the role of women age on IVF outcome for number embryos transferred, the data evidenced that in women younger than 35 years old two embryos transfer, most of which were of top quality, performed better than three embryos transfer, with a significant differences in implantation rate. The multiple pregnancy rate was the same in both groups showing that in order to achieve a significant reduction of multiple pregnancy, a further decrease in the number of embryos transferred is needed, at least in younger women. In women aged less than 35, which in turn have the higher expectancy of successful pregnancy and the higher risk of multiple pregnancy, the single embryo transfer is a suitable choice.

On the other hand, in women older than 34 years our data showed that there is a better pregnancy rate when more than two embryos were transferred with similar rate of multiple pregnancies. In older women the reduction of embryos transferred significantly decreases the chance of pregnancy, and the policy of single embryo transfer would be further detrimental. In these patients the choice of the number of embryo to transfer should be evaluated case by case.

References

1) Society for Assisted Reproductive Technology; American Society for Reproductive Medicine

2004. Assisted reproductive technology in the United States: 2000 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril.; 81:1207-20.

2) Roseboom TJ, Vermeiden JP, Schoute E, et al. 1995 The probability of pregnancy after embryo transfer is affected by the age of the patient, cause of infertility, number of embryos transferred and the average morphology score, as revealed by multiple logistic regression analysis. Hum Reprod.; 10: 3035-41.

3) Yaron Y, Amit A, Kogosowski A, et al. 1997 The optimal number of embryos to be transferred in shared oocyte donation: walking the thin line between low pregnancy rates and multiple pregnancies. Hum Reprod. 12: 699-702.

4) Gerris J, De Neubourg D, Mangelschots K, et al. 2002 Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod.; 17: 2626-31.

5) Martin PM, Welch HG. 1998 Probabilities for singleton and multiple pregnancies after in vitro fertilization. Fertil Steril.;70: 478-81.

6) Schieve LA, Peterson HB, Meikle SF, et al. 1999 Live-birth rates and multiple-birth risk using in vitro fertilization. JAMA.; 282: 1832-8.

7) Huisman GJ, Fauser BC, Eijkemans MJ, et al. 2000 Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. Fertil Steril; 73:117-22.

8) Blake DA, Proctor M, Johnson NP. 2004 The merits of blastocyst versus cleavage stage embryo transfer: a Cochrane review. Hum Reprod.; 19: 795-807.

9) Gardner DK, Surrey E, Minjarez D, et al. 2004 Single blastocyst transfer: a prospective randomized trial. Fertil Steril.; 81:551-5.

10) Palermo G, Joris H, Devroey P, et al. 1992 Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340, 17:8.

11) Veeck LL, An Atlas of human gametes and conceptus. 1999 London, UK: Parthenon Publishing,

Table I. IVF results related to number of embryo transferred.

	1 embryo	2 embryos	3 embryos	4 embryos
N of cases	79	103	215	16
E_2 levels HCG day	-	2025±398	2279±512	2460±471
N M2 oocytes	1.5+0.4	8.2±2.4	8.9±3.1	9.2±3.0
% Top embryo	18.9	37.9	39.5	32.2
Pregnancy rate	11.4	44.6	41.9	43.7
Implantation rate	11.4	28.2*	15.8	14.0

*P<0.01

Table II. Age related results in 2 or 3 embryos transfer.

	2 embryos	3 embryos
<35 years:		
n cases	51	82
pregnancy rate	58.8*	45.1
implantation rate	34.3*	18.3
multiple pregnancy rate	20.0	21.6
≥35 39≤years:		
n cases	38	100
pregnancy rate	34.2	42.0
implantation rate	23.7	16.6
multiple pregnancy rate	15.4	16.7
≥40 years:		
n cases	14	33
pregnancy rate	21.4	33.3
implantation rate	17.8	15.1
multiple pregnancy rate	0	9.1

*P<0.01