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Pregnancy outcome after blastocyst transfer as compared to early cleavage stage embryo transfer

Peter Schwärzler[1,4], Herbert Zech[2], Margherita Auer[1], Karin Pfau[1], Georg Göbel[3], Pierre Vanderzwalmen[2] and Nicolas Zech[2]

[1]Department of Obstetrics and Gynecology, University of Innsbruck, Innsbruck, [2]Institute for Reproductive Medicine and Endocrinology, Bregenz and [3]Institute for Biostatistics and Documentation, University of Innsbruck, Innsbruck, Austria

[4]To whom corresondence should be addressed at: Department of Obstetrics and Gynecology, University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria. Tel: +43 512 504 3051; Fax: +43 512 504 3055; E-mail: peter.schwarzler@uibk.ac.at

BACKGROUND: Retrospective cohort study to evaluate differences in outcome when embryo transfer was performed either on day 2–3 (cleavage stage, CS-group) or on day 4–5 (blastocyst stage, BS-group). METHODS: A total of 1259 consecutive cycles yielding 500 live born babies performed at a single centre in Bregenz, Austria, were included. Main outcome measures were implantation and (multiple) pregnancy rates and neonatal outcome including birth defects. RESULTS: Total Pregnancy rate was 44% vs 28% ($P < 0.001$) and the total 'take home baby rate' was 37% vs 22% in the BS-group and the CS-group, respectively. Rate of multiple gestations (34% vs 17%, $P = 0.001$) was significantly higher among the BS-group, resulting in a higher rate of preterm deliveries <36 weeks (26% vs 17%, $P = 0.045$). Female factor causing infertility (40% vs 21%, $P < 0.001$) was significantly higher among the BS-group. For the CS-group, rate of singleton pregnancies (83% vs 66%, $P = 0.001$) and idiopathic cause of infertility (34% vs 22%, $P = 0.012$) were significantly higher. No statistically significant differences were found in sex, Caesarean section rate, Apgar score and umbilical artery pH-values, total mean birth weight, admission rate to intensive care unit, days of hospitalization and number of minor and major birth defects. CONCLUSIONS: Our data suggest that blastocyst transfer may lead to a higher pregnancy rate with an overall better take-home baby rate (THBR) at the cost of higher rates of multiples and preterm deliveries.

Key words: ART/birth defect/blastocyst transfer/early cleavage stage/pregnancy outcome

Introduction

Development of sequential tissue culture media that attempt to give the growing embryo its metabolic and chemical needs has resulted in an extension of the embryo culture period to the blastocyst stage. Reportedly this has led to significantly higher implantation rate per embryo transferred (Gardner *et al.*, 1998). The safety of prolonged culture in human has not been strongly ascertained (McEvoy *et al.*, 2000; Sinclair *et al.*, 2000). Several reports from ruminant studies described unusually large offspring, some with congenital abnormalities, following transfer of embryos cultured *in vitro*, although such a phenomenon has not been reported in human to date (Lonergan *et al.*, 2003).

Although the human genome is activated between the 4 and 8 cell stages (Braude *et al.*, 1988) there is only a minimal level of transcription during the initial stages of zygote formation and early cleavage divisions. Therefore, the mature oocyte must contain a storage pool of proteins and/or mRNA transcripts already at the time of ovulation. The cleavage cycle during which the zygote genome is activated (4 to 8 cells in humans) is the longest among pre-implantation development. Any developmental delay at this time will result in a decrease in the level of mRNA below a critical threshold. Correct timing of blastocyst formation is an initial indication of embryo quality as it shows that the embryos have passed the narrow gap of maternal to zygotic transition.

It was demonstrated that uterine contractility decreases at the time of blastocyst transfer when compared to early stage transfers on day 2 (Fanchin *et al.*, 2001). The rhythm of contractions is reduced by half to 1.5 contractions per minute.

Furthermore, the endometrium on day 5 post-conception seems to be optimal for embryos to implant and mirrors the implantation time in natural conceptions (Gardner and Lane, 2003).

However, whilst many IVF (*in vitro* fertilization) clinics report their results in terms of pregnancy per oocyte pick-up or embryo transfer, patients frequently demand to know the 'take-home baby rate' (THBR), which is defined as babies per implanted embryos—and they want to evaluate the risks they and their children might face when they choose to use ART (Artificial Reproductive Techniques).

The purpose of this retrospective study was to evaluate differences in fertility and pregnancy rates and neonatal outcome when embryo transfer was performed either on day 2–3 (cleavage stage, CS-group) or on day 4–5 (blastocyst stage, BS-group).

Materials and methods

Study population

In this retrospective cohort study, a total of 1259 cycles of ART performed at a single centre from December 1999 to April 2001 have been evaluated. Embryo culture to the fifth day was proposed to patients with a larger amount of follicles and one previous failure of implantation. Of those patients, who were successfully treated ($n = 385$) a total of 264 patients accepted embryo transfers (ET) on day 4–5 (BS-group) and 121 patients with low responders or who did not agree with the day 5 protocol had embryo replacement on day 2–3 (CS-group).

Total pregnancy rate was recorded after serum pregnancy tests were performed 11 and 13 days after oocyte retrieval.

A total of 500 live born children resulted from these pregnancies, some of which were multiple birth deliveries. Forty-two women did not want to participate in the study and 19 women were lost for follow up.

Following written informed consent, patients' characteristics and medical history were evaluated by questionnaire after ART-treatment and delivery. Neonatal outcome of the two study populations was evaluated by collection and analysis of all birth records, in cases of referral to intensive care unit (ICU), neonatology discharge notes were reviewed. Endpoints for neonatal outcome were sex, birth weight in relation to term- and preterm delivery, Apgar-score, transfer rate to ICU and birth defects.

Variables of interest compared between the two study groups were pregnancy rate, number of vital pregnancies, rate of multiple pregnancies, sex, birth weight of the newborns and numbers of low-birth-weight infants, the latter were subdivided into term and preterm infants. Preterm delivery was defined as delivery at <36 completed weeks of gestation. Low birth weight was defined as 2500 g or less at term and preterm. Transfer rate to ICU and hospital stay of neonates and prevalence of major birth defects diagnosed in the first days after delivery was recorded. Birth defects were defined as abnormalities that are most likely of prenatal origin, including structural, chromosomal and genetic defects.

Treatment

Women undergoing assisted reproduction treatment received the combination of daily 0.1 mg triptorelin s.c. (Decapeptyl; IPSEN Biotech, Paris, France) starting in the midluteal phase of the preceding cycle. Three days after the onset of menstruation an ultrasound evaluation was performed. If downregulation (endometrium <3 mm, no ovarian cysts >10 mm) was demonstrated, 150 IU HP hMG (Menopur; Ferring Pharmaceuticals A/S, Ørestad City, Denmark) and 75 IU rFSH (Gonal-F; Serono Pharmaceuticals Ltd, Feltham, UK) were administered. When a cohort of >2 follicles reached a diameter of 19 mm, 10 000 IU hCG (Profasi; Serono Pharmaceuticals Ltd, Feltham, UK) were given. Luteal phase support consisted of administering 50 mg progesterone (Streuli, Richterswil, Switzerland) daily i.m. starting from the day of oocyte retrieval until 16 days thereafter. This was continued until pregnancy occurred, which was defined as the presence of gestational sac(s) with fetal heart activities at 30 days after transfer. Oocytes were collected 34–35 h after the administration of 10 000 IU of hCG and were incubated in 0.5 ml IVF 20 medium (Scandinavian Science, AB products, Gothenborg, Sweden). In patients entering the IVF program for male factor or unexplained failure of fertilization, cumulus and corona cells were removed from the oocytes 2–3 h after oocyte retrieval by incubation in 25 IU/ml hyaluronidase (Type VIII; Sigma Chemical Co., St Louis, MO) and before performing ICSI. Further details are described elsewhere (Vanderzwalmen *et al.*, 1996).

In vitro culture of embryos

Oocytes were incubated in IVF 20 medium (Scandinavian Science, Gothenborg, Sweden) and embryo culture was carried out in 4-well multi-dishes (Nunc, Roskilde, Denmark) containing each 500 µl of sequential media or in 40 µl microdrops under mineral oil (Cryo Bio System, Paris, France) at 37°C in a humidified atmosphere of 5% O_2 in air. Sixteen to twenty hours after insemination or ICSI, the oocytes were assessed for the presence of two pronuclei and after rinsing they were transferred into G1-2 medium (Scandinavian Science, Gothenborg, Sweden) for an additional period of 48 h.

For blastocyst culture, embryos were placed in wells or microdrops of CCM medium (Scandinavian Science) on day 3 and further cultured to the blastocyst stage.

Statistics

Associations between categorical variables were tested with the Pearson's chi square test and the Fisher's exact test. Differences between nonparametric distributed variables were examined with the Mann–Whitney *U* test. A *P*-value of <0.05 was considered statistically significant. Significance levels of multiple tests were adjusted using Bonferroni correction. All statistical calculations were performed using SPSS, version 11.0, for Windows.

Results

A total of 1259 consecutive cycles of ART were analysed—549 transfers were performed after day 2–3 and 710 after day 4–5—following which 468 pregnancies were reported; the abortion rate before 24 weeks of gestation was 16% and six fetuses (1%) died after 24 weeks of gestation. A total of 500 live born children was achieved, 143 of which were born in the CS-group and 357 in the BS-group. Total pregnancy rate and THBR were significantly higher in the BS-group. A total of 83% of the infants in the CS-group were singletons, 17% were twins and 1% were triplets, resulting in a total rate of multiple pregnancies of 17%, compared to 34% in the BS-group (Table I).

Table II shows details of maternal and paternal characteristics in the two groups.

Characteristics of the 500 newborns and details of mode of delivery are given in Table III. There were no statistical differences in mean gestational age at birth for singletons, twins and triplets and preterm delivery rate at birth in the CS- and BS-groups, respectively. Total mean birth weight was 2880 g in the CS-group and 2736 g in the BS-group. Percentage of infants with low birth weight in the CS-group was 12% for singletons, 68% for twins and 100% for triplets and 10% for singletons, 56% for twins and 100% for triplets in the BS-group, respectively. There were no statistically significant differences in APGAR-scores and pH-values (Table III).

Table I. Characteristics of the two study populations: early cleavage stage transfer day 2–3 (CS-group) vs blastocyst transfer day 4–5 (BS-group)

Variable	Total	CS-group	BS-group	*P*-value
Total number of transfers	1259	549	710	
Pregnancy rate	468 (37%)	156 (28%)	312 (44%)	<0.001
Miscarriages	77 (16%)	32 (21%)	45 (14%)	n.s
Stillbirths	6 (1%)	3 (2%)	3 (1%)	n.a.
Babies per implanted embryo (THBR)	385 (31%)	121 (22%)	264 (37%)	<0.001
Singleton in vital pregnancy	274 (71%)	100 (83%)	174 (66%)	0.001
Twins in vital pregnancy	107 (28%)	20 (17%)	87 (33%)	0.001
Triplets in vital pregnancy	4 (1%)	1 (1%)	3 (1%)	n.s.
Total of multiple pregnancies n_o	111 (29%)	21 (17%)	90 (34%)	0.001
Number of transferred embryos (SD)		2.09 (±0.51)	1.99 (±0.32)	n.s.

Forty-two patients did not want to participate in the study and 19 patients were lost for follow-up, thus resulting in a study population of 385 women and 500 live-births.

Table II. Characteristics of 385 women, who conceived with early cleavage stage embryo transfer on day 2–3 vs blastocyst transfer day 4–5

Characteristic	CS-group $n = 121$	BS-group $n = 264$	*P*-value
Mean age of mother	34 (±4.5)	31 (±4.2)	n.s.
Mean age of father	37 (±6.2)	36 (±6.0)	n.s.
Parity			
0	100 (83%)	215 (81%)	n.s.
1	20 (17%)	38 (14%)	
>2	1 (1%)	11 (4%)	
Primary cause of infertility	25 (21%)	105 (40%)	$P < 0.001$
Female factor	41 (34%)	70 (27%)	n.s.
Male factor Combined	14 (12%)	32 (12%)	n.s.
Idiopathic	41 (34%)	57 (22%)	$P = 0.012$
Mean number of previous procedures of ART (range)	1 (1–9)	1 (1–8)	n.s.

Table III. Mode of delivery and characteristics of 500 infants conceived with early cleavage stage embryo transfer on day 2–3 vs blastocyst transfer on day 4–5

Variable	CS-group $n = 143$	BS-group $n = 357$	*P*-value
Cesarean section	58 (50%)	133 (48%)	n.s.
Male sex	70 (49%)	188 (53%)	n.s.
Preterm delivery <37 weeks:	20 (17%)	68 (26%)	0.045
Singletons	10 (10%)	19 (11%)	n.s.
Twins	9 (45%)	46 (53%)	n.s.
Triplets	1 (100%)	3 (100%)	n.a.
Gestational age (mean) (weeks)	38 (±3.2)	37 (±3.1)	n.s.
Singletons	39 (±2.5)	39 (±2.3)	n.s.
Twins	36 (±2.9)	36 (±2.8)	n.s.
Triplets	31 (n.a.)	33 (n.a.)	n.s.
Birth weight: mean, g	2880 (±794.1)	2736 (±738.9)	n.s.
Low birth weight <2500 g	42 (30%)	123 (35%)	n.s.
Singletons	12 (12%)	17 (10%)	n.s.
Twins	27 (68%)	97 (56%)	n.s.
Triplets	3 (100%)	9 (100%)	n.a.
Missing	1	6	
APGAR, score			
1 min (range)	8 (1–10)	8 (1–10)	n.s.
5 min (range)	9 (4–10)	9 (4–10)	n.s.
Ph value of umbilical artery (range)	7.27 (7.01–7.47)	7.27 (7.04–7.42)	n.s.

Table IV. Neonatal outcome, days of hospitalization of infants in the two study groups

Variable	CS-group $n = 143$	BS-group $n = 357$	*P*-value
Transfer to intensive care unit (ICU) after delivery	32 (22%)	95 (27%)	n.s.
Singletons	15 (15%)	28 (16%)	n.s.
Twins n_o	14 (35%)	61 (35%)	n.s.
Triplets n_o	3 (100%)	6 (67%)	n.a.*
Mean days in ICU	12.7 (±9.3)	13.0 (±14.1)	n.s.
Major birth defects	6 (4%)	8 (2%)	n.s.
Minor birth defects	11 (8%)	17 (5%)	n.s.

n.a.: not applicable; n.s.: not significant.
*: the number is to small for statistical analysis.

A total of 32 (22%) and 95 (27%) newborns were admitted to ICU in the CS- and BS-groups, respectively. Mean duration of hospitalization and number of congenital malformations was similar in both groups (Table IV).

A classification of all observed birth defects is listed in Table V. Birth defects were classified according to the criteria of the 'National Center on Birth Defects and Developmental Disabilities' Center of Disease Control, US Department of Health and Human Services (Dimeglio *et al.*, 1995).

One would need 12267 and 2332 cases, respectively, to be 80% sure that the observed proportions of minor and major, respectively, birth defects differ significantly between both groups at the 0.05 level.

Discussion

There is growing evidence that blastocyst culture might increase opportunities for choosing more viable and genetically normal embryos. Prolongation of culture to day 5 may allow chromosomally competent embryos to develop to the blastocyst stage, thereby promoting intact embryos (Jones *et al.*, 1998). It is true that the use of blastocysts can not be considered as a way to select embryos without chromosomal abnormalities. However, although 40% of aneuploid day 3 embryos are still capable of developing further, most of them are destined to experience a further blockage of development and implantation failure (Magli *et al.*, 2000; Sandalinas *et al.*,

Table V. Congenital birth defects

	CS-group		BS-group	
	Major	Minor	Major	Minor
Heart:	Ventricular septal defect (VSD)	VSD without hemodynamic relevance	VSD perimembranous and trabecular	VSD without hemodynamic relevance, syndactyly, amniotic band syndrome
	Double inlet left ventricle, VSD, transposition of the great arteries, anomalous pulmonary venous connection		Secundum atrial septal defect (ASD)	
	VSD, Tricuspidal insufficiency, cystic fibrosis, perianal fistula		VSD trabecular	
	VSD perimembranous		Tetralogy of Fallot, multiple malformations	
Chromosomal:	Trisomy 21			
Limbs:		Talipes equinovarus I°: 4* Talipes equinovarus II°: 2 Talipes equinovarus III°: 1 Neonatal dysplasia of the hip		Talipes equinovarus I°: 3 Talipes equinovarus II°: 2 Hemangioma Stiff interphalangeal joints 4/5 Neonatal dysplasia of the hip
Other:	Left renal arterial malformation and stenosis, hypertension		Microcephaly, Alopezia areata	Hemangioma: 5
				Lymphangioma
			Stilling–Türk–Duane Syndrome	Undescended testis
			Malrotation causing volvolus 3 weeks after delivery, short bowel syndrome postoperatively	

*Grading according to Dimeglio *et al.* (1995).

2001). Consequently, culture to the blastocysts stage does identify more developmentally competent embryos than culture to day 2 or 3. However, at the moment we are lacking studies looking at a sufficient number of subjects and following their neonatal/fetal outcome of birth defects, or doing preimplantation genetic diagnosis (PGD) on CS and blastocysts confirming this presumption.

It could also be demonstrated that extending embryo culture to day 5 before transfer increased clinical and ongoing pregnancy rates for patients >35 years of age, whilst decreasing miscarriage rate (Levron *et al.*, 2002; Wilson *et al.*, 2002). Our data showed a much higher pregnancy rate in the BS-group with 40% vs 25% in the CS-group ($P < 0.001$). Accordingly, THBR were also significantly different with 37% and 22%, respectively ($P < 0.001$). The group of patients for blastocyst culture in our study is younger as compared to the CS group, even though no significant difference is observed. However, with time we have enlarged the population of patients entering the blastocyst group. We observed that the numbers of oocytes and zygotes are factors that impaired the development of embryos to the blastocysts stage more than women's age. For example the pregnancy rate in a group of 40-year-old women producing an average of eight zygotes was 25%; in the same population but with a transfer on day 3, the pregnancy rate reached only 13% (data not shown).

Gardner (Gardner *et al.*, 1998; Gardner, 2000) reported significantly higher implantation rates of blastocysts compared to ET on day 2–3. Thus, acceptable pregnancy rates can be achieved by transferring fewer blastocysts. This was confirmed by a prospective randomized study by Van der Auwera (2002). However, other groups did not find a difference in implantation and pregnancy rates in patients receiving day 3 transfer and those receiving extended-culture embryo replacement (Coskun *et al.*, 2000; Levron *et al.*, 2002). Prolongation of embryo culture did not improve clinical outcome in terms of THBR (Lundqvist *et al.*, 2002). With regard to the implantation rate our results demonstrate the beneficial effect of blastocyst transfer, showing a higher percentage of embryos implanting in the BS-group. The higher pregnancy rate in this group might reflect the possibility of choosing intact embryos for transfer, since there was no difference in mean age of the parents, mean number of cycles, mean number of transferred embryos. However, this was a non-randomized retrospective cohort study and particularly at the beginning of the time line, patients with fewer embryos were certainly biased towards the CS-group.

According to the latest Society for Assisted Reproductive Technology (SART) report available, in 2001, multiple births accounted for 35.8% of all ART deliveries (Wright *et al.*, 2004). Multiple pregnancies do not only mean an increased risk for mother and fetuses, they also are responsible for economic and social burden for the parents as well as for the health care system. Transferring embryos at the blastocyst stage has been hypothesized to increase pregnancy and implantation rates, while potentially increasing the multiple pregnancy rate. In a small randomized controlled trial in patients with good prognosis, Frattarelli could demonstrate superior implantation and pregnancy rates with blastocyst transfer compared to ET on day 3, even though fewer embryos were transferred (Frattarelli *et al.*, 2003). In our population, the BS-group yielded a significantly higher rate of twins (33% vs 16.5%), which reflects the higher implantation rate per embryo compared to the CS-group. The total higher

number of multiple pregnancies in the BS-group (34% vs 17%) is mainly caused by the higher number of twins in this group. A possible explanation is that those embryos were chosen that have progressed past embryonic genome activation, thus believed to be more developmentally competent.

A main endpoint of this study was fetal outcome. Sex ratios, birth weight in relation to term- and preterm delivery, APGAR-scores, umbilical artery pH-values, transfer rate to intensive care unit, days of hospitalization and number of birth defects were compared in the two study populations: in some animal species (mice and calves) it has been observed that male embryos reach the blastocyst stage faster than female embryos, resulting in a higher proportion of male offspring (Tsunoda *et al.*, 1985; Avery *et al.*, 1991). However, in humans, these findings could not be uniformly reproduced (Tarin *et al.*, 1995). Also, our data showed no statistically significant difference in male sex in the two study populations.

Previous data demonstrated a higher birth weight after blastocyst transfer in ruminants (Thompson *et al.*, 1995); the authors speculate that the culture system possibly could have had an impact. Also, our data showed a trend towards higher birthweight in the BS-group, but without reaching significance.

Newborns with low birth weight ($\leq$2500 g)—either because of preterm delivery or because of fetal growth retardation—are at increased risk for disabilities and death. The increased risk of low birth weight associated with the use of ART has been attributed largely to the higher rates of multiple pregnancies. Interestingly, singleton infants conceived with ART are also at increased risk for low birth weight at term, relative to singletons in the general population (Schieve *et al.*, 2002). The authors suggest that the 2.6-fold increased risk of low birth weight in those singletons may be directly related to treatments for infertility. Among twins conceived with ART, the risks of term and preterm low birth weight were similar to those in the general population of twins.

In general, studies have not shown an increased risk of major birth defects in children conceived after both IVF and ICSI (Van Steirteghem, 1998), but it seems that methodological problems may have led to an underestimation of the birth defect prevalence among children conceived by ART. Hansen *et al.* (2002) reported, that by 1 year of age, a major birth defect had been identified in 9% of babies following ART, compared to only 4% for those conceived spontaneously. Differences were found in most categories, but were significant for musculoskeletal and cardiovascular defects assuming that infertility and not necessarily ART increases birth defects rates. A major flaw of the above cited study is the lack of an appropriate control group, however, a potential association of ART and a higher rate of birth defects cannot be ruled out.

Despite higher birth weight discordance and more ICU admissions among IVF/ICSI twins, neonatal outcome in IVF/ICSI twins seems to be comparable with that of non-IVF/ICSI twins, when only dizygotic twins were considered in the comparisons (Pinborg *et al.*, 2004).

Our data, though small, do not give any evidence of a higher risk of congenital malformation by prolonging *in vitro* culture to day 5.

In conclusion, blastocyst transfer leads to increased implantation and pregnancy rates with an overall better THBR but with increased multiple pregnancies and preterm delivery rates compared to day 2–3 transfer. This might reflect increased competence of the blastocysts and better uterine synchronization. There were no differences observed in the two study groups in mean gestational age, in the rate of Caesarean section, Apgar and umbilical cord pH-values, total mean birth weight, percentage of low birth weight and admission to ICU. The incidence of minor and major birth defects in both study groups was equal but slightly higher to what is normally seen in the general population. However, final proof for the benefits of blastocyst transfer can only be obtained from prospective, randomized clinical trials.

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