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ORIGINAL PAPER

Single blastocyst transfer after ICSI from ejaculate spermatozoa, percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE)

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Abstract *Purpose*: To investigate the outcome of IVF following intracytoplasmic sperm injection (ICSI) from ejaculate, percutaneous epididymal sperm aspiration (PESA) and testicular sperm extraction (TESE), with subsequent blastocyst culture and single blastocyst transfer.

Methods: Single blastocyst transfer was performed after ejaculate ICSI (oligozoospermia) in 587 patients, TESE/PESA (azoospermia) in 31 patients, and standard IVF in 680 women.

Results: There were only minor differences in IVF characteristics between the standard IVF and the PESA-TESE couples. Couples where ejaculate ICSI were performed seemed to represent a slightly poorer prognostic group. A viable fetus after the 12th gestational week, i.e. ongoing pregnancy, was present in 41.4% after ICSI/ET, 51.6% after PESA-TESE/ET and in 40.4% after standard IVF/ET (no significant differences).

Conclusion: Single blastocyst transfer after ejaculate ICSI or after PESA/TESE appears to give similar results as conventional IVF blastocyst culture.

Keywords In vitro fertilization · Single embryo transfer · Blastocyst · Intracytoplasmic sperm injection · Percutaneous epididymal sperm aspiration · Testicular sperm extraction

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Introduction

Single embryo transfer (SET) is an increasing option in IVF in order to avoid fetal and maternal complications of multiple pregnancies, as well as social and economical burden [1]. Birth of a single, healthy baby has been recommended not only by professional fertility associations [2], but also in regulations by governmental authorities, such as the National Board of Health and Welfare in Sweden.

Most authors agree that there should be some selection of couples when SET should be recommended (reviewed in 2). In women with a poor prognosis, such as high age, poor embryo quality, or many IVF failures the risk of having twins when double embryo transfer is performed appears to be low [3].

The introduction of sequential culture for day 5 blastocyst culture almost ten years ago [4] renewed the interest for blastocyst transfer. Advocates claim higher implantation and birth rates, as compared to day 2–3 transfer, which could be attributed to a better embryo selection, synchronization between the embryo and the endometrium and improved possibilities for preimplantation genetic diagnostics [5], while others see no such advantages [6].

There is still some controversy whether oocytes fertilized through intracytoplasmic sperm injection (ICSI) are feasible for blastocyst culture. Sperm defects might make it less likely that the fertilized oocyte would develop to a good-quality blastocyst with high implantation potential. Discrepancies between different studies are large. Some studies reported less blastocyst formation and a detrimental outcome after ICSI and blastocyst culture [7–9], as those who reported no

difference, as compared to standard IVF [10–12]. There are, however, few, if any, prospective randomized studies, where blastocyst culture with either of the two fertilization methods have been compared.

The ultimate challenge in the above respects would be ICSI from males with non-obstructive and obstructive azoospermia, where spermatozoa were retrieved through percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE), with subsequent blastocyst culture *and* single blastocyst transfer. The present study reports on the outcome of such regimen.

Material and methods

All blastocyst cultures ($n = 2079$) during 2002 and until December 2005 were retrospectively studied. These constituted 56.4% of all IVF procedures at the clinic. Inclusion criteria for blastocyst culture instead of day 2 transfer were woman's age below 39 years and at least five fertilized oocytes. In addition, women, that despite not fulfilling the inclusion criteria, but who were still judged to have a good prognosis, were recommended blastocyst culture.

Controlled ovarian stimulation and oocyte retrieval was made according to a standard "long" gonadotropin-releasing hormone agonist protocol, or in about 10% a standard antagonist protocol. For ovarian stimulation a recombinant follicle stimulating hormone was used [13].

ICSI was performed with standard procedures, in general because of oligozoospermia. In azoospermia, epididymis was first palpated and fine needle aspiration was made (PESA) after funicular anesthesia. If there was no recovery of spermatozoa, TESE immediately followed, using a commercial instrument (BIP – High Speed multi) with a core tissue biopsy needle (Bard Magnum). Scrotal incision was thus avoided. Spermatozoa were microscopically recovered manually from the testicular biopsy.

Sequential embryo cultures were in general performed with media from Medicult^R (Blast Assist System) with some exceptions, where media from Vitrolife^R was used. Zygotes/embryos were transferred to Medium 1 and Medium 2 on day one and three, respectively. The embryos were cultured no more than five together in dishes without oil, in incubators with 5–6% CO_2 adjusted to a pH of 7.30–7.35.

Only fresh blastocysts were used for transfer in this study. Blastocyst transfer was performed in all cases where the best embryo was at least a morula. Where not even a morula was present, the transfer was cancelled.

Blastocyst quality was scored from 30 to 34, where 30 and 31 were top quality blastocysts. Score 31 was an expanded blastocyst with an evenly distributed trophoectoderm. If hatching had occurred it was judged as grade 30. A less expanded blastocyst was regarded as score 32. Score 33 was a blastocyst where there was only a small sign of cavitation. Score 34 was an early blastocyst.

The results were calculated on the JMP 6.0 statistical program (SAS Institute). Chi^2 test (likelihood ratio) and t-test were made as appropriate. Logistic regression (loglikelihood test) were used when adjustment for possible confounding factors was made, and for estimation of odds ratios (OR) and 95% confidence intervals (95% CI).

Results

Most blastocyst cultures lead to blastocyst transfer, i.e. 98.2% with standard IVF, 97.6% with ICSI and 100% with TESE/PESA. Single blastocyst transfer was performed in 66.9% of all standard IVF transfers (680/1016), 60.5% of ICSI (587/971) and 66.0% (31/47) of TESE/PESA transfers. The remaining were double double blastocyst transfers. The couples that had single transfers were on average expected to have a better prognosis than those who received two blastocysts. Among ICSI patients, mean age for women with single transfer was 32.3 years compared to 34.4 years ($p = 0.0001$) for women who had two embryos, IVF cycle rank was 1.9 versus 2.9 ($p = 0.0001$), and development of at least one high quality blastocyst was 70.0% vs. 46.4% ($p = 0.0001$). There were no differences in number of oocytes retrieved or fertilized.

Ongoing pregnancy rate/transfer, defined as a viable fetus after 12th gestational week, was 41.4% in women having one blastocyst, compared to 33.9% among those who had two (Odds ratio 1.88, 95% CI 1.42–2.48). When age, IVF cycle rank and blastocyst quality were introduced in a multivariate analysis, there was no longer a significant difference in ongoing pregnancies between those who had one, and those who had two, embryos transferred (odds ratio 1.10, 95% CI 0.88–1.36).

The outcome of single blastocyst transfer with PESA/TESE and ICSI, as compared to standard IVF are given in Table 1. The ongoing pregnancy rate with IVF- and ejaculate ICSI single blastocyst transfer was very similar (40% vs. 41%). The ICSI patients were, however, significantly younger, but had a higher IVF cycle rank, more number of retrieved oocytes, and had in a lower frequency at least one high-quality blastocyst. Those factors were included in a multivariate analysis. The results were unchanged, with crude odds ratio 1.04 in favour of ICSI and adjusted OR 1.03 (95% CI 0.81–1.30).

The ongoing pregnancy frequency was 11% higher with PESA/TESE as compared to standard IVF, but did not reach statistical significance. A logistic regression analysis that included the above variables was made. Crude odds ratio (1.57, 95% CI 0.76–3.26) increased to 1.76 after adjustments for PESA/TESE as compared to standard IVF

Table 1 Outcome of single blastocyst transfer with ICSI, PESA and TESE as compared to traditional IVF

	Single blastocyst transfer				
	IVF	ICSI		PESA/TESE	
	$n = 680$ (%)	$n = 587$(%)	p-value[a]	$n = 31$ (%)	p-value[a]
Mean age	32.9	32.3	0.004	31.6	0.08
IVF cycle rank	1.7	1.9	0.004	1.6	0.86
Mean no oocytes retrieved	10.9	11.4	0.03	11.1	0.79
Mean no oocytes fertilized	7.5	7.5	0.86	7.0	0.41
At least one high quality blastocyct	534 (78.5)	411 (70.0)	0.0005	27 (87.1)	0.23
Cryopreservation (any)	460 (67.9)	326 (55.6)	0.0001	21 (67.7)	0.99
Postive pregnancy test	362 (53.2)	295 (50.3)	0.29	17 (54.8)	0.86
Viable fetus at ultrasound	322 (47.4)	267 (45.5)	0.51	17 (54.8)	0.42
Ongoing pregnancy	275 (40.4)	243 (41.4)	0.73	16 (51.6)	0.22

[a] p-value as compared to conventional IVF.

patients, but the difference was non-significant (95% CI 0.66–6.09).

Discussion

The study has shown very encouraging results not only with blastocyst culture as such after PESA or TESE, but also for the clinical outcome. Also, in as much as two thirds of all cycles where PESA/TESE was performed, a single blastocyst was transferred.

ICSI with spermatozoa retrieved from the epididymis or testes has been performed for more than 10 years [14]. Studies on day 2–3 transfer have shown good results as compared to traditional IVF, with clinical pregnancy rates in general between 30–40% with multiple embryo transfer [15–17]. Small differences have been found between PESA and TESE. The results have, however, been better with motile spermatozoa [15, 17] and with fresh, as compared to thawed, sperm [17].

As to our knowledge, there is no previous study that reported on single blastocyst transfer, i.e. when only one blastocyst was transferred even when there were spare high-quality blastocysts, where sperm had been recovered from epididymis or testis. In two previous studies multiple blastocyst transfer after PESA/TESE was analysed [18, 19]

Balaban et al. [18] reported in a retrospective study transfer of on average 3.3 blastocysts after PESA/TESE or ejaculate ICSI. Blastocyst transfer was performed in approximately 90% cycles and pregnancy rates varied insignificantly between the groups of patients (59–65%), but there was a tendency of worse outcome when the male had TESE because of non-obstructive azoospermia (48%). Multiple pregnancy rates were above 50%, except in the latter group (29%).

In a randomised study, Virant-Klum et al. [19] assigned TESE patients to day 2 day or day 5 culture. On average two embryos were transferred in both groups. Clinical pregnancy rates per started cycle were only 16% and 13%, respectively. The disappointing outcome for day 5 transfer was due to the results of blastocyst culture, where at least one embryo reached blastocyst stage in only 24% of the cycles. Thus, clinical pregnancy rate per embryo transfer was 55% with blastocysts, as compared to 20% with day 2 culture. The present study cannot be compared with the above studies, as none included single blastocyst transfer.

The rationale behind supporting blastocyst culture are the possibility of better selection of high-quality embryos, 'survival of the fittest', which might give a better implantation rate [6]. When preimplantation diagnostics is in clinical routine blastocysts in additionalso be more suitable to analyse than day 2–3 embryos. The evidence that blastocyst transfer yields a better outcome than day 2–3 transfer is conflicting [5, 20]. However, if the theory that the selection of embryo is more adequate with blastocysts, than with day 2–3 embryos is true, a real difference between the two culture methods would be particularly apparent when only one embryo is transferred. Previously we [20], and others [21] have shown promising results with single blastocyst transfer. There is, however, only one randomised study that compared single day 2–3 and blastocyst transfer. Thus, Papanikolau et al. [22] planned a randomised study including 702 patients to have elective single day 3 or blastocyst transfer. The study was terminated when half the patients had been recruited, as an interim analysis showed an unacceptable large and significant difference in birth rates between the two groups, in favour of blastocyst transfer (32% vs. 22%).

Studies that compared blastocyst culture after standard IVF and ICSI shows contradictory results. Those with better results with standard IVF, as compared to ICSI, often used surplus embryos for further culture from day 2–3 to day 5. The use of surplus embryos indicates that their routine procedure was day 2–3 transfer. Blastocyst culture needs experience, which makes it difficult to interpret results where it is not a routine at the IVF clinic. Authors who had worse results with ICSI than with standard IVF blastocyst

culture hypothesize that it is due to poor semen parameters, sperm quality and chromosomal abnormalities associated with oligozoospermia, sperm motility and morphology, or the technical procedure in ICSI [6].

Several studies have shown increased spermatozoa abnormalities in males with oligoasthenozoospermia [23]. Aneuploid [24] or mosaic [25] spermatozoa in testicular spermatozoa is found in a higher frequency in men with azoospermia, in particular non-obstructive [26] than in normal sperm. Also, chromosome Y deletions will be inherited by male offspring [36]. Poor quality spermatozoa lead to a reduced number of mature blastocysts, but once reaching the blastocyst stage the IVF outcome is similar as in those with high-quality sperm [27]. Hardarson et al. [28] compared blastocyst culture of day 2 embryos considered as morphological suboptimal, with embryos of good quality. Seven chromosomes were analysed. Blastocycts from suboptimal embryos had a higher frequency of chromosomal aberrations and poor morphology, known for a low implantation potential, than those from good quality embryos. These findings support the 'survival of the fittest' theory, but it has been questioned if blastocyst transfer does prevent inheritance of paternal chromosomal defects [29].

Our study showed that single blastocyst transfer after PESA or TESE in azzospermic males yields similar results as ejaculate ICSI due to oligozoospermia, and as far as comparisons are possible, standard IVF. These findings represent a further step towards single embryo transfer as the rule, and multiple transfer as the exception.

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