Zygote Versus Embryo Transfer: A Prospective Randomized Multicenter Trial

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Purpose: To determine the efficiency of transferring human zygotes as opposed to human day 2 or 3 embryos.

Methods: A prospective, randomized, Multicenter trial. Patients were randomized into zygote or embryo transfer. Patients were prepared for oocyte retrieval using standardized protocols. Oocyte retrieval was performed under general anesthesia. Oocytes and spermatozoa were treated using standard laboratory techniques. All protocols were coordinated by the coordinating center.

Results: A total of 386 patients were included in the trial. Pregnancy rates were 36.5% after zygote transfer and 42% after embryo transfer. Implantation rates were equivalent (17%) in both groups.

Conclusions: No general difference was observed for zygote or embryo transfer. The results suggest that zygote transfer is a valid alternative to embryo transfer.

KEY WORDS: Human oocyte; intracytoplasmic sperm injection; in vitro fertilisation.

INTRODUCTION

The transfer of cleaving human preimplantation embryos into the uterus of the patient on day 2 or 3 after fertilisation remains the technique most widely used in assisted reproduction protocols after in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) (1–3). However, pregnancies have been obtained in the human uterus after the transfer of

injected oocytes, zygotes, and blastocysts (4–12), suggesting that the human uterus is not stage-selective for embryo development. The morphological analysis and selection of human zygotes for transfer into the patient's uterus has been reported to be highly successful after both IVF and ICSI (9,10). These data suggest that the extended culture of human embryos is unnecessary in many cases. In fact, the successful use of zygote transfer technology would be highly beneficial in countries such as Switzerland or Germany, where the law prevents the culture of multiple embryos and the consequent selection of the highest-quality embryos for transfer (13).

Zygote transfer could be advantageous for patients attending their first IVF cycle, for many reasons. First, zygote transfer enables the completion of the IVF cycle in a rapid timescale, alleviating patient stress. Second, human zygotes tend to survive cryopreservation better than cleaving human embryos or blastocysts (14,15), enabling the patient to repeat IVF

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with frozen material if necessary. Third, the culture of human zygotes is very simple and avoids problems associated with extended culture such as embryo fragmentation or the complete block of development. However, there are two possible disadvantages of human zygote transfer. First, the loss of the possibility to analyze human embryos for the selection of the best quality for transfer may lead to a reduction in pregnancies after zygote transfer. Second, the human uterus may not be physiologically adapted to the development of human preimplantation embryos, although current evidence suggests that this is not the case (4–10). In this paper, we test the hypothesis that no difference exists between the transfer of human zygotes or cleaving embryos. We compare the transfer of human embryos with that of human zygotes for patients attending IVF centers for a first attempt at IVF. We performed a Multicenter trial using data gained from four separate IVF centers. We show that little difference in pregnancy, implantation, and live-birth rates are obtained when zygote transfer is performed instead of the replacement of cleaving embryos. The data suggest that zygote transfer is a valid option for patients initiating IVF treatment. We further examined the use of zygote or embryo transfer for defined patient groups in order to test whether one protocol offered more advantages under certain conditions.

MATERIALS AND METHODS

Patients

Patients were attending the IVF centers for a first cycle of in vitro fertilisation including standard IVF and ICSI techniques between March 1998 and February 1999. The trial was strictly coordinated from the IVF center in Naples, Italy. No exclusion criteria were implemented; however, patients in defined categories were grouped with respect to pathology, age, and body mass index [$\text{BMI} = \text{weight (kg)}/\text{height (m}^2\text{)}$] and then randomized into transfer protocols using computer-generated random number tables. Doctors followed standard, defined protocols for ovarian hyperstimulation, and the Naples, Italy, group of biologists coordinated all biological work. Patients were prepared by downregulation of the pituitary gland with a GnRH agonist (Decapeptyl, Ipsen, Italy) followed by ovarian stimulation with exogenous recombinant FSH (Gonal-F, Serono, Italy). Starting doses of FSH were in the range 225–300 IU, followed by adjustment of the stimulation protocol

after day 5, dependent on the ovarian response, as determined by levels of serum estradiol and echographic visualization of folliculogenesis. Oocyte retrieval was performed transvaginally, 36 h after the administration of 10,000-IU β -hCG, when two to three follicles of 18–20-mm diameter were observed by ultrasound examination, and blood 17β -estradiol levels reached 150–200 pg/mL per follicle over 18 mm.

In Vitro Fertilisation and Embryo Transfer Techniques

In vitro fertilisation techniques followed standard protocols for oocyte and sperm preparation. As previously mentioned, the Naples, Italy, group of biologists coordinated all biological work, ensuring standardization of protocols. Oocytes were washed immediately after retrieval in in vitro fertilisation medium (IVFM, Medicult, Copenhagen, Denmark) to remove blood and other residues, and cultured for 3 h prior to preparation for IVF or ICSI. The preparation of sperm for IVF included washing in sperm preparation medium (Medicult) followed by swim-up. Oocytes were inseminated 4 h after retrieval in a concentration of $(0.5\text{--}1) \times 10^6$ sperm per mL. Preparation of oocytes for ICSI involved denudation of the cumulus–oocyte complex in hyaluronidase 80 IU/mL (Medicult) 3 h after oocyte retrieval. ICSI was performed a further hour after denudation. ICSI procedures were performed with a Nikon inverted microscope (Nikon, Florence, Italy) to which were installed Narishige micromanipulators. Sperm was pipetted into 10% PVP solution (Medicult), and Eppendorf microtools were used for the ICSI procedure. Oocytes were cultured in IVFM (Medicult). The fertilisation of inseminated or microinjected oocytes was checked 16–20 h after insemination or ICSI to determine the presence of two clear pronuclei. Embryos were then washed into fresh IVFM for further culture (Medicult). A morphological and development check was performed at 24-h intervals. Embryos were transferred on day 2 or 3 according to standard morphological criteria (3). Previous data has failed to show any difference between day 2 and 3 transfer and therefore these groups were categorized as one (16). Zygotes were selected for transfer according to pronuclear and nucleolar morphology (9) where possible. Both zygote and embryo transfer was performed using a soft embryo transfer catheter (Edwards-Wallace, SIMS Portex, UK) in a medium containing 75% patient serum 25% IVFM (Medicult).

Statistics

All data are presented as means plus/minus standard deviations except where stated otherwise. Statistical analysis was performed using Sigma Stat (SPSS, Erkrath, Germany). The z test was performed to test for the significance of the differences in proportions between groups and Yates' correction was applied where necessary.

RESULTS

Zygote Vs Embryo Transfer

Four hundred and seven patients attending for IVF and ICSI cycles in four separate IVF centers were preselected for either zygote or embryo transfer. Of these, 402 oocyte retrievals were performed (Table I). Two patients from the zygote transfer group and 14 from the embryo transfer group were not included after oocyte retrieval due to the absence of oocytes. In total, 203 patients had zygotes replaced and 183 patients had embryo transfer on day 2 or 3 after fertilisation. Two thousand and forty-one oocytes were retrieved for the zygote transfer and 2011 oocytes were

obtained for embryo transfer (Table I). After IVF or ICSI, 1572 zygotes formed in the zygote trial and 1610 zygotes were left in culture for patients selected for embryo transfer (Table I). In total, 611 zygotes and 741 cleaving embryos were transferred (Table I). Excess material was either cryopreserved or eliminated from further clinical use. We obtained an overall pregnancy rate after zygote transfer of 36.5% as opposed to a pregnancy rate of 42% for embryo transfer. However, overall implantation rates (17%) were equivalent in the total sample of patients. Interestingly, birth rates were similar in the two groups although the transfer of cleaving embryos resulted in a larger proportion of twins than zygote transfer did (Table I). These data suggest no overall difference occurs in the outcome after transfer of human zygotes as opposed to cleavage-stage embryos after both IVF and ICSI.

Indications for Zygote Transfer

To examine whether one protocol could be more reliable under certain pathologies, we categorized the patients according to the major cause of infertility. Broad categories were selected to minimize the risk of misgrouping patients because of differences in the diagnostic accuracy of the major cause of infertility. In the patient population selected for the trial, the diagnosis of female factor infertility was divided into three categories: blocked tubes (49 patients), endometriosis (66 patients), and idiopathic (61 patients) (Table II). Male factor categories were split for convenience into three categories: no cause (61 patients); male factor, ejaculated sperm (115 patients); and male factor, testicular sperm (50 patients) (Table II). The male factor of testicular sperm category excluded patients with nonobstructive pathologies because of the extremely low pregnancy rates achieved with these patients (17,18). Zygote transfer was performed in 20 patients where tubal factor was diagnosed, 35 patients suffering from endometriosis, 20 patients where the maternal age was over 37 years, 67 patients where the only cause of infertility was male factor, 30 patients requiring testicular biopsy, and 31 cases of idiopathic infertility (Table II).

We compared the results after zygote or embryo transfer in each of these patient categories to determine which technique was more suitable under the diverse conditions. Although pregnancy rates were often greater after embryo transfer, implantation rates were not significantly different (Table II). The differences in pregnancy rates is probably attributable to

Table I. Results of Zygote and Embryo Transfer

	Zygote transfer	Embryo transfer
No. of patients selected for trial	205	202
No. of patients at oocyte retrieval	205	197
No. of patients at transfer	203	183
Mean age	33.8 $\pm$ 4.5	32.7 $\pm$ 3.5
Mean no. of oocytes retrieved	9.5 $\pm$ 5.4	10.9 $\pm$ 6.4
No. of oocytes injected/inseminated	2041	2011
No. of zygotes formed ^a	1572 (77)	1609 (80)
No. of zygotes/embryos transferred	611 (3.2 $\pm$ 1.0)	741 (3.6 $\pm$ 0.8)
Clinical pregnancies/transfer (pregnancy %)	74 (36.5) ^b	77 (42.0) ^b
No. of fetal heartbeats (implantation rate %)	104 (17.0) ^b	126 (17.0) ^b
Live births (% of transferred embryos)	102 (16.6) ^b	123 (16.6) ^b
Singletons (% pregnancies)	51 (68.9)	37 (48.1)
Twins (% pregnancies)	20 (27.0)	36 (46.6)
Multiple orders (% pregnancies)	3 (4.1)	4 (5.2)

^a Values in parentheses are percentages.

^b No significant difference (z test with Yates' correction factor, $p > 0.05$, $\alpha = 1.0$).

Table II. Indications for Zygote Transfer by Infertility Diagnosis

Suspected main cause of infertility	Transfer protocol	No. of oocytes injected/ inseminated	No. fertilized ^a	No. transferred	Pregnancies (pregnancy rate/ transfer %)	Foetal heart beats (implantation rate %)	Live births
Tubal defects	Zygote (n = 20)	236 (9.9 ± 2.2)	154 (65.3)	72 (3.3 ± 1.0)	10 (50) ^b	11 (16.4) ^b	11
	Embryo (n = 29)	376 (11.0 ± 1.1)	277 (73.7)	133 (4.4 ± 2.0)	16 (52) ^b	9 (6.7) ^b	9
Endometriosis	Zygote (n = 35)	373 (9.3 ± 4.2)	278 (74.5)	112 (3.2 ± 0.6)	16 (43) ^b	37 (31.6) ^b	36
	Embryo (n = 31)	359 (12.2 ± 3.3)	295 (82.2)	147 (4.5 ± 1.5)	14 (42) ^b	42 (26.7) ^b	42
Male factor—ejaculated spermatozoa	Zygote (n = 67)	745 (10.0 ± 2.5)	642 (86.2)	222 (3.4 ± 1.8)	38 (54) ^b	38 (16.2) ^b	37
	Embryo (n = 58)	624 (11.9 ± 3.8)	512 (82.1)	277 (4.5 ± 1.8)	29 (50) ^b	53 (18.1) ^b	51
Male factor—testicular spermatozoa	Zygote (n = 30)	327 (8.9 ± 4.4)	244 (74.6)	105 (3.2 ± 1.2)	3 (10) ^b	8 (7.3) ^b	8
	Embryo (n = 20)	272 (11.4 ± 2.4)	223 (81.9)	82 (4.0 ± 1.0)	4 (20) ^b	8 (9.4) ^b	8
Idiopathic infertility	Zygote (n = 31)	360 (10.2 ± 5.5)	254 (70.6)	100 (3.3 ± 0.8)	7 (22) ^b	10 (10) ^b	10
	Embryo (n = 30)	380 (9.8 ± 1.3)	302 (79.5)	102 (3.2 ± 1.2)	14 (43) ^b	14 (13) ^b	13

^a Values in parentheses are percentages.

^b No significant differences were found between zygote and embryo transfers (*z* test with Yates' correction where applicable, *p* > 0.05).

the higher number of embryos selected for transfer in many cases (Table II). These data confirm the hypothesis that little gain can be achieved in terms of success rates by selecting one protocol over another in certain defined pathologies.

Maternal age is another parameter with a strong effect on pregnancy and implantation rates. We tested whether this was due to a factor that could be overcome by replacing human embryos at the zygote stage.

Again, zygote transfer had no positive effect on the outcome of IVF when compared with embryo transfer (Table III). The well-noted trend toward lower pregnancy rates was observed in both protocols, and no significant differences were observed between groups in any case (Table III). These data suggest that no difference in outcome can be obtained by the application of one of the transfer protocols tested here in cases of advanced maternal age.

Table III. Success Rates of Zygote and Embryo Transfer According to Maternal Age

Maternal age (years)	Transfer protocol	No. of oocytes injected/ inseminated	No. of oocytes fertilized	No. transferred	Pregnancies (pregnancy rate/transfer %)	Foetal heart beats (implantation rate %)	Live births
<29	Zygote (n = 64)	585	484	214 (3.5 ± 1.2)	32 (50.0) ^a	44 (21) ^a	44
	Embryo (n = 48)	544	448	198 (4.0 ± 0.8)	27 (56.3) ^a	40 (20) ^a	39
30–34	Zygote (n = 115)	1211	908	313 (2.8 ± 2.0)	36 (31.3) ^a	49 (16) ^a	48
	Embryo (n = 113)	1206	969	456 (4.0 ± 1.4)	44 (38.9) ^a	78 (17) ^a	78
>35	Zygote (n = 24)	245	180	84 (4.0 ± 1.1)	6 (25) ^a	11 (13) ^a	10
	Embryo (n = 22)	261	192	87 (4.0 ± 1.0)	6 (27.3) ^a	8 (9.1) ^a	6

Note. Maternal age refers to age at the time of oocyte retrieval.

^a No significant differences were found between zygote and embryo transfers (*z* test with Yates' correction where applicable, *p* > 0.05).

Table IV. Indications for Zygote Transfer by Response to Stimulation Protocol

	Transfer protocol	No. of oocytes injected/inseminated	No. of oocytes fertilized ^a	No. transferred	Pregnancies (pregnancy rate/transfer %)	Foetal heart beats (implantation rate %)	Live births
Normoresponders	Zygote (n = 162)	1840	1441 (78)	480 (3.0 ± 1.4)	64 (40) ^b	88 (19.2) ^b	88
	Embryo (n = 132)	1767	1457 (83)	589 (4.2 ± 2.0)	67 (51) ^b	108 (20) ^b	107
Poor responders	Zygote (n = 41)	201	131 (64)	131 (3.2 ± 1.0)	10 (27) ^b	16 (13.1) ^b	14
	Embryo (n = 51)	244	152 (63)	152 (3.1 ± 1.2)	10 (19) ^b	18 (12) ^b	16

Note. Poor responders were characterized by a requirement for over 50–75-IU ampoules of recombinant FSH during the stimulation protocol and the retrieval of less than five oocytes at pick-up.

^a Values in parenthesis are percentages.

^b Differences in pregnancy and implantation rates are not significant between zygote and embryo transfers (z test with Yates' correction).

Another factor that determines the success rate after in vitro fertilisation is the efficiency or response of the ovary to the exogenous stimulus applied by FSH (19,20). We tested whether the application of zygote or embryo transfer was more suitable under defined responses to the stimulation protocol. We examined whether zygote transfer was more indicative of success in poor responder patients. We defined poor responder patients in the present study as patients requiring over 50 ampoules of 75-IU FSH in order to achieve a response of five oocytes or less at pick-up. Little difference was observed with either protocol in this group of patients (Table IV). Pregnancy rates were slightly higher after zygote transfer, but implantation rates were equivalent.

DISCUSSION

The transfer of human two-pronuclear zygotes has been proposed as an alternative to embryo transfer for many years. Advantages of this technique are the completion of the laboratory phase of the IVF cycle in a rapid timescale and the elimination of the in vitro culture of human embryos and the selection of this material for uterine replacement, which has inherent risks and ethical considerations. Diverse reports have discussed the merits of this technique and attempted to define morphological features that can aid in the selection of human zygotes for transfer. In this report, we have tested whether human zygote transfer is a real alternative to embryo replacement for patients attending an initial cycle of IVF, and examined whether zygote or embryo transfer is more advantageous in certain pathologies.

An overall comparison between the replacement of human zygotes and embryos suggests little difference in the results obtained. Although a slight, nonsignificant reduction in pregnancy rates was observed, no difference in implantation or live-birth rates was noted after the transfer of 741 embryos and 611 zygotes. We compared the results center-by-center and confirmed the above data, demonstrating that, in our hands, little difference exists globally between the transfer of human zygotes and embryos. The results suggest that little advantage can be gained for patients attending their first cycle of IVF by culturing embryos to day 2 or 3 after fertilisation followed by selection and transfer. However, these data give little insight into the differences between the two protocols in specific patient groups. We therefore tested whether this was the case for diverse types of infertility diagnosis.

The diagnosis of female factor infertility is commonly divided into diverse groups. We tested whether any specific diagnosis affected the results after zygote or embryo transfer. In our hands, no form of female factor infertility resulted in a significant difference between the two transfer techniques. IVF outcome, when grouped according to maternal age, was also not improved by the transfer of zygotes. Neither did male factor infertility cause any significant change in IVF outcome after zygote transfer. In fact, we were unable to find any specific infertility diagnosis where the prognosis could be improved after the transfer of zygotes.

The data then strongly suggest that zygote transfer is a valid alternative to embryo transfer in most groups of patients attending IVF centers for a first

attempt at IVF. The simplicity and reliability of the technique have huge advantages over extended culture, both for the alleviation of patient stress and the possibility of cryopreservation of excess material. Extended culture, including culture of embryos to the blastocyst stage, could be reserved for patients with a prior history of poor embryo development or defined pathologies in which the extended culture of embryos is deemed necessary.

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