Cycle Characteristics of Day 3 Embryo Transfers with 4-Cell Embryos Only

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Purpose: Patient and cycle characteristics of day 3 transfers with developmentally lagging 4-cell embryos only were analyzed and related to the outcome of a live birth.

Methods: Day 3 transfers with either 4-cell embryos only (study group; $n = 138$) or 8-cell embryos only (control group; $n = 282$) were compared retrospectively.

Results: The total dose of FSH per treatment was higher, while the number of oocytes, zygotes, and transferred embryos was lower in the study group cycles compared to controls. The implantation, pregnancy, and live birth rates were dramatically lower in the study group compared to the control group. In the study group, the few cycles resulting in a live birth were characterized by a normal ovarian response to stimulation, similar to that of control group cycles with- or without a live birth.

Conclusions: In cycles characterized by intensive ovarian stimulation, but poor response, the chance for a live birth is extremely low after day 3 transfer of 4-cell embryos.

KEY WORDS: Day 3 embryo transfer; embryo cleavage rate; implantation rate; ovarian response; pregnancy outcome.

INTRODUCTION

Following an IVF or an ICSI treatment cycle, the most frequent question asked by the couple is their chances of giving birth to a child. Estimating the chances for a successful pregnancy following embryo transfer and the influence played upon this estimate by clinical factors is of fundamental importance in infertility treatment.

It is generally accepted that a relationship exists between embryo quality and pregnancy rate (1–3) and for cleavage stage embryos, pregnancy rate is well known to be negatively influenced by transfer of slowly cleaving embryos. Indeed, in day 2 transfers, patients with early cleaving 2-cell embryos have been shown to have higher pregnancy rates than patients

without early-cleaving 2-cell embryos (4) and transfer of 4-cell embryos result in significantly higher implantation and pregnancy rates compared with transfers of 2- and 3-cell embryos (5). In cycles with day 2 or 3 embryo transfer, early-cleaving embryos result in significantly higher birth rates than late-cleaving embryos (6). In transfers of embryos of different cleavage stages and morphology, embryos with optimal implantation potential were characterized by four or five blastomeres on day 2, seven or more blastomeres on day 3, absence of multinucleated blastomeres and $\leq 20\%$ embryo fragmentation (7).

Cell number was found to be the strongest predictor of pregnancy in day 3 embryos in a scoring system based on cell number, fragmentation, and other morphological criteria deemed specific to day 3 embryos (8). Using another scoring system, rate of development alone was found to be predictive of success after IVF while a poor correlation was observed between embryo morphology and predictability (9).

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In our department, embryo transfer is performed on day 3 after oocyte retrieval, and embryos are selected for transfer on the basis of their cleavage stage and morphology. Blastomere number is, however, given the greatest emphasis and 4-cell embryos, therefore, are only transferred when other options are absent.

In this report, day 3 transfers with developmentally lagging 4-cell embryos only were analyzed retrospectively. Characteristics of the patients and the cycles were analyzed and related to the outcome of a live birth, in order to optimize treatment in a future cycle.

MATERIALS AND METHODS

Study Design

All cycles with fresh embryo transfer of maximum two embryos on day 3 after either IVF or ICSI in 1998–2000 were analyzed retrospectively. The study group which consisted of all day 3 transfers ($n = 138$) with developmentally lagging 4-cell embryos only was compared with a control group, consisting of all day 3 transfers ($n = 282$) with 8-cell embryos only, the normally expected and preferred stage on day 3. Hence, only transfers with embryos of identical cleavage stage, either 4-cell or 8-cell embryos, were analyzed.

Clinical Procedures

The woman's age limit for IVF or ICSI treatment was 38 years of age. The patients were treated with the long protocol using 600 $\mu\text{g/day}$ of either intranasal buserelin acetate (Suprefact; Hoechst, Frankfurt am Main, Germany) or nafarelin acetate (Synarela; Farmacia, Caguas, Puerto Rico) for down regulation for at least 14 days. Ovarian stimulation was achieved by 75–300 IU/day of recombinant human follicle stimulation hormone (rhFSH) (Gonal-F; Serono, Geneva, Switzerland or Puregon; Organon, Oss, The Netherlands). Individual adjustment of the starting dose was performed according to the same rules in both groups. The initial dose of FSH was generally 150 IU/day for patients up to the age of 35 and 200–225 IU above this age. Known poor responders, with insufficient follicle development in a previous cycle after 225–300 IU FSH daily treatment and 0–3 oocytes retrieved, started with 300–450 IU FSH. Cycles were monitored using a combination of vaginal ultrasound and serum estradiol concentrations. Ovulation was induced with 10,000 IU human

chorionic gonadotrophin (hCG) (Profasi; Serono or Pregnyl; Organon) when at least two follicles with a mean diameter of 17 mm were visualized. Transvaginal ovum pick up (OPU) was performed 34 h later. Collected oocytes were fertilized in vitro by IVF or ICSI (10,11). All patients received luteal phase progesterone support either as vaginal suppositories (600 mg daily) (Progestan; Organon) or i.m. injections of progesterone in oil (25 mg daily) (National Hospital Pharmacy, Oslo, Norway), starting the day of OPU and lasting for 14 days.

Embryo Culture

At approximately 18 h after insemination, oocytes were scored for the presence of pronuclei. Oocytes with normal fertilization were cultured further in a commercially available culture medium (Universal IVF Medium; Medicult, Copenhagen, Denmark). It is the policy of our laboratory to culture each embryo separately to provide us with the opportunity to follow the developmental progression of individual embryos at 24 h (day 2) and 48 h (day 3) after observation of pronuclei. On day 3, a maximum of two embryos was selected for transfer on the basis of cleavage stage and morphology. Morphological assessment of embryos prior to transfer was performed as follows: grade 1.0—equally sized blastomeres and no fragments; grade 2.0—uneven sized blastomeres and no fragments; grade 2.1—embryos with <10% blastomeric fragmentation; grade 2.2—embryos with 10–20% blastomeric fragmentation; grade 3.1—20–50% blastomeric fragmentation; grade 3.2—>50% blastomeric fragmentation (12). When selecting embryos for transfer, blastomere number was given the greatest emphasis. Hence, an embryo including the 8-cell stage was preferred to an embryo with lower degree of fragmentation, but fewer blastomeres. Embryos at the 4-cell stage were therefore solely transferred in the cases where no other embryos were available. Embryos which showed cleavage arrest or total fragmentation, were not transferred. Fourteen days after oocyte retrieval, plasma β -hCG was measured and a pregnancy was defined by β -hCG > 25 IU/L. Ultrasound evaluation 5 weeks post-OPU was performed to ensure the presence of an intrauterine gestational sac. All patients were monitored until pregnancy loss or delivery. Pregnancy loss included biochemical pregnancies, spontaneous abortions, legal abortions, and ectopics. The implantation rate was defined as the fraction of transferred embryos resulting in an implanted embryo.

Statistical Analysis

Prior to statistical analysis, continuous data were tested for Gaussian distribution and then analyzed by Student's *t* test or by the Mann–Whitney test as data were found to be normally distributed or not. Although the standard deviations were not used in nonparametric statistics, they have been appended to the mean values in the tables. Categorical data was analyzed by χ^2 test. Differences were considered significant at $p < 0.05$.

RESULTS

Patient Characteristics

The 138 embryo transfers in the study group were performed in 126 patients. In the control group, the 282 transfers were performed in 263 patients (Table I). No differences among patients in the study group and control group were exposed concerning age, body mass index, primary versus secondary infertility, duration of infertility or type of treatment (IVF or ICSI) (Table I). However, the distribution of causes of infertility was different. There were fewer tubal infertility cases and more ovarian endometriosis cases in the study group compared to the control group (Table I).

Characteristics of the Cycles

In the study group, the total dose of FSH (IU) per treatment cycle was significantly higher and the du-

Table I. Characteristics of Patients in Study Group (Day 3 Transfer with 4-Cell Embryos Only) and Control Group (Day 3 Transfer with 8-Cell Embryos Only)

Variable	Study group	Control group	<i>p</i> value
Patients (<i>n</i>)	126	263	
Age (years)	32.7 ± 3.6	32.3 ± 3.5	ns
Body mass index	24.5 ± 4.7	23.5 ± 3.9	ns
Primary infertility (%)	77 (61)	149 (57)	ns
Duration of infertility (years)	6.1 ± 2.6	5.5 ± 2.1	ns
Type of treatment (IVF/ICSI)	80/46	179/84	ns
	<i>n</i> (%)	<i>n</i> (%)	
Infertility diagnosis			<i>p</i> < 0.05
Tubal factor	28 (22)	89 (34)	
Ovarian endometriosis	8 (6)	5 (2)	
Peritoneal endometriosis	16 (13)	23 (9)	
Unexplained	18 (14)	33 (13)	
Ovulation disorders	6 (5)	22 (8)	
Male	47 (37)	90 (34)	
Others	3 (3)	1 (0)	

Note. Values are given as means ± SD.

Table II. Ovarian Stimulation and Response in Study Group (Day 3 Transfer with 4-Cell Embryos Only) and Control Group (Day 3 Transfer with 8-Cell Embryos Only)

Variable	Study group	Control group	<i>p</i> value
No. ET	138	282	
Total dose of FSH (IU)/cycle	2439 ± 1091	1971 ± 743	<i>p</i> < 0.001
Duration of stimulation (days)	11.9 ± 2.8	11.2 ± 2.6	<i>p</i> < 0.01
No. of oocytes/retrieval	6.9 ± 3.9	10.1 ± 5.2	<i>p</i> < 0.001
No. of normal fertilized oocytes/retrieval	3.4 ± 2.0	6.3 ± 3.5	<i>p</i> < 0.001
No. of transferred embryos	1.56 ± 0.4	1.86 ± 0.3	<i>p</i> < 0.001
	<i>n</i> (%)	<i>n</i> (%)	
Morphology of transferred embryos ^a			<i>p</i> < 0.001
Grade 1	3 (1)	19 (4)	
Grade 2.0	22 (10)	81 (15)	
Grade 2.1	65 (30)	232 (44)	
Grade 2.2	55 (26)	143 (27)	
Grade 3.1	41 (19)	41 (8)	
Grade 3.2	30 (14)	9 (2)	

Note. Values are given as means ± SD.

^aGrade 1—equally sized blastomeres and no fragments; Grade 2.0—uneven sized blastomeres and no fragments; Grade 2.1—embryos with <10% fragments; Grade 2.2—embryos with 10–20% blastomeric fragmentation; Grade 3.1—20–50% blastomeric fragmentation; Grade 3.2—>50% blastomeric fragmentation.

ration of stimulation significantly longer, while the number of oocytes, zygotes, as well as the number of transferred embryos was lower compared to the control group (Table II).

Furthermore, there was a significantly higher degree of fragmentation in embryos transferred in the study group compared to the control group and 33% of transferred embryos in the study group had ≥20% fragmentation compared to 10% in the control group (Table II).

Outcome

The pregnancy and live birth rates were significantly lower in the study group and the pregnancy loss rate was increased (Table III). Hence, the live birth rate per transfer was 8% in the study group compared to 34% in the control group. The implantation and live birth rates per embryo transferred were significantly higher in the control group compared to the study group (Table III). The multiple birth rate was 31% in the control group and 9% in the study group. One 2-embryo transfer in the control group resulted in a triplet pregnancy. The difference in birth weight of singletons in the study group and control group (3181 g ± 1008 vs. 3408 g ± 580) was not significant.

Table III. Outcome of Transfers in Study Group (Day 3 Transfer with 4-Cell Embryos Only) and Control Group (Day 3 Transfer with 8-Cell Embryos Only)

Variable	Study group (%)	Control group (%)	<i>p</i> value
No. ET	138	282	
Pregnancy rate per ET	27/138 (19)	128/282 (45)	<i>p</i> < 0.001
Pregnancy loss rate	16/27 (59)	32/128 (25)	<i>p</i> = 0.001
Live birth rate per ET	11/138 (8)	96/282 (34)	<i>p</i> < 0.001
Implantation rate per embryo	28/216 (13)	158/525 (30)	<i>p</i> < 0.0001
Live birth rate per embryo transferred	12/216 (5)	127/525 (24)	<i>p</i> < 0.01
No. children born	12	127	
No. live births	11	96	
Singleton	10 (91)	66 (69)	
Twin	1 (9)	29 (30)	
Triplet		1 (1)	

Pregnancy loss (*n* = 16) in the study group comprised biochemical pregnancies or early abortions only. In the control group, pregnancy loss (*n* = 32) consisted of biochemical pregnancies or early- or late abortions (*n* = 29), legal abortions due to malformations of the fetus (*n* = 2) and one ectopic pregnancy.

The outcome was also analyzed comparing transfers with the same number of embryos; 1-embryo transfer in both the study group (*n* = 60) and the control group (*n* = 38) and 2-embryo transfers in both the study group (*n* = 78) and the control group (*n* = 244).

The outcome of the two comparisons was similar: the pregnancy and live birth rates were significantly lower and the pregnancy loss rate was increased in the study group compared with the control group (data not given). Hence, the outcome was similar to the outcome presented in Table III.

Within the group with only one embryo transferred, however, the observation numbers were low; 2–10 observations. For statistical reasons, therefore, further analysis between these very small groups of patients was not performed.

Characteristics of the Cycles in Relation to the Outcome of a Live Birth

Within the study group, embryo transfer cycles resulting in no live birth had significantly different profiles from cycles with a live birth. The total dose of FSH (IU) per treatment cycle was higher, while the number of oocytes retrieved and zygotes was lower (Table IV). No difference, however, was observed between patients in cycles with- or without a live birth concerning age (32.8 vs 32.7) or BMI (23.8 vs. 24.5).

Within the control group, no differences in embryo transfer cycle profiles between cycles with- or without a live birth were observed and these were similar to those of the study group cycles that resulted in a live birth (Table IV).

DISCUSSION

Day 3 transfers with developmentally lagging 4-cell embryos only (study group) were analyzed retrospectively and compared with day 3 transfers with 8-cell embryos only (control group). Characteristics of the patients and the cycles were analyzed and related to the outcome of a live birth.

Cycles resulting in transfer of 4-cell embryos only were characterized by a poor response to ovarian stimulation as the total dose of FSH per treatment was higher, while the number of oocytes, zygotes, as well as the number of transferred embryos was lower compared to the control group cycles. The implantation, pregnancy, and live birth rates were dramatically lower in the study group compared to the control group. However, within the study group, the few cycles resulting in a live birth were characterized by a normal ovarian response to stimulation, similar to control group cycles with- or without a live birth.

It is established that ovarian response to stimulation correlates with ovarian reserve and women with

Table IV. Ovarian Stimulation and Response in Relation to the Outcome of a Live Birth in Study Group (Day 3 Transfer with 4-Cell Embryos Only) and Control Group (Day 3 Transfer with 8-Cell Embryos Only)

Variable	Study group		Control group	
	No. live birth	Live birth	No. live birth	Live birth
No. ET	127	11	186	96
Total dose of FSH (IU) per treatment cycle	2491 ± 1112 ^a	1827 ± 510	1965 ± 799	1981 ± 624
No. of oocytes/retrieval	6.6 ± 3.7 ^a	9.9 ± 4.0	10.1 ± 5.6	10.2 ± 4.4
No. of normal fertilized oocytes per retrieval	3.2 ± 1.9 ^a	5.5 ± 2.1	6.1 ± 3.7	6.5 ± 2.9

Note. Values are given as means ± SD.

^a *P* < 0.05 (significantly different from study group cycles with a live birth and to control group cycles with- and without a live birth).

a declining ovarian function are known to respond poorly to ovarian stimulation and to have low pregnancy rates (13,14). In this analysis, the poor response to ovarian stimulation in the study group was not age related. Women above the age of 38, however, were usually not offered IVF treatment in our department as the implantation rate is known to decrease dramatically after the age of 38 (15).

The age-related decline in fertility may already start in the late twenties (16) and thus biological ovarian age may be more important than chronological age (17). Reduced biological ovarian age, therefore, could explain the poor outcome in the study group, characterized by a poor response to ovarian stimulation and developmentally lagging 4-cell embryos only available for transfer on day 3.

Within the study group, the few cycles resulting in a live birth were characterized by a normal response to ovarian stimulation. Hence, transfer of developmentally lagging 4-cell embryos on day 3 may result in a live birth in cycles characterized by a normal ovarian reserve. The reason for the slow cleavage of these viable embryos cannot be explained by this study. Not only the oocyte, but also the sperm may possibly affect the very early onset of human embryo development; fertilization with sperm from certain individuals has been shown to result in zygotes that tend to cleave slowly (18).

The distribution of causes of infertility varied between the study group and the control group. Tubal factor was the most frequent cause of infertility in the control group, while patients in the study group more often had a diagnosis of ovarian endometriosis and therefore, possibly impaired ovarian responsiveness (19,20). Diagnosis such as unexplained infertility may also represent an early loss of ovarian reserve (21).

In addition to a reduced number of recoverable oocytes due to reduced ovarian reserve, an impairment in oocyte quality may contribute to the poor outcome of the study group. The limited oocyte pool hypothesis, proposed by Warburton *et al.* (22), states that an oocyte in a suboptimal state of development could become the dominant follicle because of the small number of oocytes available. Hence, the quality of the recovered oocytes, the resulting embryos and the outcome of those embryos that do implant, may be impaired. In agreement with this hypothesis, we observed a highly reduced implantation rate per embryo in the study group. Furthermore, the likelihood that an implanted 4-cell embryo would result in a live birth was significantly decreased.

The high rate of pregnancy loss observed in the study group may be due to an increase in chromosomal anomalies in the developmentally lagging 4-cell embryos. Altered maternal meiotic spindle formation and meiotic nondisjunction is thought to be the key factor for the increased aneuploidy rate in oocytes, reflecting depletion of the primordial follicle pool (23–26).

The selection for embryo transfer is important to reduce the possibility of replacing a chromosomally abnormal embryo. Embryo cleavage rate and morphology may indicate the chromosomal status of the embryo. In this analysis, when embryos were selected for transfer, cell number was given the greatest emphasis and 4-cell embryos, therefore, were transferred only when other options were not available. The 4-cell embryos, however, showed a significantly higher degree of fragmentation than 8-cell embryos in the control group. Hence, a correlation between embryo fragmentation and developmental competence was observed. Only 67% of transferred 4-cell embryos, in contrast to 90% of transferred 8-cell embryos, had less than 20% fragmentation, a fragmentation burden which has most likely no effect on human embryonic development (27).

In conclusion, embryos derived from IVF or ICSI have different potential for implantation and live birth. The chance for a live birth is dramatically low after day 3 transfer of 4-cell embryos in cycles characterized by intensive ovarian stimulation, but poor response. As ovarian response to stimulation is well known to correlate to ovarian reserve, declining ovarian function may explain the poor outcome in the study group. Therefore, maximizing the stimulation protocol could not possibly compensate for the poor outcome, as the impaired quality of the recovered oocytes and resulting embryos is attributed to the diminished ovarian reserve.

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