Embryo Selection by the First Cleavage Parameter Between 25 and 27 Hours After ICSI

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Purpose: *The objective of this study was to assess first embryo cleavage (FEC) 25–27 h after intracytoplasmic sperm injection (ICSI) as a parameter for the embryo selection process.*
Methods: *From January 1998 to December 1999, a total of 670 patients were subjected to the ICSI programme at the Centre for Human Reproduction, Sinhá Junqueira Maternity Foundation, and the FEC parameter was evaluated in three situations.*
Results: *In the first, a total of 300 zygotes were analyzed on the basis of a score (16–18 h after ICSI) and observed for the presence or absence of FEC (25–27 h after ICSI). A significant ($p < 0.02$) presence of FEC was observed in zygotes with a score of 15 (ideal score). In the second, a total of 200 patients were selected and divided into two groups matched for age and laboratory performance. Group I ($n = 100$) was subjected to transfer of embryos with the absence of FEC only (since in this cycle no embryos with FEC were detected within 25–27 h after ICSI) and Group II ($n = 100$) was subjected to transfer of embryos with the presence of FEC only. The age of Group I patients (33.8 ± 4.2 years) did not differ significantly ($p = 0.50$) from that of Group II patients (33.5 ± 4.3 years). The number of embryos transferred was similar ($p = 0.07$) for Group I (2.7 ± 1.1) and Group II (2.9 ± 0.88). In Group II, the 17.5% implantation rate was significantly higher ($p < 0.01$) than the 5.9% rate obtained for Group I. The pregnancy rate for Group II was significantly higher ($p < 0.01$) (33%) than that for Group I (12%). The incidence of abortion was 16.6% in Group I as compared with 6% in Group II. In the third situation, we observed the frequency of embryos with FEC in 36 patients whose implantation rate was 100% (ideal result) and obtained a value of 82%.*
Conclusions: *The data suggest that the presence of the FEC parameter that was evaluated 25–27 h after ICSI could be used to select embryos with a higher implantation power. The data reported here may justify routine analysis of embryos with FEC for the process of embryo selection after ICSI.*

KEY WORDS: Embryo selection; first cleavage; ICSI; implantation rate; pregnancy.

INTRODUCTION

The efficiency of the embryo implantation process is directly linked to the ability to produce and select the best embryos in culture. Several groups currently state that increasing the time of embryo permanence in vitro and selecting them during the blastocyst phase may yield better results than those obtained by the common selection and transfer of embryos on Day 2 or Day 3 (1). Embryo selection on Day 2 or Day 3 is based on morphological observation (2,3), although other groups use cleavage stage (day two or three) only (4,5,6) or the combination of these two parameters assessed on the basis of a score system (7,8,9).

In 1997, Shoukir *et al.* (10) proposed a new morphological evaluation based on the time of identification of first cell division. Thus, embryo selection in a programme of in vitro fertilization (IVF) was based on the presence or absence of early cleavage in the two cell-stage 25–26 h after oocyte insemination.

In 1998, Sakkas *et al.* (11), using a similar criterion (early cleavage by 27 h) for embryo selection after intracytoplasmic sperm injection (ICSI), observed a significant increase in implantation rates in a group with early embryo cleavage (14%) as compared with a group with absence of cleavage (3.2%). However, when there were only one or two early cleavage embryos, the embryo transfer group was completed by

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adding the best one or two embryos from the no early cleavage group.

The objective of the present study was to analyze the power of the first embryo cleavage (FEC) parameter evaluated 25–27 h after ICSI for the identification of embryos with a potential for implantation.

MATERIAL AND METHODS

Patient Selection

From January 1998 to December 1999, a total of 670 patients were subjected to the ICSI programme at the Centre for Human Reproduction, Sinhá Junqueira Maternity Foundation. During this period, the criterion for embryo selection for transfer on Day 2 was preferentially based on FEC identification, 25–27 h after ICSI. This parameter was analyzed in three situations:

I. Analysis of zygote characteristics versus later presence or absence of FEC.

   A total of 300 zygotes were evaluated 16–18 h after sperm injection, assigned a partial Scott and Smith (12) score, and then observed for the occurrence of FEC. The score consisted of 5 points for the presence of joined or aligned pronuclei, 5 points for polarized nucleoli, and 5 points for the presence of a heterogeneous cytoplasm with a clear halo at the extremity.

II. Analysis of clinical and laboratory data concerning patients subjected to transfer of embryos with the presence of FEC only or embryos with the absence of FEC only.

   Among the 670 patients subjected to the ICSI programme, 100 (Group I) were subjected to transfer of embryos with the absence of FEC only (since no embryo observed during the cycle analyzed presented FEC 25–27 h after ICSI). This population was then matched for age to 100 patients (Group II) who were subjected to transfer of embryos with the presence of FEC only.

III. Analysis of the frequency of embryos with FEC among patients with a 100% implantation rate.

   Among the 670 patients subjected to the ICSI programme, 36 had a 100% embryo implantation rate (ideal embryo pattern). The frequency of embryos with the presence of FEC was evaluated in this population.

Ovarian Stimulation

For ovarian stimulation, the second phase was blocked with leuprolide acetate at the dose of 0.5 mg/day (Lupron, Abbott). With blockade established 14 days after the use of the analogue, treatment with FSH (Gonal-F, Serono, Brazil) was started at a fixed dose of 150 IU or 225 IU for a period of 7 days. On the eighth day of ovarian stimulation, monitoring of follicular development was started using transvaginal ultrasonography and the FSH dose was adapted to the ovarian response. When at least one follicle measuring $\geq$17 mm was observed, human chorionic gonadotropin (hCG) was administered at the dose of 10,000 IU. Oocytes were retrieved by vaginal ultrasound and follicle puncture was performed 34–36 h after hCG administration.

ICSI Procedure

After identification in the follicular fluid, the oocytes were classified for maturity. The cumulus-corona complex was removed by exposure to a Type IV hyaluronidase solution (H-4272, Sigma Chemical, CO,USA). Denuded oocytes were incubated in IVF 50 medium (Scandinavian IVF Science AB, Sweden) until the time for ICSI. Semen was washed using a discontinuous Sperm-prep100 TM (Scandinavian IVF Science AB, Sweden) prepared with 40 and 90% fractions. ICSI was performed according to the method of Svalander *et al.* (13).

The injected oocytes were observed for 16–18 h (Day 1) after ICSI to determine the absence or presence of pronuclei (evaluated on the basis of a partial Scott and Smith (12) score). The embryos were examined for the presence or absence of FEC (2-cell stage) 25–27 h after ICSI. On Day 2, the embryos were evaluated for number, symmetry, and fragmentation of blastomeres and transferred. In all cases, the FEC embryos were transferred when available.

The pregnancy test was performed on the 14th day after transfer and clinical pregnancy was confirmed during the sixth week by the presence of a gestational sac and an embryo with a heart beat. Data were analyzed statistically by the Whitney and Fisher tests.

RESULTS

Table I shows that zygotes with a score of 15 (16–18 h after ICSI) later presented a significant ($p < 0.02$) presence of FEC (25–27 h after ICSI).

Table II presents the clinical and laboratory data for the patients who received only embryos with the

Table I. Relationship Between Zygote Score (ZS) and the Presence or Absence of FEC

ZS	No of embryos	
	Presence of FEC	Absence of FEC
15	28	19
<15	102	151

Note. $p < 0.02$

absence of FEC (Group I = 100 patients) versus those obtained for the patients who received only embryos with the presence of FEC (Group II = 100 patients). The age of Group I patients (33.8 ± 4.2 years) did not differ significantly ($p = 0.50$) from the age of Group II patients (33.5 ± 4.3 years). The number of embryos transferred was similar ($p = 0.07$) for Group I (2.7 ± 1.1) and Group II (2.9 ± 0.88). The 17.5% implantation rate for Group II was significantly higher ($p < 0.01$) than the 5.9% rate for Group I. The pregnancy rate was significantly higher ($p < 0.01$) for Group II (33%) than for Group I (12%). The incidence of abortion was similar ($p = 0.26$) for Group I (16.6%) and Group II (6%).

Table III shows the clinical and laboratory characteristics of the population of 36 pregnant women with a 100% implantation rate. The frequency of embryos with FEC in this population was 82%.

DISCUSSION

In 1998, Scott and Smith (12), in a retrospective analysis of 114 patients subjected to IVF, observed that a score based on the characteristics of the zygote (joined pronuclei, aligned nucleoli, and cytoplasmic halo) and on the presence of early cleavage could be used for early identification of embryos that reached an implantation rate of 28%. In 2000, Ludwig *et al.* (14) analyzed 74 ICSI cycles partially, using the scoring system of Scott and Smith (12) (not including the first cleavage division). The data obtained revealed a 4% pregnancy rate when the mean score for the zygotes was <13 and a 22% pregnancy rate when the mean score for the zygotes was ≥13. On the other hand, our data demonstrated a significant relationship between zygotes with a score of 15 (16–18 h after ICSI) and the identification of FEC 25–27 h after ICSI. This fact suggests that embryos with high scores (with a greater implantation power) during the zygote phase should present their first cell division 25–27 h after ICSI. On the other hand, morphological criteria are usually employed in ICSI programmes for the selection of the best embryos for transfer, such as early cleavage (11) or the quality and stage of embryo cell division (15). In 1997, Shoukir *et al.* (10) proposed a new method for embryo selection during a period of 25 h after oocyte insemination. There was a significant (0.04) increase in clinical pregnancy rate (33.3%) in the group with early cleavage as compared with the group with absence of cleavage (14.7%). Sakkas *et al.* 1998 (11) selected embryos in an ICSI programme on the basis of early cleavage to the two-cell stage 27 h after sperm injection. A total of 88 cycles were studied (34 of them with the absence of cleavage and 54 with the presence of cleavage) and an implantation rate of 14% was obtained for the group with early cleavage and 3.2% for the group without early cleavage. The clinical pregnancy rate was significantly higher (0.04) in the group with early cleavage (25.9%) compared with the group without early cleavage (5.9%). There was no difference between the two situations in terms of the following parameters: age, stimulation protocol, and sperm characteristics. However, these investigators routinely transferred a maximum of three embryos but when only one or two embryos with early cleavage were formed, the ideal number of embryos was completed by the addition of one or two of the best noncleaved embryos. In contrast, in the present study the groups were pure, that is, Group I was subjected to transfer of embryos with the absence of FEC only, and Group II was subjected to transfer of embryos with the presence of FEC only.

Despite these differences, our data show that the implantation rate (17.5%) was significantly higher ($p < 0.01$) among the patients subjected to transfer of

Table II. Laboratory and Clinical Data of the Patients Who Received Only Embryos With the Absence of FEC (Group I) or With the Presence of the FEC (Group II)

	Group I	Group II	*p*
No of patients	100	100	
Age	33.8 ± 4.2	33.5 ± 4.3	0.50
No of embryos transferred	2.7 ± 1.1	2.9 ± 0.88	0.07
Implantation rate	5.9%	17.5%	<0.01
No of clinical pregnancies	12	33	
Clinical pregnancy rate	12%	33%	<0.01
Abortions	16.6%	6%	0.28

Table III. Frequency of Embryos With FEC in a Patient Population With a 100% Implantation Rate

Patients	36
Age	31.1 ± 4.0
Total number of embryos transferred	78
Total number of embryos implanted	78
Mean number of embryos transferred	2.17 ± 0.84
FEC	82%

embryos with the presence of FEC only than among the patients subjected to transfer of embryos with the absence of FEC only (5.9%). Similarly, the clinical pregnancy rate was significantly different ($p < 0.01$) between the two groups (presence of FEC = 33%; absence of FEC = 12%). The mean number of embryos transferred did not differ significantly between groups.

Analysis of the patients with 100% implantation rates demonstrated that the frequency of embryos with FEC was 82%. This fact demonstrates a strong correlation between the identification of the presence of FEC and an ideal embryo performance.

Thus, the present data suggest that analysis of the presence of FEC 25–27 h after ICSI identifies embryos with a greater implantation power. However, few groups routinely include this parameter in embryo selection on Day 2 or Day 3.

In the process of embryo selection, the morphological parameters are evaluated in a separate manner (16,17,18) or are grouped into scores (7,8,9). However, these analyses are transverse and almost always reflect the situation of the embryo at the time of transfer (Day 1, Day 2, or Day 3). Theoretically, an efficient early embryo selection should include a score of the ideal morphological characteristics based on a longitudinal analysis (e.g., I—time: 16–18 h; zygote (joined pronuclei, aligned nucleoli, and cytoplasmic halo), II—time : 25–27 h; first embryo cleavage; III—time : 48 h; 4 cells (blastomere symmetry, $\leq 20\%$ fragmentation, absence of multinucleation); IV—time: 72 h 8 cells (blastomere symmetry, <20% fragmentation, absence of multinucleation). Thus, morphological scores based on longitudinal information may give the embryologist a better view of the real "health" conditions of the embryo on the day chosen for transfer, in which the analysis of the FEC parameter 25–27 h after ICSI should be obligatory.

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