A multicenter prospective study to assess the effect of early cleavage on embryo quality, implantation, and live-birth rate

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Objective: To investigate the impact of early cleavage (EC) on embryo quality, implantation, and live-birth rates.
Design: Prospective cross-sectional study.
Setting: Multicenter study.
Patient(s): Seven hundred embryo transfers and 1,028 early-stage human embryos.
Intervention(s): None.
Main Outcome Measure(s): Implantation according to the presence of EC and embryo quality.
Result(s): The presence of EC is associated with embryo quality, especially in cycles with autologous oocytes. However, the use of EC as an additional criterion for selecting an embryo for transfer does not appear to significantly improve likelihood of implantation. Furthermore, embryos that presented EC had live-birth rates per implanted embryo similar to those that did not show any sign of cleavage.
Conclusion(s): At least for conventional embryo culture and morphologic evaluations, the additional evaluation of EC in embryos may not be valuable to improve embryo implantation. (Fertil Steril® 2014;101:981–7. ©2014 by American Society for Reproductive Medicine.)
Key Words: Early cleavage, embryo quality, implantation, live-birth rates

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Early cleavage, understood as the first embryo mitosis at 25–27 hours after insemination, has been considered to be an embryo quality parameter (1–9). Over the past decade, numerous studies have associated its presence with embryonic morphology on days 2 and 3 (1–4), development until the blastocyst stage (5), chromosome anomalies (6), embryo viability (7–9), implantation rate (2, 10), and abortion rate (11). However, the conclusions drawn are too contradictory to establish their use. Despite that, many publications advise using early cleavage (EC) as a "secondary parameter" to decide between embryos of similar quality.

More recently, however, time-lapse studies demonstrate that the EC time variable does not have sufficient predictive value to help embryo selection (12). Therefore, other more novel variables, such as first cytokinesis duration (13), the time when the embryo has five cells, or the synchrony between the second and third mitotic embryo cleavage, seem to be more important when predicting evolution for the blastocyst stage. Strangely enough, they are unable to forecast blastocyst morphology.

The Istanbul consensus group leaves to the laboratory the decision of whether or no to include the EC

Received March 5, 2013; revised December 19, 2013; accepted December 20, 2013; published online February 4, 2014.
M.J.d.l.S. has nothing to disclose. G.A. has nothing to disclose. A.B. is employed by and has had travel expenses paid by Somdex. G.C. has nothing to disclose. J.C. has nothing to disclose. M.V.H.d.M. has nothing to disclose. M.M. has nothing to disclose. R.H. has nothing to disclose. A.O. has nothing to disclose. C.P. has nothing to disclose. J.T. has nothing to disclose. M.A.V. has nothing to disclose. M.J.F. has nothing to disclose.
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Fertility and Sterility® Vol. 101, No. 4, April 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.12.043

variable in embryo selection (14). In this context, the Spanish Association of Reproduction Biology Studies (ASEBIR) considered conducting a multicenter study with several Spanish centers to evaluate the effect of this variable on embryo quality and implantation capacity to add, or not, the use of this variable to our recommendations for embryo selection.

MATERIALS AND METHODS

Study Patients

A multicenter prospective study, promoted by ASEBIR, was carried out from January to June 2011. Twenty centers initially participated in this study, which included 780 embryo transfers and 2,076 embryos. The participation of all interested centers was anonymously requested through the ASEBIR website and e-mail address. Institutional Review Board approval was obtained. The inclusion criteria were first or second in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles with autologous or donor oocytes. Implantation rates were calculated from those embryos originating from cycles with a 100% or a 0% implantation rate, or from homogenous embryo transfers for EC, that is to say, those embryos which, despite presenting a different evolution on later embryo development days, were similar in morphology terms when considering the EC parameter. Embryo transfers were done on both day 2 and day 3. After eliminating any incorrectly enterered implantation data, the sample size was 700 transfers and 1,028 embryos with identified implantation.

Evaluating Early Cleavage

The EC parameter was established at 25–27 hours after insemination by determining the following stages: visible pronuclei, syngamy, or 2- or 3-cell cleavage.

In this interval, the embryos with two cells were classified as EC embryos, and could present two cells or more (2C, >2C). Those that had not divided were classified as non–early-cleavage embryos (Non-EC).

Day 2 and Day 3 Embryo Morphology

On days 2 and 3, embryos were evaluated at 43–45 hours and 63–65 hours after IVF or ICSI, respectively. Embryo quality was determined based on the number of blastomeres (0, 1, 2, 3, 4, 5, 6, >6), the percentage of fragmentation (<10%, 11%–25%, 26%–35%, >35%), blastomere symmetry (equal, similar, different), vacuoles (absent, scarce and/or diameter <5 μm, abundant), the zona pellucida (normal;-abnormal), and the presence of multinucleated cells. The day 2 embryos were classified into four categories (A, B, C, D), where category A gave the best and category D the worst prognosis for a combination of the various aforementioned morphologic parameters. To classify the day 3 embryos, all four categories were assigned according to the evolution of the embryos from day 2 to day 3 (14).

Culture Conditions System

Embryo culture was performed under CO_2 concentration ranging from 5% to 6% CO_2 in air. Three different types of culture media were also used: Global, Sage, and Vitrolife.

Statistical Analysis

In order to determine variability among the participating centers, the groups participating in the multicenter study first did an external consistency test to evaluate the homogeneity among groups regarding fertilization, EC, and the ASEBIR morphologic classification.

All of the centers assessed a video containing 25 films on the embryonic development of 25 embryos from ICSI to 65 hours after ICSI. This video stated the time since insemination so that the participating users could analyze the images within the requested time ranges; based on this, fertilization was evaluated, as were the embryonic evolution parameters (i.e., EC) and the remaining embryo morphological parameters on days 2 and 3 (number of cells, fragmentation, symmetry, vacuoles, zona pellucida, and multinucleation). This video came with a data collection document in which the embryonic evaluation data were stored. The ranges set to observe different events were 17–19 hours for fertilization, 25–27 hours for EC, 43–45 hours for day 2, and 63–65 hours for day 3. The consistency index among the participating centers for all of the evaluations was measured by kappa statistics. Values of ≥ 0.6 were considered to be good.

To make a comparison between the groups of dichotomous variables, a χ^2 test was used. Implantation rates were expressed as percentage probabilities with 95% confidence interval (CI). The effect of other covariates (i.e., the ASEBIR embryo scoring system, day of embryo transfer, age range, and oocyte insemination type) on implantation was assessed by a forward logistical regression analysis. A power analysis calculation for the raw EC data was also performed by means of the Statistical Power Calculator Tool Kit on the DSS Research web page (www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx.)

RESULTS

The etiologies of the studied cycles were distributed as follows: age in 27.2%, endometriosis in 7.1%, infertility of unknown origin in 23.7%, male factor in 28.6%, tubal factor in 4.2%, ovarian failure in 1.9%, and polycystic ovary in 5.3%. The insemination techniques used were IVF in 7.2%, ICSI in 77.8%, and mixed IVF/ICSI in 14.3%.

Of all the transfers, 35.9% (n = 251) corresponded to day 2 and 64.1% (n = 449) to day 3; 29.6% (n = 207) of the embryo transfers were done with one embryo, 63.7% (n = 446) with two embryos, and 6.7% (n = 47) with three. Of all 700 transfers, 443 were cycles with autologous oocytes and 257 with donor oocytes.

Measuring Consistency among Centers

After evaluating each analyzed parameter, a satisfactory consistency index for each consulted parameter was found among the groups: fertilization, EC, and ASEBIR embryo