Is the Timing of Implantation Affected by Zona Pellucida Micromanipulation?

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Purpose: Our purpose was to examine the timing of implantation and early embryo development following uterine transfer of oocytes/embryos previously subjected to zona pellucida micromanipulation.

Methods: A total of 68 singleton pregnancies resulting from IVF and embryo transfer with/without micromanipulation. Patients were divided into four groups according to the type of micromanipulation technique: assisted hatching, embryo biopsy, intracytoplasmic sperm injection, and no micromanipulation (control group). Serial serum β -hCG levels were measured between 10 and 25 days after fertilization and log-transformed. Linear regression analyses were performed and extrapolated to $\text{hCG} = 10 \text{ mIU/ml}$ (hCG_{10}) to estimate detectable implantation. The slopes of the regression lines were used to estimate the rising speed of hCG, an indirect sign of embryo development.

Results: There were no significant differences among groups with respect to hCG_{10} , the slopes or intercepts of the regression lines.

Conclusions: Various oocyte/embryo microsurgical procedures used in ART involving zona pellucida manipulation do not appear to affect the timing of implantation or early embryo development.

KEY WORDS: hCG; implantation; micromanipulation; zona pellucida.

INTRODUCTION

The serial determination of serum β -human chorionic gonadotropin (hCG) levels in maternal blood is a valu-

able marker to predict pregnancy outcome. The appearance of hCG in maternal serum has been previously used to assess the time of clinically detectable implantation (1–4). Furthermore, because hCG production is a marker of trophoblastic activity, its serum doubling time has also been used as an indicator of embryo quality (5,6). We have previously reported that in ART patients subjected to various ovarian stimulation protocols, using gonadotropins with and without GnRHa adjunct therapy, there were no differences in implantation time or embryo quality as assessed by serum hCG levels (7). We then suggested that embryo development in early pregnancy follows a preprogrammed-timing schedule that depends mainly on the embryonic age of the healthy, successfully implanted conceptus (7).

In the present study we used a similar approach to answer the question: Do the various oocyte/embryo micromanipulation procedures performed in ART have an impact on the timing of implantation and early embryo development? In a previous report, assisted hatching was found to facilitate earlier implantation (8). In addition, some singleton pregnancies established following embryo biopsy performed for preimplantation genetic diagnosis were found to have lower initial hCG serum levels which rose steeply to match normal pregnancies by 16 days after oocyte recovery (9).

Here we examined 68 singleton pregnancies achieved by IVF and embryo transfer. Cases were selected from our computerized data based on the establishment and delivery of a singleton pregnancy and with a minimum of two determinations of serum hCG levels between 10 and 25 days after fertilization. Four groups were analyzed: patients who had assisted hatching, embryo biopsy, intracytoplasmic sperm injection (ICSI), and no micromanipulation (control group). The serum levels of β -hCG were measured from 10 to 25 days after fertilization and log-trans-

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formed; linear regression analyses of the log values of hCG versus the days after fertilization were performed and compared for the different groups. The regression lines were extrapolated to hCG = 10 mIU/ml, which was used as an estimate of detectable implantation (1–4,7). The slope of each regression line was used to estimate embryo development (5–7).

MATERIALS AND METHODS

These studies were performed following approval of the Institutional Review Board of Eastern Virginia Medical School. A total of 68 patients who achieved a normal singleton pregnancy resulting in a live birth at the Jones Institute for Reproductive Medicine and who were treated between January 1993 through December 1997 were studied. The inclusion criteria were (a) delivered singleton pregnancies with a single intrauterine gestational sac detected by ultrasound at 6–8 weeks of gestation, (b) 10,000 IU hCG (Steris Labs, Phoenix, AZ) was administered for triggering ovulation in all cases, (c) all patients received 50 mg/day of progesterone in oil i.m. for luteal support starting the day of embryo transfer and no extra hCG supplementation was given, (d) all patients had a previous normal uterine evaluation based on hysterosalpingogram and endometrial biopsy, and (e) all patients had a minimum of two determinations of serum hCG levels between days 10 to 25 following fertilization (day of fertilization was estimated to be the day of oocyte retrieval and insemination).

Controlled ovarian stimulation was accomplished by a combination of a GnRHa (Lupron, Tap Pharmaceuticals, Abbott Park, IL) started in the luteal phase and follicle-stimulating hormone [(FSH) Metrodin or highly purified FSH (Fertinex), Serono Laboratories, Randolph, MA]. Gonadotropin doses were adjusted in an individualized fashion and continued until the day of hCG administration. Uterine transfer of embryos was performed approximately 70–72 hr post-sperm injection or insemination (day 3 transfers) except for the embryo biopsy cases, where transfer was performed on day 4. Four groups of patients were studied.

The first group included patients where assisted hatching was performed. The indications for performing assisted hatching in this group of women were maternal age ≥ 36 and/or implantation failure following two or more previous embryo transfer attempts. The hatching procedure was performed on the evening of day 2, about 55 hr after insemination. The hatching technique employed the release of acidified Tyrode's

medium (pH 2.5) against the zona pellucida to create an opening about 20 μm in diameter in four-cell embryos (10). The second group included patients in whom embryo biopsy was performed for the purpose of pre-implantation genetic diagnosis (two cases of Tay–Sachs disease and one each of cystic fibrosis and X-linked muscular dystrophy). The embryo biopsy was carried out on day 3 after insemination and one or two blastomeres were biopsied from the 6- to 10-cell embryos through a zona pellucida opening of approximately 40 μm (11).

The third group of patients included cases where intracytoplasmic sperm injection (ICSI) was performed due to either the presence of a severe male factor or previously failed fertilization. In these cases, a 10- μm opening of the zona pellucida was performed at the time of sperm injection (12). The fourth group of patients represented the control subjects where IVF and embryo transfer was performed in the absence of any micromanipulation technique. The assisted hatching, embryo biopsy, ICSI, and IVF laboratory techniques used have been previously described in detail (10–13).

Quantitative measurement of serum β -hCG was performed with the IMX Total β -hCG assay [a microparticle enzyme immunoassay (MEIA); Abbott Laboratories, Abbott Park, IL]. The interassay and intraassay coefficients of variation have remained 4.6 and 6.4%, respectively, throughout the study period (7). Basal cycle serum FSH levels were also measured with the IMX-MEIA (Abbott) as reported previously (13).

Statistical Analysis

A simple linear regression model can provide a best estimate for log-transformed hCG levels at a particular gestational age (5). Serum hCG values were measured between 10 and 25 days after oocyte retrieval. Within this range, the log hCG–time relationship can be treated as log-linear (6). The regression analysis was performed taking into account hCG data as repeated measures (7). Two reference points were set to analyze the increasing level of hCG in early pregnancy: (a) fertilization day (D_0 ; day = 0)—fertilization was defined as the day of oocyte retrieval and insemination; and (b) hCG₁₀—the day of hCG = 10 mIU/ml postconception, was determined by the extrapolation of the regression line of log-hCG versus time. hCG₁₀ was used to estimate the day of clinical detectable implantation. The slope (S) of the regression line is an estimate of the rising speed of hCG and treated as a maker of embryo quality. Data were analyzed as dependent

Fig. 1. Regression of log hCG on cycle day (day 0 = day of fertilization) and their respective 95% confidence intervals for assisted hatching and IVF (control) groups.

observations. The methodology of this analysis has been discussed previously (7).

Analysis of variance was used where appropriate. Results are presented as mean $\pm$ standard deviation.

RESULTS

Table I presents number of patients examined per group, females' age, basal serum FSH levels, and number of embryos transferred. Groups were well matched regarding basal cycle day 3 serum FSH levels and number of embryos transferred. The average age was significantly higher in the assisted hatching group ($P < 0.01$).

Table I. Characteristics of the Cycles of the Four Groups of ART Patients Examined^a

Group	<i>n</i>	Age	D ₃ FSH	No. ET	hCG data
Assisted hatching	20	37.7 $\pm$ 2.0*	8.4 $\pm$ 2.7	4.2 $\pm$ 1.3	46
Embryo biopsy	4	32.7 $\pm$ 4.5	8.8 $\pm$ 2.8	4.0 $\pm$ 0.8	8
ICSI	22	34.1 $\pm$ 4.2	6.3 $\pm$ 2.0	3.9 $\pm$ 1.2	47
Control	22	34.4 $\pm$ 3.4	7.2 $\pm$ 1.5	4.0 $\pm$ 0.9	53

^a *n*, number of patients per group; D₃ FSH, basal cycle day 3 serum FSH (mIU/ml); ET, number of embryos transferred; and hCG data, number of data points between 10 and 25 days after fertilization.

* Significantly different from control ($P < 0.01$).

Table II presents the estimated day the log of hCG equaled 1 (hCG₁₀) and the slopes and intercepts of the regression lines. The day of the log hCG equaled 1 (hCG₁₀) and the slopes and intercepts of the regression lines were not significantly different among groups. Although hCG₁₀ was a day earlier in the assisted hatching group compared to the controls, this difference did not attain significance (Fig. 1). This study had 80% power to detect intercepts in the assisted hatching and ICSI groups that were greater than in the control group by a factor of about 2 (i.e., an intercept of -0.6).

DISCUSSION

This study was designed to determine whether micromanipulation techniques performed in ART affect implantation and early embryo growth. In order

Table II. Days from Fertilization to Detectable Implantation (hCG₁₀) and Regression Coefficient for hCG Measurements in the Various Groups of Patients Assessed

Group	hCG ₁₀ ^a	Slope ^a	Intercept ^a
Assisted hatching	9.2	0.200	-0.837
Embryo biopsy	10.8	0.217	-1.345
ICSI	9.4	0.202	-0.910
Control	10.2	0.225	-1.293

^a Not significant.

to answer these questions, we performed regression analyses of log-transformed hCG levels in maternal serum obtained between 10 and 25 days after oocyte retrieval (estimated to be the day of fertilization). Within this range, the log hCG–time relationship can be treated as log-linear and offers a good approach for a global analysis of embryonic growth during early stages postconception. Furthermore, extrapolation to hCG₁₀ can be used as an estimate of detectable implantation.

In a previous study, we reported that implantation and postimplantation development of embryos was not affected by the type of ovarian stimulation protocol (gonadotropins with/without concomitant GnRHa in IVF/embryo transfer or IUI therapies) or by uterine transfer of embryos performed days 2 or 3 postfertilization (7). Here we demonstrated that various oocyte/embryo micromanipulation procedures used in ART appear to have no impact on the day of detectable implantation or early embryonic growth as assessed by the analysis of serum hCG patterns.

Liu *et al.* (8) communicated that implantation occurred significantly earlier in couples subjected to assisted hatching compared to standard IVF. These authors concluded that assisted hatching may enhance embryo implantation not only by mechanically facilitating the hatching process but also by allowing earlier embryo–endometrium contact. Their report was based on the assessment of the day in which hCG appeared in the serum. Although in our study implantation occurred approximately 1 day earlier in the assisted hatching group compared to the control group, this difference was not statistically significant. Here, only singleton pregnancies were analyzed and clinically detectable implantation was determined by regression analysis of serum hCG levels between day 10 and day 25 followed by extrapolation to hCG₁₀.

Soussis *et al.* (9) reported on eight singleton pregnancies resulting from embryos biopsied for preimplantation genetic diagnosis. Although they occasionally observed reduced levels of circulating hCG (probably as a result of a reduction in cell mass following embryo biopsy), these authors concluded that such pregnancies behaved similarly to control IVF pregnancies (by serum hCG and ultrasound criteria). Our results are in agreement with these findings. Albeit with a smaller sample size, we demonstrated here that singleton pregnancies following embryo biopsies show similar clinically detectable implantation and embryo growth curves compared to nonmicromanipulated embryos (IVF controls).

It has been demonstrated that implantation and overall pregnancy outcome following ICSI are similar to IVF. Oocyte micromanipulation and the use of spermatozoa obtained from infertile men with a variety of abnormal semen parameters do not seem to affect outcome (12,14–16). Nevertheless, the potential for the transmission of chromosomal/genetic disease is present (17–20). The present analysis corroborated that the timing of implantation and early embryo growth are not affected by ICSI.

The oocyte/embryo micromanipulation methods compared in the present study are methodologically different; they involve a variety of microsurgical interventions at the level of not only the zona pellucida but also the oolemma, ooplasm, and blastomere, and are also divergent in their temporal relationships with fertilization. The procedures create zona pellucida openings of various diameters using various pipettes and chemical conditions that may affect oocytes/embryos in a dissimilar fashion. Nonetheless, we can conclude that such ART procedures having in common micromanipulation of the zona pellucida do not appear to have an impact on the timing of implantation or on the early embryonic growth of the normally implanted concepti.

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