Fertilization rate is an independent predictor of implantation rate

Mitchell P. Rosen, M.D.,[a] *Shehua Shen, M.D.,*[a] *Paolo F. Rinaudo, M.D., Ph.D.,*[a] *Heather G. Huddleston, M.D.,*[a] *Charles E. McCulloch, Ph.D.,*[b] *and Marcelle I. Cedars, M.D.*[a]

[a] Department of Obstetrics, Gynecology and Reproductive Sciences, and [b] Department of Epidemiology and Biostatistics, University of California, San Francisco, California

Objective: To determine whether fertilization rate serves as a biological assay, reflects oocyte quality, and may be used to help predict patient implantation rate.
Design: Retrospective cohort study.
Setting: Academic center.
Patient(s): Couples undergoing 3603 in vitro fertilization (IVF) cycles from 2001 to 2007.
Intervention(s): None.
Main Outcome Measure(s): We compared the implantation rate among cycles with high versus low fertilization rate. Univariate analyses were performed to determine the association of implantation rate with potential confounding variables: age, day-3 follicle-stimulating hormone level, day-3 estradiol level, antral follicle count, oocyte number, cycle attempts, embryo grading, and number of embryos transferred. Multivariate analysis was then performed to determine whether the fertilization rate remained an independent predictor.
Result(s): Cutoffs for fertilization rate were 50% for intracytoplasmic sperm injection (ICSI) and 75% for conventional insemination. Higher ICSI fertilization was statistically significantly associated with the implantation rate (25.2% vs. 17.8 %). After adjusting for variables associated with implantation rate, fertilization rate for ICSI remained a strong independent predictor of implantation. Higher conventional insemination fertilization was statistically significantly associated with implantation (32.1% vs. 25.7%) and remained a statistically significant predictor after adjustment.
Conclusion(s): Fertilization is a strong, independent predictor of implantation rate and may be useful in modeling to guide decision making for the number of embryos to transfer. (Fertil Steril® 2010;94:1328–33. ©2010 by American Society for Reproductive Medicine.)

Key Words: Fertilization rate, IVF, predictors, pregnancy, implantation rate, ART, oocyte quality, ICSI

The high incidence of multiple births is a significant complication of in vitro fertilization (IVF) treatment. The balance between the desires to maintain pregnancy rates while decreasing multiple births is often times difficult to achieve. The inability to accurately predict whether an individual embryo will implant often leads to the transfer of multiple embryos to ensure implantation of at least one. The most common method of determining the number of embryos to transfer is limited to few patient characteristics, embryo morphologic criteria, and number of prior cycles (1). Using these criteria, the current guidelines from the Society for Assisted Reproductive Technology (SART) have resulted in a decrease in the number of higher order pregnancies but without a significant reduction in the twinning rate (2).

To maintain the pregnancy rate and further minimize the number of embryos transferred, additional important predictors of implantation need to be identified. Much attention has focused on the morphologic characteristics of the oocyte or embryo destined for transfer. For example, various morphologic characteristics of the oocyte, such as zona pellucida thickness, appearance of the cytoplasm, and polar bodies have been investigated (3–6). However, the literature is conflicting, and it is difficult to assess the actual impact of each of these parameters (7, 8). Embryo grading does correlate with pregnancy outcome and is the most widely used assessment to determine which embryos to transfer, although with obvious limitations. Other than standard morphology, investigators have determined that assessment at the zygote stage, evaluation of embryo behavior at early cleavage, or extended culture performed to day 5 improves pregnancy outcomes (9, 10). With advances in technology, there is active research at the molecular level to increase our prediction of implantability (11). However, as we await the development of predictive molecular markers, recommendations for embryo transfer number will depend on the inclusion of multiple covariates and will likely include the combination of patient characteristics, ovarian response, and laboratory findings both at the cohort level and the individual embryo level.

In our practice, we have observed considerable variability in fertilization rates. Therefore, we questioned whether fertilization could serve as a potential predictor of implantation at the cohort level. With conventional insemination, the

Received October 16, 2008; revised May 8, 2009; accepted May 11, 2009; published online June 27, 2009.
M.P.R. has nothing to disclose. S.S. has nothing to disclose. P.F.R. has nothing to disclose. H.G.H. has nothing to disclose. C.E.M. has nothing to disclose. M.I.C. has nothing to disclose.
Reprint requests: Mitchell P. Rosen, M.D., UCSF Center for Reproductive Health, 2356 Sutter Street, 8th floor, Box 0916, San Francisco, CA 94115 (FAX: 415-353-7744; E-mail: rosenm@obgyn.ucsf.edu).

0015-0282/$36.00

doi:10.1016/j.fertnstert.2009.05.024

variables that contribute to fertilization rate are numerous. Intracytoplasmic sperm injection (ICSI) has alleviated the impact of sperm quality, and has removed the nuclear immature oocyte as an etiology of failed fertilization. However, even with ICSI, there remains considerable variation in fertilization rate. The etiology has been attributed to a number of factors. Most notably, the fertilization rate depends on the skill level of the ICSI technician, the cause of the male infertility, and/or the sperm origin (i.e., testis, epididymis, or ejaculate) (12–15). Additionally, some propose that DNA damage may have an impact on fertilization with ICSI (16–19). Others suggest that inherent oocyte quality or failure of cytoplasmic maturation may contribute to fertilization failure (6, 20–22). Likely, the variation in fertilization rate is a composite of multiple factors, each having some independent effect and potentially predictive of oocyte and/or sperm health.

We were interested in whether the fertilization potential was a function of the oocyte cohort quality. Therefore, in this study we initially explored the predictive value of fertilization rate after ICSI on implantation. We chose ICSI to eliminate confounding with immature oocytes and any negative impact of sperm function. Once we noted a difference with ICSI cycles, we repeated separately an analysis with conventional insemination, aware that there are additional factors beyond oocyte quality that impact successful fertilization with conventional insemination. We included all cycles, separated by type of insemination and adjusted for known variables that were associated with the likelihood of implantation, to determine the magnitude of the impact that fertilization rate has on implantation.

MATERIALS AND METHODS

Data from couples undergoing IVF/ICSI from 2001 to 2007 were reviewed. A total of 3603 cycles were analyzed. Cases where sperm retrieval was required were excluded from the analysis. The study was approved by the institutional review board at the University of California, San Francisco.

Treatment Regimen

All patients underwent standard ovarian stimulation protocols (59% long luteal, 15.2% antagonist, and 25.8% microdose flare). Transvaginal ultrasound assessment of follicular growth and endometrial thickness commenced on day 4 or 5, and serum estradiol levels were obtained at each clinic visit. Thereafter, the physician, based on the ultrasonographic findings and serum estradiol levels, adjusted the frequency of monitoring and the amount of gonadotropins.

The day of the human chorionic gonadotropin (hCG) trigger (and thus the total days of stimulation) was based on follicle size and number in addition to the serum estradiol level. The dose of hCG was 5000 or 10,000 units, depending upon the risk for hyperstimulation. The egg retrieval was performed 36 hours after administration of hCG. The embryos were transferred on day 2 through 5, depending on clinical scenario (20% day 2, 77% day 3, and 3% day 4 or 5). All cycles had luteal-phase support with 50 mg intramuscular progesterone in oil beginning 2 days after the retrieval, and 2 mg of estradiol beginning 6 days after hCG administration.

ICSI

After oocyte retrieval, the cumulus was denuded 38 to 42 hours after hCG administration. Stripping was accomplished by placing the cumulus–oocyte complex in 80 IU/mL of hyaluronidase (Sage Biopharma, Bedminster, NJ) for 30 to 60 seconds, rinsing five to six times in working solution (mHTF + 10% SSS or GMOPS + 5% HSA), and then mechanically removing the cumulus from the oocyte using a plastic pipette (ID = 135 μm; MidAtlantic Diagnostics, Inc., Mount Laurel, NJ).

The sperm was first visualized under $\times$40 magnification and then was immobilized in 7% polyvinylpyrrolidone (PVP; Irvine Scientific, Santa Ana, CA) using the tip of the injection needle (Humagen Fertility Diagnostic, Inc., Charlottesville, VA). It was then aspirated tail-first into the injection needle. The oocyte was grasped with a holding pipette at 9 o'clock using gentle suction, then was rotated such that the first polar body was located at either the 6 o'clock or 12 o'clock position. The injection pipette was pushed through the zona at the 3 o'clock position and advanced to the outer surface of the oolemma. The oolemma was penetrated by direct penetration technique. Entry into the oocyte was confirmed by free flow of cytoplasm into the injection pipette. The cytoplasm was then slowly injected back into the oocyte until the sperm was seen to pass the tip of the injection pipette and slide into the cytoplasm. The procedure was concluded by gently removing the injection pipette.

Conventional Insemination

Oocytes undergoing conventional insemination were grouped in 200-μL drops and fertilized with approximately 100,000 spermatozoa/mL 4 hours after the retrieval.

Evaluation of Fertilization

Normal fertilization was identified by the presence of two pronuclei (2PN) at the time of fertilization assessment, 16 to 19 hours after ICSI or conventional insemination.

Statistical Analysis

A Lowess plot was performed to determine the relationship between ICSI fertilization rate (2PN/number of oocytes injected), conventional insemination fertilization rate (2PN/number of oocytes inseminated), and implantation rate. A cutoff was established for each method of fertilization where the change in implantation approximated a plateau in the Lowess plot, thus distinguishing the low and high fertilization rates. Variables considered to be predictive of implantation (a priori based on the literature) were described separately by high or low fertilization rate. These included