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Stimulation day-six serum estradiol: A predictive indicator for the probability of embryo cryopreservation in IVF/ICSI cycles

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Abstract

Objective: To evaluate the predictive value of stimulation day six serum estradiol (E2) for the probability of embryo cryopreservation after fresh embryo transfer in intracytoplasmic sperm injection (ICSI) cycles.

Subjects and Methods: The study included 282 ICSI cycles for different causes of infertility, provided that the age of the female partner was <40 years and her basal follicle stimulating hormone <10 IU/L.

Setting: Alexandria IVF/ICSI center.

Main Outcome Measures: Primary outcome measures are stimulation day-six serum E2, and rate of embryo cryopreservation, after transfer of three good-quality embryos. Secondary outcome measures are pregnancy rate per fresh embryo transfer, and other intermediate variables of the ICSI cycle.

Results: Patients were stratified into three groups according to day-six serum E2 levels: Group I with values <400 pg/mL; Group II, between 400 and 900; and Group III with values >900. The mean number of oocytes retrieved was 6.3, 8.9, and 12.4; the mean number of obtained embryos was 3.3, 4.8, and 6.7; and pregnancy rates were 18.1, 36.2, and 44.7% in the three groups, respectively. Rate of embryo cryopreservation, after transfer of three good-quality embryos was 70.7% in Group III, and 26.5% in Group I. ($P = 0.01$). The negative predictive value of day-six E2 < 400 pg/mL for freezing was 83% while day-six serum E2 > 900 pg/mL has a sensitivity of 55%, specificity of 72% and positive predictive value of 50% for embryo freezing.

Conclusion: Higher stimulation day-six estradiol was associated with a higher probability of cryopreservation, and a higher pregnancy rate.

Key words: embryo cryopreservation, prediction, stimulation day six serum estradiol.

Introduction

Although embryo storage by cryopreservation has been in routine use in many *in vitro* fertilization (IVF) laboratories for many years, counseling about its possibility after transfer of fresh embryos is rarely considered until the time of embryo transfer or at best slightly earlier. Since anxiety, depression and hostility in the patients are highest before the embryo transfer,[1] discussing new issues at this time adds stress to an already stressful situation. Therefore, the possibility of embryo freezing is more appropriately discussed earlier with the couple to allow time to consider its financial, ethical, psychological, and religious implications. This 'preventive counseling concept' is useful in all of the steps of assisted reproductive technology

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(ART) to allow for coping with the stress of the procedure,[2] and has actually been found to reduce the level of anxiety and depression, and increase the level of satisfaction.[3]

Raising the issue of freezing early during stimulation might also have a positive psychological impact on the couple knowing that the chance of pregnancy extends to another cycle. Adequate counseling and stress reduction before ART has also been correlated with a successful outcome.[4] However, if the possibility of freezing appears remote, addressing the issue might add to the pressure on the couple by providing unnecessary information.

A reliable predictive test might be helpful to select a group of patients that should receive counseling about cryopreservation in order to allow for informed decision-making on the issue.

Currently this is important because embryo cryopreservation has become an integral part of ART, and indeed it is a very useful practice. Nowadays the true efficacy of any IVF–embryo transfer (ET) program should rely on calculating the pregnancy rate per oocyte harvest rather than the primary pregnancy rate after fresh transfer.[5] Elective cryopreservation of all or some embryos can also be a helpful tool in prevention of the two major complications of ART namely ovarian hyperstimulation syndrome (OHSS)[6] and high-order multiple pregnancy (HOMP).[7] Preservation of embryos for a limited time makes it possible to transfer fewer embryos per cycle, thus minimizing the occurrence of multiple pregnancies while increasing the effectiveness of a single IVF procedure. If the attempt at IVF fails, freezing embryos has the advantage that the couple can have at least one, if not more, attempts at transfer without repeated infertility treatments being required.[8] Good-quality embryos might even have a better chance to implant in freeze–thaw cycles than the initial controlled ovarian hyperstimulation cycle. This is probably because of the more physiological endocrine milieu.[9] The increasing practice of embryo cryopreservation dictates attention to all of its details including refinements of the techniques of freezing and thawing, optimization of the transfer cycle, and patient counseling to achieve its maximum potential in the ART program.

In our study, we sought to evaluate the reliability of day six stimulation serum estradiol (E2), as an early predictive indicator for the probability of having embryos suitable for freezing after fresh embryo transfer in IVF/intracytoplasmic sperm injection (ICSI) cycles.

Subjects and Methods

The data of 282 ICSI cycles conducted between 1 January 2007 and 31 December 2007 for couples with different causes of infertility in the Alexandria IVF/ICSI center were analyzed. The mean age of female partners was 31 years, and their basal follicle stimulating hormone (FSH) was <10 IU/L. Couples in which the age of female partner was >40 years and her basal FSH >10 IU/L were excluded from the study.

Controlled ovarian hyperstimulation was carried out using long luteal agonist protocol with subcutaneous decapeptyl starting from day 19 of the previous cycle. When pituitary desensitization was achieved (evidenced by menses and E2 < 50 pg/mL), stimulation was started using 225IU of hMG, and one amp. of recombinant FSH (300IU). Cycles were monitored using transvaginal sonography and serum E2 starting from stimulation day six. Surplus embryos were frozen at the 4–8 cell stage using (FREEZE-KIT 1, Vitrolife kungsbacka, Sweden), and placed in a controlled-rate freezing machine (Cryologic Australia, 23 108 s). Embryos were thawed using the cryoprotectant (THAW-KIT 1, Vitrolife kungsbacka, Sweden) diluted in a step-wise procedure, 5 min each. Basal FSH, basal E2, day-six E2 of stimulation, and final E2 were obtained. Laboratory data (number of oocytes retrieved, percentage of fertilization and cleavage, number of cryopreserved embryos and pregnancy rate) were collected on all cases and subsequently analyzed.

Patients were divided into three groups, according to stimulation day-six E2 level into: Group I ($n = 99$) values <400 pg/mL; Group II ($n = 78$) between 400 and 900 pg/mL; and Group III ($n = 105$) with values >900 pg/mL.

Statistical Analysis

Statistical analysis of data was done on an intention-to-treat basis, using SPSS for Windows package release 10.0 (SPSS INC. Chicago, IL, USA). *P*-value <0.05 was considered statistically significant.

Results

Causes of infertility were male factors in 64.3% of the cases; tubo-peritoneal factors in 17.2%; ovarian factors in 13.4%; and 5.1% had unexplained infertility. Stimulation day-six E2 levels ranged from 170 to 3700 pg/mL. The mean number of oocytes retrieved was: 6.3, 8.9, and 12.4 and the mean number of embryos

Table 1 Intracytoplasmic sperm injection data in the three studied groups

	Group I	Group II	Group III	*P*
Final estradiol (pg/mL)	1432.66 ± 98	2378 ± 110	3625.75 ± 240	0.00
Oocytes (No.)	6.3 ± 2.2	9.0 ± 3.1	12.5 ± 4.6	0.00
Embryos (No.)	3.3 ± 0.8	4.9 ± 1.1	6.7 ± 1.9	0.00
No. of Class A embryos	1.65 ± 0.5	3.02 ± 1	3.4 ± 0.9	0.02
No. of Cryo embryos	0.54 ± 0.1	1.7 ± 0.2	2.8 ± 0.7	0.00
Pregnancy rate (%)	19.1	36.2	44.7	0.02
Cryopreservation (%)	26.5	55.8	70.7	0.01

P-value for student *t*-test between Groups I and III.

Table 2 Cryopreservation data in the three studied groups

	Cryo. (No.)	No Cryo. (No.)	Total
Group I	17 (FN)	82 (TN)	99
Group II	25	53	78
Group III	52 (TP)	53 (FP)	105

Group I: estradiol <400 pg/mL, Group II: estradiol 400–900 pg/mL and Group III: estradiol >900 pg/mL. FN, false negative; TN, true negative; TP, true positive; FP, false positive.

obtained was 3.3, 4.8, and 6.7 in the three groups, respectively. A significant difference was found between Group I and Group III regarding the number of retrieved oocytes ($P = 0.001$), and the number of embryos obtained ($P = 0.001$) (Table 1). The rate of embryo cryopreservation was 71% in Group III, 55.8% in Group II and 26.5% in Group I. The only statistically significant difference was between Group I and III ($P = 0.001$). Positive correlation was found between day-six E2 and the embryo cryopreservation rate (Spearman's Correlation Coefficient = 0.308). In Group III, the mean number of cryopreserved class A embryos was 3.4 ± 0.9, this was significantly higher than Group I (1.65 ± 0.5) ($P = 0.02$), but not Group II (3.02 ± 1). The pregnancy rate was 45% in Group III versus 19% in Group I and this was statistically significant ($P = 0.02$).

The negative predictive value of day-six E2 < 400 pg/mL for freezing was 83% while day-six serum E2 > 900 pg/mL has a sensitivity of 55%, specificity of 72% and positive predictive value of 50% for embryo freezing. (Table 2)

Discussion

Having embryos stored after ART trial should be good news for the treated couple, and their IVF team, because it means that they had a good embryo transfer, which predicts a high primary pregnancy rate, and that they have another chance for achieving their hope without going through the long road of preparation again. However, telling this news at the wrong time might waste or even reverse its positive impact. Therefore, judiciously early counseling on the issue is desirable, but this should rely on realistic knowledge that freezing is strongly predicted. This knowledge can only be obtained when response to gonadotropin stimulation predicts a good oocyte yield and subsequently a good number of embryos.

The earliest response to stimulation in terms of follicular growth and E2 rise appears after a clinically mute latent phase ranging between 3 and 7 days.[10] Making use of data obtained at the beginning of the subsequent active phase can be helpful not only in the prediction of freezing but also in the management of the rest of the cycle, including counseling on performance, and modification of treatment. There are data in the published reports on the prognostic value of serum E2 starting from day 3 of the controlled ovarian hyperstimulation cycle, however, early data were contradictory indicating that they reflect basal condition rather than stimulation response.[11–15] Therefore, in our study, we used day-six E2 levels, because they are more representative of the response after the end of the latent phase. We also chose a 'cut-off E2 < 400' for a low response, because in our program, values lower than this range were associated with less than optimal transfer and a significantly lower pregnancy rate, while values >900 were chosen as a cut-off for a good response because they were significantly associated with a good transfer defined as transfer of ≥3 good-quality embryos, and a significantly higher pregnancy rate.

The results of this study showed that day-six serum E2 values ranged between 170 and 3700 pg/mL. The group with the highest serum E2 (>900 pg/mL) on day six had the highest pregnancy rates (44.7%) and the highest possibility of freezing (70.7%). A comparable pregnancy rate of 47.4% in couples who had cryo-embryos was reported by Ubaldi *et al*.[16] In the same study, the cumulative pregnancy rate after transfer of thawed embryos reached 74%. Back to our study where

cases with lower E2 values (<400) had about a 19% pregnancy rate and about 27% probability to store at least one embryo. These differences in pregnancy rates and the possibility of freezing were highly significant. Furthermore, 30% of Group III women had 'good-quality freezing', that is, three or more class A embryos frozen, while in Group I and II the incidence was 11% and 20%, respectively. This could add 'quality' to the value of early counseling. Day-six E2 > 900 pg/mL had a sensitivity of 55%, and a specificity of 72% and a positive predictive value 50% for predicting embryo freezing. This relatively low sensitivity and positive predictive value might be explained by the impact of sperm factor on the number and quality of embryos produced from a given number of oocytes. Nevertheless this is good for a prognostic test and it is enough to raise the issue with the couple so that they might consider embryo cryopreservation.

In conclusion, day-six E2 is a good predictor of the possibility of freezing. High responders should receive early explanation and counseling on all of the implications of embryo cryopreservation.

References

1. Ismail WA, Menezes MQ, Martin CW, Thong KJ. A comparison of psychological functioning in couples undergoing frozen-thawed embryo replacement in various stages of treatment using the Mean Affect Adjective Check List (MAACL). *J Assist Reprod Genet* 2004; **21**: 323–327.
2. Emery M, Darwiche BJ, Oppizzi L *et al.* Results from a prospective, randomized, controlled study evaluating the acceptability and effects of routine pre-IVF counseling. *Hum Reprod* 2003; **18**: 2647–2653.
3. Terzioglu F. Investigation into effectiveness of counseling on assisted reproductive techniques in Turkey. *J Psychosom Obstet Gynaecol* 2001; **22**: 133–141.
4. de Klerk C, Hunfeld JA, Heijnen EM *et al.* Low negative affect prior to treatment is associated with a decreased chance of live birth from a first IVF cycle. *Hum Reprod* 2008; **23**: 112–116.
5. Katsoff B, Check JH, Choe JK, Wilson C. A novel method to evaluate pregnancy rates following in vitro fertilization to enable a better understanding of the true efficacy of the procedure. *Clin Exp Obstet Gynecol* 2005; **32**: 213–216.
6. D'Angelo A, Amso N. Embryo freezing for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev* 2007; **18**: CD002806.
7. Heijnen EM, Klinkert ER, Schmoutziguer AP, Eijkemans MJ, te Velde ER, Broekmans FJ. Prevention of multiple pregnancies after IVF in women 38 and older: A randomized study. *Reprod Biomed Online* 2006; **13**: 386–393.
8. Michelmann HW, Nayudu P. Cryopreservation of human embryos. *Cell Tissue Bank* 2006; **7**: 135–141.
9. Simon C, Cano F, Valbuena D *et al.* Clinical evidence for a detrimental effect on uterine receptivity of high serum estradiol concentrations in high and normal responder patients. *Hum Reprod* 1995; **10**: 2432–2437.
10. Lunenfeld B, Insler V. Follicular development and its control. *Gynecol Endocrinol* 1993; **7**: 285–292.
11. Phelps JY, Levine AS, Hickman TN, Zacur HA, Wallach EE, Hinton EL. Day 4 estradiol levels predict pregnancy success in women undergoing controlled ovarian hyperstimulation for IVF. *Fertil Steril* 1998; **69**: 1015–1019.
12. Hershlag A, Asis MC, Diamond MP, DeCherney AH, Lavy G. The predictive value and the management of cycles with low initial estradiol levels. *Fertil Steril* 1990; **53**: 1064–1067.
13. Khalaf Y, Taylor A, Braude P. Low serum estradiol concentrations after five days of controlled ovarian hyperstimulation for in vitro fertilization are associated with poor outcome. *Fertil Steril* 2000; **74**: 63–66.
14. Smotrich DB, Widra EA, Gindoff PR, Levy MJ, Hall JL, Stillman RJ. Prognostic value of day 3 estradiol on in vitro fertilization outcome. *Fertil Steril* 1995; **64**: 1136–1140.
15. Licciardi FL, Liu HC, Rosenwaks Z. Day 3 estradiol serum concentrations as prognosticators of ovarian stimulation response and pregnancy outcome in patients undergoing in vitro fertilization. *Fertil Steril* 1995; **64**: 991–994.
16. Ubaldi F, Rienzi L, Baroni E. Cumulative pregnancy rates after transfer of fresh and thawed embryos. *Eur J Obstet Gynecol Reprod Biol* 2004; **115** (Suppl 1): S106–S109.

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