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Oocyte and Embryo Quality in Patients with Excessive Ovarian Response During In Vitro Fertilization Treatment

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***Purpose*:** This study examined oocyte and embryo quality in patients having excessive ovarian responses during assisted reproduction treatment.

Methods: Two hundred and Seventy-eight women of age <40 years using a long protocol of pituitary downregulation in their first intracytoplasmic sperm injection cycle indicated for severe male factors were retrospectively evaluated. Those with serum estradiol concentration on the day of HCG <10,000, 10,000–20,000, and >20,000 pmol/L were classified into Group A, Group B, and Group C, respectively.

Results: The percentage of metaphase II oocytes (85%), fertilization rate (60–66%), and distribution of blastomere number per embryo were similar among the three groups. The proportion of transferable embryos was not reduced in Group C when compared to those of Groups A and B.

***Conclusion*:** Excessive ovarian response does not compromise oocyte and embryo quality in humans. Freezing of all embryos is recommended in these patients in view of associated impaired endometrial receptivity.

KEY WORDS: Embryo quality; estradiol; excessive ovarian responses; oocyte quality.

INTRODUCTION

High serum estradiol (E2) concentrations resulting from excessive responses to ovarian stimulation may adversely affect the outcomes of assisted reproduction cycles (1–3). We have recently shown significant impairment in pregnancy rates during fresh cycles when the serum E2 concentration on the day of HCG was >20,000 pmol/L (3). Oocyte quality appeared not to be affected by the extremely high E2 concentrations as the incidence of fertilization failure, the fertilization, and cleavage rates were similar, irrespective of serum E2 concentrations on the day of HCG (human chorioniod gonadotrophin). Surplus embryos from cycles with different E2 concentrations also had comparable implantation and pregnancy rates in frozen–thawed transfer cycles.

On the basis of these results, we postulated that reduced implantation in cycles with high serum E2 concentrations was most likely to be related to an adverse environment in the endometrium. Delayed glandular maturation and advanced stromal morphology were further demonstrated in endometrial biopsies taken from these patients with excessive ovarian responses (4). However, it is still unclear in the literature whether excessive ovarian responses have any direct effects on oocyte and embryo quality.

The objective of this study was to evaluate and compare oocyte and embryo quality in patients with serum E2 concentration on the day of HCG <10,000, 10,000–20,000, and >20,000 pmol/L.

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MATERIALS AND METHODS

A retrospective study of infertile patients attending the Assisted Reproduction Unit at Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, for in vitro fertilization (IVF) treatment requiring intracytoplasmic sperm injection (ICSI) from early 1997 to December 2000 was undertaken. Ethical approval was not required for this retrospective analysis. Before the couples were enrolled into our program, they all underwent a standard protocol of investigations including conventional semen analysis at least on two occasions, which was performed according to WHO criteria (5).

Patients had to fulfil the following criteria before they were included in this study: (a) the first treatment cycle; (b) age of women <40 years; (c) long protocol of pituitary downregulation; and (d) ICSI indicated for severe male factors, i.e., <100,000 motile spermatozoa recovered after sperm preparation or surgically retrieved spermatozoa from epididymis/testis in case of azoospermia. Exclusion criteria were (a) short protocol of pituitary downregulation and (b) absence of mature spermatozoa after testicular sperm extraction.

The details of the long protocol of ovarian stimulation regimen, gamete handling, ICSI, and assessment of oocyte/embryo quality at our center have been previously published (3,6).

Ovarian Stimulation

In short, all women were pretreated with Buserelin (Suprecur, Hoechst, Frankfurt, Germany) nasal spray 150 μg four times a day from the midluteal phase of the cycle preceding the treatment cycle. After pituitary downregulation was confirmed, human menopausal gonadotrophin (HMG, 75 IU FSH and LH; Pergonal, Serono, Geneva, Switzerland) injections were then started. Human chorionic gonadotrophin (HCG, Profasi, Serono, Geneva, Switzerland) 10,000 IU was given intramuscularly when the leading follicle reached 18 mm in diameter and there were at least three follicles >15 mm in diameter. Coasting was not performed in all these cycles and cycles were not canceled because of excessive ovarian response. Blood was taken on the day of HCG for serum E2 concentrations, which were measured using a commercially available RIA kit (Diagnostic Products Corporation, LA). The inter- and intra-assay variability on high-level control (E2: 1082 pg/mL, conversion factor to SI unit, 3.671) were 4.2 and 4.0% respectively.

Assessment of Oocyte and Embryo Quality

Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 h after the HCG injection. About 2 h after the retrieval, the oocytes were denuded of their surrounding cumulus and corona radiata cells by using hyaluronidase and aspirated oocytes through a fine capillary. Denuded oocytes were cultured for 2 h. Prior to microinjection, each denuded oocyte was assessed according to Veeck (7) and the nuclear maturity was classified into (a) metaphase II; (b) metaphase I/germinal vesicle; and (c) atretic.

A maximum of three normally cleaving embryos were replaced into the uterine cavity 48 h after the retrieval. Excess good-quality embryos were frozen. All fresh good-quality embryos were cryopreserved if serum E2 on the day of HCG exceeded 30,000 pmol/L or features suggestive of ovarian hyperstimulation syndrome (OHSS) were present. Immediately before transfer or cryopreservation, embryos were examined for the number/regularity of blastomeres and the degree of fragmentation. Embryos were graded according to the following criteria: Grade 1—blastomeres of equal size, no cytoplasmic fragments; Grade 2—blastomeres of equal size, minor (<25%) cytoplasmic fragments; Grade 3—blastomeres of distinctly unequal size, no cytoplasmic fragments; Grade 4—blastomeres of distinctly unequal size, minor cytoplasmic fragments; Grade 5—blastomeres of equal or unequal size, major cytoplasmic fragments; and Grade 6—few or no recognized blastomeres, major cytoplasmic fragments. Grade 5 or 6 embryos were discarded because of poor quality.

Luteal phase was supported by 1500 IU HCG injections on the day of ET and 6 days later. Vaginal pessaries (Cyclogest 400 mg twice daily; Cox Pharmaceuticals, Barnstaple, U.K.) were used instead from the day of ET for 10 days when serum E2 level on the day of ovulatory HCG was above 18,000 pmol/L.

Statistical Analysis

The primary outcome measures were the percentage of metaphase II oocytes and transferable embryos. Pregnancy and implantation rates were also assessed in fresh cycles. Only clinical pregnancies were considered and are defined by the presence of one or more gestation sacs or the histological confirmation of gestational product in miscarriages. Ongoing pregnancies were those pregnancies beyond 10–12 weeks of gestation, at which stage the patients were referred out for antenatal care. Mean implantation rate was

the proportion of embryos transferred resulting in an intrauterine gestational sac. Continuous variables were not normally distributed and were given as median (2.5th–97.5th centiles), unless indicated. Statistical comparison was carried out by Kruskal–Wallis U test and χ^2 test, where appropriate. P value (two-tailed) of <0.05 was taken as significant.

RESULTS

Cycles with serum E2 <10,000, 10000–20000, and >20,000 were classified into Groups A, B, and C, respectively. Two hundred and seventy-eight cycles were included: 154 cycles in Group A, 86 cycles in Group B, and 38 cycles in Group C. Table I summarizes the ovarian responses and incidence of moderate or severe OHSS. Patients in Group C were significantly younger than those in Groups A and B. Despite similar duration and dosage of gonadotrophin used, significantly more follicles were aspirated in Group B, resulting in higher numbers of cumulus–oocyte complexes, metaphase II oocytes, fertilized oocytes, cleaving embryos, and embryos available for cryopreservation. The oocyte retrieval rate was comparable for both groups. Significantly more moderate or severe OHSS was encountered in Group C than in Groups A and B.

A total of 1208, 1360, and 769 cumulus–oocyte complexes were examined in Groups A, B, and C, respectively for nuclear maturity. The details of nuclear maturity of oocytes and pronuclear status after ICSI are given in Table II. The proportion of metaphase II oocytes and fertilization rate (the percentage of two pronuclei after ICSI) were similar among the three groups. They had similar distribution of blastomere number per embryo (Table III). The proportion of transferable embryos (Grades 1–4) in Group C was comparable to that of Group A but significantly higher than that of Group B (83.6% vs. 70.4%, $P < 0.001$, χ^2 test). The fertilization rate, the proportion of metaphase II oocytes, and transferable embryos were comparable regardless of the number of oocytes obtained and the presence of moderate or severe OHSS (data not shown).

Embryo transfer was performed in 147, 86, and 19 cycles in Groups A, B, and C, respectively. No significant differences were observed in the pregnancy rate, implantation rate, multiple pregnancy rate, and pregnancy outcome among the three groups, although there was a trend of reduced pregnancy and implantation rates in Group C (Table IV).

DISCUSSION

Evidence in animal studies clearly suggests that ovarian stimulation impairs oocyte and embryo quality. Using an embryo donation model, Ertzeid and Storeng (8) demonstrated that both oocyte/embryo quality and uterine receptivity in mice were compromised after gonadotrophin stimulation. Significantly lower number of embryos developed into blastocysts from superovulated mice, when compared with the control group. Similarly, embryos from superovulated hamsters had significantly reduced mean cell numbers than the controls (9). In an in vitro embryo adhesion assay using human endometrial cells from fertile

Table I. Ovarian Responses and Incidence of Moderate/Severe OHSS

Parameters (Median (2.5–97.5th centiles))	Group A ($N = 154$)	Group B ($N = 86$)	Group C ($N = 38$)	P value (Kruskal–Wallis U test)
Age (years)	33.0 (25.0–38.1)	32.0 (24.2–37.8)	31.0 (25.0–36.0)	0.001
Days of gonadotrophin	11.0 (8.0–22.4)	11.0 (8.0–21.0)	11.0 (8.0–15.0)	ns
Dosage of gonadotrophin (IU)	1800 (1200–4547)	1650 (1076–5400)	1800 (1500–2925)	ns
Serum estradiol (pmol/L)	5720 (1322–9579)	14224 (10088–19541)	25716 (20008–61608)	<0.001
No. of follicles aspirated	10.5 (1.9–30.3)	20.5 (8.4–46.1)	29.0 (8.0–81.0)	<0.001
No. of cumulus–oocyte complex	7.0 (1.9–21.0)	15.0 (4.2–33.0)	20.5 (6.0–56.0)	<0.001
No. of metaphase II oocytes	6.0 (0.9–18.0)	13.0 (2.4–30.8)	18.0 (6.0–43.0)	<0.001
No. of eggs fertilized	5.0 (0–13.0)	9.0 (2.0–22.7)	11.0 (3.0–30.0)	<0.001
No. of cleaving embryos	5.0 (0–13.0)	8.0 (2.0–20.8)	10.5 (3.0–28.0)	<0.001
No. of embryos frozen	1.0 (0–9.0)	3.0 (0–15.0)	7.0 (0–23.0)	<0.001
Oocyte retrieval rate (%)	70.0 (17.5–112.7)	73.6 (33.9–115.4)	71.8 (28.6–110.3)	ns
Moderate or severe OHSS[a]	1.3% (2/154)	12.8% (11/86)	39.5% (13/38)	<0.001[b]

Note. Group A: E2 <10,000 pmol/L; Group B: E2 = 10,000–20,000 pmol/L; Group C: E2 >20,000 pmol/L; ns—not significant.
[a] Percentage of cycles.
[b] χ^2 test.

Table II. Nuclear Maturity of Oocytes and Pronuclear Status After ICSI

Parameters (N (%))	Group A	Group B	Group C
Nuclear maturity			
Metaphase II	1029 (85.2)	1160 (85.3)	648 (84.3)
Metaphase I/Germinal vesicles	161 (13.3)	134 (9.9)	99 (12.9)
Atretic	18 (1.5)	66 (4.8)	22 (2.8)
Total	1208 (100)	1360 (100)	769 (100)
Degenerated after ICSI	100 (9.7)	99 (8.5)	51 (7.9)
Pronuclear status			
0 PN	147 (14.3)	205 (17.7)	142 (21.9)
1 PN	78 (7.6)	108 (9.3)	57 (8.8)
2 PN	679 (66.0)	726 (62.6)	390 (60.2)
>2 PN	25 (2.4)	22 (1.9)	8 (1.2)
Total	1029 (100)	1160 (100)	648 (100)

Note. Group A: E2 <10,000 pmol/L; Group B: E2=10,000–20,000 pmol/L; Group C: E2 >20,000 pmol/L; PN—pronuclei number.

Table IV. Outcomes of Fresh Treatment Cycles

Parameters	Group A ($N = 154$)	Group B ($N = 86$)	Group C ($N = 38$)
No. of embryo transfer	147	84	19
No. of embryos replaced			
One	12	0	0
Two	87	46	10
Three	48	38	9
Pregnancy rate/cycle (%)	20.1 (31/154)	16.3 (14/86)	7.9 (3/38)
Pregnancy rate/transfer (%)	21.1 (31/147)	16.7 (14/84)	15.8 (3/19)
Implantation rate (%)	13.3 (44/330)	9.7 (20/206)	8.5 (4/47)
Multiple pregnancy rate (%)	38.7 (12/31)	35.7 (5/14)	33.3 (1/3)
Pregnancy outcome			
Abortion	1	2	0
Ongoing	30	12	3

Note. Group A: E2 <10,000 pmol/L; Group B: E2=10,000–20,000 pmol/L; Group C: E2 >20,000 pmol/L.

women and mouse embryos, high E2 levels are shown to be deleterious to embryo adhesion because of a direct toxic effect on the embryo at the cleavage stage (10). Little information, however, exists in the literature with regard to oocyte and embryo quality in women having excessive ovarian responses during assisted reproduction treatment.

Pellicer *et al.* (11) found that retrieval of >10 oocytes in women was correlated with oocytes of lower quality, as manifested by a decreased fertilization rate. The same group (12) further demonstrated lower estradiol concentrations per follicle, lower follicular volume, and a higher incidence of diploid oocytes and cytoplasmic immaturity in the high responders. Poor oocyte quality has been associated with severe OHSS following stimulation (13,14). Aboulghar *et al.* (13) found that the percentage of good-quality oocytes and the fertilization rate were significantly lower in patients with severe OHSS than in the control group, following stimulation for conventional IVF or ICSI. The mean serum E2 concentration was 6700 ± 2350 pg/mL (±standard deviation) in the OHSS group. There were no significant differences in the quality of embryos transferred or the implantation rate between two groups. Similarly, Akagbosu *et al.* (14) reported very poor fertilization rate following ICSI for severe male factors in a patient during the cycle complicated with OHSS but normal fertilization rate in the subsequent cycle with a normal ovarian response.

Our previous study (3) including conventional IVF and ICSI cycles suggested that oocyte and embryo quality was not impaired in patients with serum E2 >20000 pmol/L on the day of HCG. To further examine oocyte and embryo quality in patients with excessively ovarian responses, the first ICSI cycles in patients suffering from severe male factors and receiving a long protocol of pituitary downregulation were retrospectively analyzed. The removal of the corona–cumulus complex before the ICSI procedure allows a more precise determination of nuclear maturity of the oocyte (15). The use of a short protocol may result in a

Table III. Blastomere Number per Embryo and Embryo Grading

Parameters (N(%))	Group A ($n = 679$)	Group B ($n = 726$)	Group C ($n = 390$)
Blastomere number			
0	3 (0.4)	19 (2.6)	4 (1.0)
1	39 (5.8)	51 (7.0)	16 (4.1)
2	241 (35.5)	260 (35.8)	151 (38.7)
3	87 (12.8)	114 (15.7)	51 (13.1)
4	254 (37.4)	232 (32.0)	136 (34.9)
5	44 (6.5)	39 (5.4)	22 (5.6)
6	9 (1.3)	10 (1.4)	9 (2.3)
7	2 (0.3)	1 (0.1)	1 (0.3)
Embryo grading			
Transferable embryos	543 (80.0)	511 (70.4)[a]	326 (83.6)[a]
Grade 1	165 (24.3)	114 (15.7)	67 (17.2)
Grade 2	295 (43.4)	281 (38.7)	203 (52.1)
Grade 3	21 (3.2)	32 (4.4)	10 (2.5)
Grade 4	62 (9.1)	84 (11.6)	46 (11.8)
Nontransferable embryos	136 (20.0)	215 (27.6)	64 (16.4)
Grade 5	100 (14.7)	161 (22.2)	47 (12.0)
Grade 6	36 (5.3)	54 (7.4)	17 (4.4)

Note. Group A: E2 <10,000 pmol/L; Group B: E2=10,000–20,000 pmol/L; Group C: E2 >20,000 pmol/L.
[a] Group C vs. Group B: $P < 0.001$, χ^2 test.

significantly lower proportion of metaphase II oocytes than a long protocol (16).

In this study, patients in all three groups had similar percentages of metaphase II oocytes and of two pronuclei after ICSI. This finding suggested that excessively high E2 concentrations might not affect nuclear maturity of oocytes and the fertilization rate. The distribution of blastomere number per embryo was comparable for three groups and the proportion of transferable embryos in Group C was not lower than those of Groups A and B. Better embryo quality in Group C may be related to younger age in this group. Similar findings were demonstrated in oocyte donation cycles (17).

We could not show any impairment of oocyte and embryo quality in those patients complicated by OHSS as well. Our findings in this study differ from those of Aboulghar *et al.* (13) and Akagbosu *et al.* (14) although most patients suffering from severe OHSS also had excessively high E2 concentrations. The reason for the discrepancy is unknown. Patients having polycystic ovaries are at risk of developing severe OHSS or excessively high E2 concentrations after ovarian stimulation. Ovarian morphology as shown by transvaginal scanning was not available in this study but polycystic ovaries are uncommon in our population (18). Although Aboulghar *et al.* (13) showed significantly lower percentage of high-quality oocytes and reduced fertilization rate in patients with polycystic ovaries regardless of OHSS, it remains unclear whether oocyte and embryo quality is impaired in patients with polycystic ovaries (19–21).

There was a trend of reduced pregnancy rate and implantation rate in patients with serum E2 >20,000 pmol/L, compared with those with 10,000 and 10,000–20,000 pmol/L. This finding was consistent with our previous observation (3). The difference was not statistically significant probably because of a smaller number of subjects in this study.

In conclusion, this retrospective study demonstrated a similar percentage of metaphase II oocytes and fertilization rate in patients with serum E2 >20,000 pmol/L on the day of HCG during cycles requiring ICSI for severe male factors, when compared to those with serum E2 (10,000/L and 10,000–20,000 pmol/L). Embryo quality was not compromised by the excessively high E2 concentrations as well, thus suggesting that an excessive ovarian response does not compromise oocyte and embryo quality in humans. In view of the high rate of OHSS and the impaired endometrial receptivity, freezing of all embryos is highly recommended in patients with serum estradiol levels >20,000 pmol/L.

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