Does high serum progesterone level on the day of human chorionic gonadotropin administration affect pregnancy rate after intracytoplasmic sperm injection and embryo transfer?

FOAD AZEM, JOSEPH B. LESSING, MIRA MALCOV, DALIT BEN-YOSEF, BENI ALMOG, & AMI AMIT

The Sara Racine IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

(Received 28 May 2007; revised 10 January 2008; accepted 15 January 2008)

Abstract
Objective. The present study was conducted to evaluate the effect of serum progesterone (P) levels on the day of human chorionic gonadotropin (hCG) administration on embryo quality and pregnancy rate in intracytoplasmic sperm injection (ICSI) cycles.
Design and setting. This was a retrospective analysis conducted in the in vitro fertilization (IVF) unit of a tertiary hospital.
Patients. Two hundred and one patients who underwent a total of 280 IVF treatment cycles allocated to ICSI during routine IVF/embryo transfer treatment.
Results. In cycles with elevated serum P, higher estradiol levels were noted (1915 pg/ml vs. 1256 pg/ml; \( p < 0.05 \)), more oocytes were retrieved and manipulated, and more embryos were available for transfer. Embryo grading was comparable between the two groups. The average age was lower in the group with elevated P; but the pregnancy rate was significantly lower (16.4% vs. 27.6%, \( p = 0.03 \)).
Conclusions. Our data demonstrate no deleterious effect of elevated P on embryo quality. However, high serum P adversely affects implantation and pregnancy rates.

Keywords: Embryo quality, intracytoplasmic sperm injection, implantation, progesterone

Introduction
The effect of plasma progesterone (P) on pregnancy rates in in vitro fertilization (IVF) is a controversial issue [1]. Previously we and others reported that high P levels on the day of human chorionic gonadotropin (hCG) administration are associated with a decrease in pregnancy rate [1–3]. Conversely, other researchers have found no adverse effects of P on pregnancy rate [4,5]. The influence of P is related to adverse effects on endometrial receptivity, or unfavorable effects on oocyte or embryo quality [1,3].

Several studies demonstrated a deleterious effect of high levels of P on fertilization by increasing the tendency toward polyspermy. Furthermore, a higher fertilization rate was reported once P level was < 0.9 ng/ml [6,7].

As sperm encounter with the oocyte is bypassed in intracytoplasmic sperm injection (ICSI), this will eliminate the effect of P on fertilization. Therefore the present retrospective study was conducted to examine the effect of elevated serum P on ICSI outcome.

Materials and methods
Patients
Two hundred and one patients in our unit underwent a total of 280 IVF treatment cycles allocated to ICSI from April 2000 to December 2000. The indications for ICSI are shown in Table I.

Ovulation induction
Ovulation induction was performed using a routine protocol of gonadotropin-releasing hormone analog (buserelin acetate nasal spray, 900 µg/day) (D-Ser
[TBU] 6-ethylamide-luteinizing hormone-releasing hormone, Suprefact<sup>®</sup>; Hoechst AG, Frankfurt, Germany) and three ampoules of human menopausal gonadotropin (Pergonal<sup>®</sup>; Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel). When the follicles reached a mean diameter of 17 mm, 10 000 IU hCG (Chorigon<sup>®</sup>; Teva Pharmaceutical Industries Ltd) was administered. Oocyte retrieval, using an ultrasound-guided transvaginal approach, was scheduled 34–36 h after hCG administration.

**Micromanipulation**

The cumulus–oocyte complexes were isolated in IVF medium (Medi-Cult, Copenhagen, Denmark). Cumulus cells were removed with hyaluronidase (80 IU/ml, H-3757; Sigma, Rehovot, Israel) in flushing medium (Medi-Cult). Denuded oocytes were assessed for their specific meiotic stage according to the presence or absence of germinal vesicle and polar bodies, and metaphase II oocytes were prepared for injection.

The ICSI procedure was performed as previously described [8]. Spermatozoa were assessed continuously for any indication of movement (i.e. ‘twitching’ of head or tail) in the sperm medium droplet without polyvinylpyrrolidone (PVP). A single motile spermatozoon was aspirated from the separate sperm droplet into the injection pipette and then transferred to the 10% PVP droplet to separate it from attaching cells and debris.

After the ICSI procedure, the oocytes were returned to the culture dish for further incubation, and were inspected under a stereomicroscope (Olympus; SZH, Tokyo, Japan) for survival and fertilization 16–20 h later.

**Embryo quality**

Embryo cleavage and quality were evaluated 46–48 h after ICSI. Embryo morphology was graded 1–4 according to the shape of the blastomeres and the amount of detached anuclear fragments: grade 1 = excellent (embryos containing intact and symmetrical blastomeres with no anucleate fragments); grade 2 = good (asymmetric cleavage or <20% embryo fragmentation); grade 3 = fair (embryo fragmentation 20–50%); grade 4 = poor (embryo fragmentation >50%) [9]. Clinical pregnancies were confirmed by sonographic demonstration of a gestational sac.

Embryo transfer (ET) was performed 48–72 h after retrieval. A maximum of four embryos were transferred and the remaining ones were cryopreserved. A serum β-hCG pregnancy test was performed 12 days after ET. In the event of a positive result, vaginal ultrasonography was carried out 3 weeks later to determine the viability and number of gestational sacs.

**Progesterone assay**

Serum P was determined for three consecutive days before and on the day of hCG administration. Serum P was measured using ligand-labeled competitive chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and inter-assay coefficient of variance for P at 0.81 ng/ml and 0.93 ng/ml (conversion factor to SI units, 3.185) was 16%.

**Statistical analysis**

An elevated P level was arbitrarily defined as 0.9 ng/ml; this cut-off facilitated comparison with other reported data. Comparisons were made by Student’s t test, χ<sup>2</sup> analysis and Pearson’s correlation coefficient test where applicable; p<0.05 was considered statistically significant.

**Results**

Patients were divided into two groups according to the level of P on day of hCG administration. Group A comprised 92 (33%) cycles in which the P level was ≤0.9 ng/ml and group B comprised 188 (67%) cycles with P levels >0.9 ng/ml. Mean age was significantly lower in group B than in group A (31.3 years vs. 33.2 years; p=0.009).

In cycles with elevated P levels, higher estradiol (E2) levels were observed (1915 pg/ml vs. 1256 pg/ml; p<0.05), more oocytes were retrieved (12.8 vs. 8.7; p<0.05), more oocytes were manipulated (9.4 vs. 6.9; p<0.05), and more embryos were transferred (3.8 vs 3.4; p=0.02) (Table II).

No differences were found between the two groups in the etiology of infertility, the protocol of controlled ovarian stimulation, fertilization rate (65% vs. 65.8%; p=0.78) or embryo quality (grade 1 and 2 embryos: 68.5% vs. 74.0%; p=0.27).

In cycles with lower P levels, the clinical pregnancy rate was significantly higher (27.6% vs. 16.4%; p=0.03). Furthermore we found an inverse relationship between P level and pregnancy rate. Figure 1 shows the correlation between pregnancy rate per
embryo transfer and level of plasma P. Pregnancy rate consistently declined with the elevation of plasma P. No pregnancy was achieved in cycles with serum P level \( < 4 \) ng/ml on the day of hCG administration. Contrary to our postulation we found no statistically significant difference in pregnancy rate in patients \( \leq 35 \) years old (14.7%), patients aged \( 35-\leq 40 \) years (26.7%) and those \( \geq 40 \) years old (20.0%; \( p = 0.083 \)).

Plasma levels of P and E\(_2\) tended to decline with age (\( p = 0.053 \)).

Table III shows that 72.4% of patients aged \( \leq 35 \) years had P levels \( > 0.9 \) ng/ml, compared with 62.2% and 54.3% in patients aged \( > 35-\leq 40 \) years or \( \geq 40 \) years, respectively.

### Discussion

Controversy continues to exist regarding the effect of elevated P on the day of hCG administration and IVF/ET and ICSI/ET outcome. Schoolcraft and colleagues [10] were the first to report low pregnancy rates in women with P \( > 0.5 \) ng/ml. These findings were subsequently supported by Mio and associates [6], who reported low fertilization rate, low rate of normal-morphology embryos and low pregnancy rate in oocytes exposed to P levels \( > 1 \) ng/ml, compared with oocytes exposed to P levels \( < 0.9 \) ng/ml. Furthermore, Fanhin and co-workers [2] reported low fertilization rate with P levels \( > 0.9 \) ng/ml, but found no significant difference in fertilization rate, percentage of mature oocytes or rate of cleaved embryos. Hofmann and collaborators [11] found that oocytes exposed to different P levels, which were donated to women with comparable P levels, yielded no significant difference in pregnancy rate. These authors concluded that a high P level affects endometrial receptivity, rather than oocyte or embryo quality. Silverberg’s group [1], who found similar pregnancy rates after freezing and thawing of embryos exposed to different levels of P in plasma, further supported this assumption.

Previously our group reported that P levels \( > 1.9 \) ng/ml resulted in lower pregnancy rate for the donors and significantly lower pregnancy rate for the recipients [3]. Because the endometria in the recipients were prepared uniformly, the authors concluded that this was the result of endometrial effects of P on oocyte or embryo quality.

Conversely, the results of the present study demonstrated that high levels of P, prior to hCG administration, adversely affected the pregnancy rate in ICSI cycles. However, the embryo quality, as determined by embryo grading, appeared to be unaffected by elevated serum P levels. In addition, we found a linear decline in pregnancy rate concomitantly with rising P level, and with P \( > 4 \) ng/ml no pregnancy was attained.

These findings are in contradiction to those of other authors [4,5] that showed no adverse effect of high serum P on the day of hCG administration on implantation rates after ICSI/ET. However, these authors did not examine the effect of high P on embryo quality.

Theoretically, high levels of P may affect IVF/ET outcome at different stages: ovulation and oocyte...
quality, fertilization process, embryo quality or endometrial receptivity.

Nagai and colleagues [12] showed that pig oocytes exposed to high levels of P demonstrated a significantly lower meiosis rate compared with oocytes cultured in low P concentration. Furthermore, Franchimont and associates [13] demonstrated that follicular-fluid P concentration was significantly lower in follicles that resulted in pregnancy. However, these findings were not supported by other studies [1,7,14] which demonstrated that although the number of retrieved oocytes was significantly higher in cycles with high P level, the percentages of atretic and mature oocytes were comparable. Similarly, in our study, we found that the number of retrieved oocytes was higher in cycles with raised P levels than in those with low P levels.

Several studies have demonstrated a deleterious effect of P on fertilization. Hartshorne [15] confirmed high polyspermic fertilization in oocytes retrieved from follicles with high P levels. Givens [7] and Mio [6] and co-workers found a higher fertilization rate when P levels were <1 ng/ml and 0.9 ng/ml, respectively.

No statistically significant difference in fertilization rate was found between our two study groups. In a previous study, Yovel and collaborators [3] postulated that high P levels may depreciate the quality of embryos. In the current study, we found no difference in embryo quality in the study groups. These findings confirm those of other authors [1,11,16] who found no deleterious effect of P on embryo quality.

We examined embryo morphology around the four- to six-cell stage. However, high P levels may alter embryo morphology in later stages of cleavage [9].

The pregnancy rate was significantly lower in the group with high P levels, despite the fact that more ETs were performed in these patients. Furthermore, the average age of patients in this group was significantly lower than that of patients with low P levels. Based on this finding we conclude that high P levels adversely affect endometrial receptivity. It was suggested that elevated P levels might affect the synchrony of implantation processes, namely apposition and adhesion. In mice, P administration caused closure of the uterus with only primary apposition, and estrogen supplementation was essential for successful implantation [17,18].

In our study, we found lower E₂ and P levels and fewer oocytes and embryos in elderly patients compared with younger women. However, embryo quality, determined by morphological grading, was similar. Because high P levels were the only significant parameter, we concluded that these levels mask the effect of age and higher number of transferred embryos.

In conclusion, although we found no effect of high levels of P on embryo morphology, the pregnancy rates were significantly lower in women with high P levels. A prospective, randomized study is warranted in order to clarify this controversial issue.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


