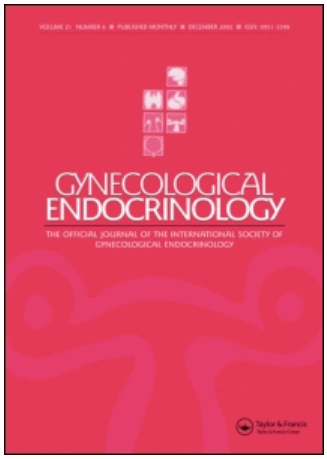

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ORIGINAL ARTICLE

The influence of estradiol/follicle and estradiol/oocyte ratios on the outcome of controlled ovarian stimulation for *in vitro* fertilization

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Abstract

Objective. The aim of the study was to evaluate the influence of the ratios of estradiol (E_2) to either the number of follicles >14 mm on the day of human chorionic gonadotropin administration (E_2/follicle) or the number of oocytes retrieved (E_2/oocytes) during controlled ovarian hyperstimulation (COH) with gonadotropin-releasing hormone (GnRH)-agonist (agonist group) and GnRH-antagonist (antagonist group), on the outcome of *in vitro* fertilization (IVF) cycles.

Patients and methods. All consecutive women aged <35 years admitted to our IVF unit during a 6-year period with normal to high response to COH were retrospectively studied. Ovarian stimulation characteristics, number of oocytes retrieved, number of embryos transferred and pregnancy rate were assessed.

Results. Six hundred and ninety consecutive IVF cycles were evaluated, 301 in the agonist group and 389 in the antagonist group. The ratios of E_2/follicle and E_2/oocyte were significantly higher in the agonist group ($p < 0.001$ for both). Moreover, while pregnancy rates within E_2/oocyte ratio of 100–200 pg/ml were comparable between the agonist and antagonist groups, when E_2/oocyte ratios were <100 pg/ml or >200 pg/ml, pregnancy rates were significantly higher in the agonist group. Furthermore, no difference in pregnancy rates was observed within the agonist group between different E_2/oocytes ratios, while within the antagonist group, higher pregnancy rates were observed when comparing those with E_2/oocyte ratio of 100–200 pg/ml with those with E_2/oocyte ratio <100 pg/ml or >200 pg/ml.

Conclusion. While E_2/oocyte ratio cannot predict the success of GnRH-agonist protocol, patients undergoing GnRH-antagonist protocol should reach E_2/oocyte ratio within the 100–200 pg/ml range in order to achieve the best IVF outcome.

Keywords: *Gonadotropin-releasing hormone agonist, gonadotropin-releasing hormone antagonist, estradiol/oocyte ratio, outcome of* in vitro *fertilization, pregnancy*

Introduction

Controlled ovarian hyperstimulation (COH) is apparently a key factor in the success of *in vitro* fertilization–embryo transfer (IVF-ET). The ability of co-treatment with a gonadotropin-releasing hormone (GnRH)-analog to prevent a premature increase in luteinizing hormone during COH has made it the standard of care worldwide. Studies comparing GnRH-agonist long protocols with GnRH-antagonist protocols have yielded conflicting results for pregnancy rate [1–4] and have led to an ongoing debate in the medical community [5,6]. Most studies related the lower pregnancy rate observed during GnRH-antagonist cycles to 'the center's inexperience' or their use in cycles with an unfavorable prognosis *a priori*, i.e. repeated failures and elderly low responders.

Loumaye and colleagues [7] studied the ratio of estradiol (E_2) level on the day of human chorionic gonadotropin (hCG) administration to the number of oocytes retrieved (E_2/oocyte ratio) in patients undergoing the long GnRH-agonist suppressive protocol. They found that the E_2/oocyte ratio is a strong index for the success of an IVF cycle, with the highest pregnancy rate observed in patients with E_2/oocyte ratio of 70–140 pg/ml. Yang and associates [8] showed that patients undergoing the flare GnRH-agonist protocol with an elevated E_2/oocyte ratio demonstrated lower pregnancy and implantation

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rates. Accordingly, we [9] observed that lower E_2/oocyte ratio predicts higher pregnancy rate during an IVF cycle in elderly patients aged 43–45 years.

Prompted by these findings, we sought to examine both the ratio of E_2 to the number of follicles >14 mm on the day of hCG administration (E_2/follicle) and the E_2/oocyte ratio in GnRH-agonists versus GnRH-antagonists COH protocols, and to evaluate their influence on IVF cycle outcome. These findings may help to clarify the proper approach to GnRH-analogs in COH and to aid fertility specialists and their patients in the decision-making process.

Patients and methods

We reviewed the computerized files of all consecutive women aged <35 years admitted to our IVF unit during a 6-year period, who reached the ovum pick-up stage. The elimination of bias in this selection, for the purposes of the present study, was achieved by excluding poor responders: women who achieved an E_2 level <500 pg/ml on the day of hCG administration and/or women in whom <5 oocytes were retrieved. Other exclusion criteria were use of donor oocytes or transfer of frozen–thawed embryos, and use of other than a mid-luteal long GnRH-agonist (triptorelin, 0.1 mg daily, subcutaneously; Ferring Lapidot, Netanya, Israel) suppressive protocol (agonist group) or the flexible multidose GnRH-antagonist (cetrorelix, 0.25 mg daily, subcutaneously; Serono Laboratories, Aubonne, Switzerland) protocol (antagonist group). The selection of type of analog used was the decision of the treating physician and largely dependent on the fashion at the time. In both protocols, gonadotropins were administered in variable doses, depending on patient age and/or ovarian responsiveness in previous cycles, and further adjusted according to serum E_2 levels and vaginal ultrasound measurements of follicular diameter, obtained every two or three days. hCG was administered for final maturation of oocytes when at least three mature (>17 mm) follicles were identified by transvaginal scan, combined with appropriate peripheral serum E_2 levels. Oocytes were aspirated by the transvaginal ultrasonographic route approximately 34 h after hCG injection. Routine IVF/intracytoplasmic sperm injection was then performed as appropriate. For luteal phase support, patients received either 50 mg progesterone intramuscularly (Gestone®; Ferring Lapidot) daily or 600 mg micronized progesterone soft gel vaginal capsules (Utrogestan®; Besins Iscovesco CTS, Petach Tikva, Israel) in three divided doses daily.

Data on patient age, cause of infertility and infertility-treatment-related variables were collected from the files. Ovarian stimulation characteristics, number of oocytes retrieved and number of embryos transferred per cycle were recorded. Outcome was defined as the proportion of cycles with oocyte retrieval that led to pregnancy.

Results are presented as mean ± standard deviation. Differences in variables were analyzed statistically with the non-parametric Wilcoxon signed rank test, Student's t test or the χ^2 test, as appropriate. A p value of less than 0.05 was considered significant.

Results

Six hundred and ninety consecutive IVF cycles were evaluated, 301 in the agonist group and 389 in the antagonist group. The clinical characteristics of the IVF cycles in the two study groups are shown in Table I.

Pregnancy was achieved in 111 patients in the agonist group (pregnancy rate, 36.9% per cycle) and 114 patients in the antagonist group (pregnancy rate, 29.3% per cycle); this difference was statistically significant ($p < 0.05$), despite the higher number of oocytes retrieved ($p < 0.02$) and embryos transferred ($p < 0.001$) in the antagonist group. Moreover, the E_2/follicle and E_2/oocyte ratios were significantly higher in the agonist group ($p < 0.001$ for both). As expected, the agonist group used significantly more gonadotropin ampoules ($p < 0.01$), required longer stimulation ($p < 0.001$) and had higher E_2 levels on the day of hCG administration ($p < 0.001$). There were no differences between the groups in terms of patient age, day-3 follicle-stimulating hormone level, peak progesterone level, number of follicles >14 mm on the day of hCG administration or fertilization rate (Table I).

Patients were further divided into three subgroups according to their E_2/oocyte ratio (A, <100 pg/ml; B, 100–200 pg/ml; C, >200 pg/ml) (Table II). When E_2/oocyte ratio ranged between 100 and 200 pg/ml (subgroup B), similar pregnancy rates were observed in the agonist and antagonist groups. On the other hand, higher pregnancy rates were demonstrated in the agonist group compared with the antagonist group when the E_2/oocyte ratios were <100 pg/ml (subgroup A) and >200 pg/ml (subgroup C) ($p < 0.02$ for both). Furthermore, while no difference in pregnancy rates was observed within the agonist group between the three subgroups, within the antagonist group, lower pregnancy rates were observed when comparing subgroups A (E_2/oocyte ratio <100 pg/ml) ($p < 0.2$) and C (E_2/oocyte ratio >200 pg/ml) ($p < 0.05$) with subgroup B (E_2/oocyte ratio 100–200 pg/ml). Subdivision of patients according to different comparable ranges of E_2/follicle ratio did not reveal any difference between the agonist and antagonist groups or within each group (Table III).

Table I. Comparison between IVF cycles in the GnRH-agonist and GnRH-antagonist groups.

	Agonist	Antagonist	*p* Value
Number of cycles	301	389	
Patient age (years)	29.2 ± 3.4	29.3 ± 3.5	
Day-3 FSH level (IU/l)	6.18 ± 2.0	6.16 ± 2.2	
Number of gonadotropin ampoules used	35.5 ± 15.7	32.5 ± 13.7	<0.01
Length of stimulation (days)	10.6 ± 2.0	9.4 ± 1.6	<0.001
Peak E_2 level on day of hCG administration (pg/ml)	2284 ± 1052	1994 ± 967	<0.001
Progesterone level on day of hCG administration (ng/ml)	0.8 ± 0.4	0.9 ± 0.7	
Number of follicles >14 mm on day of hCG administration	11.4 ± 2.2	12.1 ± 4.8	
Number of oocytes retrieved	13.3 ± 6.7	15.1 ± 7.9	<0.02
Fertilization rate (%)	55 ± 31	53 ± 27	
Number of embryos transferred	2.1 ± 0.7	2.5 ± 0.8	<0.001
E_2/follicle ratio (pg/ml)	213 ± 121	176 ± 87	<0.001
E_2/oocyte ratio (pg/ml)	194 ± 103	150 ± 73	<0.001
Pregnancy rate (%)	36.9	29.4	<0.04

IVF, *in vitro* fertilization; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; E_2, estradiol; hCG, human chorionic gonadotropin; data are presented as mean ± standard deviation.

Table II. Pregnancy rates according to the different E_2/oocyte ratio subgroups.

E_2/oocyte ratio (pg/ml)	Agonist group	Antagonist group
<100	18/39 (46)*	25/97 (26)*
100–200	49/148 (33)	73/216 (34)†
>200	44/114 (39)‡	16/76 (21)†‡

E_2, estradiol; data are presented as *n*/*N* (%); *†‡significant difference between the subgroups ($p < 0.05$).

Table III. Pregnancy rates according to the different E_2/follicle ratio subgroups.

E_2/follicle ratio (pg/ml)	Agonist group	Antagonist group
<100	9/19 (47)	16/52 (31)
100–200	52/141 (37)	65/213 (31)
>200	50/141 (35)	34/124 (27)

E_2, estradiol; data are presented as *n*/*N* (%).

Discussion

In the present study of normal-to-high responder young patients (<35 years old), we clearly observed a significantly higher clinical pregnancy rate in those undergoing the mid-luteal long GnRH-agonist suppressive protocol than in those undergoing the flexible multidose GnRH-antagonist protocol. While these results are in accordance with some reports [2,4], other studies [10–14], including a recent meta-analysis [3], failed to confirm this difference in outcome between the treatment options. These conflicting results have led to an ongoing debate in the medical community, which related the inferiority of GnRH-antagonists to their consideration only in poor responder patients or as a second treatment option in COH [5,6]. Yet, although cycles/patients with an unfavorable prognosis were excluded from the present study, we still observed a significantly lower pregnancy rate with GnRH-antagonists.

In the present study we found E_2/follicle ratio to be significantly higher in the agonist compared with the antagonist group. Moreover, E_2/follicle ratio could not predict IVF cycle outcome. This latter observation challenges the use of serum E_2 measurement for the routine follow-up during COH [15,16]. Moreover, it actually supports the suggestion of abandoning E_2 measurements during COH for IVF, since it does not compromise treatment results [17] nor assist in the prediction of severe ovarian hyperstimulation syndrome [18].

We also observed significantly higher E_2/oocyte ratios in the agonist group than in the antagonist group. Moreover, pregnancy rates were comparable between the different ranges of E_2/oocyte ratio (A, <100 pg/ml; B, 100–200 pg/ml; C, >200 pg/ml) within the agonist group and subgroup B (100–200 pg/ml) of the antagonist group. However, subgroups A and C (<100 pg/ml and >200 pg/ml, respectively) of the antagonist group showed significantly lower pregnancy rates. These observations support the suggestion that the inferiority of cycles using GnRH-antagonist may be related to 'the center's inexperience' [13] and suggest that in order to gain a higher pregnancy rate – comparable to that in the agonist group – patients undergoing the GnRH-antagonist COH protocol should achieve an E_2/oocyte ratio in the range of 100–200 pg/ml.

Three previous reports demonstrated a reduced pregnancy rate with increasing E_2/oocyte ratio. Loumaye's group [7] studied patients undergoing COH for IVF using the long GnRH-agonist suppressive protocol, with different GnRH-agonist types and modes of administration. While pregnancy rate was found to be the highest whenever E_2/oocyte ratio ranged between 70 and 140 pg/ml, it was not different from pregnancy rates in patients with

E_2/oocyte ratio exceeding 210 pg/ml. Yang's group [8] studied the influence of various E_2/oocyte ratios on reproductive outcome in women undergoing IVF using the flare-up GnRH-agonist protocol. They found that IVF cycles with an elevated E_2/oocyte ratio correlated with lower pregnancy and implantation rates. Accordingly, while studying the results of IVF cycles in women aged 43–45 years, we [9] observed that successful IVF cycles can be expected in those patients who reached the stage of ET with at least two good-quality embryos and in the presence of low ratios of E_2/follicle or E_2/oocyte. It may be therefore suggested that while E_2/oocyte ratio has no predictive role in the success of IVF cycles in normal-to-high responder patients undergoing the long GnRH-agonist suppressive protocol, it negatively correlates with IVF outcome in low-responder elderly patients, those undergoing the GnRH-antagonist COH protocol or those undergoing the GnRH-agonist flare protocol. The negative effect in these latter groups of patients may be explained by the concomitant high intrafollicular level of androgen, the precursor of estrogen, which has a deleterious effect on follicular maturation and development [19]; a detrimental effect on endometrial receptivity; or, alternatively, by a high proportion of degenerative oocytes (the 'empty follicle' syndrome).

It may be concluded that while neither E_2/follicle nor E_2/oocytes ratio has a role in the prediction of IVF outcome of normal-to-high responder patients undergoing the long suppressive GnRH-agonist protocol, E_2/oocyte ratio correlates with pregnancy rates in low responders and in patients undergoing the GnRH-antagonist COH for IVF. More attention should be directed to the appropriate timing of hCG administration in these patient groups in order to achieve optimal success rates.

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