Peri-ovarian adhesions interfere with the diffusion of gonadotrophin into the follicular fluid

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In previous studies, patients with severe peri-ovarian adhesions have been found to show low pregnancy rates and a poor response to gonadotrophin stimulation during in-vitro fertilization (IVF) treatment. The purpose of this retrospective pharmacokinetic study was to assess the diffusion of exogenous human chorionic gonadotrophin (HCG) in patients with peri-ovarian adhesions by examining the concentration of exogenous HCG in the follicular fluid in patients undergoing down-regulation and IVF due to infertility. The patients underwent laparoscopic examination for the scoring of peri-ovarian adhesions (using the classification of adnexal adhesions adopted by the American Fertility Society, a score of 0 means no adhesions, and a score of 32 represents bilateral expanded dense adhesions). Oocytes were recovered after human menopausal gonadotrophin–human chorionic gonadotrophin (HMG–HCG) stimulation with gonadotrophin-releasing hormone agonist. Serum and follicular fluid were collected at the time of oocyte recovery for measuring the HCG ratio (the follicular HCG concentration to the serum HCG concentration; a reflection of the diffusion of exogenous human chorionic gonadotrophin) by time-resolved fluoro-immunoassay. A negative correlation was found between the number of oocytes recovered and the peri-ovarian adhesion score ($r = -0.62, P < 0.01$). In a given patient, the follicular HCG concentration was always lower than the serum HCG at the time of oocyte recovery. The HCG ratio in all samples was 0.9 or less ($0.51 \pm 0.20$; range, 0.09–0.90). Significant negative correlations were found between the peri-ovarian adhesion score and both the follicular HCG concentration ($r = -0.80, P < 0.01$) and the HCG ratio ($r = -0.75, P < 0.01$). In conclusion, severe peri-ovarian adhesions interfered with the diffusion of exogenous gonadotrophin into the follicular fluid during IVF treatment. Thus, the diffusion of exogenous gonadotrophin into the follicular fluid may represent a new parameter in the assessment of ovarian blood flow and IVF outcome.

Key words: adhesion/gonadotrophin/infertility/IVF/ovary

Introduction

Peri-adnexal adhesions can cause infertility by encapsulating the terminal end of the tube, the ovary, or both. They can thus prevent oocyte retrieval by the fimbria as a consequence of the resulting disruption of the ovarian–fimbrial relationship. It has been demonstrated that the pregnancy rate decreases as the severity of peritubal and peri-ovarian adhesions increases in humans (Caspi et al., 1979). One further reason for the low pregnancy rate in patients with peri-ovarian adhesions can be the presence of an anovulatory disorder. Indeed, Hamilton et al. (1986) found a high incidence of unruptured follicles in a group of patients with adnexal adhesions. In-vitro fertilization (IVF) and embryo transfer might be expected to lead to conception in patients with peri-ovarian adhesions that cause follicles to fail to rupture. However, in our recent study, patients with severe peri-ovarian adhesions showed low pregnancy rates together with a poor response to gonadotrophin stimulation during IVF treatment (Nagata et al., 1997). Furthermore, it has been noted that peri-ovarian adhesions impede folliculogenesis (Mahadevan et al., 1985).

It has been reported in the rabbit that, when the vasculature between the genital tract and the ovary is disrupted, there is a reduction in the number of ovaulations (Byeth and Winston, 1981). However, there is no attendant reduction in the number of ovaulations when the fimbriae were resected but the ovarian vessels were preserved (Coppo et al., 1984). McComb and Delbeke (1984) further demonstrated the importance of the ovarian blood vessels and, as an incidental observation, they showed that peri-ovarian adhesion formation also greatly affected the number of ovaulation sites in the rabbit model.

In view of these previous observations in humans and rabbits, it seems likely that peri-ovarian adhesions produce their effect by disrupting the ovarian blood flow. If the ovarian blood flow were to be decreased by the presence of peri-ovarian adhesions, changes would occur in pharmacokinetics. Research in the field of human IVF treatment could help us resolve the many unsettled questions. Endogenous gonadotrophins are suppressed during superovulation in IVF treatment cycles involving pituitary down-regulation using gonadotrophin-releasing hormone (GnRH) agonists. Patients undergoing IVF treatment lose the endogenous luteinizing hormone (LH) surge and human chorionic gonadotrophin (HCG) has to be injected to mimic it and so induce oocyte division and luteinization of granulosa cells. Study of the pharmacokinetics of injected HCG in women undergoing down-regulation is a useful method of assessing gonadotrophin diffusion, because endogenous HCG is never seen at the time of oocyte recovery in non-pregnant women.
The purpose of this pharmacokinetic study was to assess the diffusion of exogenous gonadotrophin (HCG) in patients with peri-ovarian adhesions. The study examined the concentration of exogenous HCG in follicular fluid in patients undergoing down-regulation and IVF–embryo transfer. The diffusion of HCG from serum to follicular fluid was examined and related to the degree of peri-ovarian adhesions.

Material and methods

Twenty-six patients with tubal infertility underwent a total of 26 cycles of IVF–embryo transfer treatment at Fukuoka University Hospital between November 1995 and October 1996. All 26 patients underwent laparoscopic examination in the 12 months before IVF treatment or just after. In each patient, the laparoscopic examination was performed in the late follicular phase. Peri-ovarian adhesions were scored using the American Fertility Society (AFS) classification of adnexal adhesions, as shown in Table I (American Fertility Society, 1988). The sum of the scores given to left and right peri-ovarian adhesions was taken as the ‘peri-ovarian adhesion score’. Patients in whom peri-ovarian adhesions could not be scored, for reasons such as prior oophorectomy or salpingo-oophorectomy, were excluded from this study. None of the patients had an anovulatory disorder. All the patients were routinely offered an IVF cycle with a GnRH agonist (buserelin acetate; Hoechst, Tokyo, Japan) starting 7 days before menstruation or on the first day of the menstrual cycle. Follicle growth was stimulated on day 3 of the menstrual cycle by injecting human menopausal gonadotrophin (HMG) (Humegon; Organon Japan, Tokyo, Japan). The response to HMG was monitored from day 8 by a daily measurement of follicle diameter. When the two largest follicles had each reached a mean diameter of at least 18 mm, an injection of 10 000 IU of exogenous gonadotrophin (HCG; Teikoku Co. Ltd., Tokyo, Japan) was given i.m. to mimic the normal LH surge. Oocytes were recovered 34 to 37 h after the administration of HCG following transvaginal ultrasound-guided puncture of the follicles.

Venous blood samples and follicular fluid were collected at the time of oocyte recovery, all follicles with a diameter of more than 10 mm being punctured. After the venous blood samples and the follicular fluid collected from all the punctured follicles had been centrifuged at 1861 g for 10 min, serum samples and follicular fluid were collected without cellular structures. Serum samples and follicular fluid samples were frozen at −20°C until assayed for the determination of HCG. Serum and follicular HCG were measured by time-resolved fluoroimmunoassay (HCG-β subunit; DELFIA hCG kit; Walac Oy, Turku, Finland), the intra- and inter-assay coefficients of variation being 3.3% and 7.4%, respectively (conversion factor to SI units, 1.0). The serum HCG concentration, the follicular HCG concentration and the HCG ratio (the ratio between the follicular HCG concentration and the serum HCG concentration) were evaluated to give an indication of the diffusion of exogenous gonadotrophin.

The correlation between the interval after an HCG injection and the serum HCG concentration, the follicular HCG concentration or the HCG ratio was assessed by linear regression analysis. The diffusion of exogenous gonadotrophin was evaluated by assessing the relationship between it and the peri-ovarian adhesion score. To this end, the correlation between the peri-ovarian adhesion score and either follicular HCG concentrations or HCG ratio was assessed by linear regression analysis. A P-value of 0.05 or less was considered to indicate statistical significance. Data analysis was carried out using Microsoft Excel software (Microsoft Excel for Macintosh, version 4.0; Microsoft Corporation, Redmond, WA, USA).

Results

The mean age of the 26 patients at the time they underwent IVF–embryo transfer treatment was 32.5 ± 3.8 years (range, 25–40 years). Nineteen of the 26 patients had primary infertility. The mean duration of their infertility was 52.5 ± 44.7 months (range, 8–180 months). The mean body mass index was 20.1 ± 1.5 (range, 17.5–22.6). The mean score given to their peri-ovarian adhesions was 13.9 ± 10.6 (range, 0–32). The mean score given to their peritubal adhesions (the score being calculated in each patients as the sum of left and right peritubal adhesion scores) was 24.3 ± 8.6 (range, 6–32). Seven of the 26 patients underwent laparoscopic examination before IVF–embryo transfer treatment, and 19 after IVF–embryo transfer treatment.

A negative correlation was found between peri-ovarian adhesion score and the number of oocytes recovered, as shown in Figure 1 (r = -0.62, P < 0.01, y = -0.2x + 10.5). In other words, as the peri-ovarian adhesion score increased, the number of oocytes recovered decreased.

Serum and follicular HCG concentrations are shown in Figure 2. At the time of oocyte recovery, the mean serum HCG concentration for the 26 IVF–embryo transfer cycles was 196 ± 62 mIU/ml (range, 99–306 mIU/ml), the mean follicular HCG concentration was 104 ± 58 mIU/ml (range, 17–214 mIU/ml) and the mean HCG ratio was 0.51 ± 0.2 (range, 0.09–0.90). In a given patient, the follicular HCG concentration was always lower than the serum HCG concentration. The HCG ratio in all samples was 0.90 or less (range, 0.09–0.90). Figure 2 also shows the relationship between the various HCG concentrations and the interval after HCG injection. None of the variables (the serum HCG concentration, the follicular HCG concentration, or the HCG ratio) showed a significant correlation with peri-ovarian adhesions.

Table I. Scoring of peri-ovarian adhesions^a,b

<table>
<thead>
<tr>
<th>Adhesions^c</th>
<th>&lt;1/3 Enclosure</th>
<th>1/3–2/3 Enclosure</th>
<th>&gt;2/3 Enclosure</th>
</tr>
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<tbody>
<tr>
<td>Ovary</td>
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<td>Filmy</td>
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<tr>
<td></td>
<td>Dense</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
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<td>Left</td>
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<tr>
<td></td>
<td>Dense</td>
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<td>8</td>
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^bSum of the scores given to left and right peri-ovarian adhesions was taken as the ‘peri-ovarian adhesion score’.
^cFilmy adhesions can be lysed by scissors, electrocautery, or laser without undue risk of bleeding or injury to adjacent organs.
Figure 1. Correlation between peri-ovarian adhesion score and ovarian response to exogenous gonadotrophin. There was a negative correlation between the peri-ovarian adhesion score and the number of oocytes recovered ($n = 26$, $r = -0.62$, $P < 0.01$, $y = -0.2x + 10.5$).

Figure 2. Exogenous gonadotrophin concentration in serum and follicular fluid. The serum human chorionic gonadotrophin (HCG) concentration, the follicular HCG concentration and the HCG ratio showed no correlation with the time interval after HCG administration ($r = 0.05$, $r = 0.06$ and $r = 0.01$, respectively).

Discussion

Studying the pharmacokinetics of injected HCG in women undergoing down-regulation is a useful method of assessing gonadotrophin diffusion. In this study, the HCG ratio (the ratio between the follicular HCG concentration and the serum HCG concentration) was used as a reflection of gonadotrophin diffusion. In our data, the HCG ratio in all samples was 0.90 or less and it covered a wide range (from 0.09 to 0.90). The interval after HCG administration (34–37 h) bore no relation to the serum HCG concentration, or follicular HCG concentration, and the HCG ratio.

In our previous study, patients with severe peri-ovarian adhesions showed low pregnancy rates together with a poor response to gonadotrophin stimulation during IVF treatment (Nagata et al., 1997). In the present study, we found that, as the peri-ovarian adhesion score increased, the number of oocytes recovered decreased. The presence of peri-ovarian adhesions might be expected to be a significant factor adversely affecting the diffusion of gonadotrophin. In fact, as the peri-ovarian adhesion score increased, the follicular HCG concentration and the HCG ratio both decreased in this study.
These results suggest that peri-ovarian adhesions do indeed affect the diffusion of gonadotrophin into the follicular fluid. The AFS classification of adnexal adhesions has been used to allow differentiation between filmy and dense adhesions on the basis of their score. The total peri-ovarian adhesion score reflects both the degree and the extent of the adhesions, the highest scores being an indication of extensive dense adhesions. In the present study, the presence of extensive dense peri-ovarian adhesions was associated with a poor diffusion of exogenous gonadotrophin and a poor response to the gonadotrophin.

Exactly how peri-ovarian adhesions affect the diffusion of exogenous gonadotrophin is not clear. One relevant factor is likely to be the blood flow to and within the ovary. A previous power Doppler ultrasonographic study in women undergoing IVF treatment showed that a poor follicular blood flow was associated with a poor outcome, and that pregnancies only occurred in women with a good follicular blood flow (Chui et al., 1997). Peri-ovarian adhesions, involving attachments to any surface among the pelvic organs, might compromise the ovarian blood supply by contortion of the adnexal structures. Peri-ovarian adhesions involving fibrosis and scarring might also have a constrictive effect, anatomically limiting the ovarian blood supply. McComb and Delbeke (1984) studied the role of the ovarian blood vessels and demonstrated the importance of the vasculature. It was later reported that the blood flow in the ovary changes during the menstrual cycle (Hata et al., 1990), the changes being especially marked around ovulation (Bourne et al., 1991; Weiner et al., 1993). Indeed, Balakier and Stronell (1994), using colour Doppler examination, showed a significant increase in the ovarian blood flow following HCG injection. A restriction on blood flow at this time might have a deleterious effects on the diffusion of gonadotrophin.

Angiogenesis is also a crucial step in physiological processes such as the development of the corpus luteum, which includes neovascularization of the luteinizing granulosa layer of the follicle following ovulation (Stouffer, 1996). Granulosa cells contribute to the intra-follicular environment of the developing oocytes (Gregory and Leese, 1996) and have been shown to express vascular endothelial growth factors (VEGF) which have been implicated in the neovascularization of the follicle (Shweiki et al., 1993; Dissen et al., 1994). Indeed, VEGF is a strong promoter of vascularization. Recent studies have demonstrated VEGF mRNA expression by granulosa cells in the human ovary (Ferrara et al., 1992; Yan et al., 1993; Kamet et al., 1995) and VEGF mRNA was localized to human follicular granulosa cells in the peri-ovulatory follicle in vivo by Kamet et al. (1995). Neulen et al. (1995) demonstrated that HCG caused a dose-dependent increase in VEGF mRNA expression in human luteinized granulosa cells, and Lee et al. (1997) found that such cells produce significant quantities of VEGF in a process that is regulated directly by gonadotrophin (HCG).

On the base of the above discussion, a possible scenario is as follows. Peri-ovarian adhesions might initially compromise the ovarian blood supply anatomically by contorting adnexal structures. Thereafter, poor expression of VEGF mRNA and VEGF in the follicular fluid together with a low HCG concentration might lead to delayed angiogenesis in follicular granulosa cells. The presence of poor angiogenesis might exacerbate effects of a compromised ovarian blood supply. It seems reasonable to assume that the fall in the HCG ratio at higher peri-ovarian adhesion scores reflects a reduction in the functional blood supply following anatomical changes in and around the ovary and possibly a reduced angiogenesis among follicular granulosa cells.

A number of factors could lead to patients with severe peri-ovarian adhesions showing low pregnancy rates together with a poor gonadotrophin diffusion during IVF treatment. In the normal menstrual cycle, the LH surge stimulates the final maturation of the egg within the follicle, induces the preovulatory changes in the follicle and initiates luteinization of the granulosa cell. In our study, an LH surge deficiency occurred in patients with severe peri-ovarian adhesions, patients who also had a low concentration of HCG in their follicular fluid. A luteal-phase defect occurs following such as LH surge deficiency, but this study did not evaluate the relationship between peri-ovarian adhesions and any luteal-phase defect. Further work will be necessary to investigate the relationship between such a luteal-phase defect and poor gonadotrophin diffusion. Recently, Van Blerkom et al. (1997) examined the diffusion of oxygen into the follicular fluid, and suggested that severe intrafollicular hypoxia may influence the normality of chromosomal organization and segregation in the oocyte. Poor gonadotrophin diffusion might reflect poor blood flow and consequent intrafollicular hypoxia leading to the production of a non-viable oocyte. This might help explain the low pregnancy rate in patients with severe peri-ovarian adhesions.

In conclusion, the presence of severe peri-ovarian adhesions interferes with the diffusion of exogenous gonadotrophin into the follicular fluid during IVF treatment. The present study thus suggests that the diffusion of exogenous gonadotrophin into the follicular fluid may represent a new parameter that needs to be considered in the assessment of both the ovarian blood flow and IVF outcome. If patients with severe peri-ovarian adhesions show poor responses to exogenous gonadotrophin, and we want to achieve a good outcome with IVF treatment, we may have to consider either the adhesiolyisis around the ovary prior to the IVF programme or giving the patient a high dose of exogenous gonadotrophin during IVF.

References
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