Human Reproduction vol.12 no.8 pp.1789–1793, 1997

Concentrations of prostaglandin $F_{2\alpha}$ in follicular fluid from women with endometriosis*

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Previous data have indicated that prostaglandins (PG) play an important role in the ovulation process and significant quantities have been found in follicular fluid. The synthesis of PG is influenced by ovarian steroids as well as inflammatory processes. Increased PG has been shown in peritoneal fluid from women with endometriosis. The aim of this study was to see whether $PGF_{2\alpha}$ concentration in follicular fluid varies according to the presence or absence of remaining endometriotic lesions in the pelvis of women with endometriosis. Follicular fluid was collected at visual puncture of the follicles at laparoscopy of 26 women with diagnosed endometriosis. Visible lesions were found in 10 women and in 16 women no lesions could be seen macroscopically. $PGF_{2\alpha}$ was determined using radioimmunoassay. The results showed no significant difference in the concentrations of $PGF_{2\alpha}$ between women with visible and not macroscopically visible endometriotic lesions and there was no significantly linear correlation with endometriotic lesions in the follicle punctured. These data are in accordance with clinical results showing that fertility rate does not increase in endometriotic women even if the lesions disappear after treatment.

Key words: endometriosis/follicular fluid/infertility/$PGF_{2\alpha}$/prostaglandins

Introduction

Prostaglandins (PG) have been identified in many different tissues in humans, where they appear to play an important role, both directly and indirectly, in modulating other local as well as circulating hormones. In-vivo studies of prostaglandins in humans are difficult to perform because of their instability. $PGF_{2\alpha}$ is formed from arachidonic acid via the cyclo-oxygenase pathway and it is broken down in the lung to 15-keto-$PGF_{2\alpha}$ which still has some biological activity.

A large body of contradictory data has been published regarding the physiological significance of prostaglandins in the ovaries. Prostaglandins are synthesized in both granulosa, theca and stromal compartments and have been identified in significant quantities in follicular fluid (Jeremy *et al*., 1987; Abrahamsson *et al*., 1990; Gelety and Chaudhuri, 1992). The ovarian hormones on the other hand influence the synthesis of prostaglandins. Most information has been obtained from animal studies, where follicular prostaglandins increase dramatically during the hours after the preovulatory gonadotrophin rise and some studies in laboratory animals suggest that the preovulatory increase of prostaglandins plays a critical role (Le Maire *et al*., 1975; Koos and Clark, 1982; Dennefors *et al*., 1983). Several prostaglandins such as PGE_2, $PGF_{2\alpha}$, thromboxane (TxB_2) and 6-keto-$PGF_{1\alpha}$ have been identified in follicular fluid, both from hyperstimulated human ovaries as well as from spontaneous cycles (Ylikorkala and Tenhunen, 1984; Priddy *et al*., 1990; Watanabe *et al*., 1993). Granulosa cells produce prostaglandins, important for the follicle rupture. Prostaglandins, produced by the granulosa cells, are primarily inflammatory mediators and play a fundamental role in the mechanism of follicle wall rupture (Priddy and Killick, 1993; Adashi, 1996). $PGF_{2\alpha}$ and also PGI_2 appear to have a stimulatory role in ovulation whereas PGE_2 has the opposite effect. Prostacyclin may stimulate proteolytic activity in the follicular wall, particularly collagenase, possibly via activation of tissue plasminogen activator and plasmin (Miyazaki *et al*., 1991).

Infertility problems are well recognized in women with endometriosis (Luciano and Metzger, 1991). A local inflammatory reaction is evolved in the pelvic cavity, involving macrophage activation. Whether the macrophages in follicular fluid also have an elevated activity is not known. Macrophages are important producers of prostaglandins. Sano *et al*. (1994) found that macrophages represent 75–85% of the cells in the peritoneal fluid, and that peritoneal fluid cells have an elevated phospholipase A2 activity in women with endometriosis. An increased concentration of prostaglandins in peritoneal fluid has also been shown in women with endometriosis, above all during the follicular phase (DeLeon *et al*., 1986; Fraser, 1992). An excessive concentration in the peritoneal fluid might have a deleterious effect on ovulation (Anderson, 1993). After treatment of endometriosis the inflammatory process may decrease. One can postulate that the prostaglandin concentrations in the peritoneal cavity might influence the concentrations in the follicular fluid. Whether the prostaglandin concentrations in the follicular fluid from women with endometriosis differ from healthy women is not known. However, it has been shown that the production of PGE and PGF from the isthmic and ampullar part of the uterine tube is higher in women with endometriosis compared with healthy controls (Nabekura *et al*., 1994).

In a pilot study we assayed $PGF_{2\alpha}$ and PGI_2 in follicular fluid

*Presented in part at the Third World Congress on Endometriosis, Brussels, Belgium, 1992.

and peritoneal fluid from eight patients with endometriosis. The results suggested a difference between patients with macroscopic and not macroscopically visible endometriosis for $PGF_{2\alpha}$ but not for PGI_2 (unpublished data). Therefore, the primary aim of the present study was to investigate whether the concentration of $PGF_{2\alpha}$ in women with endometriosis is different in the follicular fluid at the time of oocyte retrieval when there are visible endometriotic lesions in the pelvis compared to when the lesions have apparently disappeared. The second aim was to study whether the prostanoid production in the follicles of endometriotic women influence the results of in-vitro fertilization (IVF). For ethical reasons a control group of patients with no endometriosis could not be included in this invasive study because our patients do not usually have laparoscopic egg collection.

Materials and methods

Follicular fluid was collected at the University Hospital in Malmö by visual puncture of the ovarian follicles at laparoscopy of 24 consecutive women with infertility and previously diagnosed endometriosis. No patient had been on acetylsalicylic acid (ASA), cortisone or non-steroidal anti-inflammatory drug (NSAID) preparations within two months before puncture. All women in the study had been treated with danazol or gestagens, but not for the last three months before the ovarian stimulation. This was initiated using the standard stimulation protocol for IVF procedures. The patient was given clomiphene citrate (Pergotime®; Serono, Geneva, Switzerland) 100 mg daily cycle days 2–6. Administration of human menopausal gonadotrophin (HMG, Pergonal®; Serono) 225 μg daily was started on cycle day five. Depending on the daily serum oestradiol concentrations, the dose of HMG was adjusted if needed. Ovulation was induced with 5000 IU human chorionic gonadotrophin (HCG, Profasi®; Serono) two days after the serum oestradiol concentration had reached 2500 pmol/l. Oocyte retrieval was performed 35 h later. The study was approved by the local ethics committee and all women had given their verbal consent.

At the laparoscopic scrutinization of the pelvic structures, eight cases were regarded as active endometriosis, all having non-fibrotic lesions, and 16 had no macroscopically visible endometriosis. In four cases of visible endometriosis, fluid from one follicle in each ovary was collected; in three cases, fluid from two follicles from the same ovary (two right-sided and one left-sided); and in three cases fluid from only one follicle in the right ovary was collected. Of the cases with no visible endometriosis, eight had fluid from one follicle from each ovary collected, one had fluid from two follicles from the right ovary collected and seven had fluid from only one follicle collected, in four cases from the right side and in three cases from the left side. The fluid was sampled separately from each follicle. The fluid samples were collected in empty plastic tubes and centrifuged immediately. They were transferred to 5 ml plastic tubes, frozen at –70°C within 30 min and kept at this temperature until analysed within one year. The oocytes were subsequently used for IVF treatment according to routine procedures.

Extraction procedure

Prior to radioimmunoassay, the prostanoids were extracted by using a solid phase extraction method described by Powell (1980) in order to remove proteins and fatty acids that could cross-react with the antibodies in the assay.

Radioimmunoassay

Determination of $PGF_{2\alpha}$ was performed using a competitive binding radioimmunoassay with ^{3}H-labelled tracer. After separation with dextran-coated charcoal (Amersham International plc, Little Chalfont Bucks, UK) the sample was processed for 5 min in a β-counter (Wallac 1410; Wallac AB, Sollentuna, Sweden). The radioactivity was plotted against a freshly made standard curve (range 3.1–200 pg/tube). The detection limit of the assay was 6.2 pg/ml. The percentage cross-reactivity towards other prostanoids was: PGD_2 2.74, 6-keto-$PGF_{1\alpha}$ 1.5, TxB_2 0.7, PGE_1 0.07, PGA_2 0.07, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ 0.03, PGE_2 0.5 and $PGF_{1\alpha}$ 1.0. All samples were run in duplicate and when the difference between the duplicates (i.e. intra-assay coefficient of variation) was >15%, the samples were re-assayed. Seventeen samples were run in two different assays with an interval of 15 months in order to check the reproducibility. The interassay coefficient of variation was 33 ± 7%. The correlation factor was high ($r = 0.96$, $P < 0.0001$). Data are expressed as pg/ml and given as median and range.

Statistical methods

Statistical analyses were performed using the Statview® package for Apple Macintosh. Comparisons between the two groups were made with Mann–Whitney *U*-test. $P < 0.05$ was considered significant. Assuming a 50% difference between the groups of 10 and 16 patients respectively, and with $P = 0.05$, the power of this test was calculated to be 0.783.

Results

The median age at puncture was 32 years (range 24–36) in the group with visible and 29 years (range 22–38) in the group without visible endometriosis. In all cases the sperm sample was within normal range for IVF.

Endometriosis was diagnosed a median duration of 14 months (range 0–9 years) before the puncture in the group with visible endometriosis and 10 months (range 6–24) in the group with no visible endometriosis. All women with visible endometriosis had peritoneal endometriosis. One woman also had an endometriotic cyst and appendix lesions and another had lesions in vagina and the inguinal region. All but one woman without visible endometriosis had had peritoneal lesions and three also had had ovarian, two vaginal and one appendix endometriosis, respectively. One woman had only had minor ovarian lesions and one had had an endometriotic cyst in the contralateral ovary. Only one of the 10 women (10%) with visible endometriosis had been pregnant before but she had aborted. The median infertility period for this group of women was 3.5 years (range 1–11). Among the 16 women without visible endometriosis, eight (50%) had been pregnant before, but three had aborted, two had had spontaneous abortion, one had had a missed abortion and one an extrauterine pregnancy. Only one had given birth to babies, having had three normal deliveries. The median duration of infertility in this group was 3 years (range 1–8). Thus, altogether nine of the 26 women had been pregnant before.

There was no statistically significant difference between the concentration of $PGF_{2\alpha}$ in follicles from women without visible endometriosis, being 2600 pg/ml (median, range 390–4500) compared to that in follicles from patients with visible endometriosis at puncture, being 3325 pg/ml (median, range

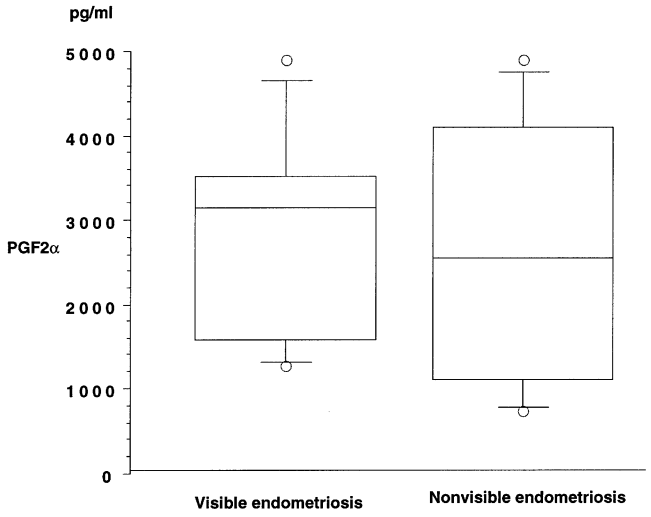

Figure 1. Median and range (pg/ml) of prostaglandin (PG) $F_{2\alpha}$ in follicular fluid from patients with visible and without macroscopically visible endometriosis. Data are presented as 10th, 25th, 50th, 75th and 90th percentiles. There was no significant difference between the groups.

850–8500) (Figure 1). The correlations between follicles from the left and the right ovary in patients without visible endometriosis (r = 0.586) and in patients with visible endometriosis (r = 0.07) were not statistically significant. There was no correlation between the amount of $PGF_{2\alpha}$ in the follicles and the presence of endometriosis on either ovary. Only two patients became pregnant after the specific IVF treatment and their $PGF_{2\alpha}$ concentrations did not differ from those not pregnant after the treatment.

Discussion

In the present material consisting of 26 consecutive women with endometriosis and infertility, only seven (27%) had been pregnant before and the duration of infertility in the group was between one and 11 years. The group with visible endometriosis had had their diagnosis for a longer period of time than the women without visible endometriosis and the fertility in the former group was lower, 1/10, compared to the later 6/16. These data might contribute to the association between endometriosis and infertility.

The present study failed to show any differences in $PGF_{2\alpha}$ concentration in follicular fluid from women with a diagnosis of endometriosis, when women with visible lesions at ovum retrieval were compared to women without visible lesions. However, that does not rule out that patients with endometriosis have a different concentration of follicular $PGF_{2\alpha}$ than normal subjects. The absence of difference might depend on the concentration of prostaglandins in the ovarian surroundings not influencing the follicular content of prostaglandins or it may be that the production of prostaglandins in the pelvis of women with endometriosis is not dependent on the phase of the disease. It is also possible that microscopic lesions may have been present in the group with no visible lesions, which of course would not have been visible to the naked eye. Furthermore we could not find any relation between the concentration of $PGF_{2\alpha}$ and the success of IVF. This may, however, be due to the low number of successful IVF cycles in this study. The group of women with visible endometriosis was somewhat older, included some women with a long history of endometriosis and they had experienced fewer pregnancies than the group with no visible endometriotic lesions. Although these factors did not differ significantly between groups, it cannot be excluded that that would be the fact in a larger number of women. These data are in accordance with several clinical studies showing that the pregnancy rate in women with endometriosis does not increase even if the visible endometriotic lesions have disappeared after treatment (Luciano and Metzger, 1991).

The reproducibility of the results of samples stored for 15 months indicates that the shorter storage of the samples used in the present study does not influence the results. This also indicates that there is no significant degradation of samples outside the living body.

It has been shown that, in women with endometriosis in its early phase, the inflammatory reaction with activation of pelvic macrophages results in an increase of both IL-1β and $PGF_{2\alpha}$ in the peritoneal fluid (Keenan *et al*., 1995). In a pilot study we assayed $PGF_{2\alpha}$ in both peritoneal fluid and follicular fluid from the same patient but we did not find any significant differences between the two compartments (unpublished data). One source for prostaglandins in follicular fluid might also be macrophages. Loukides *et al*. (1990) found that macrophages represent 5–15% of the cell population in follicular fluid and Lachapelle *et al*. (1996) found that the concentration of monocytes–macrophages is increased in follicular fluid from infertile women with endometriosis compared with women with a tubal factor diagnosis or idiopathic infertility. The activation of macrophages at follicle puncture and aspiration of the follicular fluid may not be excluded, but an immediate influence on the prostaglandin concentration in the follicular fluid aspirate is not probable. When doing an ultrasound-guided puncture of follicles, the risk for contamination with peritoneal fluid is minimal, and even less so when the puncture is performed visually at laparoscopy. However, it may never be guaranteed that fluid is not obtained from both compartments into the same sampling tube.

One must always take into consideration that follicular fluid from more than one follicle could be collected in each tube. When punctured transvaginally, the possibility should always be considered that fluids from follicles of different maturation states are mixed in the same sample, being one factor contributing to the absence of significant differences between groups and low correlation between different follicles in a woman. This possibility was by-passed in this study where puncture of each follicle was performed visually at laparoscopy. Unfortunately, however, no ultrasound examination was conducted at laparoscopy and hence we have no information about follicle size at puncture. The interesting absence of correlation between the right and left ovary in women with visible endometriosis might be an indication of more heterogeneous maturation of follicles when endometriosis is in the pelvic cavity compared to when the lesions have disappeared — whether for a temporary period or permanently is not known.

IL-1 has been shown to enhance the production of different prostaglandins in different cell systems (Vallitutti *et al*., 1989; Bull and Dowd, 1992; Hertelendy *et al*., 1993; Knott *et al*., 1993). Both forms of interleukin-1, IL-1α and IL-1β, have an inductive effect on prostaglandin synthesis by stimulating the arachidonic acid release (Knott *et al*., 1993). IL-1β stimulates the production of immunoreactive PGE_2 and $PGF_{2\alpha}$ from human ovarian granulosa cells to culture medium in a time and dose dependent manner (Kokia *et al*., 1992; Brännström *et al*., 1993; Watanabe *et al*., 1994). A distinct amount of IL-1β mRNA was demonstrated in monocytes from follicular fluid that could not be demonstrated in peripheral monocytes (Loukides *et al*., 1990) and IL-1 increases immediately before ovulation (Adashi, 1996).

We did not find a significant difference in follicular fluid from the right and left ovary, respectively, in the same woman. Thus this study gave no support to the idea that prostaglandins are secreted from the endometriotic lesions and influence the ovary where the lesion is located.

It has been suggested that prostaglandins are involved in oocyte maturation (Gelety and Chaudhuri, 1992). However, in a carefully performed study Watanabe *et al*. (1994) found that the concentration of IL-1β in follicular fluid tended to increase gradually in association with oocyte maturation. There was a positive correlation between IL-1β and PGE_2 and $PGF_{2\alpha}$, respectively, but no significant correlation between 6-keto-$PGF_{1\alpha}$ and TxB_2, respectively, with oocyte maturation or concentration of IL-1β. In a pilot study we assayed 6-keto-$PGF_{1\alpha}$ and TxB_2 in samples from eight women with visible or not visible endometriosis, respectively, but no significant difference was found between the two groups (unpublished data). There appears to be an association between higher follicular prostanoid concentrations and the chance of aspirating an oocyte–cumulus complex of adequate maturity for successful fertilization in IVF programmes (Priddy and Killick, 1993). Thus the concentration of IL-1 and prostaglandins in follicular fluid seems to be of importance for oocyte maturation and follicle rupture and the concentrations might be influenced by a pelvic inflammatory process, resulting in a disturbed follicle growth and rupture.

One possible agent for lysis of the follicular wall is plasmin. Plasminogen activator, that converts plasminogen to plasmin, is produced in both granulosa and theca cells, increases in concentration prior to ovulation and is stimulated by PGE_1 and PGE_2. NSAID is associated with a decrease in the synthesis of ovarian eicosanoids and indomethacin has also been shown to prevent conversion of plasminogen to plasmin, probably important for the lysis of the follicular wall (Le Maire *et al*., 1975; Priddy and Killick, 1993). Thus the consumption of NSAID because of pain at ovulation might be deleterious in endometriotic women.

In amniotic fluid the concentration of PGE_2 and $PGF_{2\alpha}$ rise at the initiation of labour (Cox *et al*., 1993) and one critical question has been what initiates this rise. IL-1β has also been shown to appear in the amniotic fluid during labour and the question has been raised whether micro-organisms and bacterial toxins in the vaginal fluid might produce these inflammatory mediators. Cox *et al*. (1993) have shown that the IL-1β concentrations in vaginal/cervical fluids were significantly greater during labour than before labour. This has to be kept in mind when the reason for the defective immunological reaction to retrograde menstruation in women developing endometriosis is discussed, as a vaginal infection as an inductor has been suggested.

In a well-controlled retrospective study on IVF outcome in women with endometriosis, we found a significantly lower fertilization rate in women with diagnosed endometriosis compared to women without a diagnosis of endometriosis but tubal damage (Bergendahl *et al*., 1997). The hypothesis that visible endometriosis compared to non-macroscopically visible endometriosis after treatment is a possible indication of a more aggressive form of the disease is attractive but it has never been shown convincingly.

In conclusion, in this very well-controlled study with visual puncture of each follicle and visual evaluation of the endometriotic status at the time of follicular puncture, it was shown that the existence of endometriotic lesions in the pelvis of women with macroscopically visible endometriosis did not influence $PGF_{2\alpha}$ concentration in the follicular fluid or the IVF outcome. These data strengthen the indications that the cause of infertility in women with endometriosis is not related to the existence of macroscopic endometriotic lesions but might be related to genetic factors, persistent local inflammatory process in the pelvis or changes in the oocyte itself, which has been indicated in different studies (Jansen, 1986; Luciano and Metzger, 1991; Moen and Magnus, 1993).

Acknowledgements

Dr Per Sundström is acknowledged for collating the follicular samples and Agneta Enander for her prompt handling of them. The study was performed with grant B91-17X-09511-01A from the Swedish Medical Research Council and the Karolinska Institute foundations, Stockholm.

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Received on December 23, 1996; accepted on May 23, 1997