Utero-ovarian arterial blood flow and hormonal profile in patients with polycystic ovary syndrome

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Introduction
In stimulated as well as in unstimulated cycles the vascular impedance in the uterine artery decreases during the pre-ovulatory period. Likewise the vascular impedance remains low during the luteal phase on account of the increased diastolic flow (Hata et al., 1990; Steer et al., 1990; Kurjak et al., 1991; Schurz et al., 1993). In patients with polycystic ovary syndrome (PCOS) the vascular impedance in the uterine artery is described as being unchanged throughout the cycle (Kurjak et al., 1991; De Ziegler et al., 1992).

The vascular impedance in the ovarian artery is considered to be linked to the phenomenon of massive angiogenesis influenced by luteinizing hormone (LH) in unstimulated cycles or by human chorionic gonadotrophin (HCG) in stimulated cycles (Koos and Le Maire, 1983). The increased serum concentrations of oestrogens cause vasodilatation in the ovarian artery and consequently a decrease in the vascular impedance. The ovarian artery is also influenced by the oestrogens synthesized locally in the ovary itself. In PCOS patients vascular impedance in the ovarian artery remains high throughout the cycle. This may be explained by inappropriate oestrogen synthesis in the polycystic ovary (Hata et al., 1990). Additional characteristics of the polycystic ovary might be provided by measuring the ovarian stromal blood flow (Battaglia et al., 1995; Zaidi et al., 1995).

Through the empirical evidence showing that ovulation induction is more successful in patients with hypothalamic amenorrhoea than in patients with PCOS, the co-administration of gonadotrophin hormone releasing agonists (GnRHa) for ovulation induction in PCOS was based on the knowledge that it reduces high LH concentrations and therefore eliminates premature luteinization, increases pregnancy rates and reduces miscarriage rates (Homburg et al., 1993).

The aim of this study was the evaluation of a possible association between the hormonal profile and haemodynamic parameters in patients with PCOS compared with patients with normal menstrual cycles (NMC) treated with GnRHa in an in-vitro fertilization (IVF) and embryo transfer programme.

Materials and methods

Patients
Forty-two patients undergoing IVF were included in the prospective controlled study: 20 patients with PCOS represented the study group, and 22 patients with NMC the control group.
The inclusion criterion for the study group was at least 10 follicles of <8 mm in diameter in the subcapsular region around hyperechogenic central stroma, found in each ovary by the ultrasound scan. The study group was further divided into PCOS patients with ovulatory and anovulatory menstrual cycles. In 10 anovulatory PCOS patients the ovulation was confirmed by ultrasonography and endometrial biopsy in the previous cycle. The length of the previous menstrual ranges from 28 to 34 days. In 10 anovulatory PCOS patients the absence of ovulation in the previous cycle was confirmed by ultrasound, and/or the occurrence of oligomenorrhoea (cycle length >5 days) and/or elevated serum LH concentration (>5 IU/l) in the early follicular phase.

The inclusion criteria for the control group were tubal infertility and normal ovulatory menstrual cycle, confirmed by ultrasound and endometrial biopsy in the previous cycle. The cycle length ranged from 28 to 30 days.

In all patients the ovarian stimulation was performed using GnRHα (Suprefact; Hoechst AG, Frankfurt/Main, Germany) applied from day 22 of the cycle in a daily dose of 0.6 ml (600 pg) s.c. After 14 consecutive days pituitary desensitization was checked by oestradiol determination and by ultrasound. Once the criteria for desensitization were fulfilled (oestradiol <0.05 nmol/l and follicles <5 mm in diameter), ovarian stimulation with human menopausal gonadotrophins (HMG, Pergonal; Serono S.A., Aubonne, Switzerland) was started. The daily dosage was adjusted individually.

The administration of GnRHα was continued until the HCG (Primogynyl; Schering AG, Berlin, Germany) in a dose of 10 000 IU was applied 34–36 h prior to follicular aspiration. Fertilization, incubation and embryo transfer were performed as standard procedure on an outpatient basis (Gersak et al., 1994). The vascular impedance in the utero-ovarian arteries was measured on days 4, 13, 22 of the cycle, 14 days after the introduction of GnRHα administration and at the time of HCG administration. Simultaneously serum concentrations of LH, FSH, oestradiol, androstendione and sex hormone binding globulin (SHBG) were determined.

**Hormone assays**

LH and FSH were assayed by luminometric assay (LIA) with Byk-Sangtec LIA kits (Byk-Sangtec, Konstanz, Germany) on LIA-mat System 300 (Byk-Sangtec, Konstanz, Germany). The LIA-mat LH (FSH) is a one-step assay for the immunoluminometric determination of LH (FSH) in the serum. The LH (FSH) present in the sample reacts with a monoclonal antibody (mouse) bound to the tube wall and a monoclonal antibody labelled with isoluminol. The anti-LH (FSH) tracer conjugate consists of an antibody and a covalently bound isoluminol derivative. The tracer LH (FSH) complex bound to the tube wall is detected by a light reaction. The sensitivity of the assay for LIA determination of LH was 0.5 IU/l. The intra-assay coefficients of variation (CV) were 4.8, 5.9 and 6.5% for pool sera at 28.8, 53.9 and 164 IU/l, and the interassay CV were 10.6, 8.1 and 10% for pool sera at 6.2, 30 and 60 IU/l. The sensitivity of the assay for LIA determination of FSH was 0.5 IU/l. The intra-assay CV were 5.4, 6.2 and 7.7% for pool sera at 7.8, 19.7 and 42.5 IU/l, and the interassay CV were 8.1, 6.5, 7.9% for pool sera at 8.3, 20.8 and 42.9 IU/l.

Androstendione was measured with commercially available radioimmunoassay kit (Biomedica, Saluggia, Italy). The assay is based on the competition between labelled oestradiol and oestradiol in samples for a fixed and limited number of antibody binding sites. The sensitivity was 0.40 nmol/l, the intra-assay CV were 9.8, 4.8 and 4.3% for pool sera at 0.19, 1.38 and 11.45 nmol/l, the interassay CV were 16.6, 3.9 and 5.5 for pool sera at 3.2, 9.6 and 31.6 nmol/l.

Oestradiol was measured with commercially available radioimmunoassay kit (Milab, Malmoe, Sweden). The assay is based on the competition between labelled SHBG and SHBG in samples for a fixed and limited numbers of anti-SHBG antibodies. The sensitivity of the assay for radioimmunoassay determination of SHBG in serum was 0.535 nmol/l, the intra-assay CV were 4.1 and 6.5% for pool sera at 5.4 and 73.8 nmol/l, the interassay CV were 8.3, 7.2 and 6.5% for pool sera at 6, 35.3 and 115.6 nmol/l.

**Statistical evaluation**

The data in the PCOS and in the NMC group were compared at given time-points by unpaired Student’s t-test. Correlations were performed using Pearson’s correlation coefficient. Significance was defined as P < 0.05.

**Results**

The patients in the PCOS group were further divided into anovulatory (n = 10) and ovulatory (n = 10) PCOS patients, whereas in the NMC group one patient was anovulatory in the studied cycle, but had been included in the NMC group because of previous ovulatory cycles.

**Haemodynamic parameters**

The dynamics of vascular impedance in the uterine artery in the anovulatory PCOS patients was constant regarding various
phases of the unstimulated cycle. On day 22, the RI was lower in ovulatory NMC cycles ($n = 21$) compared to anovulatory PCOS cycles ($n = 10$) ($0.82 \pm 0.04$ versus $0.86 \pm 0.04; P < 0.05$) (Figure 3).

After 14 days of GnRHa administration, the RI remained almost the same in anovulatory PCOS cycles ($n = 10$); it increased from $0.86 \pm 0.04$ to $0.88 \pm 0.04$, but this difference was not significant. In ovulatory NMC cycles ($n = 21$), however,
The RI increased from 0.82 ± 0.04 to 0.86 ± 0.04 (P < 0.05) (Figure 3).

On the day of HCG administration, a negative correlation was noted between serum oestradiol concentrations and RI in the uterine artery in the NMC group [r = −0.48, n = 20 (one patient excluded after 14 days of GnRHa administration because of endometrial polyp); P < 0.05] and in the PCOS group [r = −0.527, n = 18 (excluding one patient who conceived during the observation period and another because of failed desensitization); P < 0.05]. Considering only anovulatory PCOS cycles (n = 9; one patient excluded because of failed desensitization: see above) this correlation was not significant (r = −0.534; P = NS).

By measuring vascular impedance in the hilum of the left ovary (LO) and the right ovary (RO) at different stages of studied cycles, no differences were noted in the anovulatory PCOS group (P = NS) (Figure 4, lower panel).

In contrast, in the NMC group vascular impedance in the hilum of the active ovary was found to be lower compared to the inactive one on day 4 (0.87 ± 0.04 versus 0.89 ± 0.05, n = 17 (three patients excluded because measurements on one ovary failed); P < 0.05), on day 13 (0.84 ± 0.06 versus 0.91 ± 0.05, n = 20; P < 0.05), on day 22 (0.81 ± 0.08 versus 0.90 ± 0.04, n = 20; P < 0.05) and after 14 days of GnRHa administration (0.90 ± 0.04 versus 0.93 ± 0.03, n = 20; P < 0.05) (Figure 4).

A negative correlation between serum oestradiol concentrations and vascular impedance in the hilum of the ovary was found for the anovulatory PCOS cycles (n = 9; again, the patient with failed desensitization was excluded) on the day of HCG administration (left ovary: r = −0.8364, P = 0.005; right ovary, r = −0.8349, P = 0.05). For the ovulatory NMC cycles no correlation was not found (P = NS).

Ovarian stromal blood flow in both ovaries was found in 17 (85%) PCOS patients, whereas in the NMC group it was present only in one patient. The RI was 0.62 ± 0.11. After 14 days of GnRHa administration the stromal blood flow still persisted in PCOS patients. On the day of HCG administration the RI increased from 0.82 ± 0.04 to 0.86 ± 0.04 (P < 0.05) (Figure 3).

Hormonal measurements on days 4, 13, 22 (introduction of GnRHa), and on the day of HCG administration are shown in Figure 5.

Serum LH concentrations were significantly higher in the PCOS (n = 20) than in the NMC group (n = 22) on day 22 (9.40 ± 9.72 versus 2.08 ± 3.45 IU/l; P < 0.05), and after 14 days of GnRHa administration (0.87 ± 0.77 versus 0.41 ± 0.27 IU/l; P < 0.05). By comparing only the anovulatory PCOS cycles (n = 10) with the ovulatory NMC cycles (n = 21), the same was true also on day 4 (not shown; 4.14 ± 3.64 versus 1.99 ± 1.08 IU/l; P < 0.05).

Serum FSH concentrations on day 22 were significantly higher in the PCOS (n = 20) than in the NMC group (n = 22) on day 22 (5.76 ± 2.96 versus 2.67 ± 1.16 IU/l; P < 0.05). By comparing the anovulatory PCOS cycles (n = 10) with the ovulatory NMC cycles (n = 21), lower serum FSH concentrations were registered in the PCOS group on day 4 (not shown; 5.50 ± 1.36 versus 7.33 ± 2.57 IU/l; P < 0.05).

As for the serum oestradiol concentrations, significant differences between the groups were found neither in unstimulated part of the cycles nor in stimulated part of the cycles. However, the results were different when comparing anovulatory oestradiol concentrations in the PCOS group (n = 10) with ovulatory oestradiol concentrations in the NMC group (n = 21) on day 13 (not shown; 43 ± 0.15 versus 0.92 ± 0.33 nmol/l; P <
gonadotrophin releasing hormone agonist.

significant differences between the PCOS and NMC groups on day 22 (not shown; 33.20 ± 7.44 nmol/l; P < 0.05). HCG = human chorionic gonadotrophin, GnRHa = gonadotrophin releasing hormone agonist.

and on the day of HCG administration in stimulated cycles (not shown; 11.57 ± 7.44 versus 5.50 ± 7.44 nmol/l; P < 0.05).

Serum androstendione concentrations were higher throughout all phases of the cycle, unstimulated and stimulated, in the PCOS group compared to the NMC group (P < 0.05).

In the PCOS group serum SHBG concentrations appeared lower in all parts of the cycle, unstimulated and stimulated, but the difference was not statistically significant. However, it became significant when comparing the anovulatory PCOS cycles (n = 10) with the ovulatory NMC cycles (n = 21) on day 22 (not shown; 33.20 ± 15.50 nmol/l versus 54.48 ± 23.88 nmol/l; P < 0.05).

Discussion

Vascular impedance in the uterine artery

Vascular impedance in the uterine artery is decreased by the end of the follicular phase, being the lowest just before ovulation (Kupesic and Kurjak, 1993). Considering the menstrual cycle as a whole, the vascular impedance is the lowest in the luteal phase on account of the diastolic flow in both uterine arteries (Hata et al., 1990; Steer et al., 1990; Kurjak et al., 1991; Schurz et al., 1993). These observations were also confirmed in this study. On the other hand, Deichert et al. (1994) found that the impedance in the uterine artery in midluteal phase was significantly lower on the side of the corpus luteum than on the opposite side.

In the PCOS group vascular impedance in the uterine artery remained unchanged throughout the cycle. On day 22 of the cycle, vascular impedance and serum LH concentrations were significantly higher than in the NMC group. The relationship between high LH concentrations and vasoactive substances has not so far been defined. Nevertheless, we know that at the time of ovulation, when the LH surge coincides with the prostaglandin F2α and oxytocin surges, vascular impedance in the uterine artery is increased (Koullapis and Collins, 1980; Mitchell et al., 1980; Amico et al., 1981).

As for the correlation between the serum oestradiol concentration and the vascular impedance in the uterine artery in the follicular phase, there are different opinions. The studies by Goswamy and Steptoe (1988) imply that the RI decreases simultaneously with the increasing serum oestradiol concentrations during the early follicular phase, increases in the peri-ovulatory period and keeps increasing synchronously with the post-ovulatory fall of oestradiol concentration in the early luteal phase. In contrast, Steer et al. (1990) found a positive correlation between serum oestradiol concentrations and vascular impedance on days 7–14 of the cycle. In this study, the correlation between oestradiol and RI could not be determined, as only one measurement on day 4 was performed. Considering the results of the PCOS group, neither the dynamics in serum oestradiol concentrations and the vascular impedance in the uterine artery regarding the phase of the cycle, nor a correlation between oestradiol and RI were found. A contrasting situation, found on day 22 of the cycle in PCOS patients, when the vascular impedance in the uterine artery was significantly higher than in normally cycling women, may be due to substances synthesized within the ovary, although serum oestradiol concentrations in both groups were similar. Research has been focused on the functioning of the ovarian renin–angiotensin system (Erman et al., 1996), androgens (De Ziegler et al., 1992), prostaglandins (Koullapis and Collins, 1980) and on vascular endothelial growth factor (VEGF) which is increased in parallel with the increased ovarian vascularity (Robertson et al., 1995).

The difference in haemodynamic parameters and hormonal profile between the PCOS and the NMC group was detected also after 14 days of GnRHa administration. The suppressive effect of the 14 day GnRHa administration was reflected in a significant decrease of serum androstendione concentration in the normally cycling women (3.89 ± 1.48 to 2.87 ± 1.17 nmol/l, n = 21, P = 0.018; Figure 5), but not in the PCOS patients (6.94 ± 2.06 to 5.38 ± 1.97 nmol/l, n = 10, P = 0.101; Figure 5). However, Cheung et al. (1997) reported on the suppressive effect of GnRHa administered over a period of 3 months. In our study the high serum androstendione concentration was significantly decreased only on day 22 of the menstrual cycle in PCOS patients, when the vascular impedance was found to be significantly lower than in the NMC group.

Figure 5. Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, androstendione and sex hormone binding globulin (SHBG) in the patients with polycystic ovary syndrome (PCOS, ◦) and in patients with normal menstrual cycles (NMC, *), considering the unstimulated part of the cycle (on days 4, 13 and 22) and the stimulated part of the cycle (14 days after GnRHa administration and at the time of HCG application). Data are mean ± 2 SE. Asterisk denotes statistically significant differences between the PCOS and NMC groups (P < 0.05).
concentration coincides with high vascular impedance in the uterine artery of patients with PCOS on day 22, and GnRHa administration did not change it, probably because it was already maximal at the beginning of the studied cycles. In the NMC group the measurement of the vascular impedance in the uterine artery reflects the suppressive effect of GnRHa administration. Weiner *et al.* (1993) found a positive correlation between serum oestradiol concentrations and low vascular impedance in the uterine artery on the day of HCG administration in NMC and in PCOS cycles. Taking only anovulatory PCOS cycles into consideration, the above correlation was not confirmed in this study. Apparently, the vascular impedance in the uterine artery depends on other, vasoactive or gonadotrophin-related substances produced by polycystic ovaries.

There are two theories concerning the vascular impedance in the uterine artery proposed by De Ziegler *et al.* (1992). One is the so-called oestrogen theory which is in agreement with Goswamy and Steptoe (1988) and suggests negative correlation between serum oestradiol concentrations and uterine vascular impedance with respect to various phases of the cycle. This theory does not explain the mechanism present in the ovarian stimulation situation where high serum oestradiol concentrations poorly match good uterine blood flow. This particular phenomenon could be explained by the anti-oestrogen theory suggested by De Ziegler *et al.* (1992). The theory explains that the lack of oestrogen receptors or excess of vasoactive substances and androgens could oppose the vasodilatory oestrogen effect.

**Vascular impedance to ovarian arterial blood flow**

The ovary receives the blood supply from the ovarian and uterine arteries running in the hilum of the ovary. In this study, the topographic locations of ovaries and their vessels were changed in most of the patients, because of previous surgical adnexal procedures. We overcame these problems by measuring the lowest vascular impedance in the hilum of the ovary. In this way the vessels nourishing follicles and corpus luteum were selected (Weiner *et al.*, 1993). The dynamics of vascular impedance in the hilum of the ovary are comparable with those of vascular impedance in the ovarian artery in the NMC group (Hata *et al.*, 1990; Kurjak *et al.*, 1991).

Studies performed by various authors (De Ziegler *et al.*, 1993; Battaglia *et al.*, 1995; Zaidi *et al.*, 1995) have analysed the ovarian stromal blood flow in PCOS patients. Our results are in agreement with those of Battaglia *et al.* (1995) showing that the presence of stromal ovarian vascularization with low resistance has a generally high diagnostic value for PCOS.

Various studies (Taylor *et al.*, 1985; Scholtes *et al.*, 1989; Hata *et al.*, 1990; Kurjak *et al.*, 1991) have shown that in the active ovary of NMC patients the diastolic flow gradually increases during the late follicular phase, while the impedance to blood flow in the luteal phase remains low due to vasodilatation. In the sonograms of the inactive ovary of NMC patients, the diastolic flow is absent while high vascular impedance persists throughout the cycle.

Hata *et al.* (1990), Kurjak *et al.* (1991) and Sladkevicius *et al.* (1993) in their studies of the normal menstrual cycle did not prove the difference between the ovaries in the vascular impedance to ovarian blood flow during the early follicular phase, while Taylor *et al.* (1985) did. Our results are in agreement with those of Taylor *et al.* (1985) considering the fact that in normally cycling patients the vascular impedance to ovarian blood flow on day 4 was significantly lower in the ovary which later proved to be active than in the inactive one. By measuring the vascular impedance to ovarian blood flow during the early follicular phase one can therefore establish which ovary is about to ovulate. This may mean that the colour and pulsed Doppler method is preferable to the methods based on recognition of the dominant follicle and peri-ovulatory endometrium with the B-mode ultrasound scan.

In the NMC patients there was an increased vascular impedance in the inactive ovary in comparison to that in the active ovary, and was the same as in polycystic ovaries. The possible explanation may be that in PCOS patients, although demonstrating similar serum oestradiol concentrations as NMC patients, the increased vascular impedance may result from either decreased or inadequate local synthesis of oestrogens in the ovary itself. De Cherney and Laufer (1984) claim that a normal menstrual cycle is conditioned by an intact oestriadiol synthesis. A defect in the enzyme aromatase system, which causes an imbalance in peripheral oestrogens, plays an important role in improper ovarian circulation (Hutchinson-Williams and De Cherney, 1987). The results of this study confirm the above theory, considering that negative correlation between the vascular impedance to the ovarian arterial blood flow and serum oestradiol concentration in the unstimulated part of the studied PCOS cycles was not found. There was negative correlation between the serum oestradiol concentration and the vascular impedance to the ovarian blood flow on the day of HCG administration in anovulatory PCOS cycles, the serum oestradiol concentrations being extremely high. This correlation was not found in normal stimulated cycles. According to these findings we presume that the vascular impedance to the ovarian blood flow is more influenced by locally synthesized oestrogens than by serum oestradiol concentrations. Therefore, the estimation of the correlation between serum oestradiol concentrations and vascular impedance to the ovarian blood flow has no value.

Considering our results, the high serum LH concentration coincides with the presence of stromal blood flow throughout PCOS cycles. Besides, the elevated serum LH concentration during the follicular phase may be responsible for stromal hyperplasia with consequent androgen overproduction (Yoshino *et al.*, 1992; Buyalos *et al.*, 1993), and for increased stromal vascularization (Kurjak *et al.*, 1991; Battaglia *et al.*, 1995; Tekay *et al.*, 1995). Our finding that stromal vascularization is caused by endogenous and exogenous gonadotrophins is in agreement with De Ziegler *et al.* (1993). This might be confirmed by the presence of stromal blood flow in normally cycling women in stimulated cycles. Coloured areas provided by colour Doppler ultrasound in the ovarian stroma of normally cycling women and in PCOS patients on the day of HCG administration resemble those observed in the PCOS patients during the follicular phase. The flow velocity waveforms obtained from the intra-ovarian arteries always show a low impedance to blood flow with a continuous end-diastolic flow.
We agree with Tekay et al. (1996) that it is difficult to evaluate minor changes in intra-ovarian blood circulation during the stimulated cycles in a form that could be detected by colour and pulsed Doppler methods.

In PCOS patients the 14 day GnRHa administration does not increase vascular impedance to the ovarian blood flow in either ovary, and the stromal blood flow persists. The situation is different in the ovaries of normally cycling women, where the vascular impedance to the ovarian blood flow increases significantly both in the active and in the inactive ovary. These results confirm the presumption that polycystic ovaries permanently secrete vasoactive substances and androgens (Buyalos et al., 1993; De Ziegler et al., 1993), which may increase the vascular impedance to such an extent that the addition of GnRHa does not have any effect on the vascular impedance to the blood flow at all.

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