Human endometrial perfusion after tubal occlusion

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We examined variations in human endometrial microvascular perfusion across one menstrual cycle in women who had undergone tubal ligation and did not report unusual menstruation. Endometrial red blood cell flux was monitored by laser Doppler fluxmetry via a fibroptic probe atraumatically inserted transvaginally into the uterus of each of 13 conscious volunteers. The observations obtained have been compared with those previously reported from a matched control group of women (B.J. Gannon et al., \textit{Hum. Reprod.}, 12, 132–139 (1997)). Women who had undergone tubal occlusion for sterilization exhibited greater endometrial perfusion during menstruation (cycle days 0–5), at the time of ovulation (cycle days 13–16) and in the late secretory phase (cycle days 23–28) than occurred in controls. In addition, vasomotion in the study group was lower than that in controls in the early and late secretory phase (cycle days 17–22 and 23–28). Tubal occlusion appeared to alter endometrial perfusion. It is possible that the reported menstrual changes in women following tubal ligation are a consequence of altered endometrial perfusion; a possible causative relationship is discussed.

Key words: endometrium/laser Doppler fluxmetry/menstrual cycle/tubal ligation/uterine perfusion

Introduction

The past few decades have seen tubal occlusion become a popular form of contraception, yet there is considerable debate concerning the sequelae of this procedure (Noble, 1978; Rulin et al., 1985; Martinez-Schnell et al., 1993; Rulin et al. 1993; Kovacs, 1994). It has been suggested that there is a causal relationship between tubal sterilization and subsequent menstrual dysfunction, pelvic pain and higher hysterectomy rates, and there is a consensus view that tubal ligation is associated with an increase in the incidence of post-surgical dysmenorrhoea or menorrhagia (Neil et al., 1975; Noble, 1978; Wright, 1980; Wilcox et al., 1992; Goldhaber et al., 1993; Martinez-Schnell et al., 1993). Notwithstanding this, a correlation between tubal occlusion and subsequent menstrual disturbance is contentious and arguments with regard to causation are challenging.

There is evidence of hormonal differences between control women and those who have undergone tubal occlusion (Berger et al., 1978; Radwanska et al., 1979; Donnez et al., 1981; Verhoeven et al., 1982; Cattanach, 1985; Cattanach and Milne, 1988); such hormonal differences may lead to changes in uterine perfusion, but it is not clear how these may be manifest. Uterine and ovarian blood flow (as assessed by colour flow Doppler techniques) during the mid-follicular phase were reported as unchanged after tubal ligation (Geber and Caetano, 1996), although the relevance of this methodology to assessment of endometrial blood flow has yet to be demonstrated (Gannon et al., 1997). To date, there has been no specific investigation of the effect of tubal ligation on endometrial perfusion.

The present study reports measurements of endometrial perfusion in women who had undergone tubal occlusion, using laser Doppler fluxmetry. This minimally invasive technique provides a measure of the flux of red blood cells through a small sphere of tissue immediately adjacent to a probe placed directly over the endometrium. Gannon et al. (1997) utilized this technique to show temporal patterns of endometrial perfusion exhibiting two basic frequencies, one consistent with heart rate, and another with a slower frequency (usually 5–8 cycles/min) presumed to be vasomotion. There were variations in mean endometrial perfusion across the normal menstrual cycle, which was highest in the early follicular and secretory phases. Furthermore, it appeared that the technique provided representative estimates of endometrial blood flow. The data obtained in the present study indicate that women who have undergone tubal occlusion have endometrial perfusion patterns across the menstrual cycle that are significantly different from controls.

Materials and methods

Institutional Research and Ethics Committee approval was sought and obtained from Modbury Hospital, South Australia, where these studies were performed. Informed, witnessed consent was obtained in writing for all subjects.

Subjects

Thirteen women, who had had tubal ligations $5.7 \pm 1.3$ (mean $\pm$ SE) years previously, provided a gynaecological and menstrual history, including the date of the last menstrual period. Endometrial perfusion measurements were made in each volunteer once per week over 4 weeks (usually of one cycle) without anaesthesia or analgesia. All measurements were made in the morning to avoid possible variation in uterine blood flow due to circadian rhythms (Zaidi et al., 1995).
The volunteer was supine on an examination couch with hips and knees flexed as for a cervical smear; the vestibule and vagina were cleansed with antiseptic soaked swabs (povidone iodine, Betadine; Faulding Pharmaceuticals, Salisbury, South Australia). The vagina was gently dilated with a speculum, and the tip of the flexible fiberoptic laser Doppler probe (PR-436; Vasamedics, St Paul, MN; USA; 2.1 mm diameter) was placed at the external cervical os with plain forceps; by the application of gentle pressure along the probe shaft, the probe tip was passed without difficulty through the cervix and advanced into the uterus to 5 cm from the external cervical os [this distance was chosen from preliminary measurements of regional differences in perfusion in anaesthetized patients, Gannon et al. (1997)]. Brief measurements (15–30 s) were made at several adjacent sites by minor advancing/withdrawal/rotation of the probe until three consecutive, stable measurements within 20% of each other were made; typically this occurred within 5 measurements. The last of these sites was used for extended monitoring over 10–15 min to assess temporal variations in local endometrial perfusion. This time was a reasonable limit in terms of subject comfort and compliance. Flux measurements did not appear to be greatly affected by relocation of the probe.

Equipment
A TSI Laserflo BPM 403A laser Doppler instrument (Vasamedics, St Paul, MN; λ = 780 ± 20 nm, 2 mW at probe tip) was equipped with a thin flexible endoscope probe measuring at 90° to the probe axis (Vasamedics PR-436; 2 m long×2.1 mm diameter). The probe was sterilized by immersion in a 2% glutaraldehyde solution (Cidex; Johnson & Johnson Medical, Australia) for 10 min, then rinsed in sterile water prior to each use. Indelible black marks, 1 cm apart on the external white polymer casing of the probe, allowed ready estimation of the extent of probe advancement/withdrawal through the cervix. ‘Flow’ measurements from the instrument (averaging time 0.1 s) were charted on the instrument’s built-in chart recorder (100 mm/min; sensitivity ×4), and recorded on an IBM-XT clone computer (ASI, Taiwan) via a CODAS Analog to Digital data recording system (Datag Corp., Akron, OH, USA) at 10 samples/s. All measurements reported here were made using the same fiberoptic probe; values are reported as laser Doppler flow ‘units’ (mL/g/min/100 g tissue). These are uncalibrated instrument units, which the instrument manufacturer indicates are equivalent to local tissue blood flow in mL/min/100 g tissue. The instrument recorded 25 ± 1 (mean ± SE) TSI ‘units’ (gain at ×1) when the probe was placed in a vial of colloidal motility standard (Periflux PF-100; Perimed Inc., Sweden; this standard provides a constant reflectance and Doppler shift, based on Brownian motion of the suspension, to allow calibration of laser Doppler flowmeters). We have previously reported the ‘biological zero’ of laser Doppler measurements in endometrial tissue (Gannon et al., 1997); these were relatively low (1–2 units on average), and consequently no correction was made to the measurements reported in this study.

Data analysis
Average flux values for the sampling period were obtained from the Codas software, and verified by comparison with the TSI chart recorder output. This output was visually inspected for clearly discernible peaks of 12 cycles/min or less (Figure 1), which were interpreted as vasomotion, and are reported as cycles/min. Data are presented as mean ± SE; data analysis was by analysis of variance (ANOVA) and post-hoc testing, or two-sample t-tests, as appropriate.

The day of the measurement (relative to the onset of menstruation) was calculated from the beginning of the last reported menstrual period. Measurements of endometrial perfusion were grouped accord-

![Figure 1. Individual laser Doppler perfusion records of endometrial perfusion in (a) a normal woman on day 2 of her cycle (mean perfusion = 18.3 LDFU, 3.3 vasomotion cycles/min), and (b) a woman with a tubal occlusion on day 1 of her cycle (mean perfusion = 50 LDFU, 7.0 vasomotion cycles/min). Both traces show regular fluctuations in perfusion which are consistent with vasomotion (indicated by the arrow; see also Gannon et al., 1997). Both women were menstruating. There was a significant difference in mean endometrial perfusion between normal control women and those with tubal occlusion (see Figure 2). LDFU = laser Doppler flux units; see text.]

### Table 1. Biological and reproductive status of control versus tubal ligation groups in this study

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 19)</th>
<th>Tubal ligation (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35 ± 4.6 (21–46)</td>
<td>37 ± 2.1 (27–45)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.5 ± 4.5 (42–107)</td>
<td>61.8 ± 4.2 (40–83)</td>
</tr>
<tr>
<td>Gravida</td>
<td>2.0 ± 0.4 (0–7)</td>
<td>2.4 ± 0.3 (0–5)</td>
</tr>
<tr>
<td>Parity</td>
<td>1.6 ± 0.3 (0–5)</td>
<td>1.9 ± 0.2 (0–3)</td>
</tr>
<tr>
<td>Duration of cycle (days)</td>
<td>27.5 ± 4.0 (23–30)</td>
<td>26.8 ± 0.7 (21–30)</td>
</tr>
<tr>
<td>Duration of menstruation (days)</td>
<td>4.8 ± 0.3 (2–8)</td>
<td>5.2 ± 0.4 (3–8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 2 (95–140)</td>
<td>115 ± 4 (95–150)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>69 ± 1 (55–80)</td>
<td>70 ± 2 (60–90)</td>
</tr>
</tbody>
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Data are given as mean ± SE; the range is given in parentheses.

ing to the phases of the menstrual cycle used by Fraser et al. (1987), and the results for each phase were averaged [menses phase (days 0–5), early (days 6–9) and late (days 10–12) follicular phase, ovulatory phase (days 13–16) and early (days 17–22) and late (days 23–28) secretory phases]. However, because of the small variation in lengths of the menstrual cycles reported by our volunteers, we chose not to correct the cycle day of measurement to an idealized 28 day cycle. Laser Doppler measurements of endometrial perfusion were compared to a previously reported control group of women volunteers (Gannon et al., 1997), who had normal menstrual cycles and reproductive status, and who were matched for age, weight and reproductive status (Table 1).

### Results
Placement of the measuring probe was routinely accomplished in all volunteers without significant discomfort, bleeding, or other evidence of tissue trauma; uterine perforation or other complication did not occur in any subject.

The endometrial perfusion patterns recorded by laser Doppler
fluxmetry typically showed cyclic variations at two basic frequencies, one consistent with heart rate, and another with a slower frequency (usually 5–8 cycles/min) and of more variable amplitude, presumed to be vasomotion. These perfusion patterns were evident in both control women and those with tubal occlusion, although there were considerable differences in magnitude or frequency of the patterns in individuals, including those at the same stage of the menstrual cycle (Figure 1).

Mean endometrial red blood cell (RBC) flux was influenced by phase of cycle (ANOVA, $F_{5.62} = 6.9, P < 0.001$) in control women with normal menstrual cycles, being highest in the early follicular and early secretory phases, as previously reported (Figure 2). By contrast, mean RBC flux was not significantly different across the cycle in women who had previously had tubal occlusion (ANOVA, $F_{5.42} = 1.9, P=0.11$). Mean RBC flux was significantly higher in the tubal ligation group during menses (control versus tubal occlusion, $17.5 \pm 1.5$ versus $43.0 \pm 8.7, t_{17} = 4.2, P = 0.001$), ovulation ($16.1 \pm 2.1$ versus $26.4 \pm 4.4, t_{16} = 2.0, P = 0.05$), and the late secretory phase ($18.1 \pm 1.7$ versus $24.8 \pm 2.9, t_{20} = 2.1, P = 0.047$).

The frequency of the vasomotion was not significantly different over the phases of the menstrual cycle in control women (Figure 3; ANOVA, $F_{5.62} = 0.63, P = 0.67$), but was marginally different in women with tubal occlusion (ANOVA, $F_{5.42} = 2.5, P = 0.04$) due to a reduced frequency of vasomotion in the early and late secretory phases. These frequencies were significantly less in the tubal occlusion group than in control women (Figure 3; control versus tubal occlusion, early secretory, $6.1 \pm 0.8$ versus $2.3 \pm 0.8, t_{26} = 3.13, P = 0.004$; late secretory, $7.5 \pm 0.6$ versus $5.8 \pm 0.4, t_{20} = 2.04, P = 0.05$).

Discussion

It is apparent that some women who undergo tubal sterilization subsequently experience menstrual disturbances (which include menorrhagia and dysmenorrhoea) although this is difficult to quantify (Kasone and Bonnar, 1976; Gannon et al., 1996). Women who have undergone tubal ligation seem more likely to be admitted for menstrual problems and to undergo a hysterectomy (Wilcox et al., 1992; Goldhaber et al., 1993), but the reason for this is not clear. The decision to undergo tubal occlusion, the subsequent reporting of menstrual dysfunction and the election to undergo further surgery (for example endometrial ablation or hysterectomy) may reflect an attitude or perception on the part of women utilizing this form of contraception that lead them more readily to undergo gynaecological interventions. Nevertheless, there is a real possibility that there is some pathophysiology arising from tubal occlusion itself. The pathophysiological reasons for altered menstrual function after tubal occlusion are also difficult to identify. It does not seem directly related to the amount of tube destroyed in the operative process; indeed menstrual disorders seem more common after tubal clip occlusion procedures (Wilcox et al., 1992). What is required is an investigation of the effects of tubal occlusion on endometrial perfusion patterns across the menstrual cycle and at the time of menstruation, in particular.

The data presented here reveal that women who have undergone tubal occlusion (sterilization), and who did not subsequently report menstrual dysfunction, exhibited greater mean endometrial perfusion during menstruation (cycle days 0–5) at the time of ovulation (cycle days 13–16) and in the late secretory phase (cycle days 23–28) than occurs in controls. In addition, vasomotion in the study group is lower than that in controls in the early and late secretory phase (cycle days
17–22 and 23–28), and there was an overall reduction in variation of vasomotion frequency across the menstrual cycle. Thus, there appears to be some difference in endometrial perfusion between those women with tubal occlusion compared to controls.

Although the women in this study reported no menstrual disturbance, tubal occlusion appears to have led to altered endometrial perfusion; how may this occur? It has been suggested that the vascular circuit comprising the uterine and ovarian arteries and the corresponding veins allows counter current exchange of material flowing through them; furthermore, there is the possibility of utero-tubal and tubo-ovarian vascular and perivasular communication. This complex vascular arrangement, reviewed by Verco (1991, 1993), may so alter concentrations of bioactive substances (e.g. ovarian steroids, prostaglandins, early pregnancy factors) such that target tissues (in this case, endometrial microvessels) are exposed to higher local levels than the generally circulating peripheral levels. The existence of ovarian vein-to-artery counter current exchange would enable the local regulation of uterine, tubal and ovarian function, with these reproductive organs exposed to ovarian hormone levels higher and/or more variable than circulating hormone concentrations (Kryzmowski et al., 1982; Bendz et al., 1982). The tubal occlusion procedure would not only destroy a (small) tubal segment but would disrupt the vascular communication along and immediately subjacent to the tube (Diamond, 1977; Eddy and Pauerstein, 1980). The amount of tube destroyed would, therefore, not necessarily be relevant. The less disruptive/reactive the tubal occlusion, the less likely vascular anastomoses might be to re-establish as a consequence of the healing process. It follows that the counter current exchange of biologically active factors may be disturbed either because these factors did not enter the venous drainage on the appropriate side of the blockage and/or the arterial supply is unable to deliver these factors at greater than general circulating levels to their target organs. This may explain the lack of significant variation in endometrial perfusion across the menstrual cycle in women with tubal occlusion, observed in this study, in contrast to control women. It is also possible that other genital tract communication is disrupted by tubal occlusion; for example, transmural flow between uterine cavity and tubal lumen is blocked (generally at the extramural isthmus). At the same place, epithelial intercellular communication or subepithelial lymphatic communication is blocked, in addition to intra- or peri-vascular communication. Alternatively, reduced luteal phase production of progesterone (Radwanska et al., 1979; Donnez et al., 1981) or reduced midluteal oestrogen secretion observed after tubal ligation may explain our observations. These changes could follow ovarian necrosis which may be due to ovarian arterial hypertension (Catapanach, 1985): equally plausible is ovarian hypotension which could contribute to ovarian dysfunction.

The present study identifies significant alteration in endometrial perfusion in women who have undergone tubal sterilization, but who did not report subsequent menstrual dysfunction. When coupled with published reports of the longer term consequences of tubal sterilization, these data indicate that further exploration of the potential pathophysiology associated with this procedure is warranted.

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