Surface vascularization and endometrial appearance in women with menorrhagia or using levonorgestrel contraceptive implants. Implications for the mechanisms of breakthrough bleeding

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BACKGROUND: Women using progestogen-only contraceptives are commonly troubled by irregular bleeding. Endometrial vessel breakdown and repair is thought to be locally regulated under the indirect influence of sex steroids. Most information about endometrial vessels is derived from blind biopsies taken in an outpatient setting. Hysteroscopy allows in-vivo observation of the whole endometrial surface, including vessel morphology, distribution and areas of bleeding, as well as information about non-vascular structures that may not be accessible from biopsies.

METHODS: Hysteroscopies were performed on 34 women using the levonorgestrel contraceptive implant system (Norplant™) and in a comparison group of 20 women complaining of menorrhagia due to ovulatory dysfunctional bleeding. The images were captured and vascular appearances assessed using image analysis. RESULTS: The percentage of the superficial endometrium covered with blood vessels was found to be significantly greater in Norplant users compared with the comparison group (\( P = 0.0006 \)). More superficial vessels were seen in those with recent frequent or prolonged bleeding and spotting (\( P < 0.0001 \)). In Norplant users, but not in the comparison group, superficial vascular distribution was predominantly patchy (\( P < 0.0001 \)). Unusual vascular appearances classified as ‘neovascular’ (\( P < 0.0001 \)) and ‘mosaic’ (\( P < 0.0001 \)) patterns were commonly seen in Norplant users but not in the comparison group. The hysteroscopic appearance of the endometrial epithelium, glands and stroma also differed between Norplant users and women with menorrhagia. Apparent shedding of the superficial endometrium was commonly seen by hysteroscopy in Norplant users during a bleeding episode (\( P < 0.0001 \)). Prominent gland openings with thick mucus were commonly seen in Norplant users and small sessile polyps were seen in six cases. CONCLUSIONS: These hysteroscopic observations provide further evidence that exposure to progestogens alters the superficial endometrial vasculature and may interfere with angiogenesis.

Key words: breakthrough bleeding/endometrium/hysteroscopy/Norplant

Introduction

As the use of progestogen-only methods of contraception continues to increase worldwide, the problem of the vaginal bleeding disturbances that these methods induce is becoming of increasing public health relevance (d’Arcangues, 2000). Irregular bleeding clearly results in significant disruption and anxiety for users, which may then lead them to discontinue these preparations. Increasing evidence points to a disruption in local endometrial control of microvessel integrity as a key mechanism in breakthrough bleeding (Fraser et al., 1996). Alterations in microvessel structure in conjunction with changes in vasoactive factors in the immediate environment are believed to act together to undermine vessel integrity and lead to breakthrough bleeding (BTB) (Smith, 2000).

Most information collected on progestogen users is based on endometrial biopsy specimens obtained from small outpatient groups. Not surprisingly, very few hysterectomy specimens are available from women using low dose, long-acting progestogen contraceptives. These small samples might allow detailed examination of the morphological, structural and molecular characteristics of adjacent sections of tissue, but have a number of limitations. The endometrium is often atrophic following progestogen exposure and sufficient tissue may not be available from a proportion of patients. Endogenous steroid hormone profiles differ from progestogen users from whom adequate tissue cannot be obtained at outpatient endometrial biopsy (Hadisaputra et al., 1996). Also, the biopsy instruments unavoidably crush some of the tissue, which may alter the
The effect of progestogens on endometrial vasculature

Materials and methods

Thirty-four women aged between 18 and 40 years requesting Norplant insertion with regular menstrual cycles and a recent normal cervical smear were recruited (Hickey et al., 1998, 1999a,b, 2000a,b,c). Subjects were excluded if they did not require long-term contraception, or were taking non-steroidal anti-inflammatory drugs (NSAID). Volunteers were informed of the likely occurrence of bleeding irregularities in counselling and in written information. They were advised that the implants could be removed at any time, but that this would require a minor surgical procedure. Their informed consent was obtained before any investigations commenced. The institutional ethics committees of The Population Council (New York, NY, USA) and Family Planning NSW, Australia gave approval to this study.

Twenty women referred to the hysteroscopy outpatient clinic complaining of essential menorrhagia with cycle lengths of between 21 and 35 days were used as a comparison group. One subject gave a history of regular cycles lasting 37 days and one 40 days. This comparison group was chosen in order to compare the origins of abnormal bleeding. All were fully informed of the study and written consent for participation was obtained. Women with menopausal symptoms and menstrual irregularity, those with fibroids and women who had regularly been taking sex steroid hormones, antifibrinolytics or NSAID in the previous 3 months were excluded. When endometrial pathology was revealed at hysteroscopy or endometrial biopsy, subjects were excluded. This group fitted the clinical criteria of ovulatory dysfunctional uterine bleeding (DUB). All subjects were weighed and measured and their body mass index (BMI) calculated.

Peripheral blood samples were allowed to clot, and centrifuged at 200 g for 10 min within 2 h. Serum was stored at −20°C until analysis. Estradiol and progesterone assays were performed using a chemiluminescent immunoassay (Immulite®). Diagnostic Products Corp., Los Angeles, CA, USA). These assays have a reporting range of 73–734 pmol/l for estradiol and 0.6–127 nmol/l for progesterone. The lower limits for detection are 44 pmol/l and 0.28 nmol/l respectively and the interassay variability in our laboratory is 10% for both steroid hormones. A progesterone level of >10 nmol/l in at least one sample was considered to be indicative of luteal activity.

Norplant users prospectively recorded ‘bleeding’ or ‘spotting’ on a menstrual chart. Bleeding was defined as ‘any bloody vaginal discharge that requires the use of such protection as pads and tampons’ and spotting as ‘any bloody vaginal discharge that is not large enough to require sanitary protection’ (Belsey, 1991).

The presence of any bleeding or spotting on the day of the hysteroscopy was recorded, as was the number of days of bleeding and spotting during the previous 30 days. In all subjects, the date of the first day of the last bleeding episode was recorded.

The hysteroscopic procedure has been previously described in detail (Hickey et al., 2000a). Normal saline at room temperature was instilled at pressures of ~100 (± 10) mmHg to distend the cavity. A standard 5-point scale was chosen for assessment of the endometrial blood vessel and surface appearance. The apparent density and morphology of the endometrial surface vessels were studied in detail using video records of each examination and captured images from Polaroid photographs. Superficial vascular formations were classified as ‘regular’, where the visible formations were composed of individual fine or moderate calibre surface vessels with some terminal branching but not multiple branches (Figure 1); ‘neovascular’ with a single vessel stem and multiple fine branching smaller vessels in an arborising pattern (Figure 2); or ‘mosaic’ with a fine reticular pattern of interlinking vessels (Figure 3).

An attempt was made to quantify the frequency of each of these vascular appearances at each hysteroscopy. Regular, neovascular and mosaic appearances were recorded as ‘1’ (not present), ‘2’ (present in slight quantity), ‘3’ (present in slight to moderate quantity), ‘4’ (present in moderate to large quantity) or ‘5’ (present in large quantities) by two observers, one of whom was blind to the characteristics of the patients.

In addition, the distribution of surface vessels was recorded as ‘regular’ where superficial vessels were distributed evenly through the cavity with no obvious areas of increased or decreased vessels and ‘patchy’ where some areas of the endometrial surface had many superficial vessels and other areas only scanty or no vessels.

In order to try to quantify the proportion of the endometrial surface covered with intact vessels, we selected the area with the greatest number of superficial vessels at hysteroscopy and captured this image on a Polaroid photograph using a Sony video laser printer (Sony, Australia). The images were then digitized onto an image analysis system at 512×512 pixels (TN-8502; Noran, Madison, WI, USA).

Using image analysis, the percentage of the surface area covered by intact vessels in the captured area could then be calculated, using precise intrauterine measurement techniques (Hickey et al., 1998), and this was termed ‘superficial vascularity’. Subepithelial haemorrhages were also present in many Norplant users (Hickey et al., 1996, 1998). These areas of petechiae and ecchymoses were avoided when capturing these images to determine superficial vascularity, so ‘superficial vascularity’ refers to the percentage of the surface in the captured image covered by distinct blood vessels and not by subepithelial haemorrhages. The study by Hickey et al. (1998) demonstrated that the combined magnifying and distorting effects of the hysteroscope can alter the apparent size of images viewed by >50%. Using image analysis, a correction method was devised to adjust for these magnifying and distorting factors which reduced the effects of image magnification on the apparent size of an object viewed through the hysteroscope to 7%, and the effect of distortion to 3%. The correction method was developed by viewing a grid of known dimensions through the hysteroscope to measure its magnifying and distorting effects. These images were then digitized onto the image analysis system. A repeat image of the grid was obtained through an undistorted camera, and software was written to superimpose the distorted image on top of the undistorted image so that the two images matched as...
closely as possible in the area of least distortion. The undistorted image was then digitized, and an equation was defined to transform the distorted image to closely match the undistorted image. This effectively ‘flattened’ the curved image created by the fish-eye lens into a flat image. A custom third order polynomial geometric transform was developed for the image analysis system to correct for the hysteroscope distortion. Software was written in the computer language ‘C’ to apply a distortion correction and this was applied to the hysteroscopic measurements. Hence accurate measurements could be made of images viewed through the hysteroscope.

Two observers blind to the characteristics of each subject were used, and the results presented represent the mean scores of these observers. Interobserver variability was 12%. The area of endometrium measured varied from 0.06 to 117 mm² (SD 16.28 mm²).

During the procedure, a mild stress test was applied by allowing the cavity to collapse by draining out the distending saline, and then slowly redistending the cavity under direct vision (Hickey et al., 2000a).

Two hysteroscopies were scheduled in each Norplant user and were timed to provide information about the endometrial appearance during the first 6 months of use when bleeding problems are most common. In order to provide this information, one-third of subjects were hysteroscoped at 1 month of Norplant use, a further third at 2 months and the remaining third at 3 months. The second examination was scheduled for 3 months after the first. In this way, information was gathered about the endometrial appearance during the first 6 months of Norplant use in conjunction with prospective data on menstrual bleeding patterns. Hysteroscopies in the menorrhagia group were performed on the day of referral to the clinic and were not timed through the cycle. However, in view of the history of heavy bleeding, hysteroscopy was not performed during menstruation.

**Statistical analysis**

Statistical analysis was performed using the SAS program JMP (Carey, Raleigh, NC, USA), on a Macintosh 6200/75 computer. Linear regression was used to assess the relationship between two continuous variables. Analysis of variance (ANOVA) was used to assess whether the relationship between these variables was significant. Ordinal logistic regression was used to analyse the relationship between an ordinal dependent variable (such as hysteroscopic appearance of the endometrial vessels) and a continuous independent variable (such as estradiol and progesterone levels). χ² was used to test whether the logistic model fit was significantly different from that of a fixed response rate across the whole sample. ANOVA was used to test whether there was a significant difference in means between two or more groups, with the Tukey–Kramer post-hoc test being used to indicate significant differences in means between groups. P < 0.05
was taken to indicate statistical significance. Results are reported as mean and SEM.

Results
The average age of the 34 Norplant acceptors was 28.1 years (range 19–40 years). All reported regular menstrual cycles before Norplant insertion. Sixteen women were nulliparous. None gave a history of major systemic medical or gynaecological conditions. All subjects reported a normal cervical smear in the last 2 years and bimanual pelvic examination was normal.

There was no significant difference in BMI between Norplant users (mean BMI 26 kg/m²) and comparison subjects (mean BMI 28 kg/m²). However, the comparison group subjects were significantly older than the Norplant users. The mean age of the 20 women in the menorrhagia comparison (DUB) group was 40 years, and the Norplant group 28 years (range 26–41 years, \( P < 0.0001 \)). There was also a difference in parity between the groups. Only one woman in the comparison group was nulliparous compared with 16/34 (47%) of Norplant users. Hysteroscopy was performed in this group at various times through the menstrual cycle (mean day 17, range day 5–30). None was performed during menstruation, but in 6/20 cases (30%) hysteroscopy was performed in the perimenstrual period (days 24–05 of a 28 day cycle). Eighty-six hysteroscopies were performed over a 1 year period; 66 were in Norplant users (at 2.6–41.5 weeks of exposure to the implants) and 20 in menorrhagic women. Sixty-six rather than 68 hysteroscopies were performed in Norplant users because two women from the Norplant group declined a second hysteroscopy because of discomfort during the first procedure.

Twenty-four out of 66 hysteroscopies in Norplant users (36%) were performed during a bleeding episode. In a further 15 (23%), bleeding was observed to start during the procedure. None of the menorrhagia group presented for hysteroscopy during a bleeding episode, but bleeding was observed to start during the procedure in 7/20 subjects (35%). Four of these subjects were perimenstrual.

An increase in the mean number of bleeding and spotting days was observed following Norplant insertion, from an average of 5 per 30 days before insertion to 9 per 30 days. This did not change significantly over the 1 year follow-up period, but the number of spotting days tended to decrease with time of exposure. During the 30 days preceding the hysteroscopy, Norplant users bled or spotted for 0–30 days (mean 8.5 days, SEM 8.5) and from 2 to 9 days in the comparison group (mean 4.8 days, SEM 1).

**Superficial endometrial vascularity**
We were able to assess superficial vascularity using Polaroid photographs of the endometrium in 59/66 (89%) of the treatment group hysteroscopies and all hysteroscopies in the DUB group. In the remainder, active bleeding or profuse subepithelial haemorrhages made superficial vascularity impossible to delineate.

Endometrial superficial vascularity in the captured images was significantly greater in Norplant users than in the comparison group (\( P = 0.013 \)). When the stress test was applied, by deflating and then re inflating the cavity, there was a significant increase in superficial vascularity in both groups, but this was more marked in the Norplant users (\( P < 0.0001 \)) than in the menorrhagia group (\( P = 0.02; \) see Figure 1).

A statistically significant relationship was observed between superficial endometrial vascularity at hysteroscopy and recent vaginal bleeding patterns. The endometrial surface was pale in appearance and lacking in obvious vessels in those who had been amenorrhoeic (see Figure 4). Vascularity was most marked in those subjects who reported more days of bleeding/spotting in the 30 days prior to the hysteroscopy.

Using the measurement techniques described by Hickey et al. (1998), we calculated that <10% of the endometrial surface was covered in vessels in those who reported 0–3 days of bleeding/spotting in the previous 30 days, compared with 70–95% in those with >17 days of bleeding or spotting (\( P < 0.0001, r = 0.9939 \)). This association between duration of bleeding and superficial vascularity was not seen in the comparison group (Figure 5).

Superficial endometrial vascularity in Norplant users was not related to circulating levels of estradiol or progesterone. In the comparison group, there was a non-significant trend of increasing vascularity through the menstrual cycle (\( P < 0.058 \)).

**Vascular distribution**
In almost all observations of Norplant users at hysteroscopy (63/66, 95%), the superficial vascular distribution was ‘patchy’ throughout the endometrium. This definition describes areas of marked vascularity next to pale endometrium with an uneven distribution of surface vessels in the cavity. This patchy appearance was not seen in the comparison group, where vascular distribution was uniform in 18/20 subjects (\( P < 0.0001 \)).

**Vascular morphology**
A substantial amount of variation was seen both within and between subjects. In many cases more than one vascular pattern was seen at each hysteroscopy. However, some generalizations can be made about the vascular morphological appearances.

Regular superficial vascular patterns were seen less commonly in Norplant users compared with menorrhagia subjects. Regular vessels were seen in only 36% of Norplant users and in 11/20 (54%) of the comparison population (\( P < 0.0001 \)). Vessel appearances classified as ‘neovascular’ were seen in 61/66 (92%) of hysteroscopies in Norplant users and in only one of the comparison group, during the late secretory phase of the cycle (\( P < 0.0001 \)). There was a significant increase in the number of ‘neovascular’ patterns observed in the superficial endometrial vessels with increased time of exposure to Norplant (\( P = 0.008 \)).

Superficial mosaic vascular patterns with a reticular formation of vessels were seen in 45/66 (67%) of Norplant users and in 3/20 (15%) of the comparison group during the secretory phase of the menstrual cycle (\( P < 0.0001 \)).
Figure 5. Superficial endometrial vascularity at hysteroscopy.

Endometrial epithelium, glands and stroma

Apparent shedding of the superficial endometrium was observed in 25/66 (38%) of hysteroscopies in Norplant users, and in 4/20 (20%) of the comparison group. Shedding was superficial and focal, with sheets of endometrium detaching from the fundus, anterior and posterior endometrial surfaces and circulating in the cavity or disappearing up the Fallopian tubes (see Figure 6). In Norplant users, endometrial shedding was seen in 84% of those who presented for hysteroscopy during a bleeding episode compared with 16% who were not bleeding on the day of the procedure ($P < 0.0001$). Shedding was also positively associated with the number of days of bleeding and spotting in the previous 30 days ($P < 0.0001$). Endometrial shedding was not associated with time of exposure to the implants.

Prominent endometrial glandular openings were observed in 28/66 (42%) of Norplant users and 8/20 (35%) of comparison subjects, particularly during the secretory phase. In 22/66 (32%) hysteroscopies in Norplant users, thick mucus was noted in the uterine cavity. This was not seen in the comparison group.

Small sessile endometrial polyps of unusual appearance were observed in 6/66 (9%) of Norplant hysteroscopies (see Figure 7). Using a precise measurement technique (Hickey et al., 1998) these polyps were measured. The polyps were small, with an average width of 941 µm (± 236) and an average length of 1605 µm (± 422). The average area of the polyp was 1.6 mm² (± 0.66). The presence of these polyps was not related to serum oestrogen or progestogen levels, bleeding patterns or to time of exposure to Norplant.

Serum estradiol and progesterone levels

There was no significant change in mean estradiol levels following insertion of Norplant implants. Before insertion mean estradiol was 280 pmol/l and after insertion 198 pmol/l. All but three subjects showed progesterone values suggestive of luteal activity (>10 nmol/l) in the control cycle. Following insertion of Norplant implants the mean progesterone level was 2.1 nmol/l (± 0.09) and only two subjects showed progesterone levels of >10 nmol/l.

Discussion

These hysteroscopic observations in Norplant users provide further support for the theory that exposure to low dose progestogens alters endometrial blood vessels and that this may contribute to BTB.

An apparent increase in superficial endometrial vascularity would not normally be expected in the presence of atrophic endometrium (Hamou, 1980). However, physiological endometrial atrophy, such as the post-menopause, is not normally associated with bleeding. The histological classification of endometrial atrophy only refers to the appearance of glands and stroma and not to blood vessels (Noyes et al., 1950). Previous studies have indicated that endometrial vascular density is increased in Norplant users compared with a control population (Rogers et al., 1993; Hickey et al., 1999b). A regression of endometrial glands and stroma with preservation or proliferation of the vessels may create a situation where vessels are not adequately supported and hence are more fragile. This would be augmented by the reduction in endometrial microvascular basement lamina components seen in Norplant users (Hickey et al., 1999a) and a reduction in supporting smooth muscle α-actin (Rogers et al., 2000). Increased vascular fragility is likely to promote BTB (Fraser et al., 1996; Hickey et al., 2000c).

The hysteroscopic images captured and assessed in the present study inevitably represent only a proportion of the endometrial surface. We have observed that vascular distribution appears to be patchy in these subjects, and this makes
selecting one or two representative areas problematic. When there are large areas or superficial haemorrhage, or where previously intact vessels start to bleed during the hysteroscopy, it can be difficult to gauge the vascular morphology in the surrounding areas of the endometrium.

Recent evidence suggests that progestogens, including levonorgestrel (used in Norplant) may stimulate endometrial angiogenesis (Hague et al., 2002). Although it is not possible to confirm angiogenesis on vascular appearance, the neovascular patterns commonly seen in Norplant users in this study imply ongoing angiogenesis. Dilated vessels on the endometrial surface in Norplant users have previously been described (Hickey et al., 1998; Runic et al., 2000). In other tissues, such as the retina, changes in local perfusion and oxygenation are known to stimulate angiogenesis, dilated surface vessels, neovascular formations and capillary fragility (Pierce et al., 1995). The role of aberrant angiogenesis in BTB requires further investigation, since angiogenic inhibitors may potentially have a role in the prevention or management of bleeding.

Normal endometrial vascular distribution is thought to be regular in pattern (Shaw et al., 1979), and this was confirmed in our comparison group. The patchy distribution of vessels in Norplant users was an unexpected observation. It is unclear whether this vascular appearance is due to an uneven distribution of vessels in the endometrium or to uneven perfusion. Preliminary studies of endometrial vascular perfusion in Norplant users have demonstrated marked variation between different sites in the same uterine cavity in superficial perfusion (Hickey et al., 2000b). This may account for the poor association between endometrial changes seen from blind biopsies and bleeding patterns. Recently, biopsies taken from bleeding areas in the endometrium at hysteroscopy have shown variations in tissue factor between bleeding and non-bleeding sites (Runic et al., 2000). Tissue factor plays an important role in endometrial angiogenesis as well as haemostasis and hence may contribute to the neovascular patterns observed (Abe et al., 1999).

Endometrial shedding is associated with normal menstruation (Noyes et al., 1950). In Norplant users, shedding was seen during bleeding episodes, suggesting that tissue as well as blood is lost at the time of BTB. Analysis of the tissue content of this loss might provide further information about the cellular content of vaginal loss during BTB and how this differs from that lost during normal menstruation. It is possible that the appearance of endometrial shedding may also have represented artefact due to the hysteroscopy procedure and the use of a distention medium, although it was infrequently seen in the comparison group.

The observation of endometrial shedding was associated with low endogenous progestogen levels, comparable with normal menstruation, which is preceded by a fall in circulating progesterone and the activation of proteolytic factors such as matrix metalloproteinases able to breakdown the endometrial extracellular matrix and provoke bleeding (Marbaix et al., 1995; Salamonsen et al., 2000). It is difficult to determine whether low levels of endogenous progesterone in conjunction with continued exogenous low dose levonorgestrel exposure will mimic this situation. Recent data have demonstrated that endometrial endothelial cells do express the progesterone receptor (PR) (Rodriguez-Manzaneque et al., 2000) and that prolonged activation of PR could destabilize the vascular basement membrane leading to vessel breakdown.

Endometrial polyps were observed in five Norplant users at hysteroscopy. No polyps were seen in the comparison group, as subjects were excluded if there was any gross or histological abnormality of the endometrium. However, the Norplant polyps did not resemble endometrial polyps sometimes seen at hysteroscopy in non-progestogen-treated women, and the clinical significance, if any, of these extrusions is unknown. One study (Brechin et al., 2000) reported that endometrial polyps may be a source of unscheduled bleeding in progestogen users, but in our study there was no association between polyps and BTB. The prominent endometrial glandular openings seen in almost half the Norplant users examined were also an unexpected finding, since endometrial atrophy is common in these subjects.

The comparison group subjects used in this study were not ideal and do not represent a true control population. However, it was not considered ethically acceptable to subject normal patients to hysteroscopy for research purposes. It is recognized that that group who complained of menorrhagia is likely to represent subjects with normal and excessive menstrual blood loss, had this been objectively tested (Chimbira et al., 1980; Fraser et al., 1984). In addition, the subjects were not matched for age or parity, and these factors may have influenced endometrial vascular appearance.

In summary, these detailed observations of endometrial vascular and non-vascular appearances in Norplant users demonstrates that a number of potentially important differences can be seen in this population when compared with a group of women with ovulatory DUB. These differences may relate to changes in vascular growth and integrity in Norplant users and hence to BTB.

References

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Submitted on November 21, 2000; resubmitted on March 12, 2002; accepted on May 1, 2002.