Blood to the cornual area of the uterus is mainly supplied from the ovarian artery in the follicular phase and from the uterine artery in the luteal phase

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BACKGROUND: The blood supply to the uterus is provided by the uterine and ovarian arteries, which form anastomoses. Yet the flow direction through this anastomoses and the primary source of blood supply to the tubes and uterine cornua remains unknown. To clarify this issue, we studied the spatial propagation of temperature changes following cooling of the upper vaginal area. METHODS: A thermocatheter with eight measurement points at 5-mm intervals was inserted into the uterus of nine women in the follicular phase and 11 in the luteal phase. The distal tip was positioned in the cornual area and temperatures were registered every 2 s. The vagina was then cooled for 7 min with 25°C saline. RESULTS: The pattern of uterine cooling based on local counter-current transfer differed between the follicular and luteal phase. Cooling of the cornual area was significantly lower in the luteal phase compared with the follicular phase, indicating a shift in the prevailing source of arterial supply in that area following ovulation. CONCLUSIONS: The divide between the territories irrigated by the uterine and ovarian arteries moves between the follicular and luteal phase. This constitutes the first description of a functionally determined shift in the territorial divide of two vascular systems, and has numerous practical implications.

Key words: blood supply/local transfer/temperature/uterus/vagina

Introduction

The uterine artery is the major contributor to the blood supply reaching the uterus. Moreover, as branches of the uterine artery form anastomoses with branches of the ovarian artery, the cornual part of the uterus may receive blood from both arteries. However, the precise location of the territorial divide between the two vascular systems (uterine and ovarian) still eludes our scrutiny and remains a matter for theoretical debate. Recently, Einer-Jensen et al. attempted to clarify this issue further using a methodology based on studying temperature changes that follow a temporary local cooling of uterine blood (Einer-Jensen et al., 2001, 2002). The first step of this original approach consists of locally cooling a given territory by applying cold saline. The local cooling that ensues the temporary application of a balloon flushed with 25°C fluid cools the venous blood that flows from the given area. Subsequently, the local drop in the temperature of venous blood cools the arterial blood flowing next to it (but in reverse direction) through counter-current temperature exchanges, a general principle common to all heat exchanges systems (physiological and man-made apparatus). The net result of the counter-current cooling of arterial blood is therefore a drop in temperature in the territory irrigated by the arterial system that passes in the vicinity of the cooled venous territory. Therefore, local cooling of uterine venous flow allows us to ‘map’ the uterine arterial flow by delineating the territorial extension of the territory affected by the temperature drop. These early studies were conducted in menopausal women not exposed to ovarian hormones (untreated). The results indicated that the territorial divide between the areas supplied by the ovarian and uterine arteries lay ~1 cm down from the tubal ostia. Consequently, in this experimental set up, the cornual area of the uterus was mainly supplied by uterine arteries.

The aim of the present study was to evaluate whether in premenopausal women, the changes in ovarian function occurring throughout the menstrual cycle affect the location of the territorial divide between areas supplied by ovarian and uterine arteries. We therefore investigated the temperature changes occurring in the cornual area of the uterus following local cooling of a territory, affecting the temperature of uterine arterial flow as a result of cooling of the uterine venous flow, in cycling women who were in either the follicular or luteal
phase. For this, we used an eight measurement-point thermal probe catheter in order to obtain a precise topographic definition of the temperature changes occurring in that area.

Determining the possible modification throughout the menstrual cycle of the territorial divide between areas irrigated by uterine and ovarian arteries is clinically important. Recently, a host of data have accumulated to indicate that drugs administered vaginally also benefit from a vascular-dependent counter-current-mediated preferential transport toward the whole territory irrigated by the uterine arterial system (Cicinelli et al., 1998, 2002; Cicinelli and de Ziegler, 1999). Consequently, possible changes in the limits of the territory irrigated by the uterine artery will modify the area affected by the preferential delivery of drugs (hormones, etc.) administered vaginally. Hence, the current study was undertaken to determine whether the extent of the preferential delivery to the uterus of drugs administered vaginally might be affected by functional changes occurring in the ovary.

Methods

We enrolled 27 women, aged 28–41 years, who required a hysteroscopy because of minor menstrual cycle disturbances (pre- or post-menstrual spotting). The local ethics committee approved the study protocol, and participating women gave their informed consent. All women had ovulatory menstrual cycles, as documented by reports of regular menses and cyclical clinical signs of ovulation (mid-cycle increase in cervical mucus, pelvic pain, premenstrual breast tension and dysphoria, etc.) during the prior 6 months. Exclusion criteria included the suspicion of intrauterine overgrowth on transvaginal sonography, and the presence of uterine myomas, ovarian cysts, genital malignancy or extensive uterine prolapse.

Women were conscious during the investigation, and no sedative or painkillers were administered. The trial followed safe gynaecological procedures. Before the procedure, women had a transvaginal ultrasound in order to assess the phase of the menstrual cycle and determine the side of the dominant follicle or corpus luteum. A blood sample was collected for measurement of estradiol (E2) and progesterone. After careful disinfection of the external and internal genitalia, the custom-designed autoclavable thermal probe (ELLAB, Roedovre, Denmark; www.ELLAB.com) was inserted into the uterine cavity through the cervical canal. The probe consisted of a semi-rigid instrument with a diameter of 3 mm and a length of 60 cm, with a slightly bent tip, as most uterine instruments have. The probe was equipped with 8 Cu/CuNi thermosensitive elements. One was located at the distal tip of the instrument (point 1), while the seven others were positioned at 5-mm intervals from the tip.

The probe was passed throughout the canal of a Foley catheter (20 G) after the tip was cut off close to the balloon. The probe was inserted into the uterine cavity blindly and directed towards the uterine cornual area ipsilateral to the ovary carrying the developing follicle or corpus luteum, as detected by transvaginal ultrasound. The probe was gently pushed inside the uterine cavity until its tip came into direct contact with the tubal ostia. The Foley catheter was then pushed inside the vagina using the semi-rigid probe as a directing guide until the tip of the catheter was in contact with the cervix and the balloon in the upper third of the vagina (Figure 1). In all cases, the probe was positioned as desired on the first attempt.

After a few minutes of temperature recording in order to obtain sets of baseline temperature values, the balloon of the catheter was filled with 20 ml saline at room temperature. During the investigation, temperature measurements were carried out every 2 s with an eight-channel ELLAB TM9604 electronic thermometer. Output from the thermometer was transferred to a Compaq Armada 300 with ELLAB TSII software. Recording was continued for 10 min, after which the probe and catheter were removed.

In order to investigate the underlying clinical problem and verify the correct positioning of the probe, all patients subsequently had a hysteroscopy using a ‘vaginoscopic’ approach at the end of the investigation. A 2.9 mm lens-based hysteroscope (Karl Stroz, Tuttinglen, Germany) with single-flow diagnostic sheath was used. Distension was obtained by pressure flushing of normal saline solution. At the time of hysteroscopy, the presence of a dent left by the probe on the endometrial mucosa surface was looked for, and it was determined whether the dent lay on the same side as the ovary carrying the dominant follicle or corpus luteum. In order to avoid the possibility that the hysteroscope itself could leave a dent on the endometrial surface, we explored the cavity by placing the tip of the hysteroscope just beyond the internal cervical ostium. We also verified the correct positioning of the probe by measuring the distance between the dent and the tubal ostia.
ostia. We considered for calculation only cases in which the distance between the ostium and tip did not exceed 2 mm. After the position of the dent was established, hysteroscopic investigation was performed.

All temperature recordings were printed and inspected visually. In addition, the temperature was recorded before, and 2, 5 and 7 min after the start of vaginal cooling.

Data were stratified according to the phase of the menstrual cycle as assessed by serum hormone determination and ecographic findings. Data are reported as mean ± SD. Temperature variations at different points at 2, 5 and 7 min in the follicular and luteal groups of women were evaluated by ANOVA followed by post hoc Student–Neuman–Keul’s test; parallel comparisons at each point between the two groups and at different times were performed by means of unpaired Student’s t-test. A P-value <0.05 was considered significant.

Results
Based on the endometrial, hormonal and ultrasound evaluations, 14 patients were in the follicular phase at the time of their investigation. Serum E2 and progesterone levels were 87.6 ± 13.1 pg/ml and 0.3 ± 0.1 ng/ml, respectively. A dominant follicle (follicle diameter 15.23 ± 2.68 mm) was detected on ultrasounds in 13 cases. Thirteen women were found to be in the luteal phase (serum E2 and progesterone levels 102.0 ± 14.8 pg/ml and 14.5 ± 3.8 ng/ml, respectively). In nine out 13 women, a corpus luteum was positively detected. In all these latter cases, the endometrium showed a typical secretory pattern.

During the hysteroscopic investigation, the dent on the endometrial surface was easily identified in all cases. In five cases conducted in the follicular phase and in two women in the luteal phase, the hysteroscopy revealed incorrect placement of the probe, as the endometrial dent left by the probe was too far from the tubal ostium; these cases were excluded from calculations. Of the remaining 20 cases, nine were in the follicular phase and 11 in the luteal phase. In eight of nine women in the follicular phase, the position of the probe was confirmed to be ipsilateral to the dominant follicle; in one woman no sonographic demonstration of a dominant follicle was obtained. Similarly, in three of 11 women of the luteal group, ultrasonography was unable to demonstrate a corpus luteum; in all remaining cases the furrow of the probe was found to be in the same side as the corpus luteum. In order to rule out the possibility that the results were influenced by eventual differences in blood flow variations depending on whether the cornual side involved was ipsi- or contralateral to the functional ovarian structure, we did not consider for calculation the above-mentioned four patients in whom ecography was unable to detect the side of ovulation. Therefore, the results from only 16 women (eight women in the follicular and eight in the luteal phase) were considered for statistical analysis.

Hysteroscopy revealed no abnormalities in these 16 patients. Cooling was rapid, as maximal decrease in temperature was detected at 2 min, while the cooling effect was progressively diminished after 5 and 7 min (Figure 2).

The cooling effect on the endometrium varied in the different uterine regions (Figure 2). After 2 min of cooling in women in the follicular phase, the temperature changes recorded at the different points were significantly different (P < 0.0001); they were also significantly different at 5 min (P < 0.0001) and at 7 min (P < 0.005). After 2 and 5 min of cooling, the temperature decreases recorded from point 2 to point 7 were significantly smaller than that recorded at point 8; after 7 min of cooling the temperature decreases from point 1 to 6 were significantly different compared with point 8. In women in the luteal phase, the temperature decreases at different points were statistically different at 2 and 5 min, but not at 7 min (P < 0.0001 and P < 0.01, at 2 and 5 min, respectively). At 2 min the decreases in temperature recorded from point 2 to 6 were
functioning corpus luteum. Concomitantly, the secretory `attract' blood supply from the uterine artery on the side of the from the corpus luteum, and therefore low resistance, may artery is rather limited, one can speculate that high demand Jensen and McCracken, 1981). Since the calibre of the ovarian very high, probably amongst the highest in the body; ¯ows up to 1 ml/g tissue/min have been measured in sheep (Einer-
The capillary blood ¯ow through an active corpus luteum is its possible physiological function are not yet fully clari®ed. To the best of our knowledge, our results constitute the ®rst

Discussion
To the best of our knowledge, our results constitute the first indication that the territorial divide between the areas irrigated by uterine and ovarian arteries varies with functional changes occurring in the ovary. During the follicular phase, the cornual area is primarily supplied by the ovarian artery, whereas in the luteal phase, blood is supplied to this area by the uterine artery (Figure 3). The changes in blood supply to the cornual area of the uterus occurring between the follicular and luteal phases of the menstrual cycle may result from either hormonal effects exerted locally or functional changes taking place in the ovary.

The mechanisms at the origin of the shift in the territorial extension of areas irrigated by uterine and ovarian arteries and its possible physiological function are not yet fully clarified. The capillary blood flow through an active corpus luteum is very high, probably amongst the highest in the body; ¯ows up to 1 ml/g tissue/min have been measured in sheep (Einer-Jensen and McCracken, 1981). Since the calibre of the ovarian artery is rather limited, one can speculate that high demand from the corpus luteum, and therefore low resistance, may ‘attract’ blood supply from the uterine artery on the side of the functioning corpus luteum. Concomitantly, the secretory transformation of the endometrium and the vasodilatation of uterine vessels that has been amply described (Salle et al., 1994; Tan et al., 1996; Ziegler et al., 1999) is likely to significantly increase blood flow through the uterine artery and its branches. The resultant lowering of the resistance will increase the pressure in the ovarian–uterine ramus in the uterine end, and thus tend to move the border between blood supplies towards the ovary. In the luteal phase, a decline in pulsatility index and an increase in blood flow velocity have been demonstrated in either both uterine arteries (Salle et al., 1994; Ziegler et al., 1999), or in only that which is ipsilateral to the corpus luteum (Tan et al., 1996).

Numerous differences in cooling pattern were observed 5 and 7 min after vaginal cooling between women in the follicular and luteal phase of the menstrual cycle. Specifically, recordings from points 1 and 2 showed a significantly greater rate of cooling at 5 and 7 min in women in the luteal phase compared with those in the follicular phase. Conversely, in the luteal phase, recording at point 8 showed lower cooling, although not significant, compared with the follicular phase. Hence, we hypothesize that this could reflect higher basal body temperature and changes in the uterine blood flow occurring during the luteal phase (Salle et al., 1994; Tan et al., 1996; Ziegler et al., 1999). Therefore, it is plausible that the warmer vaginal environment of the luteal phase (higher basal body temperature) could warm the saline contained in the balloon more rapidly and, in turn, ‘smooth’ the cooling effect recorded at point 8. In contrast, at the other measurement points, owing to the above-mentioned haemodynamic variations, the greater counter-current cold transport efficiency of the uterine artery will result in a greater cooling effect compared with the follicular phase, notwithstanding the higher basal temperature. Alternatively, as we measured temperatures at the endometrial surface, we can speculate that differences in the local blood supply to the endometrium in the secretory compared with the proliferative phase may account for the lower cooling effect observed at point 8 in the secretory phase.

The data from the present study demonstrate the presence of a preferential vagina-to-uterus distribution in younger women, as previously established in post-menopausal women. The rapid cooling of areas distant from the vagina (cornual area of the uterus) and the differences in cooling pattern between the two phases of cycle confirm that the cooling of the uterus did not result from a simple local diffusion of cold. Our data also indicate that the mechanism responsible for this direct vagina to uterus transport is, at least in part, influenced by the hormonal and functional environment.

Localized cooling of a vaginal area was achieved by filling the balloon of a Foley catheter with cold saline (25°C). Since the tip of the catheter was cut off before insertion and applied against the cervix, the cold saline was positioned in the vaginal fornix. Compared with previous experience in which we directly flushed the vagina with cold saline, this new method represents an improvement for two reasons: (i) the cooling is localized to the deepest part of the vagina, close to cervix; and (ii) the cooling is dry, eliminating any suspicion that some liquid can ascend through the cervical canal. Moreover, the method is also easier to apply and more acceptable to the patient. The application of cooling close to the cervix may explain the rapid start of the cooling, since this is a preferential

Figure 3. Schematic diagram of displacement of the boundary between the territories irrigated by the uterine and ovarian arteries in the follicular and luteal phases.
area for transfer to uterus (Cicinelli et al., 2003). The relative rapid return to basal temperature after 5 min may be due to a more limited cooling capacity when saline is placed in the balloon compared with the continuous flushing in the previous investigation, and to a higher endometrial blood flow in the premenopausal women compared with the post-menopausal women. A similar ‘dry’ method with localized cooling (a saline-containing plastic tube) was used to show transfer from vagina to urethra (Cicinelli et al., 2001).

Transmission of cold was rapid, reaching maximum effect 2 min after initiation of the cooling stimulus and diminishing after 5–7 min. These results differ somewhat from those previously observed in post-menopausal women, where the maximal drop in temperature was observed 5–9 min after the cooling stimulus. The shape of the instruments used in the past and present trials were similar (Einer-Jensen et al., 2001), but their diameter differed. In the early study a catheter 0.8 mm in diameter was used (Einer-Jensen et al., 2002). In contrast, the 8-points uterine catheter used in the present trial is semi-rigid, and 3 mm in diameter. We believe that use of the latter type of catheter, having a closer contact with the endometrial surface because of its larger diameter, can, in part, explain the faster transmission of the cooling stimulus (1–2 min) observed in the present trial. The higher blood flow through the active premenopausal endometrium is also likely to have been instrumental in increasing the speed with which the temperature decreased in the affected territory.

When comparing measurements from individual points in each group, we observed that in the follicular phase at 2, 5 and 7 min, points 1–7 cooled significantly less than point 8, the most external one. In the luteal phase, 2 min after initiating the cooling stimulus, the temperature at points 1–6 was significantly higher than at point 8, but after 5 min only points 1–4 were significantly different from 8. Finally, after 7 min, no statistical difference was found between the different points of temperature measurement. This suggests a more even distribution of the cooling effect to the uterine cavity in the luteal phase as compared with the follicular phase.

The cooling effect reached a maximum after 2 min and began to diminish thereafter at all points of measurement. Yet, in the luteal phase, we observed a dichotomic pattern as the cooling effect diminished at point 7 and 8, while it increased significantly at point 1 after 5 min. The reason for this delayed cooling effect in the cornual area is not obvious, but is probably connected to the increase in and redistribution of the uterine blood flow during the luteal phase. The cooled vaginal venous blood will mix with the uterine vein blood through the shared utero-vaginal venous plexus. The increased uterine blood flow will be followed by an increased venous outflow through both the uterine vein and the utero-ovarian vein. Hence, while the temperature will significantly drop in the uterine vein, the magnitude of the decrease in temperature is likely to be less, because of the important mixing with venous blood originating from the tube and ovary. The utero-ovarian vein and the ovarian artery form a plexus where transfer of gasses and hormones takes place (Einer-Jensen and McCracken, 1981; Einer-Jensen, 1988). Transfer of heat has never been investigated, but it is likely to parallel the heat transfer observed in the male pampiniform plexus. The end result will be some cooling of the ovarian arterial blood, which may translate into a drop in temperature at points 1 and 2, in the tubal corner. The possible existence of counter-current transport mechanisms in the female pelvis from the utero-ovarian veins to the ovarian artery is in accordance with previous experiences gained with vaginal administration of danazol (Mizutani et al., 1995). It suggests a preferential distribution of danazol from vagina to not only the uterus, but also the ovary. Specifically, danazol concentration in the ovary and uterus after daily vaginal administration of a suppository containing 100 mg danazol were comparable to those seen after daily oral administration of 400 mg danazol, while serum danazol concentrations were markedly lower (Mizutani et al., 1995).

As thermal probes were inserted blindly, one can question the correctness of their positioning inside the uterine cavity with the tip lying close to the tubal ostium. To verify the proper positioning, hysteroscopic exploration of the uterine cavity served to identify the presence of a characteristic dent on the endometrial surface. This method of control was chosen because neither the ultrasonographic nor hysteroscopic guidance of the positioning was feasible. As the semi-rigid thermoprobe occupied the vagina, it was impossible to concomitantly insert a vaginal ultrasonic probe without displacing the thermo-probe from its intrauterine positioning. Moreover, our previous experience suggests that transabdominal ultrasonography does not provide precise enough information about distance between the tip of the probe and tubal ostium. Finally, the use of hysteroscopic guidance to position the thermo-probe was also impossible, because the shear diameter of the probe (3 mm) is larger than all operative hysteroscopic channels.

Our experimental protocol also allowed us to exclude the possibility that a visible dent on the endometrial surface recognized at the post-trial hysteroscopy could be generated by the hysteroscope itself. This was permitted because the early exploration of the uterine cavity was conducted while the tip of the hysteroscope remained at the level of the internal ostium of the cervix.

Physiologically, the results of this study suggest that during the follicular phase, the cornual part of the uterus receives a greater part of the blood supply from the ovarian artery, while in the luteal phase the influence of the uterine artery may prevail. Our hypothesis is in accordance with previous investigations on the effects of sex steroids on intrapelvic arterial blood flow. It is known that flow through the uterine artery is regulated mostly by the estrogen/progesterone ratio in arterial blood. $E_2$ increases blood flow in ovarian and uterine, as well as in systemic, arteries (De Ziegler et al., 1991). Owing to local transfer mechanisms, the ovarian steroids reach concentrations in the ovarian artery and tubal arterial arcade that exceed those measured in systemic circulation (Bendz et al., 1979; Einer-Jensen, 1988; Einer-Jensen and Hunter, 2000). It is therefore conceivable that $E_2$ produced by the large follicle is transferred locally from the ovarian vein blood to the ovarian arterial blood, reaching a high concentration in the local arterial blood and inducing an increased blood flow to the organs supplied (ovary, tube, tubal part of the uterus). The
increased flow will move the border between the two supplying arteries towards the corpus uteri, and the first points of measurements will therefore tend to be in the 'ovarian supply area'.

The territorial divide between the uterine and ovarian arteries' territory moves in the direction of the ovary under the influence of progesterone. This means that during the luteal phase, arterial flow in the ovarian artery is either reduced or relocated toward the corpus luteum, or that the uterine artery blood flow increases in the luteal compared with the follicular phase. In parallel to the high local estrogen concentrations in the ovarian arcade, the high local concentration of progesterone during the luteal phase may tend to lower blood flow through the ovarian artery to non-ovarian structures. An alternative hypothesis is that progesterone may have vasodilatory effects on uterine arteries. In accordance with this latter hypothesis, Tan et al. (1996) demonstrated that uterine artery pulsatility index on the side of the developing follicle declines during the midluteal phase, and was significantly lower than on the contralateral side. These authors suggested that the decline in uterine artery resistance during the midluteal phase may be instrumental in triggering optimal conditions for implantation of the blastocyst (Tan et al., 1996).

The physiological importance of the movement of the partition between the vascular territories may be the ipsilateral impact of the ovarian E2 on the sperm transport to the tube on the ovulatory side (Kunz et al., 1998). The process will 'guide' the sperm to the tube with the egg. The preimplantary nutrition and transport of the fertilized egg in the tube is favoured by the ipsilateral increased concentration of progesterone due to local transfer. The data also indicate that the ipsilateral effect of progesterone on the tubal corner of uterus is smaller than on the tube itself. This may seem somewhat strange, and warrants further investigation, as implantation tends to take place in the uterine wall in the same side as the ovulation.

From a clinical point of view, the finding that the vagina-to-uterus distribution also exists in premenopausal women supports the idea of using the vaginal route for delivering drugs preferentially to the uterus. Moreover, the finding that blood supply from the uterine artery covers a larger part of the uterus during the luteal phase has clinical implications. It allows us to conclude that substances or hormones (such as progesterone) administered vaginally to premenopausal women will reach all areas of the endometrium, a fact that is critical for the effective administration of progesterone during luteal phase support.

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References

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