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Sonographic evidence for the involvement of the utero-ovarian counter-current system in the ovarian control of directed uterine sperm transport

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TABLE OF CONTENTS

Sperm transport from the cervix into the tube is an important uterine function within the process of reproduction. This function is exerted by uterine peristalsis and is controlled by the dominant ovarian structure via a cascade of endocrine events. The uterine peristaltic activity involves only the stratum subvasculare of the myometrium, which exhibits a predominantly circular arrangement of muscular fibres that separate at the fundal level into the fibres of the cornua and continue into the circular muscles of the respective tubes. Since spermatozoa are transported preferentially into the tube ipsilateral to the dominant follicle, this asymmetric uterine function may be controlled by the ovary via direct effects utilizing the utero-ovarian counter-current system, in addition to the systemic circulation. To test this possibility the sonographic characteristics of the uterine vascular bed were studied during different phases of the menstrual cycle. Vaginal sonography with the measurement of Doppler flow characteristics of both uterine arteries and of the arterial anastomoses of the uterine and ovarian arteries (junctional vessels) in the cornual region of both sides of the uterus during the menstrual phase of regular-cycling women demonstrated significant lower resistance indices of the junctional vessels ipsilateral to the side of the dominant ovarian structure as compared with the corresponding arteries contralaterally. By the use of the perfusion mode technique, it could be observed that vascular perfusion of the fundal myometrium was significantly increased ipsilateral to the dominant follicle during the late follicular phase of the cycle. These results show that the endocrine control of the dominant ovarian structure over uterine function is not only exerted via the systemic circulation but also directly, most probably utilizing the utero-ovarian counter-current system.

Key words: Doppler sonography/sperm transport/uterine peristalsis/utero-ovarian counter-current system

Introduction

There is experimental evidence that rapid and sustained sperm transport through the female genital tract into the tubes is provided by uterine peristaltic contractions (Lyons *et al*., 1991; Kunz *et al*., 1996). Furthermore, by placing labelled albumin macrospheres of sperm size at the external os of the cervical canal and following their path through the genital tract by means of hysterosalpingoscintigraphy (Williams *et al*., 1993; Kunz *et al*., 1996), it has been shown that sperm transport is preferentially directed into the tube ipsilateral to the dominant follicle. Directed sperm transport into the 'dominant tube' must be a genuine function of the uterus since, with labelled albumin macrosphere experiments, inert particles were used, thus excluding mechanisms such as chemotaxis as being responsible for the accumulation of spermatozoa in the tube on the side of expected ovulation.

The utero-ovarian mechanisms that govern directed sperm transport are not understood. Most probably, endocrine signals from the dominant follicle reach the

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uterus directly via the utero-ovarian counter-current system (Einer-Jensen, 1988) in addition to the systemic circulation. Such a direct access of signals from the ovary to the uterus could cause a gradient of signal expression within the uterine compartment and thus be responsible for asymmetric uterine functions such as directed sperm transport into the dominant tube. In order to examine this hypothesis, studies of the uterine vascular bed were performed utilizing Doppler sonography and perfusion mode techniques.

Sonographic analyses in regularly-cycling women

Patients

Thirteen patients (mean age 29 years; range 23–41 years) entered the study. Each had a history of regular cycles and was suffering from andrological sterility. The studies were performed in all 13 patients during the midfollicular (day 8 of the cycle) and late follicular (days 12 and 13) phases, and in 11 of the 13 patients during the midluteal phase (day 20), respectively, of the cycle. All patients gave their informed consent.

Sonography

All sonographic measurements were performed using a 7.5 MHz vaginal probe with colour and pulsed Doppler facilities (Logiq 500; Kranzbühler, Solingen, Germany). After each scan a venous blood sample was drawn to monitor of serum concentrations of oestradiol, progesterone and luteinizing hormone.

Doppler sonography

After placing the probe in the dorsal fornix of the vagina, each examination started with the assessment of the localization and size of the dominant ovarian structure. The uterine arteries were then visualized lateral to the isthmical regions of the uterus, and a pulsed Doppler range gate was applied across the vessel after yielding a sagittal section of the ascending branches of the uterine arteries. The angle between the Doppler beam and the vessels approached 0°. The Doppler range was confined to a minimum velocity of 2.2 m/s. After obtaining the flow velocity waveforms, the resistance indices (RI) and the maximum (V_{max}) and minimum (V_{min}) blood flow velocities of each uterine artery were determined. The RI were calculated from selected good quality waveforms according to the formula: RI = V_{max}–V_{min}/V_{max}. Following these procedures, a transverse view of the fundal region of the uterus was adjusted and flow velocity waveforms of the arteries lateral to the cornual regions of the uterus were obtained, thus representing the anastomoses between the uterine branches of the ovarian arteries with the ovarian branches of the uterine arteries (junction vessels). After minimizing the angle between the Doppler beam and the vessels, the RI and blood flow velocities were determined as described above.

Perfusion mode technique

In order to demonstrate quantitatively the vascular perfusion of the upper third of the fundal myometrium, the perfusion mode technique was used (Sohn, 1995). The range of perfusion was confined to a minimum of 2.2 m/s. The data were quantified on the basis of visual impression of the perfusion and expressed as ipsilaterally or contralaterally increased, or symmetrically distributed ultrasound signs of vascularization in relation to the site of the dominant ovarian structure.

Hormone measurements

Serum concentrations of oestradiol and progesterone were determined with a commercially available radioimmunoassay kit (Serono Diagnostics GmbH, Freiburg, Germany).

Statistical analysis

Statistical analysis was performed using Student's *t*-test and the χ^2 test. Significance was assumed when $P < 0.05$.

Vascular flow in different menstrual phases

Doppler sonography

Figures 1 and 2 summarize the findings obtained following Doppler sonography. The RI in the ascending branches of the uterine arteries did not show any significant side differences, although during the late follicular and midluteal phase of the cycles a tendency towards a lower RI in the uterine arteries ipsilateral to the dominant ovarian structure could be observed. However, a significantly lower RI in the junction vessels ipsilateral to the dominant ovarian structure could be demonstrated during the late follicular and midluteal phases of the cycles and only just failed to become significant ($P = 0.07$) during the midfollicular phases as compared with the corresponding vessels contralaterally. The RI of the junction vessels ipsilateral to the dominant ovarian structure were significantly lower as compared with the RI of the ipsilateral uterine arteries during the mid- and late follicular phases and just failed to become significant during the midluteal phase of the cycle ($P = 0.07$).

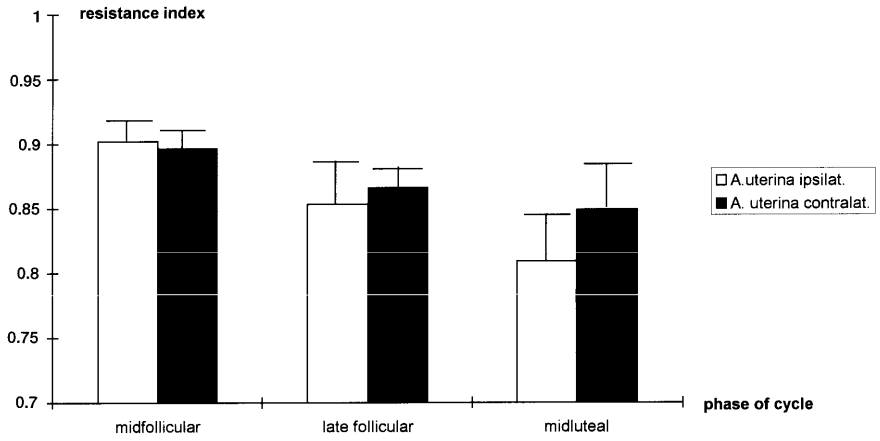

Figure 1. Distribution of resistance indices (RI; mean ± SEM) of the uterine arteries in relation to the phase of the cycle and to the site of the dominant structure as judged from Doppler sonography. The RI in the uterine arteries decreased with progression of the menstrual phase, with predominantly lower values obtained from the vessels ipsilateral to the dominant follicle and corpus luteum as compared with the contralateral arteries, though differences were not significant.

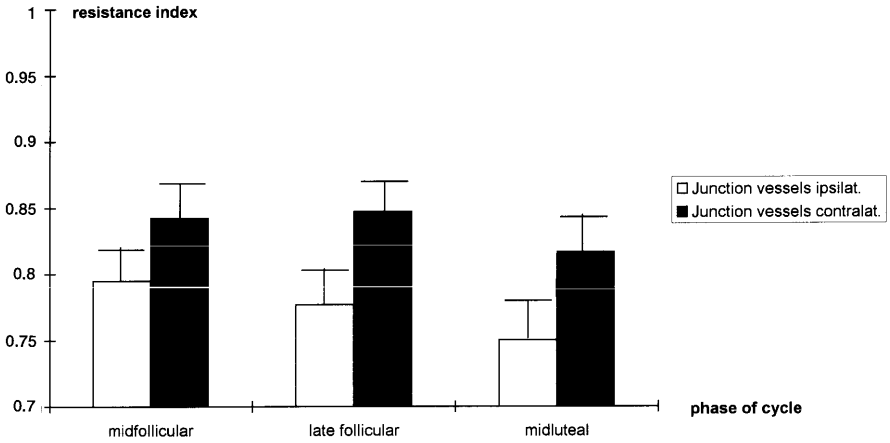

Figure 2. Graphical indication of the resistance indices (RI; mean ± SEM) in arteries that represent anastomoses between uterine branches of the ovarian arteries and ovarian branches of the uterine arteries (junction vessels). During the late follicular and midluteal phases of the cycles, significantly lower RI in the junction vessels ipsilateral to the site of the dominant ovarian structure could be demonstrated as compared with the contralateral vessels.

Perfusion mode technique

Figure 3 summarizes the findings obtained following vaginal sonography using the perfusion mode technique. A significantly increased sonographic representation of vessels in the fundal myometrium ipsilateral to the site of the dominant follicle during the late follicular phases of the cycles was found as compared with the contralateral myometrium. These side differences could also be obtained from the midfollicular and midluteal phases, but failed to become significant. The asymmetry of the vascular perfusion in a patient during the midluteal phase of the cycle with the corpus luteum in the right ovary is shown in Figure 4.

Hormone measurements

All women displayed ovulatory cycles as judged from vaginal sonography and measurements of hormone concentrations. The mean serum concentrations of oestradiol and progesterone during different phases of the menstrual cycles, as well as mean follicular diameters during the follicular phases of the examination cycles, are shown in Table I.

The ovarian control of uterine sperm transport

Directed sperm transport into the tube ipsilateral to the dominant follicle is not dependent upon the capability of

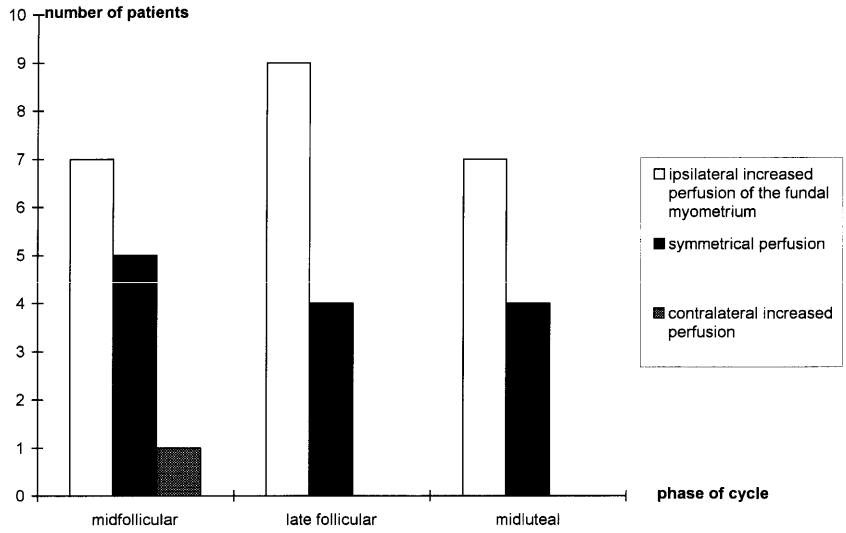

Figure 3. Histogram showing the distribution of the vascularization of the fundal regions of the uteri during the menstrual cycle, by means of the perfusion mode technique. The extent of perfusion was related to the site of the dominant ovarian structure and described as ipsilateral increased or contralateral increased or symmetrically distributed ultrasound signs of perfusion. Despite a tendency towards elevated perfusion of the fundal myometrium in relation to the site of the dominant ovarian structure being seen in all phases of the cycle, a significant difference was only seen in the late follicular phases.

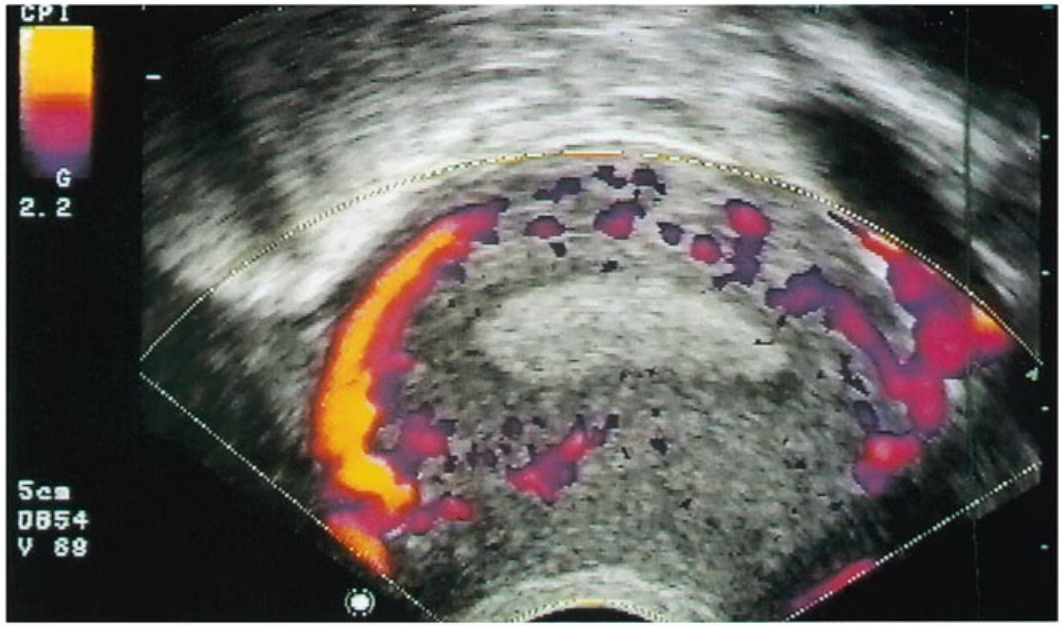

Figure 4. Transverse view of the fundal myometrium using the perfusion mode technique during the midluteal phase of the cycle in a patient with the corpus luteum located in the right ovary. The coloured structures within the myometrium are blood vessels. The dominance of the relatively increased perfusion ipsilateral to the corpus luteum as compared with the contralateral side can be clearly seen. The partly dark and reddish-coloured structure at the right corner is the left iliacal vein.

spermatozoa to move, or on mechanisms such as chemotaxis, but rather constitutes a genuine uterine function (Kunz *et al*., 1996). It is evident that directed sperm transport requires a specific morphological structure enabling the uterus to transport spermatozoa into one of the tubes, and a specific stimulation that regulates this unilateral function.

It had been shown that rapid—and presumably also sustained—sperm transport through the female genital tract is provided by uterine cervicofundal peristalsis (Kunz

et al., 1996). Uterine peristaltic activity is confined to the stratum subvasculare (Birnholz, 1984; De Vries *et al*., 1990; Lyons *et al*., 1991; Kunz *et al*., 1996), which is the innermost layer of the muscular uterine wall (Wetzstein, 1965) and composed of muscular fibres with a predominantly circular arrangement (Werth and Grusdew, 1898; Wetzstein, 1965). The stratum subvasculare is the muscular component of the endometrial–subendometrial unit or 'archimetra', of which the other components are the endometrial stroma and the endometrial epithelium (Werth and Grusdew, 1898; Noe *et al*., 1998). Being of paramesonephric origin, the archimetra is phylogenetically and ontogenetically the oldest part of the uterus (Werth and Grusdew, 1898; Noe *et al*., 1998). In humans, during embryological development of the uterus, the primordial archimetra is formed when the paramesonephric ducts have fused at the 8th week of gestation. It is a morphological feature of the archimetra that the stratum subvasculare of the myometrium retains, at least in the fundal and cornual region of the uterus, the characteristics of an initially paired organ. In this respect the circular muscular fibres surrounding the lower and middle portion of the uterus separate totally at the fundal level into the fibres of the cornua and continue from there into the circular muscles of the respective tubes. Thus, with this structure of the subendometrial muscular layer in the fundal and cornual region, the unpaired human uterus may still have the capability to function as a paired organ (Noe *et al*., 1998) and direct spermatozoa into one of the tubes. The morphologically paired character of the uterus is also reflected in its blood supply (Figure 3).

Table I. Diameters of the dominant follicles, and oestradiol and progesterone serum concentrations during the mid- and late follicular and midluteal phases of the patients' cycles. Values are mean ± SD

Phase of cycle	n	Follicular diameter (mm)	Serum oestradiol (pg/ml)	Serum progesterone (ng/ml)
Midfollicular	13	13 ± 2	100 ± 68	0.4 ± 0.3
Late follicular	13	19 ± 2	159 ± 94	1.0 ± 0.8
Midluteal	11	–	100 ± 50	10 ± 5

The frequency and intensity of uterine peristalsis depend upon the phase of the cycle (Lyons *et al*., 1991; Kunz *et al*., 1996) and are each controlled by the dominant ovarian structure. The changing pattern of uterine peristalsis during the proliferative phase of the cycle could be completely mimicked by the administration of exogenous oestrogen to hypogonadal women that resulted in peripheral blood oestradiol concentrations similar to those of the normal cycle (Kunz *et al*., 1998). Thus, oestradiol secreted from the dominant follicle constitutes the primary signal for uterine peristalsis. In this respect, oestradiol probably induces a cascade of endocrine and paracrine events such as the upregulation of oxytocin mRNA in the endometrium (Zingg *et al*., 1995) which may result in an increased stimulation of the underlying myometrium. In our own studies (Kunz *et al*., 1998), it could be demonstrated that exogenous oxytocin is able to increase the frequency of peristaltic contractions during the follicular phase of the cycle.

Exogenously administered oestradiol only simulates the amount of ovarian oestradiol that reaches the uterus systemically. A unilaterally enhanced peristaltic activity responsible for directed sperm transport would require an additional stimulus for peristaltic activity confined to this region of enhanced peristalsis and being superimposed on the stimulus provided systemically. Since uterine peristaltic activity is correlated with the level of oestrogenic stimulation (Kunz *et al*., 1998) it is conceivable that a gradient of oestradiol concentration within the uterus could result in respective differences in peristaltic activity within the organ itself.

Both, the specific structure of blood supply of the upper portion of the uterus that is provided mainly by the ovarian arteries and the concept of the utero-ovarian counter-current system (Einer-Jensen, 1988) are pertinent in this respect. The significance of the counter-current system was mainly viewed in a uterine effect on ovarian function such as inducing ovine luteolysis. However, the reverse situation may also be of physiological significance in that the ovary may act directly on the uterus utilizing this system. By means of the counter-current mechanism, oestradiol or any other ovarian endocrine signal such as oxytocin from the preovulatory follicle (Schaeffer *et al*., 1984; Ivell *et al*., 1985; Tjugum *et al*., 1986; Peek *et al*., 1987; Fortune and Voss, 1993) may be increased in the vessels supplying the fundal and cornual sections of the uterus.

Our data not only support this conjecture but also demonstrate that the dominant follicle increases the efficiency of the counter-current system on its side. The blood supply to the fundal and cornual regions adjacent to the dominant ovarian structure is increased in comparison with the contralateral side, as indicated by the enhanced perfusion of the high fundal and cornual regions of the myometrium and the lower RI in the junctional vessels as compared with that of the corresponding vessels of the contralateral side, or to the uterine arteries ipsilaterally. With respect to the RI, similar data have been obtained by Santolaya-Forgas (1992) which demonstrate a larger postovulatory decrease of the pulsatility index of the uterine arteries at the level of

the uterine cornua ipsilateral to the side where ovulation had taken place.

The mechanisms by which perfusion of the uterine region adjacent to the dominant structure is increased are not known. Oestradiol increases endometrial blood flow (de Ziegler *et al*., 1991; Achiron *et al*., 1995; Heger-Mahn *et al*., 1996; Dören *et al*., 1997) and acts directly on the vascular tone (Batra, 1994). Furthermore, increased local levels of oestradiol may further increase perfusion by inducing the production of factors that increase local vascularization, such as vascular endothelial growth factor (Charnock-Jones *et al*., 1993).

Conclusions

Directed uterine sperm transport into the tube ipsilateral to the dominant follicle results from the fact that the uterus, though an unpaired organ in the human, may act as a paired organ on the basis of the morphological structure of the stratum subvasculare of the myometrium, and on the blood supply in the fundal and cornual regions of the uterus which also conserves the morphological characteristics of a paired organ. The utero-ovarian counter-current system may provide higher oestradiol concentrations in the cornual region of the uterus ipsilateral to the dominant follicle, thus increasing unilaterally the endocrine signals within the functional cascade of uterine peristalsis.

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