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Associate Editor's comment on 'Endometrial and sub-endometrial perfusion are impaired in women with unexplained subfertility' by Raine-Fleming *et al.*

Uterine Doppler studies: technology driven data, or answers to our pathophysiological queries?

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Getting over the teething of new technologies

Knowledge in medicine has always depended on the investigative tools available at any given time. This explains that our understanding of pathophysiology has most often progressed by frog leaps, reflecting the successive technological innovations bestowed upon the various fields of medicine, such as ours. The integration of new technological innovations normally follows a standard process whereby the new tools are first tested and their potential virtues tried out in sets of pilot and feasibility trials. This preliminary step normally offers a clearer view of the novel technologies' actual merits, potential clinical applications and usefulness for answering outstanding pathophysiological queries.

In the case of uterine Doppler however, we seem to be still at the first step of this process, dealing today with proof of principle trials that attempt to delineate the potential uses and applications of uterine Doppler in gynecology. It sometimes seems that uterine Doppler never succeeded in leaving the teething stage and either reaching maturity and clinical usefulness, or falling forever into oblivion. And this is particularly odd, as in the mean time, Doppler technologies have become widely available to all of us, being a common adjunct to most gynecological ultrasound machines available today.

Menopause and controlled ovarian stimulation (COH): divergent data with unanswered questions

In 1991, Gangar *et al.* reported that uterine artery resistance was high in estrogen deprived menopausal women, but rapidly decreased after exposure to exogenous estrogens. This was taken as evidence for the vasodilative properties of estrogens. We concomitantly made similar findings in young (20–40-year-old) women prematurely deprived of ovarian function (de Ziegler *et al.*, 1991). In Gangar's paper, the dose of transdermal estrogen used (0.05 mg/24 h) yielded the relatively low levels of plasma E2 achieved in estrogen therapy prescribed to menopausal women. In our experimental paradigm, we used larger amounts (~4-fold greater) of transdermal E2, ranging from 0.1 to 0.4 mg/24 h, in order to duplicate the levels of E2 encountered in the menstrual cycle, including the pre-ovulatory rise. Yet in spite of the difference in age and doses of transdermal E2 used, the lowering effect of estrogen therapy on uterine artery impedance (PI index measured while on estrogen) was similar. Taken together, these two studies indicated therefore that maximum vasodilation of uterine vessels is already achieved by the relatively low levels of E2 reached with postmenopausal hormone replacement.

But confusingly, investigators from Gangar's institution (including one common co-author) also reported that a sizeable fraction of women undergoing controlled ovarian hyperstimulation (COH) had high pulsatility index (PI) values (>3), in spite of the high E2 levels that characterize COH (Steer *et al.*, 1992). This latter publication drew a lot of attention because the authors reported that no pregnancies occurred in women whose PI readings exceeded 3 on the day of oocyte retrieval. Logically therefore, these authors recommended that embryos be cryopreserved and transferred at a later stage when uterine artery Doppler showed PI <3.

Unfortunately, these authors failed to underscore the divergence between their highly publicized findings of high PIs in some women undergoing COH and those (probably less read) indicating that low levels of E2 induce maximum vasodilation of uterine arteries in menopausal women. This omission hampered our understanding of the mechanisms at play in those women whose PI remains high in COH in spite of high E2 levels. How could it be that minimal amounts of E2 induce a maximal decrease in uterine artery PI values (not surpassed by higher quantities of E2) when some women undergoing COH have high PI scores in spite of E2 levels that largely surpass those archieved with menopausal therapies? This question drew little attention and remained unanswered to this date.

The '3rd factor hypothesis' for explaining the low perfusion cases encountered in COH

The observation that low estrogen levels induce maximal vasodilation of uterine arteries in women whose ovaries are

inactive is in contradiction with findings that a fraction of women exposed to the high E2 levels of COH retain elevated uterine artery resistance. To reconcile these seemingly incompatible observations, we hypothesized that a 3rd ovarian factor, i.e. a factor other than E2 and P, causes the increase in uterine artery resistance seen in some infertile women in the menstrual cycle or when undergoing COH (de Ziegler *et al.*, 1993; de Ziegler 1995). One putative candidate for this 3rd ovarian factor affecting uterine perfusion is the ovarian production of androgens, which is also influenced by the degree and type of gonadotropin stimulation.

According to this '3rd factor hypothesis', if the ovaries are suppressed, such as with a GnRH agonist (GnRH-a), the putative factor will be neutralized. This should restore an ideal perfusion of the uterus when it is under the sole influence of exogenous hormones. In these women therefore, uterine perfusion should be normal during E2 and P replacement, as prescribed for frozen embryo transfers (FET) on the model of replacement cycles originally designed for recipients of donor oocyte IVF.

On the contrary, if the high uterine artery resistance found in some infertile women results from an intrinsic uterine factor (i.e. a lack of response to the vasodilative properties of E2), Doppler data will remain equally altered during the menstrual cycle (Goswamy and Steptoe, 1988; Goswamy *et al.*, 1988), COH and E2 and P replacement cycles prescribed for FET.

The existence of a specific uterine factor responsible of altering blood perfusion and, in turn, receptivity to embryo implantation is likely to exist in women whose uteri have been exposed to radiation therapy. In this latter highly specific group however, we showed that high E2 levels could restore normal perfusion and receptivity, at least in some women (Rio *et al.*, 1994).

Differences in uterine Doppler data linked to fertility vanishing with time, while Doppler measurements moved down the vascular tree

Following the original paper by Steer *et al.* (1992), numerous groups tried to integrate uterine artery Doppler in their IVF practice with the intent of singling out women whose chances of getting pregnant were either nil, or so low that cryopreservation of embryos would be warranted. But no groups could confirm the existence of a cut-off value, such as that of 3 for PI proposed by Steer *et al.* (1992) that allowed barring certain patients from undergoing fresh embryo transfers because of predictably poor chances of achieving pregnancy.

On the contrary, a number of articles later reported similar uterine artery resistance data measured by Doppler on the day of hCG administration and/or embryo transfer in women who got pregnant or not through IVF (Yuval, 1995; Schild, 1999; Pierson, 2003). These publications reported uterine Doppler measurements performed on sub-endometrial vessels as well as on uterine artery trunks. Admittedly, other publications persisted in claiming that IVF failures were associated with uterine perfusion disorders that could be identified or hinted at by uterine artery Doppler (Kupesic, 2002; Chien *et al.*, 2004) but without clearly identifying any clinically usable cut-off value for PI.

It was puzzling to us that papers reporting similar uterine PI values in IVF patients irrespective of whether they became pregnant or not, showed low PI values across the board (Yuval, 1995; Schild, 1999). Hence, it was as if the high PI values that had been measured in a sizeable fraction of COH patients, and found to predict poor IVF outcome as reported by Steer *et al.*, had vanished. Could it be that technical differences, and notably, improvements in Doppler sensitivity that allow measurements to be taken further down the uterine vascular tree, have been responsible for the lack of differences seen in the latest studies between women who got pregnant or not? We can't ascertain this, but it certainly seems that as technology has become more sophisticated, the early differences reported between pregnant and non-pregnant patients undergoing ART have got smaller, if not vanished altogether, in the recent publications.

3-D power Doppler: a story revisited with quantified and reproducible measurements

Admittedly, early measurements of endometrial and sub-endometrial blood flow suffered from methodological weaknesses, being equipment and operator dependant. The selection of endometrial and sub-endometrial vessels identified on color Doppler mode was arbitrary. Hence, extrapolations from PI results implied the assumption (which was far from proven) that the vessels selected for Doppler measurement were representative of the whole endometrial and sub-endometrial blood flow.

Yang *et al.* (1999) attempted to quantify color Doppler visualization of endometrial and sub endometrial blood flow by direct computation of power Doppler visualization of blood flow. The computed index, or Endometrial Power Doppler Area (EPDA), was based on computer-assisted identification of color pixels. Using this approach, the authors observed that women who achieved pregnancy had a significantly higher EPDA score (8.8 mm^2) than those who did not (5.8 mm^2), whereas uterine artery PI values were similar at 1.65 and 1.67 in these two groups.

Wu *et al.* (2003) pursued this approach one step further by analyzing power Doppler findings of endometrial and sub-endometrial blood flow in 3-D power Doppler reconstructions. In their hands, 3-D power Doppler analysis was the best available predictor of IVF outcome. This approach allowed the whole territory of interest, the endometrium and sub-endometrial area isolated in an 3-D electronic matrix, to be reviewed. It revived the interest in uterine blood flow measurements and the hope of singling out women in whom embryos need to be cryopreserved and transferred at a later time.

In a recent publication, Raine-Fenning *et al.* (2004a) used quantified 3-D power Doppler angiography for studying changes in endometrial and sub-endometrial blood flow occurring during the menstrual cycle. For this, the authors used a 730 GE-Voluson ultrasound machine with defined Doppler settings and conducted their analysis on acquired

3-D power Doppler matrices. The endometrial–stromal interface was delineated with the help of a specially designed virtual organ computer-aided image-analysis program (VOCAL). This approach allows the electronic delineation of the endometrial volume in which blood flow is analyzed by quantifying the Doppler signal recorded within the specific volume. Sub-endometrial blood flow was similarly assessed in a different volume constructed by shelling a secondary virtual interface set at an arbitrary distance, 5 mm, from the defined endometrial–myometrial interface. In each 3-D volume, endometrial and sub-endometrial, pulsed Doppler data were expressed as vascular index (VI) reflecting the percent color pixel over total pixel count, flow index (FI) reflecting the intensity of the power Doppler signal and a combination of these two parameters or VFI. Using this sophisticated technique, Raine-Fenning *et al.* (2004b) observed an increase in all parameters of endometrial and sub-endometrial blood flow in the late follicular phase, peaking 3 days prior to ovulation. This was followed by a decrease after ovulation with a nadir reached in the mid luteal phase.

In the current issue of *Human Reproduction*, Raine-Fenning *et al.* (2004c) pursued their study, and compared their menstrual cycle data obtained in presumably fertile women with new data mustered in a group of women suffering from unexplained infertility. They observed that in the latter group, endometrial and sub-endometrial blood flow was significantly decreased throughout the menstrual cycle. The comparison was made in non-smoking controls, as these authors previously showed that blood flow was significantly decreased in smokers. The authors concluded that endometrial and sub-endometrial blood flow are significantly decreased in unexplained infertility, despite similar E2 and progesterone levels throughout the menstrual cycle.

Back to the future of Doppler studies and the long-lived pilot trial time

When discovering that 3-D Doppler data could be automatically measured within the electronically designed limits of a virtual shell volume that precisely delineates a given fraction of the sub-endometrial tissue as reported by Raine-Fenning *et al.* (2004b), c), we were tempted to feel excitement. Yet once over the astonishment generated by the technological marvel, Raine-Fenning's report leaves us a little hanging; with our desire for an understanding of the mechanisms at play behind their uterine Doppler findings unsatisfied.

We would have liked to know, for example, whether the altered perfusion identified in infertile women persisted or disappeared once their ovarian function was suppressed and replaced by physiological amounts of exogenous E2 and progesterone (as done for FET). This would have indicated whether the factor(s) responsible for the decreased perfusion was of uterine (intrinsic alteration) or hormonal origin (ovarian factors other than E2 and progesterone such as possibly androgens).

Raine-Fenning's data on smoking and uterine perfusion are also puzzling and in many ways, raise questions about the true value of Doppler alterations as markers of uterine receptivity. From their findings, endometrial vascularity is more profoundly altered in smokers as compared with non-smokers, than it is in infertile as compared with fertile women. Should we deduce that smoking has absolute contraceptive effects, or are we forced to speculate that the altered vascularity due to smoking has different consequences on fecundity than those affecting infertile women?

In short, we would like to have understood more, so that our knowledge of the mechanisms at play stops trailing in the wake of the spectacular marvels of 3-D Doppler technology. But Raine-Fenning's paper tells us that we'll have to wait a little longer before these questions are addressed; as in spite of the technological advancements and the fairly long uterine Doppler history, we are still dwelling in pilot trial times, as far as uterine Doppler data are concerned. That, thanks to 3-D technology, uterine Doppler seems to have seriously reduced the variability of its measurements is definitely a matter for rejoicing.

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