The endometrium and IVF

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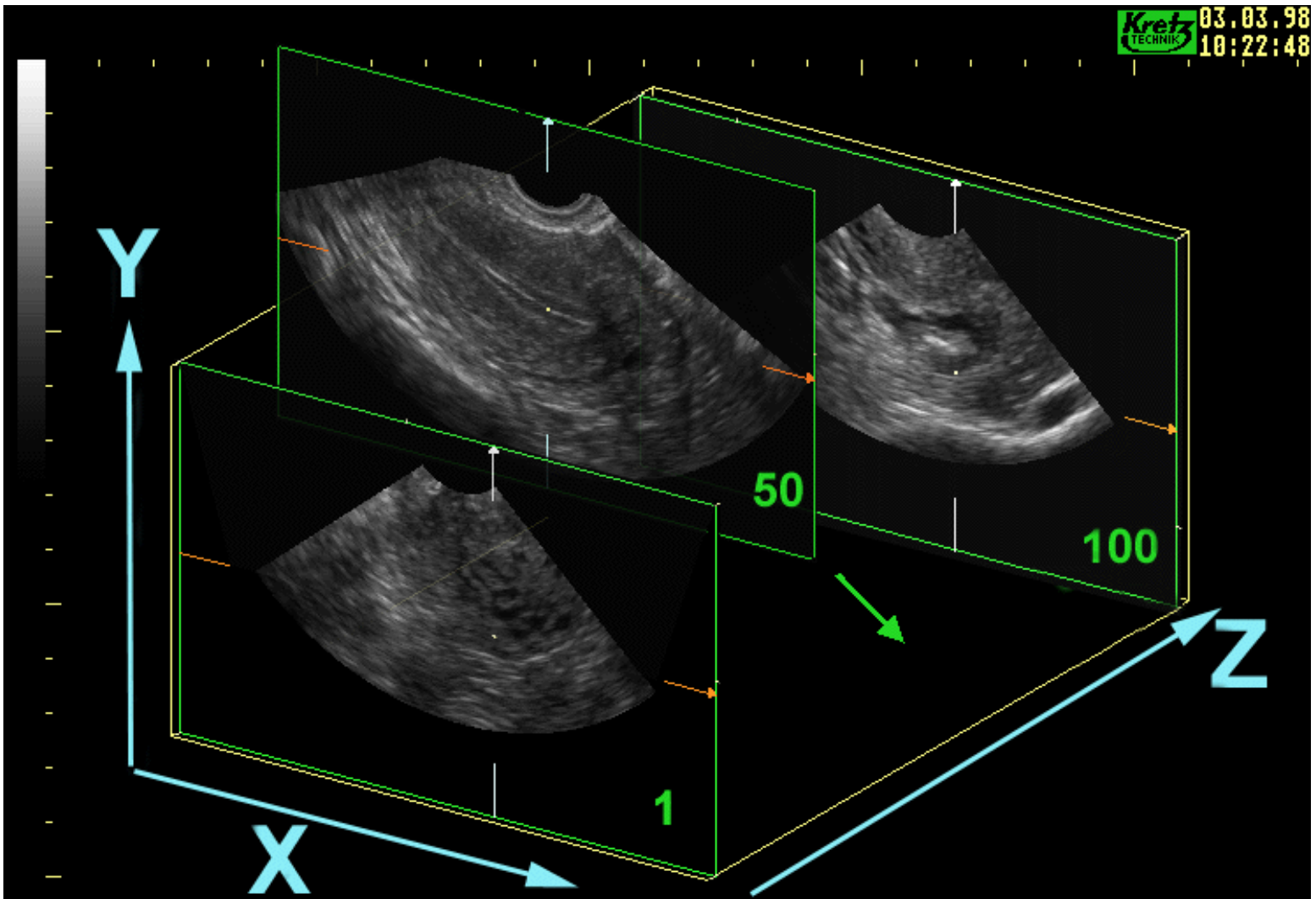
Table of content

9 Practical measure to optimize endometrial receptivity [*]

9.1 Routine assessment of the endometrium before IVF [*]

9.2 Minimize endometrial effects of androgens [*]

9.2.1 OC pill pretreatment [*]

9.2.2 Minimize the amount of FSH used in late stimulation [*]

9.2.3 Use of dexamethasone [*]

9.3 Fluid in the endometrium [*]

9.4 The "too thin" endometrium [*]

9.5 Contractility [*]
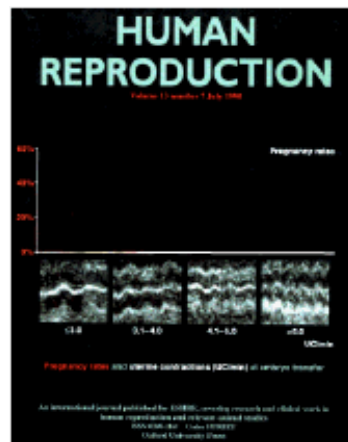
1 E2 and progesterone effects: the donor-egg IVF lesson

Take home message
In women deprived of ovarian function, replacement of only E2 (oral or transdermal) and progesterone (IM or vaginal) suffices to induce optimal endometrial receptivity with PR usually slightly higher in donor-egg IVF than in regular IVF programs. The E2 priming step duplicating the follicular phase only needs to be sufficient and great tolerance exists for duration (6 days-2month) and doses of E2 used. After E2 priming, progesterone induces a sequence of changes in endometrial glands and stroma that are primarily time-dependant and little affected by the dose of progesterone used. Optimal receptivity (window of transfer) for up to 8 cell embryos occurs on the 3rd and 4th day of progesterone exposure. Higher progesterone levels may compensate the deleterious effects of high E2 levels on UC frequency.

1.1 Only E2 and progesterone are necessary for optimal receptivity

It has been known "for ages" that the endometrium must first be primed by E2 during the follicular phase before responding to E2 and progesterone produced by the corpus luteum during the luteal phase. Yet, it is only recently through donor egg IVF that we started to precisely know the respective roles of each of these hormones. Also, despite the production of numerous non-steroidal products by the ovary (relaxin, Inhibit, etc.), donor-egg IVF thought us that the sole replacement of E2 and progesterone suffices to provide optimal receptivity in women deprived of ovarian function. Donor-egg IVF thought us also the degree of flexibility that exists in amounts and duration of treatment for maintaining optimal endometrial receptivity. In this respect, the degree of forgiveness of E2 and progesterone cycles has been astounding.

1.2 The follicular phase: sufficient E2 priming
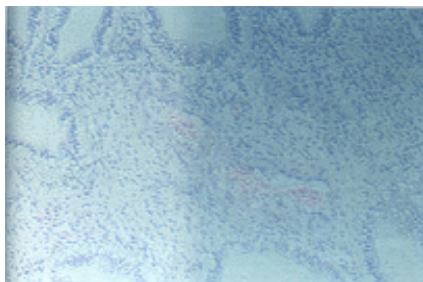
During the follicular phase, E2 induces a proliferation of endometrial glands and stroma. This translates in an increase in endometrial thickness on UTZ imaging. The crucial step in the endometrial effects of E2 is the development of E2 (ER) and progesterone receptors (PR). The role of PR priming for later facilitating progesterone effects on the endometrium is illustrated by a study by Gibbons et al. (Am J Obstet and Gynecol 1986;154:456-61). These investigators showed unequivocally that the degree of E2 priming influences the magnitude of the endometrial response to progestins. In their work, the endometrial effects of 5 mg of MPA (x12 days) assessed by various morphometric parameters were equivalent to those of 10 mg of MPA, when "E2 priming" was with 1.25 mg of CEE rather than 0.625 mg. Hence, the mere vision that because progesterone antagonizes the proliferative effects of E2, the dose of progesterone needs to be adjusted to the amount of E2 present does not appear valid. On the contrary as shown by Gibbons, the endometrial effects of progesterone are facilitated by a higher degree of E2 priming.

When estrogen priming appears insufficient as suggested for example, by a too thin endometrium, one has the possibility to revert to vaginal administration of E2. Confirming our observation of a direct vagina-to-uterus transport of vaginally administered substances or *"first uterine pass effect"*, Tourgeman et al. (Am J Ostet Gynecol 1999;180:1480-3) showed that vaginal administration of E2 (2mg BID) results in markedly higher endometrial tissue to plasma level ratio than when the same dose is administered orally. Also, because the vaginal route avoids metabolism during the first liver pass effect inherent to oral administration, serum E2 levels are nearly 10 times higher than after the same dose administered orally. Yet as in both cases the liver is exposed to the same E2 load (the total amount of E2 ingested, 2mg BID), SHBG and other parameters of hepatic effects of E2 were similar with both routes of administration (Fertil Steril 2001;75:200-2). Hence, vaginal administration of E2 is a viable option each time it appears clinically indicated to enhance the endometrial effects of E2. Furthermore, in spite of the high levels achieved, this remains as safe as administrating 2mg of E2 BID, orally. Vaginal E2 is in particularly indicated when endometrial thickness is insufficient.

1.3 The luteal phase: progesterone induces a sequences of transformation
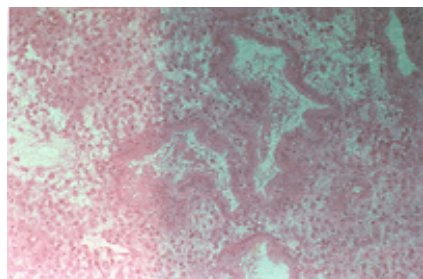
Ever since Noyes et al. published their classical paper on the characteristic changes observed in the endometrium throughout the menstrual cycle, we have been sold to the concept of "endometrial dating", particularly during the luteal phase. This implies that endometrial changes occurs at a specific (or "normal") pace so regularly that an endometrial specimen obtained during the luteal phase can be *"dated"* by reference to the physiological sequence of changes occurring in the menstrual cycle. Today, in the light of 15 years of experience with donor-egg IVF we realize that the sequences of changes is not affected by the various forms of hormonal treatments used nor by the duration of the follicular phase. This led to the concept that endometrial changes follow a logic and timed sequence that is programmed in the endometrium itself and little influenced by the nature (dose, route of administration, etc.) of the treatment, provided that sufficient amounts of hormones are received.

1.4 Endometrial glands and the stroma: two steps in the sequence of endometrial changes.

The endometrial changes retained by Noyes et al. for dating the first half of the luteal phase occur in the glands. The most characteristic of them is the development of sub-nuclear vacuoles at the base of glands that provides the "*palisade*" aspect. This is the emblematic yet transitory sign of the early luteal phase that soon disappears with the return of vacuoles toward the base of cells.

Endometrial effects of progestin (MPA) are affected by the degree of estroginazation. As stated earlier, Gibbons and Moyer showed that a higher degree of estrogen priming enhances the endometrial effects of MPA (Am J Obstet and Gynecol 1986;154:456-61).

The endometrial changes characteristic of the second half of the luteal phase are observed in the stroma. The most characteristic of them

is the predecidual transformation of stromal cells first occurring in the vicinity of endometrial vessels (spiral arteries).

We know from experimental manipulations done in donor-egg IVF cycles that endometrial glands and stroma have different sensitivities to progesterone. Exposure to minimal amounts of progesterone suffices for inducing the full array of changes in the glands and thus, results in the normal sequence of endometrial transformation during the early luteal phase (up to day 20). The stroma however is less sensitive to progesterone and a more profound impregnation is necessary for the development of full predecidualization (day 24). This provides an explanation for the long held belief that only anomalies in late luteal endometrial biopsies were diagnostic of "luteal phase defect". Hence, when "mock" cycles are performed in donor egg IVF, biopsies should always be performed toward the end of the luteal phase (day 24-26), despite implantation normally occurring earlier.

1.5 E2 to progesterone ratio

It has been a long held belief that the E2 to progesterone ratio prevailing during the luteal phase of the menstrual cycle must be respected for optimal endometrial receptivity. In IVF, the excessive levels of E2 and the resulting alterations in E2 to progesterone ratio have been incriminated in the prevailing sub-optimal receptivity (by comparison to donor-egg IVF, for example).
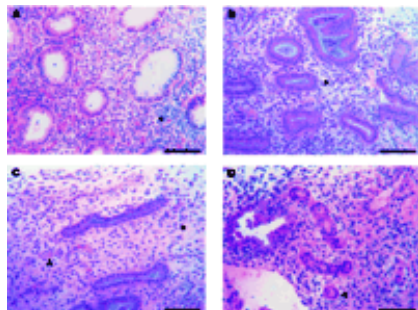
Morphology of the endometrium. In normal cycles (A) and high IVF responders (C and D) where delayed glandular and stromal develop can be seen

Puzzled by the little knowledge on the role of luteal E2 (one of the arm of the E2 to progesterone ratio), we elected to study the effect of interrupting E2 administration after progesterone was initiated in E2 and progesterone cycles designed for donor-egg IVF. Much to our surprise, early (day 20) and late (day 24) endometrial morphology was not affected at all by interrupting E2 administration as soon as progesterone was started on day 15 in experimental E2and progesterone (mock) cycles (de Ziegler et al. J Clin Edocr Matab 1992;74:322-31). We then conducted the reciprocal experiment and administered large amounts of E2 (E2-benzoate 2mg/day, IM). Here again, there was no impact seen on day 20 and 24 endometrial morphology (de Ziegler and Bouchard, Curr Opin Obstet Gynecol 1993;3:378-88). From these data later confirmed by others we concluded that even the most extreme alterations in the E2 to progesterone ratio did not influence endometrial morphology. As discussed later I this syllabus, this vision must now be readjusted a little. Whereas endometrial morphology is not, uterine contractility may be influenced by the E2 to progesterone ratio. When E2 levels are elevated as in IVF cycles, this induces a relative resistance of the uterus to the myorelaxant properties of progesterone with higher contractility during the luteal phase. In IVF this may have deleterious consequences.

If luteal E2 has no action on endometrial morphology (no changes when absent or at very high levels), it is not without effect on reproductive endocrinology. In physiological replacement cycles, E2 administered alone induces a partial decrease of gonadotropin levels whereas, E2 and progesterone normalize the levels to less than 10 mIU/mL. In patients in whom E2 administration was discontinued after the onset or progesterone administration (day 15), progesterone in the absence of "luteal" E2 became incapable of normalizing gonadotropins. In these patients, FSH and LH promptly returned to menopausal levels prevailing before treatment. Hence, E2 is a necessary cofactor of progesterone for its anti gonadotropin properties.

At the core of our concern for the practical consequences of the changes in E2 to progesterone ratio is our fear that COH may negatively impact on uterine receptivity. Despite the number of publications that have been dedicated to this topic over the years, the issue of possible deleterious effect of COH on uterine receptivity still remains a matter for discussion. Bringing fresh arguments in this debate, Basir GS et al. (Human Reprod 2001;16:435-40) looked at the impact on the endometrium of the high E2 levels achieved in IVF by morphometric analysis of the secretory changes achieved and glands and stroma. In their study, 38 infertile women who did not have an embryo transfer because of failed fertilization or fear of OHSS underwent an EMB 7 days after hCG. Endometrium specimens were measure by morphometric analysis using the following criteria: (i) volume fraction of the endometrium occupied by glands, (ii) maximal glandular diameter, (iii) height of the glandular epithelium, (iv) number of subnuclear vacuoles, (v) amounts of secretion in gland

lumen, (vi) amount of stromal edema, and (vii) number of venules in the stroma. 12 women were studied in their menstrual cycle and 26 women received COH for IVF. Of these, 11 had E2 levels <20'000 pmol/L. The other 15 women in whom ET was differed because of fear of OHSS E2 levels were >20'000 pmol/L. Morphologic comparisons were conducted between these 2 groups. Normal cycle endometrial biopsies showed "in-phase" glandular development and lowest amounts of endometrial edema. High responders demonstrated gland-stromal dyssynchrony with delayed glandular development and highly edematous stroma. In the high responders, it is possible that reduced glandular development and lack of glandular secretion are indicators of sub-optimal endometrial environment.

In their interesting paper, Basir et al. obtained evidence of diminished secretory transformation (in glands and stroma) in high IVF responders. The authors pointed at E2 as the designated culprit of the endometrial alterations observed in high responders. We believe that identifying morphological alterations in high responders does not necessarily imply that these changes are induced by high E2 levels. Together with E2, the ovary produces a multitude of other factors that are also controlled by gonadotropins. Hence, substances other than E2 and also produced in larger amounts in COH may mediate the possible deleterious effects of COH on uterine receptivity. We formulated this possibility a few years ago in a concept we called the *"third factor hypothesis".* Amongst the potential candidates for this ovarian factor(s) (other than E2) that is(are) increased in COH and has(have) deleterious effects on endometrial receptivity ("third factor") are the androgens. This issue and the practical measures it implies for optimizing uterine receptivity in IVF will be further discussed in section 5 of this syllabus. For now, it remains important to keep in mind that E2 is not the only ovarian factor produced in excessive amounts in COH.

2 Regimens for frozen embryo transfers

The successes of donor-egg IVF and the predictability with which E2 and progesterone cycles duplicate the morphological parameters of the late luteal phase have led to use those preparation cycles for priming endometrial receptivity for frozen embryo transfers.

Originally, temporary suppression of ovarian function was induced with a GnRH-a in order to duplicate the conditions prevailing in donor-egg recipients (ovarian failure) and avoid that "uncontrolled" ovulation "ruins" the attempt to synchronize embryo and endometrial maturation. These regimens were cumbersome however, particularly when pregnancy did not occur because return to normal menstrual cyclicity was sometimes delayed.

We observed that E2 treatment (oral Estrace, 2mg BID or transdermal E2, 0.1mg) administered starting on cycle day 1 prevents the inter-cycle FSH elevation and the resulting follicular recruitment for up to 3 weeks, with a reliability >95% observed in a 100-patient study. Using this model, ET can be scheduled during the 3rd (or 4th) week of the replacement cycle, on the 3rd or 4th day of progesterone administration. The adequacy of follicular maturation blockade is verified by one single blood measurement of progesterone on the last day of E2 only administration. If progesterone is <1 ng/ml on the eve of starting progesterone administration, endometrial maturation controlled by the duration of exposure to progesterone will be appropriate when ET is scheduled on the 3rd or 4th day of progesterone treatment (5th day in case of blastocyst transfers). We also perform an UTZ on the last day of E2 priming (at the time of progesterone measurement) to verify that E2 priming is sufficient by assessing endometrial thickness (thickness >7 mm). Using this E2 and progesterone regimen, we documented that implantation and pregnancy rates were identical to those achieved when embryos were either transferred in the natural cycle or after GnRH-a and E2 and progesterone treatment. This approach has later been verified by others (Simon A et al. Fertil Steril 1999;71:609-13 and Simon A Human Reprod 1998;13:2712-7).

We recently refined our E2 and progesterone regimen by starting E2 treatment (2mg BID) a few days before menses, i.e., in the preceding cycle, on day 25 or 3 days before the anticipated menses. This was motivated by evidence that in some cases the inter-cycle FSH elevation starts already a few days before menses. We had previously shown (Le Nestour et al. J Clin Endocr Metab 1993;77:439-42) that late luteal onset of E2 treatment does do delay the occurrence of menses (induced by P withdrawal) and is harmless (in spite of warning boxes in the package insert) in case of pregnancy. When suspicion of accelerated hepatic metabolism of E2 exists such as in smokers and chronic takers of neuro medications, transdermal (or vaginal) administration of E2 is preferred.

> **Take home message**
> E2 an progesterone cycles are as efficient and markedly more convenient than monitoring the menstrual cycle for priming endometrial receptivity in preparation for frozen embryo transfers. Today, there is ample evidence that it is not necessary to suppress ovarian function with a GnRH-a (Luperon). E2 treatment (Estrace, 2mg BID) is started on cycle day 1 (or better, on cycle day 25 of the previous cycle). Then, 2 to 3 weeks after the onset of menses a single evaluation is done for UTZ assessment of endometrial thickness (>7 mm) and serum progesterone measurement (must be <1 ng/mL). ET is scheduled appropriately for the degree of embryo development (on the 3rd or 4th day of progesterone treatment for "cell" stage embryos, and 5th day forblastocysts).

3 Luteal E2 supplementation

> **Take home message**
> In 2 similar pathophysiological studies conducted in E2 and progesterone cycles by 2 distinct investigators, there were no visible consequence on endometrial morphology of interrupting E2 treatment after starting progesterone administration. This led to believe that luteal E2 had no impact on endometrial morphology in humans.
> Recently however, there have been reports showing a positive impact (on pregnancy rates) of E2 supplementation in the luteal phase in IVF cycles. The literature should be followed for conclusive arguments on this topic.

The physiological role of luteal E2 has been discussed in a prior section of this syllabus (1.6). Here, we are addressing the possible value of supplementing E2 during the luteal phase of IVF cycles. Several groups have attempted to supplement E2 during the luteal phase. Classical work by the Belgian team of Devroey and van Sterteghem recently reviewed (Posaci et al Human Reprod 2000;15:1435-9) concluded that E2 supplementation during the luteal phase (together with progesterone) provided no benefit on PR. This clinical finding was in line with our own patho-physiological study on luteal E2 that concluded at the absence of endometrial effects. Furthermore, confirmation of our own work by another team (Younis JS et al, Fertil Steril 1994;62:103-7) and documentation in monkeys that luteal E2 supplementation is not necessary in donor egg IVF recipients concurred to build the dogma that luteal supplementation of E2 is not necessary.

Recently however, a couple of publications have pointed at the apparent value in IVF of E2 administration during the luteal phase

> **Jung H and Roh HK** (J Assist Reprod Genet 2000;17:28-33) studied the effect of E2 supplementation (2mg P.O., BID) throughout the stimulation cycle, from COH day 1 to the end of the luteal phase. Patients were prospectively attributed to either the E2 supplementation or control group. Implantation and pregnancy rates in the 58 cycles receiving E2 were markedly higher at 26% and 48.3% in women receiving E2 as compared to the 27 control cycles (10% and 25.9%, respectively).

> In a different trial, **Weissman FJ et al** (Fertil Steril 2000;73:761-6) prospectively studied the effects of E2 treatment during the luteal phase. Serum E2 levels and PR were higher in patients receiving E2. The authors claim that E2 supplementation is warranted (together with progesterone) in women who cannot receive luteal support from hCG because of excessively high E2 levels (>2500 pg/ml).

The issue of luteal support should be followed in the literature for conclusive arguments. It seems a little premature to make practical recommendations at this stage.

4 Late follicular phase progesterone elevation.

> **Take home message**
> Elevation of plasma progesterone > 0.9 ng/ml on the day of hCG administration carries a poor prognosis particularly, if the overall response of the ovary to hMG/FSH is weak. Studies in donor egg recipients and frozen embryo transfers revealed that deleterious effects of premature P elevation are exerted on the endometrium and not the oocyte. The ominous character however, may be lost (or minimal) in case of hefty (good) ovarian response to COH.

4.1 Progesterone >0.9 is deleterious particularly, in poor responders

Before GnRH-a times, an elevation of progesterone during the late follicular phase reflected premature lutenization and ovulation with catastrophic consequences on IVF outcome (premature ovulation, no oocytes collected, etc.). Yet, despite complete blockage of gonadotropins with GnRH-a, some patients still show a slight increase in plasma progesterone occurring before hCG administration. This however, does not result from GnRH-a escapes.

A florid controversy has existed from incept about the consequences of late follicular phase progesterone elevation. Some authors including Schoolcraft, Meldrum and ourselves have observed poorer outcome in case of premature progesterone elevation but others failed to confirm these results. Offering an original insight for explaining the controversy, Fanchin et al. (Fertil Steril 1997;68:799-805) showed that the deleterious effects of late follicular phase progesterone elevation is primarily seen in case poor overall response to COH whereas, this disappeared in the good responders.

4.2 Late follicular phase progesterone elevation is not LH dependant

In GnRH-a cycles, late follicular elevation of plasma progesterone does not reflect an escape from GnRH-a blockade but follows the ovarian response to gonadotropins. The effects of hMG/FSH on progesterone (and androgens) cumulate approximately 12 hours after FSH administration with similar results obtained after hMG and FSH treatment (Fanchin et al. Fertil Steril 1995;64:796-801). Hence, the late follicular increase in progesterone represents the end result of step by step increments occurring each day of COH treatment.

Adonakis G. et al. Fertil Steril 1998;69:450-3.
These authors studied patients who received GnRH-a and FSH for the initial part of their COH and who were later randomized to receive either FSH (300 IU/day) or hMG (FSH and LH) for the final 2 days before hCG administration. The authors observed no difference in progesterone increase between the 2 groups. They conclude that the late follicular phase increase in progesterone is unrelated to any luteinizing process attributable to LH.

5 Effects of androgens

Take home message
Androgens interfere with estrogens at the level of the endometrium and may seriously hamper endometrial receptivity. Androgens are elevated by COH. In normal cycling women plasma T approximately doubles at the end of COH. This increase may be of larger amplitude in certain women (PCOD, etc). Pretreatment with OC pill may be beneficial on the endometrium by decreasing ovarian and circulating androgen levels. In some women adrenal suppression with dexamethasone may be contemplated to minimize the overall exposure to androgens.

5.1 Endometrial effects of androgens
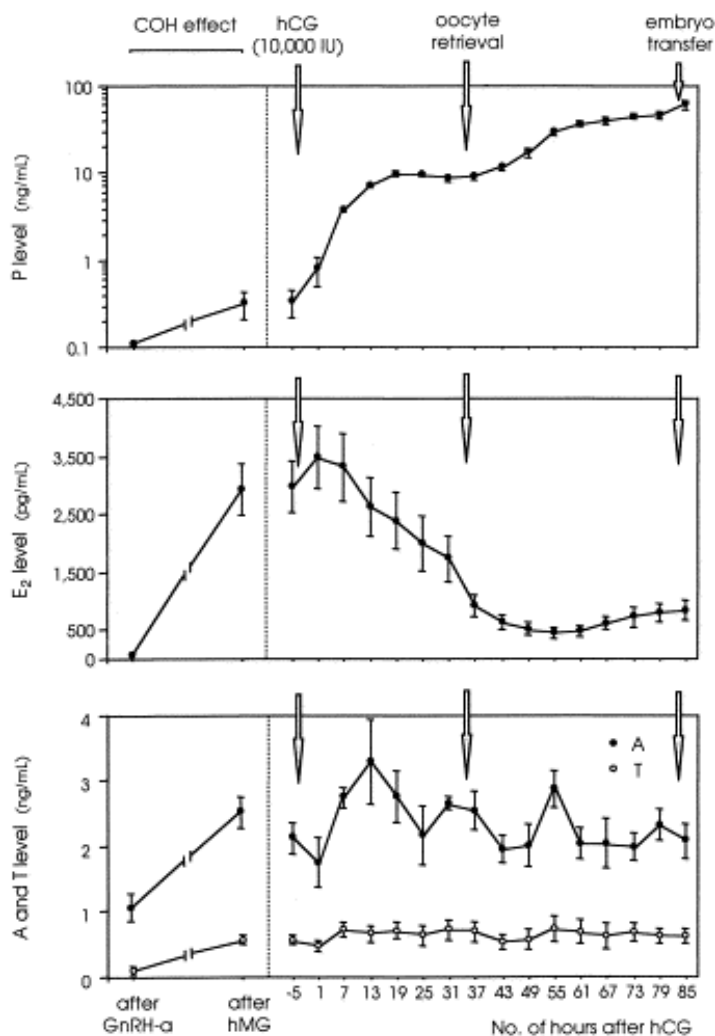
In the menstrual cycle, the ovary produces more testosterone (0.7 mg/24h) than E2 (from 0.05 to 0.5 mg/24h). The description of an increase in circulating testosterone ad mid cycle led to incriminate a role played by the LH surge.

The endometrial effects of androgens have not been entirely elucidated. Yet there is ample evidence indicating that androgens and notably, testosterone are deleterious for a proper development of the endometrium where they antagonize the effects of E2.

Tuckerman EM et al. (Fertil Steril 2000;74:771-9) studied the effects of androstenedione (A4), testosterone (T), dihydrotestosterone (DHT) and DHEA on endometrial epithelial cells. These authors confirmed the presence of androgen receptors in their endometrial epithelial cells grown in culture. In their hands, A4 hampered H3-thymidin uptake and glycodelin secretion by endometrial epithelial

cells. On the contrary, T, DHEA or DHT had no effect on cultured endometrial epithelial cells. These authors conclude that A4 can inhibit human endometrial epithelial cell growth and secretory activity in vitro. These findings are consistent with the hypothesis that adverse reproductive outcome in women with hyper-androgenemia (notably, PCO) may be in part due to a direct detrimental effect of androgens on the endometrium. (see further section of this syllabus on practical measures recommended when detrimental effects of increased androgens are feared).

5.2 The source of hyper-androgenemia In IVF: the actual culprit (FSH) was not the designated suspect (LH).

During the course of COH, there is an approximately 50% increase in circulating testosterone and adrotenedione (A4). Defying our earlier thoughts and beliefs, there is now ample evidence that it is FSH rather than LH that is responsible for this increases circulating androgens. In COH, A4 and T rise by daily increments culminating approximately 12h after hMG/FSH administration and entirely returning to baseline before the next hMG/FSH administration. We showed that prior suppression of adrenal function with dexamethasone decreases baseline (pre COH) and post COH (day of hCG) levels of A4 and T but not the absolute increment occurring during COH (Fanchin et al. Fertil Steril 1997;67:115-9).

Biberoglu K. et al. ASRM 2000 P-54
These authors conclude that low dose dexamethasone (0.25 mg/day) is a reasonable option for the treatment of hyperandrognenic patients with or without COH.

6 Clinical assessment of the endometrium

6.1 Endometrial biopsy (EMB)

Take home message
Classically EMB was the gold standard that served to define the entity of luteal phase defect (LPD). Today, numerous markers identified by immuno-cytochemistry have complemented the conventional H&E analysis. Yet despite this great sophistication (i.e, integrins), we must humbly recognize that little progress has been made toward finding identifiable markers of endometrial receptivity that can be identified on histological specimens.

6.1.1 Limits of endometrial biopsies (EMB):

Classically, endometrial biopsies have been performed in the late luteal phase, looking for signs of hampered or delayed secretory transformation. A delay of > 2days constitutes an evidence of luteal phase defect. The possibility of easily obtaining endometrial tissue has generated great hopes that EMB would provide definitive information on endometrial receptivity. Today, 1) we must admit that the subtleties of endometrial receptivity still evade visualization on histology and 2) there has been no definitive finding of "unreceptive endometrium" (short of status post radiation therapy).

Dubowy RI et al. ASRM 2000 P-279

These authors reported on the use of "objective" markers of histological changes. They retained cyclin E (normally expressed by follicular phase glands) and P-27, normally only expressed by luteal phase glands. Their objective was to compare the results to histological analyses performed by commercial pathological laboratories and reproductive endocrinologists (RE). Mid luteal endometrial biopsies were obtained from 66 infertile patients. In 26/60 (43%) EMBs, H&E assessments differed between the commercial laboratory and REs by more than 2 days (and in 15%, by more than 4 days). Glandular-stromal dyssynchrony was noted by 6.7% of commercial labs, and 53% of REs. Cyclin E staining was inappropriate in dyssynchronous endometrium.

Raymond EG et al. Human Reproduction 2000;15:2351-5.

Doctors Bruce Lessey's and Markku Seppala's groups have recently reported data that pointed at the limitations of EMBs. In their protocol, these investigators studied endometrial specimens obtained 8-10 days after ovulation in women who received EE and norgestrel starting on the day of LH surge following the "Yuzpe" emergency contraception regimen. There were no differences in either endometrial dating as per Noye's criteria. There were no differences in pinopode density between treated and untreated cycles. Endometrial b3 integrin sub-unit and LIF expression in the epithelial component of the endometrium as measured by immuno-cytochemistry were also not significantly reduced in the treated cycles. Interestingly, serum E2 concentrations were significantly lower in treated cycles, but serum progesterone and glycodelin were not affected by treatment. This also confirmed that the emergency contraception treatment did not interfere with the occurrence of ovulation. Mean endometrial thickness was significantly lower in treated (7.6 mm) as compared to untreated cycles (9.8 mm). These investigators were however incapable of finding any difference in endometrial "pattern" between treated and control cycles. This study conducted by renowned investigators casts serious doubts over the ability of endometrial biopsies of assessing endometrial receptivity, even when b3 integrins are taken into account (by immuno-cytochemistry).

Personal comment: The mechanism of action of the Yuspe regimen of emergency contraception remains unknown. Yet as ovulation is

not prevented with the Yuspe regimen, it has been believed that the primary mode of action of this regimen of emergency contraception had to be at the endometrium level. The lack of evidence for any endometrial effect is therefore puzzling particularly, considering that these renowned investigators conducted an exhaustive series of all the most pertinent potential markers of endometrial receptivity.

Glandular-stromal dyssynchrony: As discussed in section 1.4, a characteristic dyssynchrony in glandular and stromal changes has been described almost unanimously by investigators reporting on day 20 histology of E2 and progesterone cycles conceived for donor-egg IVF recipients. And despite this apparent anomaly, endometrial receptivity in donor-egg IVF is "*as good as it gets"*. Hence, no practical conclusion can be drawn from this histological peculiarity.

6.1.2 Ultrastructure

Pinopodes are sponge like smooth membrane projections that arise from the entire surface of endometrial cells lining the uterine cavity around the presumed time of blastocyst implantation. Their presence can solely be identified by scanning electron microscopy (SEM).
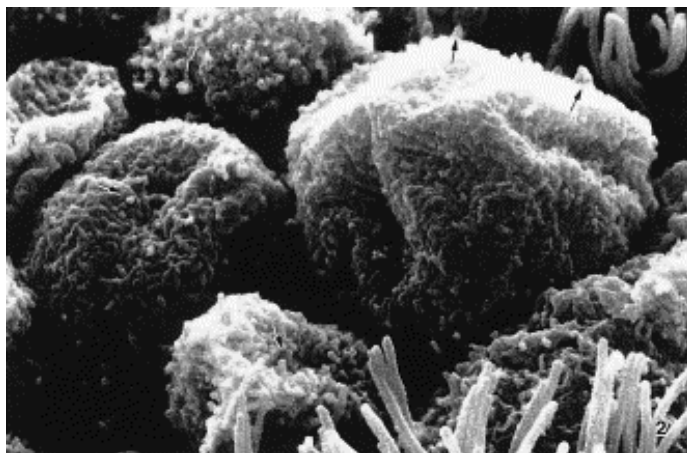
Apical protrusions occurring around the time of implantation have been identified in various species and notably, in mice and rats. The pinocytic function of these protrusions has been demonstrated by Enders and Nelson (Am J Anat 1973;138:277-300.) who showed that an electron dense tracer, ferritin, introduced in the cavity was taken up by the projections (pinopodes). This demonstrated unequivocally a pinocytic function for the projections and the term "pinopode" or drinking foot was coined. Since then, the original studies done in mice and rats have been extended in other species. In all of them, some form of pinopode like projections have been described in the mid-luteal phase. Yet morphological differences have been described between the structures observed in other species (including humans) and the pinopode of pycnocytic function originally described in mice and rats. In a recent debate, Murphy CR (Human Reprod 2000;15:2451-4.) suggested that the apical protrusions bulging at the surface of uterine epithelial cells are only somewhat similar to pinopodes described in mice except that in humans pynocytosis is part of their function.

Another criticism of the value of pinopodes as marker of endometrial receptivity comes from studies conducted in E2 and progesterone cycles designed for donor-egg IVF recipients. In these cycles a relative lag of morphological changes occurring in endometrial glands has long been described but without practical consequences on receptivity (PR in donor-egg IVF are excellent). In an unpublished study (Psychoyos, personal com.), a parallel delay in pinopode formation was observed in E2 and progesterone cycles thus, raising doubts about the value of pinopodes as marker of the receptivity window as previously proposed.

Our personal view is that apical protrusions (called pinopodes or otherwise) probably reflect the fine endometrial morphology as it flourishes in the luteal phase (possibly altered after PIDs?) but are not markers of the exact timing of endometrial receptivity (window of implantation) as previously claimed.

Develioglu OH et al. Fertil Steril 2000;74:767-70.

These authors proposed that apical cytoplasmic projections identified on H&E slides correspond to the pinopodes identifiable by SEM only. In this study, 38 endometrial samples were obtained on days 14-24 from oocyte donor undergoing COH. Part of the tissue specimen was prepared for SEM and the rest was fixed in 10% formalin for H&E evaluation. The luminal surface of the endometrium and the apical projections were scored as few, moderate or abundant. In the specimens, apical protrusions were qualified as few, moderate and abundant by "blinded" resders in 56%, 33% and 11%, respectively. Moreover, 35 and 58% of patients with few and moderate apical protrusions, respectively displayed pinopode expression by SEM and all those who showed abundance of these surface structures. In the subset of patients with positive pinopodes by SEM, H&E was not helpful in defining the stage of pinopode expression. The authors conclude that apical protrusions include structures other than true pinopodes.

6.2 Ultrasound

6.2.1 Endometrial thickness

> **Take home message**
> Endometrial thickness measured on UTZ imaging reflects the degree on endometrial priming by estrogen. An endometrial thickness "truly" <5mm warrants postponing ET and embryo freezing. Between 7 and 5 mm (double thickness measurement), the situation is a little more delicate. We have found that the presence and quantity of **cervical mucus** in the cervical canal is a handy accessory parameter for assessing the quality of estrogen priming, particularly when endometrial thickness is borderline.

Endometrial thickness increases in response to E2 but is not influenced by progesterone. With this patho-physiological mechanism in mind, we see that a too thin endometrium is likely to reflect insufficient priming by E2. Yet endometrial thickness is the net result of the compounding effects of E2 dose and duration of exposure. Also, in the absence of hyperplasia maximal thickness appears to be reached at menstrual cycle levels of E2 with no further development thereafter. Commonly, there are no differences in endometrial thickness between the menstrual cycle and COH (Epiney et al ASRM 2000). Today, there is a wealth of publication describing the ominous character of a too thin endometrium. By all means, endometrial thickness <5 mm (and probably <7 mm) is of poor prognosis.

Endometrial thickness has not been shown to vary significantly between the follicular and luteal phase of the menstrual cycle, indicating that the endometrial edema that characterizes of the luteal phase has no impact on UTZ imaging. Interestingly, endometrial thickness has been strongly correlated to uterine size in both the follicular and luteal phase.

There are also reports of negative impact of excessive endometrial thickness. Yet, recently this fear has been found unjustified by Casper's group. These investigators (Dietterich et al ASRM 2000, Abs P-7) studied 1245 IVF cycles. Endometrial thickness was >14 mm in 106 cases (8.5%). Clinical PR was 31.9% and 38.7% when endometrial thickness was 8-14 mm and >14 mm, respectively. Implantation rates were 14.4% and 19.5%, respectively. Spontaneous abortion rates were also similar at 15.1% and 14.6%, respectively. Hence, in contrast to prior studies (Weissman Fertil Steril 1999), there was no decrease in endometrial receptivity when the endometrial thickness is >14 mm.

When the endometrium is too thin, a possible mechanism explaining this finding must be sought. In E2 and progesterone cycles designed for donor-egg IVF or frozen embryo transfers, a too thin endometrium is found when the metabolism of oral E2 is accelerated through enzymatic induction. This is notably the case in smokers or in women chronically taking medications known to be inducers of P-450 enzymes (involved in steroid metabolism) such as notably, neuro-psychotropic drugs. Non-oral administration of E2 most often corrects the problems linked to enzymatic induction. Maximal uterine exposure to E2 is achieved with vaginal administration of E2.

In a recent study, Lesny et al. (Human Reprod 1999;14:1593-8) simultaneously analyzed endometrial thickness, junctional zone, myometrium and uterine diameter in 30 consecutive patients who conceived by ART and 30 consecutive patients who did not, during the same observation period. Measurements were made at baseline, on the 8th day of COH, day of hCG and day of ET. There were no differences in endometrial thickness between patients who got pregnant and those who did not. Myometrial and whole uterine thickness were larger on the day of hCG in patients getting pregnant. Thickness of the junctional zone decreased between baseline and day 8 of COH in both groups but, on day 8 the junctional zone was significantly thinner in patients who became pregnant. After the day of hCG, the thickness of the junctional zone re-increased. In patients who failed to become pregnant, the changes in junctional zone thickness were less pronounced and a return to initial thickness was less likely to occur. To this date, there are no clear patho-physiological explanation for the changes in junctional zone thickness observed throughout COH cycles and for the amplification of these changes in women who became pregnant through ART. According to Lesny et al., measurements made by UTZ imaging appeared equivalent to those made by the more complex MRI.
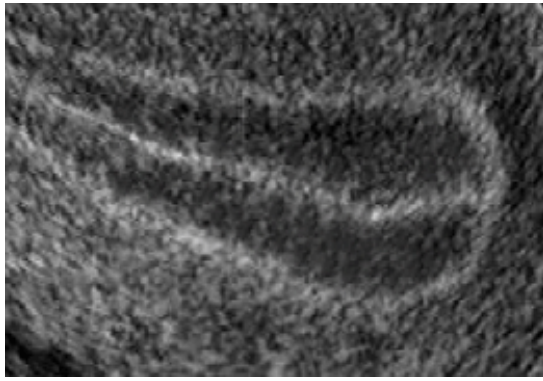
6.2.2 Endometrial echogenicity

> **Take home message**
> 1. Hyper-echogenic endometrium on the day of hCG is of poor prognosis and may warrant postponing ET. One should be aware however, of the frequent erroneous measurements of endometrial echogenicity in case of intermediate positioning of the uterus.
> 2. Unequal echogenicity evokes the possibility of polyps and/or endometrial hyperplasia and should warrant hysteroscopic evaluation.

Echogenicity is the property of a given tissue to reflect ultrasound beams. Low echogenicity tissues commonly appear "black" whereas high echogenicity tissues are "white". At the endometrium level, echogenicity is known to change throughout the menstrual cycle.

Typically, during the follicular phase endometrial echogenicity is low (endometrium appearing "black" between the endometrium-myometrium (2) and endometrial cavity (1) interphases. This corresponds to the *"3 lines"* aspect.

During the luteal phase, an increase in endometrial echogenicity is seen that gives the characteristic *"all-white"* aspect. Using the donor-egg E2 and progesterone cycle as model for studying the folliculo-luteal change in echogenicity, Grunfeld et al. (Obstet Gynecol 1991;78:200-4) showed that full hyper-echogenicity was achieved as early as on the 4th day of progesterone exposure. These (and other) authors clearly described that the increase in echogenicity induced by progesterone first occurs at the basis of the endometrium and progressively expands upward toward the surface. On the 2nd day of exposure to progesterone, the hyper-echogenic changes affect already the inner 50% of endometrial thickness (basal endometrium).
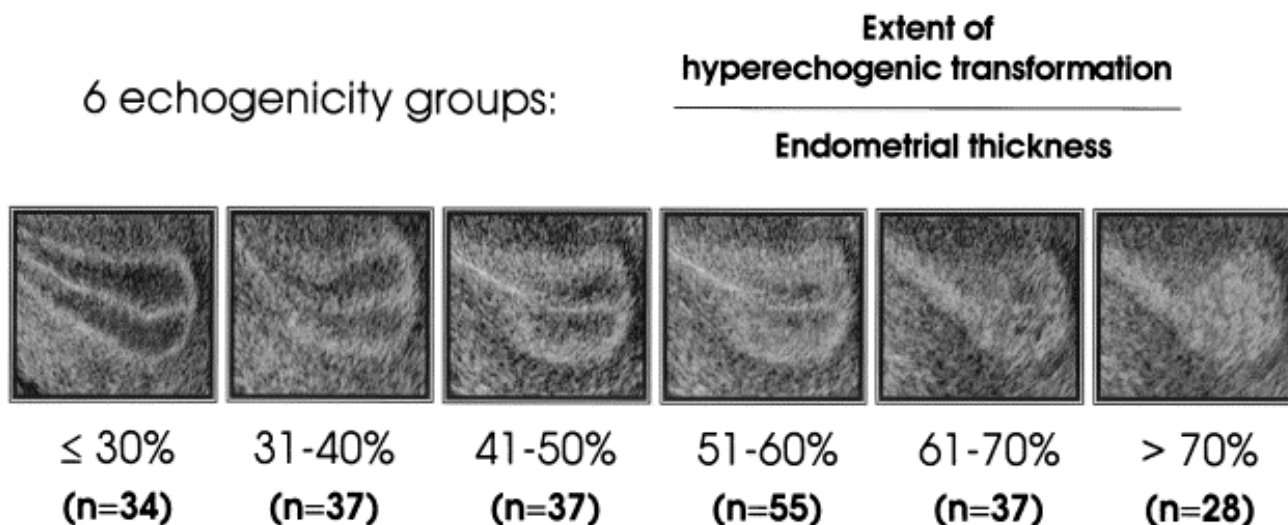
The patho-physiological mechanisms responsible for the changes in echogenicity induced by progesterone remain a topic for discussion. In their paper, Grunfeld et al. (Obstet Gynecol 1991;78:200-4) proposed that the increase in endometrial echogenicity is the early echographic manifestation of stromal edema culminating in histological specimens 2 to 4 days later. We rather believe that the hyper-echogenic changes result from coiling of endometrial glands induced by progesterone. According to this latter hypothesis, the sound waves are little reflected by straight glands running parallel to the beam during the follicular phase thus providing a hypo-echogenic appearance. After ovulation and exposure to progesterone, the sound beam will bounce on glands filled with mucus and profoundly coiled. This will provide the endometrium with its characteristic hyper-echogenic *"white"* appearance. In support of this latter mechanism is the concomitant occurrence of the changes in echogenicity and coiling of endometrial glands.
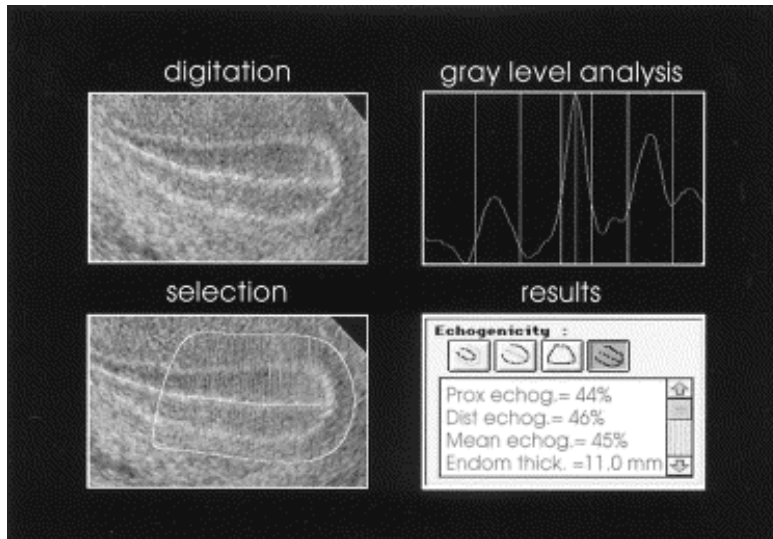
One common cause of falsely hyper-echogenic endometrium is an intermediate positioning of the uterus. Here, during the follicular phase the glands may be straight but run perpendicular to the sound beam (rather than parallel in both ante and retroverted uterus) thus, providing an enhanced echogenic appearance.

Numerous papers have attempted to correlate **endometrial echogenicity and IVF outcome**. In most papers, measurements consisted in subjective evaluations of endometrial echogenicity on ultrasounds performed between the day of hCG and day of ET. Findings were usually sorted in 3 typical aspects numbered differently according to various authors. For some, the changes were rated from I to III for endometria of increasing echogenicity. For others, the rating was in reversed order, or with letters A to C, in both orders. This cacophony of nomenclature used for describing the changes in endometrial echogenicity have not helped the clarification of the patho-physiological mechanisms involved. By and large however, the common denominator of all UTZ reports is that early finding of

increased echogenicity is of ominous nature for IVF outcome. Some authors have indicated that a frankly hyper-echogenic endometrium on the day of hCG administration was associated with no chances at all of pregnancy and should therefore, warrant embryo freezing.

6 echogenicity groups:

Extent of hyperechogenic transformation
_____
Endometrial thickness

≤ 30%	31-40%	41-50%	51-60%	61-70%	> 70%
(n=34)	(n=37)	(n=37)	(n=55)	(n=37)	(n=28)

We believe that hyper-echogenicity on the day of hCG is indeed of poor prognosis if the common causes of erroneous measurement have been excluded. The primary cause of erroneous finding of increased endometrial echogenicity is an intermediate positioning of the uterus

We attempted to quantify the changes in echogenicity observed in IVF and correlate our findings with IVF outcome (Fanchin et al. Fertil Steril 2000;74:274-81). Endometrial echogenicity was assessed as the extent of the hyper-echogenic transformation developing from the base of the endometrium upword over the total endometrial thickness. On the day of hCG, increasing endometrial echogenicity was associated with decreasing implantation and pregnancy rates.

The mechanism underlying the early hyperechogenic change of the endometrium (on the day of hCG) remains unclear. We documented that this is merely due to an early slight increase in palsma progesterone. On the contrary, we showed that in case of progesterone > 0.9 ng/mL on the day of hCG the hyperechogenic transformation is hastened with higher echogenicity on the day of ET but not on the day of hCG. Today, we speculate that androgens may play a role in the early hyperechogenic transformation of the endometrium on the day of hCG.

6.2.3 Doppler assessment of vascular resistance

Take home message.

1. Early reports provided great hopes that Doppler assessment of uterine blood flow could be predictive of endometrial receptivity. Many recent publications however, have concurred to infirm this with low over- resistance values reported in all the clinical groups and no difference anymore between pregnant and non-pregnant patients.

2. Recently, the assessment of sub-endometrial flow rendered possible with newer vaginal probes equipped with power Doppler has revived the original hope to master a non-invasive marker of endometrial receptivity as sub-endometrial blood flow appears higher in IVF patients who got pregnant.

Color and pulsed Doppler are refinements of UTZ imaging that have been incorporated to vaginal probes starting, some 10 years ago. With color Doppler the uterine arteries are easily identified on each side of the uterus. We showed that assessing uterine artery resistance with pulsed Doppler and calculation of the pulsatility index (PI) permits to identify profound changes induced by E2 and progesterone (de Ziegler et al. Fertil Steril 1991;55:775-9). In women deprived of ovarian function, PI is high before treatment. A profound decrease is observed as early as 2 weeks after exposure to E2, with maximal effects already observed with minimal (HRT) doses of E2. Furthermore, the addition of vaginal progesterone for 14 days did change the mean PI value.
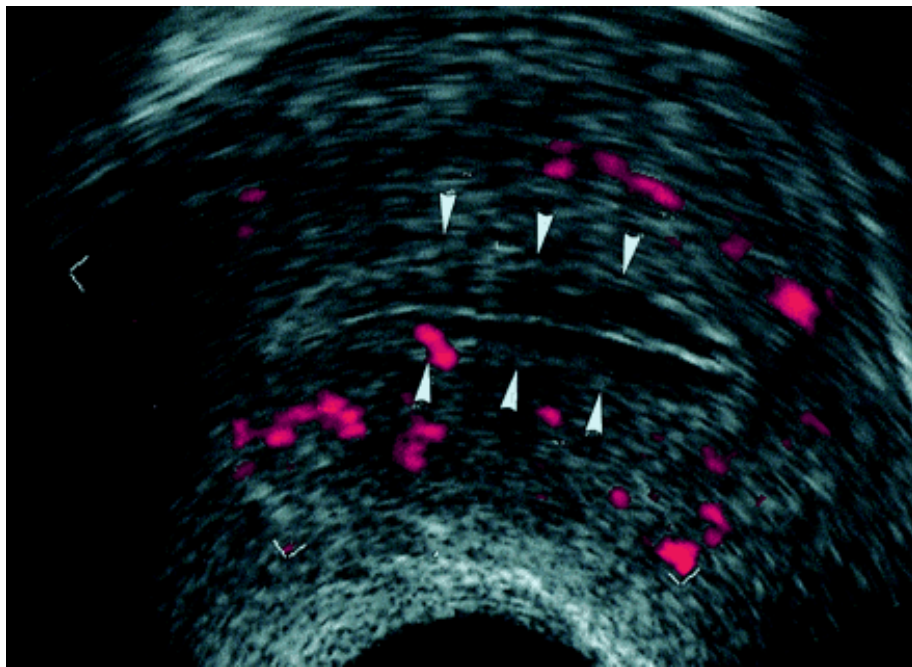
In IVF, Steer et al. have shown in a classic paper (Fertil Steril 1992;57:372-6) that despite high E2 levels a relatively large fraction of IVF patients presented PI > 3 with no pregnancies ensuing in this group. The rather peremptory findings of Steer et al. have been challenged however, as those findings could not be reproduced. Since then, the Doppler and uterine receptivity have remained a most debated topic. In this debate we see 2 primary lines of thoughts.
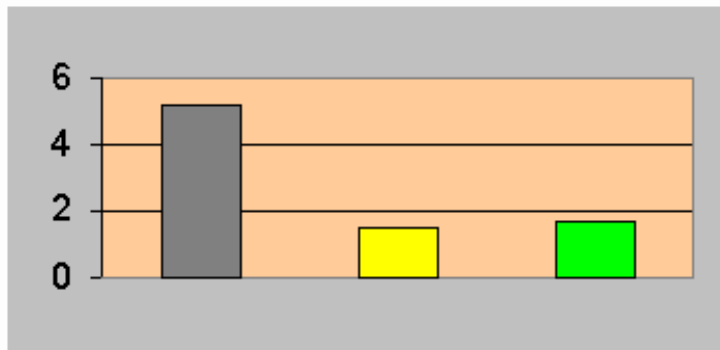
First, many recent papers have failed to confirm such a large difference (or even any difference) in Doppler PI values between pregnant and non-pregnant IVF women. One particularly well documented publication is by Yuval et al. (Human Reprod 1999;14:1067-71). These authors evaluated prospectively 156 IVF cycles. Patients were evaluated on the day of retrieval and ET. On the day of retrieval, PI was 0.997 and 0.994 in patients who conceived or did not, respectively. On the day of ET, these values were 1.096 and 1.104. The authors conclude that blood flow does not seem to correlate with pregnancy rate in IVF.

Doppler: BL and dav hCG
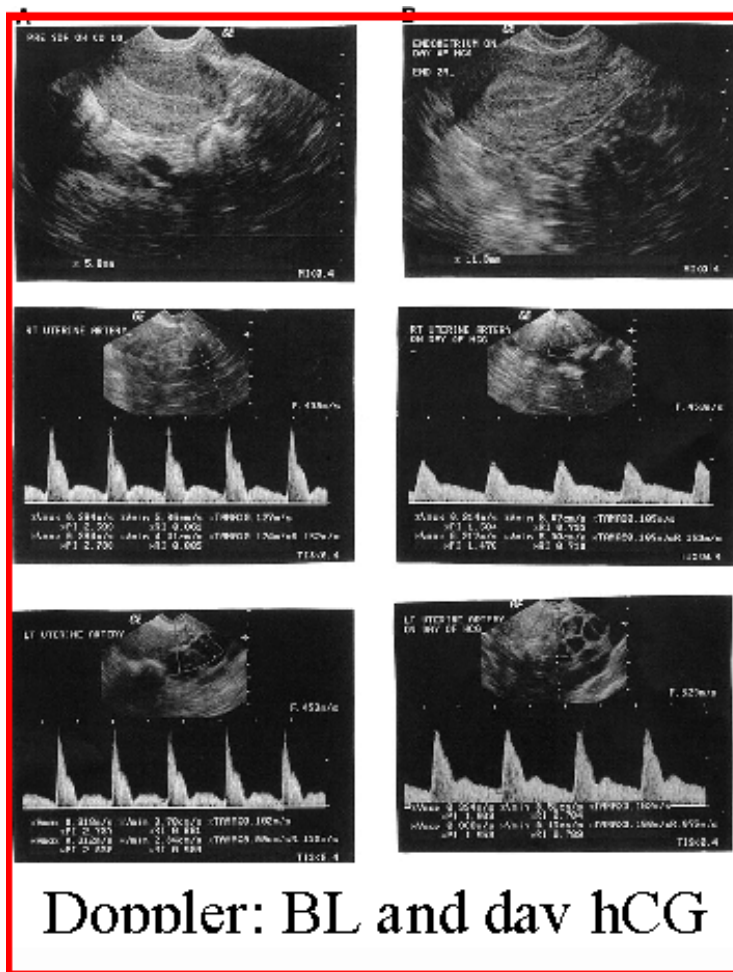
Schield et al. (Fertil Steril 2001:75:361-6) also failed to find differences in blood flow between patients becoming pregnant or not. Most authors do not conclude on the reason for the difference between their results and those of Steer for example.

We are puzzled however, by the markedly lower PI values reported in the recent publications as in that of Yuval et al., for example compared to the data of Steer. Yuval found that all patients groups had mean PI values of approximately 1 whereas, Steer et al. found values >3 in many women not becoming pregnant. We postulate that with more sophisticated equipment diastolic flow has been recorded in all patients resulting in lower PI values recorded in all. Hence, with the sensitive equipment available today, the differences reported by early Doppler studies are not found anymore.

Second, using more sophisticated techniques, recent papers however have found differences in **sub-endometrial** blood flow between patients becoming pregnant or not. Kupesic et al. (J Ultrasound Med 2001;20:152-34) report significantly lower resistance index (RI) and higher flow index measured by 3D histograms in sub-endometrial vessels in patients who became pregnant.

One possible measure available for improving uterine blood flow is the use of low dose aspirin. (see section 6.5).

6.2.4 *«3-D»* Assessment

> **Take home message**
> "3D" is a refinement of UTZ imaging. For studying the endometrium,"3D" permits to reconstruct the **frontal plan** of the endometrium, which facilitates the study of the inter-relationships between the endometrium and the surrounding myometrium. Yet no study described "3D" findings that are not really accessible by regular UTZ imaging. Hence, 3D assessment of the endometrium offers no practical advantages over thickness measurement.

"3-D" is a recent refinement of utrasound imaging and notably, of transvaginal ultrasound. Typically there are 2 types of "3-D" reconstruction available today.
1. built-in "3-D" reconstruction with automatic and calibrated sweeping. Here the probe sweeps through the area of interest at the command of a button. As a result, an electronic matrix is acquired through which new UTZ cuts can be conducted and various reconstructions such as notably, the frontal plane of the endometrium drawn.
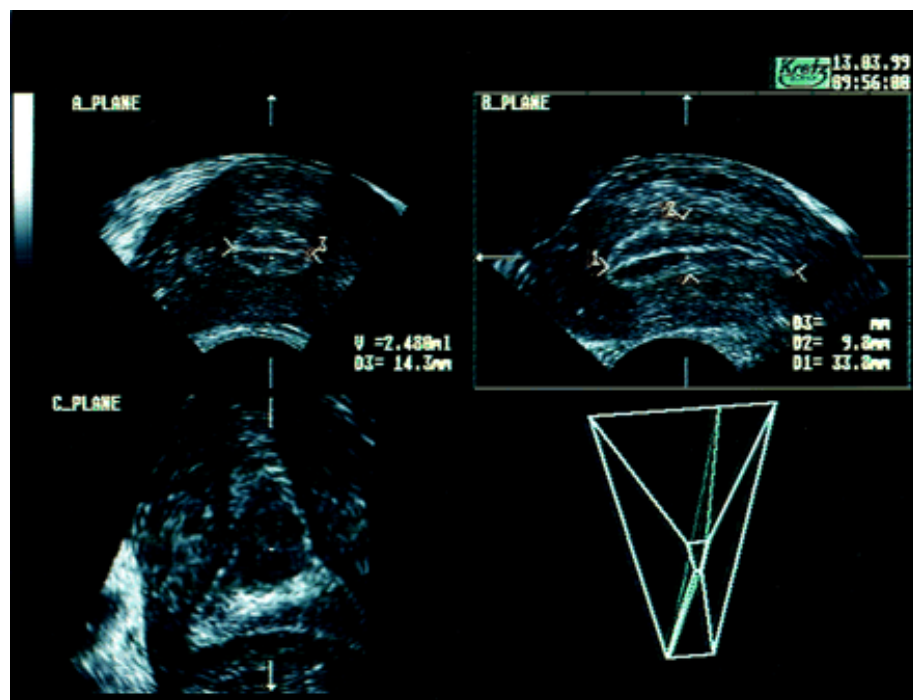
2. Off-line "3-D" reconstruction. Here a computer-assisted system acquires a sequence of images generated by the UTZ machine. The 3rd dimension is obtained by manually sweeping the probe through the area of interest. Yet, because sweeping is manual, the 3rd dimension (or Z-axis) is not calibrated. Some improvements have consisted in adding a sensor on the ultrasound probe that can detect the spatial displacement, which is later integrated in the image reconstruction in order to calibrate the 3rd dimension or z-axis of the electronic matrix.

With either technique, the most interesting aspect of *"3-D"* reconstruction in uterine imaging is the electronic reconstruction of the *"frontal plane"* of the uterus and endometrium. This permits to visualize the endometrium as it is depicted in illustrations of medical manuals which helps understanding its position relative to structures such as fibroids and/or polyps.

Several publications have looked at the potential advantages offered by *"3-D"* reconstruction for evaluating the endometrium in preparation for IVF.

Yaman C. et al. (Human Reprod 1999;14:2604) assessed the validity and reproducibility of 3D based endometrial volume measurement. Volume measurements were conducted prospectively in 57 consecutive IVF cases using either the full planar or 3-distance method. The authors conclude that both methods are valid.

Schield RL (Human Reprod 1999;14:1255-8.) conducted "3D" reconstruction of the endometrium in 49 IVF cases on the day of oocyte retrieval. Endometrial volume was 4.9 (2.2) and 5.8 (3.4) ml (SD) in 15/47 pregnant and 32/47 non-pregnant patients, respectively. The authors conclude that 3D endometrial volume estimation on the day of oocyte retrieval has no predictive value for conception in IVF cycles.

6.2.5 Enhanced contrast ultrasound (sonohysterography)

Take home message
Intra-uterine instillation of NaCl (negative) or positive contrast (Echovist or equivalent) enhances UTZ contrast and facilitates the diagnosis of polyps and/or sub-mucosal fibroids or other uterine anomalies such as endometrial synechiae. When OC pill is used prior to IVF, this represents an ideal time for an enhanced UTZ assessment because the normally thin endometrium helps visualizing intrauterine anomalies. Pre-IVF sonohysterography should be prescribed very liberally.

The idea to infuse a contrast enhancing solution in the uterine cavity came from visualizing the remarkable resolution with which CNS structures are seen in early pregnancy. As illustrated, in early pregnancy the amniotic fluid creates this contrast enhancement responsible for the spectacular quality of UTZ imaging.
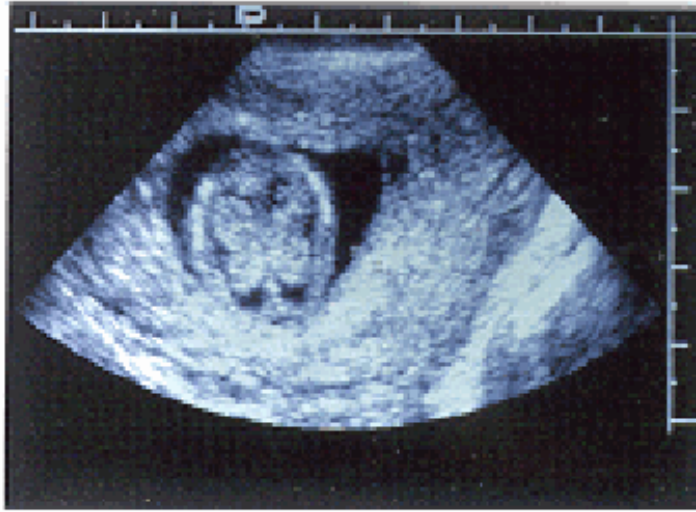
Early pregnancy:
AF serves as contrast
enhancer

In an effort to duplicate the conditions prevailing in early pregnancy, negative and positive contrast solution have been infused in the uterine cavity. NaCl, the most commonly use negative *("black")* contrast solution, is ideal for visualizing the uterine cavity. The only draw back is the need to constantly infuse the NaCl solution in the cavity during the UTZ procedure because the liquid rapidly flows out. This makes sono-hysterography a rather cumbersome "3-hand" procedure.
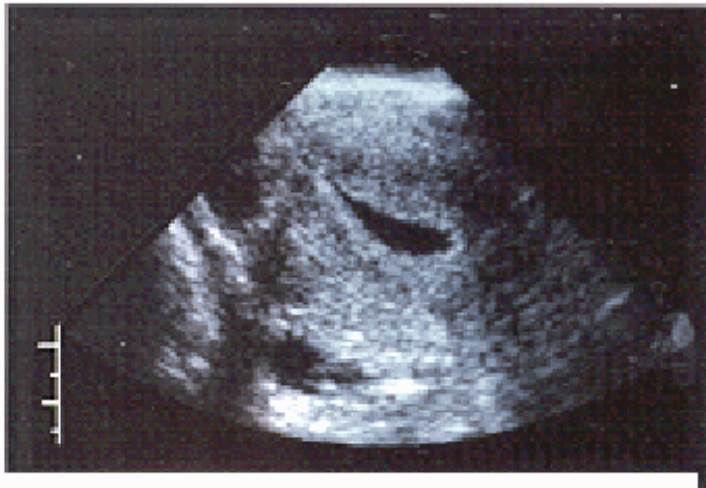
Negative contrast

Positive contrast solutions appearing *"white"* on UTZ were primarily developed for cardiac sonograms. Their usage in gynecology has been attempted with the intent of obtaining information on tubal status and possibly replacing HSGs. While the proximal segment of the Fallopian tubes can be easily seen, the UTZ images provide far less information on tubal status than HSGs. Understandably therefore, the interest for positive contrast hystero-sonograms has constantly declined over the past few years. For visualization of the uterine cavity, positive contrast hystero-sonography is inferior to negative contrast. This renders the use of expensive positive contrast solutions not justified when better images can easily (and cheaply) be obtained with negative contrast (NaCl) hystero-sonograms.

Diaferia D. et al. (ASRM 2000 O-083) prospectively studied 98 consecutive IVF cases. These authors compared regular UTZ and NaCl sonohysterography to hysteroscopy for diagnosing intrauterine pathology. UTZ and hysteroscopy were concordant in 76.5% of the cases, whereas concordance increased to 93% for sonohysterography.
The authors conclude that sonohysterography will become the primary diagnostic tool for detecting intra-uterine pathologies and hysteroscopy remaining indicated solely for the difficult cases. Today, the limiting factor for sonohysterography is the cumbersome character of this "3-hand" procedure.

Senoh D et al. Human Rprod 1999;14:2600

These authors used a very high frequency (20 MHz)-resolution **intrauterine** probe in conjunction with saline infusion. The miniature intrauterine probe (2.4 mm) allowed to perform the examination on outpatient basis, not requiring anesthesia nor cervical dilation. All women had at least 2 years of infertility and were studied during the proliferative, and early or mid secretory phases. By comparison to regular vaginal ultrasounds, intrauterine sonography was found to provide improved vision of the endometrial texture in both the proliferative and secretory phases of the menstrual cycle. Because of the very-high-frequency probes used, the depth of penetration of the UTZ beam was fairly limited, making examination of large endometrial lesions impossible.
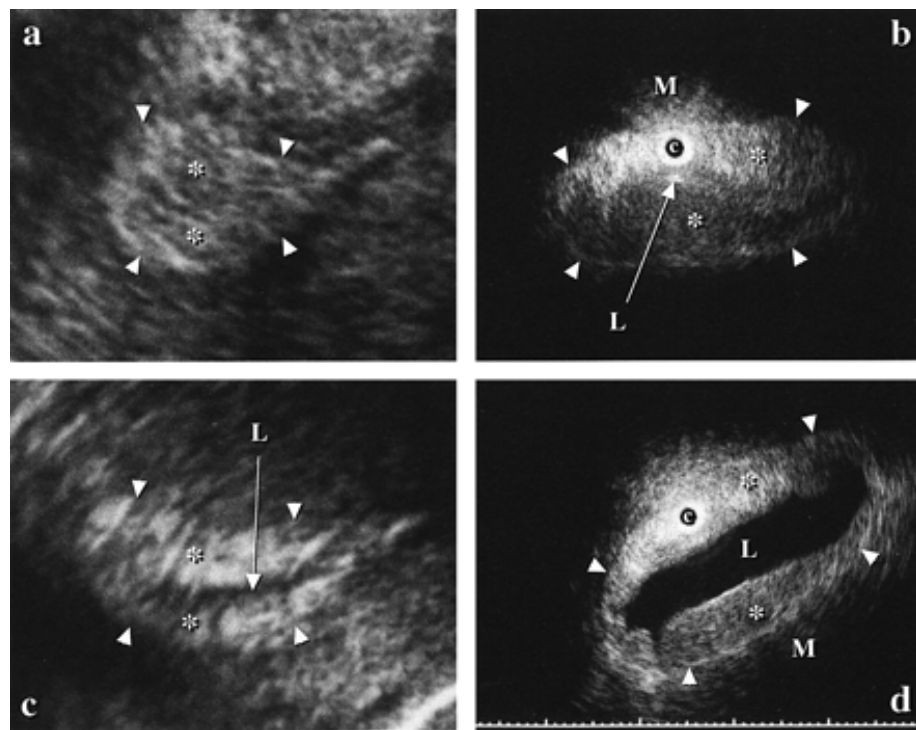
6.2.6 Fluid in uterine cavity

> **Take home message**
> The abnormal presence of fluid in the uterine cavity is sometimes observed at times other than during menses (when it is normal), leaving the clinician with a puzzling dilemma.
> When fluid is present in the uterine cavity throughout the menstrual cycle, we recommend:
>
> 1. To look for a possible hydrosalpynx responsible of constantly dripping fluid into the cavity. In these cases, the fluid found in the cavity is aqueous.
> 2. If the fluid is viscous, perform a D&C (to rule out a mucoid tumor) and initiate a 10-20 day course of antibiotic therapy (empiric approach).

Fluid in the uterine cavity is a common finding at the time of menses when menstrual blood still present in the cavity can be easily visualized. At other times of the menstrual cycle however, fluid should not be seen in the uterine cavity. Yet, this is sporadically found in rare patients in whom it can be a persistent (and very puzzling) finding throughout the menstrual cycle.

Attempts to aspirate the intrauterine fluid will determine its consistence. If the fluid found in the uterine cavity is watery, it may result from constant leakage from dilated hydrosalpinges. The hydrosalpynx should be identified (best during the late follicular phase) and surgically removed (or tightened).

Levi AJ et al. ASRM 2000 O-036
The purpose of this study was to assess a possible relationship between the appearance of endometrial fluid during ART cycles and clinical outcome. 843 cycles were analyzed. If present, intra-cavitary fluid was aspirated at the time of oocyte retrieval. Presence of hydrosalpinges was recorded. Intra-cavitary fluid was found in 6.8% of cases. PR was 26.3% in these patients compared to 42.4% in controls.

At other times, the fluid is viscous. When mucoid fluid is found in the uterine cavity, the situation is a little more delicate. The diagnosis of autoimmune disease such as Sjogren syndrome must be contemplated (see section 6.5.2) and specific antibody testing undertaken. Possibly a0 specific treatment may need to be contemplated.

In all cases of mucoid fluid in the uterine cavity, a D&C must be p0erformed to rule out a mucoid tumor. If histology is negative, we and others (Laufer, personal communication) have empirically prescribed 3 weeks of antibiotics (broad spectrum) with reasonable success.

6.3 Hysterosalpingography

> **Take home message**
> HSG remains the primary diagnostic tool for tubal pathologies and very useful for assessing the intracavitary extension of submucosal fibroids. 2 new "improvements are worth mentioning:
>
> 1. **For diagnosis of intra-uterine pathologies such as polyps and fibroids a double-contrast technique can be helpful.**
> 2. **We have shown that gentle IUI-like deposit of 0.5-1 CC of contrast medium with an embryo transfer catheter allows to visualize the tubes in > 50% of cases after they become opacified by spontaneous retrograde transport.**

De Ziegler et al. ASRM 2001. We have used 0.5-1 cc of X-ray contrast medium for performing "mock IUIs" on the day of LH surge and assess uterine contractility. Over 100 patients have now been studied. In a little over 50% of women with open tubes, the contrast medium is promptly (minutes) "expelled" toward both tubes and bilateral spillage is seen. In the remaining cases the contrast medium comes out of the uterus toward the vagina. In <!0% of cases, contrast medium remains in the uterine cavity for over 10 minutes. Mock IUI is informative of uterine contractility and may predict the likelihood of retrograde sperm transport and for some women offer a painless option for visualizing the Fallopian tubes.

Calderon I et al. (ASRM 2000 O-034) describe an interesting new double-contrast HSG for evaluating the uterine cavity: These authors investigated the possibility of applying double contrast techniques commonly used in GI radiology for enhancing the quality of HSG imaging. Air was injected after the contrast medium was flushed away, leaving a fine layer of contrast medium coating the uterine cavity. The authors conclude at the superiority of double contrast HSGs for visualizing intrauterine structures.

Having attempted the double contrast HSG technique ourselves, we observed occasional serious cramping when air is infused through a contrast filled uterine cavity using a balloon catheter. In IUI-like_procedures (using an embryo transfer catheter), after the contrast is expelled in the pelvic cavity or back in the vagina, insuflation of a few CCs of air is painless and can be quite useful for a very fine delineation of the cavity's contour.
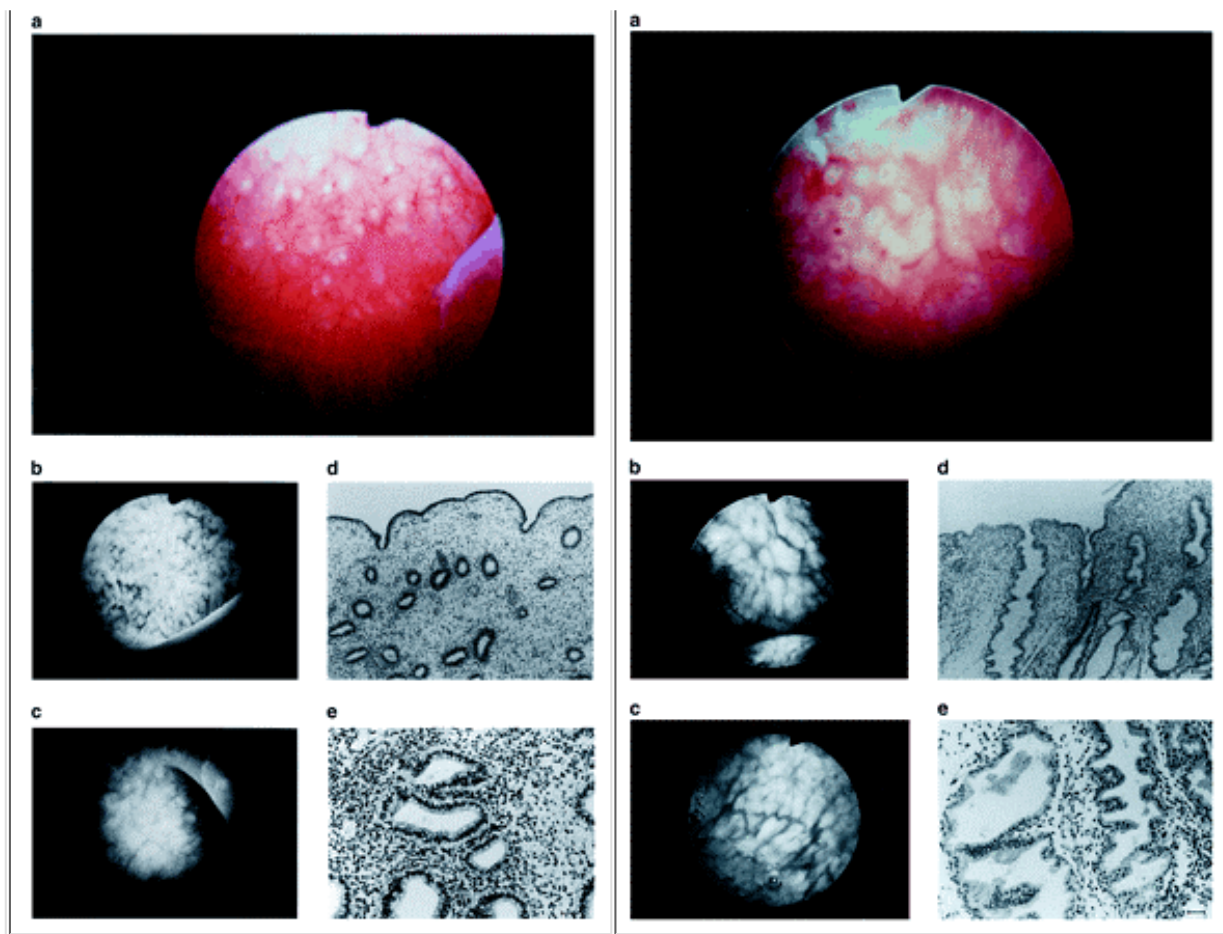
6.4 Hysteroscopy

Take home message

Hysteroscopy has been perfected and should be either part of all pre-IVF workups or used only in cases selected by hystero sonography.

Recent work suggest that mid-secretory hysteroscopy carries a predictive value for implantation and early pregnancy outcome.

Masamoto H et al. Human Rerod: 2000;15:212-8

A total of 172 patients who underwent hysteroscopy assessment of the endometrium and then became pregnant were analyzed retrospectively to explore the relationship between endoscopic findings and pregnancy outcome after implantation. Hysteroscopy was performed 7-9 days after ovulation. The procedure was carried out under local anesthesia (para-cervical block). A rigid hysteroscope with a 4.5 mm outer diameter and a 30º oblique vision was used and the cavity was expanded by irrigation of a 5% glucose solution.

Mid secretary endometrium was defined as "good" when ring-type openings showed maximum glandular secretion and well-developed varicose-like vessels. Mid secretary endometrium was defined as "poor" when glandular openings were characterized as "dot" (no secretory) and/or ponctuate type (early secretory activity) and vasculature was described as "fine". Previous publications suggested that IVF outcome was significantly higher when mid-secretory endometrium was qualified as "good". **Results:** Of 160 patients retained for analysis, 38.8% were classified as having a good mid-secretory endometrium and 61.3% as poor. The mean age of the "poor" group was almost identical to that of the "good" group. The frequency of patients with early abortion was significantly higher in the "poor" group at 25.5% than in the "good" group (8.1%, P=0.0059). There were no difference in either the frequency of patients with infertility factors or in the distribution pattern of infertility factors between the 2 groups. Histologic analysis of endometrial biopsies was "in phase" in 100% and 65% of "good" and "poor" hysteroscopy groups, respectively.

Comments: This publication appears important in that it sheds new lights on the interest of hysteroscopy in infertility workups. Not only hysteroscopy serves to rule out the presence of intra uterine anomalies such as polyps and sub-mucosal fibroids but it also serves to assess the quality of mid-secretory endometrium with a apparent predictive value for IVF and early pregnancy outcome. One important question remains. Are the differences seen between the "good" and the "poor" mid secretory endometrium the result of an intrinsic problem of the endometrium or the result of the ovulation quality (and correctable by exogenous gonadotropins). The literature must be followed for further data on this topic.

6.5 Biology

> **Take home message**
> Alterations of certain coagulation factors (i.e., Factor V Leiden mutation and activated protein C) and evidence of autoimmune processes may be responsible of sub-optimal endometrial receptivity and/or repeated miscarriages.

6.5.1 Alteration of coagulation factors and endometrial quality

A key component in the anticoagulation pathway is protein C, which when activated inhibits the action of coagulation factors V and VIII. Resistance to the anticoagulation properties of activated protein C (or, ACP resistance) may either be congenital or acquired. Congenital ACP resistance is almost exclusively du to a single point mutation at nucleotide position 1691 in the factor V gene (factor V Leiden). Mutated factor V is resistant to inactivation by ACP, resulting in increased thorombin generation. ACP resistance is associated with lupus anticoagulant and high concentration of factor VIII. Both factor V Leiden (congenital) and acquired ACP resistance are risk factors for systemic venous thrombosis.

Rai R. et al. Factor V Leiden and recurrent miscarriages (Human Reprod 2001;16:961-5)

These investigators studied the prevalence of factor V Leiden and acquired ACP resistance in 1111 consecutive Caucasian women with a history of either recurrent early miscarriage or at least one late miscarriage or in controls with previous history of adverse pregnancy outcome. Acquired AC resistance was significantly more common among both women with early (8.8%) or late miscarriages (8.7%) compared to controls (3.3%). In contrast, the incidence of factor V Leiden was similar in all groups. Hence, acquired but not congenital ACP resistance is associated with both early and late miscarriage. The role of ACP resistance in implantation failure remains to be determined.

6.5.2 <u>Auto-immune condition</u>

There have been numerous publication pointing at an elevated prevalence of auto-antibodies in women in whom IVF-ET had repeatedly failed. Hasegawa et al. (FS, 1998;70:1044-8.) looked at the effects of prednisolone and low dose aspirin on IVF outcome in women with evidence of autoimmune condition. These authors studied the incidence of autoantibodies in 307 women undergoing 607 consecutive IVF cycles. ANA titer of >1:160 was considered positive. Antiphospholipins (anticardiolipins) were positive (APA +) when measured at > 3SD above the mean value of 80 normal people. In ANA+/APA+ (n=18 cycles), PR (implantation rate) was 11.1%(5%). In 15 similar cycles but after patients received prednisolone and LDA, PR (implantation rate) was 33.3%(11.9%). Treatment with prednisolone 10 mg/day and aspirin 80 mg/day was started on the first day of hMG treatment. Patients however were not randomized between the treatment and no-treatment groups.

Rubistein M et al. Fertil Steril 1999;71:825-9)

These authors conducted a prospective, randomized, double blind placebo-controlled assay for evaluating the effect of systematic low dose aspirin treatment in 298 COH-IVF cycles, starting on day 21 of the preceding cycle. Mean PI of left and right uterine and ovarian arteries were calculated. In the treatment group (aspirin), uterine artery PI decreased from 1.98 at baseline to 1.22 on the day of hCG and from 2.1 to 1.18 in the ovarian artery. In the placebo group no significant decrease was seen in either arterial system. Implantation ad clinical pregnancy rates were 17.8% and 45%, and 9.2% and 28% in the aspirin and placebo groups, respectively. The authors conclude that low dose aspirin treatment significantly improves uterine and ovarian blood flow and implantation and pregnancy rates in women undergoing IVF-ET.

7 Uterine contractility and receptivity

Take home message:

There are 3 patterns of uterine contractility in the menstrual cycle:

1. During the end follicular phase UC frequency is elevated (5/min) with primarily retrograde contractions (sperm transport). UC are nevr perceived by women.

2. During the luteal phase, there normally is a profound decrease in UC frequency down to approximately 2.5 UC/min, as early as on day 18. This decrease in UC frequency appears to be hampered in IVF where the high E2 levels induce a relative resistance to the utero relaxing properties of progesterone. Early progesterone replacement (starting on day of retrieval) may correct this problem. Alternatively, delaying ET until blastocyst development can also be advantageous in IVF cases with high UC activity.

3. At the end of the luteal phase, there is a sharp increase in UC amplitude and resting tone, in response to progesterone withdrawal, just before and at the time of menses. These UC are perceived and even sometimes, painful.

In IVF, increased UC frequency at the time of ET carries a poor prognosis for embryo implantation. Early vaginal progesterone may be beneficial. An alternative measure is to delay ET until blastocyst development.

7.1 Background data

Numerous studies have reported on contractile activity of the non-pregnant uterus at various phases of the menstrual cycle and in IVF. The regain of interest for contractility of the non-pregnant uterus stems from the possibility of directly visualizing of the contractile activity of the uterus on ultrasound scans that provided high resolution trans-vaginal probes.

Late follicular phase	Retrograde (sperm transport) Approx. 5/min Sub-endometrial layers, not perceived Retrograde (sperm transport) Approx. 5/min Sub-endometrial layers, not perceived
Mid luteal phase	Uterine quiescence Embryo placement Pattern altered in IVF

| Luteo-follicular transition	Antegrade, all layers involved perceived, sometimes painful

Lyons E.A. et al. Fertil Steril 1991;55:771

Uterine contractions increase in frequency, amplitude and % with retrograde displacement throught the follicular and preovulatory phases. The pattern was essentially reversed during the luteal phase. The authors concluded that there is a definite identifiable pattern of sub-endometrial myometrial contractility that varies with the phases of the menstrual cycle and recurs with a similar fashion from cycle to cycle.

7.2 Our contribution

7.2.1 In E2 and progesterone cycles

During the follicular phase uterine contractions (UC) are mainly retrograde with propagation from the cervical to the uterine fundus. UC frequency increases throughout the follicular phase in response to the rising E2 levels. Max UC frequency is approximately 5/min. Higher E2 levels do not increase UC frequency.
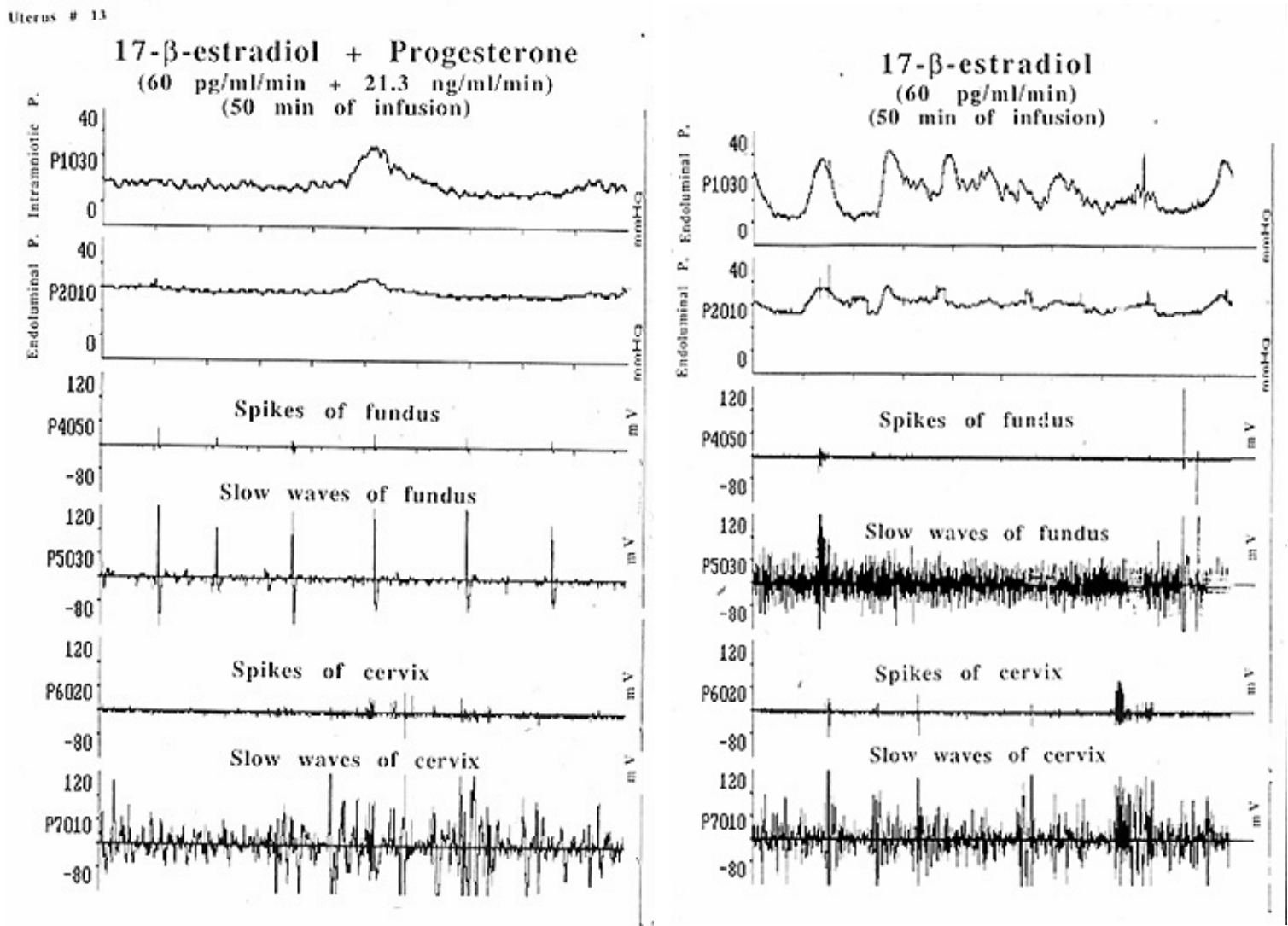
Progesterone induces a prompt decrease in uterine contraction frequency with the apparition of bi-directional contractions originating simultaneously from both uterine ends and meeting in the mid uterine area. UC frequency during mid mock luteal phase is approximately 2.5/min.

7.2.2 In the menstrual cycle

In the menstrual cycle, the patterns of UC contraction observed during the follicular and luteal phases are similar to the observations made in the E2 alone and E2 and progesterone phases of mock cycles designed for donor egg IVF, respectively.

### 7.2.3 In IVF, increased contractility at the time of ET is deleterious

At the time of hCG administration UC frequency is not different in IVF from findings made in the late follicular phase of the menstrual cycle, indicating that higher E2 levels do not further increase UC frequency.

In IVF cycles however, the decrease in UC frequency normally observed after ovulation is significantly damped, leading to higher UC frequency at the time of ET than seen in the menstrual cycle, with deleterious consequences on IVF outcome.

High UC frequency at the time of ET can be avoided by either advancing progesterone administration or delaying ET until blastocyst formation (Fanchin et al., Fertil Steril).

7.2.4 Comparison of menstrual cycle Vs. IVF: Relative resistance to progesterone

In a prospective trial we compared (Epiney M et al., ASRM 2000) the early luteal phase changes in UC pattern observed in the menstrual cycle preceding IVF and in the IVF cycle itself. The results speak for a resistance to the utero-relaxing properties of progesterone probably linked to the pharmacologically high levels of E2 in IVF. In the menstrual cycle end follicular UC frequency was similar in the menstrual and IVF cycles. Hence, the high E2 levels of IVF did not further enhance uterine contractility or the effects exerted by E2 in the menstrual cycle. In the luteal phase, the patterns were different however. In the menstrual cycle UC frequency promptly decreased and was negligible on day 18 (day 14 = day of LH surge). In the same patient undergoing IVF, the "luteal" decrease in UC frequency was much more sluggish. On the 4th day post hCG (equivalent to day 18), UC frequency was still high (at end-follicular phase levels). UC frequency decreased later, notably at the time of blastocyst transfers.

UC on day of ET after late (top) and early vag. P4 treatment (bottom).
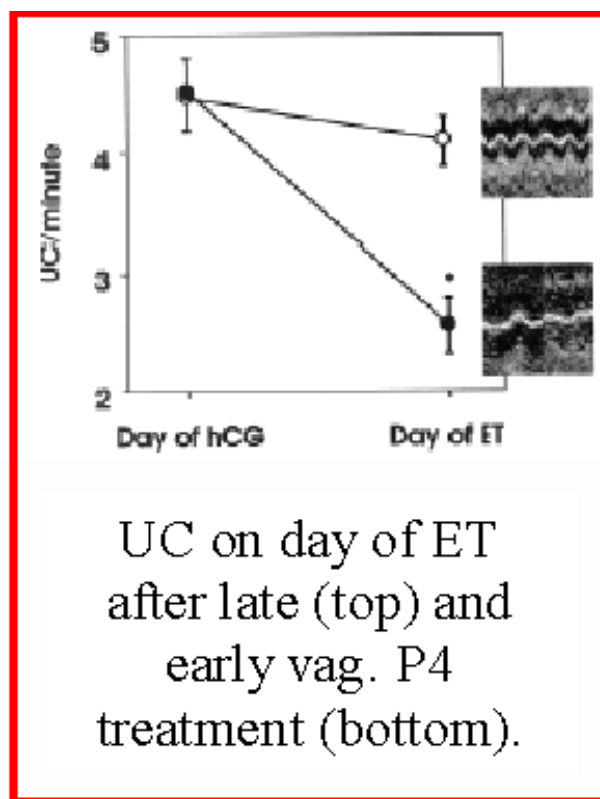
Hence, all indicates that in IVF the high levels of E2 do not modify end follicular phase UCs but induce a relative resistance to the utero-relaxant properties of progesterone. As discussed later, when excessive UC appears to be problematic in IVF, 2 options exit: (i) delaying ET to blastocyst time when UCs have usually abased. (ii) Starting vaginal progesterone early as suggested by data recently published by Fanchin.

7.3 Methodological consideration

### 7.3.1 Ultrasound based techniques: ideal for UC frequency measurement

High-resolution ultrasound probes permit real-time visualization of contractile activity of the non-pregnant uterus. Yet despite great hopes placed in this technique, only measurements of UC frequency have been validated.
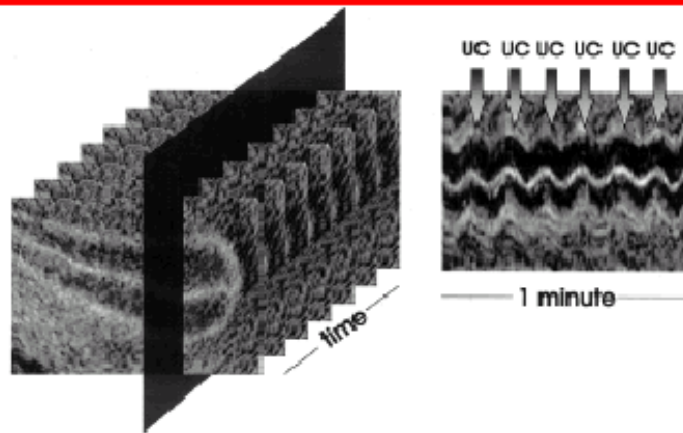
Serial acquisition of 2D images overtime allows reconstructing TM graphs where the Z-axis is time

Attempts at assessing UC amplitude and direction have been inconclusive to date. Hence, UTZ based studies concluding on these parameters must be looked at circumspectly. In IVF, UC frequency appears to be the most relevant parameter, making the non-invasive UTZ based methods ideal at this time.

UTZ based measurements have been validated against the reference, IUP changes. Results showed evidence of good correlation between UTZ and IUP. Interestingly, despite our hopes and beliefs in the "3D" derived computerized approach, assessment of UC frequency on fast play of image sequences or 3D derived measurements were equivalent with even, a lesser dispersion of measurements inwith the fast play method.
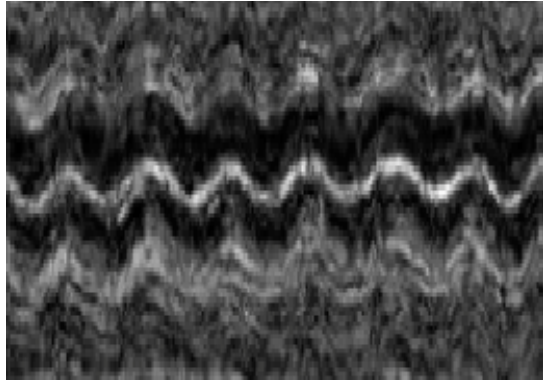

7.3.2 <u>IUP: Invasive but measures UC amplitude and resting tone</u>

Despite the great hopes placed in UTZ measurements of UC, IUP remains today the sole tool for measuring UC amplitude and resting tone, the 2 primary parameters of dysmenorrhea. Mid-cycle contractility is never perceived by patients despite representing the time of the cycle when UC frequency is the highest. In contrast, at the time of menses the increase in contractility brought by progesterone withdrawal primarily affects UC amplitude and resting tone with little effects on UC frequency. Hence, the 2 parameters pertinent to contractility at the time of menses are not measurable by UTZ imaging.

It was originally hoped that visualization of uterine contractility on UTZ would permit to delineate the various layers of myometrium involved in the contractile process. This would have possibly allowed to single out the characteristics of uterine dyskinesia (painful, ineffective uterine contractions) that are often described in women suffering from endometriosis. Unfortunately, UTZ data have not been of much help so far for understanding the characteristics of uterine dyskinesia.

7.3.3 <u>Measurement of UC direction: must follow the displacement of intrauterine markers</u>

Unfortunately, despite our hopes for this fascinating approach, we have to admit that to this date UTZ based assessments of the direction of contractility have remained *"non-validated impressions"* from which it is impossible to draw any meaningful conclusion.
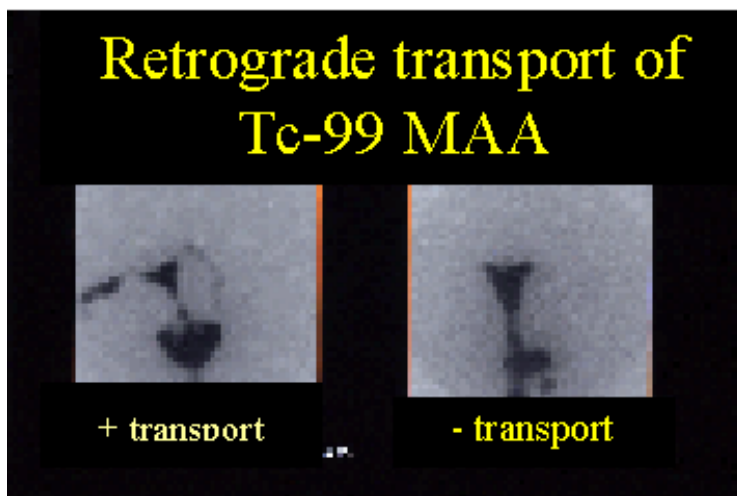
IUP recording from multiple tip catheters can provide some indication on the direction of displacement of the contractile wave, but this approach is cumbersome and does not inform on the actual displacement of uterine content. Hence, meaningful approaches for studying the direction of uterine contractility must revert to studying the actual displacement of a marker placed in the uterine cavity and followed along.

"Mock IUI" showing retrograde transport of contrast medium into the Fallopian tubes
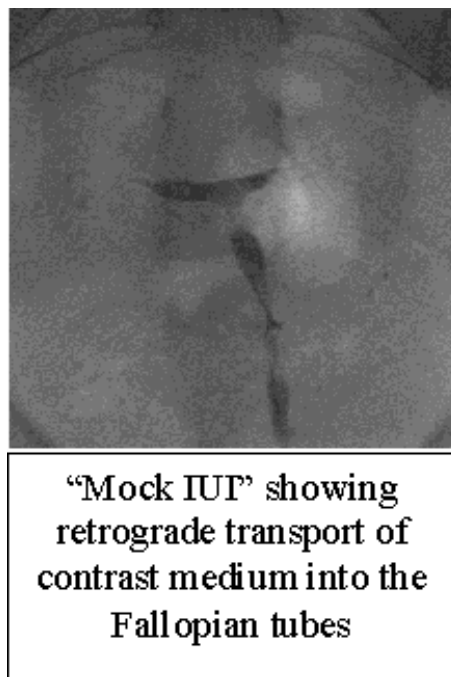
A team led by Leyendecker and Wildt has studied the displacement of Tc-99 macro albumin aggregates placed in the uterine cavity and followed along with a gamma counter. With this technique they studied the displacement of Tc-99 MAA throughout the follicular phase and identified problems in retrograde transport prevailing at this stage of the menstrual cycle particularly, in women suffering from endometriosis.

Other markers used for studying the displacement of uterine content are radio-opaque contrast media placed in the uterine cavity and followed along with x-rays. Using this and other approaches with various forms of beads, early studies have concluded that a sizable fraction of embryos placed in the uterine cavity at the time of ET were found either in the tubes or worst, in the cervical canal or in the vagina within minutes of ETs. The original interpretation for these observations (embryos found in the cervix and vagina minutes after ET) was that they were held attached to the catheter by surface tension when it was pulled out. Today, we would rather postulate that antegrade ("expelling") uterine contractility is the primary factor responsible the rapid expulsion of a fraction of the embryos transferred.

8 Intercourse and endometrial receptivity

> **Take home message**
> Intercourse results in the intra-vaginal delivery of semen PGs and other factors that are transported preferentially to the uterus. This represents the primary "raison d'être" of the "first uterine pass effect" discovered while studying the vaginal administration of progesterone (and E2). Seminal factors may have beneficial effects on the endometrium and embryo.

Because animal data have pointed at a facilitating role of semen on embryo implantation Tremellen KP et al. (human Reprod 2000;15:2653-8) looked if intercourse had an impact on IVF outcome. These authors observed a trend for better pregnancy rates in women who had intercourse within 2 days of ET. The authors speculate that immuno-active compounds such as TGFb and prostaglandin E, both present in high concentration in human semen, may be responsible for the effect. In mice, exposure of the uterine epithelium to seminal TGFb induces synthesis of pro-inflamatory cytokines including granulocyte-macrophage factors reported to accelerate pre-implantation embryo cleavage and hatching in both mouse and human embryos.

In the early days of IVF, Sher G. was already advocating the value of "inseminating" either a fresh ejaculate or the supernatant of the sperm preparation just after the oocyte retrieval. The literature should be followed for this interesting and potentially important topic.

9 Practical measure to optimize endometrial receptivity

9.1 Routine assessment of the endometrium before IVF

We believe that the uterine cavity should be routinely assessed before IVF. This can be achieved by routine hysteroscopy or, better we think, by hystero-sonography and hysteroscopy in dubious cases. If temporary OC treatment is prescribed before IVF cycles, the time on OC pill is ideal for uterine investigations (atrophic endometrium).

The literature should be followed for the value of mid-luteal hysteroscopy as predictor of IVF and early pregnancy outcome. In case of abnormal findings, attempts should undertaken to correct the hysteroscopic appearance by various hormonal supplementation (vaginal E2, temporary OC pill use, dexamethasone, etc.) and/or treatments (aspirin, vasodilators such as NO donors, etc.).

9.2 Minimize endometrial effects of androgens

9.2.1 OC pill pretreatment

Temporary use of OC pill has been reported to decrease ovarian androgens and may have beneficial effects on oocyte and/or endometrial quality.

9.2.2 Minimize the amount of FSH used in late stimulation

The increase in plasma testosterone and A4 is directly proportional to the FSH stimulation reaching the ovary and the ovarian responsiveness (sensitivity). Hence, reducing FSH exposure toward the end of COH may be beneficial in women more prone to increase the androgens (PCOD like, past history of implantation failure). Some have even advocated the value of coasting (originally solely conceived for avoiding OHSS).

9.2.3 Use of dexamethasone

Dexamethasone induces a complete blockade androgen production by the adrenals. This does not diminish the absolute increase in testosterone and A4 induced by COH but reduces the overall androgen levels achieved at the end of COH (by subtracting the adrenal component). While dexamethasone has been primarily prescribed for suppressing the immune system, it is well possible that the beneficial effects reported by numerous studies results in part from lowering androgen levels. A study of the effects of dexamethasone pretreatment in women more prone to increase their androgen levels in response to COH (PCOD/PCOD like) is necessary.

9.3 Fluid in the endometrium

Two possibilities:

> Aqueous fluid: look for hydrosalpynx
>
> Mucoid fluid: perform a D&C to rule out a mucoid tumor and propose a cours of broad spectrum antibiotics (doxycyclin).

9.4 The "too thin" endometrium

Determine the cause. In E2 and progesterone cycles, use transdermal E2 if a cause of accelerated hepatic metabolism can be identified (smoking, meds, etc.). Consider the possibility of using vaginal E2 (oral Estrace tablets, 2mg at night or BID).

9.5 Contractility

Use of utero-relaxants is currently being studied. Betamimetics (terbutalin) oral tablets can be administered vaginally at the dose of 2.5 mg once a day to TID without side effects (tachycardia, tremor) because of the local delivery characteristics.

Alternate utero relaxants are NO donors. Tri and tetra nitrates are directly effective and therefore, constitute reasonable options. Recently, the use of nitroprusside gel (0.5 g of 1%gel) has been reported in obstetrics (for cervical ripening) without encountering side effects (Facchinetti et al. Human Reprod 2000 ;15 :2224-7). This work speaks for the probable safety of this utero relaxant.

In case of poor implanters:

- Assess embryo quality through culturing to the blastocyst stage.
- Conduct pre IVF hysteroscopy and/or hysterosonography after hormonal preparation.
- Limit the magnitude of COH stimulation and possibly, consider coasting..
- Suppress adrenal androgens with dexamethasone.
- Induce temporary suppression of ovarian function with 1-2 week treatment with the OC pill.
- In case of too thin endometrium, administer E2 vaginally during the last days of COH.
- In case of PCOD/PCOD-like, consider sequential FSH and LH stimulation. If recLH is not available, use low dose hCG (50-100 IU/day).
- In case of excessive UC frequency at the time of ET, consider either delaying ET to blastocyst stage or administer luteal support with vaginal progesterone starting at time of oocyte retrieval.

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