ASYNCHRONOUS GLANDS IN THE ENDOMETRIUM OF WOMEN WITH RECURRENT REPRODUCTIVE FAILURE. OBSERVATIONS OF A COMMON ABNORMALITY.

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Short title: Asynchronous glands in secretory phase endometrium

SUMMARY

Of 969 non-consecutive endometrial biopsies performed for investigation of recurrent reproductive failure, 20 cases (2.1%) showed the striking presence of retarded or asynchronous endometrial glands in otherwise unremarkable mid or late secretory endometrium. These glands were characterised by tall columnar cells with crowded nuclei showing increased reactivity for proliferative markers (MIB-1), occasional mitoses, greatly reduced or absent secretion, and persistent expression of oestrogen receptors and usually progesterone receptors and their isoforms typical of late proliferative phase endometrium, but no down-regulation of PTEN. The nearby endometrial stromal cells exhibited no discernibly reduced reactivity for calretinin.

These changes were seen in single glands (even portions of glands), or clusters of glands, adjacent to normal late secretory type endometrial glands and set in pseudodecidualised stroma characteristic of late luteal phase. Some examples also displayed adjacent glands with intermediate features and it is speculated that firstly, this is a relatively common phenomenon with an unknown potential to affect implantation. Secondly, it is an intrinsic defect of the endometrium. Thirdly, it differs from previously described patterns of so-called luteal phase defect or deficient secretory phase.

Key words: asynchronous glands, discordant glands, luteal phase endometrium, deficient luteal phase, ER, PR, abnormal receptor expression, secretory endometrium, immunohistochemistry, pathology.

INTRODUCTION

So-called luteal phase defect (LPD) has, from time to time, been claimed as a cause, or at least a correlate, of infertility(Jones 1976, Wentz 1980, Wentz, Kossoy et al. 1990) recurrent miscarriage and abnormal uterine bleeding.(Mazur and Kurman 2005) Yet, it is an ill-defined concept at best and clinically still controversial.(Bukulmez and Arici 2004, Shivapathasundram, Kwik et al. 2011) The most widely understood interpretation of the entity is of central (hypothalamic or pituitary) dysfunction with downstream effects on the adequacy of corpus luteum hormone production. This translates into an inadequate or abbreviated endometrial response during the secretory or luteal phase of the cycle(Dallenbach-Hellweg 1984, Witten and Martin 1985), impairing its ability to support implantation and embryonic development. While some efforts have been expended in establishing diagnostic protocols, it is well accepted that identical clinical and laboratory findings occur in normal fertile as well as infertile women(Shivapathasundram, Kwik et al. 2011) and that LPD may be implicated in a few as 5% of women with infertility.(Wentz, Kossoy et al. 1990) In LPD, as defined above, the secretory endometrium appears abnormal only to the extent that it is morphologically variable from area to area or insufficiently developed for the stage of the cycle as determined clinically or biochemically. Its management is empirical and based on a presumption of normal functioning endometrium, so much so that recent sentiment borne of active intervention with artificial reproductive techniques seeks to render the entity redundant and not worthy of diagnosis.(Bukulmez and Arici 2004, Shivapathasundram, Kwik et al. 2011)

What, however, if the endometrium is not normal? Some studies indicate that the endometrium is not always "normal" in women with "deficient secretory phase".(Thornburgh and Anderson 1997) In this study, glands with elongate, hyperchromatic epithelial cell nuclei and diminished or no secretory product were distinguished immunohistochemically by reduced oestrogen receptor (ER) and progesterone receptor (PR) expression. Sometimes, such glands were the dominant histological pattern in the endometrium while in other cases they were only focally present. Whether these cases are a subset of LPD or a separate entity altogether is unclear. Equally unclear is whether the receptor status of the glands is a cause or an effect of the pathological process. Another, more recent

study(Mai, Teo et al. 2009) describes similar endometrial glandular changes in women with dysfunctional uterine bleeding and defined the glandular asynchrony as >4 day variance from the remainder of the endometrial glands in the biopsy material. These asynchronous glands showed increased proliferative activity (MIB-1 nuclear reactivity) and the nearby endometrial stroma showed, in most cases, reduced calretinin reactivity. The ER and PR status of the glands was not studied. These investigators, in noting the important role of endometrial stromal cells in inducing endometrial glandular epithelial differentiation, postulated that they, and not the glands, were the cells exhibiting the primary dysfunction.

There are a number of morphological changes observed during normal and abnormal luteal phase, as well as biochemical profiles said to contribute to the failure of successful implantation and pregnancy.(Dimitriadis, Nie et al. 2010) Whether any of these is significant or indeed a real entity is not known.

As part of a large clinical service assessing endometrial pathology and immune cell populations of women being investigated for recurrent miscarriage/IVF failure, we have encountered several cases with readily apparent focal features in endometrial gland morphology and receptor status, not dissimilar to those described in the two studies above.(Thornburgh and Anderson 1997, Mai, Teo et al. 2009) We have endeavoured to examine the clinicopathological features of these cases.

MATERIALS AND METHODS

Study population

Over an 18 month period from September 1st 2010 to February 29th 2012, 969 women attending the clinics of Genea (Sydney), IVF Australia (Sydney) and Repromed (Adelaide and Darwin) for the investigation and management of recurrent miscarriage or recurrent IVF failure, underwent diagnostic endometrial biopsy (either formal dilatation and curettage or Pipelle endometrial sampling), and had formalin-fixed tissue referred for histological assessment by a single pathologist [PR] at the GynaePath subspecialty unit of Douglass Hanly Moir Pathology in Sydney which were adjudged to represent histologically normal endometrium from various stages of natural menstrual cycles (i.e. not

programmed cycles) with adequate material for assessment. The majority of patients had been investigated for other maternal causes of recurrent pregnancy loss or IVF failure, such as parental karyotype abnormalities, maternal structural uterine abnormalities, antiphospholipid and familial thrombophilia syndromes, and genital tract infections. They were part of a larger series of patients being examined for the status of immune cells and macrophages in the endometrium,(Russell, Sacks et al. 2013) and of 969 biopsies since the index case, 20 showed discordant (asynchronous) endometrial glands set within otherwise normal mid to late secretory phase stroma and amongst normal appearing secretory phase glands (Figure 1).

Clinical information was obtained from the treating physicians of these 20 patients concerning reproductive history, any relevant hormonal data during the biopsied cycle, recent hormonal therapy, other reproductive tract or pelvic pathology (e.g. endometriosis). The study was approved on 31st May 2012, by the Sydney & South Western Sydney Local Health Districts Ethics Review Committee (Royal Prince Alfred Hospital Zone - Protocol X12-0156).

Material

All biopsy material was fixed in 10% neutral buffered formalin and all was processed into paraffin within 48 hours. Sections were cut at 4μm and stained with haematoxylin and eosin (H+E) according to a standard protocol. H+E stained sections were examined to determine the endometrial dating against a standardised 28 day cycle with ovulation, by convention, occurring at the end of the 14th day(Noyes, Hertig et al. 1975) and to identify any pathological features present. Assessment was performed only in tissue fragments oriented so that the surface epithelium was present and only in the superficial 1mm of the functional layer (avoiding basal endometrium) and also only in endometrium in which appropriate luteal phase changes were widely present (i.e. tissue from corpus/fundus and not lower uterine segment).

Immunohistochemistry

Immunostains were prepared on serial sections, also cut at 4μm and floated on SuperFrost Plus coated glass slides (Menzel-Glasner, Germany), and commercially available monoclonal antibodies for ER,

PR, MIB-1, PTEN and calretinin were used in an auto-immunostainer (Ventana Benchmark XT) using ULTRA view® DAB detection kit). Ali and Marina: Immunostains for ER-α, ER-β, PR-A, PR-B, using protocols detailed in Table 1. Grading of nuclear expression of ER and PR (including isoforms) in the glandular epithelium of the affected glands as well as nearby normal-appearing glands and the surface endometrial epithelium was performed according to the technique of Kinsel et al,(Kinsel, Szabo et al. 1989) in which intensity of nuclear staining is graded 0-3 and this is multiplied by the percentage of positive nuclei (to give a count of 0-300). All immunostaining batches included appropriate controls.

RESULTS

The mean age of the patients was 37.3 years (range 28-42 years). This did not differ materially from the ages of the large cohort of patients previously reported by us(Russell, Sacks et al. 2013) and from which series the present cases were extracted. The stage of the menstrual cycle of the study patients, however, was skewed significantly towards late luteal phase, with only three of 20 patients in mid luteal and 17 (85%) in late luteal or menstrual phase, whereas 619 (64%) of the 969 submitted endometrial biopsies were from the late luteal or menstrual phase. The study cases thus represented 20 of 969 (2.1%) of the total and 17 of 619 (2.7%) of those in late luteal or menstrual phase. All patients had been biopsied during a natural cycle (i.e. none had hormonal therapy in the study cycle – an exclusion criterion).

The main feature of these 20 cases, and the sole selection criterion for this study, was the variable presence of endometrial glands within the superficial functionalis which, despite apparently normal luteal phase changes in the immediately adjacent stroma and glands, were characterised by simple rounded contours, elongated and crowded nuclei, occasional mitoses and minimal or unapparent luminal secretion, resembling the glands of the proliferative phase (Figure 1). The change usually affected whole glands in cross section (Figure 2a) but sometimes only portion of a gland (Figure 2b). Most commonly there were small aggregates of affected glands in the superficial functionalis (Figure

3) with direct contiguity between the affected glandular epithelium and the endometrial surface, but this varied in intensity up to involvement of about 40% of the glands in one case (Figure 4).

While most endometria exhibited a sharp distinction between affected and nearby "normal" glands (Figures 1-4), six cases additionally showed transitional patterns in the degree of discordance of the gland epithelium perhaps reflecting a spectrum of severity. This was more widespread and more easily appreciated in the immunostains for receptors (Figure 5) than in the H+E sections. Broad immunohistochemical details of the 20 cases are given in Table 2.

All cases exhibited uniform and strong expression of ER in the affected glands, highlighting a clear discrimination from the nearby normal glands that, in turn, displayed variable down-regulation of ER expression typical of luteal phase endometrium (Figure 6a). ER expression in the overlying surface endometrial epithelium was usually mid-way between the affected and normal endometrial glands. This discrepancy between asynchronous and normal glands was strongly reflected in persistence of isoform ER-α (Figure 6b) and to a lesser extent in the expression of ER-β (Figure 6c), in each instance.

By contrast, expression of PR and its isoforms displayed two distinct patterns. In 16 of 20 cases, the asynchronous glands displayed persistent strong and diffuse expression of PR, PRA and PRB (Figure 7) while in the remaining four cases there was variable expression with some glands showing persistent expression and other glands showing no or negligible expression (see Table 2), focally within the same biopsy material. In each and every instance, there was concordance between PR and both PRA and PRB isoforms (Figure 8).

The proliferative marker MIB-1 was variably expressed in the asynchronous glands (Table 2), occasionally reactive in up to 40% of epithelial cell nuclei (Figure 9), while nearby glands exhibited minimal reactivity. This finding correlated with occasional mitoses being observed in such glands (Figure 1). Calretinin was expressed in the endometrial stromal cells and there was no measurable difference between the stroma around the asynchronous glands and nearby normal secretory glands (Figure 10).

DISCUSSION

The dramatic alterations in the endometrium that occur during the menstrual cycle are primarily by oestrogen and progesterone acting via their own nuclear receptors (ER-α, ER-β, PRA, and PRB).(Sereepapong, Chotnopparatpattara et al. 2004) They induce production of molecules that act locally in an autocrine or paracrine fashion,(Jabbour, Kelly et al. 2006) or via receptor-independent pathways, as suggested by Hou et al.(Hou, Tan et al. 2004) At least some of this is via glandular/stromal interaction(Pierro, Minici et al. 2001) through which, for example, oestrogenic stimulation of stromal cells to produce growth factors such as insulin-like growth factor 1 and epidermal growth factor that bind receptors expressed on the epithelial cells induces glandular proliferation. By contrast, progesterone secretion leads to down-regulation of ER expression in both the glands and stroma, rendering the endometrium less responsive to oestrogen in the second half of the cycle, as well as down-regulation of progesterone receptors.(Mote, Balleine et al. 2000) Human PR exists in two isoforms (PRA and PRB) with both expressed in more or less equal amounts in the glandular epithelium but PRA predominating in the stroma.(Mote, Balleine et al. 1999) Progesterone initially inhibits proliferation in both the glands and the stroma (a rapid cessation of mitotic figures is seen) and then induces secretory conversion of the glands and major alterations of the stroma. Examination of the morphology and receptor status of the cases in the present study reveals an apparent paradox to this model.....

Thoughts:

I think this has to be a clonal process, unrelated to systemic hormonal factors. I have seen it in sequential biopsies in one patient (not in this series). The changes are too sharply circumscribed to individual glands (and even parts of glands) to be a local growth factor problem. The importance of observing the change in the superficial functionalis is in making sure these are not basal glands caught up some plane of section artefact. The similarities in receptor profiles to those in the surface epithelium (which also has a different endocrine profile and phenotype from ordinary endometrial glands) might support the change being clonal.

The presence of transitional glands in some cases, not immediately apparent in H+E sections, makes me think it is part of a spectrum and I am seeing only the most obvious, most severe or most evident cases. By that last point I mean the change is most definite in late secretory phase because the distinction from late secretory phase glands is most marked then. Earlier in the cycle, the change might still be there but just not so easy to pick up and impossible to see in proliferative phase.

While if there are only occasional glands involved, this might be just a strange peculiarity of glandular differentiation, if the change is more marked or if it induces functional changes in the nearby glands (? the transitional glands) that are inimical to implantation, then it might be a significant finding in these women – wouldn't know what to do about management!!!!!!

What are your thoughts?

Peter

Table 1. Summary of primary antibodies and immunohistochemical technique.

Antibody	Source	Clone	Dilution	Antigen retrieval
ER	Ventana	SP1	pre-diluted	CC1(Ventana)
ER-α				
ER-β				
PR	Ventana	1E2	pre-diluted	CC1(Ventana)
PR-A				
PR-B				
MIB-1	Cell Marque	SP6	1/50	CC1(Ventana)
PTEN	Novocastra	NCL-PTEN	1/50	CC1(Ventana)
Calretinin	Cell Marque	rabbit	1/50	CC1(Ventana)

Table 2 Details of cases with asynchronous endometrial glands in luteal phase and the expression of receptors in the affected glands

Case No	Age (yrs)	Day*	Asynch. Glands[#]	ER~	ER-α	ER-β	PR	PR-A	PR-B	MIB-1
1	39	25	15%	300	250	250	300	150	150	0-5%
2	40	25	5%	300	300	200	200	200	200	0-10%
3	40	20	10%	300	250	200	250	150	150	20-80%
4	37	24	1%	300	250	200	300	200	200	0-30%
5	34	22	5%	300	300	200	250	180	180	0-5%
6	33	24	15%	300	250	250	200	200	200	0-5%
7	33	M	1%	300	300	200	0	0	0	0%
8	28	22	3%	300	200	200	200	250	250	0-15%
9	38	23	5%	300	300	200	250	250	200	0-5%
10	41	24	5%	300	300	250	300	250	200	0-5%
11	40	23	5%	300	200	200	250	200	200	0-5%
12	39	23	5%	300	200	200	300	100	100	40%
13	37	25	1%	300	300	200	300	250	250	25-30%
14	35	23	2%	300	250	250	250	250	250	0-1%
15	42	23	5%	300	300	250	100	100	150	10-15%
16	31	23	1%	300	200	200	100	25	25	0-1%
17	42	24	1%	300	200	200	250	200	250	2-5%
18	41	22	2%	300	250	200	50	0-50	0-50	0-1%
19	40	24	40%	300	300	200	300	200	300	30-50%
20	36	24	5%	300	200	200	50	0-50	0-50	0-1%

*day of cycle (against standardised 28 day cycle with ovulation occurring at the end of the 14th day); M=menstrual phase. [#]approximate percentage of endometrial glands showing asynchronous maturation. ~approximate scoring of expression of receptors, including isoforms, in affected asynchronous glands according to the protocol of Kinsel et al(Kinsel, Szabo et al. 1989)

LEGENDS FOR FIGURES

Figure 1. High power of index case (Case 1) with three asynchronous endometrial glands resembling proliferative phase glands, two of which show mitoses (upper right), set in pseudodecidualised stroma and adjacent to a late secretory gland (lower left). H+E x200

Figure 2. (a) Asynchronous glands immediately adjacent to, but discrete from, a typical late secretory endometrial gland (Case 13). H+E x 100. (b) Asynchronous gland epithelial differentiation occurring in only part of a late secretory phase endometrial gland (Case 10). H+E x 200

Figure 3. A small superficial clutch of asynchronous glands set in otherwise unremarkable day 23 secretory endometrium (Case 15). This was the most commonly observed pattern. H+E x 40

Figure 4. Low power of Case 19, the most severely affected, in which almost one half of all glands were affected by the process. H+E x 20

Figure 5. Case 8 in which asynchronous glands were clustered in late secretory endometrium and several adjacent glands showing an intermediate phenotype. This "altered differentiation" is much more readily appreciated in the immunostains for receptors than in the H+E sections. Immunostain for ER x 40

Figure 6. Persistent expression of ER in asynchronous glands in mid and late secretory phase endometrium, compared to adjacent normal glands. (a) Case 19. Immunostain for ER x 100. (b) Case 17. Immunostain for ER-α x 100. (c) Same field in Case 17. Immunostain for ER-β x 100

Figure 7. Field of late secretory endometrium with numerous asynchronous glands (Case 19) and showing a dramatic difference in expression of PR compared to the adjacent normal glands. Immunostain for PR x 40

Figure 8. Two patterns of PR expression were identified in asynchronous glands, sometimes in the same biopsy as here from Case 12. Null expression was seen in some asynchronous glands (centre right) but with complete concordance between PRA (a) and PRB (b). Elsewhere, there was persistent

over-expression of PR in asynchronous glands (right), again with concordance between PRA (c) and PRB (d). x 100

Figure 9. Nuclear reactivity for the proliferative marker MIB-1 in asynchronous glands was often slightly elevated but sometimes dramatically so, as here from Case 12. Immunostain for MIB-1 x 200

Figure 10. Calretinin immunostaining did not reveal any appreciable difference in reactivity of endometrial stromal cells in the vicinity of asynchronous glands compared to those surrounding normal late secretory type glands. Variation in this image (Case 18) is due to stromal oedema and negative staining of cells in the included spiral arterioles. Immunostain for calretinin x 100

Figure 11. Immunostaining for ER (a) and PR (b) in normal mid-proliferative endometrium. x 100

REFERENCES

Bukulmez, O. and A. Arici (2004). "Luteal phase defect: myth or reality." Obstet Gynecol Clin North Am **31**(4): 727-744, ix.

> Although the diagnosis of luteal phase defect (LPD) has been described convincingly in the research setting, it remains a controversial clinical entity. Apart from many uncertainties that surround the diagnosis of LPD, there is no convincing evidence that LPD is associated with infertility and recurrent abortion. Once diagnosed, the treatment options are empiric and include those that are recommended for unexplained infertility. The efforts to diagnose LPD in patients who have infertility or recurrent abortion are not justified.

Dallenbach-Hellweg, G. (1984). "The endometrium of infertility. A review." Pathol Res Pract **178**(6): 527-537.

> Almost all functional disturbances involved in sterility result in morphological changes in the endometrium. Since hormone levels fluctuate depending upon various biorhythms, the histological examination of the endometrial biopsy is the most reliable parameter for evaluating the cause of infertility. A major subdivision can be made into anovulatory and ovulatory infertility. The anovulatory cycle is most frequently caused by a polycystic ovary syndrome, less frequently by gonadal dysgenesis, receptor or enzyme deficiencies, ovarian neoplasms, autoimmune reactions, or the luteinized unruptured follicle syndrome. Histologically the endometrium, depending upon the amount of estrogen secreted and reacting, is atrophic, resting, deficiently or irregularly proliferated or hyperplastic at the end of a menstrual cycle. A limited and deficient luteinization within a non-ovulating insufficient follicle may also result in abortive secretion which must be differentiated from deficient secretion following ovulation. In ovulatory infertility an absolute or relative progesterone deficiency results in a deficient secretory phase with delayed maturation of glands and stroma. The delay in maturation may be dissociated in the presence of an insufficient corpus luteum with absolute progesterone deficiency or coordinated when progesterone secretion is normal but counteracted by too much estrogen, as from a preceding persistent follicle. Since in these instances ovulation is delayed, the coordinated delay in maturation of the endometrium is only apparent. A truly delayed coordinated maturation may occur when the progesterone cannot fully act because of a preceding deficient proliferation. In addition, cyclic asynchrony with shortened or prolonged menstrual cycles may cause infertility by altering the endometrium so the blastocyst cannot implant. Rare non-functional endometrial changes as causes of infertility may be tuberculous endometritis, polyps or true neoplasms.

Dimitriadis, E., et al. (2010). "Local regulation of implantation at the human fetal-maternal interface." Int J Dev Biol **54**(2-3): 313-322.

> Embryo implantation and formation of a functional placenta are complex processes that require a plethora of regulatory molecules. In recent years, many of these mediators have been identified, often from studies in experimental animals. Furthermore, their expression patterns at the embryo-maternal interface in women have been characterized and provide clues to their potential actions. What has been missing in most cases is any experimental demonstration of their function. Proteases, cytokines and chemokines are among the molecules identified at the embryo-maternal interface. Functional studies of the protease, proprotein convertase (PC)6, the gp130 cytokines, leukemia inhibitory factor (LIF) and interleukin (IL)11 and the chemokines, CX3CL1 and CCL14 demonstrate potential actions within the uterine cavity. These actions include: enhancing blastocyst development,

modifying adhesive properties of the blastocyst and the uterine epithelial surface, and providing chemotactic guidance to the blastocyst. As implantation proceeds, PC6 and IL-11 also act to drive decidualization. The products (proteases, chemokines and cytokines) produced by these decidual cells provide a unique environment. This is important for both directing and restraining trophoblast invasion and for leukocyte trafficking into the decidua until the placenta is fully established.

Hou, X., et al. (2004). "Canonical Wnt signaling is critical to estrogen-mediated uterine growth." Mol Endocrinol **18**(12): 3035-3049.

Major biological effects of estrogen in the uterus are thought to be primarily mediated by nuclear estrogen receptors, ERalpha and ERbeta. We show here that estrogen in an ER-independent manner rapidly up-regulates the expression of Wnt4 and Wnt5a of the Wnt family and frizzled-2 of the Wnt receptor family in the mouse uterus. One of the mechanisms by which Wnts mediate canonical signaling involves stabilization of intracellular beta-catenin. We observed that estrogen treatment prompts nuclear localization of active beta-catenin in the uterine epithelium. We also found that adenovirus mediated in vivo delivery of SFRP-2, a Wnt antagonist, down-regulates estrogen-dependent beta-catenin activity without affecting some of the early effects (water imbibition and angiogenic markers) and inhibits uterine epithelial cell growth, suggesting that canonical Wnt signaling is critical to estrogen-induced uterine growth. Our present results provide evidence for a novel role of estrogen that targets early Wnt/beta-catenin signaling in an ER-independent manner to regulate the late uterine growth response that is ER dependent.

Jabbour, H. N., et al. (2006). "Endocrine regulation of menstruation." Endocr Rev **27**(1): 17-46.

In women, endometrial morphology and function undergo characteristic changes every menstrual cycle. These changes are crucial for perpetuation of the species and are orchestrated to prepare the endometrium for implantation of a conceptus. In the absence of pregnancy, the human endometrium is sloughed off at menstruation over a period of a few days. Tissue repair, growth, angiogenesis, differentiation, and receptivity ensue to prepare the endometrium for implantation in the next cycle. Ovarian sex steroids through interaction with different cognate nuclear receptors regulate the expression of a cascade of local factors within the endometrium that act in an autocrine/paracrine and even intracrine manner. Such interactions initiate complex events within the endometrium that are crucial for implantation and, in the absence thereof, normal menstruation. A clearer understanding of regulation of normal endometrial function will provide an insight into causes of menstrual dysfunction such as menorrhagia (heavy menstrual bleeding) and dysmenorrhea (painful periods). The molecular pathways that precipitate these pathologies remain largely undefined. Future research efforts to provide greater insight into these pathways will lead to the development of novel drugs that would target identified aberrations in expression and/or of local uterine factors that are crucial for normal endometrial function.

Jones, G. S. (1976). "The luteal phase defect." Fertil Steril **27**(4): 351-356.

In summary, the luteal phase defect is a deficiency of corpus luteum progesterone steroidogenesis, either in amount or duration, or both. The clinical manifestations include either primary infertility or repeated first trimester abortions. The diagnosis can only be made clinically on the basis of a well-timed endometrial biopsy that is read histologically as 2 or more days out of phase with the next period in at least two cycles.

Kinsel, L. B., et al. (1989). "Immunocytochemical analysis of estrogen receptors as a predictor of prognosis in breast cancer patients: comparison with quantitative biochemical methods." Cancer Res **49**(4): 1052-1056.

Biochemical quantitation of estrogen receptors has been used to predict prognosis in breast cancer. Immunocytochemical analysis of estrogen receptors correlates with biochemical analysis but has very few follow-up studies in the literature to validate it as a prognostic indicator. 257 patients were followed for up to 10 years (median, 6.2 years) after primary surgical treatment. Estrogen receptor analysis using both biochemical and immunocytochemical techniques was performed on their tumor specimens. Patients with positive estrogen receptor values had longer survival than patients with negative values. This was demonstrated by both methods in the first 5 years of follow-up but only by immunochemistry after 5 years. The relationship between estrogen receptor status and disease-free interval was less strong than with survival. This study demonstrates that immunocytochemical estrogen receptor analysis was of prognostic significance.

Mai, K. T., et al. (2009). "Histopathogenesis of endometrium with asynchronous glands in dysfunctional uterine bleeding." Histopathology **55**(1): 126-130.

Mazur, M. T. and R. J. Kurman (2005). Dysfunctional uterine bleeding. Diagnosis of endometrial biopsies and curettings. A practical approach. M. T. Mazur and R. J. Kurman. New York, Springer: 100-120.

Necrotic debris or old blood is sometimes present in the lumens of otherwise normal endometrial glands (Fig. 5.11). This debris seems to result from abnormal breakdown with entrapment of the debris within glands. The association with abnormal endometrial breakdown and bleeding is poorly defined, however, and often no definite abnormalities are present when luminal debris is seen. Without other morphologic abnormalities, luminal debris is a nonspecific finding with no known clinical significance.

Mote, P. A., et al. (1999). "Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle." J Clin Endocrinol Metab **84**(8): 2963-2971.

The human progesterone receptor (PR) is expressed as two isoforms, PRA and PRB, that function as ligand-activated transcription factors. In vitro studies suggest that the isoforms differ functionally and that the relative levels in a target cell may determine the nature and magnitude of response to progesterone. However, it is not known whether the two isoforms are normally coexpressed in vivo. To understand the functional significance of relative PR isoform expression in normal physiology, it is essential to determine whether PRA and PRB are coexpressed in the same cell. This study reports the development of a dual immunofluorescent staining technique to demonstrate PRA and PRB proteins by single cell analysis in the same tissue section of human endometrium during the menstrual cycle. PRA and PRB are coexpressed in target cells of the human uterus. In the glands, PRA and PRB were expressed before subnuclear vacuole formation and glycogenolysis, implicating both isoforms in this process, whereas persistence of PRB during the midsecretory phase suggested its significance in glandular secretion. In the stroma, the predominance of PRA throughout the cycle implicates this isoform in post-ovulatory progesterone-mediated events. These results support the view that PRA and PRB mediate distinct pathways of

progesterone action in the glandular epithelium and stroma of the human uterus throughout the menstrual cycle.

Mote, P. A., et al. (2000). "Heterogeneity of progesterone receptors A and B expression in human endometrial glands and stroma." Hum Reprod **15 Suppl 3**: 48-56.

The human progesterone receptor (PR) is expressed as two isoforms, PRA and PRB, which function as ligand-activated transcription factors. In-vitro studies suggest that the isoforms differ functionally and that their relative expression in a target cell may determine the nature and magnitude of response to progesterone. We have shown recently that PRA and PRB are co-expressed in target cells of the human endometrium. The purpose of this study was to investigate the homogeneity of expression of PRA and PRB in target cells of the human uterus throughout the menstrual cycle. In the functionalis, PRA and PRB were expressed in comparable levels in glandular epithelium during the proliferative phase of the cycle, whereas there was persistence of PRB but not PRA in the glands during mid-secretory phase. In the stroma, there was predominance of the PRA isoform throughout the cycle. There was remarkable homogeneity in the relative expression of PRA and PRB in adjacent cells within the same tissue compartment, suggesting that the mechanisms regulating relative PR isoform expression are similarly active in these cells. By contrast, heterogeneity between glands was observed under some circumstances in the functionalis of the endometrium, suggesting PR isoform down-regulation by progesterone to be asynchronous. Heterogeneity was also seen between the glands of the basalis and functionalis of the endometrium implying region-specific responses to hormonal stimuli. This study demonstrates adjacent cell homogeneity in the relative expression of PRA and PRB in normal human endometrial tissue and a differential response to ovarian steroid hormones between cell types and between different regions within the same tissue.

Noyes, R. W., et al. (1975). "Dating the endometrial biopsy." Am J Obstet Gynecol **122**(2): 262-263.

Pierro, E., et al. (2001). "Stromal-epithelial interactions modulate estrogen responsiveness in normal human endometrium." Biol Reprod **64**(3): 831-838.

The coculture of endometrial epithelial cells (EEC) with stromal cells (ESC) allows achievement of an improved in vitro system for studying interactions between cells via soluble signals. The purpose of this study was to investigate whether 17beta-estradiol and insulin can induce proliferation of EEC through ESC-secreted factors. No evidence of estrogen-induced EEC proliferation has been reported so far in the conventional culture methods. To this end, we used an in vitro bicameral coculture model where human EEC were grown on extracellular matrix-coated inserts applied in dishes containing ESC. Proliferation was assessed by tritiated thymidine incorporation. Homogeneity of endometrial cell populations was ascertained immunocytochemically. 17beta-estradiol did not induce any proliferative effect on EEC cultured alone. Endometrial epithelial cell proliferation was significantly enhanced in EEC/ESC cocultures; moreover, it was further increased by 17beta-estradiol addition. Insulin increased proliferation in EEC cultured alone, but again the effect was more pronounced in EEC/ESC cocultures. Coincubation of 17beta-estradiol and an antibody against insulin-like growth factor I (IGF I) led to neutralization of ESC-mediated EEC proliferation. This work provides evidence that the effect of 17beta-estradiol on human EEC proliferation may be mediated at least in part through ESC-secreted IGF I. We also showed that insulin effect is also partially due to ESC activation.

Russell, P., et al. (2013). "The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure. III: Further observations and reference ranges." Pathology **45**(4): 393-401.

AIM: : Abnormally functioning immunocompetent cells in the endometrium are thought to be responsible for at least some cases of recurrent reproductive failure [recurrent miscarriage or recurrent in vitro fertilisation (IVF) failure], but their detailed investigation has been hampered by a lack of a standardised protocol of counting such cells in study or control patients. The purpose of this study is to use a standardised protocol for the assessment of immune cells in the endometrial biopsies of a large cohort of women with recurrent reproductive failure and establish relevant reference ranges. METHOD: : In a recent study, we reported the presence and distribution of selected immune cells and macrophages in the endometria from 222 women who had a routine endometrial biopsy for investigation of recurrent miscarriage or IVF failure. Since the completion of that study, a further 1767 cases have been examined, using the same assessment parameters of the earlier study. RESULTS: : This updated analysis of 1989 endometrial biopsies provides reference ranges for CD8, CD163, CD56 and CD57 cells for individual 'days' of a normalised menstrual cycle. CD8 T-cells displayed a modest (50%) increase in numbers in the luteal phase and periglandular aggregation was a useful indicator of a subtle focal endometritis, possibly of infective origin, and generally not identified in H&E sections. A rapid accumulation of CD163 macrophages occurs in the superficial stroma after day 22 of the cycle, while a significant number of cases displayed single or clustered macrophages within glandular lumens of the superficial endometrium in luteal phase, especially after day 20 of the cycle. The significance of this change is unclear but may relate to a macrophage response to abnormal glandular secretion or to bleeding occurring at the time of ovulation. CD56 uterine natural killer (uNK) cells show such a dramatic rise in both absolute numbers and percentage of stromal cells from day 22 of the standardised 28 day cycle that this needs to be taken into account in all clinical studies or individual assessments of endometrial biopsies. CD57 NK cells are seen in small numbers in most cases and cell counts of greater than 10 per mm are regarded as abnormal. CONCLUSIONS: : This large database provides a daily range which is the most accurate survey yet of uNK cell numbers. Co-location of CD8 T-cells and CD56 uNK cells in perviascular aggregates has been demonstrated.

Sereepapong, W., et al. (2004). "Endometrial progesterone and estrogen receptors and bleeding disturbances in depot medroxyprogesterone acetate users." Hum Reprod **19**(3): 547-552.

BACKGROUND: Depot medroxyprogesterone acetate (DMPA) is a very popular hormonal contraceptive. Unpredictable bleeding disturbances are the main reasons for discontinuation and may be mediated through endometrial hormone receptors. This study was aimed to compare the expression of progesterone and estrogen receptors in the endometrium of bleeding DMPA users with that of amenorrhoeic DMPA users. METHODS: Subjects were recruited between April 2000 and January 2001. On the day of the third DMPA injection, 42 amenorrhoeic DMPA users and 42 DMPA users who had frequent or prolonged endometrial bleeding and were bleeding on that day were matched by age and body mass index. Endometrial biopsies were collected for immunohistochemical study of progesterone receptor A plus B (PRAB) and B alone (PRB) and estrogen receptor alpha (ERalpha) and beta (ERbeta) expression. RESULTS: There were 23 adequate endometrial samples from each group. There were no differences in any of a series of comparisons of PRAB, PRB, ERalpha and ERbeta expression in glands or stroma between the groups. Serum estradiol and progesterone levels were also not different between the groups.

CONCLUSIONS: Endometrial PRAB, PRB, ERalpha and ERbeta expression in glands and stroma was not different between DMPA users who had frequent or prolonged bleeding and amenorrhoeic DMPA users.

Shivapathasundram, G., et al. (2011). "Luteal phase defect: part of the infertility zeitgeist or relic from the past?" Hum Fertil (Camb) **14**(1): 60-63.

Luteal phase defect (LPD) or short luteal phase is a controversial entity that has been variously defined over the years. There are a number of potential causes for LPD all of which are associated with inadequate progesterone secretion throughout the luteal phase which impairs endometrial development and is thus thought to cause infertility. However, the relationship between LPD and infertility is complex, with LPD found in both fertile and infertile women. Attempts have been made at treating LPD with a number of regimens including progesterone supplementation and ovulation induction using clomiphene citrate, however, problems with study design have prevented conclusive evidence for the efficacy of these treatments being drawn. Practically, with the more interventionalist and aggressive approaches to managing couples with unexplained infertility, LPD may have become an irrelevant entity.

Thornburgh, I. and M. C. Anderson (1997). "The endometrial deficient secretory phase." Histopathology **30**(1): 11-15.

The deficient secretory phase is a functional abnormality of the endometrium that has hitherto been poorly recognized. Endometrial curettings form 34 cases were examined in detail by histology. We also performed morphometric analyses of epithelial cell nuclei and assessed the oestrogen and progesterone receptor status of these cases compared with controls. Clinicopathological correlations were examined. The cases showing the deficient secretory phase were characterized histologically by the presence of elongated, hyperchromatic glandular cell nuclei, diminished or no secretory activity in the second half of the menstrual cycle and poorly developed stroma. Morphometry confirmed that the nuclei were different in shape from those seen at any time during the normal menstrual cycle and from basal endometrium. Nuclear expression of oestrogen and progesterone receptors in formalin-fixed and paraffin-embedded sections was reduced. It is apparent from this study that the endometrial appearances described represent a definable condition that may be linked to menstrual irregularities and, in some circumstances, infertility; it should be more widely recognized.

Wentz, A. C. (1980). "Endometrial biopsy in the evaluation of infertility." Fertil Steril **33**(2): 121-124.

One hundred and forty-nine patients presenting with infertility underwent two hundred and ten endometrial biopsies as part of a routine infertility evaluation. The initial biopsy was out of phase in 44 (29.5%), confirmed by subsequent biopsy to yield a total of 28 (19%) patients with luteal phase inadequacy. Of 44 patients taking clomiphene citrate, 13 (29.5%) had out-of-phase biopsies. Although hyperprolactinemia, recurrent miscarriages, extremes of reproductive life, and clomiphene citrate administration have been associated with an increased incidence of the defect, in this series a predisposing cause could not be detected in approximately one-half of the patients. The routinely obtained endometrial biopsy provides a safe, reproducible, and adequate means of providing histologic evidence for normal endometrial development for subsequent implantation.

Wentz, A. C., et al. (1990). "The impact of luteal phase inadequacy in an infertile population." Am J Obstet Gynecol **162**(4): 937-943; discussion 943-935.

A retrospective analysis of patients evaluated and treated for infertility was performed. Luteal phase inadequacy was diagnosed when the late luteal endometrial biopsy pattern was greater than 2 days out of phase in two cycles; in patients treated with clomiphene citrate therapy was changed if one biopsy was out of phase. One hundred ninety-seven patients underwent 242 biopsies. Among 137 women no treated with clomiphene citrate, 24 (17.5%) had out-of-phase biopsy specimens; 7 of 24 (29.2%) repeat biopsies were out of phase, with luteal phase inadequacy in 7 of 137 (5.1%) women. The probability of an out-of-phase biopsy occurring by chance alone was 4.2 of 137 or 3.1%. No woman was diagnosed to have luteal phase inadequacy as the single infertility factor. Fifty-three pregnancies (41%) occurred in 130 women without luteal phase inadequacy and in 2 of 7 (28.6%) diagnosed to have luteal phase inadequacy with other infertility factors. In clomiphene citrate-treated patients, pregnancy occurred in 15 of 26 (57.7%) with corrected luteal phase inadequacy and in 21 of 34 (61.8%) without luteal phase inadequacy. In this population the diagnosis of luteal phase inadequacy was not made more frequently than by chance alone. Moreover, fecundity in patients with treated luteal phase inadequacy is comparable to that in patients without this diagnosis.

Witten, B. I. and S. A. Martin (1985). "The endometrial biopsy as a guide to the management of luteal phase defect." Fertil Steril **44**(4): 460-465.

The endometrial biopsy serves as a useful and valuable tool in the diagnosis and treatment of luteal phase defect (LPD). Eighty patients were diagnosed as having an LPD by endometrial biopsy. The subjects were divided into four equal groups, and different treatment protocols were introduced according to the histologic pattern found in the endometrial biopsy specimen. Patients in group I had glandular stromal asynchrony and were treated with clomiphene citrate therapy. Progesterone suppositories were administered to those patients in group II who showed glandular stromal synchrony. Groups III and IV had the same histologic pattern as groups I and II, but a reversal of the treatment protocol was made. The raw pregnancy rate was 85% and 80% for groups I and II, respectively. Groups III and IV had a raw pregnancy rate of 40% and 30%, respectively. Life-table analysis projected the pregnancy rate based on the protocol of therapy administered. This confirmed our findings and strengthened our belief that the endometrial biopsy is an invaluable guide in the treatment of LPD. This article addresses two distinct endometrial patterns within the framework of LPD and proposes a structured therapeutic regimen to treat this defect.