Human Reproduction, pp. 1–9, 2021
https://doi.org/10.1093/humrep/deab258

human reproduction

OPINION

Peritoneal fluid progesterone and progesterone resistance in superficial endometriosis lesions

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Submitted on August 31, 2021; resubmitted on October 22, 2021; editorial decision on November 03, 2021

ABSTRACT: Peritoneal fluid in ovulatory women is an ovarian exudate with higher estrogen and progesterone concentrations than in plasma. In the follicular phase, progesterone concentrations are as high as plasma concentrations in the luteal phase. After ovulation, estrogen and progesterone concentrations in the peritoneal fluid are 5–10 times higher than in plasma, both in women with and without endometriosis. The histologically proliferative aspect without secretory changes of most superficial subtle lesions is not compatible with the progesterone concentrations in the peritoneal fluid. Therefore, we have to postulate a strong progesterone resistance in these lesions. The mechanism is unclear and might be a peritoneal fluid effect in women with predisposing defects in the endometrium, or isolated endometrial glands with progesterone resistance, or subtle lesions originating from the basal endometrium: the latter hypothesis is attractive since in basal endometrium progesterone does not induce secretory changes while progesterone withdrawal, not occurring in peritoneal fluid, is required to resume mitotic activity and proliferation. Hormone concentrations in the peritoneal fluid are an important factor in understanding the medical therapy of endometriosis. The effect of oestro-progestin therapy on superficial endometriosis lesions seems to be a consequence of the decreased estrogen concentrations rather than a direct progestin effect. In conclusion, the peritoneal fluid, being a secretion product of the ovarian follicule, deserves more attention in the pathophysiology and treatment of endometriosis.

Key words: endometriosis / peritoneal fluid / progesterone resistance / endometriosis pathogenesis / endometriosis pathology / endometriosis etiology / endometriosis in young women

Introduction

Endometriosis is histologically defined as 'endometrium like glands and stroma outside the uterus'. It is surprising that 'only endometrial-like gland profiles and stroma are required to make the diagnosis, which contrasts with many other diseases where a wide range of histological features contribute to both diagnosis and stratification of treatment' (Colgrave *et al.*, 2020). Attempts were made to improve histologic interpretation through immunohistochemistry for CD 10 (Nikoo *et al.*, 2014), by morphometry in quantitative histology (Rahman and Itakura, 1996) or by measuring specific histologic features (Colgrave *et al.*, 2020).

The pathophysiology of endometriosis remains enigmatic. The metaplasia theory postulates the differentiation of a stem cell or the transformation of a partially differentiated precursor cell into another differentiated cell. The retrograde menstruation and implantation theory of endometrium (Sampson, 1921) or basal endometrium (Leyendecker *et al.*, 2002, 2004) were based on the histological similarity of endometriosis to endometrium (Sampson, 1921). The many biochemical differences between endometriosis tissues and endometrium can be interpreted as the consequence of the endocrine or immunological environment of endometriosis, or used to question the similarity of endometrium to endometriosis tissues (Koninckx *et al.*, 2020b).

The plasma estrogens and progesterone concentrations during the menstrual cycle and the corresponding histological changes of the endometrium are well known (Noyes et al., 1975). Although such changes were expected in endometriosis cells, the synchronicity of endometriosis tissue and endometrium is limited, as reviewed and confirmed recently (Colgrave et al., 2020). The active and proliferative aspect of subtle, especially red, lesions without secretory transformation (Nisolle and Donnez, 1997) was interpreted as progesterone resistance (Donnez, 2021), a feature demonstrated biochemically in some superficial and deep endometriosis lesions (Bulun et al., 2019).

Peritoneal fluid is an ovarian exudate (Koninckx et al., 1980d) with high concentrations of estrogens and progesterone (Koninckx et al., 1980b), as reviewed previously (Koninckx et al., 1998). This feature of peritoneal fluid might be important for understanding the initiation and growth of endometriosis lesions and the lack of synchronicity with the endometrium.

Steroid hormones in peritoneal fluid and endometriosis

The peritoneal cavity and peritoneal fluid

The intact peritoneal cavity, containing the bowels and the internal female genital organs, is a virtual cavity, without vascularization, and with different temperature regulations than the core body temperature of the chest and the brain (Corona et al., 2011). The peritoneal cavity has, in women, a direct connection with the outside world. It, therefore, is considered a cavity that is outside the body, similar to the mouth or vagina. The cavity is lined by the peritoneum, composed of a 1.7 m^2 single layer of mesothelial cells, a basal membrane and some loose connective tissue resting on tissues with vascularization (Koninckx et al., 2016; Mutsaers et al., 2016). The peritoneum is a semi-permeable membrane with fast diffusion of small molecules, but a slower diffusion of larger molecules. This explains why the concentrations in the peritoneal fluid are some 40% lower than in blood for substances with a molecular weight around 60 000 kD, such as LH, FSH and albumin (Koninckx et al., 1980b), while large molecules, such as factors V and VIII (Pattinson et al., 1981), are virtually absent. This also explains why peritoneal fluid does not coagulate and that locally secreted macromolecules, such as glycodelins and Ca125, accumulate (Koninckx et al., 1992b). The peritoneum is actively regulated by gap junctions and cellular transport, exchange with blood vessels and the migration of immuno-competent cells, and even the diffusion rates of gases such as CO_2 and N_2O (Koninckx et al., 2016). Therefore, the peritoneal cavity has a specific micro-environment that differs from plasma with, in addition, a specific microbiome originating from the uterus and upper genital tract as well as the bowel, by transmural migration (Schweinburg et al., 1950). The microbiome and oxidative stress of retrograde menstruation could contribute to the initiation of endometriosis (Koninckx et al., 2019b). Following minor trauma the large flat mesothelial cells react within seconds by retraction, exposing the intercellular matrix and inducing an acute inflammation with diapedesis of neutrophils (Koninckx et al., 2016) and angiogenesis. Without the peritoneal barrier the peritoneal cavity thus becomes part of the body, which is an effective defence mechanism against infection.

In the absence of follicular growth, as in men, postmenopausal women and women taking oral contraceptives, the peritoneal cavity is a cavity containing a minimal amount of fluid. In ovulatory women, peritoneal fluid originates from ovarian exudation (Koninckx et al., 1980d) as previously reviewed (Koninckx et al, 1998). Fluid volumes increase exponentially during the follicular phase to 20 ± 6 ml (SD) and occasionally up to 400 ml (Fig. 1). Exudation originates predominantly from the growing follicle, probably because of the high follicular estrogen concentrations (Bouckaert et al., 1986), and from the corpus luteum after ovulation. It thus is not surprising that the concentrations of estrogens and progesterone are higher in peritoneal fluid, especially after ovulation (Fig. 1; Koninckx et al., 1980b). Considering the 40% lower sex hormone-binding globulin concentrations in peritoneal fluid, free estrogen concentrations are slightly higher than in plasma during the follicular phase. However, after ovulation estrogen concentrations acutely rise to 1000 pg/ml, which is more than three times higher than in plasma. In the follicular phase, peritoneal fluid progesterone concentrations are comparable to luteal phase plasma concentrations, and are thus much higher than plasma concentrations which are very low. After ovulation, progesterone concentrations rapidly increase by 10- or 20-fold. It was estimated that after ovulation estrogen and progesterone mainly leak into the peritoneal cavity and are resorbed from there, instead of being secreted into the bloodstream (Cicinelli et al., 2009; Koninckx and Gomel, 2016). That peritoneal fluid thus probably has higher concentrations around the ovulating ovary might even be a mechanism orienting the ampulla during oocyte pick-up (Koninckx et al, 1998). This concept was recently confirmed indirectly by finding higher progesterone concentrations in the peritoneal cavity at the site of ovulation (Cicinelli et al., 2009).

In women with endometriosis, the volume and the steroid hormone concentrations of peritoneal fluid were comparable to women without endometriosis. However, in women with a luteinized unruptured follicle, associated with typical and severe endometriosis but not with subtle lesions (Koninckx, 1998), the postovulatory increases in progesterone and estrogen concentrations did not occur (Koninckx et al., 1980a; for review (Koninckx et al, 1998)). It was speculated that the much lower peritoneal fluid progesterone concentrations in these women might be a cofactor in the initiation of endometriosis (Koninckx et al., 1980c; for review (Koninckx et al, 1998)) and the growth of superficial endometriosis (Koninckx et al., 2021).

In 1980, the assays of oestrogen and progesterone were performed after extraction, thin-layer chromatography and recovery, thus being highly specific assays (Koninckx et al., 1980b). Potentially important was the presence of a high amount of 20α-hydroxyprogesterone and, on thin-layer chromatography, at least three other substances with a blue fluorescence under UV (Koninckx et al., 1980b). These substances were not identified, but the characteristics suggest high concentrations (i.e. they were visible) of delta-4 3-keto steroids, since they showed UV fluorescence-like progesterone and androstenedione, and they bound to transcortin. The most fluorescent substance was less polar than progesterone suggesting a progesterone-like molecule without oxygen at position 20. Since these substances were not identified, and since identification with mass spectrometry was not available, our impression of an association with endometriosis was not investigated in 1980.

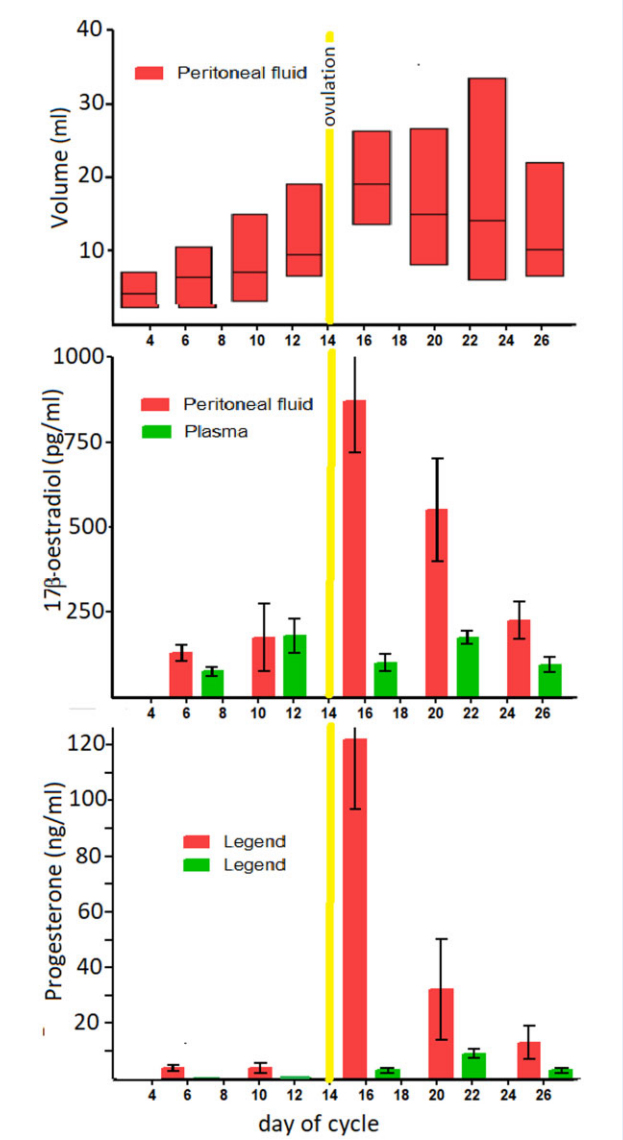

Figure 1. Changes in peritoneal fluid, estrogens and progesterone in plasma and peritoneal fluid during the menstrual cycle. Peritoneal fluid is mainly an ovarian exudate from the growing follicle or the corpus luteum. The volume increases during the follicular phase. Free estrogen concentrations are higher in peritoneal fluid than in plasma and progesterone concentrations are as high as during the luteal phase in plasma. After ovulation, the steroid hormone concentrations abruptly increase and are 5–10 times higher than in plasma with a different time course Adapted from Koninckx *et al.* (1980d) and Koninckx *et al.* (1980b).

Histology of superficial endometriosis lesions

Small, superficial and poorly vascularized peritoneal endometriosis lesions, such as red and white vesicles are mainly influenced by peritoneal fluid. The influence of peritoneal fluid decreases with the depth of invasion (Koninckx *et al*, 1998). We, therefore, were not surprised to find that endometriosis lesions deeper than 5 mm were active and in-phase in 68% and 74% of lesions, respectively, in contrast with 25% and 38%, respectively, in lesions of 2 to 4 mm deep or typical lesions (Cornillie *et al.*, 1990). These differences were interpreted as reflecting the diffusion gradient of steroid hormones in peritoneal fluid into the tissues. Deep endometriosis being our focus of interest, the observation that very superficial—subtle lesions were more active in 58% and in-phase in 57% was not discussed (Cornillie *et al.*, 1990). To the best of our knowledge, peritoneal fluid concentrations of estrogens and progesterone have never been taken into account when interpreting the histology or presence of steroid hormone receptors of superficial endometriosis. Metzger concluded that the hormonal responsiveness of endometrial implants was unpredictable and inconsistent (Metzger *et al.*, 1988) since in ovulatory women only 13% of 438 superficial endometriosis lesions, and in early pregnancy and menopause only 50% and 31%, respectively, of endometrial implants, were histologically in phase. The non-pigmented or subtle endometriosis lesions in particular (Stripling *et al.*, 1988) were repeatedly described as active and proliferating without secretory changes (Nisolle and Donnez, 1997). Also, the first description of non-pigmented endometriosis was illustrated with images of active lesions (Jansen and Russel, 1986). All red lesions were proliferative with a similar proliferation index as proliferative endometrium without secretory changes (Donnez *et al.*, 1996; Nisolle and Donnez, 1997) and only 13% were considered in phase with the eutopic endometrium, notwithstanding apparently normal estrogen and progesterone receptors on histology. Typical or black powder burn lesions in a white sclerotic area were less active, rarely in phase and have been considered burnt out lesions. Also, the recent quantitative histological analysis confirms the variable morphology of endometriotic lesions, which are rarely in phase with the endometrium during the menstrual cycle (Colgrave *et al.*, 2020).

Estradiol and progesterone receptors in the functional and basalis layer of the endometrium and endometriosis showed a strong and prolonged decline at the end of the menstrual cycle. However, in the basalis of the endometrium (basalis IV (Padykula *et al.*, 1989)) and pelvic endometriosis lesions, the decline was less and recovered at the end of the cycle. This was observed in epithelial (Fig. 2) and stromal cells (Leyendecker, *et al.*, 2002). Estrogen and progesterone receptors of endometriosis are thus in phase with basalis endometrium but not with the functional layer. Potentially important is the rhisosome structure of the basalis endometrium (Yamaguchi *et al.*, 2021).

Cystic ovarian endometriosis will not be discussed here, since intra-ovarian estrogen and progesterone concentrations are not well known, while concentrations in the chocolate fluid (Koninckx *et al.*, 1992a) range from normal to very high.

Progesterone and progesterone resistance

In the endometrium, the induction of progesterone receptors by estrogens, and the secretory changes and the decidualization induced by progesterone, are well known and used for endometrial biopsy dating (Noyes *et al.*, 1975) as well as the diagnosis of luteal phase insufficiency (Thornburgh and Anderson, 1997). In basal endometrium, in contrast, progesterone does not induce secretory changes, but progesterone withdrawal stimulates the resumption of mitotic activity and endometrial regeneration (Coudyzer *et al.*, 2015).

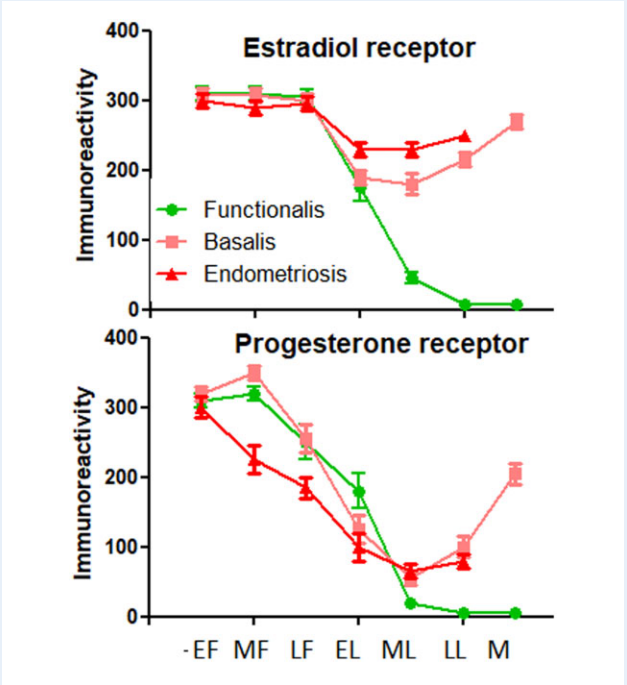

Figure 2. Steroid receptor immunoreactivity during the menstrual cycle. Estradiol and progesterone receptors in epithelial cells of the endometrial functionalis and basalis and of endometriosis during the early (EF), mid (MF) and late follicular phase (LF) and the early (EL), mid (ML) and late luteal phase (LL) of the menstrual cycle and during menstruation (M). Immunoreactivity scores are shown in arbitrary units. Adapted from Leyendecker, *et al.* (2002).

The similarity of the endocrine responses of basalis layer and endometriosis resulted in the suggestion that endometriosis might derive from basal endometrium (Leyendecker *et al.*, 2002) and progesterone resistance was suggested as the underlying mechanism (Leyendecker *et al.*, 2002). Following the demonstration of progesterone resistance in women treated for breast cancer (Simpson *et al.*, 1998), the exploration of endometrium and endometriosis began. In superficial endometriosis, a defect in stromal cells comprising impaired 17β-hydroxysteroid dehydrogenase-2 induction and estradiol inactivation results in the absence of progesterone-B receptor and progesterone resistance in epithelial cells. However, the endometrium of women with endometriosis has a variable impairment of 17β-hydroxysteroid dehydrogenase-2 induction, and thus a variable level of progesterone resistance, although secretory changes, assessed by histology, looked normal (Attia *et al.*, 2000; Bulun *et al.*, 2006). Unfortunately, these biochemical data are obtained from tissue homogenates and cannot resolve whether the endometrial resistance to progesterone is homogeneous or in some glands only, as described histologically in 1997 (Thornburgh and Anderson, 1997). Also, biochemical assays did not detect cyclical variations in progesterone receptors (Attia *et al.*, 2000), as observed by histology (Leyendecker *et al.*, 2002).

Molecular biological pathways of progesterone resistance have been described in several recent comprehensive reviews (Patel *et al.*, 2017; McKinnon *et al.*, 2018; Bulun *et al.*, 2019; Yilmaz and Bulun, 2019; Taylor *et al.*, 2021). The emerging picture shows a series of epigenetic abnormalities in the expression of progesterone receptors in stromal and epithelial cells of the endometrium and endometriosis, that harbor several cancer driver mutations. Remarkably, using passenger mutations, only the epithelium (not the stroma) of endometriosis was found to be clonal (Lac and Huntsman, 2018). In addition, many other mechanisms of progesterone resistance have been described, such as a series of epigenetic mechanisms (Wu *et al.*, 2006; Al-Sabbagh *et al.*, 2012; Dyson *et al.*, 2014; Meyer *et al.*, 2014; Chen *et al.*, 2020), the association with immunophilin FKBP52 deficiency (Tranguch *et al.*, 2007), progesterone receptor coactivator Hic-5 (Aghajanova *et al.*, 2009), dioxin (Bruner-Tran *et al.*, 2010), immunologic changes (Barrier, 2010), polycystic ovaries (Li *et al.*, 2014), mitogen-inducible gene 6 upregulation (Xu *et al.*, 2015), KRAS oncogene activation and SIRT1/BCL6 overexpression (Yoo *et al.*, 2017). Less attention was paid to the observations that progesterone resistance could be induced by peritoneal fluid from women with endometriosis (Chae *et al.*, 2016, McKinnon *et al.*, 2018) and that progesterone resistance of the endometrium can be a consequence of endometriosis (Fazleabas, 2010).

Subtle lesions and the pathophysiology of endometriosis

The importance of non-pigmented or subtle lesions has been debated since their description. With retrograde menstruation and the implantation theory in mind, these small and superficial lesions were considered early manifestations after implantation. Since most women have retrograde menstruation (Koninckx *et al.*, 1980c) containing viable tissue, implantation was suggested to be a physiological phenomenon occurring intermittently in all women (Koninckx, 1994). Subtle endometriosis was, therefore, considered to be normal implanted endometrium (Koninckx *et al.*, 1994), a concept still prevailing in 2005 (Evers *et al.*, 2005) although then emphasizing the importance of larger tissue fragments. However, other authors considered subtle lesions as pathology, especially in adolescent women (Martin *et al.*, 1989; Martin and O'Conner, 2003), since they were associated with pain and, by conscious pain mapping, 40% of subtle lesions were painful, which is similar to typical lesions (Demco, 2000). The clinical significance of subtle endometriosis continued to be debated and the asynchrony between endometrium and endometriosis lesions was not understood. In addition, microscopic endometrium-like tissues found in peritoneum, the bowel at a distance from deep nodules and in lymph nodes do not seem to develop into more severe lesions. More recently, the genetic epigenetic theory postulated that some additional events were needed to initiate the progression of these subtle lesions into more severe endometriosis (Koninckx *et al.*, 2020a).

Our view on subtle lesions recently changed when realizing that the active and proliferative histology of most red and many subtle lesions without secretory changes (Nisolle and Donnez, 1997) is incompatible with the peritoneal fluid concentrations of 5 ng/ml progesterone in the follicular phase and 50–100 ng/ml after ovulation. With these concentrations, superficial lesions should show secretory changes even in the follicular phase and *a fortiori* in the luteal phase: therefore, these lesions must have severe progesterone resistance. Moreover, the fact that

progesterone withdrawal does not occur in peritoneal fluid might explain why these lesions do not resume proliferation, as occurs in basalis endometrium.

Discussion

A first observation is the different worlds of clinical, histological and molecular biological research. Clinicians emphasize subtle, typical, cystic ovarian and deep endometriosis, but in the literature on progesterone resistance, the type of lesions investigated are hardly mentioned. Research seems limited to typical and a few deep endometriosis lesions. Although histologic images demonstrate inactive typical lesions and active deep endometriosis (Bulun *et al.*, 2019) this was not discussed. Not surprisingly, the small and difficult to biopsy subtle lesions have not been investigated specifically. Also, although very different from plasma concentrations, the peritoneal fluid concentrations of estrogens and progesterone are not mentioned in the discussion of progesterone resistance. It should, moreover, be realized that to collect peritoneal fluid a dedicated laparoscopic surgeon starting laparoscopy in slight anti-Trendelenburg position is needed.

Steroid hormone concentrations in superficial endometriosis lesions and the depth of diffusion of steroid hormones have not been measured and, thus, are not known. However, steroid hormones are apolar substances and are thus poorly soluble in water but highly soluble in fat. Therefore, they need to be transported in plasma by binding proteins with only a small free fraction. Polarity also explains why estriol (three hydroxyl groups) can be taken by mouth, but that estradiol (two hydroxyl groups) needs micronization to be absorbed sufficiently for clinical use. Estrone (one hydroxyl group) and progesterone (two oxygens but no hydroxyl group) are too apolar to be resorbed after oral intake. Diffusion through the cellular membrane, being a lipid bilayer, is very fast for small apolar substances, like steroid hormones, but slower for large or ionized molecules. Peritoneal fluid steroid hormones, therefore, are expected to diffuse readily into the underlying tissues creating a diffusion gradient until cleared by the circulation. The observations that deep endometriosis histology changes after 5 mm of depth (Cornillie *et al.*, 1990) and the biphasic frequency distribution of depth of endometriosis lesions with a nadir around 5 mm (Koninckx *et al.*, 2019a) suggest 5mm a reasonable depth for diffusion to occur. Superficial tissue layers thus must be influenced mainly by peritoneal fluid steroid concentrations, although local concentrations might vary with vascularization.

The proliferative aspect of subtle lesions without secretory changes, notwithstanding concentrations of 5 (follicular phase) to 100 ng/ml (luteal phase) of progesterone, suggests strong progesterone resistance. Instead of explaining why and how some deep endometriosis lesions acquire progesterone resistance, we need to understand how most deep lesions acquire progesterone responsiveness. That subtle lesions are not histologically heterogeneous, with areas with secretory changes and areas without, might suggest that they are already clonal lesions, as demonstrated for typical (Wu *et al.*, 2003), cystic ovarian (Tamura *et al.*, 1998; Yano *et al.*, 1999) and deep endometriosis (Mayr *et al.*, 2003) using laser microdissection (Zhao *et al.*, 2016). If subtle lesions already harbor cellular changes, it is less surprising that they elicit an immunologic and inflammatory response and that most of them are cleared (Wiegerinck *et al.*, 1993). Equally attractive is the hypothesis that subtle lesions result from implanted basalis endometrium since progesterone does not induce secretory changes, and progesterone withdrawal, needed to start proliferation, does not occur in the peritoneal cavity.

It is a surprise that subtle lesions occur so frequently, in up to 60–70% of women with pain or infertility (Koninckx *et al.*, 1991) However, frequencies vary with recognition by the surgeon (Martin *et al.*, 1989). This suggests a vulnerable, epigenetic progesterone-resistance switch that is turned on and off easily, for example in basal endometrium or by peritoneal fluid. This peritoneal fluid effect in women with endometriosis was demonstrated *in vitro* and suggested to be caused by the pro-inflammatory state (Chae *et al.*, 2016; McKinnon *et al.*, 2018). The fact that, in addition, the endometrium of women with endometriosis exhibits some progesterone resistance (Chae *et al.*, 2016), or can carry some cancer driver mutations (Suda *et al.*, 2018; Yachida *et al.*, 2021) could be interpreted as a hereditary predisposition to develop endometriosis. However, it cannot be excluded that the peritoneal fluid itself may cause endometriosis to develop in some women, for reasons that are currently unknown. An alternative hypothesis is that only some endometrial glands have progesterone resistance, as observed histologically (Thornburgh and Anderson, 1997). Heterogeneity of glands might explain the biochemically variable degree of progesterone resistance in the endometrium of women with endometriosis (Bulun, *et al.*, 2019). However, it cannot be excluded that the endometrial progesterone resistance is not the cause but the consequence of endometriosis, the result of endometriosis cells that migrated to the endometrium (Weimar *et al.*, 2013).

The steroid hormone concentrations in peritoneal fluid make it logical that bleeding in endometriosis lesions was rarely observed during laparoscopy and by microscopy. As concluded by Colgrave *et al.* (2020), 'propositions that endometriotic lesions bleed during menstruation need to be re-considered'.

The concentrations of steroid hormones in peritoneal fluid are also important for understanding medical therapy of endometriosis with oestro-progestagens, progestogens only or GnRH therapy (Donnez and Dolmans, 2021). These therapies induce ovarian suppression with steroid hormone concentrations in peritoneal fluid probably comparable to plasma concentrations. Unfortunately, these concentrations cannot be measured since the volumes of peritoneal fluid are too low to be collected. The clinical efficacy of medical therapy (Giudice, 2010; Bedaiwy *et al.*, 2017) has been understood as an individually variable (Becker *et al.*, 2017) lack of estrogens during GnRH therapy and a progesterone effect when taking oestro-progestins. However, it seems unlikely that progestins in medical treatment can compensate for or override the decrease in the high progesterone concentrations that occur during the natural cycle. However, it cannot be excluded that synthetic progestins could have a specific effect on endometriosis since, in comparison with progesterone, progestins have a lesser effect on the endometrium (Clauberg test) and a greater effect on the hypothalamus-hypophysis (ovulation inhibition effect). Therefore, the effect of oestro-progestins or progestins only might instead be linked to the low estrogen concentrations in peritoneal fluid, as indirectly supported by the decrease in the mitotic index in red lesions after GnRH or lynestrenol therapy (Donnez *et al.*, 1996). The role of ethinylestradiol can only be speculated upon. Ethinyl estradiol is probably not specifically important for medical therapy since the clinical effect or progestagens only and oestro-progestins is rather similar. However, it

may be that ethinyloestradiol diffuses poorly into the peritoneal cavity because of the mesothelial cells. This, unfortunately, will be difficult to demonstrate because of the low volumes of peritoneal fluid, the technical difficulties of the ethinylestradiol assay, and the low concentrations of ethinylestradiol owing to low plasma binding. To understand medical therapy, it seems important to have data on the effects on endometriosis tissue as well as plasma and peritoneal fluid concentrations of ethinylestradiol and synthetic progestins.

We did not discuss steroid hormone concentrations in the uterus and the endometrium: they are considered to be similar to plasma concentrations. However, countercurrent exchanges between the ovarian vein and artery occur (Einer-Jensen and Hunter, 2005), which signal directional sperm transport to the side of ovulation (Cicinelli, 2004; Mueller *et al.*, 2006). Also, the increased uterine steroid concentrations after the application of drugs in the upper third of the vagina (Cicinelli *et al.*, 2004; Cicinelli, 2008) was explained by counter-current exchanges.

Conclusion

Most subtle superficial endometriosis lesions are histologically proliferative and must have a strong progesterone resistance, considering the progesterone concentrations in peritoneal fluid. The mechanism of resistance is unclear and the hypotheses vary from being basal endometrium, to an effect of peritoneal fluid, to genetic and epigenetic changes in some endometrial glands, which might be clonal developments of basal endometrium. The endometriosis-associated progesterone resistance of the endometrium can be viewed both as a cause and a consequence of endometriosis. However, to understand what is cause and what is consequence, we should have data on women who are going to develop endometriosis. The similarity in cyclical changes of estrogen and progesterone receptors in basal endometrium and superficial endometriosis lesions is unexpected, considering that the former is influenced by plasma and the latter by peritoneal fluid concentrations. Finally, the mechanism of action of medical therapy of endometriosis needs to be interpreted using the knowledge that peritoneal fluid is an ovarian exudate with high concentrations of estrogens and progesterone. Specific steroid hormone concentrations in the endometrium resulting from counter-current systems also need to be explored further.

Acknowledgements

We do thank Dr Gebhart Leyendecker for important discussions contributing to this manuscript.

Authors' roles

The manuscript, triggered by the recent discussion in *Human Reproduction* on progesterone resistance, was written by P.K. and reviewed and updated by all authors.

Funding

No specific funding was received.

Conflict of interest

None of the authors has a conflict of interest to declare.

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