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Introduction

This theory of transition from endometrial or other stem cells to endometriosis is an extension of the endometriotic disease theory with both endometrium and endometriosis coexisting in the same patient as observed by Sampson (Am J Path. 1927, 3:93-110.43). The genetic-epigenetic theory is not dependent on the cell of origin or method of dissemination. The set of genetic and epigenetic incidents transmitted at birth are hereditary aspects that predispose to the endometriosis-associated changes in the endometrium, immunology, and placentation. However, to develop typical, cystic ovarian or deep endometriosis lesions, a variable series of additional transmissible genetic and epigenetic incidents are required to occur in a precursor cell. Subtle lesions are viewed as endometrium in a different environment until additional incidents occur. Typical cystic ovarian or deep endometriosis lesions are heterogeneous and represent three different diseases.

Published abstract.

Objective: To study the pathophysiology of endometriosis.

Design: Overview of observations on endometriosis.

Setting: Not applicable.

Patient(s): None.

Interventions(s): None.

Main Outcome Measure(s): The hypothesis is compatible with all observations.

Result(s): Endometriosis, endometrium-like tissue outside the uterus, has a variable macroscopic appearance and a poorly understood natural history. It is a hereditary and heterogeneous disease with many biochemical changes in the lesions, which are clonal in origin. It is associated with pain, infertility, adenomyosis, and changes in the junctional zone, placentation, immunology, plasma, peritoneal fluid, and chronic inflammation of the peritoneal cavity. The Sampson hypothesis of implanted endometrial cells following retrograde menstruation, angiogenic spread, lymphogenic spread, or the metaplasia theory cannot explain all observations if metaplasia is defined as cells with reversible changes and an abnormal behavior/morphology due to the abnormal environment. We propose a polygenetic/polyepigenetic mechanism. The set of genetic and epigenetic incidents transmitted at birth could explain the hereditary aspects, the predisposition, and the endometriosis-associated changes in the endometrium, immunology, and placentation. To develop typical, cystic ovarian or deep endometriosis lesions, a variable series of additional transmissible genetic and epigenetic incidents are required to occur in a cell which may vary from endometrial to stem cells. Subtle lesions are viewed as endometrium in a different environment until additional incidents occur. Typical cystic ovarian or deep endometriosis lesions are heterogeneous and represent three different diseases.

Additional endometriosis concepts and theories are at:
www.EndometriosisConcepts.com
www.danmartinmd.com/files/endotheory.pdf
The PDF covers concepts and theories beginning about 1855 BC with an increased focus in 1860 with the first microscopic description of what we now call endometriosis.

The genetic-epigenetic and endometriotic disease theories are theories of transition from endometrial or other stem cells to endometriosis. It is not dependent on cell of origin or method of dissemination. Theories can be divided into:

- Cell of Origin
  - Endometrium as Müllerian Tissue - Degree of Differentiation
    - Whole Tissue Endometrial Fragments
    - Precursors in normal whole tissue endometrial fragments
    - Precursors in traumatized endometrium
    - Mesenchymal Cells
    - Stromal Stem Cells
    - Epithelial Stem Cells
  - Embryonic Müllerian Remnants
    - Organized Fragments
    - Stem Cells
    - Müllerian Remnants (any congenital)
    - Müllerianosis (organoid)
    - Mülleriosis (non-organoid and projected to include transition)
  - Metaplastic Theories (non- Müllerian)
    - Peritoneal / Coelomic / Mesenchymal Stem Cells
    - Bone Marrow Stem Cells
    - Endometrial Stem Cells
- Dissemination (Metastasis)
    - Retrograde Menstruation
    - Hematogenous Dissemination
    - Lymphatic Dissemination
    - Traumatic / Surgical Dissemination
    - Embryonic Dissemination
      - The primary Müllerian area is in the usual location, not disseminated.
      - A theoretical secondary Müllerian System is used to explain dissemination.
        - Pelvic peritoneal area
        - Other body areas
- Transition

  The transition from endometrium to endometriosis appears to hold the most potential for future research and therapeutic options and is the subject of *Pathogenesis of endometriosis: the endometriotic disease or the genetic-epigenetic theory*. Transition involves the cellular, histological, biochemical, immunological, epigenetic, genetic, and other changes that distinguish endometriosis from the endometrium. Those changes involve the local environment, inflammation, epigenetic changes, genetic changes progenitor cell differentiation, biochemical changes immunologic changes, apoptosis, autophagy, reactive oxygen species, fibrosis, muscular metaplasia, macrophage migration inhibitory factor, clonality, microRNA, signaling, nerve activation, cancer-associated driver mutations, fibroblast to myofibroblast transdifferentiation, neurogenesis, angiogenesis, genetic dysregulation and more that are covered in this document.

Author's Draft

Pathogenesis of endometriosis: the endometriotic disease or the genetic-epigenetic theory

Running title: Pathogenesis of endometriosis

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Abstract

Endometriosis or endometrium like tissue outside the uterus has a variable macroscopical appearance and a poorly understood natural history, Growth is generally stimulated by estrogens and inhibited by progestogens. It is a hereditary disease with many biochemical changes in the endometriotic lesions which are clonal in origin. It is associated with pain and infertility, with adenomyosis, with changes in the junctional zone and in placentation, with numerous immunologic and other changes in plasma and in peritoneal fluid and with a chronic inflammation of the peritoneal cavity.

The Sampson hypothesis of implanted endometrial cells following retrograde menstruation, angiogenic spread or lymphogenic spread, cannot explain all observations. Also the metaplasia theory cannot explain all observations if metaplasia is defined as cells with reversible changes and an abnormal behaviour/morphology because of the abnormal environment.

To explain all observations on endometriosis with one hypothesis we propose a poly-genetic/poly-epigenetic hypothesis. The set of genetic and epigenetic incidents transmitted at birth could explain the hereditary aspects, the predisposition and the endometriosis associated changes in the endometrium, in immunology and in placentation. In order to develop typical, cystic ovarian or deep endometriosis lesions a series of additional transmissible genetic and/or epigenetic incidents are required to occur in a body cell which may vary from endometrial to stem cells. Subtle lesions can be viewed as the expression of the stress of the environment until additional incidents start their development into typical, cystic ovarian or deep endometriosis, which represent 3 different diseases. Extra pelvic endometriosis can be explained in a similar way.

Keywords: endometriosis, pathogenesis, classification, heredity, genetics, epigenetics

Capsule

A poly-genetic/poly-epigenetic pathogenesis can explain all observations of endometriosis. Typical, cystic ovarian and deep endometriosis are 3 different diseases.

Introduction

Endometriosis is an enigmatic disease and the pathophysiology remains debated with hypotheses, theories and speculation. The pathophysiology of endometriosis is important, since understanding the mechanisms involved will help to orient prevention, diagnosis and therapy.

For endometriosis there is no animal model which is sufficiently similar to the human myometrium, junctional zone (JZ) and endometrium. Similarly there is no animal model to permit to study adequately human placentation and pregnancy disorders as pre-eclampsia. Therefore, our views on the pathophysiology of endometriosis are based on clinical, histological and biochemical observations and on research of endometriotic tissues.

Several hypotheses have been formulated to explain the pathophysiology of endometriosis. Each of them were logical and consistent with the observations when formulated. Additional observations can reinforce a hypothesis or make new theories necessary. Only when the exact mechanisms are elucidated, a hypothesis becomes like a law of physics.

We therefore will summarise the observations made on endometriosis, followed by a description of the theories of the pathophysiology of endometriosis as they were sequentially developed over the last century. Finally we will discuss the clinical implications for prevention, diagnosis and therapy of the pathophysiology of endometriosis as understood today.

Observations on endometriosis

Definition of endometriosis lesions.

In the 19th century endometrium like tissue was described in the myometrium by Rokitanski (1) and in the rectovaginal septum by Cullen who called this entity an adenomyoma (2-5). Soon thereafter similar lesions were described in the bladder (6) and endometrium like tissue was found in 'hemorrhagic (chocolate) cysts in the ovaries' in1922 (7). The word endometriosis was introduced by Sampson in 1927 (8, 9).

"Endometrium like glands and stroma outside the uterus" has become the definition of endometriosis, without necessarily specifying the nature of the clinical pathology. Also our progresses in genetics, epigenetics and molecular biology did not affect this definition based on microscopical appearance. This explains why entities, such as stromatosis (10) and Müllerianosis (11), are not considered endometriosis notwithstanding similarities.

Macroscopical and microscopical appearances and prevalences of endometriosis lesions.

The first reports described severe clinical lesions as adeno-myotic nodules (1-5) and cystic ovarian lesions (7). These observations were subsequently confirmed and similar lesions were described in many other different locations in the abdomen and the thorax. Also smaller black puckered 'powder burn' superficial peritoneal lesions in sclerotic areas were repetitively observed during surgery. Later these were called "typical' lesions. Only after the introduction of diagnostic laparoscopy in the seventies, the high prevalence of these superficial typical lesions in women with pain and/or

infertility was realized. Although non-pigmented lesions with glands and stroma had been described before (7, 12-15) the observation of frequent non-coloured peritoneal lesions in 1986 (16) and the observation that retrograde menstruation occurred in almost all women (17, 18) started the search for early and small lesions. Together with other lesions such as polypoid and flame like lesion, they were subsequently called subtle lesions (19, 20). Also microscopical endometriosis lesions were found in the peritoneum and later in lymphoid glands and in the bowel at distance from deep endometriosis (21).

When during CO2 laser excision of endometriosis some lesions were found to be much deeper, "deep endometriosis" was introduced in 1990 to describe adenomyosis externa lesions which were associated with severe pain (22). These are microscopically similar to the Cullen's (2-4) adenomyoma lesions with endometrial glands and stroma in fibromuscular tissue (22). The definition of deep endometriosis as lesions deeper than 5mm under the peritoneum was suggested since the frequency distribution of depth of lesions in women with pain or infertility (23) indicated 2 populations overlapping at 6 mm of depth (Fig 1). A second argument was that at depths deeper than 5 mm glands were more active (24) and this was considered compatible with the depth where deep endometriosis had escaped from the inhibition by the high progesterone concentrations in peritoneal fluid (25). However, this change from a histological definition to a 5 mm depth definition has caused confusion. Since the 2 populations overlap, some typical lesions fit the definition of deep endometriosis; in addition the inaccurate surgical estimation of depth permits the inclusion of many more typical lesions. Today we would consider what we described as type I lesions of 1992 (24) as deeper typical lesions. Unfortunately solid histological data do not exit to substantiate this. 'Adenomyosis externa' would have been a better definition of deep endometriosis (26) since adenomyosis externa lesions are generally unique (occasionally 2 and rarely 3 in number) and larger than 1 cm in diameter, mainly in the pouch of Douglas and, with frequent invasion into the muscle of the bowel wall; (deep) endometriosis nodules, although not described explicitly as adenomyosis externa, are found in the diaphragm; they occasionally invade nerves (27); they have a neurotropic effect (28, 29), and some 20% are associated with lymph node involvement (30, 31).

The natural history and epidemiology of endometriosis

Endometriosis is often considered a progressive disease since larger lesions must have developed over some period of time before they are diagnosed. However, progression has been challenged recently (32). Progression has not been observed clinically, not from subtle and not from typical to cystic lesions or deep lesions and not from cystic lesions to deep lesions. The only evidence is that superficial endometriosis , assessed by points in the rAFS classification, regressed and progressed slightly in 42% and 29% respectively (33). In addition, when diagnosed most lesions seem clinically to

no longer rapidly progress. For typical lesions absence of progression of mature lesions is consistent with their burnt-out aspect on pathology. Most cystic lesions can remain unchanged over longer periods as demonstrated by long term observation with repeat assessments with ultrasound. Most, rectovaginal deep endometriosis lesions that were not operated did not grow rapidly (clinical observations) (34).

Endometriosis is often considered a recurrent disease (35) although most studies deal with recurrence of symptoms instead of recurrence of endometriosis lesions (36). Recurrence rates of cystic ovarian endometriosis following stripping vary with the surgeon (37) and with the technique used. Recurrence rates are less than 20% within 6 months but increase with time (38, 39). Recurrence rates of deep endometriosis lesions after complete deep endometriosis excision are rare and less than a few percent (36) The recurrence rates of typical lesions and subtle lesions are estimated to be much higher but the data are limited. In addition it is unclear whether recurrences are a consequence of incomplete excision instead of the formation of new lesions.

The epidemiology of endometriosis is unclear since the laparoscopic diagnosis, especially of subtle and deep lesions varies with the expertise of the surgeon with subsequent diagnostic uncertainties in hospital based discharge records (40). Clinical observation by deep endometriosis surgeons suggests that the prevalence and severity of deep endometriosis has markedly increased during the last 20 years (34). Subtle endometriosis lesions decrease with age whereas typical, cystic and deep lesions increase with age, at least till menopause (34).

The peritoneal cavity is the most frequent localization of endometriosis lesions, which are more frequent on the left side of the pelvis (41, 42) and on the right side of the diaphragm as expected from the circulation of peritoneal fluid.

Endometriosis is a heterogeneous disease.

Although most women with deep endometriosis have severe pain especially during menstruation (43) it is remarkable that some (estimated at 5%) large and visible lesions are not painful during palpation. Although most deep endometriosis lesions do not (or very slowly) progress over time when diagnosed; occasional lesions can be fast progressive (unpublished observations). Progestagenic therapy and pregnancy stop growth and/or cause decidualisation of the endometrium and decrease endometriosis associated pain as expected. During pregnancy however, some endometriosis lesions behave differently causing polypoid bladder lesions (44), bowel (45, 46) or bladder (47) perforations or peritoneal bleedings (48). Although endometriosis is considered an hormonally responsive disease requiring estrogens to stimulate growth, bowel perforations occur

during estro-progestin treatment (49) and estrogen independent growth has been observed in postmenopausal women (50) and in men (51).

Heredity of endometriosis.

The risk of developing endometriosis is 6% to 9% higher in first degree relatives of women with endometriosis (52, 53) and 15% higher when they had severe disease (54, 55). Familial clustering of endometriosis in the human (56) and primates (57) is reasonably well demonstrated although not 100% conclusive (58). In twin sisters the prevalence (59-62) and the age of onset (63) of endometriosis are similar. It is estimated that hereditary factors account for some 50% of endometriosis (64-66).

However, genome wide scanning and linkage analysis did not identify unequivocally the genes involved and their coding errors (67). Linkage analysis found 2 aberrant loci but the LOD scores were too low for 1 major gene. Genome-wide association studies have identified 12 single nucleotide polymorphisms at 10 (68) or 15 (69) independent genetic loci. Most of these were more strongly associated with severe endometriosis (classes III/IV of the revised American Fertility Society) and they are located in DNA sequences known to play a role in the regulation of target genes (70), which have not yet been identified (69). A recent meta-analysis identified five novel loci, implicated in sex steroid hormone pathways, and five secondary association signals and 19 independent single nucleotide polymorphisms robustly associated with endometriosis (71). Other observations highlight gene polymorphism (72) or mitogen-activated protein kinase signaling (73). Thus we are far from understanding the mechanisms involved and from developing a diagnostic marker (74). Attractive is the first hit- second hit hypothesis of 1971 (75). If a second genomic hit in a carrier with a first hit, would express endometriosis, this can explain the hereditary character. The many studies that tried to identify a specific hereditary predisposition, especially those investigating detoxication failed (76).

Biochemical and molecular biological changes in endometriosis lesions.

All individual endometriosis lesions (77), especially deep (78) and cystic ovarian (79-81) endometriosis are clonal in origin and multiple lesions in one woman derive from different progenitor cells (77).

Local estrogen production within the lesion, aromatase activity and/or progesterone resistance were demonstrated in larger endometriosis lesions (82), microscopic and subtle lesions being too small for analysis. Progesterone resistance (83-90) was suggested as an argument for the basal endometrial origin of endometriosis (91). Numerous molecular biochemical changes exist such as mitogen-activated protein kinase (73), transcription-3 signaling (92), genetic variants expression(93) and the Hoxa10/HOXA10 gene(94), cytokines(95, 96) (97, 98), dentritic cells(99),

vitamin D (100), mast cells(101, 102), hypoxia inducible factor(103), high Mobility Group Box-1 and Toll-Like Receptor 4(104), matrix metalloproteinase promoter polymorphisms(105), galectin-3 expression(106), promoter polymorphisms of MMPs genes(107), progesterone receptor expression(108), GF-I (109), activing–A (110), Smad3/4 (111) or leptin (112) stimulated activation of aromatase activity and the expression of numerous cancer associated mutation (113). Interestingly, most of these changes are increasingly viewed as the result of genetic or epigenetic polymorphism or changes (82, 114-116).

Epigenetic changes, eventually during fetal life (117), have become a focus of interest over the last decade(118-122). They comprise methylation and demethylation of DNA (119, 123, 124), modifications in histone code in endometriosis tissue in comparison with the endometrium. Many aberrations have been described, leading to lots of speculation about mechanisms but without a comprehensive view yet.

Observations associated with endometriosis

A significant correlation of observations occurs when one causes the other, or when both are the consequence of a common factor, .

Association with pain and infertility

As discussed recently(21), it is unclear whether microscopical endometriosis in the peritoneum, in the bowel at distance from deep endometriotic nodules and in lymph nodes cause pain or infertility. Subtle lesions do not commonly cause pain given the high prevalence in women with infertility only (23). There is no direct evidence that they cause infertility. On the contrary, the luteinized unruptured follicle syndrome is associated with typical but not with subtle endometriosis lesions (125). Typical, endometriosis is estimated to cause minor pain in 50% of affected women but half of them are pain-free as estimated in women with infertility only (23) , Cystic ovarian endometriosis causes (severe) pain in over 80% and deep endometriosis causes (very severe) pain in the large majority of women (23). Notwithstanding the 30% to 50% cumulative pregnancy rates after surgical excision (126), it remains unclear whether and how typical and deep endometriosis cause infertility. That cystic ovarian endometriosis is a cause of infertility is not surprising since associated with adhesions.

Association with adenomyosis

In contrast with the widely held belief of the association of endometriosis with adenomyosis, the data demonstrating this association are limited (127) and the studies are small. Focal adenomyotic nodules are more frequent in women with deep endometriosis diagnosed by laparoscopy (128, 129). Studies based on imaging only, and thus limited to cystic ovarian

endometriosis or larger deep endometriosis, report a strong association of endometriosis and adenomyosis, defined as JZ thickening or diffuse adenomyosis or a focal adenomyotic nodule with prevalences of 80.6 % endometriosis in adenomyosis and 91.1 % of adenomyosis in endometriosis (130).

Associations with changes in the uterus

Several hundred minor biochemical changes in the endometrium (131-135) of women with endometriosis have been described. Contractility of the uterus is modified in women with deep endometriosis and/or adenomyosis (136). Endometriosis, especially cystic ovarian and deep endometriosis (137, 138), and adenomyosis (139, 140) is associated with abnormal placentation, insufficient physiologic changes in the spiral arteries, and an increased risk of preterm birth, small for gestational age (SGA) babies, and pre-eclampsia (141). Abundant retrograde menstruation (142) seems to be associated with endometriosis.

Association with changes in plasma

Numerous reports have identified immunologic changes in plasma of women with endometriosis (143-151). That the low NK activity in plasma remains low whereas the elevated CA125 concentrations return to normal after the surgical excision of deep endometriosis is an argument that the NK cell defect is a cause and the elevated CA125 a consequence of endometriosis (152).

Other reported changes comprise lymphocytes (153), prostaglandins (154) and insulin-like growth factor I (155).

Association with changes in peritoneal fluid

Estrogen and progesterone concentrations in peritoneal fluid are much higher than in plasma, especially after ovulation (25). Women with endometriosis and the associated luteinized unruptured follicle syndrome have much lower concentrations after ovulation. Since progesterone is known to inhibit growth of endometrium, the lower concentrations in the luteinized unruptured follicle syndrome were even speculated to permit the development of endometriosis as a consequence of infertility (17). Women with pelvic endometriosis have more and more activated macrophages and an increase of their secretion products in peritoneal fluid. Numerous reports describe changes in cytokines (156-160), growth factors, acylcarnitines, phosphatidylcholines, and sphingomyelins (161), uterine leucocytes(147), vascular epithelial growth factor (162, 163), other angiogenic factors (164-183) especially of the TGFb superfamily (184).

As expected from the low permeability of the peritoneum for larger molecules, the concentrations of CA125 and of glycodelins are elevated in women with endometriosis as a consequence of the local

inflammation and of the local secretion by endometrial cells, respectively (185). Interestingly glycodelins (185) decrease NK cell activity (186) which can be viewed as an auto-protective mechanism of the endometriotic cell.

Finally, abundant retrograde menstruation will cause retraction of peritoneal mesothelial cells, which thus facilitates the implantation of endometrial cells (187, 188).

Associated with dioxin and total body irradiation.

Dioxin (189-192) and total body radiation (193, 194), are suggested to be associated with endometriosis development. Both can have genomic or epigenetic (195) effects. In addition the endometriosis that develops after total body radiation in primates develops after a delay of 5 years which suggests a genomic effect.

Associated with cancer

Endometriosis seems associated with a higher risk of cancer as recently reviewed (196, 197). The association with ovarian cancer remains debated (198).

Associated with vaginal and pelvic infections

The low grade pelvic inflammation in endometriosis was recently considered as the consequence of an initial infection and subsequent sterile inflammation (144). High risk papilloma virus infection was found more frequently in ovaries of women with cystic ovarian endometriosis (199). The incidence of Escherichia coli in menstrual blood and of lower genital or vaginal infections was higher in women with endometriosis (200).

Definitions used for genetics, epigenetics, metaplasia and redundancy.

Genetics and epigenetics can be compared to a computer's hardware and software respectively. The chromosomes contain the genetic code, but 'programs' (epigenetics) regulate transcription, and translation to proteins and post-translation processing. These epigenetic 'programs' are 'influenced' by internal signals and external factors e.g. through methylation. Some of these epigenetic changes are stable and transmitted during mitosis others not.

Mistakes in the DNA sequence are chromosomal alterations, which can occur during cell division or as a consequence of noxious agents. Most DNA mistakes are repaired by the cell and if these mechanisms fail the cell becomes apoptotic and dies. However, if the cell survives, the changes persist and will be transmitted to the next generation of cells. Activation and repression of DNA transcription and of the subsequent translation is a complex process. Stable structural changes in these regulatory mechanisms are called epigenetics (201). However, different investigators ascribe different definitions to epigenetics(202) . Some such as the NIH Epigenomics Mapping Consortium

(203) use epigenetics to explain changes in gene expression; others use it to refer to transgenerational effects and/or inherited expression (204). In order to clarify our definitions we will use genetics to indicate irreversible and transmissible chromosomal or sequencing changes and epigenetics to indicate stable and transmissible non-DNA changes.

The function and the morphologic aspects of cells and tissue are the result of the sum of activation of the different molecular biological mechanisms in given cells with their specific genetic (chromosomal) and epigenetic characteristics in a specific environment. The microscopic aspect of cells and tissues can thus change either as a result of a changed environment (205) or as a consequence of genetic and/or epigenetic incidents. Metaplasia is often used as a descriptive word without reference to the underlying mechanism. In order to clarify our definitions we will use metaplasia to indicate potentially reversible changes of one cell type into another cell type (206).

Applied to the pathophysiology of endometriosis, it is important to know whether endometriosis cells are normal endometrium like tissue with a pathologic behaviour and appearance as a consequence of the abnormal environment outside the uterus or whether the abnormal behaviour requires a series of transmissible genetic or epigenetic incidents. The environment, however, can also be a factor inducing genetic and/or epigenetic changes e.g. through oxidative stress in the peritoneal cavity (207) or by bleeding in tissues.

Functional redundancy is a characteristic of many processes in a cell. Redundancy can be compared to a roadmap. In order to transport goods from A to B the shortest motorway can be used, or an alternative longer motorway, or primary roads or eventually secondary roads. If we reach our destination, this comes at a price: the journey can take longer and/or the maximal capacity of goods transported will be less. This explains that changes in morphology and/or function of a cell requires either sufficient genetic and/or epigenetic changes, and/or molecular biological changes induced by the environment , together with a level of stress, comparable to the capacity of transport of goods. Redundant mechanisms thus can mask the phenotypic effect of mutations and epigenetic changes. (208).

A genetic and/or epigenetic alteration and a clonal origin do not exclude heterogeneity within an endometriosis lesion as demonstrated for breast cancer (209) and other cancers as recently reviewed (210, 211).

The theories or hypotheses on the pathogenesis of endometriosis

The cause of the adenomyoma's described by Cullen (2-5) was initially suggested by Meyer(212) and later by Gruenwald(213) to be due to metaplasia. Another hypothesis was their development from

Müllerian remnants (214) . Later Sampson (8, 12, 215) suggested retrograde menstruation as the etiology of cystic ovarian endometriosis.

Retrograde menstruation and implantation theory.

Retrograde menstruation is an attractive hypothesis to explain the pathophysiology of endometriosis, since menstrual fluid contains living cells, demonstrated already in 1927 (216), with implantation and growth potential as demonstrated in 1958 by subcutaneous injection(217), by growth in vitro and later on the chicken allantoic membrane (218). For the latter tissue integrity is important (218). In addition the implantation of endometrial fragments was directly observed (219) in a neonate with the McKusick-Kaufman syndrome; also pelvic endometriosis is more frequently found in the pouch of Douglas and on the left side which is compatible with gravity and with the clockwise circulation of peritoneal fluid.

Microscopical and subtle lesions are considered the initial stages after implantation. Neonatal menstruation (220-223), occurring in some 5% of neonates (224-230), especially in postmature and SGA babies, might explain premenarcheal and severe adolescent (231, 232) endometriosis. The abnormal behaviour of endometriosis lesions and the aromatase activity or progesterone resistance are speculated to be caused by an abnormal environment, by immunology or by implantation of basal endometrium.

The retrograde menstruation and implantation theories cannot explain all clinical manifestations(233). First it is unclear why not all women develop endometriosis considering that retrograde menstruation occurs rather systematically in all women. Second this theory fails to explain why endometriosis progresses to typical, cystic and deep lesions in some women only. Third this hypothesis is incompatible with the occurrence of endometriosis in women without a uterus and a Rokitansky-Mayer-Küstner syndrome (213) and in men (234). Fourth this concept is not compatible with the clonal aspect (77) of endometriosis lesions. For these reasons the retrograde menstruation and implantation theory has to be dismissed since it is at least incomplete.

Metaplasia theories

As early as in 1942 the incompleteness of the implantation theory was realised and complemented with the mesothelial cell metaplasia theory (213). More recently other metaplastic theories were formulated including metaplasia of peritoneal stem cells, of endometrial stem cells after retrograde menstruation and more recently of bone marrow cells (235-237). These concepts were supported by the frequent mesothelial-mesenchymal-transitions (MMT) and the role of bone marrow cells in peritoneal repair.

Metaplasia theories can explain the occurrence of endometriosis in men and in women without a uterus. If metaplasia is defined as metaplastic changes without permanent and transmissible genetic and/or epigenetic changes, the metaplasia theory can neither explain clonality nor why and in whom endometriosis lesions develop. If however, metaplasia is used to indicate stable and transmissible genetic or epigenetic changes this theory becomes similar to the genetic-epigenetic theory.

The original cell

The endometrium (220-223) or endometrial stem cells (238, 239), from retrograde menstruation after menarche or at birth, are obvious candidates to be the original cell. Since women without a uterus and even men can develop endometriosis, pluripotent stem cells from the peritoneal cavity (228, 235, 236, 240-248) are another possibility. In addition endometriosis may be derived directly from bone marrow cells as suggested by the observations of their direct involvement in endometrium and endometriosis (237, 249-252) and in peritoneal repair after surgery (253). Platelets (254) are suggested to play a role in this process. Recently a specific cell in the endometrium, called pale cells (255, 256) because of their appearance, and cells remaining from embryonic development (257-259) were speculated to be involved in the development of endometriosis.

The genetic/epigenetic theory or the endometriotic disease theory (EDT).

The endometriotic disease theory (Fig 2) postulated (260) that specific genetic incidents are required for the development of a disease with clinical symptoms, i.e. typical, cystic or deep endometriosis. Microscopical and subtle endometriosis were considered early lesions similar to endometrium without additional genetic changes and were considered to occur intermittently in all women (261). It was suggested to use ‘endometriosis for these ‘normal’ subtle endometriosis cells and ‘endometriotic disease’ for lesions with genetically or epigenetically abnormal cells and clinical symptoms. The development into typical, cystic or deep lesions was postulated to vary with the type of genetic or epigenetic incidents. Some subtle lesions thus contain ‘normal’ cells that will regress spontaneously whereas other will progress to more severe disease. Unfortunately today we cannot distinguish between both types of subtle lesions (21).

The genetic/epigenetic theory is an update of the EDT by adding epigenetic changes and redundancy to genetic changes. These genetic and epigenetic changes are more likely to occur in the pelvic peritoneal cavity because of the oxidative stress of retrograde menstruation (207)and eventually as the consequence of an infection (144, 199, 200). In addition, the endometrium like cells with their incidents remain in the peritoneal cavity, in contrast with the eutopic endometrium, one of the fastest growing tissues, will be eliminated each month.

The EDT or genetic/epigenetic theory is compatible with all observations made on endometriosis. Subtle or microscopic lesions will progress to more severe lesions only if additional incidents happen. This is compatible with the clinical suggestion that typical, cystic and deep endometriosis are 3 different diseases. It is fully compatible with all hereditary aspects and predisposition of endometriosis and explains why dioxin and total body radiation could increase the risk of endometriosis. It is compatible with the observation that deep and cystic ovarian endometriosis are clonal in origin, with clinical heterogeneity of endometriosis lesions, and with the molecular changes observed in endometriosis lesions and with the observed genetic and/or epigenetic aspects (67). The many molecular abnormalities in the endometrium of women with endometriosis are explained as an expression of the genetic and/or epigenetic changes transmitted at birth. Also the increased risk of pregnancy complications, the associated infertility and some immunologic alterations can be viewed as the expression of these changes inherited at birth. Even subtle lesions can be viewed as the expression of inherited changes in an abnormal environment.

It should be stressed that this view does not exclude that some observed associations are the consequence of the development of the disease. Also the final incidents starting the disease are additive to other incidents that might have occurred previously. It can explain the high prevalence in the peritoneal cavity and the increasing prevalence with age of typical, cystic and deep endometriosis. Bleeding and remodeling in the endometriosis lesions (262) are candidates to trigger additional genetic or epigenetic incidents. That many of the molecular biological alterations described in endometriosis lesions are increasingly viewed as the result of genetic and/or epigenetic incidents lends further support to the hypothesis

Some observations are more difficult to explain although they do remain compatible with the genetic/epigenetic theory. The Induction of deep endometriosis like the lesions that develop in the baboon by transplantation of functional and basal endometrium together with myometrium and junctional zone cells (263) is intriguing. First, it is unclear whether the baboon is a useful model since deep endometriosis has not been observed in primates unless after dioxin administration (264); secondly it is unlikely that intact blocks of myometrium and JZ/myometrium are the cause of deep endometriosis in the human. Also intriguing is the role of the increased nerve density and their modulation over time (265, 266). This interaction with the body can be understood both as a cause and as a consequence.

Today, we can only speculate which, which combination and how genetic and/or epigenetic incidents lead to typical, cystic or deep or extra-genital forms of endometriosis.

Growth and maturation of typical, cystic and deep endometriosis lesions.

The growth of endometriosis cells obviously varies with the local environment of plasma or of the peritoneal cavity and thus with the many differences in hormones, immune factors and growth factors. As an example, the high glycodelin concentrations in peritoneal fluid might protect early lesions from NK cell attack (267, 268). It is unclear why growth of most endometriosis lesions seems to be self-limiting with little growth of most lesions after they are clinically diagnosed.

Endometriosis lesions can be associated with recurrent local micro-bleedings similar to menstruation causing menstrual pain in deep endometriosis and probably in typical and cystic ovarian lesions. These bleeding episodes are repeated tissue injuries that are followed by repair and fibrosis, which are believed to play a role in the growth of endometriosis (269, 270). It is unclear whether these bleeding episodes are necessary for growth and why growth of most lesions is self-limiting. These micro bleedings episodes may trigger additional genetic and/or epigenetic incidents through inflammation and oxidative stress. Interestingly micro-traumas are also observed in the endometrial-myometrial JZ (255), consistent with the view of the archimetra (130, 271).

Clonality of endometriosis lesions was demonstrated in glands and surrounding stroma. It therefore is unclear how the smooth muscle and the fibrosis surrounding deep endometriosis lesions must be viewed. We suggest that the fibrosis does not belong to the disease and that fibrosis is composed of normal cells with reversible “metaplastic” changes induced by the endometriosis lesion through cell-cell interaction (272). This suggestion is based on the observation that recurrence rates after (often incomplete) excision and after large bowel resections for deep endometriosis are not strikingly different.

Clinical implications of the EDT or genetic-epigenetic theory.

Most subtle or microscopic lesions are normal endometrium like cells that will likely resolve. However, these were not studied with stromal, epithelial or other markers. When in some of these cells, before or after implantation genetic and/or epigenetic changes occur in addition to the hereditary incidents present, the development of the disease endometriosis can start. Typical, cystic and deep lesions are viewed as benign tumours, which following a period of growth generally no longer progress rapidly and do not recur after complete excision. However, new lesions can be formed after new incidents, and the probability of this happens increases with the cumulative genetic and/or epigenetic abnormalities transmitted at births and acquired during lifetime. Adolescent endometriosis becomes a genetic and/or epigenetic incident early in life.

The associated subfertility with monthly fecundity rates below 10% similar to women with unexplained infertility may be at least partly the consequence of the inherited defects, and not necessarily the consequence of endometriosis. Also the pregnancy associated problems as placenta praevia, hypertension and SGA babies, which do not improve after deep endometriosis excision (273), seem to be a consequence of the inherited defects rather than of the endometriosis (138).

Endometriotic lesions are heterogeneous. While most lesions require estrogens for their growth, estrogen independent growth exists as observed in postmenopausal women (50). Heterogeneity between lesions is consistent with the observation that occasionally some deep lesions can progress rapidly, that some do not cause pain and that some behave differently during pregnancy.

That recurrence rates are not markedly different after excision of deep endometriosis from the bowel and after bowel resection suggest that endometriosis lesions triggered by the cumulative genetic and/or epigenetic incidents might induce cell-cell mediated metaplastic changes in the 'normal' surrounding fibrosis. If confirmed this becomes an argument against being too aggressive during surgery.

A classification of endometriosis should reflect that microscopic, subtle, typical, cystic, deep and extra-genital endometriosis need to be considered as 4 or more different entities. Also the pathophysiology of adenomyosis and its relationship with endometriosis can be explained with this genetic/epigenetic concept (127).

Prevention of genetic/epigenetic incidents triggering the disease, can only be speculated about. However it seems attractive to postulate that reduction of repetitive stress by retrograde menstruation and micro-trauma's in the lesions, and prevention of pelvic inflammatory diseases may be useful in this regard.

Discussion

Many words in the endometriosis literature are not clearly defined and the same words are used to describe different things. This confusion stems from the fact that the meaning of words often changed over time and especially after new clinical and molecular-biochemical observations were added to the initial clinical, macroscopical and microscopical descriptions. Stem cell research demonstrated that changes during cellular differentiation can be stable and transmitted, but reversible. The same ambiguity exists in oncology. It is unclear whether 'metaplastic' changes preceding the development of cancer are reversible or irreversible and whether they increase the risk that another incident start the development of a malignant tumour. Metaplasia was introduced as a descriptive histological observation. Later we understood that the underlying mechanisms could be reversible or irreversible changes and that

both can be transmitted. Metaplasia is currently used to indicate both the (reversible) expression of environmental stress and the expression of stable genetic or epigenetic damage. Epigenetics is used for both reversible and stable changes that are transmitted after cleavage. When transmitted at birth they are called the epigenetic trait (274). The definition of deep endometriosis changed from microscopically adenomyotic and macroscopically spherical lesions to lesions deeper than 5mm under the peritoneum. Since the populations overlap (Fig I) some (conical) typical lesions became considered as deep lesions. Progression and regression are poorly documented since repeat laparoscopies cannot be performed for ethical reasons. We only recently realised the significant stress that the CO2 pneumoperitoneum, the surgical trauma and blood (188) causes to the mesothelial cells. Recurrence is used to indicate recurrence of pain, recurrence of the endometriosis, or requirement of repeat surgery. However, it is rarely clear, whether recurrence of endometriosis after excision is due to new lesions or to lesions missed during surgery or due to incomplete surgery.

The implantation theory was valid when formulated but is inadequate without additional clarification of how endometrial tissue converts into endometrioid tissue. The metaplasia theory when formulated in 1942 was a histological observation and did not consider genetic or epigenetic changes. Today the double meaning of metaplasia continues to create confusion since it is used to indicate both reversible and stable changes. The genetic/epigenetic theory adds epigenetics to the endometriotic disease theory. Considering typical, cystic and deep endometriosis as the consequence of a series of genetic and epigenetic incidents is compatible with all observations made until now. However, it will remain a theory until disproven by a contrary observation. Today we do not yet understanding exactly how and which irreversible genetic and/or epigenetic incidents, which hereditary incidents and which environmental factors cause a specific endometriosis lesion. Moreover, redundancy of many biological processes adds to the difficulty of identifying minor changes which remain without visible clinical effects. Similar to concepts of tumour biology it is important to distinguish between hereditary changes transmitted between generations, and additional local cellular incidents which will either express the disease or facilitate the expression of the disease after additional incidents later. This is especially important when considering the floating mesothelial and stem cells in peritoneal fluid: a first 'facilitating' incident indeed could explain the subsequent development of various forms of endometriosis in different locations. The exact mechanisms however remain unknown (275, 276).

Deep, peritoneal, and ovarian endometriosis often occur in the same women (277). This is not surprising since the common endometrium like appearances suggest some common genetic and/or epigenetic incidents. It is suggested that the same mechanisms apply to adenomyosis and to abnormalities of the junctional zone which explains the relationship between endometriosis,

defective physiologic changes or transformation of spiral arteries in early pregnancies, and pre-eclampsia and SGA babies. It also is compatible with the archimetra concept (278).

Similar to uterine myomas, endometriosis lesions can remain dormant without progression for longer periods of time. Although the mechanisms of reactivation are not understood deep endometriosis seems to be reactivated by trauma such as by IVF related needle punctures for oocyte pick-up, triggering subsequent development of severe lesions and even a frozen pelvis (32) as frequently observed.

The genetic/epigenetic theory can explain heterogeneity between different lesions; it also is compatible with micro-heterogeneity in one specific lesion similar to the micro-heterogeneity in sialic acid content and thus of half-lives of gonadotropins.

The genetic/epigenetic theory is also important for our views on non-human models of induced endometriosis, in both primates and rodents. These models remain valid to study the effect of abnormal environments on (normal) endometrium. Transplantation of human endometriosis into SCID/nude mice could be a model to study the development of (abnormal) endometriotic tissue in a normal or controlled environment.

In conclusion, the genetics-epigenetics theory permits to explain and understand all observations of this enigmatic disease called endometriosis from heredity, clonality to inflammation, mutations, progesterone resistance, aromatase and many other findings associated with the disease by the time typical, deep or cystic endometriosis have developed. Elucidating the mechanisms and pathways involved will hopefully permit the development of more specific means of prevention and therapy of this common and ravaging disease.

References in Publisher's edition.

NOTE: These are not numbered the same as the references in the draft above.

https://www.fertstert.org/article/S0015-0282(18)32135-6/references

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Video introduction

https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/39465-26548

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Conflicts of interest

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

Fig 1. Frequency distribution of the depth of endometriosis lesions in women with infertility, with pain and with pain and infertility as observed during surgical excision. The data extracted from the Leuven database spanning the years 1990 – 2010 confirm and extend previous data (23) and illustrate the overlap between the 2 populations of more superficial (typical lesions) and deeper adenomyosis externa lesions.

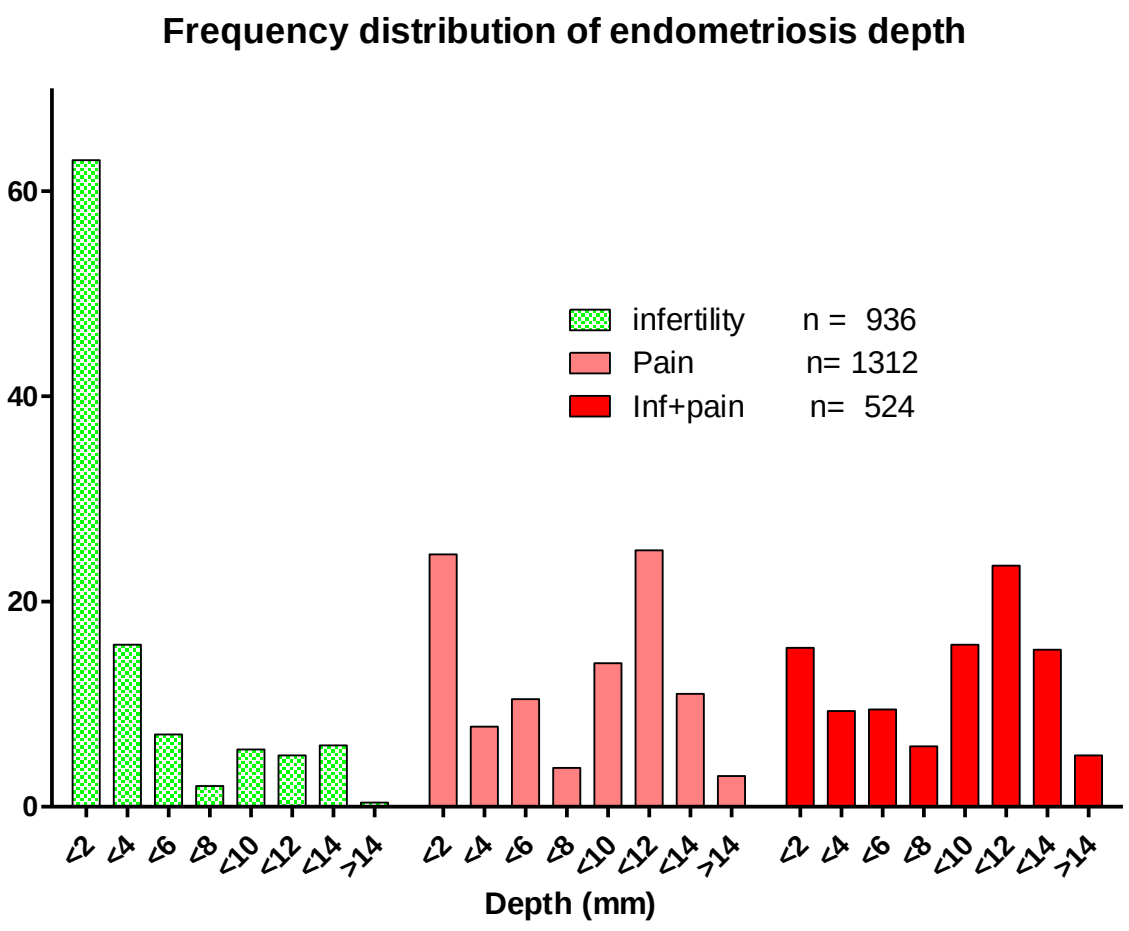

Fig 2 The updated endometriotic disease theory(260). The original cell is can be and endometrial cell or a stem cell or a bone marrow cell with their inherited genetic and epigenetic defects causing their predisposition. Follow implantation or reversible metaplasia because of the abnormal environment these subtle lesions can acquire additional defects without morphological expression. Additional genetic or epigenetic changes are required for these cells to change behaviour and to progress into typical, cystic, deep or other lesions.