THE PRESENCE OF ENDOMETRIOSIS IN THE HUMAN FETUS

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Abstract

Endometriosis is a gynecological disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. Despite the fact that this disease is very frequent and has a significant social impact, the pathogenesis, as well as the diagnosis and therapy are still not perfectly delineated. Retrograde menstruation and coelomic metaplasia are the most documented pathogenetic hypotheses. Research directed by our group and others have outlined experimental evidence indicating an alteration of the fine-tuning of the female genital system developmental program during the fetal life as the pathogenetic event prompting to the development of endometriosis later in life. Goal of this chapter is to summarize the latest evidences about the presence of endometriosis in the human fetus. The possible clinical and pathological implications of these findings will be discussed.

Keywords: endometriosis, fetus, Mullerian duct, embryology, estrogen, pathogenesis, organogenesis

Introduction

Endometriosis is characterized by the growth of endometrial glands and stroma at extra-uterine sites, most frequently over visceral and peritoneal surfaces within the female pelvis [1]. It is a very common gynecological disorder present in up to 10% of women of reproductive age [2]. The incidence rises to 30% in patients with difficulties in conceiving [3]. Deep infiltrating endometriosis is a subset of endometriosis where the lesions are predominantly located under the peritoneal surface and is associated with intense pelvic pain symptoms [4]. Endometriosis is generally accompanied by chronic pelvic pain, adhesion formation and infertility. It has been calculated that endometriosis is responsible for more than 100,000 hysterectomies each year in the United States, with significant annual health care costs attributable to this disease [3]. Moreover, the symptoms of this disease are mostly non-specific, and are very similar to those associated with other chronic pain disorders. As a result, in a great majority of cases the definitive diagnosis is reached after several years and only by invasive surgical procedures, often with an incredible time-interval between the onset of the symptoms and final diagnosis of 8-12 years [2,3]. Original non-invasive approaches for the diagnosis of endometriosis have been recently proposed by our research group, however, this therapy is not yet standard of care [5-8]. Endometriosis remains a significantly under-diagnosed and under-treated disease and is considered a "social disease", since it has an important socio-economic impact in view of the costs for the diagnosis and treatment, the loss of economic performance of the patients, and the negative impact on quality of life and capability of conceiving [2,3].

Pathogenesis of endometriosis

Thanks to the work of Benagiano & Brosen and that of Knapp, the steps in the discovery and characterization of endometriosis in the history of medicine are now very well defined [9,10]. Endometriosis has been a known entity for more than two centuries. In light of the significant social and economic impact this disease is known to have, it seems impossible that the pathogenesis of endometriosis has not been definitively elucidated. Currently, several pathogenetic theories have been proposed to explain the development and establishment of endometriosis. Table 1 summarizes the most commonly accepted theories for the histogenesis of endometriosis.

The most easily understood and widely accepted theory for the histogenesis of endometriosis is that of retrograde menstruation/transplantation, proposed one century ago by Sampson [2]. This theory hypothesizes that at menstruation some effluent flows retrograde through the lumen of the Fallopian tubes into the peritoneal cavity, causing the adhesion and growth of endometrial fragments. This

mechanism that considers endometriosis simply an auto-transplant of normal endometrial tissue in an ectopic location in the organism, explains the most common sites of endometriosis [2,3]. Moreover, retrograde menstruation is a common phenomenon with more than 90% of women having blood in their pelvis at the time of menstruation [1]. Despite the fact that researchers have tried for decades to confirm this mechanism of histogenesis for endometriosis, no conclusive evidence has been produced in favor of this theory. More importantly, it fails to explicate the presence of endometriosis in such remote areas outside the peritoneal cavity, as the lungs, skin, lymph nodes, and breasts [2,3]. Moreover, it is not an acceptable pathogenetic mechanism for endometriosis described in early puberty and in newborns [11], as well as in women affected by the Mayer-Rokitansky-Küster-Hauser, a syndrome characterized by congenital aplasia of the uterus and the upper part of the vagina [12]. Finally, it cannot be considered a valid mechanism in the event of endometriosis in male, which is a rare but very well described phenomenon with a total of 17 cases reported in the literature to date [13]. Nevertheless, elegant observations by Redwine propose that endometriotic tissue lacks characteristics of an auto-transplant [14].

The coelomic-metaplastic theory suggests that endometriosis in the pelvis and elsewhere is caused by endometrial metaplasia of the peritoneal serosa or serosa-like structures, perhaps induced by environmental factors [15]. This theory would explain the cases where retrograde menstrual flow is impossible.

The lymphatic and vascular dissemination theory suggests the spread of endometrial cells by lymphatic or hematogenous vessels [15].

The theory of circulating stem cells claims that transient pluripotent hematopoietic stem cells could differentiate into endometriotic tissue at different anatomical sites [15].

The theory of embryonic cell remnants postulates that endometriosis originates from embryonic rests of the Mullerian ducts and Wolffian ducts. The Müllerian ducts, indeed, give rise to the female reproductive tract. This organogenesis is controlled by complex spatio-temporal molecular pathways, including the anti-Müllerian hormone signaling [15]. Aberrant differentiation or migration of the Müllerian ducts during embryogenesis could spread cells in their migratory pathway across the posterior pelvic floor, thus clarifying the observation that endometriosis is commonly found in the cul-de-sac, uterosacral ligaments, and medial broad ligaments.

This mechanism of histogenesis was proposed by pioneer scientists of this disease in the late 19th and 20th century, but inexplicably forgotten after the advent of Sampson's retrograde menstruation theory [9,10]. Table 2 summarizes the most important observations supporting the embryological

theory in chronological order, as clearly described by the works of Benagiano & Brosens and Knapp [9,10]. Further supporting evidence of the fetal origin is the observation that the cells of pubertal or post-pubertal clinical endometriosis maintain some typical characteristics of fetal endometrium cells, such as ontogenic resistance to progesterone [16]. This characteristic is, obviously, not present in the mature adult endometrium.

Recently, work from our research group and others have demonstrated the presence of ectopic endometrium in a significant number of human female fetuses [17-19]. These scientific evidences clearly support the embryogenetic theory. In this chapter, we describe the most salient data produced by our research group and other scientists regarding fetal endometriosis, in order to better determine the real biological impact of this phenomenon.

Fetal endometriosis

Our research group has demonstrated the presence of ectopic endometrium in a significant number of human female foetuses (ten in one hundred one cases) analysed by autopsy in three different works [17-19]. These structures were found outside the uterine cavity and could not be attributed to any normal anatomical formation. In particular, the anatomical sites of these endometrial structures were: in the mesenchymal tissue close to the posterior wall of the uterus, in the proximity of the Douglas pouch, in the recto-vaginal septum, in the rectal tube at the level of muscularis propria, and in the wall of the uterus. Interestingly, all of these anatomical sites are very well-known locations for endometriosis in women [1]. Immunohistochemical studies were utilized to assist in the evaluation and characterization of these endometriotic structures. These organoid lesions demonstrated positive staining for CA-125, Cytokeratin 7 and Estrogen Receptor in the epithelial component, while the stromal cells displayed positive staining for both CD-10 and Estrogen Receptor. Fetal endometrium was also evaluated in these patients and revealed identical staining patterns. The exact anatomical distributions of all the endometriosis-like structures found in these three works, are depicted in detail in figure 1. An example of the histological and immunohistochemical appearance of this ectopic endometrium is reported in figures 2 to 5. Based on the anatomical location, and on the histological and immunohistochemical characteristics, these structures must be ascribed to endometrial tissue, dislocated outside the uterine cavity during the earlier steps of organogenesis and displaying a molecular immunophenotype identical to that of the endometrium present in the uterus. To the best of our knowledge, these observations have been the first direct and systematic demonstration of the theory of embryonic cell remnants as the cause of endometriosis.

After our data was published, subsequent studies also confirmed the presence of endometriotic structure in female fetuses. In the work by de Jolinière et al., the reproductive organs of seven female foetuses were analysed at autopsy [20]. In two out of seven fetuses, ectopic endometrial glands were found in the in the myometrium, while several ectopic endometrial glands surrounded by stroma were found in the uterine broad and ovarian ligaments and under the fallopian tube serosa in six fetuses. These glandular structures expressed positive staining for estrogen and progesterone receptors, while the stromal components displayed positive staining for CD-10 and vimentin [20].

Nevertheless, Schuster and Mackeen have reported a case of fetal endometriosis diagnosed as a large fetal pelvic mass at 35 weeks of gestation [21]. The mass was surgically removed on the second day of life and histological examination of the specimen confirmed the diagnosis of cystic endometriosis of the left ovary [21].

If we analyse the scientific literature preceding the above described work on foetal endometriosis, we find some anecdotal scientific observations [22,23]. Moreover, the presence of endometriosis in the fetus was hypothesized, but not demonstrated, by Batt et al with the mullerianosis theory, even if this phenomenon was considered as different from endometriosis [24]. Based on our data and the observation of others regarding the presence of ectopic endometrium in the fetus, we believe these endometriotic structures remain quiescent and asymptomatic until puberty, at which time the hormonal inputs result in activation and consequently, the onset of the symptoms of endometriosis [15]. The molecular mechanisms responsible for the histogenesis of these ectopic endometrial structures, as well as their survival until puberty, are largely unknown. As far as pathogenesis is concerned, it is possible to hypothesize that complex molecular mechanisms, also regulated according to a precise spatio-temporal scheme, can be altered by abnormal inputs that act on an adequate genetic background in a specific organogenesis window of time. We consider this phenomenon as an alteration of the fine tuning of female genital structures organogenesis caused by genetic and epigenetic factors, that would cause disruption of some organizational events associated with development of the normal neonatal uterine wall [25]. The observation that endometriosis is significantly higher in patients affected by uterine malformations, such as Müllerian anomalies, anogenital distance, myometrial structural alterations, and other genital anomalies, is indirect support of this proposed mechanism [25]. Recently, works by Makiyan [26] have suggested that the origin of endometriosis is from primordial germ cells; this observation further supports the presence of endometriosis in the fetus.

If we consider the fact that the most important hormone in the female genital tract morphogenesis is estrogen, it is likely that altered estrogenic input may be one of the factors responsible for the

histogenesis of foetal endometriosis [25]. There are important epidemiological and experimental studies that link the onset of endometriosis, as well as other changes in the female genital system, to exposure in utero to endocrine disruptors, substances capable of mimicking the action of the hormone estrogen. In this regard, it is important to remember the epidemiological work of Missmer, which demonstrated a significantly higher number of cases of endometriosis in female patients exposed in utero to diethylstilbestrol [27] as well as experimental data from our group showing an endometriosis-like phenotype in mice exposed in utero to the endocrine disruptor bisphenol [28]. Finally, through experimental approaches of genomics, we have been able to reveal a specific pattern of gene expression in the tissue of endometriotic structures compared to normal endometrial tissue. This specific gene expression pattern essentially concerned genes involved in embryogenesis and was not modified by the phases of the hormone cycle [15].

Conclusions

Endometriosis is a multi-factorial disease with many-sided features and accordingly, the pathogenesis could be slightly different in different cases. The proposed theories of pathogenesis are perhaps not mutually exclusive and may be interrelated. Nevertheless, the recent findings reviewed in this chapter lend a compelling argument to the embryogenesis theory especially when compared to the retrograde menstruation theory. The clinical and therapeutic implications are relevant. In particular, recurrence of the disease, should not be ascribed to retrograde menstruation but rather to an incomplete surgery due to the presence of microscopic foci and/or at a different timing in the growth of the various foci in the same patient. This last phenomenon is very common in diseases induced by endocrine disruptors and is probably due to individual susceptibility. Even more important, treatment of the disease with synthetic estrogens or selective estrogen receptor modulators chemical compound is, indeed, effective in reducing the symptoms, but could cause the growth of microscopic lesions and exacerbate the disease. A more complete understanding of the molecular mechanisms responsible of the pathogenesis of endometriosis could, hopefully, individuate suitable therapeutic targets for this still “uncurable” disease.

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Figure 1 – Anatomical distribution of the ectopic endometrium found in the female human foetuses in the works by Signorile et al (see references 24-26)

Representation of the pelvic organs of a female foetus at around 25 weeks of gestation, displaying the anatomical location of the endometriotic structures. The different locations are indicated by asterisks in the proximity of the Douglas pouch; in the mesenchymal tissue close to the posterior wall of the uterus; in the rectal tube at the level of muscularis propria; in the wall of the uterus; in the recto-vaginal septum.

Abbreviations used: an (anus); co (coccyx); va (vagina); re (rectum); sc (spinal column); ut (uterus); bl (bladder).

Figure 2 - Histological and immunohistochemical appearance of ectopic endometrium in a female human foetus of 25 weeks

The picture shows an endometrial structure in the recto-vaginal septum; in the inset, the immunohistochemical expression of oestrogen of this structure at higher magnification is depicted (modified from ref 24).

Figure 3 - Histological and immunohistochemical appearance of ectopic endometrium in a female human foetus of 24 weeks

The image shows an endometrial structure in the proximity of the Douglas poutch; in the inset the immunohistochemical expression of oestrogen receptor of this structure at higher magnification is depicted (modified from ref 24).

Figure 4 - Histological and immunohistochemical appearance of ectopic endometrium in a female human foetus of 18 weeks

The picture shows an endometrial structure in the rectal tube at the level of muscularis propria; in the inset the immunohistochemical expression of CA-125 of this structure at higher magnification is depicted. Note that the epithelium of the rectum is negative for CA-125 (modified from ref 24).

Figure 5 - Histological and immunohistochemical appearance of ectopic endometrium in a female human foetus of 16 weeks

The image shows an endometrial structure in the mesenchymal tissue close to the posterior wall of the uterus; in the inset the immunohistochemical expression of CA-125 of this structure at higher magnification is depicted. Note that in the wall of the primitive myometrium is present a little group of endometrial cells positive for CA-125 (indicated by an asterisk), that could represent a primitive nest of adenomyosis (modified from ref 24).

Table 1. Different theories proposed for the etiology of endometriosis

Theory	Mechanism proposed
Retrograde menstruation/transplantation	Retrograde menstruation allows implantation of endometrial glands into the peritoneal cavity
Coelomic metaplasia	Endometriosis arises in the pelvis or elsewhere by endometrial metaplasia of peritoneal mesothelium or other cell types
Lymphatic and vascular dissemination	Spread of endometrial cells happens by lymphatic or hematogenous vessels
Circulating stem cells	Transient pluripotent hematopoietic stem cells could differentiate into endometriotic tissue at different anatomical sites
Embryonic cell remnants	Endometriosis originates from embryonic rests of the Mullerian ducts and Wolffian ducts

Table 2. Observations supporting the embryological origin of endometriosis, performed by pioneer scientists of this disease in the late 19th and 20th century, as described in the work of Benagiano & Brosens [11] and Knapp [12]

Author	Mechanism proposed	Reference
Von Reckinglausen 1893	Wolffian origin	Dtsch med Wochenschir 1893; 46: 825.
Orloff 1895	Embryonic cells	Zeitschr Heilkunde 1895; 5: 121.
Pick 1897	Mesonephric origin	Arkiv f Gynäk 1897; 54: 119.
Kossman 1897	Mullerian origin	Archiv f Gynäk 1897; 54: 359.
Mayer 1903	Epithelial heterotopy	Z Geburtshilfe Gynäkol 1903; 49: 32.
Schikele 1904	Mesonephric origin	Zentralbl Allg Pathol Anat 1904; 15: 261
Cullen 1908	Mucosal theory	WB Saunders 1908
Frankl 1911	Mullerian origin	Arkiv f Gynäkol 1911; 93: 659
Lockyer 1918	Mullerian origin	MacMillan and Co, 1918