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Endometriosis etiology: Hypothesis of Maternal Microchimerism

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Abstract

Endometriosis is an oestrogen-dependant reproductive disease, with genetic, vascular, neural, inflammatory and auto-immune characteristics. There are many theories about the etiology of endometriosis, however, all of these theories have limitations and do not explain all the locations that endometriosis is found or types of patients with endometriosis.

The objective of this paper is to postulate the hypothesis that endometriosis is caused by Maternal Microchimerism, the presence of maternal cells in the fetus. A literature review was conducted, analysing the characteristics, current etiological theories of endometriosis, theory

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limitations and relationship of maternal microchimerism and endometriosis. At time of writing, there was no literature on maternal microchimerism and endometriosis.

These results suggest that Maternal Microchimerism could be a cause of endometriosis. This could account for the genetic and auto-immune characteristics seen in people with endometriosis, inducing a micro-environment for vascular, neural and epigenetic changes. This could also account for account for endometriosis seen in non-menstruating patients, such as men, fetuses and post-menopausal women and endometriosis found in non-peritoneal locations. If the hypothesis of Maternal Microchimerism is correct, endometriosis could be considered a pregnancy-related disease that could affect all humans, changing the accepted demographics of patients and potentially new diagnostic techniques and treatment options for patients with endometriosis. Further studies are needed to test this hypothesis.

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1 Introduction

Endometriosis is an oestrogen-dependant disorder (1) defined as the presence of endometrium-like tissue outside the uterus (2). It's estimated to occur in 10% of reproductive-age women although true prevalence is unknown due to definitive diagnosis requiring surgical visualisation, hampered as the severity endometriosis does not correlate with symptoms and patients because only undergo surgery when in pain and/or infertile (3).

Whilst endometriosis is primarily classed as an oestrogen-dependant disorder, multiple studies have shown evidence endometriosis has characteristics of a genetic disorder (1, 4) vascular disease, inflammatory disease (5), auto-immune disease (1, 4, 5) and neural-disorder (1, 4, 5, 6). The histology of eutopic endometrium is different in women with and without endometriosis, such as a decrease in eutopic endometrial thickness in women with endometriosis, and also the eutopic and ectopic endometrium is different in women of endometriosis (6, 7).

Endometriosis is often thought of as a peritoneal disease in menstruating women. However, evidence shows endometriosis occurring outside of the peritoneum, such as the kidneys, bladder, lungs and brain (8) and in non-menstruating people such as men, post-menopausal women (9), pre-pubescent girls and fetuses.

There are many existing theories for the etiology of endometriosis, however, no single theory definitively proves or fully explains the origin of endometriosis. In this article, we will review the immunological, biochemical and genetic characteristics of endometriosis, prevalence of endometriosis in non-uterine and non-menstruating people, the existing theories of endometriosis etiology, their limitations and proposes a new hypothesis for the etiology of endometriosis, the Hypothesis of Maternal Microchimerism (MMc). This hypothesis aims to complement the existing theories to understand the etiology of endometriosis.

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2 Endometriosis Characteristics

2.1 Immune Dysfunction and Biochemistry

Endometriosis was first theorised as an autoimmune disease by Gleicher et al. in 1987 (10). Autoimmune diseases are more common in women but are even more common in women with endometriosis (8) and studies have shown women with endometriosis have a dysfunctional immune response (1, 8, 10, 11). Ectopic endometrial cells trigger an immune response which, mixed with a dysfunctional immune response, creates a micro-environment which supports cell proliferation, cell adhesion and angiogenesis and, therefore, the development and maintenance of ectopic endometriosis (8).

Inflammation

Abnormal inflammation and elevated autoantibodies are a common characteristic of autoimmune diseases (1, 5, 8, 10, 11). Other diseases, such as cancer and tissue damage, also show inflammation and presence of autoantibodies so this does not necessarily mean that endometriosis is an autoimmune disease. Individuals with endometriosis have shown to have an abnormal immune response (5), including abnormal levels of inflammatory markers TNF-α, interleukins, Matrix metalloproteinase (MMP), Immunoglobulin G (S), IgA and IgM auto antibodies and prostaglandins (1, 5, 10, 11, 12), Natural Killer (NK) cells (1, 10, 13), Reactive Oxygen Species (ROS) (8) and abnormalities in apoptosis and autophagy (1, 8, 10, 11) processes.

TNF-α is an inflammatory cytokine produced by activated macrophages and contributes to endometriosis-associated pelvic inflammation (8, 11). TNF-α is elevated in the peritoneal fluid of women with endometriosis and correlates with the severity of endometriosis (5). TNF-α activates inflammatory leukocytes and produces other pro-inflammatory cytokines, including

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Interleukin 1 (IL-1), IL-6, IL-8, Monocyte Chemotactic Protein (MCP-1) (5, 8) and further promotion of TNF-α (10, 11), which can promote endometrial adhesion and MMP expression and may contribute to ectopic endometrium development (5). Levels of IL-4, IL-5, IL-10, Transforming Growth Factor β (TGF-β), Soluble Intercellular Adhesion Molecule (sICAM-1), Endothelin-1 and free p40 subunit of IL-12 are also elevated in the peritoneal of women with endometriosis (5).

IgG antilaminin-1 antibodies are elevated in women with endometriosis (12), especially those who experience recurrent miscarriages and infertility (1). These elevated levels can cause a disruption or embryogenesis and placental development. Prostaglandins, involved in inflammation and injury, are found in higher levels of both eutopic and ectopic endometrium in women with endometriosis and are thought to be caused by hyperactivity of COX-2 and microsomal prostaglandin E synthase (5). Prostaglandin E2 and F2α specifically are found in excess and contribute to dysmenorrhea and pelvic pain.

ROS is also found in higher levels in women with endometriosis. During menses, ectopic endometrial sites also shed and cause internal bleeding. As blood is broken down, excess haemoglobin leads to an iron overload causing redox reactions, which promotes further ROS at ectopic endometrial sites (8). ROS oxidates lipoproteins and causes DNA damage in the endometrial cells, inducing an immune response and attracting lymphocytes and activated macrophages, which produce cytokines. This promotes endothelial growth and further promotion of ectopic endometrial sites (8).

Apoptosis and Autophagy Suppression

Apoptosis, programmed cell death, is required during each menstrual cycle when the endometrium sheds. This is especially important if retrograde menstruation occurs to remove

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the endometrial cells and blood from internal ectopic sites. Increased levels of antiapoptotic and pro-proliferation has been found in ectopic endometrium, which would reduce the body's ability to remove the ectopic endometriosis (8, 10, 11), is also associated with several autoimmune diseases. Autophagy, a non-apoptotic form of programmed cell death, degrades long-lived proteins and cytoplasmic organelles and has also been shown to be reduced in ectopic endometriosis (1).

Natural Killer Cells

NK cells are large granular lymphocytes involved in pathogenesis, using receptors to differentiate between normal and malignant cells. Cells that attach to killer-activating receptors (KAR) of the NK cell promotes cytotoxic activity to kill the target cell, whereas cells that attaches to the killer-inhibitory receptors (KIR) of NK cells suppress cytotoxic activity to not kill the target cell (1, 10, 13). NK cell levels are decreased in the peritoneum of women with endometriosis, which may contribute to ectopic endometrium cells not being removed. There is also an overexpression KIR, suppressing the cytotoxic activity of NK cells (1). Myeloid Derived Suppressor cells (MDSCs), which also suppress NK cells, are found in elevated levels in the peritoneum (1, 5).

Vascular

Vascular Endothelial Growth Factor (VEGF) is a signal protein that stimulates the growth of new blood vessels (1). Oestrogen levels, hypoxia and peritoneal inflammatory cytokines rise during the menstrual cycle and stimulate the production of VEGF which induces angiogenesis. Additional growth factors, such as Insulin-like Growth Factor (IGF), Platelet Derived Growth

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Factor (PDGF) and Hepatocyte Growth Factor (HGF) (1), further induce endometrial and endothelial cell proliferation (1).

VEGF, IGF, PDGF and HGF are overexpressed in the eutopic endometrium and peritoneal fluid of women with endometriosis (1, 11, 14), providing a micro-environment that supports dysfunctional cell proliferation. There is also an increase of lympathic and blood micro-vessels in eutopic endometrium of women with endometriosis (7).

Neural

Nerve Growth Factor (NGF) is overexpressed in the ectopic endometrium and peritoneal fluid (6). Studies have confirmed a higher density of nerves in ectopic endometrium with evidence suggesting pain correlates with the increase of neural density (6, 7). There is also an increase of inflammatory cells near the endometrial nerve fibers of women with endometriosis that stimulates the nerve endings in ectopic endometrium and triggers the secretion of pro-inflammatory neuromodulator, causing peripheral neuroinflammation. This suggests a complex neural and immune association in women with endometriosis (6). Substance P (SP), which is secreted by sensory nerves and mediates neurogenic inflammation, is found in ectopic endometrium and peritoneal fluid and is suggested to maintain the ectopic endometrium, which is thought to contribute to the hypersensitivity and hyperalgesia of neurons in endometriosis (6, 7).

To summarise, the dysfunctional immune response in women with endometriosis is similar to that of an autoimmune disease. Higher levels of inflammatory markers and ROS further induce inflammation and proliferation at ectopic endometrium sites, which complements the reduced expression and cytoxic activity of NK cells and reduced level of apoptosis and autophagy

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processes. Increased levels of growth factors, such as VEGF and NGF, provide a vascular and neural micro-environment to support the ectopic sites. This all combines, in women with endometriosis, to create a micro-environment that supports the maintenance of ectopic endometrial sites. It is not clear if the dysfunctional immune responses are due to endometriosis being an autoimmune disease or if they are caused by other factors such as genetic or epigenetic changes, The Stem Cell Theory or oestrogen stimulation at pre-existing ectopic endometrial sites e.g., Műllerian Embryogenesis.

2.2 Genetic and Epigenetic Characteristics

Many studies have shown that endometriosis is a polygenic, highly heritable disease with a twin study estimating additive genetic traits of 47% (15). Endometriosis is seen in a higher rate of occurrence in first degree relatives with frequent co-occurrence in twins (8) and studies show many genes both altered and up- or down-regulated, both in women with and without endometriosis and also between the ectopic and eutopic cells of women with endometriosis (1, 4, 8, 16, 17). This is hypothesized to predispose women for a dysfunctional immune system response, biochemical changes that influence the attachment of ectopic endometrial cells at endometriosis sites and reproductive development (8). Lastly, evidence shows epigenetic modifications, described as the stable inheritance of phenotypes of cells and organisms without changes in DNA, which could be involved in the maintenance of the ectopic endometrium (4).

Genetic polymorphisms predisposing towards endometriosis have also been found in FOXP3, FCRL3, NF-κB and B lymphocyte stimulator (1, 10, 17). It is theorised that up-regulation of telomerase enzymes may alter the endometrial cell phenotype in women with endometriosis, which promotes further inflammation, angiogenesis and cell proliferation (8, 18).

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Numerous large-scale gene expression profiling studies have demonstrated many genes are dysregulated or have aberrant DNA methylation in women with endometriosis (4). Steroid Receptor (SR) and co-pressors Nuclear Receptor Co-Repressor (NcoR) and 'Silencing Mediator of Retinoic Acid and Thyroid Hormone receptor' (SMRT) have shown an abnormal expression in eutopic and ectopic endometrium (4), which could alter the regulation of steroidal hormones. Up-regulation of Inositol 1,4,5-Triphosphate Receptor Type 1 (ITPR1) and down-regulation of Protein Phosphatase 2 Regulatory Subunit B Epsilon (PPP2R5E), seen in women with endometriosis, could dysregulate oocyte meiosis (1) and DNA methylation on Steroidogenic factor-1 (SF-1), could be involved in sex development and Műllerian structures (10, 19).

Inflammatory gene regulation is altered in women with endometriosis, such as up-regulation of FCRL3-169C/T allele, which plays a role in auto-antibodies production (1, 10), and antiapoptotic and pro-survival genes in ectopic endometrial cells whilst genes that regulate the apoptosis pathway are downregulated (8).

Many genes that influence cellular differentiation, proliferation and adhesion have also been found to be altered. Protein Tyrosine Phosphatase Non-Receptor Type 11 (PTPN11) is downregulated (1) and DNA methylation is seen on aromatases, key molecules in oestrogen production, and E-cadherins, which are involved in cell differentiation, migration and adhesion (1, 10, 19). Increased levels of cytokines, MCP-1 and 'Regulated-On-Activation, Normal-T-cell-Expressed-and-Secreted' (RANTES) is seen in ectopic endometrium, correlating with the severity of the patient's endometriosis (1), and Neuronal Growth Regulator 1 (NEGR1) is upregulated, all of which are associated with increased cellular adhesion (1). Up-regulation of VEGF mRNA has been found in the eutopic endometriosis of women with endometriosis (11).

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This shows the genetics and epigenetics of endometriosis could be involved in the dysfunction of the immune system, autoimmunity, oestrogen and other steroid regulation, sex development, apoptosis and cellular adhesion, all of which play a part in the pathology of endometriosis. It is not yet understood if this is a causal factor or an effect of endometriosis via e.g., epigenetic modifications, however, the change of genetics and epigenetic expression in eutopic and ectopic endometrium suggests genetic factors do contribute to the etiology of the disease.

3 Existing etiological theories of endometriosis

3.1 Sampson's Theory of Retrograde Menstruation

Sampson's Theory of Retrograde Menstruation was proposed in 1927 and is one of the oldest and most widely accepted etiology theories of endometriosis (1, 8). Retrograde menstruation is a naturally occurring phenomenon where endometrial cells, during a menses, retrograde through the fallopian tubes into the pelvic region, rather than exiting through the vagina (8). Whilst retrograde menstruation has been show to occur at a higher volume in women with endometriosis, increasing the risk of endometrial cells being found in the peritoneum, retrograde menstruation is thought to occur in 76-90% of women and endometriosis is only found in 10% of women (8, 20). Women with endometriosis have been shown to have higher levels of refluxed blood in the peritoneum and retrograde menstruation could explain how ectopic endometrium can return following excision surgery (1). However, Sampson's Theory does not fully explain the etiology of endometriosis as retrograde menstruation occurs in women without endometriosis. Further, retrograde menstruation does not explain the biochemical differences between eutopic and ectopic endometrial cells and is not consistent in cases where endometriosis is found in non-peritoneal areas such as the lungs and brain (8). Sampson's Theory also requires patient who menstruate and therefore does not explain how

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non-menstruating patients, such as patients with medically paused periods, men, post-menopausal women or pre-pubescent women, can have sites of ectopic endometriosis.

3.2 Stem Cell Theory

Stem cells are characterised as undifferentiated cells which can self-renew and differentiate into specialised cells (8). The basalis layer of the endometrium has been proposed to be a stem cell source for re-epithelialisation and vascular generation of the endometrium. During each menses, the epithelial and vascular cells are shed but the basalis layer is not shed (8).

The Stem Cell Theory of endometriosis compliments Sampson's Retrograde Theory, in that it depends on retrograde menstruation during menstruation. However, in the Stem Cell theory, the basalis stem cells could be shed and deposited in the peritoneum via retrograde menstruation in women with endometriosis, which is not thought to happen in women without endometriosis. This could explain the ability of cells to attach and cause ectopic endometriosis sites during retrograde menstruation for women with endometriosis.

It is hypothesised that the abnormal shedding of basalis stem cells is triggered during each menses by oestrogen (8). However, as stem cells are meant to differentiate into many types of cells, it is questioned why shed basalis stem cells would still differentiate into endometrial cells if deposited in the peritoneum, rather than propagate into e.g., peritoneal cells. The Stem Cell Theory has the same limitations as Sampson's Retrograde Theory of non-peritoneal ectopic endometriosis and non-menstruating patients.

3.3 Műllerian Embryogenesis

Műllerian ducts are a pair of ducts that form in the embryo that develop to form parts of the female reproductive system, such as the uterus, fallopian tubes and uterus, including the

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endometrium. Műllerianosis is described as an organoid structure of embryonic origin e.g., endometrium, incorporated within other normal organs during embryogenesis (9). The Műllerian Embryogenesis theory is that embryogenesis of the Műllerian ducts was defective (8). Residual embryonic endometriotic cells from the Műllerian ducts grow elsewhere in the fetus, causing endometrial lesions at ectopic sites. Upon oestrogen stimulation e.g., during puberty, these cells would proliferate and patients would start showing symptoms (8).

Műllerian Embryogenesis does provide an explanation of how non-menstruating patients can have endometriosis, how endometriosis can occur in non-peritoneal sites and how eutopic and ectopic endometriosis could form to be histologically different and trigger an immune response. However, the Műllerian Embryogenesis Theory states that this is an etiology for developmental endometriosis (9), which occurs during embryogenesis, rather than acquired endometriosis which forms later in life. The Műllerian Embryogenesis Theory also states that developmental endometriosis can be surgically removed to cure the condition. Therefore, whilst this theory may be correct for some patients, it contradicts itself in patients where acquired ectopic endometrium does re-grow following excision. This limitation could be answered due to cells being missed during excision, allowing for re-proliferation and re-formation of ectopic endometriotic sites but may not account for new ectopic endometriosis sites growing at later stages of a patient's life.

3.4 Coelomic Metaplasia

Metaplasia is the change of cells to a form that does not normally occur in that tissue. The Coelomic Metaplasia Theory proposes that endometriosis originates from metaplasia of specialised coelomic (epithelial lining of the abdomen) cells into ectopic endometrial cells (8,

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11). This would require a trigger to stimulate the transdifferentiation, such as puberty or immunological factors. (8).

Coelomic Metaplasia Theory could explain how endometriosis could occur in non-menstruating people e.g., men and pre-pubescent girls, depending on the stimulation required (13), however, is limited to ectopic endometrium in the abdomen and does not explain how endometriosis can form at non-peritoneal sites.

4 Limitations in current endometriosis research

This next section reviews the evidence of endometriosis in non-menstruating patients. These cases are not as commonly researched but are fundamental to understanding an overview pathology of the disease.

4.1 Endometriosis before puberty - Fetus

Sampson's Retrograde Theory and Stem Cell Theory, as etiologies of endometriosis, require menstruating women. However, there is research documenting ectopic endometrium found in fetuses.

In 2009, Signorile et al. found misplaced endometrium in 4 out of 36 (11%) female fetal samples (20). In 2010, Signorile et al. published their next findings showing endometriosis in one fetus out of 13 female samples (7%) (21). Signorile et al. continued research in 2012 and found glandular structures which expressed oestrogen receptors, representing primitive endometrium outside of the uterine cavity, in 9 out of 101 (9%) cases female fetuses (22) and then in 2013 Batt & Yeh found primitive endometriosis in 4 out of 36 (11%) female fetuses (9). These 4 studies show evidence of endometriosis outside the uterus in female fetuses at a prevalence of 7-11% which is similar to the prevalence of endometriosis found in in female adults (10%) (3).

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4.2 Endometriosis in Men

There are documented cases of endometriosis found in males. There seems to be a common pattern of male patients with prostate disease who have received oestrogen treatment (9, 23, 24, 25) but another study showed a male patient with cirrhosis, a disease is associated with significantly increased oestradiol levels (26). However, there are also cases of males with endometriosis who have not been exposed to oestrogen treatments or have diseases which increase oestrogen (9, 27). Sampson's Retrograde Theory, Stem Cell Theory, Műllerian Embryogenesis and Coelomic Metaplasia are limited in being female diseases and do not account for endometriosis that occurs in males.

4.3 Induced Endometriosis Research Methods

Endometriosis is found naturally in some primates, however, due to the prevalence and slow progression of natural endometriosis, endometriosis is sometimes induced into primates for in vivo testing (28). A review of in vivo methods show that recipient primates often receive human endometrial cells for implantation (28), allowing the endometrial cells to attach and mimic endometriosis. Implantation of cells from another subject is a form of microchimerism, providing initial evidence that in microchimeric cells can cause endometriosis. It is of note that following microchimerism transplant, oestrogen supplementation is required for the endometriotic lesions to proliferate (8, 14, 29, 30). This gives justification to the hypothesis that endometriosis could be caused by endometrial cells being ectopically placed and stimulated later into endometriosis via puberty or oestrogen treatment.

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5 Microchimerism

Microchimerism (Mc) is the coexistence of genetically distinct cells, derived from two different individuals, in an organism (31). This can occur naturally, such as maternal and fetal microchimerism, or can be artificial such as blood transfusions and organ transplants. Mc occurs naturally during pregnancy from 4-6 weeks gestation (31) with fetal cells passing over the placenta into the mother (Fetal Microchimerism (FMc)) and maternal cells passing over the placenta into the fetus (Maternal Microchimerism (MMc) (32) and can last a lifetime. However, the purpose of Mc cells is not yet fully understood (31, 33, 34). Microchimeric cells have a high plasticity in certain situations (31), meaning they could adapt and propagate in a new environment. Injury and infection are suggested to lead to proliferation of microchimeric cells (35).

Natural Mc in humans occurs in the placenta (36), where fetal and maternal blood travels in channels in the fetal placental region. The fetal blood flows from the fetus, down the umbilical cord and into chorionic villi in the placenta, whereas maternal blood in the placenta flows in the intervillous space and into the mother's uterine circulation. These maternal and fetal blood channels are separated by the placental carrier, a layer fetal trophoblasts. The placenta is bordered by the mother's basal plate, which is adjacent to the mother's endometrium, including the mother's basalis stem cells (36). Lastly, the fetal umbilical cord enters the fetuses abdomen.

Microchimerism is often thought to be benign but has been shown to have both beneficial and detrimental effects. MMc passes maternal antibodies to the fetus, developing the fetus' immunity (32), although MMc immune cells do not replace the functionality in the receiving body as seen in a blood transfusion (34). FMc cells have also been found in mother's breasts and are thought to provide a protective role against cancer development (31). However, studies have shown high levels of MMc cells in children with skin and muscle disorders and was seen in children who died from congenital heart block (31) and sometimes Mc can be malignant or

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cause Graft Versus Host Disease (GVHD) (34), a condition which can occurs with transplant-microchimerism, but, for unknown reason, this does not always occur (31, 34, 37).

Both MMc and FMc can trigger autoimmune responses in the receiving body (31, 35) and have been found to be increased in patients with autoimmune diseases (31, 34). In thyroid cancers, FMc cells were shown to differentiate into epithelial and hematopoietic cells, which are thought to be involved in repair processes (31) and it was also found that Mc cells, normally present at low levels tolerogenic to the recipient's immune system, could trigger cell proliferation (34). Studies suggest that differentiated tissue specific MMc can be found in neonates, meaning semi-allogeneic maternal cells could be targeted of neonatal immune response and may be common in chronic idiopathic inflammatory disease (34).

Two studies so far have been carried out on endometriosis and microchimerism however both looked at FMc, where fetal cells are found in the mother. The first study by Fassbender et. al., 2015 looked for male FMc in eutopic and ectopic endometrium sites for women with endometriosis. Due to limitations in the method, the hypothesis could not be confirmed (38). Bhat et al., 2019 completed a similar experiment with a different method and confirmed that male DNA was present in all groups of patients, irrespective of endometriosis or pregnancy history, suggesting bi-directional microchimerism from other relatives e.g., siblings (39). Whilst FMc could impact existing endometriosis in women, the current research does not investigate Mc in patients prior to pregnancy.

No research on Maternal Microchimerism and endometriosis could be found. This implies that this is a new potential area of research.

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6 Discussion:

It is likely that no single theory can explain the full etiology of endometriosis, due to the complexity and multi-factor of the disease. Currently, none of the existing theories account for full the etiology of endometriosis, of note including the difference in the histology and biochemistry found between eutopic and ectopic endometriosis (7) or the occurrence of endometriosis in non-peritoneum areas or non-menstruating patients. This paper looks at the hypothesis that Maternal Microchimerism could contribute to the etiology of endometriosis. No evidence could be found of any previous research into this area of study. Two studies have been completed into FMc and endometriosis, however, this does not explain endometriosis in non-menstruating patients.

The Hypothesis of Maternal Microchimerism proposes that endometriosis may originate in neonates during pregnancy, when maternal cells cross into the fetus. The fetus' placenta is bordered by the mother's basal plate and then the endometrial basalis layer. MMc cells have been associated with auto-immune and inflammatory diseases in neonates and endometriosis is an inflammatory proposed to be an auto-immune disease with genetic aspects (31).

The Stem Cell Theory already hypothesises that endometrial basalis cells are stem cells that may migrate and proliferate in non-uterine sites. The close proximity of the mother's endometrial basalis cells to the placenta could increase the chance of the mother's endometrial cells becoming microchimeric. On the fetal side, MMc cells would pass to the fetus down the umbilical cord into the abdomen. This region is where endometriosis is often found in patients, which could explain why endometriosis is mostly prevalent on the peritoneum, however, MMc cells are found in all areas of the body which could also explain how endometriosis is found, less commonly, in non-peritoneal sites such as the brain, lungs and liver.

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MMc could explain how ectopic endometriosis is found in non-menstruating patients such as fetuses, pre-pubescent women, post-menopausal women and men. In vivo testing for endometriosis commonly uses chimeric cells and requires oestrogen supplements, which would be analogous to endometrial MMc cells first being deposited in the fetus and endometriosis symptoms showing up following exposure to oestrogens in women reaching puberty or men receiving oestrogen treatment. There is limited research on endometriosis in fetus, pre-pubescent women and men. This may be due to the assumption that endometriosis is a women's disease which only occurs in menstruating women and therefore medical investigation would not commonly consider endometriosis as a cause for non-menstruating patients.

Endometriosis has been considered to be an auto-immune disease due to the immune dysfunction response seen. Microchimerism is associated with causing auto-immune disease (31, 34). If MMc stem cells were to adhere to non-uterine sites during fetal development, stem cells could propagate in the non-uterine sites which, when coupled with a dysfunctional immune response, could explain the biochemical and immune differences of ectopic endometriosis. If this occurred from a fetal stage, this would set a precedent for the immune dysfunction seen. Also, if the immune system is perceiving MMc endometrial cells as foreign cells, this could account for the auto-immune characteristics seen in endometriosis.

This is similar to the Műllerian Embryogenesis Theory, with the fundamental difference that Műllerian Embryogenesis Theory states that ectopic endometrium sites occur only in development endometriosis and does not cause acquired endometriosis. The Műllerian Embryogenesis Theory also states that once the Műllerian cells are removed then endometriosis is cured. MMc cells have been shown to last a lifetime (31, 33, 34) and, if the MMc are maternal endometrial basalis stem cells, could explain how endometriosis re-occurs following excision and even following hysterectomy in some patients. It is also hypothesised that MMc cells proliferate in response to injury (35), which could promote the proliferation of ectopic

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endometrium in the recipient triggered by oestrogen during puberty and inducing of the dysfunctional immune response seen in women with endometriosis, even decades into the women's life.

It may be questioned why endometrial MMc cells would not cause a more extreme response in the body, such as GVHD or even cancer. Microchimeristic cells have shown they do not always induce GVHD, with hypotheses including a dysfunctional immune response (31, 34, 37). There are many types of endometriosis, from superficial peritoneal lesions and cysts (also known as endometriomas) to polypoid endometriosis (2, 40). The risk of malignant transformation of endometriosis is estimated at 1% and the risk of epithelial ovarian cancer is two to three-fold in women with endometriosis (40), which could be due to different fetal immune responses to foreign MMc cells based on the variety of genetic scenarios. These different forms of endometriosis, from superficial to cysts to cancer development may correlate with different types of MMc cells receive and adhesion locations.

Next steps in testing this hypothesis would be to analyse if ectopic endometrium cells are chimerical cells. As endometriosis is most commonly found in female patients and MMc cells derive from mothers, Y chromosome analysis would not differentiate maternal and female fetal cells. Instead, zygosity determination, which is used to differentiate between twins, could be used to show if there are genetic differences between the eutopic and ectopic endometrium from the same patient. Cell-type-independent markers, such as fetal and maternal specific HLA loci, could allow definition of candidate microchimeric cell populations for subsequent single-cells analysis (35). Note, as MMc can pass down not only maternal cells but other chimeric cells from the mother, such as the fetus' siblings, grandparents or potentially even cells from non-relatives if the mother ever received a transplant or transfusion, ectopic samples should not be tested specifically against the mother's DNA. Stahlberg et al. provides analysis for the different methods that can be used in microchimeristic testing, suggesting single-cell analysis

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with cell enrichment, isolation and cell characterisation as successful and available methods (35).

7 Conclusion:

Currently, there is no single theory that fully explains etiology of endometriosis. Sampson's Retrograde Theory, Stem Cell Theory, Műllerian Embryogenesis, Coelomic Metaplasia, auto-immune disease and genetics all have limitations and do not explain all locations of endometriosis or types of patients with endometriosis.

The newly proposed Hypothesis of Maternal Microchimerism, where maternal cells pass to the fetus during pregnancy, could explain how endometriosis occurs in all of the scenarios of endometriosis: menstruating women, non-menstruating women, men and re-occurrence of excised endometriosis. MMc could account for the histological and biochemical differences seen between eutopic and ectopic endometriosis, the dysfunctional immune response, vascular and neural response in ectopic endometriosis and the re-occurrence of endometriosis following excision and even hysterectomy.

MMc would put the point of endometriosis etiology at fetal development, with endometriosis symptoms triggered by oestrogen stimulation such as puberty, rather than endometriosis etiology starting at puberty in women. If correct, endometriosis could be considered a pregnancy-related disease that could affect all humans. This could change accepted demographic of patients and look at new, and ideally less invasive, diagnostic techniques and treatment options for patients with endometriosis. Further studies are required to test this hypothesis.

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Reference List:

1. Zhang, T., De Carolis, C., Man, G.C.W. and Wang, C.C. (2018). The link between immunity, autoimmunity and endometriosis: a literature update. Autoimmunity Reviews, 17(10), pp.945–955

2. Zondervan, K.T., Becker, C.M. and Missmer, S.A. (2020). Endometriosis. New England Journal of Medicine, 382(13), pp.1244–1256

3. Koninckx, P. R., Ussia, A., Adamyan, L., Tahlak, M., Keckstein, J., Wattiez, A., & Martin, D. C. (2021). The epidemiology of endometriosis is poorly known as the pathophysiology and diagnosis are unclear. Best practice & research. Clinical obstetrics & gynaecology, 71, 14–26. https://doi.org/10.1016/j.bpobgyn.2020.08.005 [Accessed 18 Sep.2021]

4. Guo, S.-W. . (2009). Epigenetics of endometriosis. Molecular Human Reproduction, [online] 15(10), pp.587–607. DOI 10.1093/molehr/gap064 [Accessed 18 Sep. 2021].

5. Eisenberg, V.H., Zolti, M. and Soriano, D. (2012). Is there an association between autoimmunity and endometriosis? Autoimmunity Reviews, 11(11), pp.806–814

6. Wu, J., Xie, H., Yao, S. and Liang, Y. (2017). Macrophage and nerve interaction in endometriosis. Journal of Neuroinflammation, 14(1)

7. Brosens, I., Brosens, J.J. and Benagiano, G. (2012). The eutopic endometrium in endometriosis: are the changes of clinical significance? Reproductive BioMedicine Online, 24(5), pp.496–502.

 doi:10.20944/preprints202109.0372.v1

8.Sourial, S., Tempest, N. and Hapangama, D.K. (2014). Theories on the Pathogenesis of Endometriosis. International Journal of Reproductive Medicine, 2014, pp.1–9.

9. Batt, R.E. and Yeh, J. (2013). Müllerianosis. Reproductive Sciences, 20(9), pp.1030–1037

10. Nothnick, W.B. (2001). Treating endometriosis as an autoimmune disease. Fertility and Sterility, 76(2), pp.223–231

11. Bianco, B., André, G.M., Vilarino, F.L., Peluso, C., Mafra, F.A., Christofolini, D.M. and Barbosa, C.P. (2012). The possible role of genetic variants in autoimmune-related genes in the development of endometriosis. Human Immunology, 73(3), pp.306–315

12. Menzhinskaya, I.V., Melkumyan, A.G., Pavlovich, S.V., Chuprynin, V.D., Vanko, L.V. and Sukhikh, G.T. (2020). [Autoimmune markers for non-invasive diagnosis of endometriosis in women]. Biomeditsinskaia Khimiia, [online] 66(2), pp.162–166. DOI: 10.18097/PBMC20206602162 [Accessed 18 Sep. 2021]

13. Matarese, G., Placido, G.D., Nikas, Y. and Alviggi, C. (2003). Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends in Molecular Medicine, [online] 9(5), pp.223–228. DOI: 10.1016/s1471-4914(03)00051-0 [Accessed 18 Sep. 2021]

14. Grummer, R. (2001). Peritoneal endometriosis: validation of an in-vivo model. Human Reproduction, 16(8), pp.1736–1743.

15. Saha, R., Pettersson, H.J., Svedberg, P., Olovsson, M., Bergqvist, A., Marions, L., Tornvall, P. and Kuja-Halkola, R. (2015). Heritability of endometriosis. Fertility and Sterility, [online] 104(4), pp.947–952. DOI: 10.1016/j.fertnstert.2015.06.035 [Accessed 18 Sep. 2021]

16. Wu, Y., Strawn, E., Basir, Z., Wang, Y., Halverson, G., Jailwala, P. and Guo, S.-W. (2006). Genomic Alterations in Ectopic and Eutopic Endometria of Women with Endometriosis. Gynecologic and Obstetric Investigation, 62(3), pp.148–159.

17. Rahmioglu, N., Montgomery, G.W. and Zondervan, K.T. (2015). Genetics of Endometriosis. Women's Health, 11(5), pp.577–586.

18. Hapangama, D.K., Turner, M.A., Drury, J., Heathcote, L., Afshar, Y., Mavrogianis, P.A. and Fazleabas, A.T. (2010). Aberrant expression of regulators of cell-fate found in eutopic endometrium is found in matched ectopic endometrium among women and in a baboon model of endometriosis. Human Reproduction, 25(11), pp.2840–2850.

19. Kobayashi, H., Iwai, K., Niiro, E., Morioka, S. and Yamada, Y. (2014). Fetal programming theory: implication for the understanding of endometriosis. Human Immunology, [online] 75(3), pp.208–217. DOI: 10.1016/j.humimm.2013.12.012 [Accessed 18 Sep. 2021].

20. Signorile, P.G., Baldi, F., Bussani, R., D'Armiento, M., De Falco, M. and Baldi, A. (2009). Ectopic endometrium in human foetuses is a common event and sustains the theory of müllerianosis in the pathogenesis of endometriosis, a disease that predisposes to cancer. Journal of experimental & clinical cancer research: CR, [online] 28, p.49. DOI: 10.1186/1756-9966-28-49 [Accessed 18 Sep. 2021].

21. Signorile, P.G., Baldi, F., Bussani, R., D'Armiento, M., De Falco, M., Boccellino, M., Quagliuolo, L. and Baldi, A. (2010). New evidence of the presence of endometriosis in the human fetus. Reproductive BioMedicine Online, 21(1), pp.142–147

22. Signorile, P.G., Baldi, F., Bussani, R., Viceconte, R., Bulzomi, P., D'Armiento, M., D'Avino, A. and Baldi, A. (2012). Embryologic origin of endometriosis: analysis of 101

human female fetuses. Journal of Cellular Physiology, [online] 227(4), pp.1653–1656. DOI: 10.1002/jcp.22888 [Accessed 18 Sep. 2021]

23. Martin, J.D. and Hauck, A.E. (1985). Endometriosis in the male. The American Surgeon, [online] 51(7), pp.426–430. Available at: https://pubmed.ncbi.nlm.nih.gov/4014886/ [Accessed 18 Sep. 2021].

24. Rei, C., Williams, T. and Feloney, M. (2018). Endometriosis in a Man as a Rare Source of Abdominal Pain: A Case Report and Review of the Literature. [online] Case Reports in Obstetrics and Gynecology. DOI: 10.1155/2018/2083121 [Accessed 18 Sep. 2021]

25. Endometriosis of the Male Urinary System: A Case Report. (1980). The Journal of Urology, [online] 124(5), pp.722–723. DOI: 10.1016/S0022-5347(17)55627-X [Accessed 18 Sep. 2021]

26. Jabr, F. and Mani, V. (2014). An unusual cause of abdominal pain in a male patient: Endometriosis. Avicenna Journal of Medicine, 4(4), p.99

27. Al-Obaidy, K.I. and Idrees, M.T. (2018). Endometriosis With Cystic Degeneration: A Rare Disease of Males. International Journal of Surgical Pathology, 27(3), pp.311–314

28. Laschke, M.W. and Menger, M.D. (2007). In vitro and in vivo approaches to study angiogenesis in the pathophysiology and therapy of endometriosis. Human Reproduction Update, 13(4), pp.331–342

29. Tirado-González, I., Barrientos, G., Tariverdian, N., Arck, P.C., García, M.G., Klapp, B.F. and Blois, S.M. (2010). Endometriosis research: animal models for the study of a complex disease. Journal of Reproductive Immunology, 86(2), pp.141–147

30. Grümmer, R. (2006). Animal models in endometriosis research. Human Reproduction Update, 12(5), pp.641–649

31. Fugazzola, L., Cirello, V. and Beck-Peccoz, P. (2012). Microchimerism and Endocrine Disorders. The Journal of Clinical Endocrinology & Metabolism, 97(5), pp.1452–1461

32. Jennewein, M.F., Abu-Raya, B., Jiang, Y., Alter, G. and Marchant, A. (2017). Transfer of maternal immunity and programming of the newborn immune system. Seminars in Immunopathology, 39(6), pp.605–613

33. Gammill, H.S. and Nelson, J.L. (2010). Naturally acquired microchimerism. The International Journal of Developmental Biology, 54(2-3), pp.531–543

34. Stevens, A.M. (2016). Maternal microchimerism in health and disease. Best Practice & Research Clinical Obstetrics & Gynaecology, 31, pp.121–130

35. Ståhlberg, A., El-Heliebi, A., Sedlmayr, P. and Kroneis, T. (2017). Unravelling the biological secrets of microchimerism by single-cell analysis. Briefings in Functional Genomics, 17(4), pp.255–264

36. Dawe, G.S., Tan, X.W. and Xiao, Z.-C. (2007). Cell Migration from Baby to Mother. Cell Adhesion & Migration, Cell adhesion & migration, 1(1), pp.19–27

37. Ichinohe, T. (2010). Long-term feto-maternal microchimerism revisited. Chimerism, 1(1), pp.39–43

38. Fassbender, A., Debiec-Rychter, M., Bree, R.V., Vermeesch, J.R., Meuleman, C., Tomassetti, C., Peeraer, K., D'Hooghe, T. and Lebovic, D.I. (2015). Lack of Evidence That Male Fetal Microchimerism is Present in Endometriosis. Reproductive Sciences, 22(9), pp.1115–1121

39. Bhat, M.A., Sharma, J.B., Roy, K.K., Sengupta, J. and Ghosh, D. (2019). Genomic evidence of Y chromosome microchimerism in the endometrium during endometriosis and in cases of infertility. Reproductive Biology and Endocrinology, 17(1)

 doi:10.20944/preprints202109.0372.v1

40. McCluggage, W.G. (2019). Endometriosis-related pathology: a discussion of selected uncommon benign, premalignant and malignant lesions. Histopathology, 76(1), pp.76–92.