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ASSISTED REPRODUCTION TECHNOLOGIES

The role of the *Hoxa10/HOXA10* gene in the etiology of endometriosis and its related infertility: a review

Alysson Zanatta · André M. Rocha · Filomena M. Carvalho · Ricardo M. A. Pereira · Hugh S. Taylor · Eduardo L. A. Motta · Edmund C. Baracat · Paulo C. Serafini

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Abstract
Purpose Endometriosis and its associated infertility have been the object of continuous research for over a century. To understand the molecular mechanisms underlying the disease, it has become necessary to determine the aspects of its etiology that are not explained by the retrograde menstruation theory. This could in turn elucidate how various clinical and surgical treatments might affect the evolution and remission of the disease.

Capsule The homebox gene Hoxa10/HOXA10 might be related to the embryogenic etiology of endometriosis, as well as to its associated infertility.

Methods This review is focused on the most recent clinical and laboratory findings regarding the association of HOXA10 with endometriosis and infertility.
Result The homebox (Hox/HOX) proteins are highly conserved transcription factors that determine segmental body identities in multiple species, including humans. Hoxa10/HOXA10 is directly involved in the embryogenesis of the uterus and embryo implantation via regulation of downstream genes. Cyclical endometrial expression of Hoxa10/HOXA10, with a peak of expression occurring during the window of implantation, is observed in the adult in response to estrogen and progesterone. Women with endometriosis do not demonstrate the expected mid-luteal rise of HOXA10 expression, which might partially explain the infertility observed in many of these patients. Recent studies also demonstrated HOXA10 expression in endometriotic foci outside the Müllerian tract.
Conclusions Multiple lines of evidence suggest that the actions of the *homeobox A10* (*Hoxa10/HOXA10*) gene could account for some aspects of endometriosis.

Keywords Homeobox genes · Hoxa10 · Endometriosis · Etiology · Infertility · Surgery

A. Zanatta (✉) · A. M. Rocha · R. M. A. Pereira · E. L. A. Motta · P. C. Serafini
Huntington Medicina Reprodutiva,
Av. República do Líbano, 529—Ibirapuera,
04501-000 São Paulo, SP, Brazil
e-mail: alysson.zanatta@gmail.com

F. M. Carvalho
Departamento de Patologia, Faculdade de Medicina de São Paulo,
São Paulo, SP, Brazil

A. Zanatta · E. C. Baracat · P. C. Serafini
Departamento de Ginecologia, Hospital das Clínicas,
Faculdade de Medicina de São Paulo,
São Paulo, SP, Brasil

H. S. Taylor
Division of Reproductive Endocrinology and Infertility,
Department of Obstetrics and Gynecology,
Yale University School of Medicine,
New Haven, CT, USA

E. L. A. Motta
Departamento de Ginecologia,
Universidade Federal de São Paulo,
São Paulo, SP, Brasil

Introduction

Endometriosis is considered by most a chronic, recurrent and progressive disease [1], although its natural history is not fully understood. At least 10% of the reproductive female population is estimated to be affected by this disease, and its predominate symptoms, which include pelvic pain and/or infertility [2]. Therefore, the etiology of the disease is a motivation for research and a topic of intense debate.

Sampson's theory of retrograde menstruation [3] hypothesizes that endometrial cells, derived from the endometrial

cavity, locate to the pelvic cavity during menses and implant onto the peritoneum and pelvic organs. The author based his hypothesis on the direct observation of retrograde menstruation during surgeries, as well as on the prevalence of ovary involvement and the observation that endometriotic foci distribution mimicked that of ovarian cancer lesions. Sampson also concluded that endometriosis is primarily late onset, generally after the third decade of life, because otherwise the disease would be detectable immediately following menarche. In addition, Sampson concluded that ectopic endometrial cells were likely to be identical to eutopic endometrial cells. Furthermore, as endometriosis was frequently observed in nulliparous women, it was concluded that pregnancy might protect women against endometriosis. These observations were based on the best quality of clinical evidence available at that time, and on an extensive career devoted to the topic.

However, endometrial cells are now known to be functionally distinct from those in the eutopic endometrium [4]. It is also known that endometriosis does not emerge exclusively after the third decade of life. In fact, recent studies have demonstrated that 11% of female fetuses submitted to necropsy were found to have endometriosis, with lesions located in similar regions to those found in adult women [5]. Contemporary observations indicate that the most common sites of endometriosis are the uterosacral ligaments and torus uterinus (i.e., the uterine transversal fold corresponding to insertion of both uterosacral ligaments) [6]. Furthermore, retrograde menstruation may be observed in up to 90% of women [7], while the prevalence of endometriosis is estimated to be only 10%. In addition, there is no direct evidence (e.g.: electron-microscopy images) that endometrial cells suspended in the peritoneal fluid are able to invade and adhere to the peritoneal surface [8]. Therefore, the traditional theory of retrograde menstruation [3] has not been completely proved and cannot account for many clinical aspects of the disease [9].

Alternatively, the theory of Mülleriosis [10, 11], which precedes the theory of retrograde menstruation, states that endometriosis might originate from mesenchymal embryonic cells. Those cells would be randomly distributed in the pelvis during organogenesis, throughout the route of descent of the Müllerian ducts toward the pelvis. According to this theory, endometriotic foci would result from metaplasia of these mesenchymal cells, stimulated by estradiol, and beginning at puberty.

Modern molecular biology has made it possible to understand the complex molecular machinery involved in the process of metaplasia. For instance, the homeobox A10 (HoxA10/HOXA10) transcription factor has been implicated as an important player in the development of endometriosis [12]. The homeotic genes are highly conserved genes that impart anatomical and functional identities to the various segmental body units during ontogeny [13]. The homeotic genes of vertebrates are referred to as *homeobox* (*Hox/HOX*) genes. Hoxa10/HOXA10 is involved in the embryogenesis of the uterine epithelium, stroma and muscle [14]. It is cyclically expressed in the adult endometrium in response to steroid hormones, regulating endometrial receptivity during the nidation window [15]. Several studies have suggested an impairment of implantation in patients with endometriosis, but the mechanisms underlying it are not well understood [16–18]. In accordance with HOXA10's role in implantation, it was found that women with endometriosis have altered expression of HOXA10 in the eutopic endometrium, which could account for the defective implantation observed in these women [19]. Surprisingly, the HOXA10 was also found to be expressed both in the epithelium and stroma of endometriotic lesions, although at a lower level [12]. These observations point to a role for HOXA10 in the etiology of endometriosis. In this paper, the function of the homeotic genes will be reviewed, particularly that of *HOXA10*, as well as recent studies that relate this gene to the pathogenesis of endometriosis.

HOX proteins impart developing body segment identities during embryogenesis

The mammalian homeobox genes, or "*Hox/HOX*" genes, are homologs of the fruit fly *Drosophila melanogaster* selector gene complexes *Antennapedia* and *Bithorax* [20]. Across species, these selector genes are spatially and temporally expressed during organogenesis for the determination of the body part identity along the anterior-posterior axis [21]. Namely, the homeotic genes are master regulatory genes responsible for the patterning of embryonic body segments. Their action is exerted through the encoding of transcription factors that determine the activation or repression of a myriad of downstream genes (which are not completely known), leading to the development of a determined anatomical structure. The denomination "*Hox*" is used for non-human vertebrate homeotic genes, while "*HOX*" is applied to human homeotic genes. The fruit fly has 8 homeotic genes grouped in a region of the genome known as the Homeotic complex (HOM-C) [22]. In humans and mice, there are at least 39 *Hox/HOX* genes distributed in four groups lettered A, B, C and D. These groups each comprise 9–13 genes and are distributed in the human chromosomes 7, 17, 12, and 2, respectively [21]. The presence of 4 groups of *Hox/HOX* genes confers the potential for genetic redundancy, with the identity of a determined body segment determined by the combination of *Hox/HOX* genes expressed. The homeobox, which is the defining characteristic of *Hox/HOX* genes, is formed by a highly conserved sequence of 183 base pairs, which encode a homeodomain of 61 amino acids, similar to the bacterial helix/anti-helix model. The homeodomain mediates protein

binding to promoter regions of target genes containing the sequence 5′-TAAT-3′.

One important characteristic of the *Hox/HOX* genes is collinearity, as the genes are expressed along the anterior-posterior axis in the same sequence as they appear on the chromosome. For instance, the *Hox* genes located at the 3′ extremity of the chromosome are expressed earlier and in a more cranial position in relation to those situated more 5′, which are expressed later and in more caudal regions (Fig. 1). For example, the gene located at the most 3′ end of the HOM-C complex, *labial*, is expressed in the anterior portion of the developing cephalic segment, primarily in the mandibular lobe and hypopharynx. It is also the earliest expressed gene during organogenesis [23]. In addition, correct and orderly expression of the anterior homeobox genes *HoxA1* and *HoxB1*, as directed by a retinoic acid gradient, is necessary for embryonic neurogenesis of the central nervous system [24]. If a mutation or deletion of a *Hox* gene occurs, the body segment where it is normally expressed may develop the characteristics dictated by the juxtaposed 3′ *Hox* gene, resulting in a phenotypic change in relation to its anterior structure, a phenomenon called anterior transformation. In fact, this is the mechanism responsible for uterine malformations caused by intrauterine exposure to diethilstilbestrol (DES) [25].

In the fruit fly, homeobox gene mutations cause dramatic phenotypic changes. For instance, the loss of function of the HOM-C 3′ *labial* gene results in failure of the involution of the cephalic segment of the embryo, resulting in derangement of the salivary glands and cephalo-pharynx apparatus [23]. Other examples in *Drosophila* include the development of an extra pair of wings in a segment which should have developed a pair of halters instead, or the transformation of the antenna into legs [26]. However, in mammals, the genetic redundancy provided by the presence of 39 *Hox/HOX* genes results in less dramatic transformations in cases of mutations or deletions. In mice, deletion of *Hoxa10* results in a transformation of the proximal portion of the uterine body into a tubular and narrow structure similar to the fallopian tube, which is itself under the influence of the *Hoxa9* gene [27]. Occasionally, the gene just posterior (5′) to the mutated gene acquires dominance and produces a posterior transformation.

It is clear that the *Hox/HOX* genes have a powerful ability to regulate the morphology of body segments. This regulation may influence the spatial axis in a developing organism, a temporal axis during development, or adult cellular maturation. In animals, deletion of *Hox* genes produces axial and appendiceal malformations. In humans, uterine malformations caused by the synthetic estrogen DES are likely mediated by HOXA10. If the etiology of endometriosis could be explained by the Mülleriosis theory, it is possible that Hoxa10/HOXA10 is somehow involved with it. The next sections will focus on how Hoxa10/HOXA10 might be involved in endometriosis and its related infertility.

Hoxa-10/HOXA-10 is involved in the organogenesis of the Müllerian reproductive tract

The specificity of the temporal and spatial expression of the *Hox/HOX* genes is evident in the organogenesis of the

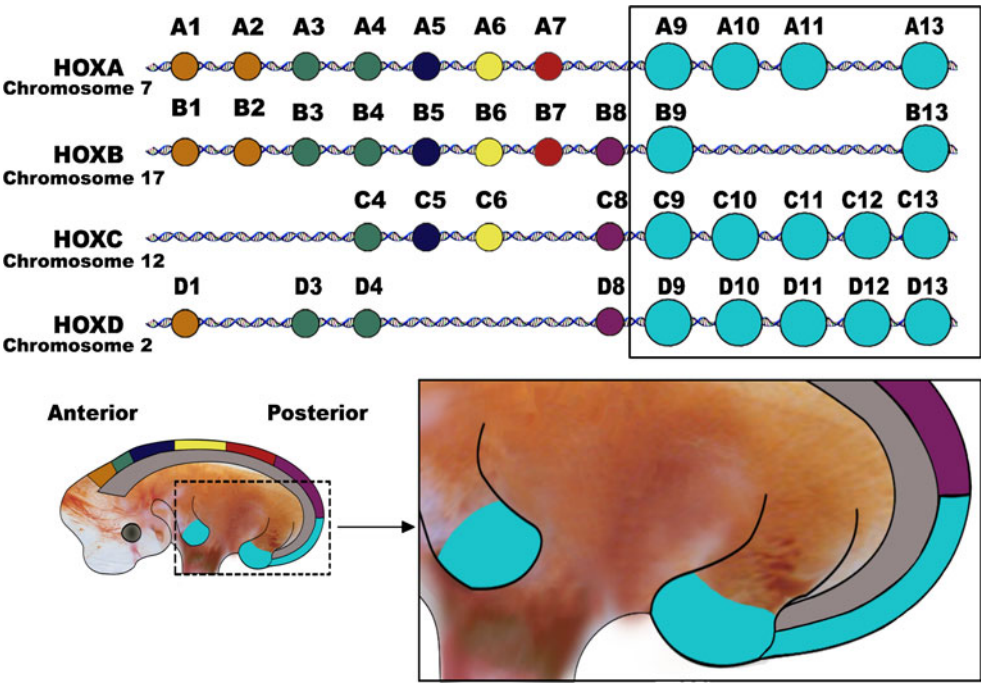

Fig. 1 The paralogs homeobox genes. In the mammalian, four groups (**a**–**d**) of paralogs homeobox genes are distributed in the chromosomes 7, 17, 12 and 2, respectively. The more 3′ genes are expressed earlier and in the rostral and anterior regions of the embryo, while the 5′genes are expressed later, caudally and in the more posterior regions. Homeobox genes 9 to 13 (inset) are expressed in the limb primordia, as also as in the posterior and distal segments of the body, including the genital system. The anterior limit of a 5′ gene overlaps the fading posterior expression of its immediate 3′precedent

female reproductive tract. The female reproductive system is derived from the paramesonephric (Müllerian) ducts, which are made up of columnar cells surrounded by mesenchymal, and remain relatively undifferentiated prior to birth. The final differentiation of fallopian tubes, uterus, cervix and anterior vagina, which primarily occur postnatally, are programmed by molecular events that are not yet completely understood.

The *Hoxa9, Hoxa10, Hoxa11* and *Hoxa13* genes are homologs of the posteriorly-expressed Drosophila gene *abdominal-B* (*Abd-B*). In mammals, these genes regulate the differentiation of the Müllerian ducts into adult genital structures. They are precociously and simultaneously expressed in the paramesonephric duct during early embryogenesis (an exception to the colinearity principle), in a phase when the Müllerian ducts lack stromal or epithelial differences [14]. These genes are highly expressed in both ductal epithelium and juxtaposed stromal cells. Two weeks after birth, a period that corresponds to the peak of the differentiation process in mice, *Hoxa9, Hoxa10, Hoxa11* and *Hoxa13* develop their characteristic spatial distribution throughout the duct. Accordingly, the expression of *Hoxa9* is limited to the fallopian tube; *Hoxa10* is expressed in the uterine epithelium, stroma and muscle; *Hoxa11* is expressed in the cervical glands and epithelium (although it is also expressed in the uterine corpus); and *Hoxa13* is strongly expressed in the vaginal epithelium [14] (Fig. 2). In situ hybridization of *Hoxa10* mRNA reveals strong expression in the uterus, but no expression in the fallopian tube, cervix or vagina. Curiously, *Hoxa10* is also expressed in the distal intestine [14]. The *Hoxa10/HOXA10* gene is expressed at lower levels in the myometrium, where it is also under the dynamic influence of progesterone, as is the case in the endometrium [28]. As expected, spatial expression of the *Hox* genes in this context corresponds craniocaudally to their 3′ to 5′ order on the chromosome. The *HOX* axis of the human genital system is identical to that of mice [14].

The female reproductive system is unique in relation to other tissues and systems, as most differentiation takes place postnatally. Estrogen seems to regulate the expression of posterior *Hox/HOX* genes during embryogenesis, similar to the way retinoic acid regulates the formation of the central nervous system. Female mice deficient in the estrogen receptor alpha (ER-α) have uterine hypoplasia without the development of adult endometrium, and are sterile [29]. Uterine malformations caused by DES are mediated by ER-α, with the "T" shaped uterus and vaginal adenosis (glandular uterine and cervical tissue present in the vagina) likely examples of anterior transformation. The plasticity of the female genital system is demonstrated by the final establishment of its spatial distribution postnatally, and by the intense transformations it undergoes during menstrual and gestational cycles.

Estrogen and progesterone regulate *Hoxa10/HOXA10* function, which might be necessary for endometrial cell differentiation and embryo implantation

Some of the *Hox/HOX* genes responsible for patterning during embryonic genitourinary tract formation are also

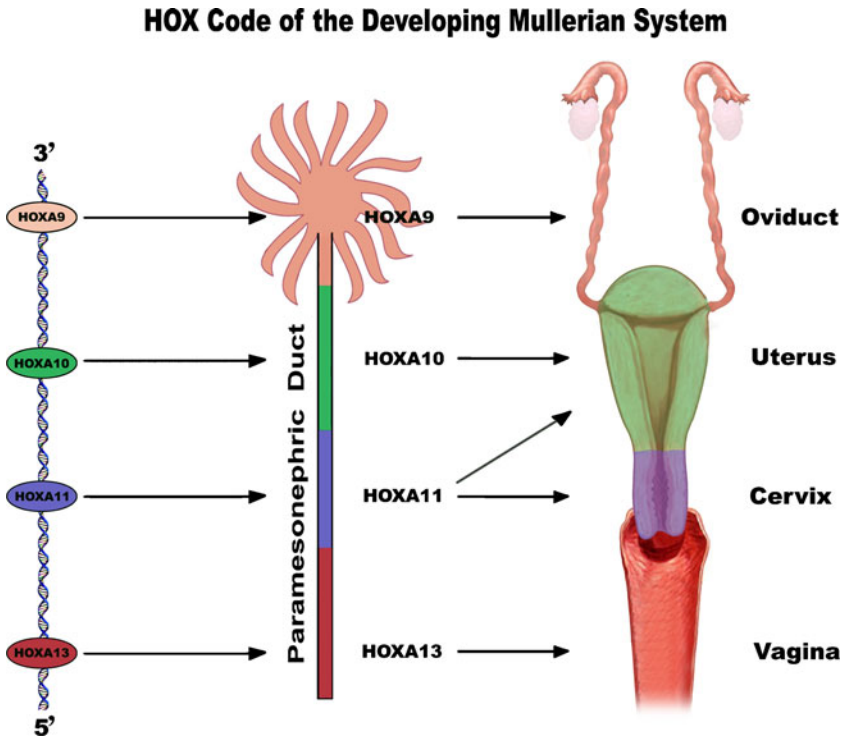

Fig. 2 Spatial distribution of the orthologs homeobox genes A9, A10, A11 and A13 in the developing paramesonephric duct. The most 3′ortholog HOXA9 gene is expressed in the oviduct anlage; the HOXA10 gene is expressed in the uterine anlage; HOXA11 is associated with both the uterine and cervix anlagen; and the HOXA13 gene is expressed in the vagina anlage. This spatial distribution is in accordance with the property of collinearity, as the genes are expressed in the paramesonephric duct in the same order as they are situated in chromosome, simultaneously during the embryo development

responsible for remodeling the adult genitourinary tract. In the adult, *Hoxa10/HOXA10* is expressed in tissues with high plasticity (bone marrow and endometrium) [15, 30], in accordance with its role in tissue differentiation. Hoxa10/HOXA10 function is essential for normal uterine embryogenesis and regulation of the menstrual cycle, because it regulates a variety of downstream genes, including cell adhesion molecules, signal transduction factors, and metabolic mediators. In the endometrium, the *Hoxa10/HOXA10* gene is expressed in a cyclical manner, under the influence of estrogen and progesterone, with maximal rise during the window of implantation [15]. Generally, Hoxa10/HOXA10 protein expression is restricted to the nucleus of both glandular and stromal cells [31]. Genes which are regulated by Hoxa10/HOXA10 include the homeotic gene *Emx2/EMX2*, *ß3-integrin*, *insulin growth factor binding protein-1 (IGFBP-1), cyclin-dependent kinase inhibitor*, genes of the *Wnt* family, *FK506 binding protein 4*, and the prostaglandin receptors *EP-3* and *EP-4*, among others [32, 33]. In the endometrium, the process of final cellular differentiation is similar to that observed during organogenesis, when functionally independent tissues arise from undifferentiated cells. The *Hox/HOX* genes are critical in this cell fate determination process.

The *Hox/HOX* genes likely regulate cellular differentiation and proliferation in the adult by mimicking their actions during embryogenesis [34]. For instance, during each menstrual cycle, endometrial cells undergo an initial period of proliferation, followed by differentiation, eventually rendering the endometrium receptive for implantation. When implantation does not occur, apoptosis and endometrial shedding initiates a new cycle. In the endometrium, both *HOXA10* and *HOXA11* are expressed in a cyclical manner, mediating some functions of the estrogen and progesterone hormones. They are expressed in the epithelium and stroma during the whole menstrual cycle, exhibiting maximal rise during the intermediate secretory phase, otherwise known as the implantation window [15]. This period corresponds to the peak of endometrial histological differentiation, and to high levels of circulating estrogen and progesterone. These steroid hormones bind to their endometrial receptors and activate the transcription of *HOXA10*, which in turn regulates cell differentiation, resulting in an endometrium receptive to embryo implantation. The expression of *HOXA10* mRNA in the epithelium and stroma is directly related to serum levels of 17-ß estradiol. Both estrogen and progesterone individually stimulate the endometrial expression of *Hoxa10/HOXA10*, and progesterone has additional stimulating effects over estrogen [15].

In addition to estrogen and progesterone, testosterone and vitamin D are also regulators of *Hoxa10/HOXA10*. The endometrium expresses testosterone receptors during the entire menstrual cycle, primarily in the endometrial functional layer [35]. Patients with hyperandrogenism secondary to polycystic ovary syndrome demonstrate lower levels of *HOXA10* mRNA in the secretory phase, which might influence fertility in this group of patients [36]. Furthermore, the female genitourinary tract also expresses vitamin D receptor, and the process of uterine decidualization may be partially influenced by the direct action of vitamin D on the expression of *HOXA10* [37].

HOXA10 may influence the formation of misplaced endometrial cells during embryonic life, originating the disease known as endometriosis

The female internal genital system is formed by the paramesonephric, or Müllerian ducts. Longitudinal folds of mesenchymal cells invaginate from the lateral abdominal walls of the embryo to form stripes that subsequently grow caudally and fuse in the midline. The cranial portions of the ducts are hollow and open freely in the celomic cavity, giving rise to the Fallopian tubes. Their caudal portion are initially solid and, after a period of central resorption, will originate the uterus, cervix and proximal third of the vagina. The broad ligaments arise from the two folds of peritoneum brought together by the fusion of the Müllerian ducts in the midline, and the development of the uterosacral ligaments follows that of the uterus [38].

Although controversial, the spatial distribution of the endometriotic lesions in the pelvis resembles, in a particular way, the caudal growth of the Müllerian ducts. Starting from the abdominal lateral parietocolic gutters, it has been observed a high prevalence of bilateral colon-to-sidewall adhesions in women with chronic pelvic pain and associated pelvic endometriosis [39]. Besides, as much as 60% of these adhesions may harbor histological proven endometriotic lesions [40]. In the Fallopian tubes, endometriosis foci maybe found adjacent to embryonic duct remnants, and the histological evaluation of those lesions suggests the existence of a gradual transformation from embryonic remnants epithelium to endometrial glands [41]. Downway into the pelvis, it is observed that the pelvic sidewalls are frequent sites for endometriotic lesions, both superficial and retroperitoneal [42, 43]. The pelvic sidewalls correspond to the posterior leaf of the broad ligaments, and, as previously stated, those are folds of peritoneum brought together by the fusion of the Müllerian ducts. Still in the pelvis, it is also known that the torus uterinus and the “so called” uterosacral ligaments are the most common sites for endometriosis [6, 44], especially the portions of the ligaments adjacent to the torus uterinus. These sites are also related to the midline fusion of the Müllerian ducts.

Taking into account the localization of endometriotic lesions nearby and related to Müllerian structures, it maybe plausible to assume a relationship between the embryonic development of the Müllerian ducts, endometriosis, and *HOXA10* (Fig. 3). Embryonic remnants are of coelomic origin, and the coelomic epithelium has the potential to differentiate into endometrial tissue [45]. The *HOX* genes have the primary role in imparting positional identity to undifferentiated tissues along all body axes, and *HOXA10* directs the development of the uterus [14]. The paramesonephric epithelium will give origin the endometrial epithelium, while the endometrial stroma will arise from adjacent mesenchymal cells. In adult women, the HOXA10 protein is preferentially expressed in endometrial stromal cells, the levels typically higher than those of the glandular cells, both in the eutopic and ectopic endometrium of fertile and infertile patients [12, 46]. This finding may be compatible to the role of *HOX* genes in directing the formation of differentiated tissues from mesenchymal cells in the embryonic period and from undifferentiated cells in adult life.

The finding of HOXA10 expression in endometriotic lesions outside its normal domain raises the supposition that *HOXA10* might be necessary for "de novo" development of endometrial tissue, both at eutopic and ectopic locations [12]. HOXA10 protein expression has been demonstrated in human peritoneal, ovarian and lung endometriosis [12], rectosigmoid endometriosis [47], and also in the distal intestine of mice [14]. Considering the possibility that the *HOXA10* gene is related to the development of endometriosis, it remains to be elucidated how and when its action would take place. Signorile et al. [5] have beautifully demonstrated the presence of endometriosis in 11% of human female fetuses, at a gestational

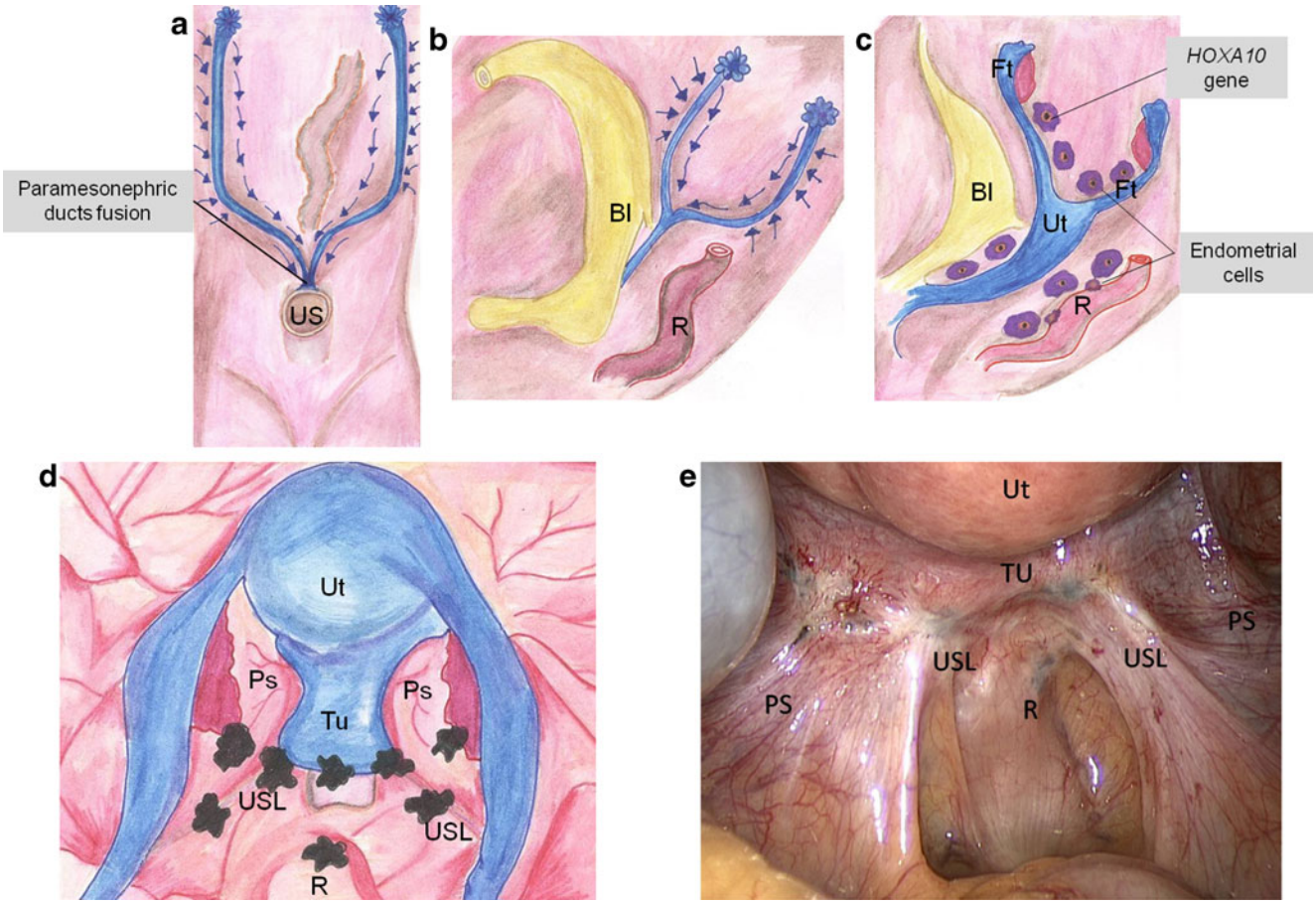

Fig. 3 Embryogenesis of the female internal genital system and proposed role of the HOXA10 in the etiology of endometriosis. Coronal (**a**) and paramedian (**b**) perspectives of the human female embryo at 6-wk gestational age. Longitudinal folds of mesenchymal cells invaginate from the lateral abdominal walls to form stripes that subsequently grow caudally and fuse in the midline (the paramesonephric ducts, depicted in blue), as indicated by small arrows. *HOXA10* gene will impart identity to the portion of the duct destined to be the uterus, including the endometrium. **c** Possibly, mesenchymal cells under the influence of the *HOXA10* gene maybe driven to originate endometrial cells at misplaced locations, including the torus uterinus *(TU)*, uterosacral ligaments *(USL)*, pelvic sidewalls, or posterior leafs of the broad ligaments *(PS)*, and rectosigmoid *(R)*, among others. These would correspond to the most common locations of endometriotic lesions (denoted as black spots) observed in the adult woman (**d** and **e**). *Ut*=uterus; *US*=urogenital sinus and developing bladder; *Bl*=bladder

age as soon as 16-wk, a prevalence that would be similar to that of the general adult population. All endometriosis foci were positive for both CA-125 and estrogen receptor. Lesions were found in the rectovaginal space, in the mesechymal tissue close to the posterior wall of the uterus, in the proximity of the Douglas pouch, and in the muscularis propria of the rectal tube. One of the fetuses also demonstrated nests of endometrial cells inside the myometrium, which likely represent adenomyosis. It may be not surprisingly that all these anatomical sites are common locations for endometriosis in women. According to these results, the authors hypothesized that these endometrial foci were somehow misplaced outside the uterine cavity during the early steps of organogenesis, and they would be undetectable until puberty when hormonal stimulus would cause its re-growth and the onset of the disease known as endometriosis. Based on the cited researches, we also believe this is a very plausible hypothesis. It is very likely that the HOXA10 gene has a central role in this sophisticated, still incompletely understood, system of organogenesis and embryonic origin of endometriosis. To further clarify the role of *HOXA10* gene, it would be important to identify if *HOXA10* is expressed in endometriosis foci in fetuses, the same manner as it has been identified in adult women.

Abnormal endometrial *HOXA10* expression is associated with infertility in endometriosis patients

The association between endometriosis and infertility is evident, but the exact mechanisms by which endometriosis causes infertility are still unknown. Tubal blockage, impaired ovulation, pelvic inflammation and decreased endometrial receptivity are possible contributors to endometriosis-related infertility. One of the major underlying causes of infertility in patients with endometriosis might be implantation failure [48]. Implantation rates are reduced in these patients, during both natural and assisted reproductive technology (ART) cycles, even in patients with minimal disease [49]. Gene profiling and microarray studies have indicated a wide variety of genes that are either up or downregulated in the endometrium of patients with endometriosis during both phases of the menstrual cycle [50–52]. The proteins encoded by these genes are primarily associated with cell adhesion, proliferation and differentiation, the extracellular matrix, and transmembrane molecules, among others. However, no single gene (or class of genes) can be considered responsible for the particular molecular derangement observed in the endometrium of these patients.

The *Hoxa10/HOXA10* gene is a master regulator that either activates or represses downstream genes, some of them related to embryo implantation. *Hoxa10* (-/-) mice ovulate normally, but implantation does not occur. However, when their embryos are transferred to wild-type mice, implantation is restored. Conversely, wild-type embryos do not implant in *Hoxa10* (-/-) mice [53]. In *Hoxa10* (-/-) mice, there is commonly hemorrhage and disorganization at the implantation site and adjacent lumen [15]. Similar defects are observed in *Hoxa11* (-/-) mice, where there is insufficient development of stromal, glandular and decidual tissues during early pregnancy [54]. During the implantation window, in vivo uterine gene transfection may alter the level of expression of *Hoxa10* in mice, and consequently the number of offspring [55].

So far it has been established that a coordinated and orderly expression of *Hoxa10/HOXA10* is necessary for implantation, but downstream target gene expression or repression driven by Hoxa10/HOXA10 is also important. For example, Hoxa10/HOXA10 represses the expression of the homeobox gene *Emx2*/*EMX2* [56]. EMX2 is cyclically expressed in the adult endometrium, where it exerts antiproliferative effects. Emx2 is also necessary for genitourinary tract development, as *Emx2* mutant animals have Müllerian duct agenesis and die in utero due to urinary malformations [57]. In the adult, *Emx2* expression drops to approximately 50% of normal levels in the peri-implantation endometrium [56], an event which does not occur in women with endometriosis [58]. Furthermore, mice transfected with *Emx2* cDNA in the peri-implantation period have a 40% decrease in litter size, an effect mediated by diminished endometrial epithelial cell proliferation [59]. These findings suggest that Hoxa10-regulated Emx2 expression is fundamental for embryo implantation.

In addition, the endometrium of patients with endometriosis exhibits other abnormalities that could further explain the abnormal fertility exhibited. For instance, progesterone-regulated, biological markers of endometrial receptivity, including glycodelin A, osteopontin, lysophosphatidic acid receptor 3, and HOXA10, are all reduced during the secretory phase in patients with endometriosis [31].

As previously stated, a mid-secretory rise of Hoxa10/HOXA10 expression normally occurs in each menstrual cycle. However, infertile patients with endometriosis do not demonstrate this rise in HOXA10 expression, nor of HOXA11, another homeobox gene involved in uterine embryogenesis and endometrial receptivity [19, 60]. This failure might be due to alterations in genomic DNA methylation at the *HOXA10* locus [61], as it has been observed that when endometriosis is experimentally induced in mice, genomic methylation is altered in eutopic endometrial cells. This suggests that endometriotic foci could modify the endometrium through intercellular

signaling pathways, likely involving progesterone [62] and/or *IGFBP-1* [34]. This is a relatively new concept that could explain how endometriosis renders the endometrium less receptive for implantation. The concept of "pelvic inflammation" caused by endometriosis could be somehow explained by direct alterations of the endometrium caused by extrauterine endometriotic foci, through a pathway in which Hoxa10/HOXA10 may have a central role. It is not known if surgical radical excision of endometriosis foci is able to restore a molecular endometrial environment most favourable to implantation in those patients with endometriosis related infertility, maybe influencing or decreasing methylation at the *HOXA10* locus.

Although histologically similar, there are significant molecular differences between the endometrium and endometriotic foci. Estrogen is the most powerful known mitogen in endometriotic foci, and both estrogen and progesterone are able to initiate expression of *Hoxa10/HOXA10*. The ectopic endometrium exhibits lower expression of 17 ß- hidroxysteroid dehydrogenase 2, aberrant expression of aromatase, and altered total levels of the progesterone receptors [4, 63]. The ß subtype of the estrogen receptor (ERß) is 140 times more highly expressed in endometriotic foci in relation to the eutopic endometrium of patients without the disease [51]. The final result is a known resistance of endometriotic foci to progesterone, in addition to an increased local production of estradiol. HOXA10 has been found to be expressed in the stromal portion of endometriotic foci, including the pelvic peritoneum, ovary and lung parenchyma [12], which is unexpected, given that these sites are outside the Müllerian axis. Based on these findings, it is possible that HOXA10 expression is necessary for the development of "de novo" endometriosis. During embryogenesis, Hoxa10/HOXA10 is necessary for the development of immature mesenchymal cells into endometrial tissue. Therefore, the gene might also be responsible for imparting the endometriotic identity to immature cells in the adult. The identification of Hoxa10/HOXA10 expression at extrauterine sites could point to possible sites that can develop the disease. It is possible that precocious "silencing" of these extrauterine genes, either medically or surgically, could pre-empt the emergence of endometriosis. Despite many controversial reports about the issue of surgery, our group recently demonstrated that surgical radical excision of endometriotic foci before an ART attempt may double the chances of achieving pregnancy during ART cycles [64], although the exact mechanism by which this occurs is unknown.

Finally, when pregnancy occurs, the uterine decidua expresses high levels of *HOXA10* mRNA [15]. *HOXA10* is also expressed in the uterine decidua at term [65]. The role of Hoxa/HOXA10 in implantation is highlighted by the observation of aberrantly high levels of *HOXA10* mRNA expression at the tubal mucosa during ectopic pregnancy, specifically at the implantation site [66]. An equally important observation is that *HOXA10* mRNA expression is directly reduced by hydrosalpinx fluid in normovulatory women [67], and surgical resection (salpingectomy) restores normal endometrial expression of *HOXA10* [68].

Final considerations

The traditional theory of retrograde menstruation does not completely explain the origin of endometriosis. A model that might prove more useful is the Mülleriosis theory. Using a simple analogy, if embryonic mesenchymal cells represent "seeds" thrown in a "field" (the pelvis, or extrapelvic locations), these "seeds" would flourish under "irrigation" (hormonal stimulation) during the woman's reproductive life. Misplaced "seeds" (mesenchymal substract) could be the source of endometriosis. The surgical resection of these "seeds" could possibly eradicate the disease. The *Hox/HOX* genes encode highly conserved transcription factors responsible for imparting functional identity to specific body segments. In mice and humans, Hoxa10/HOXA10 has a role in cell proliferation and differentiation, hematopoiesis, embryogenesis, and implantation. The *HOXA10* gene is expressed at endometriosis sites, such as the peritoneum, rectosigmoid, ovary and lung parenchyma. Hydrosalpinx fluid modifies endometrial expression of HOXA10, and surgery (salpingectomy) re-establishes its normal expression. An experimental endometriosis model demonstrated alterations in the methylation pattern and expression of the *Hoxa10* gene in the eutopic endometrium. Given these findings, it is possible that manipulation of endometrial *Hoxa10/HOXA10* gene expression could improve implantation rates in patients with endometriosis-associated infertility, as has already been suggested in laboratory studies [55]. Furthermore, in patients with endometriosis, surgical excision might restore the normal expression of HOXA10 in the eutopic endometrium. A possible mechanism by which endometriosis surgery would improve HOXA10 eutopic expression and restore fertility would be through alterations in its methylation. However, further studies are necessary to elucidate how medical and surgical treatments might influence Hoxa10/HOXA10 expression in both eutopic endometrium and endometriotic foci.

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