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REVIEW

Strategies to manage refractory endometrium: state of the art in 2016

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Abstract The endometrium is one of a number of factors involved in achieving optimal outcomes after assisted reproductive treatment. Owing to its "passive" growth following adequate ovarian stimulation, it has received virtually no attention. Only when either endometrial thickness or ultrasonographic pattern seem inadequate have different strategies been assessed to try to improve it, especially in those cases where it seems difficult or impossible to make it grow. The objective of this review is to summarize the different strategies that have been investigated in patients with inadequate endometrium, to attempt to provide solid evidence of therapies that may be beneficial and to move away from empirism. A review of the existing literature was performed by searching MEDLINE, EMBASE, Cochrane library and Web of Science for publications in English related to refractory endometrium. Most current treatments are based on anecdotal cases and not on solid data, although worldwide many doctors and patients use them. In conclusion, this review found that it is not easy to provide a pragmatic, evidence-based approach to help physicians and patients confused by the available data on how to improve a poor endometrium. Honest balanced information provided to our patients is the best that we can do.

KEYWORDS: assisted reproductive treatment, endometrial lining, IVF, refractory endometrium

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Introduction and definition of refractory endometrium

Trying to define an optimal endometrium in which to transfer a good embryo has been a goal for researchers. Since ultrasonography became available, both endometrial thickness and pattern have been intensely evaluated. Later, further investigation on uterine irrigation was conducted. Early research quickly identified that the hypoechogenic endometrium was more receptive than the iso- or hyperechogenic endometrium (Check et al., 1993). However, no agreement has been reached on endometrial thickness. Although most clinicians empirically prefer endometria >7 mm, available evidence does not support any specific thickness, as pregnancies with similar success have been described from 5 mm to more than 15 mm (Cai et al., 2011; Remohí et al., 1997). When Doppler technology became available, attempts were made to find the utility of this new technology in predicting embryo implantation, but unfortunately with very little success (Mercé et al., 2008). Nowadays it is understood that imaging technologies can provide information about endometrial receptivity up to a certain stage, as pregnancies have been described even in thin endometria <5 mm, as well as in hyperechogenic endometria. So molecular technologies will help us to further understand endometrial receptivity (Cruz and Bellver, 2014).

These new molecular technologies still need to be validated in prospective trials and new generations of the early tests in use today will be developed, and may be used across centres. Yet today clinicians judge the endometrium from the image they obtain from transvaginal ultrasonography. According to the most recent evidence, endometrial thickness under 7 mm would define a refractory endometrium with compromised success rates (Dix and Check, 2010; Kasius et al., 2014). Although prevalence is low (2.4% according to Kasius et al., 2014), it still represents a challenge today.

When endometrial growth is inadequate, diverse therapeutic approaches are proposed and tested. This review will go over the different known causes for inadequate endometrium growth, as well as the conventional and unconventional treatment options that are being assessed.

Search methodology

Refractory endometrium management is still generally being debated. This comprenhensive mini-review paper aims to present and discuss current evidence to help provide clinicians with relevant information in their decision-making process with patients.

PubMed was searched for articles published in English between January 1990 and January 2016 using the following MeSH search terms: "endometrium" OR "endometrial lining" combined with "inadequate" OR "refractory" OR "thin", and with "fertility" OR "infertility" OR "surgical" OR "pregnancy" OR "Assisted Reproductive Technology" OR "ART" OR "*in vitro* fertilization" OR "IVF" OR "intracytoplasmatic sperm injection" OR "ICSI" with restriction to humans. Data were extracted independently by two authors, who also performed an initial screening of the title and abstract of all the articles to exclude any citations deemed irrelevant. A manual search of the review articles and cross references completed the search. The data that are presented exclusively as abstracts in national and international meetings were also excluded.

Table 1 Causes of refractory endometrium.

Cause
Surgical:
dilation and curettage
partial ablation
aggressive myomectomy
post-Strassman
Radiotherapy
Infections
Congenital Müllerian anomalies
Idiopathic

Causes of refractory endometrium

Surgical aetiology

The most frequent cause of refractory endometrium lies in its surgical origin through the development of intrauterine adhesions (IUA) (**Table 1**). In over 90% of cases, these IUA are the result of cervical dilation and post-abortion obstetrical curettage (Schipper et al., 2010), shown as alterations of menstruation (hypo-amenorrhoea), sterility-infertility with implantation failure in assisted reproductive treatment. It also determines obstetric pathologies, such as recurrent abortion, preterm delivery, placenta accreta and uterine rupture (Al-Serehi et al., 2008; Shiau et al., 2005). The most severe grades are Asherman syndrome whose suspected diagnosis is made by clinical and transvaginal ultrasound, and is confirmed by hysteroscopy or hysterosonography (Taskin et al., 2000).

Pathophysiology

Any trauma or accidental resection on the endometrial lining, particularly during pregnancy, can develop fibrosis bridges or adhesions between opposite myometrial surfaces, which distorts the uterine cavity (Galliano et al., 2015; Schipper et al., 2010). In the field of reproduction, adhesions interfere negatively with embryo implantation, and also with correct sperm migration (Revel, 2012). Similarly, placenta accreta would occur because the defect in the basal decidua allows the direct attachment of the chorionic villus to the myometrium (Taskin et al., 2000).

There are three main causes of post-surgical IUA formation: cervical dilation and curettage; intrauterine surgery; and Strassman operation.

Cervical dilation and curettage

It is estimated that 15% of all pregnancies end in spontaneous abortion and about 40% of patients with IUA present obstetric curettage in their medical history, making dilation and curettage (D and C) a risk factor for adhesion formation (Hooker et al., 2014). The most common locations of IUA are

isthmic (51%) and cornual (31%). Yet in most cases this results in mild adhesions (grade 1) with no apparent reproductive consequences (Hooker et al., 2014; Salzani et al., 2007).

Asherman syndrome is a severe degree of IUA wherein the expression of the aforementioned symptom is maximum (hypoamenorrhoea, recurrent abortion, implantation failure, dysmenorrhoea). According to the study of Schenker and Margalioth, the most important risk factors for Asherman syndrome to develop in a gravid uterus are: obstetric curettage (66.7%) and puerperal curettage (21.5%), which are related directly and proportionally to the number of curettages (Conforti et al., 2013). Presence of a previous Caesarean scar has a much lower impact (2%) (Schenker and Margalioth, 1982).

Intrauterine surgery

Taskin et al. (2000) reported that most cases of adhesions in a non-gravid uterus are the result of surgery; they found IUA in 45.5% of women who underwent hysteroscopic resection of multiple fibroids or in 31.3% if a single fibroid was removed. During the resection of submucous myomas by hysteroscopy, the main trigger of post-surgical IUA development is the use of the resectoscope on the endometrial bed. Mazzon et al. (2014) concluded that cold hysteroscopic resection produces a lower rate of IUA (4.2%) compared with other published studies, such as Shokeir et al. (2008) and Guida et al. (2004), who used hyaluronic acid, or Di Spiezio Sardo et al. (2011), who used carboxymethyl glucose gel, to prevent adhesions. Other studies such as that by Touboul et al. (2009) describe a reasonable rate (7.5%) of IUA after myomectomy with a bipolar resectoscope. It does not seem that a postoperative hormone replacement therapy or the insertion of an intrauterine device after hysteroscopic metroplasty is necessary (Nawroth et al., 2002).

More recently, Yang et al. (2013) showed that the intervention most frequently associated with postoperative IUA is septoplasty (86%), followed by adhesiolysis of prior IUA (76%), myomectomy (40%), and these authors found no cases after polypectomy. They did not recommend assisted reproductive treatment for 2 months after hysteroscopic septoplasty, nor for 3 months after myomectomy or adhesiolysis. Considering time to pregnancy is a relevant parameter, it is interesting to note that a recent report concluded that patients can undergo ovarian stimulation after their next menses after a polypectomy without affecting assisted reproductive treatment outcomes (Pereira et al., 2016).

Strassman operation

The bicornuate uterus does not usually require surgery and, although not normally associated with infertility, only 65% of pregnancies reach term (Chan et al., 2011). There are several metroplasty procedures followed to create a single uterine cavity, but the Strassman technique, either abdominal or laparoscopic, is the first choice to correct this Müllerian anomaly. Although surgical treatment is controversial, most authors recommend it only for cases of complete bicornuate uterus and recurrent abortion or very preterm delivery (Khalifa et al., 1993; Lolis et al., 2005; Rechberg et al., 2009; Schipper et al., 2010), and it has only been described as a cause of IUA in very exceptional circumstances (Saeed et al., 2008).

Radiotherapy

Patients receive radiotherapy below the diaphragm owing to malignancies such as Hodgkin disease, non-Hodgkin disease, cervical cancer, uterine sarcomas, colon cancer or endometrial cancer. In these cases the uterus can suffer different side effects, resulting in the lack of a functional uterus. Induced damage, described as a result of antitumoral procedures, includes microuterus or atrophic endometria, absence of endometrial changes in response to exogenous hormonal therapies (Letur-Konirsch et al., 2002), necrosis of endometrial glands and stroma, superficial ulcers, endometrial scars and a reduction in uterine volume of up to 40% of the normal adult size (Holm et al., 1999). Similar findings have been described in a study by dynamic contrast-enhanced MRI in 10 patients who received radiotherapy (Milgrom et al., 2013).

Radiotherapy for malignancies that do not come into contact with the uterus does not seem to affect the uterine function, although some reports have shown that radiotherapy above the diaphragm at a young age produces reduced uterine volume (Larsen et al., 2004).

Radiation-induced uterine damage is also dose- and age-dependent. Premenarchal patients are more likely to be affected, as the growing uterus is probably more vulnerable to irradiation than the adult uterus. Although uterine damage is usually considered irreversible, possible improvement in uterine volume and endometrial thickness in response to hormone replacement therapy has been reported in patients who have been previously treated with total body irradiation (Bath et al., 1999). Older patients show a better response to hormone replacement therapy than younger ones. In a case report, an increase in uterine volume from 7 ml to 30 ml was achieved after 25 weeks of oestradiol therapy. The patient became pregnant with oocyte donation and delivered a healthy baby after an uncomplicated pregnancy (Krause et al., 2014).

Radiation to the uterus can interfere with implantation and uterine growth during pregnancy, which can result in poor obstetric outcome. Radiation to the uterus carries an increased risk of spontaneous abortions, premature deliveries and low birth weight (Sanders et al., 1996). Such poor outcomes may result from various described insults. Diminished endometrial thickness and shorter uterine length (3.2 cm on average), accompanied by diminished uterine blood flow, have been demonstrated in women who have been treated with whole abdominal therapy in their childhood (Critchley et al., 1992).

These effects are likely to be the result of disruption to uterine blood vasculature and decreased uterine weight and length, with the extent of damage related to the radiation field and dose. In women treated with whole abdominal radiotherapy, the mean uterine length of 10 women has been reported to be 4.1 cm, compared with 7.3 cm found in the controls (Critchley et al., 1992). Endometrial thickness diminished in women who had previously undergone abdominal radiation, and no increased thickness has been reported when treated with exogenous sex steroid replacement. In a later study, women who had undergone total body irradiation showed some improvement in endometrial thickness and uterine volume in response to sex steroids (Bath et al., 1999). It is likely that the high irradiation doses used in abdominal

and directed uterine irradiation result in irreversible damage to the uterus (Lédée-Bataille et al., 2002).

This is particularly worrisome for women who seek pregnancy, and even those individuals who decide to use oocyte donors as a result of ovarian failure must be made aware of the higher risk of miscarriage and premature deliveries (Wallace et al., 2003).

In some iatrogenic menopauses, the uterus still allows embryo implantation and development, as demonstrated in patients with Hodgkin disease who received donated oocytes (Anselmo et al., 2001; Muñoz et al., 2015). Few studies have evaluated endometrial growth after radiotherapy. Some reports of oocyte donation after the exposure of the uterus to radiation have shown a similar outcome in these patients compared with oocyte donation recipients without radiotherapy (Muñoz et al., 2015).

In fact, reproductive outcome after radiotherapy has been reported in various studies as being worse, and presenting premature delivery, high miscarriage rate and mid-trimester pregnancy loss (Signorello et al., 2006; Urbano and Tait, 2004). Consequences for procreation are related to the morphologic uterine sequelae and its altered function, mainly early miscarriages and abnormal placentation. Three studies have also shown impaired pregnancy in women after chemo- or radiotherapy (Kavic and Sauer, 2001; Pados et al., 1992; Sauer et al., 1994).

Endometritis

Endometritis refers to an inflammation of the endometrium. The influence of endometritis on fertility has not yet been fully assessed. Implantation refers to a physiological inflammation process that involves inflammatory mediators, such as leukocytes, cytokines, chemokines and other endometrial factors. These cells and their inflammatory mediators are important in the regulation of immunoresponses and trophoblast growth. Chronic endometritis (CE) may hamper endometrial receptivity and may cause infertility, as it has been demonstrated that, in the event of CE, the endometrium is characterized by an abnormal pattern of lymphocyte subsets and, consequently, by an aberrant endometrial microenvironment (Matteo et al., 2009). Indeed, untreated CE has been suggested to diminish the success rates of both spontaneous conception and IVF cycles, and can contribute to adverse obstetric outcomes (Cicinelli et al., 2015). A recent study has reported that women with CE show altered endometrial wave patterns in both the periovulatory and midluteal phases. Altered uterine contractility may explain the symptoms related to CE and infertility (Pinto et al., 2015).

CE is a subtle pathology that seems to be difficult to diagnose and treat. It is quite often asymptomatic or may appear with mild symptoms, which include pelvic pain, dysfunctional uterine bleeding, dispareunia and leukorrhoea. During hysteroscopy, these are indicated by the presence of micropolyps, focal or diffuse hyperaemia and stromal oedema; diagnostic accuracy is 93.4%. (Cicinelli et al., 2005). CE is distinguished by variable numbers of plasma cells within the endometrial stroma, and can be due to a number of processes, including infections (e.g. chlamydia, tuberculosis, other organisms related to cervicitis and pelvic inflammatory disease), intrauterine foreign bodies or growth (e.g. intrauterine contraception, submucous leiomyoma and polyps) and radiation therapy. No aetiology is identifiable in approximately one-third of patients. The uterine cavity is a non-sterile environment and the presence of micro-organisms does not necessarily mean inflammation. It is not just the presence of infectious agents within the internal genital tract, but rather the interactions between infectious agents and the endometrial environment, that are now seen as the most critical issue that determines the presence of a pathology (Romero et al., 2004).

The most frequent infectious agents detected at the endometrial level are common bacteria (*Streptococcus*, *E. coli*, *Staphylococcus*, *Enterococcus faecalis*) (58% of cases). *Ureaplasma urealyticum* has been detected in 10% of positive endometrial cultures and chlamydia in only 2.7% (Cicinelli et al., 2008). Endometrial cultures can be negative in a few cases, and this does not exclude the possibility of other microorganisms (e.g. anaerobic bacteria or viruses) also coexisting and playing a role.

Mycobacterium tuberculosis is a leading cause of infertility in endemic countries (40 – 75%). It causes implantation failure by immune modulation of the endometrium by causing hormonal imbalance and by releasing antiphospholipid antibodies. Once the latent infection is taken care of and the damaged tissue is removed from the affected organ, IVF improvement and positive pregnancy outcome result (Jindal et al., 2012).

Prevalence of CE varies vastly between 2.8% and 66%, depending on the biopsy method and the infertile patient population being investigated (Cicinelli et al., 2008; Kasius et al., 2011). In randomized controlled trials (RCT), a hysteroscopy-guided endometrial biopsy has been obtained and histologically examined in asymptomatic infertile women with a normal transvaginal ultrasound before a first IVF/intracytoplasmatic sperm injection (ICSI) treatment cycle. The authors observed that CE was rarely diagnosed and reproductive outcome was not negatively affected by CE (Kasius et al., 2011).

Yet a retrospective study has reported that CE is a frequent finding in women with repeated unexplained implantation failure (RIF) upon IVF, and that common bacteria and mycoplasmas are the most frequently involved infectious agents. The reproductive outcome upon IVF significantly improved in those patients in whom antibiotic treatment was able to normalize both hysteroscopic and histologic endometrial patterns (Cicinelli et al., 2015). Another retrospective review in patients with RIF has reported that women with CE upon endometrial sampling obtain lower implantation rates in a subsequent IVF-embryo transfer cycle. However, no differences were found in the subsequent clinical or ongoing pregnancy rates after successful antibiotic treatment (Johnston-MacAnanny et al., 2010).

There is still some controversy as to whether CE is associated with a history of recurrent early pregnancy loss (REPL) and/or fetal demise (Giakoumelou et al., 2016; Kitaya, 2011). An observational cohort study has reported high CE prevalence in these women (11%), with a cure rate of 100% after a course of antibiotics. The subsequent live-birth rates after treatment were encouraging (McQueen et al., 2014). A retrospective study has shown that in women with repeated miscarriages, CE is a frequent finding, and women who received adequate antibiotic treatment obtained a significantly higher rate of successful pregnancies compared with those who were

not treated or in whom the disease persisted (Cicinelli et al., 2014). A prospective observational study has concluded that CE is associated with REPL, and that high-sensitivity hysteroscopy is suitable for diagnosing CE in these women (Zolghadri et al., 2011).

No consensus has been reached as to optimal antibiotics, dose and duration for CE treatment. Appropriate antibiotic treatment, based on antibiogram results, appears to improve symptoms and histology in some women with CE. A course of doxycycline (100 mg orally twice daily for 10 – 14 days) is recommended for patients with CE of unknown aetiology, or azithromycin is advised, 500 mg orally on the first day and then 250 mg orally on days 2 through to 5, if patients are allergic to doxycycline. Targeted therapy is appropriate when the cause of endometritis is known.

Congenital Müllerian anomalies

The association between Müllerian anomalies and refractory endometrium is poorly documented in the scientific literature. Thus the points described are usually extrapolated from the reproductive prognosis associated with Müllerian anomalies more than with refractory endometrium.

The urogenital system develops from four sources – the intermediate mesoderm, the coelomic epithelium (mesothelium) lining of the peritoneal cavity, the endoderm of the urogenital sinus and primordial germ cells – and includes the kidneys, gonads, and the urinary and reproductive tracts. The female reproductive tract primarily develops from Müllerian ducts, which form as an invagination of the coelomic epithelium, and further develop into the upper two-thirds of the vagina, the uterus and Fallopian tubes. The endometrial tissue derives from the fused mucous membrane of the Müllerian ducts, which in the 20th week of embryonic development is totally differentiated from the endometrium. The endometrium is a highly dynamic tissue that is endowed with the capacity to undergo dramatic changes in response to steroid hormones, whose ultimate aim is to create a window of receptivity for blastocyst implantation (Kobayashi and Behringer, 2003; Lin et al., 2002).

Müllerian duct malformations represent a varied group of congenital anomalies that result from arrested development, abnormal formation or the incomplete fusion of mesonephric ducts. Uterine congenital anomalies have been related with infertility, recurrent pregnancy loss, prematurity and other obstetric complications, which increase perinatal morbidity and mortality rates, whereas these uterine malformations are asymptomatic in others (Raga et al., 1997).

Prevalence of Müllerian malformations is 1 in 200, or 0.5%. A third of anomalies are septate, a third are bicornuate uteri, 10% are arcuate uterus, 10% are didelphis and unicornuate uterus, and less than 5% are uterine and vaginal aplasia. However, there is some discrepancy that the prevalence of these abnormalities presumably relates to the application of different diagnostic methods, with variable test performance, and to the use of different classification systems to define abnormalities. Correctly diagnosing the malformation is most important, but often proves very difficult. Correct treatment can only be performed if the malformation is clear (Brucker et al., 2011). In the infertile population, this prevalence is similar to that of the general population. However, there seems to be a higher prevalence of septate uteri in the infertile population, which suggests an association. For example, the high prevalence of arcuate uteri in the recurrent miscarriage population (12.2%) highlights the potentially important role of this deformity in this clinical condition, which should never be underestimated (Saravelos et al., 2008). Yet nowadays it can be claimed that arcuate uteri are not associated with poor reproductive outcome. In fact, many authors consider arcuate uteri to be a normal variant (Galliano et al., 2015). The clinical presentation of anomalies associated with defects in fusion and reabsorption of the septum varies according to the affected duct segment. Although the association between canalization defects and suboptimal reproductive efficiency appears to be accepted generally, the exact aetiology and pathophysiological processes that underlie infertility and pregnancy loss remain uncertain. It has been suggested that an endometrium that overlies the septum is abnormal and is, thus, a poor site for implantation (Chan et al., 2011). In fact, two studies have shown that hysteroscopic resection of the septum improves the reproductive outcome of infertile women (Bakas et al., 2012; Mollo et al., 2009).

The causes of Müllerian anomalies have yet to be fully clarified. Karyotypes are normal (46 XX) in 92% of women with Müllerian anomalies and are abnormal (sex chromosome mosaicism) in 8% of these women. The majority of these developmental abnormalities are infrequent and sporadic, and are thus attributed to polygenic and multifactorial causes (Rackow and Arici, 2007). Understanding the basic genetic mechanisms of abnormal development of the female reproductive tract may help us understand these adverse reproductive outcomes, such as implantation failure, recurrent miscarriage and premature delivery. It has been suggested that Hox family genes may not only be important to understand our embryologic development, but may also play a regionally specific regulatory role in the adult female reproductive tract. For example, in adult mice, Hoxa10 expression in the endometrium has been shown to be critical for successful implantation and subsequent fertility (Connell et al., 2013). Accordingly, alterations in the expression of Hox genes in the human endometrium may explain the reproductive problems associated with Müllerian anomalies, such as refractory endometrium. Future research should aim to determine whether HOX gene expression is altered in patients with Müllerian anomalies, and if this expression reveals an association with reproductive outcomes.

Medical therapeutic strategies

High oestradiol doses

When infertile patients show inadequate endometrium that does not grow as is expected, one of the first approaches is to modify standard oral oestradiol administration. Although this strategy has not been shown to be superior to others, the need for oestrogen in the follicular phase of the cycle is beyond question, as oestrogen helps endometrial proliferation by causing spiral artery contraction and reducing oxygen tension in the functional layer, which facilitates embryo implantation (Casper, 2011; Young, 2013).

The main route for oestradiol administration is oral, and based on evidence, there are no relevant differences between

micronized oestradiol or oestradiol valerate (Lignieres et al., 1986). It seems irrelevant to do a progressive step-up increase in the dose that simulates a natural cycle (Coughlan et al., 2014; Shen et al., 2013). So patients can start with high doses (6 – 8 mg or up to 16 mg) continuously from cycle day 1, unless tolerance is poor. In general, 2 mg suffice to block the hypothalamus-pituitary-ovary axis, so there is no need to add gonadotrophin-releasing hormone (GnRH) analogues in the luteal phase as this may compromise endometrial vascular flow and proliferation. In order to increase endometrial thickness, some groups have suggested maintaining high doses for long periods, for up to 9 weeks (Chen et al., 2006; Remohí et al., 1995), with no adverse effects, such as endometrial hyperplasia or bleeding. In general, human endometrium is capable of maintaining its receptivity even in prolonged follicular phases with variable serum oestradiol concentrations. Different therapeutic strategies will most likely yield similar results, as exogenous oestrogen administration compared with, or even combined with, endogenous oestrogenic action from gonadotrophin administration (Coughlan et al., 2014; Shen et al., 2013) has not shown any superiority.

Other oestradiol administration routes are intramuscular, transdermal or vaginal, in an attempt to avoid first liver pass after oral administration, but they have obtained no clear benefit for one over another, and have achieved similar pregnancy rates (Fanchin et al., 2001). Some countries also use intranasal oestrogens, but studies on their efficacy are lacking. Oral administration is the preferred route for simplicity reasons, but transdermal or parenteral may be considered as they both obtain higher serum concentrations. Yet even in these cases, a reduced expression of oestrogen receptors, or even desensitization, may occur, which is not solved by increasing serum oestradiol concentrations.

Oral oestradiol administration has an impact on liver metabolism as it induces changes in the synthesis of renin, high- and low-density lipoprotein and coagulation factors, and therefore increases the risk of cardiovascular disease. However, these changes do not happen with parenteral or transdermal administration (Basdevant et al., 1983; Lignieres et al., 1986) as it avoids first liver pass and provides a better safety profile with a longer mean half-life and a higher oestradiol/estrone ratio, which may benefit the reproductive process (Feinman et al., 1993). The transvaginal route also avoids liver pass and offers a similar safety profile to transdermal or parenteral administration, and should be considered when dealing with high-risk patients (i.e. advanced maternal age).

The administration of oestradiol patches achieves good serum concentrations of serum oestradiol, but this could prove slightly inconvenient because several patches must be used simultaneously to achieve and maintain these serum concentrations. Transdermal oestradiol gel might mitigate some of these problems, but it is the parenteral route that obtains the highest and most stable serum oestradiol concentrations with two injections per week (8 mg/week) and offers the most favourable oestradiol/estrone ratio. However, as it is an oil-based injection, administration is painful for patients and entails a risk of abscess formation, as with any other oil-based product.

Transvaginal oestrogen administration is a very good alternative to obtain high serum and endometrial tissue concentrations of oestrogens. Using vaginal oestradiol as a hemihydrate, when combined with clomiphene citrate, has been reported to significantly increase endometrial thickness, but not pregnancy rates (Cetinkaya and Kadanali, 2012). Similarly, data from egg donation programmes, in which oral and vaginal micronized oestrogen administration were compared, proved a valid alternative in safety terms (Tourgeman et al., 2001). But this is not the only alternative, as possible interactions between oestrogens and progesterone may occur at the vaginal mucosa, as well as a drop in serum oestradiol concentrations in the luteal phase, or even a more marked difficulty of progesterone compensating higher uterine contractility due to increased oestradiol concentrations. These phenomena might have a negative impact on embryo implantation (Fanchin et al., 2001). The vaginal route may improve patient tolerance and symptoms, although RCT have not shown any improvement in endometrial thickness, not even with other co-interventions, although the study sample was limited (Check et al., 2004).

HCG injection in the proliferative phase

Human chorionic gonadotrophin (HCG) is a glycoproteic hormone produced by the implanting embryo whose main role is to stimulate progesterone production by the corpus luteum. It is one of the earliest embryonic signals that initiates and controls embryo invasion; it also regulates immune tolerance to the embryo during the implantion process. Recent observations have suggested that it is also produced by endometrial epithelial cells in the secretory phase, where HCG/LH receptor mRNA and protein have been identified (Paiva et al., 2011). HCG would play a local paracrine role in differentiation and endometrial receptivity by regulating different cytokines and growth factors. Licth et al. (2007) demonstrated that an intrauterine injection of 500 IU of HCG inhibited insulin-like growth factor binding protein-1 and macrophage colony-stimulating factor, and stimulated the secretion of leukaemia inhibitory factor, vascular endothelial growth factor (VEGF) and MMP-0.

Based on the presence of endometrial receptors for HCG, Papanikolaou et al. (2013) ran a pilot study to investigate the possibility of treating patients with repeated thin endometrium (<6 mm) with a subcutaneous HCG injection in the follicular phase. Seventeen oocyte recipients and/or frozen embryo transfers were included, with previously repeated endometrial thickness <6 mm, and in both the fresh IVF cycle and cryotransfer, who did not respond to vaginal oestradiol, sildenafil or vitamin E. In the treatment cycle, as of day 8 of oestradiol administration (17-beta oestradiol 8 mg/day), the dose of 150 IU of daily subcutaneous HCG was initiated for 7 days. After ultrasonographic endometrial measurements, HCG administration was stopped, standard progesterone supplementation was initiated (600 mg micronized vaginal progesterone) and embryo transfer was done as planned. The authors described increased endometrial thickness (5.2 versus 6 mm, $P = 0.008$), with a >10% increase in 52.9% of the patients, and >20% in 35.3% of them. They also described two pregnancies in the five patients without increased endometrial thickness, and as a group, 41% of patients had a live birth. So they concluded that HCG administration in the follicular phase increased both endometrial thickness and clinical outcome.

This effect would support the concept of paracrine action of HCG on the endometrium, which might enhance

endometrial receptivity, especially in women with a repeated poorly developed endometrium. However, in a previous study by Prapas et al. (2009), the exact opposite effect was observed: oocyte recipients with a normal endometrium, who had been treated with 750 IU HCG every third day during oestradiol priming, showed not only a thinner endometrium than the controls, but also a significantly lower pregnancy rate (13.6% versus 45.4%, $P < 0.05$). After 22 cycles, the study was prematurely suspended for ethical reasons. According to Papanikolaou et al. (2013), this discrepancy may be due to a dose-dependent effect, and they speculated that doses over a hypothetical upper limit could have a deleterious impact on endometrial receptivity.

Granulocyte-colony stimulating factor (G-CSF)

G-CSF is a glycoprotein produced in various tissues and cells, including vascular endothelium, macrophages and other immunocytes, that acts not as a growth factor, but also as a cytokine. G-CSF increases endometrial stromal cell decidualization mediated through cAMP by apocrine and paracrine action, and by inducing proliferation and differentiation of the human endometrium. A role in the initiation of endometrial lesions has also been suggested (Jensen et al., 2010).

In rats, G-CSF induced endometrial cell regeneration by mobilizing stem cells to the endometrial injury location, and by increasing endometrial growth and the expression of cytokeratin/vimentin (Zhao et al., 2013).

Mechanisms of action at the endometrial level have not yet been fully elucidated. In a recent study with *ex vivo* endometrial cell cultures of fertile and infertile women, G-CSF supplementation modulated the gene expression of crucial genes that play a role in different pathways, including endometrial vascular remodelling, local immune regulation and cell adhesion (Rahmati et al., 2014).

Limited experience in women with recurrent miscarriage (Santjohanser et al., 2013; Scarpellini and Sbracia, 2009) and repeated implantation failure (Wurfel et al., 2010) after undergoing IVF with G-CSF supplementation is available. Regarding its potential benefit in women with a recurrent thin endometrium, different groups have published isolated cases of pregnancies after intrauterine G-CSF administration (Gleicher et al., 2011, 2013; Kunicki et al., 2014; Lucena and Moreno-Ortiz, 2013). The first successful experience was described by Gleicher et al. (2011), and consisted in four pregnancies achieved after intrauterine perfusion of G-CSF, an off-label indication that not only increased endometrial thickness in the patients, but also obtained successful pregnancies after IVF in these four patients. These preliminary results stimulated authors to enroll in prospective studies. First they reported an observational study of 21 women who did not fulfill the criteria to be enrolled in a randomized prospective study, or simply refused to participate. Patients were treated with intrauterine perfusion (Tomcat catheter) of 30 IU of G-CSF (300 mcg/ml, Neupogen, Filgastrim) approximately 6 – 12 h prior to HCG injection to increase endometrial thickness. If during oocyte retrieval endometrial thickness was <7 mm, G-CSF administration was repeated. The mean endometrial thickness prior to the first G-CSF administration was significantly thinner than the thickness observed at the time of embryo transfer (6.4 ± 1.4 versus 9.3 ± 2.1 mm, $P = 0.001$). No differences in endometrial thickness were observed between the cycles that achieved pregnancy and those that did not. The mean increase in endometrial growth was 2.9 ± 2.0 mm. Although the results were encouraging, this study did not include a control group, and an RCT was still lacking.

The effect of G-CSF has been investigated in 68 women who underwent frozen embryo transfer with endometrial thickness <7 mm after 12 – 13 days of oestradiol treatment (Eftekhar et al., 2014). Although endometrial thickness was comparable between the treated and untreated women, the pregnancy rate was 32% in those patients who received G-CSF compared with 12% in the untreated patients, which suggests a beneficial effect on endometrial receptivity.

More recently, 30 patients with previously cancelled embryo transfers due to a thin endometrium received intrauterine G-CSF in subsequent frozen embryo transfers (FET) (Xu et al., 2015). Compared with previous cycles, endometrial thickness increased from 5.7 ± 0.7 mm to 8.1 ± 2.1 mm after G-CSF treatment ($P < 0.001$). Fifty-two patients underwent FET despite endometrial thickness being under 7 mm, and were included as controls. Significantly higher clinical pregnancy rates were observed in the G-CSF group compared with the control group (48.1% versus 25.0%; $P = 0.038$, respectively).

However, when a well-designed, adequately powered, proper RCT was done by the pioneers of this concept, they were unable to demonstrate any effect of G-CSF on either endometrial growth or pregnancy rates in women undergoing IVF (Barad et al., 2014).

So although case series and non-randomized studies are encouraging, the only published RCT failed to show any impact on clinical outcome. Solid evidence for the hypothetical beneficial effect of G-CSF enhancing endometrial receptivity is still lacking, but the best available evidence is not very supportive.

Autologous platelet-rich plasma

Platelet-rich plasma (PRP) collected from a peripheral vein, through activating platelets by clotting, releases cytokines and growth factors, including VEGF, transforming growth factor, platelet-derived growth factor and epidermal growth factor. It has been used in other therapeutic areas of medicine to improve tissue regeneration. Recently, Chang et al. (2015) investigated if it could benefit infertile women with endometrial thickness under 7 mm. Five patients who underwent IVF, and whose embryo transfer was cancelled due to poor endometrial growth (<7 mm), underwent PRP intrauterine infusion after 10 days on 12 mg oestradiol valerate per os; if endometrial thickness was still <7 mm after 72 h, a second PRP intrauterine infusion was done. All five patients achieved endometrial thickness >7 mm, and they were all pregnant after embryo transfer, with four ongoing pregnancies and one early miscarriage (45, XO after the analysis of the products of conception). PRP intrauterine infusion might be a new alternative for patients with poor endometrial growth, although still better solid evidence is expected.

Acetylsalicylic acid (ASA) steroids, vitamins and supplements

Aspirin

Low-dose aspirin, at least hypothetically, might enhance endometrial growth and embryo implantation via a triple mechanism of action: reducing subendometrial contractility, minimizing inflammation by inhibiting cyclooxygenase and prostaglandin biosynthesis and improving uterine endometrial blood flow (Wada et al., 1994).

Low-dose aspirin has been widely used in IVF cycles. Although no beneficial effect has been shown on embryo implantation (Dentali et al., 2012; Siristatidis et al., 2011), it is still being investigated in certain subgroups of patients, such as those with a thin endometrium. In a prospective randomized study, Weckstein et al. (1997) investigated the effect of low-dose aspirin on 28 oocyte recipients with a thin endometrium (<8 mm) after oestrogen priming. Those patients who had been treated with aspirin showed a higher implantation and clinical pregnancy rate than the untreated women. However, the better pregnancy rates were unrelated to any improvement in endometrial thickness, and the beneficial impact was associated with improved endometrial blood flow (Weckstein et al., 1997). Hsieh et al. evaluated the impact of low-dose aspirin on women who underwent intrauterine insemination with a thin endometrium. The patients who received aspirin showed a higher pregnancy rate (18.4% versus 9%), as well as an improved endometrial pattern, although the endometrial thickness and uterine vascular flow parameters remained unchanged (Hsieh et al., 2000). Thus current evidence to support the beneficial impact of low-dose aspirin in patients with a thin endometrium is scarce and controversial, as no effect on endometrial thickness or uterine blood flow has been demonstrated.

Vitamin E ± pentoxifylline

Pentoxifylline is a synthetic derivate of methlyxanthine that inhibits phosphodiesterase by increasing intracellular cAMP. It has a vasodilating effect and increases red cell membrane flexibility, while reducing blood viscosity by inhibiting red cell aggregation. It is also a mediator of the inflammatory process as it increases the phagocytic activity of leukocytes and monocytes, and by antagonizing some cytokines, such as TNF-α, interferon-γ, GM-CSF, among others, and by inhibiting fibroblast proliferation and extracellular matrix by enhancing collagenase enzyme activity (Lédée-Bataille et al., 2002).

Pentoxifylline has been clinically used in the symptomatic treatment of vascular pathologies, such as intermittent claudication, peripheral vascular diseases or lower limb ulcers, and in alcoholic hepatitis, sickle cell anaemia, diabetic neuropathy, or acute and chronic brain insufficiency. One of the most studied effects is the reduction in radiotherapy-induced lesions. It is a valid alternative for radiation-induced fibrosis and, although the precise mechanism has not yet been fully elucidated, the effect is enhanced when co-administered with vitamin E (Chiao and Lee, 2005). Recently it has been shown to be beneficial in the prevention of breast cancer radiation-induced fibrosis when initiated before treatment and for 12 months (Magnusson et al., 2009).

α-Tocopherol, or vitamin E, is an exogenous liposoluble molecule with antioxidant effects as it blocks oxygen free radicals, as well as acting as a vasodilator. Generally it has been used along with pentoxifylline to treat secondary effects induced by radiotherapy, as it facilitates the regression of the postradiotherapy-induced superficial and musculoskeletal fibrotic lesions on smooth parts (Brennan et al., 2008; Chiao and Lee, 2005) and in the pelvis. A Phase II trial in six women with chronic uterine lesions induced by radiotherapy demonstrated a significant increase in the uterine diastolic blood flow, myometrial volume and endometrial thickness (6.2 ± 0.6 mm versus 3.2 ± 1.1 mm) after being treated with vitamin E 1000 IU and 800 mg of pentoxifylline daily for at least 9 months (Letur-Konirsch et al., 2002).

These positive results have led to research attempting to improve endometrial thickness with a combined therapy of vitamin E and pentoxifylline. Letur-Konirsch treated women with premature ovarian failure for 9 months, and endometrial thickness below 5 mm increased to 7.4 mm (Letur-Konirsch and Delanian, 2003). Lédée-Bataille et al. (2002) treated 18 women, who were recruited in an oocyte donation programme, and who had a thin endometrium after oestrogen priming. After being treated with vitamin E, their endometrial thickness significantly increased (4.9 ± 0.6 versus 6.2 ± 1.4 mm). More recently in an observational, retrospective study on 20 women with thin endometrium, Acharya et al. (2009) demonstrated an increase in endometrial growth after treatment (4.37 ± 1.5 mm versus 6.05 ± 1.83 mm); in their study, endometrial thickness in 73.7% of the women increased with treatment. Doses recommended by other authors include 800 mg pentoxifyllin plus 1000 IU vitamin E for 6 – 8 months. Tolerance is usually very good, but may cause nausea, vomiting or headaches as the main secondary effects. Treatment should be suspended prior to embryo transfer.

Recently, Kitaya et al. (2014) published a case report of a patient with diffuse leiomiomatosis who entered secondary amenorrhoea after a complicated multiple myomectomy, and had a very thin endometrium, which ranged from 3.2 – 4.6 mm. This particular patient had been treated with vitamins E and C for 4 years, showed a gradual increase in endometrial thickness, and achieved a live birth after her first fresh IVF cycle.

Although numbers are quite limited and the strength of the evidence is weak, it seems that the combination of vitamin E and pentoxifylline might improve the endometrial condition, especially in women who have undergone radiotherapy. Properly designed studies are needed before any evidence-based practice recommendation can be made.

Nitroglycerin

Nitric oxide (NO) is involved in endometrial cycle control and uterine preparation for pregnancy. Drugs that release NO act as vasodilating agents, so they might be useful in women with a thin endometrium. There are no specific publications available on this topic. However, Ohl et al. (2002) investigated the impact of nitroglycerin patches administered the day before embryo transfer in women with repeated implantation failure, but these authors were unable to demonstrate any effect. There is no current evidence to support the use of nitroglycerin patches in women with a thin endometrium.

Sildenafil

Sildenafil citrate is a selective inhibitor of 5-phosphodiesterase, the enzyme that hydrolyses cGMP. More precisely, sildenafil

enhances the vasodilator effect of nitric oxide by reducing cGMP degradation. It exerts its action at the endothelial smooth muscle level, and plays a relevant role in regulating vascular structure, growth and tone. This vasodilation effect has been used to treat different pathologies, which range from pulmonary hypertension to erectile dysfunction, among others. It has also been suggested to treat placental insufficiency and intrauterine growth retardation as it increases uteroplacental flow, as shown in an animal model (Stanley et al., 2012).

Sher and Fisch (2000) described the potential use of sildenafil to treat patients with recurrent thin endometrium. In their first publication, they described four failed IVF cases with suboptimal endometrial growth in whom, after being treated with sildenafil, uterine artery blood flow increased, as did endometrial thickness. The dose administered was 25 mg/6 h in vaginal suppositories in the proliferative phase, and administration was stopped prior to HCG administration or embryo transfer. As it is a vasodilating compound, hypotension and headaches may appear, even though vaginal administration is well tolerated. These preliminary findings have been replicated by other groups in larger data sets that include patients who have difficulties achieving optimal endometrial growth, and 70% success has been achieved in endometrial thickness terms, although this is not as good as in specific pathologies, such as previous endometritis (Firouzabadi et al., 2013; Sher and Fisch, 2002). Zinger et al. (2006) described two successful cases in women with Asherman syndrome. Another effect of sildenafil has been described: on top of the endometrial growth facilitating effect, it can reduce natural killer cell activity, which may be of interest for women with recurrent miscarriages (Jerzak et al., 2008). Converserly, in their prospective, randomized study, Check et al. (2004) were unable to show any benefit of sildenafil on endometrial growth. When taking a closer look at basic studies, after treatment *in vitro* with sildenafil, endometrial epithelial cell culture has been reported to have a very moderate effect on cell proliferation, but no significant changes were found in nitric oxide concentrations (Khazaei et al., 2011). These results cast doubt on the clinical findings.

So although biological plausibility exists, any evidence for the clinical benefit of sildenafil in women with a recurrent thin endometrium is weak, and very few publications on non-randomized studies have been found, with very few patients included.

L-arginin

Arginin in an essential amino acid that plays a crucial role in multiple physiological functions, such as regulating vasodilation and vascular flow, activating the immune system and the inflammatory process. It is the main substrate for nitric-oxide synthesis via nitric oxide synthase, and for ornithine and polyamines, which are key factors in plancental angiogenesis and uterine flow regulation (Kim et al., 2011). As a general rule, arginin may increase vascular flow under conditions where endothelial dysfunction is associated with low nitric oxide production. This is why arginine and arginase inhibitors have been assessed as a potential treatment for cardiovascular ischaemic disease, as well as in limbs and the liver (Pernow and Jung, 2013; Vertiz-Hernandez et al., 2007).

Takasaki et al. (2010) evaluated the impact of arginine on nine women with a recurrent thin endometrium at doses of 6 mg/day. They described an increased vascular flow of the radial uterine arteries in 89% of their patients and endometrial growth >8 mm in 67%. Once again, however, solid data are still lacking to validate the usefulness of this approach.

GnRH analogues

The concept of adding GnRH analogues to the luteal phase support has been investigated in infertile women in recent years. Evidence is weak, particularly in women with thin endometrium. Patients with a thin endometrium (≤7 mm) were randomized to receive conventional luteal phase support, plus triptorelin, 0.1 mg on the day of oocyte retrieval, day of embryo transfer and 3 days later, or just conventional luteal phase support. According to Qublan et al. (2008), the administration of GnRH analogues significantly improved implantation and pregnancy rates. However, no other study has validated these findings.

Surgical therapeutic strategies

Role of hysteroscopy in patients with a "thin" endometrium

A possible validity of hysteroscopy in thin-unresponsive endometrium cases could be to diagnose a previously unrecognized uterine pathology. Some authors have described 18 – 50% and 40 – 43% of revealing uterine pathologies in patients prior to IVF, with or without recurrent implantation failure, and with no prior suspected abnormalities (Bozdag et al., 2008; Fatemi et al., 2010). Some of these lesions could have been produced by previous inflammatory processes, either with or without previous surgical trauma that induced intrauterine adhesions. (Conforti et al., 2013). Nevertheless, a higher incidence of adhesions has been described in patients with curettages or uterine surgery (Hooker et al., 2014). If intrauterine adhesions are suspected, hysteroscopy assessment and treatment must be performed. Hysteroscopic scissors are preferred to electrosurgery to avoid thermal endometrial damage (Galliano et al., 2015). To prevent adhesion recurrence after adhesiolis (3 – 1% to 62,5%), several methods have been proposed, such as intrauterine device, intrauterine balloon stent, hyaluronic acid and anti-adhesions barriers, hormonal treatment, stem cells and G-CSF, although few comparative studies have been published (Lebovitz and Orvieto, 2014).

Increasing live birth rates have been described after performing hysteroscopy before the first IVF cycle (Pundir et al., 2014), but only in those cases not associated with suspected uterine cavity abnormality (Bosteels et al., 2010). Some authors have described two different explanations based on biological plausibility observations. On the one hand, cervical dilatation during a hysteroscopy procedure can facilitate posterior embryo transfer (Mansour and Aboulghar, 2002). On the other hand, an immunological mechanism mediated by mechanical hysteroscopic manipulation or distension of the endometrium can play a role in increasing endometrial receptivity. One interesting view is that having performed hysteroscopy, no significant differences have been found in the treatment effect between women without pathological findings and the group with a uterine pathology (Bosteels et al., 2010).

Mobilizing endometrial stem cells from bone marrow

Endometrial reconstruction has been recently suggested as a promising option for achieving pregnancy in the uterus of women with severe endometrial damage, such as those with Asherman syndrome or atrophic endometrium. This approach is based on the regenerative properties of the human endometrium, which is a highly regenerative tissue that undergoes more than 400 cycles of growth, differentiation and shedding during women's reproductive years (Gargett, 2007; Jabbour et al., 2006).

Three main sources for endometrial cell reconstruction have been proposed. The first one is a population of clonogenic cells, resident in both the epithelial and stromal compartments of the endometrium: epithelial progenitor cells and mesenchymal stem/stromal cells (MSC) (Gargett et al., 2009). The identification of endometrial side population cells has supported the presence of adult stem cells in the human endometrium (Cervelló et al., 2010; Kato et al., 2007; Masuda et al., 2010; Tsuji et al., 2008). In fact, freshly isolated human endometrial side population cells have reconstituted endometrial tissue components and the entire endometrium when transplanted under the kidney capsule of immunocompromised mice (Cervelló et al., 2011; Masuda et al., 2010). These cells may be administered or stimulated in patients with Asherman syndrome or an inadequate endometrium to regenerate a thick hormone-responsive endometrium capable of supporting implantation (Gargett and Ye, 2012). Clonogenic multipotent MSC have also been identified in menstrual blood (Patel et al., 2008), which suggests a potential role in initiating endometriotic lesions (Sasson and Taylor, 2008).

The second described source for endometrial regeneration is bone marrow-derived stem cells. Evidence from human and mouse studies has suggested that these cells incorporate into the endometrium in small numbers and transdifferentiate into endometrial epithelial, stromal and endothelial cells (Gargett and Ye, 2012; Taylor, 2004). In fact in female recipients of male donor bone marrow transplants, male XY endometrial cells have been identified (Cervelló et al., 2012; Ikoma et al., 2009). The percentage of donor-derived endometrial epithelial, stromal or vascular cells in human bone marrow transplant recipients seems low, at around 1 – 10% (Gargett and Ye, 2012). A recent study has shown that subendometrial autologous stem cell transplantation of mononuclear stem cells obtained from the iliac crest in six amenorrhoeic women with refractory Ashermans syndrome significantly improved endometrial thickness by exogenous oral oestrogen therapy in the following months and resumed menstruation in five of these patients (Singh et al., 2014).

Human embryonic stem cells have also been suggested as another source for endometrial reconstruction. They are pluripotent stem cells that have the potential to differentiate into any cell type in the body. However, a major concern for their transplantation stems from the risk of uncontrollable differentiation into unwanted cells types, including tumours (Gargett and Ye, 2012). To overcome immunological problems related to human embryonic stem cell transplantation, induced pluripotent stem cells have been proposed as an alternative source (Takahashi et al., 2007). They can be obtained from the fibroblasts of women affected by Asherman syndrome to generate endometrial epithelial and stromal cells that can be later transplanted into the uterine damaged cavity for endometrial regeneration.

To date, only one case of human endometrial regeneration with reproductive success has been published (Nagori et al., 2011). Autologous bone marrow cells obtained from the iliac crest were instilled in the uterus of a woman with Asherman syndrome and a thin endometrium that was unresponsive to oestrogens. Cells were implanted on day 2 of the menstrual cycle after curettage. Afterward, four artificial cycles of oestrogen and progesterone therapy were administered together with low-dose aspirin to achieve a good endometrial size and pattern. Three donor oocyte IVF embryos were transferred in a 7.1-mm multilayered endometrium by adding progesterone vaginal gel to oestrogen and aspirin therapy for luteal phase support. Single gestation was achieved, and is ongoing at gestation week 8. This positive result may be the result of the transdifferentiation of bone marrow cells, of the injury-induced stimulation of endogenous stem/progenitor cells, or the combination of both.

Different research lines that focus on endometrial regeneration are currently ongoing, and this option is the last chance to achieve pregnancy in the uterus of women with severe endometrial damage, in whom surgical and medical approaches have failed to improve endometrial thickness and function.

Uterine transplantation

Absolute uterine factor infertility (AUFI) is considered an untreatable disorder. It includes non-functioning or non-existing uterus cases, such as uterine agenesis (Mayer-Rokitansky-Küster-Hauser syndrome; MRKH) or hypoplasia, previous hysterectomy and severe intrauterine adhesions. AUFI prevalence has been estimated at 1 in 500 women of childbearing age (Brännström et al., 2014). In fact, more than 12,000 women in the UK (Sieunarine et al., 2005), 200,000 women in Europe, and up to seven million women in the USA (Del Priore et al., 2013) are thought to be affected by this problem. To date the available motherhood options in AUFI include acceptance and resignation, adoption, or use of a maternal surrogate, but the last two possibilities, especially surrogacy, are not widely acceptable for ethical, economic, legal or religious reasons (Del Priore et al., 2013; Ethics Committee of the American Society for Reproductive Medicine, 2013).

Uterine transplantation has been developed in recent years as a promising option for AUFI. This is the only ephemeral human transplant in which the graft is not intended for lifelong use, but is removed after the birth of one or two healthy children in order to limit the immunosupression period (Brännström et al., 2014). Its advantages over surrogacy include: no commercial aspects, the mother assumes pregnancy risks, improved natural bonding, adequate lifestyle control during pregnancy, no complicated legal procedures and unequivocal definition of motherhood. However, uterine transplantation entails surgical and medical risks for both the mother and fetus. Medical challenges include vascular anastomosis, ischaemic preservation, blood flow evaluation in the transplanted uterus and immunosuppression problems. In addition, several ethical and social problems also surround this option, especially when we consider that both living and deceased women can be accepted as donors (Brännström, 2013;

Catsanos et al., 2013; Del Priore et al., 2013; Hafany et al., 2011; Kisu et al., 2013).

The first case in humans was conducted in Saudi Arabia in 2000 in a 26-year-old woman who underwent hysterectomy 6 years earlier because of postpartum haemorrhaging. The donor was a 46-year-old woman with benign ovarian cysts. Two menstruations were observed after transplantation, but the transplanted uterus was removed after 99 days owing to prolapse, necrosis and vascular thrombosis (Fageeh et al., 2002). This unsuccessful outcome was probably caused by not enough surgical experience and knowledge of the procedure at that time. After new attempts in different animal models, including non-human primates with menstruation recovery, and even a successful pregnancy and delivery published for the first time after autotransplantation in a cynomolgus monkey in 2012 (Mihara et al., 2012), a Turkish (Ozcan et al., 2013) and a Swedish (Brännström et al., 2014) group recently performed 10 allogenic uterine transplantations in young women. The former was carried out in August 2011 in Turkey on a woman with MRKH syndrome who received a uterus from a brain-dead 22-year-old nulliparous woman due to a traffic accident (Ozcan et al., 2013). The other nine cases were carried out in September 2012 in eight women with MRKH syndrome and one woman with a previous hysterectomy because of cervical cancer. They received a uterus from four premenopausal and five postmenopausal women, of whom five were the mothers of the recipients (Brännström et al., 2014).

In 2013 the first clinical pregnancy in humans was published after the transfer to an oestrogen-prepared endometrium of one thawed embryo from the oocytes of the above-mentioned woman was transplanted in 2011 in Turkey. In this patient a biochemical pregnancy was achieved with her first day-3 single embryo transfer, and a 5-week clinical pregnancy confirmed by vaginal ultrasound was obtained in her second day-3 single embryo transfer. However, the gestational sac failed to develop 1 week later, and aspiration and curettage were performed (Erman Akar et al., 2013). More than a decade after the beginning of animal studies, from rodents to non-human primates (Brännström et al., 2012; Johannesson et al., 2013), the first case of a healthy live birth after uterus transplantation was published in 2014 in one of the nine women transplanted in Sweden (Brännström et al., 2014). This was a 36-year-old woman with Müllerian agenesis who received a uterus from a close family friend aged 61 years. The baby was delivered by Caesarean section at 31 weeks plus 5 days due to pre-eclampsia and abnormal cardiotocography. A healthy male neonate who weighed 1775 g in breech presentation was born with a normal Apgar score and umbilical pH artery.

Hence the door is currently open for considering uterine transplantation as a promising clinical option for AUFI in the near future once experienced groups have settled the surgical, ethical and social matters related to this technique. Severe endometrial damage could become one of the main indications for uterine transplantation when other medical approaches fail.

Conclusions

Treatment of refractory endometrium still remains a challenge today. It is not easy, after reviewing the published research on this topic, to provide an evidence-based practical approach. There is a paucity of solid evidence that supports one intervention versus the other. At the same time, lack of evidence to favour any of the summarized approaches does not mean that some might work in selected particular patients. At least we have been able to identify those interventions that clearly do not work, and those that have a future ahead (**Table 2**). Initially, a hysteroscopic evaluation of the uterine cavity should be a priority; if treatments to increase endometrial growth fail, functional endometrial receptivity can be studied. In the meantime, honest balanced information provided to our patients is the best we can do.

Table 2 Summary of described efficacy of the different therapeutic options for refractory endometrium 🔴 No evidence of benefit 🟡 Unclear effect 🟢 Benefitial intervention.

Therapeutic option	*Efficacy*
Endocrine strategies	
high doses of estradiol	🔴
long courses of estradiol	🔴
vaginal estradiol	🔴
systemic HCG	🟡
intrauterine PRP	🟡
intrauterine G-CSF	🔴
GnRH analogues	🟡
AAS, vitamins & supplements	
aspirin	🟡
nitroglicerin patches	🔴
vitamin E	🟡
L-arginine	🔴
pentoxifiline	🟡
sildenafil	🟡
Surgical strategies	
hysteroscopy	🟢
stem cells	🟡
uterine transplantation	🟡

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