Strategies for managing Asherman's syndrome and endometrial atrophy: Since the classical experimental models to the new bioengineering approach

Lucía de Miguel-Gómez1,2 | Mónica Romeu3,4 | Antonio Pellicer2,5 | Irene Cervelló1

1Fundación Instituto Valenciano de Infertilidad (FIVI), La Fe Health Research Institute, Valencia, Spain
2University of Valencia, Valencia, Spain
3Reproductive Medicine Research Group, La Fe Health Research Institute, La Fe University Hospital, Valencia, Spain
4Women's Health Area, Human Reproduction Unit, La Fe University Hospital, Valencia, Spain
5IVIRMA Roma, Rome, Italy

Correspondence
Lucía de Miguel Gómez, Department of Pediatrics, Obstetrics and Gynaecology, Faculty of Medicine and Dentistry, Avenida Blasco Ibañez, 15, Valencia 46010, Spain. Email: lucia.miguel@uv.es

Funding information
Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana: (PROMETEO/2018/137); Instituto de Salud Carlos III: (CP19/00149, PI17/01039)

ABSTRACT

Endometrial function is essential for embryo implantation and pregnancy, but managing endometrial thickness that is too thin to support pregnancy or an endometrium of compromised functionality due to intrauterine adhesions is an ongoing challenge in reproductive medicine. Here, we review current and emerging therapeutic and experimental options for endometrial regeneration with a focus on animal models used to study solutions for Asherman's syndrome and endometrial atrophy, which both involve a damaged endometrium. A review of existing literature was performed that confirmed the lack of consensus on endometrial therapeutic options, though promising new alternatives have emerged in recent years (platelet-rich plasma, exosomes derived from stem cells, bioengineering-based techniques, endometrial organoids, among others). In the future, basic research using established experimental models of endometrial pathologies (combined with new high-tech solutions) and human clinical trials with large population sizes are needed to evaluate these emerging and new endometrial therapies.

KEYWORDS
bioengineering, endometrial regeneration, platelet-rich plasma, stem cells

1 | INTRODUCTION

The uterus, ovaries, and fallopian tubes compose the internal female reproductive tract. The human uterus is a hollow and inverted pear-shaped organ, while in species like rodents, ruminants, and pigs, two lateral uterine horns join distally into a single body or corpus (Rendi et al., 2012). Regardless of shape, each uterus has differentiated layers: the perimetrium (the serous and most external layer), myometrium (the thickest and muscular layer, located in the middle), and endometrium (the mucous and most internal layer) (Simón et al., 2009).

The endometrium supports embryo implantation and fetal nutrition in pregnancy and is highly dynamic and regenerative, undergoing more than 400 cyclic changes through proliferation, differentiation, and shedding (if there is no embryo) each menstrual cycle for the duration of the reproductive years of a woman (Gargett et al., 2012). In humans, in each menstrual cycle, endometrial thickness progressively increases and decreases in response to estrogen and progesterone produced by the ovaries. Estrogen causes the endometrium to grow and thicken to prepare the uterus for pregnancy during the proliferative phase and after ovulation in the middle of the cycle, progesterone increases to prepare the tissue for embryo implantation in the secretory phase. In the absence of a fertilized egg, both hormones decrease and menstruation occurs. Once the endometrial lining has completely shed, a new menstrual cycle begins (Cunningham et al., 2015). This cyclic regeneration is postulated to...
be driven by the somatic stem cell population located in the niche of the basal layer of the human endometrium (Gargett et al., 2010; Cervelló et al., 2013). In rodents (mice and rats), the main animal model used in reproductive research, the reproductive cycle is called the estrous cycle and contains four phases (proestrus, estrus, metaestrus, and diestrus), occurring every 4 to 5 days. Unlike humans and other primates, rodents do not menstruate (Goldman et al., 2007).

Endometrial anomalies impact fertility and can reduce the chance of pregnancy. Absolute uterine factor infertility results from the absence of a uterus or a nonfunctional uterus (Brännström et al., 2015) while less severe conditions, such as leiomyomas, adenomyosis, Müllerian duct anomalies, and endometrial alterations, impair reproductive outcomes but do not necessarily imply absolute infertility. Among the pathologies directly related to endometrial factor, some affect the endometrial lining, such as endometrial atrophy (EA), and others involve intrauterine adhesions (IUAs) or scar tissue formation, which in the most severe cases can completely obliterate the uterine cavity leading to Asherman’s syndrome (AS) (Galliano et al., 2015). Women with EA have a thin endometrium, usually defined by an endometrial thickness, at the time of hCG administration (in an in vitro fertilization procedure), measured by ultrasonography of less than 6–8 mm. At the same time, women with AS syndrome have a severe degree of IUAs, accompanied by menstrual disturbances, infertility, recurrent pregnancy loss, and/or placental abnormalities (Conforti et al., 2013). In any case, this thin and/or fibrotic endometrium may impair implantation and lead to early pregnancy loss or diminish the probability of pregnancy (Mahajan & Sharma, 2016; Senturk & Erel, 2008). Therefore, regenerating the endometrial tissue in EA and AS patients, either to restore the endometrial integrity from fibrotic lesions or to thicken it, is a therapeutic option to allow for embryo implantation.

As described above, animal models have been developed to elucidate possible solutions and treatments for endometrial alterations. However, effective and standardized options are lacking. Stem cells, platelet-rich plasma, and bioengineering-derived methodologies may be useful in place of traditional surgical treatment methods and hormonal treatments, but further studies are needed to bring these techniques into clinical practice (García-Velasco et al., 2016). Different models are used to test and study mechanisms of action of these treatments, as well as to understand the pathogenesis of the endometrial variations before clinical translation to humans (Andersen et al., 2018), but a well-established uterine-damaged animal model is needed to test treatment options for endometrial regeneration. Mechanical damage using a needle (Alawadhi et al., 2014; Cervelló et al., 2015), a curette (Huberlant et al., 2014; Feng et al., 2020), or an electric scalpel (Xu et al., 2018) are proposed as ways to model uterine injury. Another option is to damage the endometrium by chemical methods, such as ethanol (Jang et al., 2017), which has gained wide acceptance over the years (Sun et al., 2019).

In this review, we summarize classical and emerging advances in experimental models, mainly rodents (mice and rats) of endometrial regeneration. The therapeutic alternatives for treating AS and EA, based on animal research, are shown in Table 1. These studies are grouped into two areas: stem cell therapies, growth factors, and other molecules, and emerging therapeutic alternatives (including platelet-rich plasma, tissue engineering, bioengineering solutions, and organoids). Different variables related to either endometrial regeneration evaluation or fertility restoration verification are also listed (Table 1). Among the methods discussed, bioengineering-derived techniques are the most promising in the management of an injured endometrium, as well as in AS and EA. To translate these techniques to clinical use, a well-established model of endometrial injury is essential. All the studies cited along with this study support the effectiveness of the different treatments to regenerate the endometrium in animal models (mainly rodents) of uterine damage. However, not all of them induce uterine damage using the same protocol.

Thus, this study is focused on the importance of animal models before translating novel therapies to human patients.

2 | MATERIAL AND METHODS

The PubMed database and Google Scholar were searched to identify studies published through December 2020 assessing therapeutic options for endometrial pathologies. We used the following search terms: animal model, Asherman’s syndrome, bioengineering, endometrial atrophy, endometrium, growth factors, hydrogel, microfluidics, murine model, organoids, platelet-rich plasma, scaffold, stem cells, and thin endometrium. Additional studies were found in the bibliographies of selected works. Only original articles in English were included. Studies from bovine, murine, ovine, and porcine models were reviewed as well as some human studies.

3 | RESULTS

3.1 | Classical management of AS and EA

These classical techniques have been mainly described in humans. Hysteroscopic adhesiolysis is the most common treatment for human AS (Khan & Goldberg, 2017; Roge et al., 1997). However, surgery is not always effective, and often (20% to 62.5%) the IUAs reappear (Hanstede et al., 2015). Thus, postoperative measures are frequently needed, such as the insertion of an intrauterine device, a Foley balloon, or hyaluronic acid treatment (Amer et al., 2005; Lin et al., 2013; March, 2011). But still, a systematic review from 2017 concluded that there is no clear evidence on the safety and effectiveness of anti-adhesion treatment after hysteroscopy for improving the reproductive outcomes rates or for decreasing reappearance of IUAs (Bosteels et al., 2017).

Regarding EA patients, and also in those with AS, other less invasive therapeutic approaches have been tested, including the use of exogenous estrogens (Coughlan et al., 2014; Cheng et al., 2006; Shen et al., 2013; Tourgeman et al., 2001), a gonadotropin-releasing hormone agonist (Qublah et al., 2008), human chorionic gonadotropin (Davar et al., 2016; Papanikolaou et al., 2013), or tamoxifen (Reynolds et al., 2010).
| Therapeutic approach                                      | Study                          | Animal model | Endometrial damage method | Endometrial thickness | Fibrotic area | Endometrial glands | Blood vessels | Markers of endometrial functionality and regeneration | Fertility outcomes |
|----------------------------------------------------------|-------------------------------|--------------|----------------------------|-----------------------|---------------|-------------------|---------------|-----------------------------------------------------|-------------------|
| Stem cell therapies, growth factors, and other molecules | Bone marrow–derived stem cells | Zhao et al., 2015 | Rat | 95% ethanol injection | ↑ | NR | ↑ | ↑ | ↑ bFGF, IL6, VIM, CK, ITGβ3, LIF | NR |
|                                                           |                               |              |                             |                       |               |                   |               | ↓ TNF-α, IL1β                                      |                   |
|                                                           | Bone marrow–derived stem cells | Gao et al., 2018 | Rat | Endometrial ablation with 85°C hot water | NR | ↓ | ↑ | ↑ | ↑ CK, LIF | ↑ |
|                                                           | CD133⁺ bone marrow–derived stem cells | Cervelló et al., 2015 | Mouse | Mechanical damage (24G-needle) | NR | ↓ | NR | NR | ↑ Ki67, TSP1 | NR |
|                                                           | Umbilical cord-derived mesenchymal stem cells | Tang et al., 2016 | Rat | Mechanical damage (curette) | NR | ↓ | ↑ | NR | ↑ Ki67, VEGF, VIM, CK | NR |
|                                                           |                               |              |                             |                       |               |                   |               | ↓ COL1A, TGFβ1, FGF2, CTGF |                   |
|                                                           | Umbilical cord-derived mesenchymal stem cells | Zhang et al., 2018 | Rat | 95% ethanol injection | ↑ | ↓ | ↑ | ↑ | ↑ Ki67, VIM, CK, MMP9, VEGFA, CD31 | NR |
|                                                           |                               |              |                             |                       |               |                   |               | ↓ aSMA, TGFβ1, TNF-α, IL2, IFNγ |                   |
|                                                           | Amniotic mesenchymal stem cells | Gan et al., 2017 | Rat | Mechanical damage (surgical scalpel blades) | ↑ | ↓ | ↑ | NR | ↑ bFGF, IL6, VEGF, CK | NR |
|                                                           |                               |              |                             |                       |               |                   |               | ↓ TNF-α, IL1β, TGFβ1, PDGFB, TIMP, COL1A |                   |
|                                                           | Amniotic mesenchymal stem cells | Ouyang et al., 2020 | Rat | Mechanical damage (curette) | ↑ | ↓ | ↑ | ↑ | ↑ bFGF, VEGF, IGF1, WNT5a, SNAI2 | ↑ |
|                                                           |                               |              |                             |                       |               |                   |               | ↓ TGFβ1, TIMP1, COL1A1, PDGF-C, TSP1, CTGF |                   |

(Continues)
| Therapeutic approach | Study | Animal model | Endometrial damage method | Endometrial thickness | Fibrotic area | Endometrial glands | Blood vessels | Markers of endometrial functionality and regeneration | Fertility outcomes |
|----------------------|-------|--------------|---------------------------|----------------------|--------------|-------------------|--------------|-----------------------------------------------------|-------------------|
| Adipose mesenchymal stem cells + estradiol | Kilic et al., 2014 | Rat | Trichloroacetic acid injection | ↑ | ↓ | NR | ↑ | ↑ PCNA, Ki67, VEGF | NR |
| Menstrual mesenchymal stem cells | Hu et al., 2019 | Mouse | Mechanical damage (4G-needle) | ↑ | NR | NR | NR | ↑ VEGF, VIM, CK | ↑ |
| Stromal cell-derived factor 1 + bone marrow-derived stem cells | Yi et al., 2019 | Mouse | 95% ethanol injection | ↑ | ↓ | ↑ | NR | ↑ Ki67, CD31, LIF, IL6, ITGβ3, MMP2, MMP9 | NR |
| Stromal cell-derived factor 1 | Ersoy et al., 2017 | Mouse | Mechanical damage (needle) | NR | ↓ | NR | NR | NR | ↑ |
| Emerging therapeutic alternatives: platelet-rich plasma | Platelet-rich plasma from adult blood | Jang et al., 2017 | 95% ethanol injection | ↑ | ↓ | ↑ | NR | ↑ CK, HOXA10, VEGF, Ki67, cKIT | NR |
| Platelet-rich plasma from adult blood | Kim et al., 2020 | Mouse | Mechanical damage | NR | ↓ | ↑ | NR | ↓ TGFβ1, TIMP1, COL1A1 | ↑ |
| Platelet-rich plasma from adult and umbilical cord blood | De Miguel-Gómez et al., 2021 | Mouse | Mechanical damage (24G-needle) | NR | NR | NR | NR | ↑ Ki67, HOXA10, UBA3, THY1, STAT5a | NR |
| Emerging therapeutic alternatives: tissue engineering solutions and bioengineering | Collagen scaffolds + basic fibroblast growth factor | Sun et al., 2011 | Resection of uterine segment | ↑ | ↓ | ↑ | ↑ | ↑ aSMA, vWF, Ki67 | ↑ |
| Collagen scaffolds + vascular endothelial growth | Lin et al., 2012 | Rat | Resection of uterine segment | ↑ | ↓ | ↑ | ↑ | ↑ aSMA, vWF | ↑ |
| Collagen scaffolds + bone marrow-derived stem cells | Ding et al., 2014 | Rat | Resection of uterine segment | ↑ | ↓ | ↑ | ↑ | ↑ aSMA, vWF, bFGF, IGF1, TGFβ1, VEGF | ↑ |
| Collagen scaffolds + embryo derived stem cells | Song et al., 2015 | Rat | Resection of uterine segment | ↑ | ↓ | ↑ | ↑ | ↑ | ↑ |
| Decellularized scaffolds from rat uterus | Miyazaki & Maruyama, 2014 | Rat | Resection of uterine segment | ↑ | ↓ | ↑ | ↑ | ↑ VIM, CK, aSMA, PR | ↑ |
| Therapeutic approach | Study | Animal model | Endometrial damage method | Endometrial thickness | Fibrotic area | Endometrial glands | Blood vessels | Markers of endometrial functionality and regeneration | Fertility outcomes |
|----------------------|-------|--------------|---------------------------|----------------------|--------------|-------------------|--------------|-----------------------------------------------|------------------|
| Decellularized scaffolds from rat uterus | Hellström et al., 2016 | Rat | Resection of uterine segment | NR | NR | NR | ↑ | aSMA, eCAD, vWF, HOXA11, BCL2 | NR |
| Decellularized scaffolds from ovine uterus | Daryabari et al., 2019 | Rat | Resection of uterine segment | ↑ | NR | NR | NR | aSMA, CD31, Ki67 | NR |
| Aloe-poloxamer hydrogel + estradiol + decellularized rat uterus derived nanoparticles | Yao et al., 2020 | Rat | Mechanical damage (surgical scalpel blades) | ↑ | ↓ | ↑ | NR | ↑ Ki67, ERβ | NR |
| Hydrogels Heparin-poloxamer hydrogel + estradiol | Zhang et al., 2020 | Rat | Mechanical damage (curette) | ↑ | ↓ | ↑ | NR | ↑ bFGF, PCNA, BCL2, KISS1 | ↑ |
| Hydrogel + Stromal cell-derived factor 1 | Wenbo et al., 2020 | Rat | Mechanical damage (curette) | ↑ | ↓ | ↑ | NR | ↑ TGFβ1, VEGF, CD31, CK | ↑ |
| Hydrogel + bone marrow-derived stem cells | Yang et al., 2017 | Rat | Mechanical damage (scraping spoon) | ↑ | ↓ | ↑ | ↑ | ↑ CK, vWF | NR |
| Hydrogel + endometrial stromal cells | Kim, Park et al., 2019 | Rat | Mechanical damage | ↑ | ↓ | ↑ | NR | ↑ Ki67, CD44, PECAM, IGF1, VEGF, LIF | ↑ |
| Hyaluronic acid hydrogel + stem cell secretome | Liu et al., 2019 | Rat | Electrocoagulation | ↑ | NR | ↑ | ↑ | ↑ | ↑ |

Note: Studies are grouped into two areas: stem cell therapies, growth factors, and other molecules, and emerging therapeutic alternatives (including platelet-rich plasma and tissue engineering solutions and bioengineering). Sub-approaches are included. Different variables related to either endometrial regeneration evaluation or fertility restoration verification are also listed. BAX: apoptosis regulator BAX; BCL2: apoptosis regulator Bcl-2; eCAD: e-cadherin; CASP3: caspase 3; CD: cluster of differentiation; CK: cytokeratin; COL1A1: collagen alpha-1(I) chain; CTGF: connective tissue growth factor; ECM: extracellular matrix; EGF: epidermal growth factor; ER: estrogen receptor; bFGF: basic fibroblast growth factor; G-CSF: granulocyte colony-stimulating factor; HGF: hepatocyte growth factor; HOXA10: homeobox A10; IFNγ: interferon gamma; IGF: insulin-like growth factor; IL: interleukin; KISS1: metastasis-suppressor KISS-1; MMP: matrix metalloproteinase; NR: not reported; ITGβ3: integrin beta 3; LIF: leukemia inhibitory factor; PCNA: proliferating cell nuclear antigen; PDGF: platelet-derived growth factor; PECAM: Platelet endothelial cell adhesion molecule; PR: progesterone receptor; aSMA: alpha smooth muscle actin; SNAI2: zinc finger protein SNAI2; STAT5a: signal transducer and activator of transcription 5 A; TGFβ: transforming growth factor beta; THY1: Thy-1 membrane glycoprotein; TIMP: metalloproteinase inhibitor 1; TSP1: Thrombospondin-1; TNF-α: tumor necrosis factor alpha; UBA3: NEDD8-activating enzyme E1 catalytic subunit; VEGF: vascular endothelial growth factor; VIM: vimentin; vWF: von Willebrand factor; WNT5a: protein Wnt-5a. ↑: increased; ↓: diminished.
Other classical approaches include the use of vasoactive substances to increase endometrial blood flow in AS and EA patients (Miwa et al., 2009; Ng et al., 2007). Some studies report higher implantation and clinical pregnancy rates after administration of low-dose aspirin in patients with EA (Urman et al., 2000; Weckstein et al., 1997) while other investigations reported any improvement (Check et al., 1998; Hsieh et al., 2000). In AS, an improvement in endometrial thickness has been reported after aspirin administration, but no change in reproductive prognosis was observed (Chen et al., 2017). Before that, another group postulated that aspirin restored endometrial blood flow, preventing relapse of adhesions after surgery (Chen et al., 2016). Sildenafil citrate has also been tested. Several case studies show promising results using it in terms of endometrial thickness and reproductive outcomes in patients with EA (Sher & Fisch, 2000; 2002; Takasaki et al., 2010) and AS (Zinger et al., 2006). However, a randomized clinical trial (RCT) from 2013 reports that this drug can improve endometrial thickness but not reproductive outcomes (Dehghani et al., 2013). Another therapeutic option is pentoxifylline in combination with the antioxidant vitamin E. Synergy between these two drugs increases the therapeutic option is pentoxifylline in combination with the antioxidant vitamin E. Synergy between these two drugs increases the therapeutic

Thus, the effectiveness of the different treatments is often discordant, preventing their routine adoption in clinical practice with AS and EA patients.

### 3.2 | Stem cell therapies, growth factors, and other molecules

#### 3.2.1 | Stem cell-based therapy

Stem cell therapies may treat diseases or conditions for which few treatments exist due to the well-described regenerative potential of these types of cells (Rohban & Pieber, 2017). The success of stem cell therapy has been extensively demonstrated in other medical fields such as cardiology (Müller et al., 2018), neurology (Song et al., 2018), or orthopedics (Akpancar et al., 2016). In the reproductive field, stem cell treatments show promising results in animal models. Male mouse bone marrow–derived stem cells (BMDSCs) were transplanted into a female AS murine model (Alawadhi et al., 2014), and by detecting the Y chromosome, stem cells were shown to arrive at the damaged site in the endometrium and promote the recovery of endometrial function. This improvement was determined based on a decrease in fibrotic area and an increase in pregnancy rates in mice treated with BMDSCs. Mesenchymal BMDSCs (BMMSCs) also improved endometrial thickness in a rat model with uterine damage via migration kinetics toward the injury site and immunomodulatory properties. After treating rats with BMMSCs, an increase in endometrial thickness and higher expression of the endometrial markers, directly related to improved functionality and receptivity, vimentin, cytokeratin, integrin β3 (ITGβ3), leukemia inhibitory factor (LIF) was observed. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin (IL) 1β, were downregulated while basic fibroblast growth factor (bFGF) and IL6, both anti-inflammatory cytokines, were upregulated, promoting an immunotolerant environment (Zhao et al., 2015). In 2018, (Gao et al., 2018) described the effectiveness of mesenchymal BMDSCs in an AS murine model. Improved cell proliferation, increased expression of LIF, and reduced fibrosis in the endometrium were observed. Another study by Cervelló et al. in 2015 reported similar results in murine models using human BMDSCs positive for the CD133 antigen (CD133”BMDSCs) (Cervelló et al., 2015). These labeled stem cells were observed around endometrial blood vessels, inducing proliferation in surrounding cells and regulating the paracrine factors thrombospondin 1 and insulin-like growth factor 1 (IGF1). The CD133”BMDSCs used by Cervelló et al. came from a human pilot study of 16 AS and EA patients where BMDSCs were mobilized by G-CSF, an enhancer of the production of progenitors and stem cells by the bone marrow and their subsequent release into the bloodstream, collected through peripheral blood aphaeresis and isolated based on CD133” expression (Santamaria et al., 2016).

Although bone marrow is the most common source of stem cells for the treatment of endometrial alterations, stem cells can be derived from other tissues. Indeed, administration of mesenchymal stem cells (MSCs) derived from the umbilical cord increased glandular count, reduced endometrial fibrosis, and promoted cell proliferation in an AS murine model (Tang et al., 2016; Zhang et al., 2018). Further, MSCs derived from the amnion restored endometrial injury by reducing fibrosis, improving endometrial morphology (Ouyang et al., 2020), and exerting immunomodulatory properties (Gan et al., 2017). MSCs obtained from adipose tissue also restored endometrial tissue via increased vascularization and decreased fibrotic area in a murine model (Kilic et al., 2014), as did those obtained from menstrual blood. Hu et al. reported that MSCs derived from menstrual blood could restore endometrial function via increasing the expression of vascular endothelial growth factor (VEGF), vimentin, and keratin, and also pregnancy rates (Hu et al., 2019).

All reviewed types of stem cells, such as BMDSCs (Nagori et al., 2011; Singh & Seth, 2014; Saldin et al., 2016) and those derived from the umbilical cord (Cao et al., 2018), adipose tissue (Sudoma et al., 2019), and menstrual blood (Tan et al., 2016) have also been used in human studies. These works have reported how stem cell therapy, obtained from a variety of sources, can increase endometrial thickness allowing embryo implantation and successful pregnancies in women with AS and EA.

#### 3.2.2 | Stem cell paracrine properties

Injected BMDSCs likely act in a paracrine manner via secretion of biomolecules as final effectors (Gnecci et al., 2008; Schinkōthe et al., 2008). These molecules are as important, if not more so than differentiation and repopulation of the stem cells in modulating the composition of the local environment to evoke tissue repair. These biomolecules include cytokines, growth factors, and extracellular matrix (ECM) components (metalloproteinases, collagens) and are
involved in biological processes such as cell proliferation and migration, cytoprotection, angiogenesis, reducing fibrosis and apoptosis, ECM homeostasis, reducing inflammation, and immunosuppression (Baraniak & McDevitt, 2010; Gnocchi et al., 2008, 2016). This suggests that the application of the biomolecules secreted by stem cells, called the secretome, including lipids, free nucleic acids, soluble proteins, and extracellular vesicles, could be sufficient to activate regeneration or restoration of a specific tissue rather than necessitating the transplantation of stem cells (Beer et al., 2017). Indeed, the secretome derived from MSCs has anti-inflammatory, antiapoptotic, antimicrobial, and angiogenic properties and promotes wound healing and tissue repair (Vizoso et al., 2017). This secretome-based approach has been tested in animal models of different human diseases such as liver (Driscoll & Patel, 2019) or cerebrovascular (Maki et al., 2018) diseases tested in animal models of different human diseases such as liver (Driscoll & Patel, 2019) or cerebrovascular (Maki et al., 2018) diseases and clinical trials (Konala et al., 2016), rather than in the gynecological field. The few published works using the stem cell secretome for endometrial repair are mentioned further in the text, in the Hydrogels section.

Besides, growth factors and cytokines are secreted by human stem cells including those derived from bone marrow (Baberg et al., 2019; Oskowitz et al., 2011), umbilical cord (An et al., 2017), and adipose tissue (Chang et al., 2017; Mussano et al., 2017). Deeper investigation and identification of these paracrine factors will aid the development of noninvasive therapies that could replace stem cell therapy in gynecological pathologies. Our group has taken the first steps in this field, reporting that CD133+ BMDSCs injected in an AS murine model (Cervelló et al., 2015) activated Serpine 1, which promotes cell migration and is involved in decidualization (Lumbers et al., 2015), and Jun proto- oncogene, which promotes endometrial epithelial cell proliferation while decreasing the expression of cyclin D1, a regulator of the cell cycle, through a paracrine mechanism to aid endometrial regeneration. This creates an immunomodulatory environment in endometrial tissue that promotes regenerative processes (De Miguel-Gómez et al., 2020). Paracrine molecules can be also delivered from exosomes, nano-sized extracellular vesicles that release active paracrine molecules (Yu et al., 2014). Exosomes were successfully used to promote endometrial regeneration and restore fertility rates in a murine model (Zhao et al., 2020), implicating this delivery method as a promising treatment tool. Indeed, exosomes derived from adipose-derived MSCs may restore endometrium to normal morphology, decrease fibrosis, and increase the expression of pro-regenerative factors such as ITG8β, LIF, and VEGF, thus supporting an improvement in implantation and pregnancy rate (Zhao et al., 2020).

3.2.3 | Growth factors and other molecules

Growth factors and other molecules have therapeutic effects both individually and in combination, though most studies are in vitro or animal models and the clinical translation to human treatment is undetermined. Hepatocyte growth factor, an enhancer of in vitro proliferation and migration of human endometrial epithelial cells (Sugawara et al., 1997) and transforming growth factor β (TGF-β) isoforms promotes in vitro endometrial remodeling (Nasu et al., 2005) along with platelet-derived growth factor (PDGF) isoforms, which stimulate proliferation and migration among cultured human endometrial stem cells for endometrial tissue repair and support endometrial tissue contraction and remodeling (Matsumoto et al., 2005). Besides, epidermal growth factor (EGF), PDGF-BB, and basic fibroblast growth factor promote in vitro endometrial stromal and epithelial colony-forming units, an intrinsic characteristic of somatic stem cells (Gargett et al., 2008).

In vivo, stromal cell-derived factor 1 (SDF1α) improved stem cell engraftment in an AS mouse model receiving BMSCs therapy (Ersoy et al., 2017). Later, the synergic effect of SDF1α and BMDCSs in a murine model of AS was also reported and endometrial regeneration levels after a single application of SDF1α were similar to those from stem cells alone (Yi et al., 2019). Similarly, BMDCS therapy improved endometrial thickness and reproductive outcomes by transfecting stem cells with VEGF in a mouse model with injured endometrium (Jing et al., 2018). Molecules such as icariin and ligustriene, common plant derivatives used in traditional Chinese medicine, were tested in rat models of uterine damage. Le et al. (2017) reported the positive effect of icariin on endometrial thickness and the expression of several pro-regenerative factors. Ye et al. (2019) reported similar results with ligustriene.

The use of these factors in treating endometrial pathologies in humans has not been reported. Just the use of the G-CSF for endometrial regeneration has been described, but results are controversial. Two independent clinical trials (Barad et al., 2014; Eftelkar et al., 2014) reported that G-CSF treatment does not significantly increase endometrial thickness. However, a later meta-analysis analyzing 11 different studies concluded that intrauterine perfusion of G-CSF can improve endometrial thickness along with clinical pregnancy and implantation rates in patients with a thin endometrium (Xie et al., 2017).

3.3 | Emerging therapeutic alternatives

As discussed above, stem cell therapy is effective in inducing endometrial regeneration in animal models. However, stem cell therapy is costly, invasive, and painful, and therefore not an ideal intervention. Additionally, depending on the type of stem cells, other issues such as ethical and moral questions, risk of teratoma formation, and low retention of cells may arise (Kim & Park, 2017). The use of single molecules has not been deeply explored, as we previously detailed, and most studies use them as enhancers of stem cell action. However, the lack of consensus and the weaknesses of the published studies have promoted the emergence of other therapeutic alternatives, such as platelet-rich plasma (PRP).

3.3.1 | Platelet-rich plasma

PRP is a plasma fraction with a supra-physiologic platelet concentration consisting of biologically active biomolecules like growth factors, such as PDGF, TGFβ, or SDF1α; cytokines; and other proteins, inside α-granules platelets, which are key components for tissue repair (Anitua et al., 2012; Mussano
et al., 2016). These molecules can only be released after breaking the plasma membrane of platelets (i.e., using calcium chloride), a process called activation or degranulation (Fréchette et al., 2005). Further, PRP can be easily obtained via centrifugation to create a gradient in which the lower part of the plasma fraction is enriched in platelets from a peripheral blood sample (Dohan Ehrenfest et al., 2018). This methodology is a minimally invasive procedure appropriate for autologous treatment in AS and EA patients (Pietrzak & Eppley, 2005).

In vitro experiments based on human endometrial cell processes such as cell migration or proliferation (Aghajanova et al., 2018; Wang et al., 2018) describe a positive effect of PRP on regeneration mechanisms. Overexpression of genes and proteins related to a healthy endometrium and regeneration processes, such as estrogen (ERα) and progesterone (PR) receptors (Marini et al., 2016). VEGF, and procollagen type I (Anitua et al., 2016), are also reported.

In vivo rodent models with injured endometrium also show promising results after intrauterine administration of autologous PRP, decreasing fibrosis and increasing expression of several markers of proliferation (Ki-67), angiogenesis (VEGF), and normal endometrial function (cytokeratin, homeobox A10 -HOXA10-) (Jang et al., 2017). Further, PRP administration improves endometrial morphology, reduces the degree of fibrosis, and produces a higher number of implantation sites and live-births (Kim et al., 2020). PRP was also described as a promotor of the regenerative action of BMSCs (Zhou et al., 2020). These authors suggested that PRP enhances stem cell differentiation through the nuclear factor κB pathway. They proposed that PRP regeneration activity was based on the activation of the NF-κB p50 subunit, which induces the upregulation of the anti-inflammatory cytokine IL-10, described to be involved in endometrial regeneration after injury (Xue et al., 2019). In addition, we recently corroborated that PRP promotes in vitro endometrial cell proliferation and migration and regenerates endometrial tissue after damage in a murine model. This effect is strengthened when blood is obtained from the umbilical cord, the most undifferentiated blood source (De Miguel-Gómez et al., 2021). These results suggest that the umbilical cord could be a good source of plasma for treating endometrial pathologies and other regenerative medicine applications, as demonstrated by other groups (Castellano et al., 2017; Ehrhart et al., 2018). PRP has also been tested in women with either thin endometrium (Chang et al., 2019; Eftekhar et al., 2018; Kim, Shin et al., 2019; Molina et al., 2018; Nazari et al., 2016; Tandulwadkar et al., 2017) or AS (Javaheri et al., 2020; Zadehmodarres et al., 2017). However, not all these human works presented a robust study design. From the results obtained by those conducted as clinical trials (Chang et al., 2019; Eftekhar et al., 2018; Javaheri et al., 2020; Tandulwadkar et al., 2017), it could be concluded that PRP is an effective therapeutic option for treating patients with thin endometrium in which the cause is not IUA (this was an exclusion criterion in the majority of existing studies).

### 3.3.2 Tissue engineering solutions and bioengineering approaches

In 1988, tissue engineering was defined as the “application of engineering and life science basis toward the development of biological substitutes for improving, maintaining, or restoring tissue natural functions” (Sklak & Fox, 1988). Bioengineering is a fundamental pillar for tissue engineering based on the use of biomaterials to support tissue regeneration (Brien, 2011). Biomaterials used to model the human endometrium are typically collagen (Gentleman et al., 2009), proteoglycans (formed of glycosaminoglycans, such as heparin or keratin sulfates, covalently attached to a core protein) (Rnjak-kovacina et al., 2017), alginate (Nayak et al., 2020), and chitosan (Choi et al., 2016). They can be used alone or in combination with stem or fully differentiated cells, growth factors, or other biomolecules that work synergistically with the biomaterial (Brien, 2011).

Endometrial regeneration can occur in murine models using collagen scaffolds in combination with other approaches. Collagen and growth factors, such as bFGF (Sun et al., 2011) or VEGF (Lin et al., 2012), and stem cells, derived either from the bone marrow (Ding et al., 2014) or embryonic tissues (Song et al., 2015), have a synergic effect on endometrial regeneration in a rat model with an excised portion of one of the uterine horns. Endometrial morphology (H&E staining, endometrial thickness, and the number of endometrial glands), regeneration of muscular cells (αSMA quantification), blood vessel density (vWF quantification), and pregnancy outcomes improved after the combined treatments.

Several ongoing clinical trials are also evaluating collagen scaffolds loaded in combination with umbilical cord MSCs (National Library of Medicine US) or autologous BMSCs (National Library of Medicine US), to treat intrauterine adhesions in women. These studies have not reported results yet. However, the good results of the previously mentioned animal models using either the secretome or the stem cells themselves, and in the addition of the well-known biocompatibility of the collagen scaffolds with humans, make the expected results promising.

### Decellularized scaffolds

Scaffolds made from ECM after decellularization (removal of all cellular components of a biological scaffold while retaining the ECM structure) of tissues or whole organs have also been evaluated. Scaffolds are a relatively new concept first applied in the assisted reproduction field in 2014 in a rat uterus (Santoso et al., 2014). A longitudinal segment of a rat uterus was decellularized using two different methods, sodium dodecyl sulfate (SDS) and high hydrostatic pressure, and both supported regular pregnancies. Later, other groups reseeded the decellularized scaffolds before replanting them in animal models. Successful endometrial regeneration was noted in rat models using decellularized uterine scaffolds, obtained using SDS or Triton-X 100, grafted into an injured uterus after reseeding with rat primary uterine cells (Miyazaki & Maruyama, 2014) and mesenchymal BMSCs (Hellström et al., 2016). Both studies reported comparable reproductive outcomes in the groups with cell-seeded
scaffold transplant and control groups. Maruyama’s group also described the importance of scaffold orientation and reported that if the uterine patches were reverse oriented (luminal part in the outside while the serosal side remained in the inside, or lumen), the regenerated uterine tissue was aberrant (Miki et al., 2019).

Not only single fragments but also the decellularization of the whole uterus has been achieved in different animal models. The decellularization of an entire rat uterus using several protocols, of which sodium deoxycholate was the best for preserving ECM, was reported (Hellström et al., 2014). Similar results were also reported in a later study using a whole sheep uterus. In this study, rings from a bioengineered uterus were recellularized using sheep fetal BMDSCs and the decellularized uterus fragments maintained the reseeded cells in vitro for 2 weeks (Tiemann et al., 2020). The same recellularizing procedure using human endometrial stem cells was also applied to decellularized scaffolds obtained from a whole porcine uterus, which successfully maintained ECM and vascular network integrity (Campo et al., 2017). Further, an ovine acellular uterus scaffold was harvested in rats and became recellularized with endometrial tissue and vascular cells (Daryabari et al., 2019). Finally, decellularized endometrial scaffolds recellularized again with endometrial cells, both obtained from human samples, responded to 28-day hormone treatment in vitro, complete with the secretion of decidual markers (Olalekan et al., 2017).

**Hydrogels**

Another bioengineering approach is the use of hydrogels, which are three-dimensional hydrophilic polymer networks (Hoffman, 2002) derived from hyaluronic acid (Liu et al., 2019; Kim, Park et al., 2019) or different poloxamers (Yang et al., 2017; Zhang et al., 2020), among other biomaterials. To enhance the synergies of this novel approach and other classical therapies, artificial hydrogels have been applied in several murine models together with other factors or cells. These combinations have been performed using estradiol embedded in an aloe-poloxamer (Yao et al., 2020) and a heparin-poloxamer hydrogel (Zhang et al., 2020) for restoring endometrium in rat models of intrauterine adhesions. Both studies reported improvement in the endometrial morphology status and a reduction in fibrosis, as well as the overexpression of cell proliferation and endometrial regeneration factors. Hydrogels in combination with the chemokine SDF-1α restored the endometrium and improved endometrial thickness, fibrotic area, number of glands, and embryo implantation rate (Wenbo et al., 2020). Different types of cells or their derivatives, such as endometrial stromal cells (Kim, Park et al., 2019), BMDSCs (Yang et al., 2017), and the stem cell secretome (Liu et al., 2019) are reported to improve therapeutic effects when combined with hyaluronic acid or poloxamer-based hydrogels in murine models of endometrial damage.

Hydrogels could be directly related to decellularized scaffolds because they can also derive from decellularized amniotic ECM and combined with estradiol loaded in microspheres (Chen et al., 2020) with an increased cell proliferation rate and a higher expression of EGF and IGF-1 and its receptors after culturing Ishikawa cells (a human endometrial adenocarcinoma cell line) with this hydrogel. Further, we recently developed a biocompatible and stable hydrogel derived from decellularized porcine endometrium that supports in vitro culture of human endometrial cells, either epithelial and/or stromal, enhancing cell proliferation. This tissue-specific hydrogel may improve endometrial regeneration and pregnancy rates in a murine model by remodeling the original tissue (López-Martínez et al., 2021). Thus, hydrogels and decellularized scaffolds could be the most promising technique for regenerating the endometrium and improving classical cell culture techniques used in assisted reproduction.

**Microfluidics**

Microfluidic technology has emerged as a method to model reproductive organs in vitro and to assist in evaluating therapeutic solutions for endometrial pathologies (Campo et al., 2020). A microfluidic device included a coculture of primary human stromal and endothelial cells in which the hormonal changes occurring during the menstrual cycle were simulated (Gnecco et al., 2017); this approach allowed the study of the implication of the vascular endothelium during the decidualization process (Gnecco et al., 2017). A microfluidic platform termed EVATAR, containing reproductive tract tissues and peripheral organs mimicking a 28-day human menstrual cycle was also described (Xiao et al., 2017). These technologies will promote the in vitro study of new therapeutic options not only for the endometrium but also for the rest of the reproductive organs.

### 3.4 Endometrial organoids

In the last years, organoids, defined as genetically stable in vitro–cultured 3D structures that encompass key features of in vivo organs (Schutgens & Clevers, 2020), have emerged as an alternative to conventional in vitro cell culture systems. These 3D biological structures have been revealed as key models for several diseases, drug screenings, testing, and benchmarking for novel therapeutic approaches, as well as a potential tool of personalized medicine (Clevers, 2016). Thus, for endometrial management, not only in AS/EA patients but also in endometriosis, organoids could be a promising instrument either to better understand the pathogenesis of AS/EA or screen incoming untested therapies. This novel approach could complement or even reduce the studies performed in animal models before the clinical translation to humans.

In the last decade, organoids have been derived from different human tissues such as the liver (Huch et al., 2015) or prostate (Karthaus et al., 2014). More recently, several groups have obtained them from human endometrial tissue. These organoids exhibited the characteristics of uterine glands in vivo, expressing specific epithelial, such as epithelial cell adhesion molecule (EPCAM), and secretory, such as mucin-1, markers. These organ-like structures also responded to hormonal stimulation (estrogen and progesterone) by the overexpression of ERα and PR or the secretion of the progesterone-associated endometrial protein (PAEP) that
reveals decidualization, among other features (Turco et al., 2017). Similarly, in 2018, another group isolated organoids not only from the human endometrium but also from murine samples (Boretto et al., 2017). They reported the endometrial epithelium-like phenotype by expression of E-cadherin, ERα, and cytokeratin, by mucin-1 secretion, and by the response to ovarian hormones. This group also published organoids directly derived from human patients, opening then the door to disease modeling and personalized medicine for endometrial-associated pathologies (Boretto et al., 2019).

After the revision of all works cited along with this review and despite those describing human studies, we want to remark the importance of basic science and standard animal models in the study of novel treatments for specific endometrial disorders (AS/EA) before the clinical translation. The generally smaller size of the animal models together with the bigger litter size, short generation times, and more availability of tissue (endometrium in this case) for molecular studies are the main advantages of using animals prior testing in humans (Carter, 2020). Besides, while promising therapies and study platforms are emerging, they still need to be further explored.

4 | CONCLUSION

Classical management of AS and EA is lacking effectiveness, so new approaches have emerged for endometrial regeneration to increase fertility options when this tissue is damaged (Figure 1). Stem cell therapy is the most widely explored and different sources of stem cells have shown promising results in animal models. However, there are disadvantages to stem cells, and new alternatives that can enhance or even replace the regenerative mechanisms of stem cells are changing the field of endometrial regeneration. Adjuvants or promoters of stem cells have been proposed as treatment methods and the emergence of high-tech solutions, such as platelet-rich plasma or bioengineering-based techniques, are likely the best alternatives. Due to the relatively recent emergence of these therapeutic options, robust clinical trials are needed to corroborate the promising findings in experimental models.

Clinical translation of these new approaches will rely on the generation of a well-established animal model of endometrial injury in which to evaluate treatment options. In this context, recent studies based on the successes and limitations of these animal models have evaluated different methods for simulating AS/EA, concluding that ethanol is a better induction of endometrial damage than only mechanical curettage (Kim et al., 2018). Defining the optimal animal model for translational research could strengthen the reproducibility and globalization of these kinds of approaches. Additional studies with stronger designs (including higher population sizes or more robust control groups, to enumerate a few features to improve) dealing with important yet unresolved questions, mainly regarding the pathogenesis of AS/EA, are needed to corroborate the use of emerging options for treating endometrial pathologies and fulfill not only a successful treatment but also a complete understanding of both pathologies. Lastly, the emergence of new research tools, like organoids, can also change and improve the current methods to study AS and EA and to screen different therapies.
ACKNOWLEDGEMENT
This study was supported by the Carlos III Health Institute, grants CP19/00149 and PI17/01039 to I.C., and the Regional Valencian Ministry of Education, PROMETEO/2018/137 to L.d.M.-G., A.P., and I.C.

CONFLICT OF INTEREST
L.D.m.-G., M.R., I.C. and A.P. report no conflicts of interest.

AUTHOR CONTRIBUTIONS
Lucía de Miguel Gómez and Mónica Romeu: literature research, manuscript drafting, and critical discussion. Irene Cervelló and Antonia Pellicer: review design, manuscript drafting, and critical discussion. Irene Cervelló and An-}

REFERENCES
Acharya, S., Yasmin, E., & Balen, A. H. (2009). The use of a combination of pentoxifylline and tocopherol in women with a thin endometrium undergoing assisted conception therapies – a report of 20 cases. Human fertility (Cambridge), 12, 198-203. https://doi.org/10.3109/14647270903377178

Aghajanova, L., Houshdaran, S., Balayan, S., Manvelyan, E., Irwin, J. C., Huddleston, H. G., & Giudice, L. C. (2018). In vitro evidence that platelet-rich plasma stimulates cellular processes involved in endometrial regeneration. Journal of assisted reproduction and genetics, 35, 757–770. https://doi.org/10.1007/s10815-018-1130-8

Akpancar, S., Tatar, O., Turgut, H., Akyildiz, F., & Ekinci, S. (2016). The current perspectives of stem cell therapy in orthopedic surgery. Archives of trauma research, 5, 37976(4). https://doi.org/10.5812/atr.37976

Alawadhi, F., Du, H., Cakmak, H., & Taylor, H. S. (2014). Bone marrow-derived stem cell (BMDSC) transplantation improves fertility in a murine model of Asherman’s syndrome. PLoS one, 9(5), e96662. https://doi.org/10.1371/journal.pone.0096662

Aleyasin, A., Aghahossein, M., Mohseni, M., & Mahdavi, A. (2009). Effects of pentoxifylline and vitamin E on pregnancy rate in infertile women treated by ZIF: a randomized clinical trial. International journal of reproductive biomedicine 7, 175–179.

Amer, M. I., El Nadim, A., & Karim, H. (2005). The role of intrauterine balloon after operative hysteroscopy in the prevention of intrauterine adhesions: A prospective controlled study. Middle east fertility society journal, 10, 125–129.

An, S. Y., Jang, Y. J., Lim, H. J., Han, J., Lee, J., Lee, G., Park, J. Y., Park, S. Y., Kim, J. H., Do, B. R., Han, C., Park, H. K., Kim, O. H., Song, M. J., Kim, S. J., & Kim, J. H. (2017). Milk fat globule-EGF Factor 8, secreted by mesenchymal stem cells, protects against liver fibrosis in mice. Gastroenterology, 152, 1174–1186. https://doi.org/10.1053/j.gastro.2016.12.003

Andersen, M. D., Alstrup, A. K. O., Duvald, C. S., Mikkelsen, E. F. R., Vendelbo, M. H., Ovesen, P. G., Pedersen, M., & Michael. (2018). Animal models of fetal medicine andobstetrics. In Ibeh Bartholomew (Ed.), Animal models of fetal medicine and obstetrics. In Ibeh Bartholomew (Ed.), Experimental Animal Models of Human Diseases - An Effective therapeutic strategy (London: In).

Anitua, E., de la Fuente, M., Ferrando, M., Quintana, F., Larreategui, Z., Matorras, R., & Orive, G. (2016). Biological effects of plasma rich in growth factors (PRGF) on human endometrial fibroblasts. European Journal of Obstetrics and Gynecology and Reproductive Biology, 206, 125–130. https://doi.org/10.1016/j.ejogrb.2016.09.024

Anitua, E., Prado, R., Sánchez, M., & Orive, G. (2012). Platelet-rich plasma: Preparation and formulation. Operative techniques in orthopaedics, 22, 25–32. https://doi.org/10.1053/j.oto.2012.01.004

Baberg, F., Geyh, S., Waldérea-Lupa, D., Stefanski, A., Zilkens, C., Haas, R., Schroeder, T., & Stühler, K. (2019). Secretome analysis of human bone marrow derived mesenchymal stromal cells. Biochimica et Biophysica Acta - Proteins and Proteomics, 1867, 434–441. https://doi.org/10.1016/j.bbabap.2019.01.013

Barad, D. H., Yu, Y., Ph, D., Kushnir, V. A., Shohat-tal, A., & Lazzaroni, E., Lee, H. J., & Gleicher, N. (2014). A randomized clinical trial of endometrial perfusion with factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. Fertility and Sterility, 101, 710–715. https://doi.org/10.1016/j.fertnstert.2013.12.016

Baraniak, P. R., & McDevitt, T. C. (2010). Paracrine actions in stem cells and tissue regeneration. Regenerative medicine, 5, 121–143. https://doi.org/10.2217/rme.09.74.Stem

Beer, L., Mildenr, M., & Ankersmit, H. J. (2017). Cell secretome based drug substances in regenerative medicine: When regulatory affairs meet basic science. Annals of translational medicine, 5, 5–7. https://doi.org/10.21037/atm.2017.03.50

Boretto, M., Cox, B., Noben, M., Hendriks, N., Fassbender, A., Roose, H., Amant, F., Timmerman, D., Tomassetti, C., Vanhie, A., Meuleman, C., Ferrante, M., & Vankelecom, H. (2017). Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability. Human development, 144, 1775–1786. https://doi.org/10.1242/dev.148478

Boretto, M., Maenhoudt, N., Luo, X., Hennes, A., Boeckx, B., Bui, B., Heremans, R., Peene, L., Kobayashi, H., Van Zundert, I., Brems, H., Cox, B., Ferrante, M., Uji-I, H., Koh, K. P., D’Hooghe, T., Vanhie, A., Vergote, I., Meuleman, C., … Vankelecom, H. (2019). Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. Nature cell biology, 21, 1041–1051. https://doi.org/10.1038/s41556-019-0360-z

Bosteels, J., Weyers, S., D’Hooghe, T. M., Torrance, H., Broeckmans, F. J., Chua, S. J., & Willem, B. (2017). Anti-adhesion therapy following operative hysteroscopy for treatment of female subfertility. The Cochrane Database of Systematic Reviews, 11, CD011110. https://doi.org/10.1002/14651858.CD011110.pub3

Brännström, M., Johansson, L., Bokström, H., Kvarnström, N. M., Nöle, J., Dahm-Kähler, P., Enskog, A., Milkovic, M., Ekberg, J., Diaz-Garcia, C., Gäbel, M., Hanafy, A., Hagberg, H., Claussson, M., & Nilsson, L. (2015). Livebirth after uterus transplantation. The lancet, 385, 607–616. https://doi.org/10.1016/S0140-6736(14)61728-1

Brien, F. J. O. (2011). Biomaterials & scaffolds for tissue engineering. Materials Today, 14, 88–95. https://doi.org/10.1016/S1369-7021(11)70058-X

Campos, J., Baptista, P. M., López-Pérez, N., Faus, A., Cervello, I., & Simón, C. (2017). De- and recellularization of the pig uterus: a bioengineering pilot study. Biology of Reproduction, 96, 34–45. https://doi.org/10.1095/biolreprod.116.143396

Campos, H., Murphy, A., Ylizid, S., Woodruff, T., Cervelló, I., & Kim, J. J. (2020). Microphysiological modeling of the human endometrium. Tissue Engineering: part A, 26, 759–768. https://doi.org/10.1089/ten.tea.2020.0022

Cao, Y., Sun, H., Zhu, H., Zhu, X., Tang, X., Yan, G., Wang, J., Bai, D., Wang, J., Wang, L., Zhou, Q., Wang, H., Dai, C., Ding, L., Xu, B., Zhou, Y., Hao, J., Dai, J., & Hu, Y. (2018). Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: A phase I clinical trial. Stem Cell Research and Therapy, 9(1), 1–10. https://doi.org/10.1186/s13287-018-0904-3

Carter, A. M. (2020). Animal models of human pregnancy and placentation: alternatives to the mouse. Reproduction, 160(6), R129–R143. https://doi.org/10.1530/REP-20-0354

Castellano, J. M., Mosher, K. L., Abbey, R. J., McBride, A. A., James, M. L., Berdnik, D., Shen, J. C., Zou, B., Xie, X. S., Tingle, M., Hinkson, I. V.,
Angst, M. S., & Wyss-Coray, T. (2017). Human umbilical cord plasma proteins reactivate hippocampal function in aged mice. Nature, 544, 488–492. https://doi.org/10.1038/nature22067

Cervelló, I., Gil-Sanchis, C., Santamaría, X., Cabanillas, S., Díaz, A., Faus, A., Pellicer, A., & Simón, C. (2015). Human CD133+ bone marrow-derived stem cells promote endometrial proliferation in a murine model of Asherman syndrome. Fertility and Sterility, 104, 1552–1560. https://doi.org/10.1016/j.fertnstert.2015.08.032

Cervelló, I., Mas, A., Gil-Sanchis, C., & Simón, C. (2013). Somatic stem cells in the human endometrium. Seminars in Reproductive Medicine, 3, 69–76. https://doi.org/10.1055/s-0032-1331800

Chang, P. Y., Zhang, B. Y., Cui, S., Qu, C., Shao, L. H., Xu, T. K., Qu, Y. Q., Dong, L. H., & Wang, J. (2017). MSC-derived cytokines repair radiation-induced intra-villous microvascular injury. Oncotarget, 8, 87821–87836. https://doi.org/10.18632/oncotarget.21236

Chang, Y., Li, J., Wei, L. N., Pang, J., Chen, J., & Liang, X. (2019). Autologous platelet-rich plasma infusion improves clinical pregnancy rate in frozen embryo transfer cycles for women with thin endometrium. Medicine, 98(3), e14062. https://doi.org/10.1097/MD.0000000000014062

Check, J. H., Dietterich, C., Lurie, D., Nazari, A., & Chuong, J. (1998). A comparison of different sources of platelet-rich plasma as treatment option for infertility-causing endometrial pathologies. Fertility and Sterility, 115, 490–500. https://doi.org/10.1016/j.fertnstert.2020.07.053

De Miguel-Gómez, L., López-Martínez, S., Campo, H., Francés-Herrero, E., Faus, A., Díaz, A., Pellicer, A., Domínguez, F., & Cervelló, I. (2021). Comparison of different sources of platelet-rich plasma as treatment option for infertility-causing endometrial pathologies. Fertility and Sterility, 115, 490–500. https://doi.org/10.1016/j.fertnstert.2020.07.053

Davar, R., Miraj, S., & Farid Mojahedi, M. (2016). Effect of adding human chorionic gonadotropin to frozen thawed embryo transfer cycles with history of thin endometrium. International Journal of Reproductive Biomedicine, 14, 53–56.

De Miguel-Gómez, L., López-Martínez, S., Campo, H., Francés-Herrero, E., Faus, A., Díaz, A., Pellicer, A., Domínguez, F., & Cervelló, I. (2021). Comparison of different sources of platelet-rich plasma as treatment option for infertility-causing endometrial pathologies. Fertility and Sterility, 115, 490–500. https://doi.org/10.1016/j.fertnstert.2020.07.053

Davari, R., Miraj, S., & Farid Mojahedi, M. (2016). Effect of adding human chorionic gonadotropin to frozen thawed embryo transfer cycles with history of thin endometrium. International Journal of Reproductive Biomedicine, 14, 53–56.

Dehghani Firouzabadi, R., Davar, R., Hojat, F., & Mahdavi, M. (2013). Effect of sildenafil citrate on endometrial preparation and outcome of frozen-thawed embryo transfer cycles: a randomized clinical trial. Iranian Journal of Reproductive Medicine, 11, 151–158.

Ding, L., Sun, H., Su, J., Lin, N., Péault, B., Song, T., Yang, J., Dai, J., & Hu, Y. (2014). Transplantation of bone marrow mesenchymal stem cells on collagen scaffolds for the functional regeneration of injured rat uterus. Biomaterials, 35, 4888–4900. https://doi.org/10.1016/j.biomaterials.2014.02.046

Dohan Ehrenfest, D. M., Pinto, N. R., Pereda, A., Jiménez, P., Corso, M., Del, Kang, B. S., Nally, M., Lanata, N., Wang, H. L., & Quirynen, M. (2018). The impact of the centrifugation characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. Platelets, 29, 171–184. https://doi.org/10.1080/09537104.2017.1293812

Driscoll, J., & Patel, T. (2019). The mesenchymal stem cell secretome as an acellular regenerative therapy for liver disease. Journal of Gastroenterology, 54, 763–773. https://doi.org/10.1007/s00535-019-01599-1

Eftekhari, M., Neghab, N., Naghshineh, E., & Khani, P. (2018). Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial. Taiwanese Journal of Obstetrics and Gynecology, 57, 810–813. https://doi.org/10.1016/j.tjog.2018.10.007

Eftekhari, M., Sayadi, M., & Arabijahani, F. (2014). Transvaginal perfusion of G-CSF for infertile women with thin endometrium in frozen ET program: A non- randomized clinical trial. Iranian Journal of Reproductive Medicine, 12, 661–666.

Ehrhart, J., Sanberg, P. R., & Garbuszova-Davis, S. (2018). Plasma derived from human umbilical cord blood: Potential cell additive or cell-substitute therapeutic for neurodegenerative diseases. Journal of Cellular and Molecular Medicine, 22, 6157–6166. https://doi.org/10.1111/jcmm.13898

Ersoy, G. S., Zolbin, M. M., Cosar, E., Moridi, I., Mamillapalli, R., & Taylor, H. S. (2017). CXCL12 promotes stem cell recruitment and uterine repair after injury in Asherman’s Syndrome. Molecular Therapy: Methods & Clinical Development, 4, 169–177. https://doi.org/10.1016/j.omtm.2017.01.001

Feng, Q., Gao, B., Zhao, X., Huang, H., Yi, S., Zou, L., Liu, X., Xue, M., & Xu, D. (2020). Establishment of an animal model of intrauterine adhesions after uterine repair after injury in Asherman’s Syndrome. Molecular Biology of Reproduction, 36, 1211–1223. https://doi.org/10.1007/s10815-019-01463-4

Fréchette, J., Martinez, I., & Gagnon, G. (2005). Platelet-rich plasma: Growth factor content and roles in wound healing. Journal of Dental Research, 84, 434-439. https://doi.org/10.1177/154405910508400507
endometrial regeneration and pregnancy outcomes in a murine model of Asherman's syndrome. *Frontiers in Physiology*, 11(February), 1–9. https://doi.org/10.3389/fphys.2020.00105

Kim, Y. Y., Choi, B. B., Lim, J. W., Kim, Y. J., Kim, S. Y., & Ku, S. Y. (2018). Efficient production of murine uterine damage model. *Tissue Engineering and Regenerative Medicine*, 16, 119–129. https://doi.org/10.1007/s13770-018-0149-3

Kim, Y. Y., Park, K., Jin, Y., Suk, M., Ching, H., Rosenwaks, Z., & Ku, S. (2019). Synergistic regenerative effects of functionalized endometrial stromal cells with hyaluronic acid hydrogel in a murine model of uterine damage. *Acta Biomaterialia*, 89, 139–151. https://doi.org/10.1016/j.actbio.2019.03.032

Konala, V. B., Mamidi, M. K., Bhonde, R., Das, A. K., Pochampally, R., & Pal, R. (2016). The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration. *Cytotherapy*, 18, 13–24. https://doi.org/10.1016/j.jcyt.2015.10.008

Le, A. W., Wang, Z. H., Dai, X. Y., Xiao, T. H., Zhuo, R., & Zhang, B. Z. (2017). An experimental study on the use of iacarin for improving thickness of thin endometrium. *Genetics and Molecular Research*, 16(1). https://doi.org/10.4238/gmr16019126

Ledee-bataille, N., Olivennes, F., Lefaix, J., Chaouat, G., Frydman, R., Delanian, S., & Le, N. (2002). Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme. *Human Reproduction*, 17, 1249–1253. https://doi.org/10.1093/humrep/17.5.1249

Letur-Könisch, H., Guis, F., & Delanian, S. (2002). Uterine restoration by radiation sequela regression with combined pentoxifylline – tocopherol: A phase II study. *Fertility and Sterility*, 77, 1219–1226. https://doi.org/10.1016/S0015-0224(01)00130-5

Lin, N., Song, T., Wang, J., Meng, K., Yang, J., Hou, X., Dai, J., & Hu, Y. (2012). The effect of collagen-binding vascular endothelial growth factor on the remodeling of scarred rat uterus following full-thickness injury. *Biomaterials*, 33, 1801–1807. https://doi.org/10.1016/j.biomaterials.2011.11.038

Lin, X., Wei, M., Li, T. C., Huang, Q., Huang, D., Zhou, F., & Zhang, S. (2013). A comparison of intrauterine balloon, intrauterine contraceptive device and hyaluronic acid gel in the prevention of adhesion reformation following hysteroscopic surgery for Asherman’s syndrome: A cohort study. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 170, 512–516. https://doi.org/10.1016/j.ejogrb.2013.07.018

Liu, F., Hu, S., Yang, H., Li, Z., Huang, K., Su, T., & Wang, S. (2019). Hyaluronic acid hydrogel integrated with mesenchymal stem cell-secretome to treat endometrial injury in a rat model of Asherman’s syndrome. *Advance health care materials*, 8(14), e1900411. https://doi.org/10.1002/adhm.201900411

López-Martínez, S., Campo, H., de Miguel-Gómez, L., Faus, A., Navarro, A. T., Díaz, A., Pellinier, A., Ferrero, H., & Cervelló, I. (2021). A natural xenogenic endometrial extracellular matrix hydrogel toward improving current human in vitro models and future in vivo applications. *Frontiers in Bioengineering and Biotechnology*, 9, 156. https://doi.org/10.3389/fbioe.2021.639688

Lumbers, E. R., Wang, Y., Delforce, S. J., Corbisej de Meaultsart, C., Logan, P. C., Mitchell, M. D., & Pringle, K. G. (2015). Decidualisation of endometrial stromal cells with hyaluronic acid hydrogel in a murine model of Asherman’s syndrome. *Fertility and Sterility*, 101, 620–627. https://doi.org/10.1016/j.fertnstert.2014.06.052

Makí, T., Moroncho, A., Segundo, P. M. S., Hayakawa, K., Takase, H., Liang, A. C., Gabriel-Salazar, M., Medina-Gutiérrez, E., Washida, K., Montaner, J., Lok, J., Lo, E. H., Arai, K., & Rosell, A. (2018). Endothelial progenitor cell secretome and oligovascular repair in a mouse model of prolonged cerebral hypoperfusion. *Stroke*, 49, 1003–1010. https://doi.org/10.1161/STROKEAHA.117.019346

March, C. M. (2011). Management of Asherman’s syndrome. *Reproductive medicine online*, 23, 63–76. https://doi.org/10.1016/j.rbmo.2010.11.018

Marini, M. G., Perrini, C., Esposti, P., Corradetti, B., Bizzaro, D., Riccaboni, P., Fantinato, E., Urbani, G., Gelati, G., Cremonesi, F., & Lange-Consiglio, A. (2016). Effects of platelet-rich plasma in a model of bovine endometrial inflammation in vitro. *Reproductive Biology and Endocrinology*, 14(1), 1–17. https://doi.org/10.1186/s12958-016-0195-4

Matsumoto, H., Nasu, K., Nishida, M., Ito, H., Bing, S., & Miyakawa, I. (2005). Regulation of proliferation, motility, and contractility of human endometrial stromal cells by platelet-derived growth factor. *Journal of Clinical Endocrinology and Metabolism*, 90, 3560–3567. https://doi.org/10.1210/jc.2004-1918

Miki, F., Maruyama, T., Miyazaki, K., Takao, T., Yoshimasa, Y., Katakura, S., Hiura, H., Uchida, S., Masuda, H., Uchida, H., Nagai, T., Shibata, S., Tanaka, M., & Maruyama, T. (2019). The orientation of a decellularized uterine scaffold determines the tissue. *Biology of Reproduction*, 100, 1215–1227. https://doi.org/10.1093/biolre/ioz004

Miwa, I., Tamura, H., Ph, D., Takasaki, A., & Ph, D. (2009). Pathophysiological features of “thin” endometrium. *Fertility and Sterility*, 91, 998–1004. https://doi.org/10.1016/j.fertnstert.2008.01.029

Miyazaki, K., & Maruyama, T. (2014). Biomaterials Partial regeneration and reconstruction of the rat uterus through recellularization of a decellularized uterine matrix. *Biomaterials*, 35, 8791–8800. https://doi.org/10.1016/j.biomaterials.2014.06.052

Molina, A. M., Sánchez, J., Sánchez, W., & Vielma, V. (2018). Platelet-rich plasma as an adjuvant in the endometrial preparation of patients with refractory endometrium. *Jornal Brasileiro de Reproducao Assistida*, 22, 42–48. https://doi.org/10.5935/1518-0557.20180009

Müller, P., Lemcke, H., & David, R. (2018). Stem cell therapy in heart diseases-cell types, mechanisms and improvement strategies. *Cellular Physiology and Biochemistry*, 48, 2607–2655. https://doi.org/10.1159/000492704

Mussano, F., Genova, T., Munaron, L., Petrillo, S., Erovigni, F., & Carossa, S. (2016). Cytokine, chemokine, and growth factor profile of platelet-rich plasma. *Platelets*, 27, 467–471. https://doi.org/10.3109/09537104.2016.1143922

Mussano, F., Genova, T., Corsalini, M., Schierano, G., Pettini, F., Di Venere, D., & Carossa, S. (2017). Cytokine, chemokine, and growth factor profile characterization of undifferentiated and osteoinduced human adipose-derived stem cells. *Stem Cells International*, 2017, 6202783. https://doi.org/10.1155/2017/6202783

Nagori, C. B., Panchal, S. Y., & Patel, H. (2011). Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman’s syndrome. *Journal of Human Reproductive Sciences*, 4, 43–48. https://doi.org/10.4103/0974-1208.82360

Nasu, K., Ph, D., Nishida, M., Matsumoto, H., & Bing, S. (2005). Regulation of proliferation, motility, and contractility of human endometrial stromal cells by transforming growth factor-beta isoforms. *Fertility and Sterility*, 84, 114-1123. https://doi.org/10.1016/j.fertnstert.2005.02.055

National Library of Medicine (US). (2020). Identifier NCT02680366. Treatment of severe Asherman syndrome by collagen scaffold loaded with autologous bone marrow mononuclear cells. https://clinicaltrials.gov/ct2/show/NCT02680366?term=NCT02680366%26draw=2%26rank=1
Nayak, A. K., Mohanta, B. C., Hasnain, S., Hoda, M. N., & Tripathi, G. (2020). Effect of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: A pilot study. *International Journal of Reproductive Biomedicine, 14*, 625–628. https://doi.org/10.29252/ijrm.2011.02070.x

Ouyang, Y., You, S., Zhang, Y., Zhang, C., Zhang, G., Shao, X., He, F., & Hu, L. (2020). Transplantation of human annexin epithelial cells improves endometrial regeneration in rat model of intrauterine adhesions. *Cells and Development, 29*, 1346–1362. https://doi.org/10.1089/scd.2019.0246

Papapanikolaou, E.G., Kyrou, D., Zervakakou, G., Paggou, E., & Humaidan, P. (2013). Follicular HCG endometrium priming for IVF patients experiencing resisting thin endometrium. A proof of concept study. *Journal of Assisted Reproduction and Genetics, 30*, 1341–1345. https://doi.org/10.1077/j.1019-0129.011.0004

Rendi, M. H., Muehlenbachs, A., Garcia, R. L., & Boyd, K. L. (2012). Female reproductive system. In *Comparative Anatomy and Histology*, Treuting, P. M., Dintzis, S. M., Eds.; Academic Press, 2012; pp. 253–284.

Roges, P., Cravello, L., D’Ercole, C., Brousse, M., Bobili, L., & Blanc, B. (1997). Intrauterine adhesions and fertility: Results of hysteroscopic treatment. *Gynaecological Endoscopy, 6*, 225–228. https://doi.org/10.1046/j.1365-2508.1997.1050522.x

Rohban, R., & Pieber, T. R. (2017). Mesenchymal stem and progenitor cells in regeneration: Tissue specificity and regenerative potential. *Stem Cells International, 2017*, 513732. https://doi.org/10.1155/2017/513732

Saldin, L. T., Cramer, M. C., Velankar, S. S., White, L. J., & Badyak, S. F. (2016). Extracellular matrix hydrogels from decellularized tissues: Structure and function. *Acta Biomaterialia, 49* (February), 1–15. https://doi.org/10.1016/j.actbio.2016.11.068

Santamaria, X., Cabañillas, S., Cervelló, I., Arbona, C., Raga, F., Ferro, J., Palmero, J., Remohí, J., Pellicer, A., & Simón, C. (2016). Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman’s syndrome and endometrial atrophy: A pilot cohort study. *Human Reproduction, 31*, 1087–1096. https://doi.org/10.1093/humrep/dev042

Senturk, L. M., & Erel, C. T. (2008). Thin endometrium in assisted reproductive technolog. *Current Opinion in Obstetrics & Gynecology, 20*, 221–228. https://doi.org/10.1097/GCO.0b013e328302143c

Shen, M., Wang, C., Chen, C., & Tzeng, C. (2013). New horizon on successful management for a woman with repeated implantation failure due to unresponsive thin endometrium: use of extended estrogen supplementation. *The Journal of Obstetrics and Gynecology research, 39*, 1092–1094. https://doi.org/10.1111/j.1447-0756.2012.02070.x

Sherr, C., & Fisch, J. D. (2000). Vaginal sildenafil (Viagra): A preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Human Reproduction, 15*, 806–809. https://doi.org/10.1093/humrep/15.4.806

Sherr, G., & Fisch, J. D. (2002). Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. *Fertility and Sterility, 78*, 1073–1076. https://doi.org/10.1016/s0016-7510(02)02279-4

Simón, C., Horcajadas, J. A., García-Velasco, J., & Pellicer, A. (2009). El endometrio humano: desde la investigación a la clínica. 1–9. Editorial Médica Panamericana.

Singh, N., & Seth, T. (2014). Autologous stem cell transplantation in refractory Asherman’s syndrome: A novel cell based therapy. *Journal of Human Reproductive Sciences, 7*, 93–98. https://doi.org/10.4103/0974-1208.138864

Skalak, R., & Fox, C.F. (1988). Tissue engineering. *Anan, R., Ed.; Liss. Inc.: New York, USA.*

Song, C.G., Zhang, Y.Z., Wu, H.N., Cao, X.L., Guo, C.J., Li, Y.Q., Zheng, M.H., & Han, H. (2018). Stem cells: A promising candidate to treat neurological disorders. *Neural Regeneration Research, 13*, 1294–1304. https://doi.org/10.4103/1673-5374.235085

Sugawara, J., Fukaya, T., Murakami, T., & Yoshida, H., & Yajima, A. (1997). Hepatocyte growth factor stimulated proliferation, migration, and...
Zhao, J., Zhang, Q., Wang, Y., & Li, Y. (2015). Uterine infusion with bone marrow mesenchymal stem cells improves endometrium thickness in a rat model of thin endometrium. Reproductive Sciences, 22, 181–188. https://doi.org/10.1177/1933719114537715

Zhao, S., Qi, W., Zheng, J., Tian, Y., Qi, X., Kong, D., Zhang, J., & Huang, X. (2020). Exosomes derived from adipose mesenchymal stem cells restore functional endometrium in a rat model of intrauterine adhesions. Reproductive Sciences, 27, 1266–1275. https://doi.org/10.1007/s43032-019-00112-6

Zhou, Y., Shen, H., Wu, Y., Zhao, X., Pei, J., Mou, Z., Dong, J., & Hua, X. (2020). Platelet-rich plasma therapy enhances the beneficial effect of bone marrow stem cell transplant on endometrial regeneration. Frontiers in Cell and Developmental Biology, 8, 52. https://doi.org/10.3389/fcell.2020.00052

Zinger, M., Liu, J. H., & Thomas, M. A. (2006). Successful use of vaginal sildenafil citrate in two infertility patients with Asherman’s syndrome. Journal of Women’s Health, 15, 442–444. https://doi.org/10.1089/jwh.2006.15.442

How to cite this article: de Miguel-Gómez, L., Romeu, M., Pellicer, A., & Cervelló, I. (2021). Strategies for managing Asherman’s syndrome and endometrial atrophy: Since the classical experimental models to the new bioengineering approach. Mol Reprod Dev, 88, 527–543. https://doi.org/10.1002/mrd.23523