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human reproduction update

Bioengineering trends in female reproduction: a systematic review

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BACKGROUND: To provide the optimal milieu for implantation and fetal development, the female reproductive system must orchestrate uterine dynamics with the appropriate hormones produced by the ovaries. Mature oocytes may be fertilized in the fallopian tubes, and the resulting zygote is transported toward the uterus, where it can implant and continue developing. The cervix acts as a physical barrier to protect the fetus throughout pregnancy, and the vagina acts as a birth canal (involving uterine and cervix mechanisms) and facilitates copulation. Fertility can be compromised by pathologies that affect any of these organs or processes, and therefore, being able to accurately model them or restore their function is of paramount importance in applied and translational research. However, innate differences in human and animal model reproductive tracts, and the static nature of 2D cell/tissue culture techniques, necessitate continued research and development of dynamic and more complex *in vitro* platforms, *ex vivo* approaches and *in vivo* therapies to study and support reproductive biology. To meet this need, bioengineering is propelling the research on female reproduction into a new dimension through a wide range of potential applications and preclinical models, and the burgeoning number and variety of studies makes for a rapidly changing state of the field.

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OBJECTIVE AND RATIONALE: This review aims to summarize the mounting evidence on bioengineering strategies, platforms and therapies currently available and under development in the context of female reproductive medicine, in order to further understand female reproductive biology and provide new options for fertility restoration. Specifically, techniques used in, or for, the uterus (endometrium and myometrium), ovary, fallopian tubes, cervix and vagina will be discussed.

SEARCH METHODS: A systematic search of full-text articles available in *PubMed* and *Embase* databases was conducted to identify relevant studies published between January 2000 and September 2021. The search terms included: bioengineering, reproduction, artificial, biomaterial, microfluidic, bioprinting, organoid, hydrogel, scaffold, uterus, endometrium, ovary, fallopian tubes, oviduct, cervix, vagina, endometriosis, adenomyosis, uterine fibroids, chlamydia, Asherman's syndrome, intrauterine adhesions, uterine polyps, polycystic ovary syndrome and primary ovarian insufficiency. Additional studies were identified by manually searching the references of the selected articles and of complementary reviews. Eligibility criteria included original, rigorous and accessible peer-reviewed work, published in English, on female reproductive bioengineering techniques in preclinical (*in vitro*/*in vivo*/*ex vivo*) and/or clinical testing phases.

OUTCOMES: Out of the 10 390 records identified, 312 studies were included for systematic review. Owing to inconsistencies in the study measurements and designs, the findings were assessed qualitatively rather than by meta-analysis. Hydrogels and scaffolds were commonly applied in various bioengineering-related studies of the female reproductive tract. Emerging technologies, such as organoids and bioprinting, offered personalized diagnoses and alternative treatment options, respectively. Promising microfluidic systems combining various bioengineering approaches have also shown translational value.

WIDER IMPLICATIONS: The complexity of the molecular, endocrine and tissue-level interactions regulating female reproduction present challenges for bioengineering approaches to replace female reproductive organs. However, interdisciplinary work is providing valuable insight into the physicochemical properties necessary for reproductive biological processes to occur. Defining the landscape of reproductive bioengineering technologies currently available and under development for women can provide alternative models for toxicology/drug testing, *ex vivo* fertility options, clinical therapies and a basis for future organ regeneration studies.

Key words: bioengineering / uterus / endometrium / myometrium / ovary / fallopian tubes / cervix / vagina / female reproduction / fertility restoration

Introduction

To provide the optimal milieu for implantation and fetal development, the female reproductive system must orchestrate uterine dynamics in response to ovarian hormones. Specifically, estradiol and progesterone are produced through the processes of follicle development and luteinization in the ovary, and respectively regulate the proliferative and secretory phases in the endometrium. After ovulation, mature oocytes may be fertilized in the fallopian tubes, and the resulting zygote is transported toward the uterus, where it can implant and continue developing (if the endometrium is in an adequately receptive state). Throughout pregnancy, the cervix acts as a physical barrier to protect the fetus from external microorganisms or foreign objects that may enter through the vagina. Fertility can be compromised by pathologies that affect any of these organs or processes, and therefore, being able to accurately model them or restore their function is of paramount importance in applied and translational research.

The study of human reproduction requires multidisciplinary approaches. While animal models provide many opportunities for translational discoveries, there are inherent limitations due to differences compared to human reproductive physiology. Similarly, 2D cell or tissue culture models can provide novel insights on aspects of reproductive biology, but these models are more static and simplified and therefore do not recapitulate the dynamic, complex *in vivo* biology. These limitations underscore the need for continued research along with development of dynamic and more complex *in vitro* platforms, *ex vivo* approaches and *in vivo* therapies. This need is being filled, in part, by rapid advancements in the field of bioengineering, which applies life science and engineering principles to develop biomaterials for restoring, maintaining and/or improving tissue functions. Indeed, bioengineering is leading the way to a new dimension in the study of female reproduction by providing a wide range of potential applications and approaches for discovery.

Proposed bioengineering approaches to repair and/or improve female reproductive potential have evolved in parallel with advances in scientific knowledge and technology. Based on our systematic search, current strategies can be classified into six major categories, and these can be applied synergistically to understand reproductive biology and solve related problems: scaffold-free systems, hydrogels, decellularized extracellular matrix (dECM) or polymer scaffolds, 3D bioprinting, organoids and microfluidic approaches. Scaffold-free approaches make use of cells' ability to self-organize and synthesize their own matrices, generating structures that can be used as functional units or regenerative blocks (Hayama *et al.*, 2014; Orabi *et al.*, 2017; Kuramoto *et al.*, 2018, 2020). Hydrogels (which, for the purposes of this review, are defined by their liquid/injectable original state) can include a variety of natural and synthetic components and offer innumerable options for encapsulating or loading drugs, molecules, cells or reproductive tissues (Zhu *et al.*, 2016; Tavana *et al.*, 2016a; Yang *et al.*, 2021; Zhang *et al.*, 2021b). Selecting the most suitable hydrogel requires knowing the necessary mechanical and physicochemical properties for a given application (Kedem *et al.*, 2011; Shikanov *et al.*, 2011b). For example, animal-derived hydrogels include commercial mixtures of extracellular matrix (ECM) components, such as Matrigel and Cultrex, which are purified basement membrane extracts secreted by mouse Engelbreth-Holm-Swarm tumor cells.

In contrast, dECM scaffolds derive from tissues and organs that were processed by physical, chemical and/or enzymatic methods (Hellström *et al.*, 2014; Laronda *et al.*, 2015; Campo *et al.*, 2017; Pors *et al.*, 2019; Li *et al.*, 2021; Sargazi *et al.*, 2021; Pennarossa *et al.*, 2021a). These biocompatible scaffolds preserve the structure and

biochemical milieu of the tissue of origin (in terms of ECM signaling and migration), minimizing the risk of immune rejection after transplantation (Raya-Rivera *et al.*, 2014; Daryabari *et al.*, 2019; Yao *et al.*, 2020b; Padma *et al.*, 2021b). Notably, to facilitate transplantation/implementation, these scaffolds are often solubilized and used in hydrogel format (López-Martínez *et al.*, 2021a). Scaffolds can also be produced from other natural polymers (such as collagen and bacterial cellulose) or synthetic components (Young *et al.*, 2003; Liu *et al.*, 2007; Edwards *et al.*, 2015). Taking the fabrication of cell-loaded or cell-free scaffolds one step further, 3D bioprinting creates materials with precise shapes, textures and porosities, and offers vast applications in regenerative medicine (Laronda *et al.*, 2017; Souza *et al.*, 2017; Acién *et al.*, 2019; Tiboni *et al.*, 2021; Wu *et al.*, 2022).

Among more recent developments are organoids and microfluidics. Organoids are simplified organs or organ-like structures formed in 3D culture systems, which enable recreation of the architecture and physiology of most female reproductive tissues. Organoids provide models for healthy and diseased tissue phenotypes, making them ideal platforms for personalizing bioengineering and biomedicine through both *in vitro* and *in vivo* studies (Kessler *et al.*, 2015; Turco *et al.*, 2017; Lõhmussaar *et al.*, 2021; Oliver *et al.*, 2021). Microfluidic platforms, increasingly referred to as the 'organ-on-a-chip' concept, utilize properties of fluid dynamics in small-channelled platforms to facilitate study of the dynamic hormonal cycles and endocrine interactions that characterize the reproductive organs (Xiao *et al.*, 2017).

The majority of bioengineering studies date from the year 2000. However, innovative works from the 20th century built the foundation of this emerging field (Fig. 1). The groundwork for scaffold-free approaches included the first bone marrow transplant between twins (Thomas *et al.*, 1959), and the generation of cell-sheets (Yamada *et al.*, 1990) with regenerative potential (Pellegrini *et al.*, 1997) (Fig. 1A1). Organoids were described as early as the 1960s, when single-cell suspensions completely reconstituted whole organs (Weiss and Taylor, 1960), retinal organoids self-organized *in vitro* (Stefanelli *et al.*, 1961) and later, breast (Li *et al.*, 1987) and alveolar (Shannon *et al.*, 1987) epithelial cells aggregated to form 3D structures in Matrigel (Fig. 1A1).

Explorations in the 1980s and 1990s produced different types of *in vitro* co-culture systems (Fig. 1A2 and 3). In particular, the successful combination of hydrogels with different biological products, such as pancreatic islets (Lim and Sun, 1980), prostaglandins (Embrey *et al.*, 1980) and epithelial cells (Yannas *et al.*, 1989), encouraged the use of different biomaterials for regenerative medicine. In this regard, studies in which embryos were cultured together with trophoblastic vesicles (Camous *et al.*, 1984) or ampullary cells (Bongso *et al.*, 1989) inspired other co-culture systems. On the other hand, dECM scaffolds appeared after ECM was obtained from murine renal glomeruli (Hjelle *et al.*, 1979), liver connective tissue (Rojkind *et al.*, 1980), intact acellular matrix from porcine intestinal submucosa (Badylak *et al.*, 1995) and bladder (Chen *et al.*, 1999) (Fig. 1A2). Microfluidic platforms also emerged with micromachining capillary electrophoresis (Harrison *et al.*, 1993), and microchannel networks for cell culture (Folch and Toner, 1998). Finally, bioprinting gained popularity with the first tissue-engineered ear (Cao *et al.*, 1997), the use of 3D-printed substrates for cell adhesion (Park *et al.*, 1998) and introduction of soft lithography (Xia and Whitesides, 1998); the latter encompasses a group of techniques for fabricating or replicating structures, channels or membranes by using soft polymeric material (usually polydimethylsiloxane) stamps or molds (Kim *et al.*, 2018) (Fig. 1A3).

These six categories of bioengineering strategies promote four main translational and/or clinical applications: the development of next-generation *in vitro* platforms, or representative *in vitro* toxicology and drug screening models; the discovery of alternative therapies or new biomarkers; and improvement of tissue/organ regeneration and/or transplantation protocols (Fig. 1B). The establishment of a capillary system for sperm samples (Ulstein, 1972) is an excellent example of an innovative platform to improve ART, while the *in vitro* culture of human endometrial 3D glandular structures (Kirk and Alvarez, 1986; Rinehart *et al.*, 1988), endometrial stromal cells embedded in a collagen matrix [and covered with epithelial cells (Bentin-Ley *et al.*, 1994)] and ovarian epithelial organoids (Kruk and Auersperg, 1992) ensured the initial steps towards personalized *in vitro* screening platforms. Finally, the early development of the ESTES technique, where a portion of the ovary is transplanted into the uterus (Estes, 1909), provided a foundation for later progress in reproductive organ transplantation (Eraslan *et al.*, 1966; Winston and Browne, 1974; Scott *et al.*, 1981).

Since these initial discoveries paved the way, the bioengineering field has undergone rapid growth and expansion. Many engineered reproductive tissues and platforms are currently in different stages of clinical development; most models remain experimental, but others are in pre-clinical trials, and some are already being applied clinically. Given the quantity and heterogeneity of studies published within this specialty, the goal of this review was to systematically summarize the mounting evidence on bioengineering strategies, platforms and therapies, both currently available and under development, in the context of female reproductive medicine, including novel alternatives for fertility restoration.

Methods

Search strategy

PubMed and Embase were searched for relevant reports. The search strategy was limited to full-text articles, published in English, involving mammals or material derived therefrom, between January 2000 and September 2021. Combinations of the following keywords were used: bioengineering, reproduction, artificial, biomaterial, microfluidic, bioprinting organoid, hydrogel, scaffold, uterus, endometrium, ovary, fallopian tubes, oviduct, cervix, vagina, endometriosis, adenomyosis, uterine fibroids, chlamydia, Asherman's syndrome (AS), intrauterine adhesions, uterine polyps, polycystic ovary syndrome and primary ovarian insufficiency. Specific queries used in each database are presented in Supplementary Table SI. Additional studies were identified by manually searching the references of the selected articles and of complementary reviews.

Study selection and eligibility criteria

Literature search results were exported to an MS Excel spreadsheet and duplicates were identified using electronic and manual methods (Fig. 2). Titles, abstracts and full texts were then screened independently and in duplicate by two authors (E.F.-H. and R.L.) using the following eligibility criteria: original, rigorous and accessible peer-reviewed work published in English, on female reproductive bioengineering techniques in preclinical (*in vitro*/*in vivo*/*ex vivo*) and/or

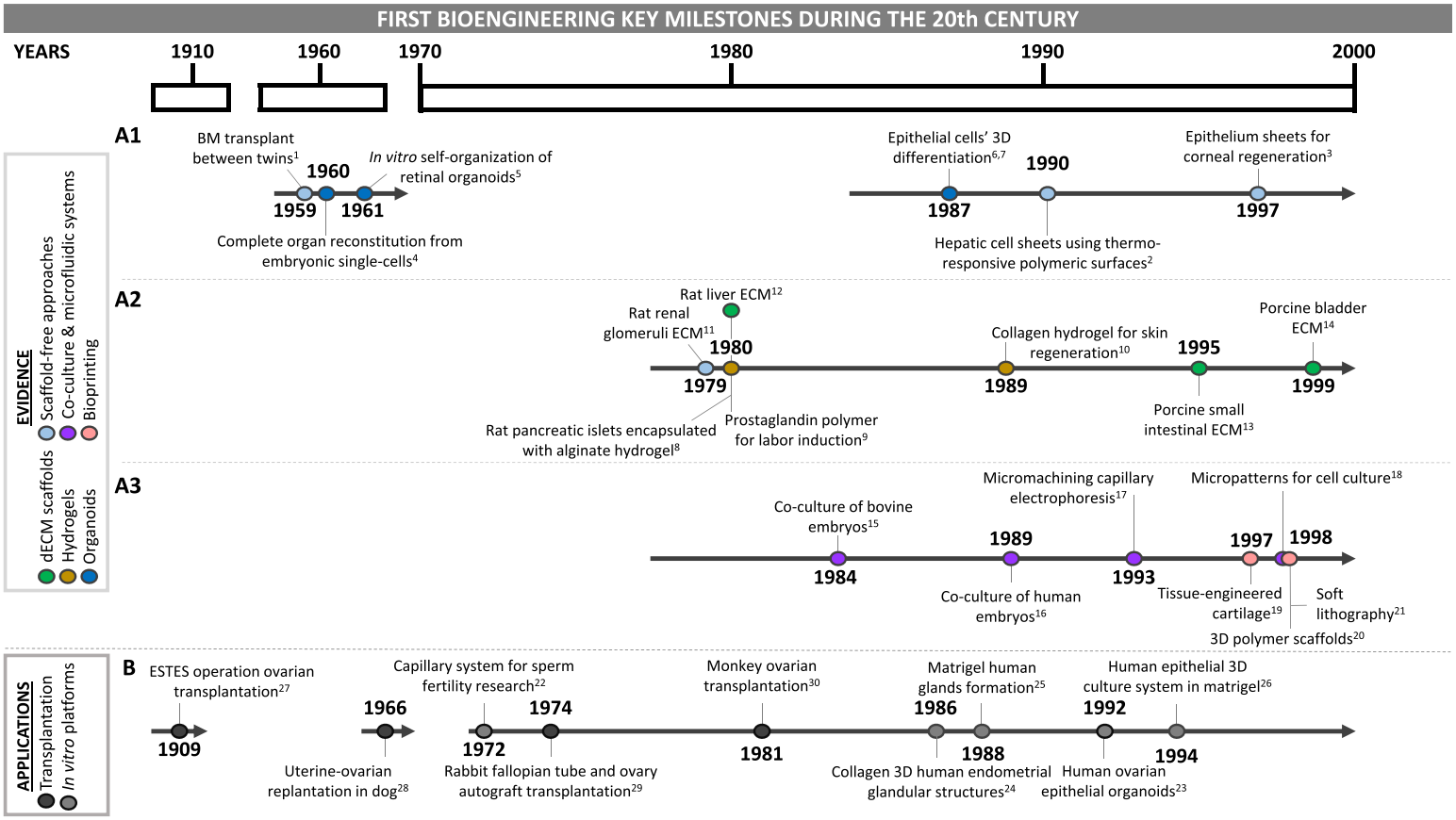

Figure 1. Key milestones during the 20th century forging the development of the bioengineering field. (**A**) Evidence. (**A1**) Advances such as the first bone marrow transplant between twins (1) (Thomas *et al.*, 1959), the control of attachment and detachment of cultured cells (2) (Yamada *et al.*, 1990) and the use of cell sheets (3) (Pellegrini *et al.*, 1997) laid the groundwork for scaffold free-approaches. Concomitantly, in 1960, the reconstitution of a complete organ from single-cell suspensions (4) (Weiss and Taylor, 1960) opened an avenue to the present organoids. The *in vitro* self-organization of retina (5) (Stefannelli *et al.*, 1961) and the 3D organization of breast (6) (Li *et al.*, 1987) and alveolar (7) (Shannon *et al.*, 1987) epithelial cells after culture with Matrigel moved this path further along. (**A2**) Some works from the 1980s reported the combination of hydrogels with different biological products such as pancreatic islets (8) (Lim and Sun, 1980), E2 (9) (Embrey *et al.*, 1980) and epithelial cells (10) (Yannas *et al.*, 1989), introducing these promising biomaterials for regenerative medicine. In parallel, obtaining ECM from renal glomeruli (11) (Hjelle *et al.*, 1979), from liver connective tissue (12) (Rojkind *et al.*, 1980), and a decade later, an intact acellular matrix from intestinal submucosa (13) (Badylak *et al.*, 1995) and bladder (14) (Chen *et al.*, 1999) provided the beginnings of the dECM scaffold approaches. (**A3**) The beginnings of co-culture systems are captured in two main works in which embryos were cultured together with trophoblastic vesicles (15) (Camous *et al.*, 1984) and ampullary cells (16) (Bongso *et al.*, 1989). Research that formed the basis of microfluidic systems was reported in the nineties; some examples are the emergence of on-chip capillary electrophoresis (17) (Harrison *et al.*, 1993) and elastomeric microchannel networks for cell culture (18) (Folch and Toner, 1998). Works from the end of the century paved the way for bioprinting: creation of a tissue-engineered ear (19) (Cao *et al.*, 1997), use of 3D printed substrates for cell adhesion (20) (Park *et al.*, 1998) and introduction of soft lithography (21) (Xia and Whitesides, 1998). (**B**) Applications. The establishment of a capillary system for sperm samples (22) (Ulstein, 1972) and the culture of human ovarian epithelial organoids (23) (Kruk and Auersperg, 1992) were the beginnings of the development of *in vitro* screening platforms. The next generation *in vitro* platforms are based on studies like those from 1986 and 1988, which established endometrial epithelial cells were co-cultured with an ECM from glandular structures (24, 25) (Kirk and Alvarez, 1986; Rinehart *et al.*, 1988) and a similar system also containing endometrial stromal cells (26) (Bentin-Ley *et al.*, 1994). Finally, the development of the ESTES technique for dog ovarian transplantation (27) (Estes, 1909) in the early 20th century provided an excellent basis for a later dog uterus replantation (28) (Eraslan *et al.*, 1966), a rabbit fallopian tube and ovary autograft transplantation (29) (Winston and Browne, 1974) and a primate ovarian transplantation (30) (Scott *et al.*, 1981). BM, bone marrow; E2, estradiol; ECM, extracellular matrix; dECM, decellularized extracellular matrix.

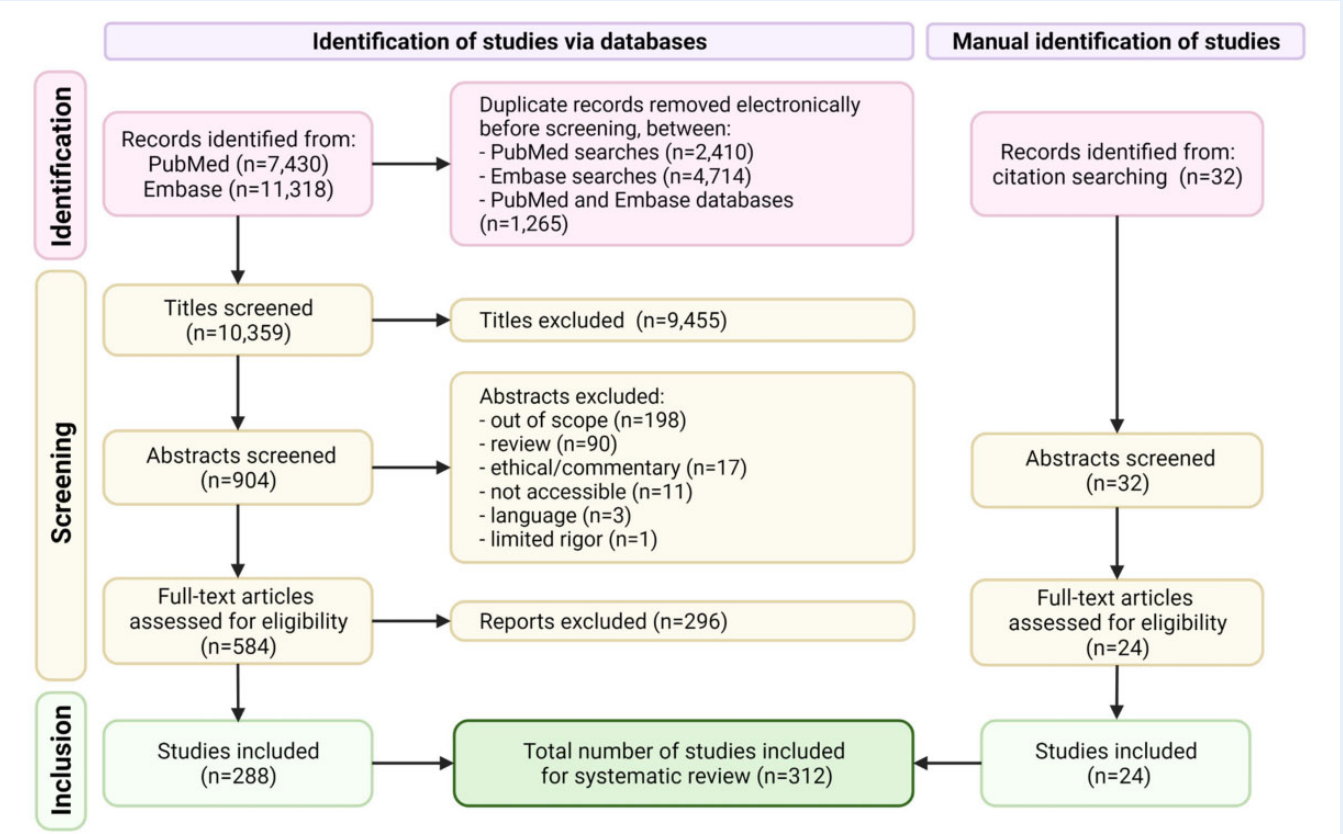

Figure 2. PRISMA flow diagram. Exact terms used for each of the database searches are detailed in Supplementary Table SI. Template adapted from Page *et al.* (2021). Created with BioRender.com.

clinical testing phases. Studies in which gels were developed for intravaginal delivery of hormones, bactericides, nucleic acids or contraceptive drugs were not considered in this review because of their pharmacological nature. Questions or disagreements were resolved by discussion (E.F.-H., R.L., A.P. and I.C.). The final list of included studies was approved by I.C.

Data extraction

Extracted data, including titles, authors, year of publication, reproductive organ (uterus, ovary, fallopian tube, cervix, vagina or full tract), bioengineering strategy, platform/biomaterial used, species, cell/tissue model, study type (*in vitro*, *in/ex vivo*, clinical) and main findings were compiled into a shared Google Sheets spreadsheet and revised by M.H., L.M.-G., S.H. and M.B.

Synthesis of results

Relevant findings extracted from each study are summarized in Table I. Due to the inability to completely detail the many articles comprising this systematic review in Table I, a comparison of *in vivo* uterine regeneration parameters (e.g. immune tolerance, recovery of thickness and muscle layer, presence of glands, angiogenesis, implantation potential and maintenance of pregnancy) is provided in Supplementary Table SII, while specific outcomes of *in vitro* follicle growth (IVFG) studies (e.g. follicle survival, initial and final follicle size, steroidogenesis, oocyte maturation rates, developmental competence and/or fertility restoration) are detailed in Supplementary Table SIII.

Studies related to gynecological pathologies, both included in the initial search terms and different ones addressed by the selected articles (such as endometriosis, uterine fibroids, AS, intrauterine adhesions, polycystic ovary syndrome, primary ovarian insufficiency and Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome) were included in Table I, however owing to the extent of relevant studies applying bioengineering techniques to create novel ovarian, uterine and cervical cancer models (published between April 2014 and September 2021), the latter were grouped separately according to their application and organ in Supplementary Table SIV. Finally, throughout the entire review we classify hydrogels as originally softer/injectable materials regardless of whether they gelify afterwards (e.g. collagen solutions), and scaffolds as their more rigid counterparts (e.g. collagen membranes).

Results

Search results

The search queries yielded 10 390 results (from a total of 18 748 titles identified) after removal of duplicates. Titles and abstracts were

Table I Main findings of bioengineering studies related to female reproductive organs.

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
ENDOMETRIUM AND MYOMETRIUM					
Scaffold-free approaches	Cell sheet	*In vivo* (rat)		Rat oral mucosal epithelial cell sheets prevented IUAs caused by endometrial damage and helped to maintain the uterine luminal structure.	Kuramoto *et al.* (2015)
				Multilayered rat endometrial epithelial and stromal cell sheet transplantation regenerated endometrial tissue, supporting pregnancy similar to normal endometrial tissue.	Kuramoto *et al.* (2018)
				Rat adipose-derived stem cell sheets transplanted into partially excised uteri promote regeneration of endometrial and muscle cells and stimulate angiogenesis.	Sun *et al.* (2018)
				Human UC-MSC sheets improved uterine incision repair in a rat hysterotomy model.	Kuramoto *et al.* (2020)
	MicroTissues 3D Petri Dish micro-mold spheroids	*In vitro*		Generation of endometrial organoids with both epithelial and stromal cells of the **human** endometrium.	Murphy *et al.* (2019)
				Human endometrial organoids containing epithelial and stromal cells responded to androgens associated with PCOS.	Wiwatpanit *et al.* (2020)
Hydrogels	DC endometrium			ECM coating from synchronous DC rabbit endometrium achieved similar results to the gold standard embryo culture conditions.	Campo *et al.* (2019)
		In vitro + *in vivo* (mouse)		Porcine endometrial ECM hydrogel supports *in vitro* culture of **human** endometrial cells in 2D and 3D conditions. Improved proliferation of EnSCs with respect to collagen and Matrigel.	López-Martínez *et al.* (2021a)
		In vivo (mouse)		Porcine endometrial ECM hydrogel loaded with growth factors enhanced tissue regeneration and restored fertility in a mouse model of endometrial injury.	López-Martínez *et al.* (2021b)
	Collagen	*In vitro*		E2 stimulation of **human** Ishikawa cells induced functional changes in HUVECs within a collagen biomaterial.	Pence *et al.* (2015)
				3D collagen gel-embedded **human** endometrial tissue slices responded to ovarian steroid hormones over 3 weeks.	Muruganandan *et al.* (2020)
				A tissue-engineered **human** endometrial stroma manifests changes in morphology and biochemical markers of decidualization, and responds to steroid withdrawal.	Schutte and Taylor (2012)
				Human endometrial stromal cells acquired contractile ability by passive loading of cyclic tensile stretch.	Kim *et al.* (2020b)
		In vivo (rat)		Collagen-binding VEGF restored fertility in a full-thickness injury model of rat scarred uterus.	Lin *et al.* (2012)
				Human UC-MSCs facilitated collagen scaffold degradation in rat uterine scars, promoting full-thickness wall regeneration and restoring fertility.	Xu *et al.* (2017c)
		Clinical		Improvement in endometrial proliferation, differentiation and neovascularization following allogeneic cell therapy using **human** UC-MSCs on collagen hydrogels in patients with IUAs.	Cao *et al.* (2018)
				Transplantation of **human** UC-MSCs on collagen hydrogels improved endometrial angiogenesis, proliferation and response to hormones in patients with AS.	Zhang *et al.* (2021b)
				Collagen-binding bFGF improved functional remodeling of scarred endometrium in infertile women.	Jiang *et al.* (2019)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels	Collagen-Matrigel	*In vitro*		3D culture model with **human** stromal and epithelial cells replicates the normal endometrium physiologically and morphologically, including stromal invasion of KLE cells.	Park *et al.* (2003)
				Co-culture model of **human** epithelial and stromal cells changed cytokine production, reducing inflammation and protease activity.	Schutte *et al.* (2015)
				Development of a 3D spheroid **human** model of endometriosis where collagen I triggers directional migration, invasion and matrix remodeling of stroma cells.	Stejskalová *et al.* (2021)
	Matrigel			Luminal and glandular epithelial cells covered the injured surface of **human** endometrial strips in a Matrigel-based *in vitro* model.	Stavreus-Evers *et al.* (2003)
				Spheroid-based 3D cell culture system consisting in endometrial adenocarcinoma and EVT cell lines mimics early implantation events in **humans**.	Buck *et al.* (2015)
		In vivo (rat)		Injectable **human** UC-MSC-laden Matrigel microspheres enhanced endometrial regeneration and improved fertility rates in a rat model with thin endometrium.	Xu *et al.* (2021)
	HA	*In vitro* + *in vivo* (rat)		Local injection of HA-danazol gel reduced size of endometrial cysts, without disrupting the estrous cycle in a rat model of endometriosis.	Nomura *et al.* (2006)
		In vivo (mouse)		HA-fibrin-encapsulated murine dEMSCs repaired the damaged endometrium, with successful implantation and normal embryo development.	Kim *et al.* (2019)
		In vitro + *ex vivo* + *in vivo* (rat)		*In situ* administration of HA gel/**human** MSC-secretome treatment repaired endometrial injury, promoting pregnancy, in a rat model of AS.	Liu *et al.* (2019)
	Collagen + HA + agar	*In vitro* + *in vivo* (mouse)		Three-layered artificial endometrium (made from **human** EnSC, stromal and vessel cells) remained functional *in vitro* for 28 days and restored fertility (with successful pregnancy and LBs) in endometrial ablation mouse model.	Park *et al.* (2021)
	Dextrin	*In vivo* (pig)		Using a dextrin-based adhesion barrier resulted in a higher percentage of adhesion-free sites compared with the controls after laparoscopy in a pig model.	Kai *et al.* (2018)
	Fibrin-agarose	*In vitro*		**Human** implantation is modeled by co-culturing human endometrial epithelial and stromal cells in a 3D system that allows JAR spheroid attachment.	Wang *et al.* (2012)
				Human epithelial-stromal interaction enhanced prolactin expression in fibrin-agarose gel. JAR spheroids invaded the epithelium and embedded into the 3D matrix under decidualization conditions.	Wang *et al.* (2013)
	PEG	**Clinical**		PEG-based sprayable adhesion barrier reduced adhesion tenacity, extent and incidence scores in patients undergoing myomectomy.	Mettler *et al.* (2004)
				Resorbable PEG-based hydrogel reduced post-operative adhesions following myomectomy.	Mettler *et al.* (2008)
		In vitro		Co-culture of **human** endometrial epithelial cells and stromal cells encapsulated in a PEG hydrogel with ECM-binding peptides remodel the synthetic matrix and display hormone-mediated differentiation.	Cook *et al.* (2017)
		In vitro + *in vivo* (rat)		L-phenylalanine-loaded PEBP/PEG hydrogel suppressed uterine fibrosis and promoted embryo implantation in a rat uterine curettage model.	Wang *et al.* (2021)
				**Human** AD-MSC exosome-hydrogel promoted neovascularization and endometrial regeneration in rats, facilitating LBs.	Lin *et al.* (2021a)
	PVA/CMC	*In vivo* (rabbit)		Reduced incidence, extent and severity of peritoneal adhesions following gynaecological surgery.	Müller *et al.* (2011)
	Pluronic F-12, Vitamin C	*In vivo* (rat)		Pluronic F-12 hydrogel encapsulating vitamin C and rat bone marrow stromal cells promoted rat endometrial regeneration by restoring the endometrial membrane and reducing inflammation.	Yang *et al.* (2017)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels	Methacrylamide-functionalized gelatin	*In vitro*		**Human** endometrial epithelial and stromal cells showed proangiogenic activity in response to E2 in gelatin hydrogels.	Pence *et al.* (2017)
	HP	*In vitro* + *in vivo* (rat)		ε-Polylysine-HP hydrogel encapsulating keratinocyte growth factor repaired the morphology of injured rat endometrium.	Xu *et al.* (2017a)
				HP hydrogel loaded with keratinocyte growth factor facilitates the morphologic and functional recovery of injured rat uteri.	Xu *et al.* (2017b)
				HP-E2 hydrogel prolongs release of E2, improving both gland numbers and fibrotic area, in a IUA rat model.	Zhang *et al.* (2017b)
		In vivo (rat)		HP-E2 hydrogel facilitated the regeneration of injured endometrium, inhibiting cell apoptosis in a IUA rat model.	Zhang *et al.* (2020b)
	Chitosan-heparin			Treating injured rat endometrium with a stromal cell derived factor-1α-loaded chitosan-heparin hydrogel restored endometrial thickness, gland number and reduced fibrosis.	Wenbo *et al.* (2020)
	Actamax adhesion barrier	**Clinical**		Spraying a degradable hydrogel adhesion barrier during gynecologic laparoscopic abdominopelvic surgery reduced postoperative adhesion development.	Trew *et al.* (2017)
	Aloe poloxamer + DC uterus nanoparticles	*In vitro* + *in vivo* (rat)		Aloe poloxamer with E2 encapsulated in DC rat uterus nanoparticles significantly recovers morphology and decreases uterine fibrosis in a IUA rat model.	Yao *et al.* (2020a)
dECM and polymer scaffolds	DC uterus	Proof of concept		Comparison of three protocols for whole rat uterus decellularization. The sodium deoxycholate protocol gave rise to a scaffold that structurally and mechanically resembled native uterus.	Hellström *et al.* (2014)
		Proof of concept + *in vitro*		Whole pig uterus decellularization produced a cytocompatible scaffold. Recellularization with **human** EnSC resulted in organoid-like structure formation.	Campo *et al.* (2017)
		In vivo (mouse)		DC uterine matrix transplantation restored all the uterine layers and fertility.	Hiraoka *et al.* (2016)
		In vitro + *in vivo* (rat)		Xenogeneic crosslinked rabbit uterine ECM achieved rat uterus regeneration and was recellularized *in vivo* after 90 days.	Yao *et al.* (2020b)
				Whole DC sheep uterus gave rise to biocompatible scaffolds with native-like biomechanical, structural and vascular properties that were recellularized *in vivo*.	Daryabari *et al.* (2019)
				Engraftment of rat MSC-recellularized DC uterine matrix on partially excised uteri yielded functional uteri with pregnancy and fetus rates comparable to the control group.	Li *et al.* (2021)
				Perfusion-recellularized uterine matrix is able to partially regenerate and reconstruct the damaged rat uteri.	Miyazaki and Maruyama (2014)
				Recellularized uterine ECM patches repair a partially defective uterus and support pregnancy.	Hellström et al. (2016)
		In vivo (rat)		Both high hydrostatic pressure and detergent-based decellularization protocols can efficiently create rat uterine matrices for uterine regeneration.	Santoso *et al.* (2014)
				The orientation of a DC uterine scaffold determines the tissue topology and architecture of regenerated uterus in rats, without affecting pregnancy.	Miki *et al.* (2019)
				Decellularization based on Triton-X100 and deionized water generated the lowest immune response after allogeneic transplantation of DC rat uterine scaffolds.	
				Rat uterus decellularization with sodium deoxycholate revealed more ECM-related damage-associated molecular patterns, and resulting scaffolds induced pro-inflammatory cytokine responses.	Padma, Alsheikh, Song, *et al.* (2021b)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
dECM and polymer scaffolds		*In vitro*		Mouse uterine DC scaffolds proved to be an adequate natural niche for **human** MenMSCs differentiation toward uterus-specific cell lineages.	Arezoo *et al.* (2021)
				Comparison of three protocols for whole sheep uterus decellularization, generating different ECM scaffolds that supported *in vitro* stem cell growth and proliferation.	Tiemann *et al.* (2020)
				Enzymatic preconditioning of sheep uterine ECM scaffolds improved recellularization compared with standard culture conditions and with the use of transwells alone.	Padma *et al.* (2021c)
	DC myometrium			Creation of allo- and xeno-neo-myometrium by culturing isolated myocytes into DC rat and **human** myometrial scaffolds.	Young and Goloman (2013)
	DC endometrium			**Human** recellularized endometrium responded to a 28-day hormone treatment by expressing E2 and P4 receptors and secreting IGF binding protein-1 and prolactin.	Olalekan *et al.* (2017)
				Comparison of different decellularization protocols for **human** endometrial fragments.	Sargazi *et al.* (2021)
	DC human amniotic membrane	*In vivo* (rat)		Engineered rat oral mucosa epithelial cells prevented progression of IUA and improved endometrial epithelium regeneration.	Chen *et al.* (2019)
				Human amniotic membrane and adipose stem cells improved regeneration, angiogenesis and receptivity in a rat IUA model.	Han *et al.* (2020)
	Urinary bladder ECM			Porcine urinary bladder matrix scaffolds improved endometrial regeneration in a rat model of intrauterine adhesions.	Zhang *et al.* (2020a)
	HA/carboxymethylcellulose membrane			Both melatonin and HA/carboxymethylcellulose membrane proved to be effective in prevention of adhesion formation in rats.	Demirbag *et al.* (2005)
	HA + mitomycin C			Mitomycin C-loaded crosslinked HA films and gels reduced formation of postoperative adhesions between uterine horns and with surrounding tissues and organs.	Liu *et al.* (2005)
	Carbylan-SX (semisynthetic glycosaminoglycan)			Carbylan-SX film and gel were efficacious in reducing postoperative intra-abdominal adhesion formation in cecum-abdominal wall and uterine horn in rats.	Liu *et al.* (2007)
	GelMA and sodium-alginate			Porous scaffold from droplet microfluidics loaded with bFGF had the ability to improve neovascularization and repair rat endometrium.	Cai *et al.* (2019)
	Alginate	*In vitro*		Development of an embryo implantation model consisting of an alginate scaffold seeded with **human** epithelial cells to which JAR spheroids are able to adhere.	Stern-Tal *et al.* (2020)
	Alginate-multivalent integrin α5β1 ligand			Alginate-multivalent integrin α5β1 ligand scaffolds enhanced **human** endometrial stromal cell growth under perfusion culture.	Li *et al.* (2011b)
	Collagen	*In vitro* + *in vivo* (rat)		Collagen scaffold loaded with **human** UC-MSCs promoted endometrial regeneration and restored fertility in a rat model.	Xin *et al.* (2019)
		In vivo (rat)		Collagen scaffolds with human bFGF improved regeneration of rat uterine endometrium and muscular cells, vascularization and pregnancy outcomes.	Li *et al.* (2011a)
				Collagen/rat BM-MSCs system increased proliferation of endometrial and myometrial cells, enhanced angiogenesis and restored fertility.	Ding *et al.* (2014)
				Collagen scaffold loaded with **human** ESC-derived endometrium-like cells regenerated the structure and function of rat uterine horns.	Song *et al.* (2015)
				Collagen scaffold loaded with **human** endometrial perivascular cells overexpressing CYR61 promoted endometrial and myometrial regeneration and induced neovascular regeneration in injured rat uteri model.	Li *et al.* (2019)
				Collagen scaffold with **human** UC-MSCs improved IUAs in rats, by increasing endometrial glands and reducing firbosis.	Liu *et al.* (2020)
		Clinical		Transplantation of collagen scaffold with autologous bone marrow mononuclear cells promoted functional endometrium reconstruction in patients with AS.	Zhao *et al.* (2017)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
	Gelatin-coated polyamide	*In vitro*		**Human** eMSCs were differentiated into smooth muscle cells or fibroblast-like cells to simulate fascial tissue composition, using an optimized gelatin-coated polyamide scaffold.	Su *et al.* (2014)
		In vivo (rat)		Seeding **human** eMSCs in SC gelatin-coated polyamide mesh resulted in enhanced collagen growth and organization.	Edwards *et al.* (2015)
	PGA-PLGA	*In vivo* (rabbit)		Subtotal uterine excisions were reconstructed with autologous constructs made from endometrial and myometrial cells. Fetal development supported to term and LB.	Magalhaes *et al.* (2020)
	Poly(glycerol sebacate)	*In vitro* + *in vivo* (rat)		Directional differentiation of rat BM-MSCs and restoration of morphology and function of wounded uteri.	Xiao *et al.* (2019)
	PLA + Pluronic F68			Novel PLA-Pluronic copolymer films prevented adhesion comparable to membranes of oxidized regenerated cellulose.	Yamaoka *et al.* (2001)
	PLA-poly(ε-caprolactone)/gelatin nanofiber	*In vivo* (mouse)		Degradable mesh with murine eMSCs promoted tissue integration and anti-inflammatory response after subcutaneous transplantation.	Mukherjee *et al.* (2019)
	PLA patch ("nanofilm")	*Ex vivo* + *in vivo* (rabbit)		PLA nanofilm sealed defects smaller than 3 mm in chorion-amnion and uterine membranes allowing intrauterine development in a rabbit model.	Pensabene *et al.* (2015)
	Polyglactin-910 mesh	*In vitro*		**Human** uterine smooth muscle myocytes proliferated and formed 3D tissues within 14 days.	Young *et al.* (2003)
	Emulsion-templated porous polymers			3D scaffolds enhanced differentiation of primary **human** endometrial epithelial and stromal cells resembling the *in vivo* architecture and function.	Eissa *et al.* (2018)
				Scaffolds with fibronectin improved adhesion, infiltration and function of primary **human** endometrial stromal cells.	Richardson *et al.* (2019)
	PTFE			Novel hormone responsive *in vitro* model of the **human** uterine wall by co-culturing smooth muscle cells and endometrial epithelial and stromal cells on a synthetic membrane.	Kuperman *et al.* (2020)
Bioprinting	Gelatin + alginate	*In vitro* + *in vivo* (rat)		3D-printed hydrogel scaffold loaded with **human** iPSC-derived MSCs promoted the regeneration of endometrial and endothelial cells, and improved endometrial receptivity in a rat model.	Ji *et al.* (2020)
	Myometrial 3D cell rings	*In vitro*		Bioprinted uterine rings created with **human** myometrial cells show origin-dependent patterns of contractility and respond differently to uterine contractility inhibitors.	Souza *et al.* (2017)
Organoid	DC endometrium			Solubilized endometrial ECM from porcine uteri enhances proliferation rates of **human** endometrial organoids.	Francés-Herrero *et al.* (2021b)
	Matrigel-based 3D culture platform			Development of a functional *C. trachomatis*-murine endometrial organoids infection model system.	Bishop *et al.* (2020)
				Establishment of a novel organotypic culture system that models the hormonal responses of the normal **human** endometrium (epithelia and stroma) *in vitro*.	Bläuer *et al.* (2005)
				Generation of long-term, hormone-responsive **human** endometrial organoid cultures from healthy and cancerous tissue.	Turco *et al.* (2017)
				Formation of **human** and murine organoid structures showing long-term expansion, and reproducing the molecular and histological phenotype of the endometrial epithelium.	Boretto *et al.* (2017)
		In vitro + *in vivo* (mouse)		Establishment of **human** organoids from endometriotic, cancerous and pre-cancerous tissues showing disease diversity and original lesions *in vivo*.	Boretto *et al.* (2019)
		In vitro		Derivation of **human** endometrial gland organoids from term placenta that express typical markers of glandular epithelia.	Marinić *et al.* (2020)
				Glandular organization, ultrastructural features, hormone responsiveness and glycodelin A expression make **human** organoids a powerful *in vitro* model for the endometrium-embryo cross-talk.	Luddi *et al.* (2020)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Organoid				Derivation of **human** endometrial organoids from menstrual flow, comparable to those derived from endometrial biopsies.	Cindrova-Davies *et al.* (2021)
				Establishment of **human** endometrial assembloids, consisting of gland-like organoids and primary stromal cells, to model the impact of decidual senescence on embryo implantation.	Rawlings *et al.* (2021)
				Co-culture of **human** iPSC-ESFs with placenta-derived endometrial epithelial cells generated hormone-responsive organoids in a model of human decidua.	Cheung *et al.* (2021)
		In vitro + *in vivo* (rat)	💊	**Human** endometrial organoids containing H9-ESC induced into EEPCs and stromal components facilitated endometrial regeneration and angiogenesis in a rat model of AS.	Jiang *et al.* (2021)
	Collagen	*In vitro*		Generation of a 3D collagen scaffold-based model of the **human** endometrium by co-culturing endometrial organoids and stromal cells.	Abbas *et al.* (2020)
	Functionalized PEG-macromers			Derivation of **human** endometrial organoids with cell specificity and apicobasal polarity in fully synthetic matrices.	Hernandez-Gordillo *et al.* (2020)
Microfluidic	PDMS chip	Proof of concept		Development of a microfluidic device for single mouse embryo co-culture with murine endometrial cells.	Kimura *et al.* (2009)
	Resin-based porous membrane; PDMS	*In vitro*		Development of a microfluidic model of the **human** endometrium, compartmentalizing culture of perivascular stroma and endothelial cells.	Gnecco *et al.* (2017)
	PDMS			Enhanced decidualization of **human** endometrial stromal cells via endothelial-derived prostaglandin E2 and prostacyclin due to the action of hemodynamic forces.	Gnecco *et al.* (2019)
	Porous glass			**Human** endometrium on-a-chip revealed insulin- and glucose-induced alterations in the transcriptome and proteomic secretome.	De Bem *et al.* (2021)
	PDMS + fibrin gel			Reconstitution of a three-layer, hormone-responsive, vascularized endometrium-on-a-chip on a 3D fibrin matrix using **human** HUVECs, Ishikawa and ESFs.	Ahn *et al.* (2021)
OVARY					
Scaffold-free approaches	Micro-molded agarose gel created with PDMS cast	*In vitro*		**Human** TCs self-assembled into complex spheroid, toroid and honeycomb microtissues. Artificial **human** ovary constructed at 72 h with TCs surrounding GC spheroids or COCs without stromal invasion or disruption.	Krotz *et al.* (2010)
	Ovary-like tissue	*In vivo* (mouse)	💊	Murine and rat PGCs and PGC-free gonadal cells can develop and reconstruct ovary-like tissue containing functional oocytes in an ectopic xenogeneic microenvironment.	Hayama *et al.* (2014)
	Qdot 655 ITK carboxyl QDs			QDs found in the ovaries do not affect mouse behavior or estrous cycles, but decreases IVF rate. QDs can downregulate FSH and LH receptors and decrease maturation rate.	Xu *et al.* (2016)
	PDMS	*In vitro* + *in vivo* (rat)	💊	Spheroid **human** PD-MSCs likely prolonged ovarian function, produced more follicles, doubled E2 levels compared to 2D culture and increased Nanos3, Nobox and Lhx8 at 1 and 2 weeks.	Kim *et al.* (2018)
	PEG-PLA versus TiO_2 nanoparticles	*Ex vivo*	💊	FSH/LH and IGF-1 supplementation rescued initial decrease of E2/P4 with PEG-PLA nanoparticles in rat ovaries. Neonatal exposure to TiO_2 nanoparticles hindered FSH/IGF stimulation.	Scsukova *et al.* (2020)
	Chitosan-based nanoparticles	*In vitro* + *in vivo* (rat)	💊	Treatment based on curcumin-encapsulated, self-assembled nanoparticles showed positive effects in reverting the symptoms of PCOS in rats.	Raja *et al.* (2021)
Hydrogels	Alginate	*In vitro* + *in vivo* (mouse)	💊	BMP4 increased number of developing porcine follicles, E2 secretion and GDF9/AMH. After xenotransplantation, hormone levels restored in ovariectomized mice and antral follicles developed.	Felder *et al.* (2019)
				1.5% alginate enhanced murine secondary follicle survival and oocyte maturation, supported normal IVF and resulted in LB after ET.	Xu *et al.* (2006)
		In vitro		Culturing multiple murine primary follicles together promoted follicle growth, rescued follicle integrity and increased transzonal projections and oocyte maturation.	Hornick *et al.* (2013)
				Co-culturing murine primary-secondary follicles with MEFs for the whole 14-day period increased survival and growth. Primary follicles had lower oocyte maturation rates than >80 μm follicles.	Tagler *et al.* (2012)
				90 μm murine follicles survived twice as much as 80 μm follicles and grew on average 29 μm more.	Tagler *et al.* (2013)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels				70 µm murine follicles survived much more than 60 µm follicles. Ascorbic acid supplementation improved structural integrity via expression of ECM and cell adhesion molecules.	Tagler *et al.* (2014)
				0.7% alginate resulted in visible TC layer and approporiate steroidogenesis of murine follicles, as well as enhanced size, pseudoantrum rate and GVBD.	West *et al.* (2007)
				0.5% alginate increased average murine follicle diameter. Antral follicles produced appropriate levels of E2+P4, a 34-fold increase in aromatase expression and elevated LH receptors.	West-Farrell *et al.* (2009)
				Cryopreservation (by slow freezing) produced murine follicles with similar survival, average follicle diameter, antral development, decreases in Cx-43 and Cx-37 expression and increases in P4/E2 and maturation rates.	Xu *et al.* (2009a)
				Cultured murine follicles had aromatase, inhibin βa, BMP15, KIT ligand, TGFβR2 expression downregulated relative to *in vivo* follicles, while their COCs had increased expression of Inhibin α and βa, decreased expression of BMP15, GDF9, KIT and similar expression of Figlα, JAG1, Mater.	Parrish *et al.* (2011)
				Murine secondary follicles mature, ovulate and luteinize *in vitro*. Progesterone agents (mifepristone and ulipristal acetate) significantly inhibited rupture.	Skory *et al.* (2015)
				Co-culture of mouse secondary follicles and ovarian cells in 0.5% alginate increased follicle survival, diameter and P4 production, while decreasing oocyte cortical granule abnormalities.	Jamalzaei *et al.* (2020)
				Normal OSE migrated and encapsulated wounded surfaces of mouse ovarian fragments. Direct effects of fetal bovine serum and bovine serum albumin on encapsulation and proliferation.	Jackson *et al.* (2009)
		In vivo (mouse)		SC murine ovarian grafts with the least amount of follicles had the highest survival. SC sites produced more mature oocytes. Higher embryo development rates after IVF versus ICSI. MDA-MB-231 cells encapsulated with follicles did not produce metastatic lesions.	Rios *et al.* (2018)
		In vitro		0.25% alginate increased survival of rat preantral follicles, average follicle diameter, antral development, ovulation and oocyte maturation compared to 2D culture.	Zhang *et al.* (2019c)
				Pre-antral canine follicles in 0.5% alginate grew faster, but had smaller diameters, and produced 5–10× less P4 than in 1.5% alginate. LH may be required to support TC differentiation and GC function.	Songsasen *et al.* (2011)
				0.25% alginate produces larger and more morphologically abnormal caprine follicles but higher E2/P4, aromatase and 3βHSD, antrum formation, growth and oocyte maturation rates.	Brito *et al.* (2014)
				Ovine secondary follicles cultured in 1% alginate increased COC expansion, maturation rates, mitochondrial activity and ROS as well as upregulated TFAM, ATP6/8 and downregulated KHDC3, NLRP5.	Mastrorocco *et al.* (2020)
				Collecting rhesus monkey follicles during the follicular phase (versus luteal phase) significantly increased survival, and average follicle diameter. Follicles grew significantly more with FSH alone versus FSH and LH.	Xu *et al.* (2009c)
				By preserving follicle viability and growth better than ethylene glycol, dimethylsufoxide can safely be used to cryopreserve **human** primordial/primary follicles encapsulated in alginate.	Camboni *et al.* (2013)
				E2, P4, inhibin A/B and activin A secretion patterns of **human** follicles *in vitro* mimicked *in vivo* serum levels. Individually cultured **human** primary–secondary follicles produced AMH approximately through the time of antrum formation.	Skory *et al.* (2015)
				Multilayered **human** secondary follicles continued to grow long term. E2/P4 positively correlated with follicle development whereas AMH transiently increased during early follicle development and then declined upon antrum formation. A total of 20% oocyte maturation and MII oocyte size was similar to germinal vesicle oocyte size.	Xiao *et al.* (2015)
				1% alginate supports survival (of oocytes and GCs) and development of small pre-antral **human** follicles from frozen-thawed OT for a week after enzymatic isolation.	Amorim *et al.* (2009)
				Human follicles in native OT remain viable for up to 24 h whereas isolated primordial follicles did not survive in 2% alginate. Encapsulating OT fragments supported antral development and surface epithelium, but not retention of follicle organization or basement membranes.	Laronda *et al.* (2014)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels	Alginate versus collagen			**Human** MenMSCs increased early secondary follicle survival, diameter, antrum formation, E2/P4 production, oocyte maturation and expression of BMP15 and GDF9 but decreased expression of Mater.	Rajabi *et al.* (2018)
	Collagen			3, 5, 7 mg/ml hydrogels supported rat follicle survival, size, integrity and GVBD better than 1 mg/ml.	Joo *et al.* (2016)
		In vivo (rat)		Transplantation of rat AD-MSCs-laden collagen scaffolds improved restoration of ovarian function and fertility outcomes in a rat model of POI.	Su *et al.* (2016)
		In vivo (mouse)		Transplantation of **human** UC-MSCs into POF mice preserved ovarian function as well as increased E2, AMH, ovarian volume, number of antral follicles, GC proliferation and CD31 expression.	Yang *et al.* (2019)
		Clinical		Primordial follicles activated *in vitro* via phosphorylation of FOXO3a and FOXO1. Transplantation to the ovaries of patients with POF rescued overall ovarian function. CP achieved in patients with POF after transplantation of **human** UC-MSC or collagen/UC-MSCs.	Ding *et al.* (2018)
	Alginate + PLO	*In vivo* (rat)		Ovarian contructs (of rat OT, GCs, TCs) restored hormone levels, in ovariectomized rats, for 90 days after transplantation. May be used as an alternative and safe cell-based hormone replacement therapy.	Sittadjody *et al.* (2017)
	Alginate versus PEG-fibrinogen ± PTEN inhibitors	*In vitro*		Alginate + bpV (pic) produced significantly more atretic follicles in **human** OT fragments than PEG-fibrinogen. Addition of 740Y-P (versus bpV(pic)) significantly increased follicle development and E2 levels.	Lerer-Serfaty *et al.* (2013)
	Alginate versus FA versus HA			FA increased survival, follicle size, antral development, oocyte maturation and embryo cleavage after fertilization but did not affect E2/P4 production.	Jin *et al.* (2010)
				Grouping five caprine multilayered secondary follicles per bead improved antral development and oocyte maturation. Alginate was better than HA and FA. FA produced 8-cell parthenotes. Cultured follicles had similar Cx43, Cx37 and 3βHSD but higher aromatase gene expression compared to non-cultured.	Brito *et al.* (2016)
	Alginate in growth factor-reduced Matrigel and alginate lyase microspheres	*In vitro* + *in vivo* (mouse)		After *in vitro* culture or grafting with murine ovarian cells, beads degraded, lost spherical shape and infiltrating blood capillaries could be observed in the grafted beads. CD34+ and CD45+ cells were found around and inside the matrix.	Vanacker *et al.* (2012)
	Alginate and/or Matrigel	*In vitro*		**Human** secondary follicles survived and developed to the antral stage. Hormones produced from individual follicles were undetectable the first week.	Xu *et al.* (2009b)
				Human small pre-antral follicles were well preserved in both groups, but encapsulation before cryopreservation improved survival and follicle size compared to cryopreservation before encapsulation.	Vanacker *et al.* (2013)
				Alginate significantly improved survival (after 1 week) and follicle development in **human** OT, compared to Matrigel, but did not affect E2 levels.	Kedem *et al.* (2011)
	Matrigel	*In vitro* + *in vivo* (rat)		Implantation of vascularized hydrogel with ovarian spheroids (made of rat GCs and TCs) in ovariectomized rats significantly aids the recovery of endocrine function, leading to full endometrial regeneration.	Yoon *et al.* (2021)
		In vitro + *in vivo* (mouse)		Matrigel loaded with **human** UC-MSCs promote GC proliferation and ovarian vascularization in a mouse model of POI.	Zhou *et al.* (2021)
	Agar versus Matrigel	*In vitro*		Agar substrate proved to be as suitable as Matrigel on growth and development of cryopreserved-thawed **human** follicles in OT culture.	Ghezelayagh *et al.* (2021)
	Alginate versus VitroGel			VitroGel improved pseudoantrum formation, E2 production, COC recovery, oocyte maturation (normal spindle and chromosome alignment and low ROS and mitochondrial membrane potential), from murine pre-antral follicles, compared to alginate.	Kim *et al.* (2020a)
	FA-IPN			FA-IPN supports murine secondary follicle survival, GC proliferation, antral formation, growth, appropriate E2/P4/Androstenedione production and improves oocyte maturation.	Shikanov *et al.* (2009)
				Alginate content can be <0.25% with the IPN. Growing murine secondary follicles secrete proteases, which degrade fibrin (to reduce compressive forces), mimicking their naturally dynamic microenvironment *in vivo*.	Shikanov *et al.* (2011b)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels	Sodium alginate bioglass	*In vitro* + *in vivo* (mouse)		Encapsulated **human** amniotic epithelial cells or its conditioned media can protecting GC function and enhance ovarian vascularization in chemotherapy-induced POF model.	Huang *et al.* (2021)
	Sodium alginate, fibrin or fibrin-HBP-VEGF	*In vivo* (mouse)		Murine hemi-ovaries in the fibrin-HBP-VEGF group had more primordial follicles, allowing mice to resume cyclicity earlier and conceived more rapidly. VEGF increased blood vessels at 3 weeks.	Shikanov *et al.* (2011c)
	HA	*In vitro*		HA increased murine secondary follicle survival and accelerated antral formation. Vitrified-warmed follicles encapsulated in HA had 54% MII compared to 57% in non-embedded follicles.	Desai *et al.* (2012)
		In vivo (rat)		Rats with HA+VEGF+bFGF-encapsulated ovaries maintained primordial follicles, but had shorter first estrous cycles, lower levels of E2 and c-Myc after autotransplantation.	Tavana *et al.* (2016b)
				OT encapsulation with HA can minimize ischemia-induced follicle loss, preserve the follicular pool, promote follicular survival, facilitate angiogenesis and restore hormone levels.	Tavana *et al.* (2016a)
				Autotransplanted vitrified OT encapsulated with HA had less intact follicles and lower FSH levels.	Taheri *et al.* (2016)
	HA gel versus PLGA/MH sponge	*In vitro* + *in vivo* (mouse)		Local delivery of **human** ESC-MPCs increased ovarian reserves, E2 and AMH levels, improving quality of oocytes, embryos and estrous cycle regularity in a POI model.	Shin *et al.* (2021)
	Fibrinogen-thrombin	*In vivo* (mouse)		Exogenous murine endothelial cells revascularized **human** OT grafts, increasing their viability and follicle development. Cells engineered to constitutively express AMH preserved primordial follicle reserves.	Man *et al.* (2017)
				F25/T4 and F12.5/T1 had similar vascular surface, CD45+ cells and supported murine preantral follicle recovery, survival and development. Isolated murine ovarian cells also survived and proliferated after grafting.	Luyckx *et al.* (2014)
				More murine secondary (than primordial-primary) follicles were proliferating. After 1 week, follicles had higher viability with 5–6% of follicles reaching the next developmental stage.	Chiti *et al.* (2016)
				Dense fibrin network encapsulated murine primary follicles, maintained physiological and morphological features, improved blood vessels around secondary follicles, but not theca parameters.	Chiti *et al.* (2017)
				Grafting of 10 or 100 **human** leukemic cells with ovarian stroma (artificial ovary) was insufficient to cause leukemia after 20 weeks, while grafting with 3 × 10(6) cells produced peritoneal masses at 4 weeks and systemic disease.	Soares *et al.* (2015)
				Human STEMPRO AD-MSCs increased partial pressure of oxygen, surface area of human CD34+ vessels, follicle survival and decreased apoptosis after xenotransplantation.	Manavella *et al.* (2018)
				Human STEMPRO AD-MSCs protected follicle reserves by modulating the PI3K/Akt pathway to maintain quiescence of primordial follicles.	Cacciottola *et al.* (2021)
				The combination of **human** ovarian graft embedding in fibrin clots and host treatment with simvastatin resulted in improved post-implantation outcomes in a mouse model.	Magen *et al.* (2020)
		In vitro		F50/T50 best mimics native **human** OT (based on fiber thickness, porosity and rigidity).	Chiti *et al.* (2018)
				F100/T4 had highest proliferation rate and least variable apoptosis, but F25/T4 and F12.5/T1 had uniform cell distribution, better homogeneity, **human** ovarian stromal cell density and reproducible fibrin degradation.	Luyckx *et al.* (2013)
	Fibrin	*In vivo* (mouse)		Initial survival of murine primordial follicles decreased but follicles developed and ovulated. Ovarian function confirmed by reduction in FSH and daily vaginal cytology.	Smith *et al.* (2014)
				>75 μg/ml bFGF improved survival, increased proliferation and protected primordial follicles (but did not affect primary and secondary follicles), in murine hemi-ovaries, with increased revascularization.	Gao *et al.* (2013)

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Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Hydrogels				Cryopreserved **human** preantral follicles, isolated and encapsulated in fibrin matrices (with or without HA) survive and grow for 7 days after xenografting in mouse.	Paulini *et al.* (2016)
				Fibrin-collagen hydrogels with murine MSCs restored cyclicity earlier but delayed follicle development in mouse OT. VP-MSC increased expression of AMH, FSH receptor, GDF9 and VEGF while BM-MSC increased expression of Ptch1.	Mehdinia *et al.* (2020)
	Laminin versus Matrigel	*In vitro*		Culturing **human** OT using laminin components of the native ovarian ECM enhanced follicle survival and proportion of secondary follicles compared to Matrigel.	Hao *et al.* (2020)
	bFGF sheet	*In vivo* (mouse)		Transplanting bFGF sheets (which released bFGF) with frozen-thawed **human** OT increased revascularization and follicle density (primordial and primary), but decreased fibrosis.	Tanaka *et al.* (2018)
	Chitosan-Silk fibroin	*In vitro*		Development of a novel *in vitro* model by encapsulating **human** ovarian stromal cells in chitosan-silk hydrogels.	Jafari *et al.* (2021)
	PEG-VS			5% hydrogel supported murine secondary follicle survival and antral development. Follicle morphology quickly diminished and deteriorated in >10% PEG solution. The YKNR plasmin substrate degraded rapidly, but supported antral formation and oocyte maturation.	Shikanov *et al.* (2011a)
				>10% hydrogel supported murine antral formation, but reduced oocyte maturation (compared to 5–7.5% hydrogel). Parthenotes with highest pronuclear and blastocyst formation in 10% hydrogel.	Ahn *et al.* (2015)
		In vivo (mouse)		Antral and mature preovulatory follicles, functional blood vessels and corpora lutea (indicated successful ovulation) after orthotopic transplant of murine primordial and primary follicles. A total of 60% of follicular reserve maintained at day 60.	Kim *et al.* (2016)
	PEG-VS versus Dual PEG-VS versus TheraCyte	*In vivo* (mouse)		Although it took twice as long, murine ovaries in Dual PEG capsules produced the greatest number of cycling mice (and functional tissue), in addition to preventing sensitization and lymphocytic infiltration.	Day *et al.* (2019)
	PEG + ECM sequestering peptides	*In vitro*		Sequestered cell-secreted ECM proteins loaded in PEG hydrogel improved murine early secondary follicle survival, growth and oocyte maturation.	Tomaszewski *et al.* (2021)
dECM and polymer scaffolds	DC ovary	Proof of concept		Comparison of three protocols of murine ovarian decellularization by agitation.	Alshaikh *et al.* (2019)
		Proof of concept + *in vitro*		Comparison of protocols for de- and re-cellularization of murine ovaries proposed sodium deoxycholate as the best detergent for this application.	Alshaikh *et al.* (2020)
				Rapid cell adhesion and aggregation of homologous fibroblasts, consistent with porcine ovarian scaffold's ability to sustain cell adherence, proliferation and differentiation.	Pennarossa *et al.* (2020)
				SDS-T-A DC scaffolds had intact ECM components/microstructure, reduced residual DNA and supported fibroblast viability and recovery of murine preantral follicles. DC **human** cortex had smaller pores and denser collagen fibers compared to the bovine ovary.	Nikniaz *et al.* (2021)
		In vitro + *ex vivo* + *In vivo* (rat)		DC porcine ovary caused minimal immunogenic response after SC xenotransplantation in rats and showed an improvement in E2 secretion *ex vivo*.	Liu *et al.* (2017)
		In vitro		Comparison of protocols for decellularization of **human** ovary and successful recellularization with **human** endometrial mesenchymal cells.	Sistani *et al.* (2021)
				Germline stem cells (isolated through magnetic activated cell sorting) can repopulate DC porcine ovarian scaffolds and differentiate into adult mature ovarian cells when stimulated.	Pennarossa *et al.* (2021b)
				Porcine ovarian ECM sustained *in vitro* cell survival and drove epigenetically-erased cell differentiation, fate and viability.	Pennarossa *et al.* (2021a)
		In vitro + *in vivo* (mouse)		DC bovine/**human** OT scaffold recellularized with murine primary ovarian cells and transplanted to initiate puberty in mice that had been ovariectomized.	Laronda *et al.* (2015)
				Peritoneum-derived MSCs in **human** OT scaffolds can produce germ cell markers (DAZL) after 1 week *in vitro*, and GDF9+ follicle-like structures 1 month after transplantation.	Eivazkhani *et al.* (2019)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
dECM and polymer scaffolds		*In vivo* (mouse)		DC **human** OT recellularized with **human** ovarian stroma cells supported survival of **human** follicles and antral formation of murine follicles after transplantation. A total of 21–25% follicles recovered at 3 weeks.	Pors *et al.* (2019)
		In vivo (rat)		Primary rat ovarian cells maintained viability and bioactivity as well as reconstructed follicle-like structures within DC **human** ovaries. Cells expressed steroid hormone receptors and GC markers and significantly increased E2/P4 in ovariectomized rats.	Hassanpour *et al.* (2018)
	DC amniotic membrane	*In vitro*		Intact **human** amniotic membrane increased murine primary–secondary follicle survival, size, E2 production, survival index and expression of Cx37, GDF9 and BMP15.	Motamed *et al.* (2017)
	Bovine ovary and uterus 'tissue papers'	Proof of concept + *in vitro* + *ex vivo*		DC ovarian 'tissue paper' supports murine follicle adhesion, viability and health *in vitro*, as well as maintains viability and hormonal function of primate and **human** OT *ex vivo* for 8 weeks postmortem.	Jakus *et al.* (2017)
	AlloDerm	*In vivo* (mouse) + **clinical**		Follicle development detected in **human** OT after 8–10 weeks and 6–8 antral follicle count achieved by 11–14 months. FSH normalized by 7 months. Embryos cryopreserved after 7–8 IVF cycles. Both patients achieved CPs after ET and one had LB.	Oktay *et al.* (2016)
	DC SIS	*In vivo* (rabbit)		Using porcine SIS to reconstruct ovarian resection reduced adhesion score and improved ovarian volume and epithelization in rabbit.	Celik *et al.* (2009)
	Collagen versus SIS	*In vivo* (mouse)		**Human** OT wrapped in **human** recombinant collagen improved grafting in mice, compared with porcine SIS.	Abir *et al.* (2020)
	hrVit versus SIS versus alginate scaffolds versus CollPlant	*In vitro*		Primordial follicle growth in **human** OT not enhanced by LIF. Despite some significant differences among the four matrices, none appeared to have a clear advantage.	Younis *et al.* (2017)
	FA versus fibrin-collagen	*In vivo* (mouse)		Fibrin encapsulation enhanced murine primordial-primary follicle survival, integration with the host tissue and resumption of estrous cycling. LBs achieved with follicles in VEGF-loaded fibrin beads.	Kniazeva *et al.* (2015)
Bioprinting	ORMOCER versus SU8	*In vitro*		ORMOCER did not improve doubling times or damage DNA, but forms gap junctions. Applying a two-photon polymerization to Ormocomp allows adherence to vertical/steep surfaces and layer formation after 3–4 days.	Ovsianikov *et al.* (2007)
	Porcine gelatin 'ink'	*In vivo* (mouse)		30° and 60° scaffolds provide corners that surround murine multilayered secondary follicles on multiple sides while 90° scaffolds have an open porosity that limits follicle-scaffold interaction. Transplant restored ovarian function and LB achieved.	Laronda *et al.* (2017)
	GelMA	*In vitro*		Cell-laden 3D printing of artificial ovaries supported follicle development and produced MII oocytes after *IVM*.	Wu *et al.* (2022)
Organoid	Matrigel			Prolonged treatment of tumor necrosis factor alpha induced phenotypic changes of **human** OSE spheroids.	Kwong *et al.* (2009)
				Generation of organoids from dissociated **human** female gonad cell suspensions in a three-layered Matrigel-based system.	Oliver *et al.* (2021)
Microfluidic	Alginate			Static conditions produced larger primordials and supported primordial-primary dog follicle transition but decreased RNA and GDF9 in (abnormal) cat follicles. 10 µl/min flow systems supported primordial-primary cat follicle transition and initial growth (D0-3), and dog follicle growth (but not normality). Preantral dog follicles had the highest growth rate in normal alginate beads (antral follicles grew the least).	Nagashima *et al.* (2018)
	Alginate versus collagen			Oxidized alginate does not support murine early secondary follicle survival. More antral follicles developed in collagen (versus alginate) core.	Choi *et al.* (2014)
	PDMS			Tp53R273H-mutated murine FTE (but not OSE) cells radially migrated out of cortical inclusion cysts. Number of invading cells and invasion distance enhanced by follicular fluid but worsened by collagen I.	Fleszar *et al.* (2018)
				Germinal vesicle-stage murine COCs denuded by passing through a microchannel (without hyaluronidase). Dynamic conditions improve oocyte maturation and glutathione, developmental competence and blastocyst formation.	Sadeghzadeh Oskouei *et al.* (2016)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
		Proof of concept		Murine COCs pass through a series of constriction–expansion units. No significant difference in fertilization or blastocyst rates after ICSI and IVF for oocytes denuded on chip versus denuded manually.	Weng *et al.* (2018)
FALLOPIAN TUBE					
Hydrogels	DC oviduct	*In vitro*		Rabbit embryos cultured on oviductal ECM hydrogel-coated wells presented a 'quieter' metabolism compared to embryos cultured under standard conditions.	Francés-Herrero *et al.* (2021a)
	Alginate			Co-culture of **human** FTE and murine secondary follicles revealed crosstalk in the reproductive cycle.	Zhu *et al.* (2016)
		Ex vivo		3D **human** fimbriae cultures retained tissue architecture and epithelial subtypes, responding to H_2O_2 and insulin exposure.	Eddie *et al.* (2015)
Organoid	Matrigel-based 3D culture platform	*In vitro*		Establishment of long-term organoid cultures from mouse FTE cells.	Xie *et al.* (2018)
				Establishment of long-term, stable 3D organoid cultures from **human** FTE that respond to E2 and P4 treatment in a physiological manner.	Kessler *et al.* (2015)
				Distal regions of **human** FTE showed increased organoid forming capacity, Wnt/inflammatory signaling and high-grade serous carcinoma signatures compared to proximal regions.	Rose *et al.* (2020)
				Co-culture of **human** FT-MSCs, HUVECs and FTE cells formed organoids that could be blocked by Wnt inhibitor DKK1.	Chang *et al.* (2020)
				Use of **human** iPSCs to establish a novel *in vitro* 3D **human** FTE organoid model.	Yucer *et al.* (2017)
	Mebiol			Murine FTE stem cells formed organoid colonies in a PEG-based 3D culture system, with some cells differentiating into secretory or ciliated cells.	Lin *et al.* (2021b)
Micro fluidic	PDMS + Nuclepore chip			Bovine oviduct-on-a-chip supported more physiological (*in vivo*-like) zygote genetic reprogramming than conventional IVF.	Ferraz *et al.* (2018)
CERVIX-VAGINA					
Scaffold free	Cell constructs	*In vitro* + *in vivo* (mouse)		Construction of a model of **human** vaginal mucosa with a capillary-like network that has the potential to become functional *in vivo*.	Jakubowska *et al.* (2020)
	Self-assembly			**Human** vaginal tissue was bioengineered using a self-assembly technique, which formed mature vaginal epithelium and matrix after *in vivo* animal implantation.	Orabi *et al.* (2017)
	Air–liquid interface	*In vitro*		Generation of a 3D **human** cervical model using ectocervical epithelilum built on a cervical stromal equivalent (that resembles native ECM) .	De Gregorio *et al.* (2017)
			💊	Generation of a 3D herpes simplex virus-2 infection model using **human** vaginal epithelial cells that reproduce basal and apical layers and shows pathological effects after virus inoculation.	Zhu *et al.* (2017)
Hydrogels	Silk-HA	*Ex vivo*		Development of an *ex vivo* pregnant-like tissue model (with **human** cervical tissue and fibroblasts) to assess silk-based hydrogels-mediated cervical augmentation.	Raia *et al.* (2020)
	Collagen derivative	*In vivo* (rat)	💊	Collagen derivative T16 hydrogel improved autologous collagen arrangement, cell proliferation and vaginal epithelium thickness in ovariectomy rat model.	You *et al.* (2020)
	Chitosan-thiogly-colic acid	*In vitro* + *in vivo* (rat)	💊	Genistein-loaded chitosan-thioglycolic acid hydrogel has high mucoadhesive properties and partially recovered the epithelial thickness of atrophic murine vagina.	Yang *et al.* (2021)
dECM and polymer scaffolds	DC vagina	*In vivo* (rat)	💊	Porcine acellular vagina matrix promoted tissue-engineered vagina reconstruction in a rat model of partial vaginectomy.	Zhang *et al.* (2017a)
		In vitro		Generation of a porcine vaginal ECM scaffold that allows attachment and growth of AD-MSCs and vaginal epithelial cells.	Greco *et al.* (2018)
	DC ectocervix			Development of three **human** ectocervical tissue models: (I) de- and recellularized ectocervix; (II) co-culture of ectocervical and ovarian explants; (II) cell-based ectocervix construct.	McKinnon *et al.* (2020)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
dECM and polymer scaffolds	DC Porcine SIS	*In vivo* (rhesus monkey)	💊	Porcine SIS scaffold loaded with **human** UC-MSCs enhanced vaginal repair in ovariectomized rhesus monkey.	Ma *et al.* (2021)
		Clinical	💊	Tissue-engineered autologous vaginal organs showed normal structural and functional variables in patients with MRKHS with a follow-up of up to 8 years.	Raya-Rivera *et al.* (2014)
			💊	Porcine SIS graft used for successful cervicovaginal reconstruction in a patient with MRKHS.	Zhang *et al.* (2019b)
	De-epidermized dermis	Proof of concept		Development of 3D **human** normal and cervical cancer tissue models via re-cellularization of dermal grafts.	Karolina Zuk *et al.* (2017)
	RENOV	**Clinical**	💊	Vaginal reconstruction with acellular **human** cadaver dermal matrix proved to be a safe, effective and minimally invasive procedure that provided near-normal sexual function for patients with MRKH.	Zhang *et al.* (2017c)
	Collagen	*In vitro*		*In vitro* **human** vaginal epithelial cell model based on collagen-coated beads recapitulated *in vivo* structural and functional properties.	Hjelm *et al.* (2010)
		In vivo (rat)	💊	Rat AD-MSCs-laden collagen scaffold promoted vaginal epithelial cell regeneration, vaginal tissue repair and improved vaginal stenosis and contracture on radiation-induced injury.	Ye *et al.* (2020)
		Clinical	💊	Anterior colporrhaphy with bovine pericardium reinforcement slightly improved success rate over colporrhaphy alone.	Guerette *et al.* (2009)
			💊	Successful creation of a neovagina in a patient with MRKHS using a bovine-derived dermis scaffold.	Noguchi *et al.* (2004)
	PEG	*Ex vivo* + *in vivo* (mouse)		PEG-coated nanoparticles diffused through **human** cervicovaginal mucus *ex vivo*, and uniformly lined the mouse colorectal and vaginal epithelium *in vivo*.	Maisel *et al.* (2016)
	Alginate + chitosan	*In vitro*		Development of an alginate/chitosan membrane that is stable in a simulated **human** vaginal environment and with the ability of releasing metronidazole over time.	Tentor *et al.* (2020)
	Alvetex			Generation of 3D **human** endocervical model that responds to E2 and P4 during a 28-day culture. Treatment with mifepristone attenuated the inhibition of IL-1β and LIF secretion.	Arslan *et al.* (2015)
	Silk			Treating **human** cervical-like constructs with P4 decreased collagen and increased the softness of the ECM over 28 days.	House *et al.* (2018)
	PLA and compact polyurethane membrane	Proof of concept		Fibrin glue could successfully adhere a PLA and polyuretane bilayer membrane to **human** cervical tissues. The membrane provides a fluid barrier and can be inserted through the cervix.	Roman *et al.* (2018)
	Oxidized cellulose	**Clinical**	💊	Vaginal reconstruction using oxidized cellulose proved to be a safe and effective procedure, with minimum complications and good success rates.	Dadhwal *et al.* (2010)
Bioprinting	PACIENA prosthesis + Interceed		💊	Good anatomical and functional results were achieved using 3D printed PACIENA prosthesis for vaginoplasties without skin grafts.	Acién *et al.* (2019)
	DC vagina bioink	*In vitro* + *in vivo* (rat)	💊	**Human** BM-MSCs could differentiate in 3D vagina tissue printed with ECM bioink of DC porcine vagina, inducing vascularization and epithelization *in vivo*.	Hou *et al.* (2021)
	Polyetherurethane	*In vitro*		3D-printed cervical implants supported **HUVECs** adhesion and growth, allowing for controlled loading and release of anti-human papillomavirus protein.	Zhao *et al.* (2020)
	Polyurethane + clotrimazole			3D-printed clotrimazole-loaded vaginal ring sustained drug release over 7 days and displayed a complete *C. albicans* growth inhibition after 5 days.	Tiboni *et al.* (2021)

Continued

Table I Continued

Strategy	Platform/ biomaterial	Type of study (model)	Disease-related	Main findings	Reference
Organoid	Matrigel			Derivation of **human** organoids from the squamocolumnar junction region of the uterine cervix, along with metaplastic squamous cells in the transformation zone.	Maru *et al.* (2020)
				Generation of **human** and **murine** ecto- and endocervical organoids, which were used in the study of the mechanisms that maintain cervical epithelial junctions and the emergence of metaplasia.	Chumduri *et al.* (2021)
	Cultrex RGF-BME type 2	*In vitro* + *in vivo (mouse)*	💊	Establishment of **human** ecto- and endocervical 3D organoids that stably recapitulate physiological and carcinogenic traits, growing as xenografts in mice.	Lõhmussaar *et al.* (2021)
Micro fluidic	PDMS	*In vitro*		Development of an organ-on-chip of the cervical epithelial layer that can recapitulate the **human** ecto- and the endocervical epithelial regions.	Tantengco *et al.* (2021)
FULL TRACT					
Microfluidic	Acrylic	*Ex vivo*		Development of a microfluidic system (with **human** FTE, endometrial cells, ecto-cervix explant and liver microtissues) that supports murine ovarian follicles and reproduces the human 28-day menstrual cycle hormone profile.	Xiao *et al.* (2017)
	PDMS	*In vitro*		Fabrication of a microwell-structured microfluidic device that allows single mouse oocyte trapping, IVF and embryo culture.	Han *et al.* (2010)
				Human endometrial (EnSCs, HUVECs and stromal cells) and ovarian (GCs and TCs) components modeled the bidirectional crosstalk between the uterus and the ovary in a 'dual reproductive organ-on-a-chip' model, which could be used for testing reproductive toxicants.	Park *et al.* (2020)

Description of the main bioengineering findings in the female reproductive system in the last 21 years based on different strategies, platforms/biomaterials, type of study (including *in vivo* models) and gynaecological related-diseases.

To note: model in type of study column only refers to *in vivo* approach; studies with human cells/tissues are marked in bold, while clinical studies are highlighted in yellow; and pill icons indicate studies carried out with patients, animal models and biological samples with female reproduction-related diseases (established cell lines have not been taken into account).

3βHSD, 3β-Hydroxysteroid dehydrogenase; AD-MSC, adipose-derived mesenchymal stem cell; AMH, anti-Müllerian hormone; AS, Asherman's syndrome; bFGF, basic fibroblast growth factor; BM-MSC; bone marrow-derived mesenchymal stem cell; BMP, bone morphogenic protein; *C. albicans: Candida albicans*; *C. trachomatis: Chlamydia trachomatis;* CD, cluster of differentiation; COC, cumulus-oocyte complex; Collplant, human recombinant virgin collagen bioengineered in tobacco plant lines; CP, clinical pregnancy; Cx37/43, connexin 37/43; CYR61: cysteine-rich angiogenic inducer 61; DAZL, deleted in azoospermia like; DC, decellularized; dECM, decellularized extracellular matrix; dEMSC, decidualized endometrial stromal cells; DKK, Dickkopf WNT Signaling Pathway Inhibitor 1; E2, estradiol; ECM, extracellular matrix; EEPC, endometrial epithelial progenitor cell; eMSC, endometrial mesenchymal stem cell; EnSC, endometrial stem cell; ESC, embryonic stem cell; ESC-MPC, embryonic stem cell-derived mesenchymal progenitor cell; ESF, endometrial stromal fibroblast; ET, embryo transfer; EVT, extravillous trophoblast; F_/T_, fibrinogen (mg/ml)/thrombin (IU/ml) concentration ratio; FA, fibrin-alginate; FA-IPN, fibrin-alginate interpenetrating network; FOXO1/3a, forkhead box protein O1/3a; FT-MSC, Fallopian tube mesenchymal stem cell; FTE, Fallopian tube epithelium; GC, granulosa cell; GDF9, growth differentiation factor 9; GelMA, gelatin-methacryloyl; GVBD, germinal vesicle breakdown; H9-ESC: human embryonic stem cell-9 line; HA, hyaluronic acid; HBP, heparin binding protein; HP, heparin poloxamer; hrVit, human recombinant vitronectin; HUVEC, human umbilical vein endothelial cell; IGF, insulin-like growth factor; iPSC, induced pluripotent stem cell; IUA, intrauterine adhesion; Jag1, jagged canonical Notch ligand 1; JAR spheroids: human choriocarcinoma (JAR) cells grown as multicellular spheroids; KHDC3, KH domain containing 3 like; LB, live birth; Lhx8, LIM homeobox 8; LIF, leukemia inhibitory factor; MEF, mouse embryonic fibroblast; MenMSC, menstrual blood mesenchymal stem cell; MH, magnesium hydroxide; MII, metaphase II; MRKH, Mayer-Rokitansky-Küster-Hauser; MSC, mesenchymal stem cell; Nanos3, Nanos C2HC-Type Zinc Finger 3; NLRP5, NLR Family pyrin domain containing 5; ORMOCER, Photosensitive organic-inorganic hybrid polymer (ORganically MOdified CERamics); OSE, ovarian surface epithelium; OT, ovarian tissue; P4, progesterone; PD-MSC, placenta-derived mesenchymal stem cell; PDMS, polydimethylsiloxane; PEBP, poly(ethylene glycol)-b-poly(L-phenylalanine); PEG, 4-polymeric poly-(ethylene glycol); PEG-PLA, polymeric poly(ethylene glycol)-block-polylactide methyl ether; PEG-VS, 4-arm poly-(ethylene glycol) tetravinyl sulfone; PGC, primordial germ cell; PLA, poly-L-lactic acid; PLGA, poly(D, L-lactide-co-glycolide); PLO, poly-L-ornithine; POF, premature ovarian failure; POI, premature ovarian insufficiency; PSC-ESF, pluripotent stem cells induced into endometrial stromal fibroblast; PTFE, polytetrafluoroethylene fluoropolymers; PVA/CMC, polyvinyl alcohol-carboxymethylcellulose; QD, quantum dots; RGF-BME, reduced growth factor-basement membrane extract; ROS, reactive oxygen species; SC, subcutaneous; SDS-T-A, sodium dodecyl sulfate-Triton-Ammonium; SIS, small intestine submucosa; SU8, negative photo-resistor based on epoxy components; TC, theca cell; TFAM, transcription factor A (mitochondrial); TGFβR2, transforming growth factor beta receptor 2; tHESC, hTERT-immortalized human endometrial stromal cell; TiO_2, titanium dioxide; UC-MSC, umbilical cord-derived mesenchymal stem cell; VEGF, vascular endothelial growth factor; VP-MSC, visceral peritoneum mesenchymal stem cell.

screened for eligibility (based on exclusion criteria presented in Fig. 2) and 584 (5.6%) full-text papers were retrieved for detailed assessment. An additional 24 studies were retrieved from manual searching of citations. We classified studies by bioengineering strategy within each organ (Fig. 3), and by their main application(s). Finally, 312 articles were included for systematic review, including 18 (5.8%) studies on scaffold-free approaches, 124 (39.7%) discussing hydrogels, 87 (27.9%) related to dECM and polymer scaffolds, 10 (3.2%) using bioprinting, 45 (14.4%) implementing organoid cultures and 28 (8.9%) exploring microfluidic techniques (Table I and Supplementary Table SIV). The majority of the bioengineering studies included *in vitro*/*ex vivo* or *in vivo* work using animal models, with only a few studies reaching clinical stages (seven uterine, two ovarian and seven cervical).

Bioengineering tools in female reproductive medicine: systematic summary of the evidence

Below we summarize studies using the six bioengineering techniques in work related to female reproductive organs.

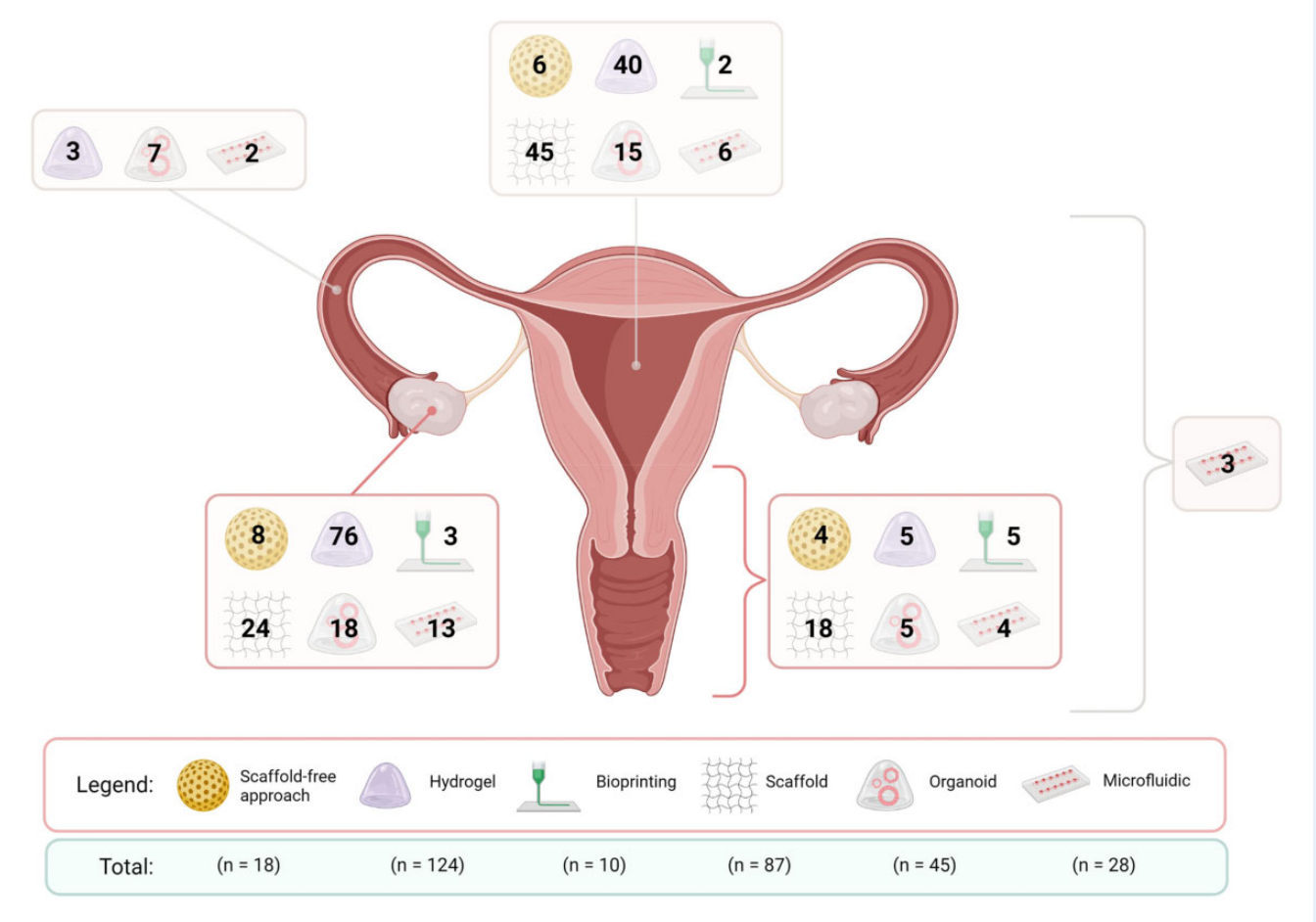

Figure 3. Organ-level overview of the bioengineering studies carried out between January 2000 and September 2021 and included in this systematic review. The studies involved the uterus, ovaries, fallopian tubes and cervix/vagina. The numbers reflect the number of studies included in Table I and Supplementary Table SIV. Created with BioRender.com.

Scaffold-free approaches

This section mainly contemplates studies based on the non-matrix-assisted self-organizing capacity of cells to generate multicellular entities. Six studies presented scaffold-free approaches applied to bioengineering of the uterus and its tissues, including four *in vivo* studies based on cell sheets (Kuramoto *et al.*, 2015, 2018, 2020; Sun *et al.*, 2018) and two *in vitro* investigations based on the generation and study of epithelial and stromal organoids (Murphy *et al.*, 2019; Wiwatpanit *et al.*, 2020). Of the eight studies involving scaffold-free approaches in ovary, one used a cell-based method involving primordial germ cells to generate ovarian tissue (OT) *in vivo* (Hayama *et al.*, 2014), four developed self-assembled spheroids (Krotz *et al.*, 2010; Chowanadisai *et al.*, 2016; Kim *et al.*, 2018; Ward Rashidi *et al.*, 2019), while toxicity of Qdot 655 ITK carboxyl quantum dots (Xu *et al.*, 2016) and nanoparticles made with chitosan (Raja *et al.*, 2021), polyethylene glycol (PEG) and polylactic acid or titanium dioxide (Scsukova *et al.*, 2020), were tested in preclinical and *ex vivo* models, respectively. Scaffold-free approaches to develop bioengineered vaginal and cervical tissues were included in four studies, which applied self-assembled vaginal constructs *in vivo* (Orabi *et al.*, 2017; Jakubowska *et al.*, 2020) or air–liquid interface techniques to generate vaginal and cervical *in vitro* models (De Gregorio *et al.*, 2017; Zhu *et al.*, 2017).

Hydrogels

This review unveiled a plethora of different hydrogel-based research involving uterine cells or tissues. Of the 40 studies compiled, 65%, 30% and 5% used natural, synthetic or hybrid hydrogels, respectively. The most commonly used natural hydrogels were based on collagen (46.2%), Matrigel (24.4%) and hyaluronic acid (HA; 15.4%). Synthetic hydrogels were based predominantly on PEG (38.5%) and poloxamer (30.7%). Studies applied these approaches for *in vitro* modeling of disease, tissue cross-talk and differentiation (Schutte *et al.*, 2015; Cook *et al.*, 2017; Pence *et al.*, 2017; Stejskalová *et al.*, 2021), *in vivo* regeneration of the endometrium and myometrium (Lin *et al.*, 2012; Yang *et al.*, 2017; Li *et al.*, 2019; Yoon *et al.*, 2021; Lin *et al.*, 2021a) and the treatment of intrauterine adhesions (Müller *et al.*, 2011; Liu *et al.*, 2020).

Encapsulation of follicles and tissue fragments is the most exploited hydrogel-based application in ovarian bioengineering. Alginate (alone or in combination with other materials) is the most commonly implemented hydrogel, with use in 47.14% of studies evaluating hydrogels for *in vitro* cultures of murine (Xu *et al.*, 2006, 2009a; West *et al.*, 2007; West-Farrell *et al.*, 2009; Jackson *et al.*, 2009; Jin *et al.*, 2010; Parrish *et al.*, 2011; Tagler *et al.*, 2013, 2014; Skory *et al.*, 2015; Zhang *et al.*, 2019c), canine (Songsasen *et al.*, 2011), caprine (Brito *et al.*, 2014), ovine (Mastrorocco *et al.*, 2020), primate (Xu *et al.*, 2009c) and human (Amorim *et al.*, 2009; Camboni *et al.*, 2013; Laronda *et al.*, 2014; Skory *et al.*, 2015; Xiao *et al.*, 2015) ovarian follicles and tissue. Natural and synthetic hydrogels such as laminin (Hao *et al.*, 2020), collagen (Joo *et al.*, 2016), fibrin (Smith *et al.*, 2014; Paulini *et al.*, 2016), Matrigel (Xu *et al.*, 2009b; Kedem *et al.*, 2011; Ghezelayagh *et al.*, 2021), VitroGel (Kim *et al.*, 2020a), HA (Desai *et al.*, 2012) or PEG (Shikanov *et al.*, 2011a; Lerer-Serfaty *et al.*, 2013; Tomaszewski *et al.*, 2021) are less frequently reported.

Twenty-four studies used hydrogels in preclinical models of IVF (Xu *et al.*, 2006), allo-/xenotransplantation of ovarian cells, follicles and fragments (Vanacker *et al.*, 2012) or ovarian function restoration (Su *et al.*, 2016; Ding *et al.*, 2018; Yang *et al.*, 2019; Yoon *et al.*, 2021) (Table I); seven applied hydrogels in oncological modeling and drug testing (Supplementary Table SIV).

Tissue-specific hydrogels based on rabbit oviductal dECM and alginate resulted, respectively, in *in vitro* models of embryo culture (Francés-Herrero *et al.*, 2021a) and crosstalk between human epithelium and murine follicles (Zhu *et al.*, 2016), or *ex vivo* models of the human fallopian tube fimbriae (Eddie *et al.*, 2015). Hydrogels based on silk-HA, collagen derivatives and chitosan could recreate an *ex vivo* pregnant-like human cervical model (Raia *et al.*, 2020), or treat vaginal atrophy *in vivo* (You *et al.*, 2020; Yang *et al.*, 2021).

Decellularized extracellular matrix and polymer scaffolds

Forty-five uterine-related studies evaluated dECM and polymer scaffolds in cytocompatibility and *in vitro* modeling experiments, as well as *in vivo* regeneration and anti-adhesions tests (Table I). Those based on decellularized (DC) matrices accounted for 46.6% of reports, while 33.3% and 20% used scaffolds based on purified natural or artificial polymers, respectively. Various studies isolated and evaluated tissue-specific ECM from whole uteri of rats (Hellström *et al.*, 2014, 2016; Miyazaki and Maruyama, 2014; Santoso *et al.*, 2014; Miki *et al.*, 2019; Padma *et al.*, 2021a,b), mice (Hiraoka *et al.*, 2016), pigs (Campo *et al.*, 2017), rabbits (Yao *et al.*, 2020b) and sheep (Daryabari *et al.*, 2019; Tiemann *et al.*, 2020; Padma *et al.*, 2021c); from rabbit (Campo *et al.*, 2019), pig (López-Martínez *et al.*, 2021a,b) and human endometrium (Olalekan *et al.*, 2017; Sargazi *et al.*, 2021); and from rat and human myometrium (Young and Goloman, 2013). Natural polymer scaffolds included alginate (Li *et al.*, 2011b; Stern-Tal *et al.*, 2020), HA (Demirbag *et al.*, 2005; Liu *et al.*, 2005) and gelatin-coated (Su *et al.*, 2014; Edwards *et al.*, 2015; Cai *et al.*, 2019) materials, while artificial polymer scaffolds were created with polyglactin (Young *et al.*, 2003), polylactide (Pensabene *et al.*, 2015; Mukherjee *et al.*, 2019), polyglycolic acid (Magalhaes *et al.*, 2020), emulsion-templated highly porous materials (Eissa *et al.*, 2018; Richardson *et al.*, 2019) and polytetrafluoroethylene fluoropolymers (Kuperman *et al.*, 2020).

In contrast, only dECM or natural polymers were used for 24 studies involving ovarian research. Several reports described the generation and *in vitro*/*in vivo* biocompatibility of DC murine (Alshaikh *et al.*, 2019, 2020), porcine (Liu *et al.*, 2017; Pennarossa *et al.*, 2020, 2021a,b), ovine (Eivazkhani *et al.*, 2019), bovine (Laronda *et al.*, 2015; Nikniaz *et al.*, 2021) and human (Hassanpour *et al.*, 2018; Pors *et al.*, 2019; Sistani *et al.*, 2021) ovaries, as well as porcine small intestine submucosa (Celik *et al.*, 2009; Abir *et al.*, 2020) or human amniotic membrane (Motamed *et al.*, 2017).

Of the 18 studies that assessed scaffolds for cervicovaginal applications, 33.3% were clinical studies of vaginal reconstruction using oxidized cellulose (Dadhwal *et al.*, 2010), bovine collagen matrices (Noguchi *et al.*, 2004; Guerette *et al.*, 2009), human acellular dermis (Zhang *et al.*, 2017c) or porcine small intestine submucosa (Raya-Rivera *et al.*, 2014; Zhang *et al.*, 2019b). The remaining studies consisted of either *in vitro* cervico-vagina models based on DC porcine vaginas (Greco *et al.*, 2018), human cervical dECM (McKinnon *et al.*, 2020) and scaffolds of collagen (Hjelm *et al.*, 2010), alginate/chitosan (Tentor *et al.*, 2020), silk (House *et al.*, 2018), Alvetex (Arslan *et al.*, 2015) and polylactide (Roman *et al.*, 2018) or preclinical models of vaginal repair (Maisel *et al.*, 2016; Zhang *et al.*, 2017a; Ye *et al.*, 2020; Ma *et al.*, 2021).

Bioprinting

The scarcity of bioprinting implementation in reproductive studies reflects the novelty of this technique. Two reports applied 3D bioprinting technology to *in vitro* studies of uterine contractility (Souza *et al.*, 2017) and *in vivo* endometrial regeneration using a gelatin-alginate hydrogel loaded with human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) (Ji *et al.*, 2020). Similarly, three studies applied bioprinting with natural polymers for *in vitro* ovarian modeling (Ovsianikov *et al.*, 2007), follicle culture (Wu *et al.*, 2022) or fertility restoration in a preclinical model (Laronda *et al.*, 2017). Finally, five groups bioprinted bactericidal vaginal rings (Tiboni *et al.*, 2021), vaginal tissue with acellular bioink (Hou *et al.*, 2021), PACIENA prosthesis for vaginoplasties (Acién *et al.*, 2019), cervical implants (Zhao *et al.*, 2020) or 3D models of cervical cancer (Gospodinova *et al.*, 2021).

Organoids

We identified 15 studies generating endometrial organoids. Matrigel was primarily used as a supportive matrix, with (Francés-Herrero *et al.*, 2021b) or without (78.5% of studies) endometrial dECM supplementation. Other studies used collagen (Abbas *et al.*, 2020), or functionalized PEG matrices (Hernandez-Gordillo *et al.*, 2020). Remarkably, in six studies, epithelial and stromal components were combined to create organoids (Murphy *et al.*, 2019; Wiwatpanit *et al.*, 2020; Jiang *et al.*, 2021) or assembloids (Bläuer *et al.*, 2005; Abbas *et al.*, 2020; Rawlings *et al.*, 2021), which simultaneously represent both endometrial populations. Ovarian spheroids or organoids were derived in 18 studies using Matrigel, Cultrex or agarose, together with diverse cell types, including ovarian epithelial cells (Kwong *et al.*, 2009; Kopper *et al.*, 2019), granulosa and theca cells (Yoon *et al.*, 2021) or embryonic gonads (Oliver *et al.*, 2021). Remarkably, 89% of the studies generated ovarian organoids, derived from patients with cancer, that reliably mimicked the original pathology (Maru *et al.*, 2019). Similarly, in five studies, human organoids were derived from the squamocolumnar junction region of the uterine cervix (Maru *et al.*, 2020) or ecto- and endocervical tissue using Matrigel or Cultrex (Lechanteur

et al., 2017; Chumduri et al., 2021; Lõhmussaar et al., 2021; Tanaka et al., 2021). Fallopian tube organoids were generated from murine (Xie et al., 2018) and human (Kessler et al., 2015; Rose et al., 2020; Zhang et al., 2021a) fallopian tube epithelial cells used alone or co-cultured with umbilical vein endothelial cells and fallopian tube-derived MSCs (Chang et al., 2020), and from human iPSC (Yucer et al., 2017) or murine fallopian tube epithelial stem cells (Lin et al., 2021b). Culture was supported by Matrigel (in five of these six studies) or Mebiol (Lin et al., 2021b). Organoid studies accounted for 58%, 13% and 13% of bioengineering platforms reported in the fallopian tubes, ovary and uterus, respectively.

Microfluidic systems

Microfluidic platforms can be used to model static, passive (created by gravity) or active dynamic (with a determined flow rate) conditions. Six studies applied microfluidics to create advanced *in vitro* models of the human endometrium (Astolfi et al., 2016; Gnecco et al., 2017, 2019; Ahn et al., 2021; De Bem et al., 2021), or co-culture embryos with endometrial cells (Kimura et al., 2009). Of the 13 microfluidic-based studies in the ovarian field, two applied dynamic follicle culture in alginate and/or collagen matrices (Choi et al., 2014; Nagashima et al., 2018), while two studies evaluated the mechanical effect of flow on oocyte denudation and maturation (Sadeghzadeh Oskouei et al., 2016; Weng et al., 2018). The rest of the studies sought to recreate oncological models, evaluate drug effectiveness or elucidate therapeutic targets (as we discuss in detail in the applications sections). Finally, four studies applied microfluidics to model the human cervical epithelial layer (Lin et al., 2017; Aziz et al., 2020; Yang et al., 2020; Tantengco et al., 2021) and bovine (Ferraz et al., 2018) and canine (Ferraz et al., 2020) oviducts, while one study modeled human uterine and ovarian endocrine crosstalk (Park et al., 2020), and two studies recreated a complete female tract in microfluidic systems, combining endometrial, ovarian, oviductal, cervix and liver tissue to model the hormonal profile of a menstrual cycle (Xiao et al., 2017) or to manipulate, fertilize and culture embryos in a single device (Han et al., 2010).

Preclinical models and clinical applications: an update

Bioengineering approaches can elucidate the normo- and pathophysiology of female reproductive organs by developing next-generation *in vitro*/*ex vivo* platforms, creating representative models for toxicology/drug screening, developing alternative therapeutic strategies, discovering new biomarkers and improving tissue/organ regeneration and/or transplantation protocols.

Development of next-generation in vitro *and* ex vivo *platforms*

The creation of *in vitro* platforms that faithfully reproduce the physiological and pathological states of higher organisms is of paramount importance in applied and translational research. Bioengineering platforms have provided novel 3D models of follicle culture (see citations in Hydrogels section), human embryo implantation (Wang et al., 2012, 2013; Buck et al., 2015; Stern-Tal et al., 2020; Rawlings et al., 2021), a three-layered endometrium that remained functional for 28 days (Park et al., 2021), endometrial cancer invasion (Park et al., 2003) and wound healing (Stavreus-Evers et al., 2003), as well as bidirectional crosstalk between the uterus and the ovaries (Park et al., 2020). Moreover, collagen scaffolds loaded with human epithelial and endothelial cells (Pence et al., 2015) or tissue slices (Muruganandan et al., 2020) respond to ovarian hormones, while collagen-embedded human stromal cells demonstrate decidualization changes (Schutte and Taylor, 2012) and contractile ability (Kim et al., 2020b). Notably, endometrial cells encapsulated in a PEG hydrogel with ECM-binding peptides remodeled the synthetic matrix and displayed hormone-mediated differentiation (Cook et al., 2017).

In vitro follicle growth produced developmentally competent murine oocytes (Xu et al., 2006; Ahn et al., 2015) that led to live births (LBs) after embryo transfer (Xu et al., 2006). Most studies (94%) implemented individual follicle culture, while others successfully demonstrated that culturing multiple follicles together substantially improves follicle survival (>80% versus <29% with individual culture) and oocyte maturation (Hornick et al., 2013; Brito et al., 2016). Interestingly, IVFG benefits from the addition of ECM sequestering peptides (Tomaszewski et al., 2021), ascorbic acid [which increases expression of ECM and cell adhesion molecules (Tagler et al., 2014)], bone morphogenetic protein 4 (Felder et al., 2019), mouse embryonic fibroblasts (Tagler et al., 2012), ovarian cells (Jamalzaei et al., 2020) and human menstrual blood MSCs (Rajabi et al., 2018), but not denuded oocytes, oocyte-secreted factors, granulosa cells (Hornick et al., 2013) or leukaemia inhibitory factor (Younis et al., 2017). VitroGel, a novel animal-origin free hydrogel, also improves IVFG parameters, outperforming alginate and producing competent oocytes in a recent study by Kim et al. (2020a). Furthermore, when used exclusively, alginate concentrations ranged between 0.25% and 3% (Supplementary Table SIII); however, as a fibrin-alginate interpenetrating network, the concentration of alginate can be reduced below 0.25%, providing a more realistic environment for follicle growth and improving oocyte maturation (Shikanov et al., 2009, 2011b). Notably, combinations of 25 mg/ml fibrinogen and 4 IU/ml thrombin or 12.5 mg/ml fibrinogen and 1 IU/ml thrombin, are suggested as the best scaffolds for human ovarian stromal cells *in vitro* (Luyckx et al., 2013) and for murine follicle development *in vivo* (Luyckx et al., 2014), while 50 mg/ml fibrinogen and 50 IU/ml thrombin best mimics the rigidity of the native human ovarian cortex (Chiti et al., 2018).

DC models also offer important advances by maintaining unique tissue-specific ECM milieus, not only providing the most realistic scaffold for each organ's endogenous cell types, but also remarkably acting as a biocompatible framework for cells from other tissues/species. For example, DC mouse uterine tissue is an adequate natural niche for human menstrual blood MSC differentiation toward uterus-specific cell lineages (Arezoo et al., 2021), DC sheep uterus stimulates rat fetal dorsal root ganglion regeneration and angiogenesis during chicken embryo development (Padma et al., 2021c) and solubilized porcine endometrial dECM enhances proliferation rates of human endometrial organoids (Francés-Herrero et al., 2021b).

The generation and increasing use of organoids are revolutionizing the field of reproductive medicine. Among other limitations, the primarily epithelial nature of these structures is noteworthy. To date, several investigations have already provided models of multicompartment tissues [i.e. endometrial and stromal compartments (Murphy et al., 2019; Rawlings et al., 2021)], ecto- and endo-cervical epithelial regions (Maru et al., 2020; Chumduri et al., 2021; Lõhmussaar et al., 2021) and heterogeneous tumors (see next paragraph and/or Supplementary Table SIV), in addition to research models for

chlamydia (Bishop et al., 2020) and herpes (Zhu et al., 2017) infections. Remarkably, organoids are able to reproduce specific uterine (Boretto et al., 2019; Bishop et al., 2020; Hernandez-Gordillo et al., 2020; Luddi et al., 2020; Marinić et al., 2020), ovarian (described in detail below) and cervical (Karolina Zuk et al., 2017; Maru et al., 2020; Lõhmussaar et al., 2021) tissue phenotypes, as well as respond to hormones (Bläuer et al., 2005; Boretto et al., 2017; Turco et al., 2017; Wiwatpanit et al., 2020; Cheung et al., 2021). These models can be established from patient biopsies (Maru et al., 2019; Lõhmussaar et al., 2021), biological fluids (Cindrova-Davies et al., 2021) or cell lines (e.g. SKOV3, H08910, OVCAR3/4/8 used in oncological studies listed in Supplementary Table SIV). Further transplantation of spheroids or organoids may restore ovarian function (Kim et al., 2018) or promote endometrial regeneration (Jiang et al., 2021).

Next-generation platforms for oncological studies include the development of 3D ovarian cancer models using scaffolds of bacterial cellulose with chitosan (Ul-Islam et al., 2019), collagen (Zheng et al., 2015), poly-DL-lactide-coglycolide-PEG (Zhou et al., 2018) or RADA16-I peptide hydrogel (Song et al., 2020). Similarly, a novel 3D cervical cancer model was created with 3D-printing, using bioinks mixed with sodium alginate (Gospodinova et al., 2021). Dynamics of cancer progression can be modeled *ex vivo* in 3D (Ajeti et al., 2017; Fleszar et al., 2018; Loessner et al., 2019; Flont et al., 2020; Fan et al., 2021), utilizing multilayered microfluidic systems (Lin et al., 2017; Flont et al., 2020) and ovarian spheroids [to study macromolecular crowding (Bascetin et al., 2021)].

Unique *ex vivo* and *in vitro* proof of concept applications include, DC bovine ovarian and uterine 'tissue papers' (Jakus et al., 2017), an *in vitro* artificial human ovary (Krotz et al., 2010), a pregnant-like cervix (Raia et al., 2020), an endocervical model that responds to hormones during a 28-day cycle (Arslan et al., 2015), automated and reliable oocyte denudation on a chip (Weng et al., 2018) and the EVATAR platform that models the dynamics of the human menstrual cycle (Xiao et al., 2017).

Realistic in vitro *toxicology and drug screening models*

Bioengineered *in vitro* platforms enable evaluation of the biocompatibility of biomaterials (Xu et al., 2016; Scsukova et al., 2020), effects of chemical toxicants [such as doxorubicin (Zhou et al., 2015; Aziz et al., 2020), or dioxin (Park et al., 2020)] or response to cancer therapies (Supplementary Table SIV). For example, 3D tumor models in ring format currently support automated and rapid personalized drug screening (Phan et al., 2019). Other drug screening models include microdissected tumor tissues in microfluidic culture (Astolfi et al., 2016) or alginate hydrogels (Salas et al., 2020), organoids of small cell neuroendocrine carcinoma of the uterine cervix (Tanaka et al., 2021) and ovarian cancer organoids, which have proven to be excellent models to test chemotherapy drugs (Maru et al., 2019; de Witte et al., 2020; Maenhoudt et al., 2020). In fact, since endometrial and ovarian organoids can be derived from each patient's biopsies (Kopper et al., 2019; Nanki et al., 2020; Bi et al., 2021; Chen et al., 2021; Espedal et al., 2021), they reflect specific tumor heterogeneity and are ideal for drug pre-screening and the development of personalized treatment regimens. Notably, ovarian cancer spheroids exhibited increased tumorigenicity and proportion of cancer stem cells after several passages (Ward Rashidi et al., 2019); chemoresistant cancer stem cells can also be generated with 3D culture of $CD44^{+}CD117^{+}$ cells (Chen et al., 2014). Fallopian tube organoids are similarly suitable for developing combination therapies for high-grade serous ovarian cancer (Zhang et al., 2021a) while multicellular spheroids derived from these cancer patients' malignant effusions enable drug screening (Chen et al., 2020).

Further, recent applications of drug-loaded hydrogels (Jamal et al., 2018; Cabral-Romero et al., 2020) and microfluidic conditions (Ran et al., 2019; Saha et al., 2020; Yang et al., 2020) evaluated targeted cytotoxicity. HA-carboxymethyl cellulose scaffolds facilitate the study of ovarian cancer persistence (Picaud et al., 2014), and ovarian constructs enable evaluation of metastatic potential of leukemic cells that could have infiltrated OT (Soares et al., 2015).

New therapeutic biomarkers and clinical strategies

The organs and tissues of the female reproductive system are not only subject to pathologies that affect reproductive capacity, but also to those that can be life threatening, such as cancers. Via recent applications, microfluidic platforms and organoid/spheroid cultures are revealing diagnostic (Wang et al., 2015; Dorayappan et al., 2019; Zhang et al., 2019a; Chung et al., 2021), and prognostic (Chowanadisai et al., 2016; Ward Rashidi et al., 2019; Chung et al., 2021) biomarkers and/or gene signatures, in addition to elucidating drivers of tissue metaplasia (Chumduri et al., 2021). These approaches are helping to establish new and alternative cancer therapies. For example, endometrial organoids allowed the identification of a menin-mixed lineage leukemia inhibitor for endometrial cancer (Chen et al., 2021), while fallopian tube organoids provided a platform to test combination therapies for ovarian cancer (Zhang et al., 2021a). Locally injectable hydrogels, such as those made of PEG and polylactic-co-glycolic acid (Shin and Kwon, 2017), PEG and poly(ε-caprolactone) polymeric micelles (Xu et al., 2018), polypeptide PC10A and silver sulfide quantum dots (Jin et al., 2019) or light-cured glycol chitosan (Hyun et al., 2019), successfully sustained delivery of drugs to ovarian or cervical tumor models, while those made of HA-danazol reduced the size of endometriosis cysts (Nomura et al., 2006). Similarly, 3D cervical models supported testing the efficacy of PEGylated lipoplexes containing silencing RNAs targeting human papillomavirus lesions (Lechanteur et al., 2017), while gold nanorods can facilitate intracellular drug delivery (Yan et al., 2016). Another bioengineering strategy that can improve clinical workflow is the encapsulation of follicles in alginate (with or without Matrigel) before cryopreservation, which not only is more time efficient, but also affords a means of improving follicle survival and development (Camboni et al., 2013; Vanacker et al., 2013).

Tissue and organ regeneration or transplantation

The complete or partial regeneration of damaged tissues and organs is arguably the application for which bioengineering is most recognized. In the reproduction field, many *in vivo* studies have tested hydrogels and scaffolds for uterine regeneration (Supplementary Table SII). Among them, polylactide nanofilm can seal defects smaller than 3 mm in chorion-amnion and uterine membranes (Pensabene et al., 2015), while degradable polylactic acid-*co*-poly(ε-caprolactone)-gelatin nanofiber meshes with endometrial MSCs promote tissue integration via an anti-inflammatory response (Mukherjee et al., 2019). Heparin-poloxamer hydrogels (Xu et al., 2017a,b; Zhang et al., 2017b, 2020b), collagen hydrogels or scaffolds loaded with bone marrow MSCs (Ding et al., 2014), basic fibroblast growth factor [(bFGF; (Li et al., 2011a)], embryonic stem cell-derived endometrium-like cells (Song et al.,

2015), vascular endothelial growth factor [VEGF (Lin *et al.*, 2012)] or human umbilical cord-derived MSCs [UC-MSCs (Xin *et al.*, 2019; Liu *et al.*, 2020)] and stromal cell-derived factor-1α-loaded chitosan-heparin hydrogel (Wenbo *et al.*, 2020) repaired morphology and restored the function of injured rat uteri. Further, improved uterine regeneration, and some restoration of fertility with successful implantations, pregnancies and LBs is achievable via transplantation of DC human amniotic membrane loaded with adipose stem cells (Han *et al.*, 2020) or oral mucosal epithelial cells (Chen *et al.*, 2019), DC uterine matrix (Santoso *et al.*, 2014; Hellström *et al.*, 2016; Hiraoka *et al.*, 2016; Miki *et al.*, 2019; Li *et al.*, 2021), or DC endometrial ECM hydrogel loaded with growth factors (López-Martínez *et al.*, 2021b) (Supplementary Table SII). Similarly, gelatin methacrylated and sodium-alginate scaffolds with bFGF (Cai *et al.*, 2019), MSC-laden Matrigel microspheres (Xu *et al.*, 2021), hydrogel-encapsulated decidualized endometrial stromal cells (Kim *et al.*, 2019), HA hydrogels (Liu *et al.*, 2019), HA-collagen hydrogels with endometrial stem cells, stromal cells and vessel cells (Park *et al.*, 2021), PEG-based hydrogels (Wang *et al.*, 2021) or poly(glycerol sebacate) scaffolds seeded with bone marrow-MSCs (Xiao *et al.*, 2019) also successfully regenerated a damaged endometrium.

One reproductive disorder prompting a search for an effective tissue regeneration treatment is AS, an acquired iatrogenic disorder characterized by adhesions within the uterine cavity or cervix. To date, patients have received treatments using collagen hydrogels loaded with UC-MSCs (Cao *et al.*, 2018; Zhang *et al.*, 2021b), bFGF (Jiang *et al.*, 2019) or bone marrow mononuclear cells (Zhao *et al.*, 2017) to improve uterine response and function. *In vivo* studies in rats demonstrated that UC-MSCs facilitate collagen degradation, regenerate uterine wall thickness and restore fertility (Xu *et al.*, 2017c), while organoids derived from human embryonic stem cells regenerate uteri of AS models (Jiang *et al.*, 2021). Furthermore, HA-based hydrogels (Liu *et al.*, 2019) and cell sheets made of rat endometrial cells (Kuramoto *et al.*, 2018), rat oral mucosa epithelial cells (Kuramoto *et al.*, 2015) or UC-MSCs (Kuramoto *et al.*, 2020) also demonstrated utility in preventing and/or repairing uterine adhesions. Other biomaterials, such as AdSpray [based on dextrin (Kai *et al.*, 2018)], Carbylan-SX (Liu *et al.*, 2007), mitomycin C-loaded crosslinked HA films and gels (Liu *et al.*, 2005), urinary bladder ECM (Zhang *et al.*, 2020a), HA/carboxymethylcellulose membranes (Demirbag *et al.*, 2005) and polylactic acid-pluronic copolymer (Yamaoka *et al.*, 2001), also prevent post-operative adhesions, while hydrogels made of PEG with or without poly(L-phenylalanine) (Wang *et al.*, 2021), aloe poloxamer with estradiol encapsulated in nanoparticulate DC uterus (Yao *et al.*, 2020a) and stromal cell-derived factor-1α-loaded chitosan-heparin (Wenbo *et al.*, 2020) prevent/reduce uterine fibrosis in preclinical models. Notably, commercial hydrogel-based adhesion barriers, such as PEG-based SprayGel [used for myomectomy patients (Mettler *et al.*, 2004, 2008)] and Actamax Adhesion Barrier (Trew *et al.*, 2017), have already proceeded to clinical use.

Research over the last two decades also yielded significant strides in reproductive organ transplantation. Although uterine and ovarian transplantation surgeries often are performed without the aid of bioengineering, recent approaches may provide benefit, particularly for some OT transplantation patients. For example, encapsulating human OT in Alloderm allowed two patients to conceive through ART (Oktay *et al.*, 2016). In mouse models, transplanted HA-encapsulated vitrified ovaries compromises follicles and FSH production (Taheri *et al.*, 2016), but encapsulating fresh ovaries with a HA-based hydrogel (with/without VEGF and bFGF) protects the follicular reserve and re-establishes endocrine function (Tavana *et al.*, 2016a,b). Similarly, co-culture of human bone marrow- or visceral peritoneal-derived MSC hydrogels with mouse OT can restore endocrine function earlier after transplantation, but delays follicle development (Mehdinia *et al.*, 2020). On the other hand, culturing OT fragments with laminin components of the native ovarian ECM enhances follicle survival and development to the secondary follicle stage (Hao *et al.*, 2020), while encapsulating follicles in PEG vinyl-sulfone hydrogels maintains the reserves to day 60 and supports antral development and ovulation (Kim *et al.*, 2016). In corroboration, encapsulating OT in TheraCyte or Dual-PEG capsules (which has a proteolytically degradable PEG vinyl-sulfone core with a non-degradable shell) restores ovarian function and follicle development after allotransplantation, without evoking an immune response (Day *et al.*, 2019). Using hydrogels to sustain local release of bFGF decreases fibrosis in human OT, in addition to improving revascularization and follicle density after xenotransplantation (Tanaka *et al.*, 2018); these findings corroborate prior work using fibrin-bFGF scaffolds, which protect murine follicular reserves and increase revascularization after transplantation (Gao *et al.*, 2013). Similarly, exogenous mouse endothelial cells engineered to constitutively express anti-Müllerian hormone (AMH) (Man *et al.*, 2017), or STEMPRO® adipose-derived MSCs (Manavella *et al.*, 2018; Cacciottola *et al.*, 2021), can preserve primordial follicles by promoting revascularization of OT encapsulated with fibrinogen-thrombin. A recent report describes improved ovarian cortex xenografting outcomes achieved by embedding OT in fibrin clots and treating mice with simvastatin (Magen *et al.*, 2020). Furthermore, encapsulation of OT with an alginate hydrogel results in developmentally competent oocytes and protects against metastatic lesions [at least short term (Rios *et al.*, 2018)].

One goal of reproductive bioengineering is to achieve artificial ovaries for alternative fertility preservation strategies. This goal remains somewhat out of reach, but initial work described the encapsulation of ovarian stromal cells in chitosan-silk hydrogels (Jafari *et al.*, 2021). Further, primordial follicles in murine ovarian fragments encapsulated with fibrin modified with heparin-binding peptide, heparin and VEGF (Shikanov *et al.*, 2011c; Kniazeva *et al.*, 2015) and follicles transplanted in bioprinted scaffolds (Laronda *et al.*, 2017) have also produced pups after natural mating. Fibrinogen and thrombin, which are clotting factors, are similarly used to encapsulate follicles (Chiti *et al.*, 2016, 2017) or OT, with or without addition of stem cells or stromal cells. On the other hand, transplantation of granulosa and theca cell constructs restores hormone function, improving bone and uterine health as well as lowering body fat, compared to pharmacological hormone replacement therapy (Sittadjody *et al.*, 2017).

Hydrogels and scaffolds provide some advantages in models of premature ovarian failure (POF) or premature ovarian insufficiency (POI). For example, human amniotic epithelial cells encapsulated within sodium alginate bioglass protect granulosa cell function and ovarian vascularization in a chemotherapy-induced POF model (Huang *et al.*, 2021). Similarly transplant of human UC-MSCs embedded in Matrigel promotes granulosa cell proliferation and ovarian vascularization (Zhou *et al.*, 2021), and adipose-derived stem cells in a collagen scaffold restore ovarian function in POI models (Su *et al.*, 2016). Notably, local delivery of embryonic stem cell-derived mesenchymal progenitor cells

in a HA gel increases the ovarian reserve, and estrogen and AMH levels, ultimately improving the quality of oocytes and embryos in mice that model POI (Shin et al., 2021).

Bioengineered materials and techniques can also be implemented during reconstructive gynecological surgeries. Recently, vaginal reconstruction was successful in a patient with MRKH syndrome, a rare congenital disorder characterized by abnormal uterine and vaginal development despite normal ovarian function and external genitalia; this approach used a DC porcine small intestine submucosa scaffold (Zhang et al., 2019b). Remarkably, this biomaterial achieves structural and functional vaginas for up to 8 years (Raya-Rivera et al., 2014). Similarly, vaginoplasty with an acellular dermal matrix (called RENOV) is safe and effective, and results in an anatomically correct vagina that provides near-normal sexual function (Zhang et al., 2017c). Neovaginas were also safely constructed using Surgicel (an oxidized cellulose scaffold) for 10 patients (Dadhwal et al., 2010), or bovine-derived dermis scaffold for another patient (Noguchi et al., 2004).

Discussion

Summary of the evidence: where do we stand?

The organs of the female reproductive system—the uterus, ovaries, fallopian tubes, cervix and vagina—work together to provide the hormonal and anatomical support necessary for the generation of offspring. As such, reproductive health is susceptible to a number of negative congenital or acquired factors, restricting fertility and quality of life. These concerns prompt a large field of research into the underlying biology as well as approaches for preventing or treating various pathologies. However, ethical and technical limitations around using and/or transplanting human tissues for research purposes requires that most studies are conducted *in vitro* or *in vivo* using animal models. While valuable, these approaches face inherent limitations in translatability, such as the complexity of recreating the anatomy, physiology and interactions of reproductive organs using classical 2D *in vitro* models, in addition to the differences between species. Thus, bioengineering has become indispensable for creating representative and reliable 3D models (for both *in vitro* and *in vivo* uses) as well as providing alternative applications for regenerative medicine. This review's systematic compilation of the extensive bioengineering advances in the context of the female reproductive system since 2000, provides a global overview of the different techniques, their pre-clinical testing and/or clinical applications and the anticipation of future trends.

Uterus

The uterus, and in particular the endometrium, is fundamental for implantation and maintenance of pregnancy (Governini et al., 2021). As such, much research is devoted to the creation of functional endometrial models and combining endogenous endometrial cell populations in different formats and biomaterials (Table I). Notable among these are paracrine models of epithelial and stromal cell co-culture (Schutte et al., 2015; Park et al., 2021), as well as models of decidualization (Schutte and Taylor, 2012; Gnecco et al., 2019), implantation (Park et al., 2003; Wang et al., 2012, 2013; Buck et al., 2015), vascularization (Pence et al., 2017), ECM interactions (Cook et al., 2017) and uterine contractility (Kim et al., 2020b). In recent years, several groups attempted to recreate the complexity of these models with organoids or assembloids (Boretto et al., 2017; Turco et al., 2017; Murphy et al., 2019; Abbas et al., 2020; Rawlings et al., 2021), which offer an apparently unlimited potential to recreate the physiological and pathological states of the endometrium (Boretto et al., 2019). In fact, organoid technology is marking a turning point in endometrial-related research. Despite having been described only 5 years ago, more than 13% of the uterus-related articles reported in this study exploit this technology. Remarkably, although most biomaterials attempt to mimic ECM interactions *in vitro*, only a few studies notably implement native ECMs (Young and Goloman, 2013; Olalekan et al., 2017; Campo et al., 2019; Arezoo et al., 2021; López-Martínez et al., 2021a; Francés-Herrero et al., 2021b).

Absolute uterine factor infertility can be treated with uterine transplantation (UTx). Taking into account scientific (Brännström et al., 2021) and media reports, as well as personal communications, we currently estimate that more than 40 LBs have been achieved from over 80 UTx procedures that have been performed thus far. The surgical success rate (defined by a viable organ within 3 months, resumption of regular menstruations within a year, successful pregnancy and LB) was 78% and 64% for live and deceased donor UTx procedures, respectively, and the cumulative LB rates in surgically successful UTx procedures were estimated to be above 80%. Despite these promising success rates, this procedure involves an invasive surgery and associated risks. Bioengineering has been used to mitigate these risks by providing alternative clinical applications. Specifically, bioengineering techniques for the uterus focus predominantly on preventing/reducing adhesions, often associated with AS (Zhao et al., 2017; Cao et al., 2018; Zhang et al., 2021b) and related to uterine factor infertility. In these and other cases of endometrial damage, the main therapeutic objectives are to regenerate tissue structure (e.g. recover endometrial thickness, angiogenesis) and consequently restore function, which ultimately allows the uterus to support implantation and carry a pregnancy to term (Hellström et al., 2016; Kuramoto et al., 2018; Li et al., 2019; Liu et al., 2019; Wang et al., 2021). Toward this end, different hydrogels and scaffolds show potential *in vivo*, by regenerating injured uteri in rodent models (Supplementary Table SII). Emerging technologies, such as 3D bioprinting and microfluidics, remain under-utilized in research applied to uterine health, but promising possibilities exist for both *in vitro* modeling (Ahn et al., 2021; De Bem et al., 2021) and *in vivo* tissue regeneration (Ji et al., 2020).

Ovary

The ovaries exert two main functions, namely to tightly regulate folliculogenesis so as to avoid premature depletion of oocytes, and to produce sufficient sex hormones (e.g. estrogen and progesterone) to support decidualization, pregnancy, breast development for lactation and even bone health (Sittadjody et al., 2017). Developing new IVFG platforms opens opportunities for oncological patients who cannot benefit from current fertility preservation strategies (specifically, OT cryopreservation) due to risk of reintroducing malignancy upon autologous re-transplantation. Culturing follicles/OT *in vitro* 'bypasses' this risk and can produce mature oocytes faster than if the OT was xenografted into a murine model [usually in 8–12 days (Supplementary Table SIII) versus weeks-months (Oktay et al., 2016)], but does not have the potential to restore endocrine function. Most IVFG studies

we included in this review successfully cultured secondary follicles to the antral stage, and some even recovered mature and competent oocytes (Supplementary Table SIII). Few groups have ventured into culturing primary follicles because these follicles tend to have lower survival and oocyte maturation rates (Tagler *et al.*, 2012, 2014; Smith *et al.*, 2014).

The success of IVFG is not only affected by initial follicle size, but also by the saturation of the biomaterial. Physiologically, the rigidity of the ovarian cortex and the 'sponginess' of the medulla play important roles in regulating folliculogenesis. In fact, the mechanical forces of the ovarian cortex ECM may maintain reserves of primordial follicles, only releasing a couple of follicles to grow in the medulla every menstrual cycle (Choi *et al.*, 2014). Nonetheless, although softer/more flexible biomaterials, such as alginate, Matrigel and VitroGel, could facilitate follicle expansion, materials that are too soft (i.e. 1 mg/ml collagen, fibrin alone or HA-Matrigel, rapidly degrading YKNR plasmin substrate) cannot provide the necessary 3D support, causing granulosa cells to erroneously proliferate and migrate into their surroundings (Shikanov *et al.*, 2009, 2011b; Desai *et al.*, 2012; Joo *et al.*, 2016). In contrast, saturated/rigid matrices [i.e. 1.5% alginate (West-Farrell *et al.*, 2009)] hinder follicle growth. Although OT transplantation has led to more than 200 human LBs so far (Dolmans *et al.*, 2021), encapsulating OT before transplantation may provide additional benefits by promoting revascularization, decreasing fibrosis, protecting follicles from "burn-out" (ischemia-induced death of follicles during the first couple of days after transplant), and ultimately, providing the best microenvironment for follicle development *in vivo*. However, in attempts to standardize OT transplantation or replacement and be able to offer these strategies to a broad population (e.g. oncological patients and/or those in need of hormone replacement therapy), the construction of an artificial ovary containing immature stimulable follicles is gaining momentum and could lead the way for the next decade. Another common ovarian bioengineering application with great potential is the development of heterogeneous and/or patient-derived organoid models to evaluate individual drug response and cancer dissemination (Supplementary Table SIV).

Fallopian tubes

Fallopian tubes (or oviducts) are the anatomical structures that connect the ovaries and the uterus, providing the space and physiological environment for fertilization and early embryo development. Few bioengineering methods exist to date to recapitulate fallopian tubes and their associated functions *in vitro*, despite their crucial supportive role during early embryo development. Derivation of human fallopian tube organoids from different cell types (Kessler *et al.*, 2015; Lin *et al.*, 2021a) provided an important breakthrough in the creation of functional *in vitro* models. Among the few other fallopian tube studies in the bioengineering field, some demonstrate the important cross-talk between the ovaries and the fallopian tubes (Zhu *et al.*, 2016), or the direct effect of oviductal ECM molecules on embryonic metabolism (Francés-Herrero *et al.*, 2021a). Microfluidic platforms, with their small channels, may be the most suitable for modeling the physiology and pathology (Ferraz *et al.*, 2020) of this tubular organ. Indeed, the implementation of a bovine oviduct-on-a-chip led to improved IVF outcomes (Ferraz *et al.*, 2018).

Cervix and vagina

The cervix and vagina play critical roles in reproduction by serving as an entryway for sperm during ovulation, physical barriers for infectious microorganisms and a pathway during childbirth. Bioengineering these tissues has provided novel multilayered organoid models to study herpes (Zhu *et al.*, 2017) and cervical cancer (Tanaka *et al.*, 2021), also enabling testing of their respective treatments. Although a functional vagina can be created by self-dilation of the vaginal dimple in a majority of patients with MRKH syndrome, vaginal scaffolds are used for reconstructive surgeries (Noguchi *et al.*, 2004; Dadhwal *et al.*, 2010; Zhang *et al.*, 2017c, 2019b; Acién *et al.*, 2019). Other bioengineering alternatives may prevent premature rupture of fetal membranes and incontinence (Roman *et al.*, 2018), or test contractility inhibitors with bioprinted uterine rings (Souza *et al.*, 2017). Moreover, hydrogels can be used as carriers for antibiotics, antivirals, antifungals, contraceptives and other drugs (Dos Santos *et al.*, 2020).

Full tract

Female reproductive function is orchestrated by multiple autocrine, paracrine and endocrine dialogues, which so far have only been studied *in vivo* in model organisms that cannot accurately reproduce the human body. To overcome the limitations of these models, there exists the need to recreate a multiorgan environment that incorporates physical, mechanical and hormonal variables. Microfluidics offers the most promising bioengineering method, having already enabled the development of an organ system-on-a-chip that combines human liver spheroids, mouse ovarian explants, human fallopian tube epithelium, human endometrium and human cervix tissues to physiologically model a 28-day menstrual cycle (Xiao *et al.*, 2017). Recently, the endocrine crosstalk between the uterus and the ovary has been modeled on-a-chip, to be able to evaluate the effects of reproductive toxicants (Park *et al.*, 2020). Another application rarely exploited to date is the possibility of combining, in a single microfluidic platform, a major portion of the workflow in assisted reproduction clinics, thereby minimally altering the environmental conditions to which gametes and embryos are exposed (Han *et al.*, 2010).

Future perspectives

New 3D *in vitro* models representing multiple cell types and/or tissue layers are not only helping to elucidate the physiological dynamics of complex biological processes within the reproductive tract (e.g. those that regulate folliculogenesis, ovulation, decidualization and cancer progression), but also improving personalized medicine (Stejskalová *et al.*, 2021). In particular, organoids generated in 3D culture can adequately mimic healthy and diseased cell-cell and cell-ECM native tissue interactions, making them ideal models for evaluating individual drug response (for cancer, endometriosis, dysmenorrhea, hormone disorders or other related issues, bacterial/viral/fungal infection, etc.) or implantation potential (Wei *et al.*, 2021). However, organoid models, especially endometrial ones, have unresolved issues, which the scientific community has started, and should continue, to investigate. Among others, the main limitations are: the lack of expandable organoid lines with stromal and immunological components; the inaccessibility to the organoid lumen; the lack of interactions with native ECM components; and the variability associated with patient tissue origin and culture handling. Automated 'lab-on-a-chip' technologies that can rapidly screen various

bodily fluids (e.g. blood, ascites or pleural fluid, urine) for specific biomarkers, cancer cells, drugs or oocytes may also efficiently and reliably refine future clinical/therapeutic decisions. Since body-on-a-chip platforms have the potential to model hormone dynamics and systemic disease (e.g. PCOS, diabetes, cancer), combining them with organoid or organ culture and ECM-based environments may provide more robust 3D models for genetic/epigenetic and pharmacokinetics testing.

Much remains to be achieved for the field to create (and eventually offer) a completely artificial female reproductive system. Nevertheless, recent advances in the creation of an artificial ovary (Krotz *et al.*, 2010; Chiti *et al.*, 2016; Sittadjody *et al.*, 2017; Jafari *et al.*, 2021; Yoon *et al.*, 2021; Wu *et al.*, 2022), uterus (Souza *et al.*, 2017; Ji *et al.*, 2020; Li *et al.*, 2021; Park *et al.*, 2021), cervix (Arslan *et al.*, 2015; De Gregorio *et al.*, 2017; Zhao *et al.*, 2020) and vagina (Orabi *et al.*, 2017; Hou *et al.*, 2021) have made promising headway toward this incredible goal. For example, the development of alternative, more natural, options for hormone replacement therapies offers promise for mitigating menopause-associated problems (Sittadjody *et al.*, 2017; Yoon *et al.*, 2021). In the race to manufacture transplantable tissues and organs, 3D bioprinting has played a discreet role so far, accounting for only 3% of the studies included in this review. Specifically, its relative novelty, limited accessibility among research groups worldwide and lack of standardized protocols and technology could be slowing down its take-off, making it an attractive and necessary niche for investment. Studies focused on bioengineering of the fallopian tubes are scarce, since their functions are bypassed in assisted reproduction clinics. However, recent work demonstrates that an artificial oviduct-on-a-chip may substantially improve IVF and early embryo culture systems by providing a more realistic microenvironment (Ferraz *et al.*, 2018). Moreover, these anatomical structures are the target of numerous studies to develop alternative contraceptive methods. Among these, artificial hydrogels based on styrene maleic anhydride (Subramanian *et al.*, 2019) and PEG (McLemore *et al.*, 2005) offer promise as contraceptive approaches through successful testing in the fallopian tubes of rats and rabbits. Finally, we note the need for greater clinical translation in reproductive bioengineering. Despite the large number of proposals described at the preclinical level, only 5% of the studies compiled in this review are clinical. Advances at the legislative level, meta-analyses to establish optimal procedures, and stronger networks of collaboration between laboratories and medical centers, could be of value.

Limitations

This systematic review identified a wealth of bioengineering-related studies in the context of female reproduction. Nonetheless, it is possible that relevant studies were not found or were excluded because of the keyword selection, subjective nature of the filtering process or reference limit. We compiled the 312 articles that we considered the most significant and representative of the current state of the field. There is an additional limitation in terms of classification of the articles by biomaterial, since the literature lacks consensus in delineating certain hydrogels and scaffolds (e.g. collagen was reported as a hydrogel and scaffold), and some studies combined bioengineering techniques (e.g. organoid or culture with hydrogel/scaffold within a microfluidic system). Therefore, we classified articles, on a case-by-case basis, in a way we deemed most appropriate. Since the original Embase search identified numerous oncology-related studies, additional searches with keywords representing reproductive diseases were conducted to ensure appropriate coverage of the latter. Finally, due to different organs under consideration and divergences in study objectives and designs, the included studies exhibit wide heterogeneity that precluded meta-analysis of the results.

Conclusion

Female reproduction is regulated by complex networks of molecular, endocrine and tissue/organ interactions. As such, substituting the entire female reproductive tract will be challenging; however, interdisciplinary work provides novel insight into the physicochemical properties necessary to support and achieve these biological processes. Advances in reproductive bioengineering technologies have redefined the landscape of fertility-restoring strategies and therapeutic options that are, or soon could be, available to patients. These translational endeavors provide substantial promise for effective treatments for a wide range of reproductive system pathologies.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Authors' roles

Conceptualization: I.C., E.F.-H., R.L., M.H., L.d.M.-G., S.H., M.B. and A.P.; systematic literature search, selection and data curation: E.F.-H. and R.L.; data review: I.C., E.F.-H. and R.L.; manuscript and figure preparation: I.C., E.F.-H., R.L. and L.d.M.-G.; manuscript review: I.C., M.H., L.d.M.-G., S.H., M.B. and A.P. All authors have agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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