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REVIEW

Regenerative Medicine Approaches in Bioengineering Female Reproductive Tissues

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Abstract

Diseases, disorders, and dysfunctions of the female reproductive tract tissues can result in either infertility and/or hormonal imbalance. Current treatment options are limited and often do not result in tissue function restoration, requiring alternative therapeutic approaches. Regenerative medicine offers potential new therapies through the bioengineering of female reproductive tissues. This review focuses on some of the current technologies that could address the restoration of functional female reproductive tissues, including the use of stem cells, biomaterial scaffolds, bio-printing, and bio-fabrication of tissues or organoids. The use of these approaches could also be used to address issues in infertility. Strategies such as cell-based hormone replacement therapy could provide a more natural means of restoring normal ovarian physiology. Engineering of reproductive tissues and organs could serve as a powerful tool for correcting developmental anomalies. Organ-on-a-chip technologies could be used to perform drug screening for personalized medicine approaches and scientific investigations of the complex physiological interactions between the female reproductive tissues and other organ systems. While some of these technologies have already been developed, others have not been translated for clinical application. The continuous evolution of biomaterials and techniques, advances in bioprinting, along with emerging ideas for new approaches, shows a promising future for treating female reproductive tract-related disorders and dysfunctions.

Keywords Female infertility · Reproductive tract dysfunction · Regenerative medicine · Stem cells · Bioengineering

Introduction

Amidst the advancements being made in medicine, there is an increasing percentage of patients who live with chronic disorders and dysfunction of various organs and tissues. To date, most research has concentrated on disorders and dysfunctions of vital organs, while less emphasis has been placed on research into dysfunction of the organs in the reproductive system. The female reproductive system is complex, and dysfunction can occur in several organs and tissues, resulting in infertility and/or hormonal imbalance. These physiological dysfunctions impact the psychological and socio-economic status of the affected individuals. Therefore, there is a critical need for developing novel medical strategies that could restore normal physiological function and improve the quality of life of affected women.

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Normal Function and Dysfunction of the Female Reproductive Tract

The female reproductive system is comprised of the ovaries, fallopian tubes, uterus, vagina, and external genitalia (**Fig. 1**), most of them are differentiated from the mesoderm-originated Müllerian duct [2]. Ovaries are known to produce (a) oocytes that fertilize during reproduction and (b) hormones that govern the various physiological processes in the body, including reproductive maturity. A long-held dogma in the ovarian biology of mammals, postulated in the early 1950s, states that in mammalian species, females are born with a defined number of eggs in their ovaries that developed during embryogenesis referred as "egg reserve" [3–7]. Egg reserve is the alternative name used for the primordial follicles that contains a meiotically arrested primary oocyte at the center and surrounded by one layer of cuboidal cells, which later (during the reproductive phase) differentiate into functional granulosa cells. During the reproductive phase, until the preformed egg reserves are depleted, a few of the primordial follicles from the definitive stock start to mature at the beginning of every ovarian cycle. These

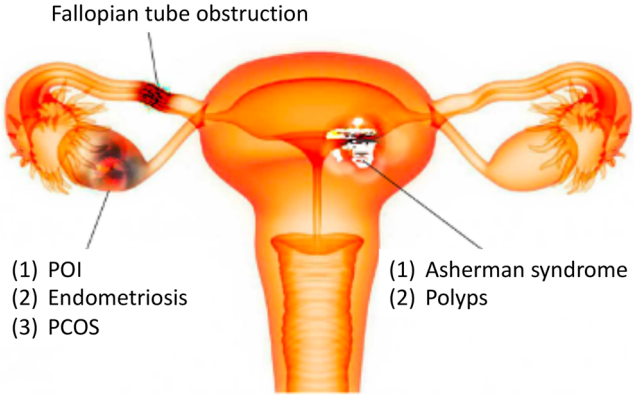

Fig. 1 The female reproductive tract includes the ovaries, fallopian tubes, uterus, and vagina. Some of the common disorders and dysfunctions of female reproductive organs are listed. Adopted from Zhao *et al*., 2019 [1]

primordial follicles develop into ovarian follicles by differentiating the cuboidal cells into granulosa cells and recruit theca cells from the stroma of the ovary. The ovarian follicles form the functional units of the organ and produce the sex steroids estradiol (E_2) and progesterone (P_4) in order to prepare the uterus for implantation of a fertilized egg. The innermost layer of the uterus, the endometrium, thickens in response to E_2 during the first half of the reproductive cycle prior to ovulation. Post-ovulation, the P_4 produced by the corpus luteum, the transformed follicular structure after ovulation, maintains the endometrium in a secretory phase. Ovarian sex steroids E_2 and P_4 play a major in other physiological roles such as mammary tissue development, bone health, and sexual functions in the female [8–10].

Menopause refers to the progressive decline of ovarian function including the production of sex hormones, primarily E_2, in women aged 45–60, resulting in cessation of menstruation [11, 12]. In addition to natural menopause due to age, younger women can also undergo induced menopause due to diseases resulting in premature ovarian insufficiency (POI) or surgically induced as a part of cancer treatments. POI is a condition that occurs with women before the age of 40, and in this condition, the ovaries do not produce normal levels of hormones (E_2) or release eggs, which leads to infertility [13]. Ovarian cancer (OC) is the 5th leading cause of cancer-related death among women [14, 15]. The American College of Obstetricians and Gynecologists suggests treating the high-risk population, such as a family history of ovarian cancer, with prophylactic chemotherapy or bilateral oophorectomy. If the ovaries are retained, many of the chemotherapy treatments are gonadotoxic [16], leading to an increasing prevalence of ovarian damage, with manifestations ranging from subfertility to premature ovarian insufficiency. Women of reproductive age that have undergone oophorectomy are at high risk of developing cardiovascular disease and osteoporosis due to premature menopause [17]. In addition to cancer therapies, other causative factors for female reproductive dysfunction include ovarian insufficiency (natural or treatment-induced), polycystic ovarian syndrome (PCOS), endometriosis, fallopian tube occlusion, Asherman syndrome (AS), and other less frequent anomalies listed in **Table 1** and **Fig. 1** [1, 18, 19], such as the use of therapeutic alkylating agents, metabolic and autoimmune disorders, viral infections, and genetic alterations [12, 20–24].

Asherman's Syndrome (AS), caused by endometriosis or repeated and aggressive curettages to remove uterine tissue, often leads to a blocked uterine cavity and/or destruction of the endometrial lining. In AS, intrauterine adhesions obliterate the uterine cavity and result in the absence of functional endometrial lining of the uterus. Infertility, resulting from dysfunctional uterine tissue, presents clinically as a recurring loss of pregnancies or hypo-menorrhea/amenorrhea [25].

Table 1 Common disorders of the female reproductive tract, their definitions, and etiologies

Disorders and dysfunctions	Definition	Etiologies
Premature ovarian insufficiency (POI)	Cessation of ovary function during the reproductive active age	Genetic disorders, autoimmune diseases, radiation, chemotherapy and infections
Polycystic ovarian syndrome (PCOS)	A multi-factorial disorder associated with infertility, hirsutism, obesity and various menstrual disturbances	Maternal PCOS, intrauterine hyperandrogenism, inflammatory adipokines, aboriginal origin, diet
Resistant ovary syndrome (ROS)	Ovarian cells resistant to gonadotrophins (FSH and LH) presenting symptoms of POI	FSH receptor block in ovarian cells, antibodies to FSH and LH, post-receptor signaling defect.
Uterine fibroids	Benign tumors that spontaneously appear in the uterus	Triggered by high levels of estrogen
Endometriosis	Presentation of endometrial tissue growth and inflammation outside the uterus	Oxidative stress, reactive oxygen species, inflammatory genetics and epigenetic factors
Fallopian tubal occlusion	Occlusion of fallopian tube due to inflammation in the fallopian tube or pelvic peritoneum	Neoplasms, inflammations, tubo-ovarian abscess
Asherman Syndrome	Absence of a normal opening of the lumen in the female genital tract from fallopian tubes to the vaginal canal	Trauma, infection, low levels of E2, repeated or aggressive curettage, severe endometritis

Fallopian tubes that connect the ovary to the uterine cavity play a pivotal role in fertilization and transfer of the embryo to the implantation site in the uterus [26]. Fallopian tube occlusion due to neoplasms, inflammations, and tubo-ovarian abscess is the common defect that has been diagnosed in the clinics. The vagina or vaginal canal inferior to the uterus, where sperm is deposited during coitus, also undergoes cyclical changes in response to ovarian physiology changes [27, 28]. The vaginal defects and disorders include infection, the Mayer Rokitansky Kuster Hauser (MRKH) Syndrome, vaginal fistula, vaginal prolapse, and various trauma/surgical scars [1, 18, 19].

Current Therapies and Limitations

The current options to treat infertility include (a) hormone-stimulated superovulation for diminishing egg reserves; (b) intra-uterine insemination (IUI) and in vitro fertilization (IVF) to improve the chance of fertilization; (c) intracytoplasmic sperm injection (ICSI) for defects in sperm motility; (d) donor eggs where depleted egg reserves are diagnosed; and (e) uterine surrogacy for women with uterine defects [29]. Some of the common factors contributing to the depletion of the egg reserve include genetic syndromes, iatrogenic factors such as chemo-, radiation- or immunosuppressive therapies [30]. Hormone replacement therapy is an option for restoring E_2 and/or P_4 in post-menopausal women. However, long-term HRT is controversial due to potential increased heart disease risks, stroke, and cancer [31, 32]. Despite current evidence suggesting that HRT may not be as risky as previously reported [33, 34], physicians and patients are hesitant to use HRT to treat menopausal symptoms. Moreover, recent data suggest that there is only a short period for treatment in which HRT is effective [35, 36]. Additional therapies for treating disorders of the female reproductive system include surgical corrections and tissue transplantation [37, 38]. An allogeneic uterus transplantation study published by Brannstrom et al. [39] reported a successful live birth. However, these approaches are not appropriate for every patient and can lead to various health issues. For example, the transplantation of donor tissues faces graft rejection by the host and requires life-long immunosuppressive treatment. In the case of uterine defects or deformities, the use of a surrogate mother can present moral and ethical issues for the biological parents and the surrogate.

Potential of Regenerative Medicine Approaches

Regenerative medicine offers a viable alternative for treating disorders that are currently lacking effective therapies and may have severe side effects. The bioengineering of female reproductive tissues, using various regenerative medicine and tissue engineering approaches, has the potential to revolutionize the way female reproductive dysfunction is treated. These technologies could be used for (a) therapeutic purposes, to repair/regenerate/replace dysfunctional tissue, (b) diagnostic purposes, and (c) investigative purposes to understand the underlying mechanisms of female reproductive physiology. The bioengineering strategies can be classified into two broad categories: (a) The transplantation of fresh or cryopreserved organs and (b) tissue engineering approaches that utilize a combination of cells, growth factors, and biomaterials that leverage the body's inherent ability to regenerate/repair reproductive organs. While whole organ transplantation has demonstrated some success [39], the source of the organ and the immunogenic effects of allografts remain challenging. Tissue engineering strategies through regenerative medicine largely avoid these issues.

The field of regenerative medicine encompasses various technologies, including cell therapies, tissue engineering, and cloning, as illustrated in **Fig. 2**. Tissue engineering utilizes several of these technologies, including cells (**Table 2**), biomaterials, and bioactive factors (**Table 3**) and bioengineering techniques (**Table 4**) to generate new tissue or organs. Each of these approaches could offer novel treatments for these disorders. With proper characterization and thorough evaluation, the bioengineered tissues have promising potential to improve the quality of life of patients with reproductive tract defects. This field is rapidly growing and evolving [119].

Bioengineered Tissues of the Female Reproductive Tract

Several approaches have been developed to bioengineer tissues of the female reproductive system. The female reproductive tract organs have different cellular, ECM and structural compositions from one another. For example, the ovary is a solid tissue, whereas other organs such as the uterus, fallopian tube, and vagina are hollow structures. Therefore, unique approaches are needed to bioengineer the different tissues of the female reproductive tract.

Ovary

The ovary's fundamental role is to produce oocytes and secrete hormones from ovarian follicles/corpus luteum during the ovarian cycle. Ovarian dysfunction can result in infertility, hormonal imbalance, cardiovascular disorders, neurological disorders, vasomotor symptoms, genital atrophy, and bone loss. In an attempt to restore ovarian function, various bioengineering strategies have been developed. These strategies are still at the experimental and preclinical stage and include (a)

Table 2 Cells used in bioengineering tissues

Cell type	Description
Embryonic stem cells (ESCs)	Pluripotent stem cells (PSCs) obtained from pre- and post-implantation embryos. The inner cell mass (ICM) of blastocyst and epiblasts of post-implanted embryo isolated and used as ESCs. ESCs exhibit various properties that other types of stem cells lack including immune evasion and the potential for differentiating into any desired cell type [40–51].
Induced pluripotent stem cells (iPSCs)	Derived by reprogramming adult somatic cells, usually fibroblasts, into PSCs by transient overexpression of a defined set of transcription factors (OCT4, SOX2, cMYC, and KLF4) to de-differentiate the cells [52–57]
PSCs through somatic cell nuclear transfer (SCNT)	To a denucleated oocyte, nucleus of somatic cell is replaced, followed by parthenogenetic activation. The early embryonic stage cells obtained as ESCs carries the patient's own genetic material [58].
Amniotic fluid stem cells (AFSCs) and Placental stem cells (PlSCs)	PSCs isolated from amniotic fluid and placenta demonstrate the pluripotent potential of the ESCs without the ethical constraints. These cells are currently being tested in experimental or pre-clinical stages [59]
Adult stem cells (ASCs)	Certain cells in adults possess stem cells properties such as mesenchymal stem cells (MSCs) that resides in various tissues [60–68]

the transplantation of partial or whole ovaries that had been cryopreserved before cancer treatments as a part of oncofertility, (b) the generation of ovarian tissue from stem cells, and (c) the use of tissue engineering methods to reconstruct ovarian tissue.

Among the two major functions of the ovary, identifying novel methods for restoring egg production has a predominant need. Oogenesis, or egg production, begins during embryonic development, where cells from the yolk sac migrate to the genital ridge and contribute to the development of the ovaries [120]. This process starts as primordial germ cells (PGCs) differentiate into oogonia. Meiosis is stimulated by retinoic acid, at which point the oogonia enter meiosis and become primary oocytes, which are arrested at the prophase phase-I [121, 122]. Meiosis resumes in the follicular phase of the ovarian cycle during adult life [120]. Until recently, it had been thought that initiation of oogenesis is completed during embryological development and that all of the available primary oocytes are present at birth, leaving no oocyte stem cells in the adult ovary [61]. Therefore, research has primarily focused on developing methods to mature existing primary oocytes into fertilizable secondary oocytes utilizing in vitro techniques and/or in vivo methods. One common method used is cryopreservation of the whole ovary or cortical strips, which are later used for in vitro methods of differentiation or through in vivo transplantation of the cryopreserved ovarian tissue to resume oogenesis. Other methods include developing technologies to promote the maturation of pre-existing follicles by using 3D cell culture methods. Alternatively, recent research has suggested that oocyte stem cells exist in the adult ovary and that these cells can regenerate the oocyte pool during adult life. Therefore, a new research area has focused on developing methods to obtain stem cells from the ovary and mature them into secondary oocytes which are competent for fertilization.

Whole Ovary or Cortical Strip Cryopreservation and Transplantation

Protocols have been developed for cryopreserving ovarian tissue (whole, partial, or as cortical strips) obtained from donors as a measure to preserve fertility in individuals who are undergoing gonadotoxic treatments such as radiation or chemotherapy. This branch of research is termed "oncofertility" [123, 124]. Ovarian tissue cryopreservation (OTC) has also been adopted for patients with benign conditions such as ovarian torsion, ovarian cysts, endocrine disorders, and autoimmune diseases [38, 125]. The main objective of OTC is to maintain the structural and functional integrity of the ovary, which could be later transplanted back into the female to restore ovarian function. This is the only fertility preservation method available for prepubertal patients since there are no protocols available for ovarian stimulation and oocyte collection for young patients [125, 126]. Autologous transplantation is part of fertility preservation for those who are undergoing gonadotoxic treatments. This approach involves transplantation of cryopreserved tissue (whole or partial) back into the patient, either in an orthotopic or heterotopic fashion. Autologous transplantation of ovarian tissue following cryopreservation has a reported success rate of 63.9% in restoring ovarian activity and a natural live birth success rate of about 57.5% [118].

Cryopreservation was initially used for female reproductive tissues in the 1980s when embryos were frozen with cryoprotectant dimethyl-sulfoxide (DMSO) [127]. Later improvements in cryopreservation were made by introducing 1,2 propanediol and sucrose as cryoprotectants [128] and developing a method for gradual cooling of the tissue to –30 °C prior to long-term storage in liquid nitrogen [129]. The cryopreservation of oocytes employs various approaches to preserve the functional integrity of the eggs during long-term storage. Among these

Table 3 Biomaterials used in bioengineering

Biomaterials	Desirable qualities and limitations
I. Natural materials	
Collagen (COL)	Biocompatibility, biodegradability, abundancy, less antigenic, resistance to distension, ability to integrate with surrounding tissues, high degree of flexibility and tensile strength. Body temperature-activated crosslinking of COL for polymerization makes it challenging in certain applications [69]
Silk	Cytocompatibility, biomaterial with toughness and ductility, suitable for fabricating various scaffold forms including nets, electrospun fibers, films, porous sponges, particles and even hydrogels [70–73]
Alginate	Cytocompatibility, biodegradability, adjustable porosity, mechanical strength, able to be functionalized using desired biomolecules. Suitable for applications such as drug delivery, wound healing, cell encapsulation and tissue engineering [74–76]
Fibrin	Biocompatibility, biodegradability, and high affinity for various biological surfaces; naturally contains cell binding sites in its molecule and thus favors cell seeding in bioengineering processes; can be fabricated into nanofibers and non-woven mesh by electrospinning methods, which could serve as a predefined scaffold for tissue engineering [77–79]
Hyaluronic acid (HA) or hyaluronan	Ability to interact with native extracellular molecules, regulation of ECM fluid, maintenance of structural integrity, and the viscoelastic nature of cartilages. HA can be used as an injectable hydrogel delivery medium or can be electrospun into fibrous scaffolds for tissue engineering purposes [80–82]
II. Synthetic materials	
Poly (a-esters): polylactide (PLA), polyglycolide (PGA), poly-lactic-co-glycolic acid (PLGA) and poly-e-caprolactone (PCL)	Biocompatible, biodegradability, thermoplastic nature of these polymers allows them to form 3D scaffolds with desirable qualities for various applications such as molding, extruding, solvent casting, electrospinning, gas foaming and phase separation techniques. Limitations include poor surface hydrophilicity, possible induction of proinflammatory reactions post implantation, and no cell-adhesion site or sequence on their surfaces [79, 83–86]
Polypropylene (PP)	High and well-controlled porosity, nontoxicity and chemical inertness, its hydrophobic surface limits its use in the field of tissue engineering, non-degradable polymers composed of hydrogen and carbon [87]
polyethylene terephthalate (PET)	Bocompatible polymers have desirable mechanical properties and are cost-effective in fabrication and therefore serves as a popular material for commercial use. The major drawback of these polymers is their surface inertness, which requires surface functionalization approaches [88–93]
III. Decellularized tissues	Extracellular matrix (ECM) components preserved include collagen, elastin, laminin, fibronectin, glycosaminoglycans (GAGs) and several other ECM molecules depending on the source of the tissue and the decellularization technique used. decellularized scaffolds serve as repository for various growth factors that direct the differentiation of the stem cells towards functional cells of the tissue. Any leftover native allogenic or xenogeneic cellular material might raise immune reaction in the recipients [94, 95]

methods, slow-freezing and vitrification are the most commonly used. Slow-freezing, also known as equilibrium freezing, is carried out in a fashion that gradually replaces the fluid between the intracellular and extracellular compartment without causing any osmotic shock or deformation to cells [130]. The advantage of this technique is the use of a low concentration of cryoprotectant. However, this approach is time-consuming and requires an expensive programmable freezing machine [131–134]. Vitrification was developed by Rall and Fahy in 1985 [135] as an alternate approach. Vitrification is a non-equilibrium freezing method, where ice crystal formation is completely eliminated through the use of a high cooling rate along with higher concentrations of cryoprotectant [136]. Vitrification has been widely used to cryopreserve retrieved human oocytes [137–141] and in vitro matured oocytes [142, 143].

Autologous transplantation of cryopreserved whole ovary or partial ovarian tissues into rabbits [144, 145], sheep [146–148], and monkeys [149] were reported to show ovarian function such as follicular development, ovulation, and

Table 4 Approaches used in female reproductive tissue bioengineering

Approaches	Description
Cell Therapy	Delivery of autologous or allogenic stem cells either by injecting directly to the affected site or by mixing the cells with a biomaterial prior to administration in order to replace dysfunctional cell population with healthy and functional cells and restore the function. Delivery material could be hydrogel supplemented with bioactive biomaterials or decellularized scaffolds. As an alternate to the scaffold-based delivery of cells, the cell-sheet engineering technology, where the therapeutic cells are cultured and grown into a 3D sheet, the seeded stem cells would synthesize their own ECM [96–105]
3D bioprinting	Cells mixed with hydrogel "bio-ink" and printed in combination with synthetic biomaterials to obtain 3D structures of native tissue [106, 107]
Organoids	3D spheroid-shaped cell aggregates measuring a size of 100-250 μm that function similarly to the native tissue. Organoids presents cell-cell interactions of native tissues and hence, retain functional and morphological features of in vivo tissues and organs. Organoids serve as tool for bioengineering, investigating physiological mechanisms, diagnostic purposes and therapeutic approaches [108–112]
Encapsulation	Tissues or cells encased in hydrogel biomaterials and immune-isolate transplanted tissues/cells from the host, while providing 3D support and allow exchange of materials. Encapsulation utilizes a variety of materials to create the hydrogel including alginate, agarose, collagen, HA and poly-ethylene-glycol (PEG) [113–117]
Tissue transplantation	Even though transplantation *per* se does not qualify as a tissue engineering, it is often used as the method of tissue restoration after cryopreservation. Autologous: For those who are undergoing gonadotoxic treatments, the reproductive tissue such as ovaries are cryopreserved (whole or partial) and transplanted back into the patient in orthotopic or heterotopic sites after the treatment. Allogeneic: Transplantation of reproductive tissues such as uterus from donors supplemented with immune suppression [118].

resumption of estrus cycle along with the restoration of hormonal balance. Kawamura et al. [150] reported a promising technique for autologous transplantation of ovary tissue that recapitulates fertility in a mouse model, where follicular growth was induced by disrupting the Hippo signaling by fragmenting ovaries followed by stimulating with Akt stimulators, which produced retrievable and fertilizable mature oocytes. This technique was termed as in vitro activation (IVA) of cryopreserved ovarian tissue. Using this technique, a 30-year-old Japanese woman was treated for infertility and successfully gave birth to a healthy baby [151]. Since, more than 130 live births have been reported worldwide using OTC techniques [152]. However, when it comes to heterologous (allogeneic and xenogeneic) transplantation, the grafted tissue requires protection from the host immune system via immune suppression.

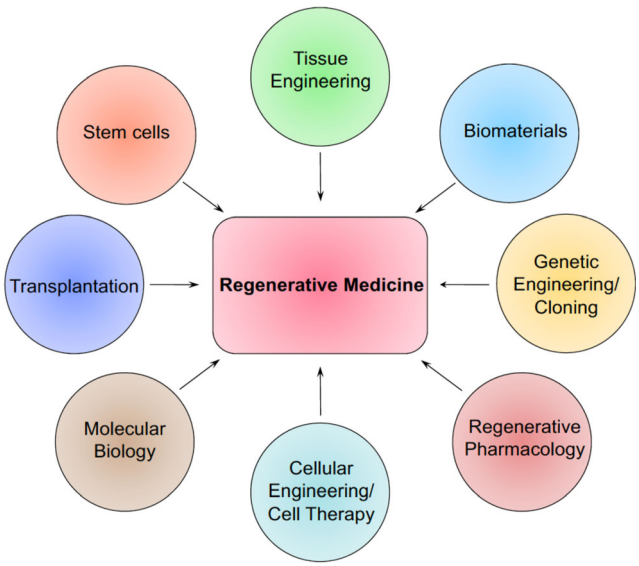

Fig. 2 Schematic representation of the various components of regenerative medicine. Adopted from Yalcinkaya *et al.*, 2014 [24], with permission

In Vitro Maturation of Oocytes

In vitro maturation (IVM) refers to techniques developed to mature (a) pre-existing primary oocytes in the center of primordial or primary follicles or (b) neo-oogenesis, which is a technique to differentiate mature oocytes from ovarian stem/progenitor cells such as germline stem cells (GSCs) and/or oogonia stem cells (OSCs). Long-standing dogma supports the idea that women lack functional adult GSCs to replenish the pool of eggs in adult life [153, 154]. However, several groups have recently reported the presence of GSCs in adult ovaries [61, 155–158]. Oocytes derived from adult GSCs, such as OSCs or stem cells obtained through iPSC technology and somatic cell nuclear transfer (SCNT) technol ogy, may serve as an alternate for cases where there is a premature depletion of egg reserve. Despite the growing body of evidence supporting the presence of adult GSCs, the concept is still actively debated [159–164]. For adult GSCs to be universally accepted, further studies will be needed to characterize various functional aspects of oocytes derived from adult GSCs, such as oocyte commitment, meiotic progression, maturation, fertilization competency, and development competency [165].

Embryonic Stem Cells and Oogenesis

During embryonic development, the zygote develops into a blastocyst containing an inner cell mass (ICM) of pluripotent cells. At a later stage, the ICM gives rise to 2 cell types: (a) epiblasts that eventually develop into the embryo and (b) hypoblasts that develop into the yolk sac. The germline cells originate from the hypoblast-derived yolk sac and not from the epiblast-derived embryo. Since all the embroyo cells, except the germline cells, are derived from epiblasts, epiblasts are considered to be embryonic stem cells with pluripotent capability. Methods have been developed to obtain pluripotent stem cells from the mouse embryo (mouse embryonic stem cells—mESCs). Later those methods were adopted to derive human embryonic stem cells (hESCs) as well. Recently, a new type of pluripotent stem cell (PSC) has been isolated from the epiblasts of post-implanted mouse embryos (mouse epiblast-derived stem cells—mEpiSCs) [166, 167]. These cells are remarkably similar to the ESCs derived from the pre-implanted blastocyst inner cell mass (ICM) [168].

There are several striking similarities and differences among the stem cells derived from the different stage embryos. Unlike mEpiSCs, the mESCs have been shown to give rise to chimera offspring, suggesting a germline transmission potential. This feature of ICM-derived mESCs, the ability to yield germ cells, is because the mouse ICM includes cells which eventually develop into the hypoblast and serve as the precursor for germ cell lineage. Even though the mEpiSCs and mESCs exhibit similar expression patterns to some degree and share key pluripotency factors, they were reported to be derived from different embryonic stages, which are primed with other upstream signaling modules. Since hESCs share the same characteristics as mEpiSCs, which belong to a later-stage (primed and committed) embryo, these cells together are termed as "primed" PSCs, whereas the ICM-derived mESCs from the earlier stage embryo, with a potential for germline transmission, has been termed "naïve" PSCs [29]. Thus, only naïve PSCs would be appropriate for use in the tissue engineering of ovaries. Gafni et al. [169] developed culture conditions to derive naïve hESCs from blastocysts, which have germline potential. Following this path, many additional investigators have started procuring naïve hESCs [54, 170, 171] in an attempt to derive oocytes.

The development of gametes in humans can be traced to the end of the third week of gestation, where the PGCs are formed in the yolk sac wall. In response to the signals of bone morphogenetic proteins (BMPs 2, 4, and 8) from the neighboring cells, the precursors of PGC start the expression of markers such as *PRDM1* (or *BLMP1*), *PRDM14*, and *IFITM3*, thereby acquiring the PGC phenotype. These PGCs then migrate and colonize the gonadal tissue around the fifth week of gestation. After migration, the PGCs still express some of the key markers of egg-progenitor cells such as *TNAP*, *OCT3/4* (*POU5F1*), *cKIT*, and *SSEA 1* and *3* [172–176]. Expression of these markers along with some additional markers such as *DDX4* (*VASA*), *NANOG*, and *SOX4*, has been used in the field of stem cells to identify and isolate the potential precursor cells for oocytes. Around the fifth week of gestation, reactivation of the X chromosome occurs in the female germ cells [177]. The PGCs further undergo what is referred to as global epigenetic reprogramming by chromosomal remodeling, genetic imprint erasure, and extensive DNA methylation [178]. Finally, the PGCs again enter a global-remethylation phase, resulting in a partially methylated oocyte genome [179]. Understanding the molecular programming of germ cells (oocytes) has led to research to derive oocytes from various PSCs. In vitro, PGCs were derived from mESCs [42, 44, 45, 180] and iPSCs [181]. Differentiation of PSCs into oocyte-like cells were reported by several groups in monolayer culture [182, 183], in a co-culture system with fetal gonads [184], or in 3D cultures such as embryoid body culture [185] based on the expression of early germ cell markers such as *VASA*, *DAZL*, *IFITM1*, *PRDM1A*, *GCNF*, *GDF3*, and *cKIT* and later stage markers such as *SCP3*, *MLH1*, *DMC1*, *GDF9*, and *ZP4* [186, 187]. Mouse EpiSCs were shown to lose their competency towards PGCs [188], which led to developing a novel approach to derive PGCs from EpiSCs. In this approach, the naïve ESCs obtained from the epiblasts of pre-implantation embryos were cultured in the presence of Activin A to derive intermediate epiblast-like cells. The activin A-exposed cells resembled embryonic cells that could give rise to PGCs in vivo and yielded functional oocytes [189].

Even though several groups have demonstrated that ESCs from the mouse can form follicle-like structures generating oocyte-like cells, none were able to progress forward to meiosis [42, 190]. Hayashi et al. [191] aggregated mEpiSC-derived PGCs with somatic cells from embryonic ovaries and then transplanted the cells under the ovarian bursa. This method of in vitro derivation of PGCs from ESCs, combined with in vivo oogenesis, yielded fertilizable oocytes. When the embryos from fertilized oocytes were transplanted into surrogate mothers, they developed into viable offspring, which showed that in vivo oogenesis from ESC-derived PGCs has a better outcome than the complete in vitro oogenesis process. The complete in vitro oogenesis produced fragile and abnormal-shaped oocytes, and follicles that lacked the supporting granulosa cells. In addition, the fertilization of such oocytes presented abnormal pronuclei, which could explain the observed low rate of post-implantation development. These defects were suspected to be the result of faulty epigenetic reprogramming that occurred during the in vitro oogenesis from PGCs [192]. On the other hand, oogenesis research using hESCs has shown only limited progress. The oocyte-like cells derived from hESCs using mouse fibroblast as feeder layer produced oocyte-like cells and expressed (a) markers related

to meiosis such as *SYCP3* and *MLH1*, (b) germ cell-related genes such as *DDX4* (*VASA*), and (c) pluripotency-related genes such as *POU5F1* (*OCT4*), *IFITM3*, and *NANOG* [193, 194]. An improved method of using ovarian fibroblasts as feeder layer for hESCs also generated large cells with oocyte markers including *VASA, DAZL, GDF3, GDF9, MLH1, SCP1*, and *POU5F1* [195]. Other than generating oocyte-like cells, there has been no success in generating human oocytes in vitro that could be applied for any assisted reproductive technologies (ARTs) utilizing these types of stem cells. Therefore, the technology of obtaining oocytes from pluripotent stem cells needs further improvement.

Adult Stem Cells (OSC) and Oogenesis

Long-standing dogma states that all the oogonia in a developing ovary either differentiate into primary oocytes that become a part of dormant primordial follicle or undergo degeneration resulting in an adult ovary that lacks any renewable source of germ cells. However, this belief was challenged by a recent study that showed adult ovaries carry mitotically active germ cells referred to as OSCs that are capable of producing new oocytes throughout adult life [61]. White et al. [196] reported that similar to adult mice, women of reproductive age contain mitotically active germ cells such as OSCs. When these putative germ cells were aggregated with dispersed adult ovarian tissue in vitro, they formed follicle-like structures and produced oocyte-like cells. Virant-Klun et al. [197, 198] obtained the OSCs from the ovarian surface epithelium and demonstrated the production of oocyte-like cells that expressed molecular markers such as *OCT4A, OCT4B, C-KIT, VASA, STELLA, and ZP2*. While some researchers do not agree with the theory of the existence of OSCs in adult ovaries, others try to offer alternate views for oocyte-like cell-producing stem cells. These theories state that OSCs could be the de-differentiated cells capable of developing into germ cells under appropriate conditions [199] or that the OSCs could be small embryonic-like stem cells that reside in the ovaries [200]. Conversely, using an endogenous genetic approach, another group demonstrated that the germ cells present in adult mice were neither capable of dividing by mitosis nor could contribute to de novo oogenesis [201]. The existing controversy and disagreement of the presence of OSCs require more studies before the technology reaches clinical applications.

Addressing Hormone Imbalance

Besides oocyte production, the ovaries secrete sex steroids such as E_2 and P_4, which play a role in reproduction and participate in various physiological processes. Follicles are the functional unit of the ovary and are responsible for producing and releasing sex steroids. An ovarian follicle encompasses an oocyte in the center surrounded by several layers of granulosa cells (GCs), which in turn is enclosed by a layer of theca cells on the periphery of the follicle (**Fig. 3a**). The regulation of the endocrine function in ovarian follicles has been explained by the two-hormone two-cell theory, where LH and FSH, from the anterior pituitary gland, stimulate theca cells and granulosa cells to produce E_2, respectively. LH, through the LH receptor, stimulates the expression of *StAR*, *HSD3B1, CYP11A1*, and *CYP17A1* (the key steroidogenic enzymes) in theca cells (TCs), which aids in the uptake of cholesterol and converts it into pregnenolone then into progesterone, which then is converted into androgens such as androstenedione or testosterone. The androgens that are produced by TCs are then converted into estrogen by the action of aromatase (*CYP19A1*) that is present in the granulosa cells (GCs). *CYP19A1* expression in GC is under the control of FSH; thereby, the two endocrine cells of the ovarian follicle operate in a tandem fashion [202]. The LH surge during ovulation induce the expression of *StAR* and *3β-HSD* in granulosa and the loss of CYP17A1 (in TCs) and CYP19A1 (in GCs). Thus, the follicle losses the ability to produce androgen and estrogen after ovulation and acquire progesterone producing capacity referred as luteinization, and the whole structure of the follicle is termed corpus luteum [120]. Therefore, the steroidogenesis predominantly produces estrogen during the follicular phase of the ovarian cycle, which then switches to progesterone production during the luteal phase of the ovarian cycle as a consequence of luteinization caused by the LH surge. The switching of E_2 secretion to P_4 secretion is unidirectional; the cyclicity of sex steroid secretion observed in physiology is due to the emergence of the next set of follicles from the alternate ovary.

The cyclicity of the ovarian cycle alternates the development of the endometrial lining and function for implantation purposes. However, the protective role of ovarian sex steroids on other organ systems is contributed by lower steroid hormone levels than what is required for reproductive function. The combination of E_2 and P_4 inhibits the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and LH from the pituitary. Similarly, inhibin, a protein hormone secreted by ovarian follicles, inhibits the secretion of the FSH-releasing pattern of GnRH through the Kissipeptin neurons and thereby FSH secretion from the pituitary. Therefore, the hypothalamus-pituitary-ovarian (HPO) axis and its negative feedback loops keep tight control on the ovarian function.

The inevitable phenomenon of ovarian failure, whether by natural processes such as age or disease or by treatment-induced dysfunction, leads to an imbalance of hormones, thereby causing various pathophysiology. The current treatment to correct the hormonal imbalance is to administer exogenous hormones, referred to as hormone replacement therapy

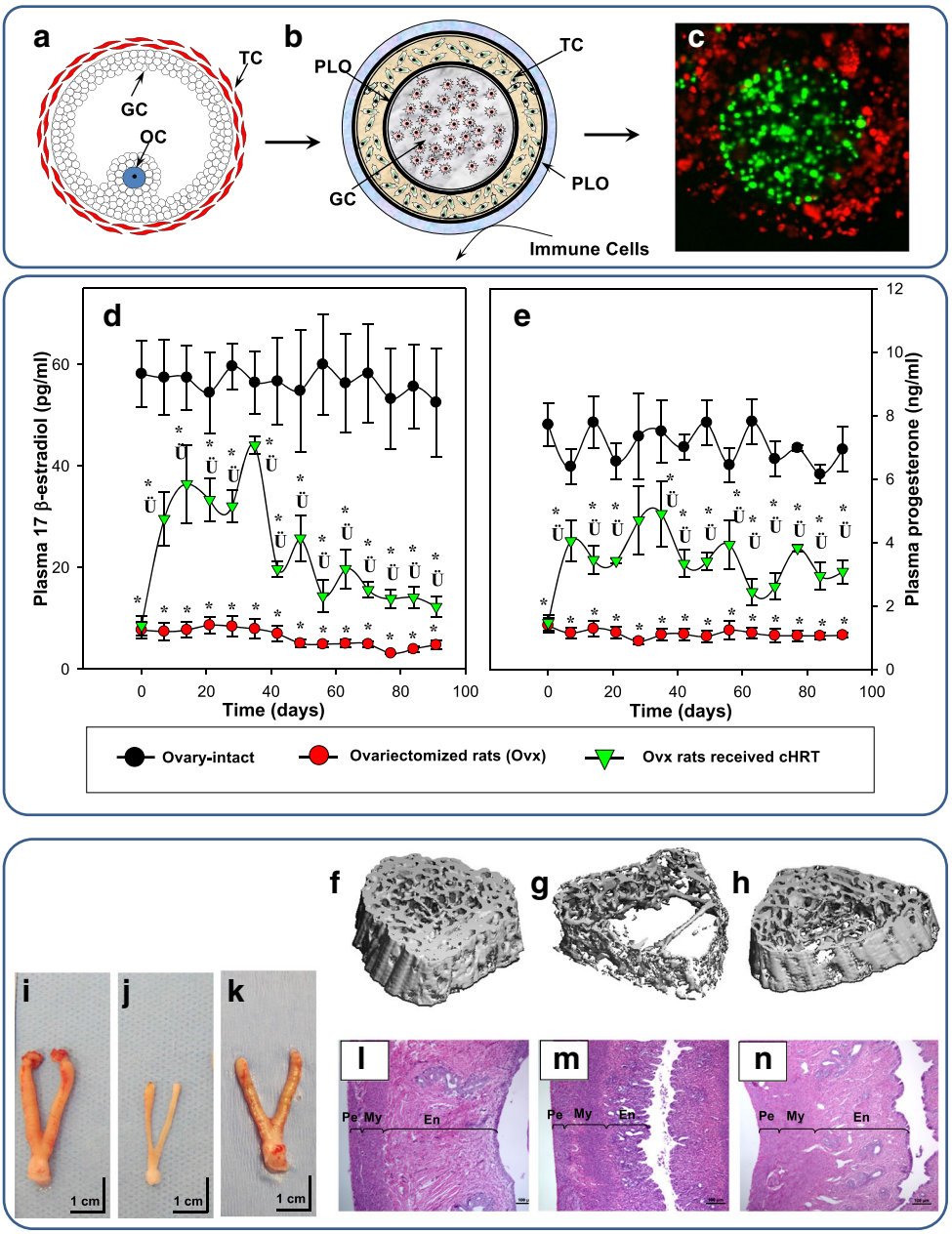

Fig. 3 Cell-based hormone therapy to correct abnormalities caused by dysfunctional ovaries. **A.** The basic architecture of ovarian follicle with OC in the center surrounded by GCs and TCs; **B.** bio-engineering design to recapitulate the structural architecture of follicles; **C.** Bio-engineered endocrine tissue of ovary with GCs (pre-stained with cell-tracker green) in the middle and TCs (pre-stained with cell-tracker red) on the periphery of the constructs; Endocrine functions of these bioengineered endocrine tissue secreted E_2 and P_4 into the plasma of ovx rats for 90 days as evident from the sustained levels of these steroids (**D** and **E**). The μCT images of femur bone shows the correction of ovarian failure-associated trabecular bone abnormalities of with the re-established hormonal level (**F-H**). The safety nature of cHRT has been demonstrated by assessing the morphometry and histology of sex steroid-sensitive uterine tissue, where it showed no signs of any hypertrophy or hyperplasia of uterine tissue (**I-N**). *GC* granulosa cell, *OC* oocyte, *Ovx* ovariectomy, *PLO* poly-L-ornithine, *TC* theca cell. Adopted from Sittadjody *et al.*, 2017 [117], with permission

(HRT), which has been reported to cause side effects ranging from cardiovascular issues to cancers. As an alternative, hormone delivery from ovarian tissue has been studied in an experimental setting. Transplanting the whole ovary in rodents was reported as early as the 1960s, where Krohn studied the young ovary's ability to restore the ovarian functions in old ovariectomized mice [203]. Later, others performed similar experiments to demonstrate the potential of ovarian transplantation to restore the hormonal imbalance in aged rodents [204–206]. Despite the evidence supporting the correction of hormonal imbalance in rodents, the practical applicability of ovary transplantation in humans is challenging. Autologous transplantation of cryopreserved ovarian tissues in heterotrophic sites was also reported to restore the endocrine function of the ovary in humans [207, 208]. Several groups have shown the restoration of ovarian endocrine function following ovary transplantation [209–215]. Notably, it was reported that the endocrine function continued for several years following transplantation of ovarian tissue [216]. While autologous transplantation may not be possible in most cases where the native ovary of the affected person is dysfunctional or diseased, the option of heterologous transplantation requires life-long immunosuppressive treatment, which is known to damage ovarian function. Regenerative medicine offers an alternative solution for such conditions. The endocrine

unit of the ovary is bio-engineered and immune-isolated by microencapsulation techniques or engineered from the patient's cells, which does not require immunosuppression.

Several groups, including ours, have developed a bio-engineered ovarian tissue to provide a more natural method of hormone production in ovarian dysfunction models [115–117, 217]. The advantage of the cell-based HRT (cHRT) over the pharmacological HRT (pHRT) is that the bio-engineered endocrine tissue has the ability to restore the HPO axis in rat models as they were shown to reduce the ovariectomy-induced increase in circulating gonadotropins (FSH and LH) [116, 117]. In addition, the bio-engineered tissue constructs can be transplanted at different orthotopic sites, including subcutaneous tissue where there is vasculature to support the survival of the grafted constructs and allow for the circulation of secreted hormones.

Osteoporosis is known to be induced by a decline in circulating sex steroids and the elevation in circulating FSH levels caused by the disrupted negative feedback loop [218]. A cHRT approach has been developed by our group through delivering endocrine cells of ovarian follicles using alginate microcapsules into an ovariectomized rodent model. The endocrine cells of ovary, namely, GC and TC, when bio-fabricated into a structure that closely mimicked the native follicular structure, demonstrated a long-term secretion of hormones and re-established the disrupted HPO axis in the ovariectomized model. This cHRT model on one hand demonstrated the protective effect of hormones on the bone, and it also showed the safe-nature of the approach as evident from the absence of any utero-trophic effects from the transplanted bio-engineered endocrine tissue (**Fig. 3**)[117, 217]. Hence, the regulation of HPO axis and its feedback loop through bio-engineered endocrine tissue provide a more physiological and natural way of correcting hormonal imbalance.

The major challenge in such cHRT approach is the limited life span of transplanted endocrine cells and the lack of a source to replenish these cells once they decline in their function. Therefore, this approach requires periodical implants. In a rat model, the bio-engineered endocrine tissue was shown to secrete hormones for up to 90 days. When this is extrapolated to the human life span, it would be reasonable to have new implants of bio-engineered ovarian tissue once or twice a year to correct the hormonal imbalance. However, it might be somewhat precocious to comment on the clinical applications of cHRT as it still requires thorough investigations on various aspects of this application, including the side effects, the fate of the implanted cells, and the immune reaction before use in the clinic.

Uterus

The uterus is the largest organ in the female reproductive tract. It is composed of a three-layered wall containing the perimetrium on the periphery, the myometrium in the middle, and the endometrium towards the lumen of the uterine cavity. The endometrium, consisting of the luminal and glandular epithelium surrounded by stroma, undergoes approximately 400 cycles of regulated cell proliferation, differentiation, angiogenesis, tissue breakdown, and shedding during the menstrual cycles throughout the reproductive life of women [219]. Additionally, the uterine tissue is also known to undergo cellular differentiation, hypertrophy, and hyperplasia associated with an increase in uterine weight up to 4–5 times that of its original weight during pregnancy and undergoes remodeling once again to return to its original condition soon after parturition [220]. Based on the physiological phenomena of uterine tissue, it is evident that this organ (particularly endometrium) has a high regenerative capacity and is believed to harbor stem cells that are responsible for this extraordinary regenerative property. These actively dividing cells and the distribution of their surrounding niche are believed to be associated with endometrial diseases such as endometriosis, endometrial cancer, and inappropriate tissue remodeling resulting in a dysfunctional uterus that affects embryonic implantation and fetal development [221].

In a dysfunctional uterus, gestational surrogacy is the only available option, which comes with its downsides, such as personal, religious, ethical, and legal factors that make this decision difficult [222–226]. Another potential option is an allogeneic uterine transplant. Brannstrom et al. reported a live birth after the whole uterus transplantation [39]. However, the shortage of donors and/or complications associated with long-term usage of immune suppression drugs makes this approach challenging [227–231]. Hence, the use of bioengineered uterine tissue (whole organ or partial tissue) could be a novel option for these patients. Bioengineering uterine tissue could be achieved by (a) cell therapy, where only stem cells are used to correct the defective tissue; (b) non-cell laden scaffold, where the scaffold provides the framework for the native stem cells of the uterine tissue to populate and repair the damaged area; and (c) the use of a cell-laden scaffold to engineer a partial or whole new organ.

The innermost lining of the uterus is known for its unique regenerative capacity during the reproductive cycle. This regenerative property of the endometrium has been attributed to a small population of adult stem cells (ASCs) in the tissue [232–235]. The ASCs present in the uterine endometrium possess the properties of MSCs, and they are known for the regeneration of the tissue both in vitro and in vivo [234, 236–240]. It is believed that the MSCs from bone marrow and circulating blood migrate to the endometrium. In addition, there prevails a niche near the perivascular region of the endometrium that is similar to that of ESCs. Therefore, any stem cell that reaches this region or exists in that niche has the potential to give rise to multiple cell types of uterine tissue. Thus, the ESC niche at the basal layer of endometrium near

the perivascular region and the migration of MSCs from bone marrow were reported to augment endometrial regeneration [241]. BMSCs have been reported as a viable source for non-hematopoietic stem cells to repair and regenerate different cellular compartments including luminal epithelium, glandular epithelium and stroma. Among the different cellular compartments, the BMSCs contribute mainly to the regeneration of the stromal compartment and, to a lesser extent, to both glandular and luminal epithelia [62–65]. Even though BMSCs have been shown to improve endometrium regeneration in mouse models [242–245], they failed to deliver positive outcomes in either a non-human primate model or in human studies [246]. Alternatively, stem cells native to the endometrial lining were reported to correct the defects in a pre-clinical model [247]. There is currently no effective stem cell therapy for endometrial pathologies; however, approaches using the stem cells native to endometrium hold great opportunity in regenerate uterine tissue.

Synthetic polymer scaffolds created by various techniques, including electrospinning or decellularized uterus, have been used to provide a 3D network for the native stem cells to populate and regenerate tissue [248, 249]. When stromal cells of endometrium origin were obtained from human samples and embedded in COL-1-enriched Matrigel followed by seeding with endometrial epithelial cells, a 3D structure was formed that resembled the endometrium of the uterus [250]. Schutte et al. [251] reported a similar 3D bioengineered cell construct. In their study, immortalized human endometrial stromal cells were embedded in COL-Matrigel and then layered with epithelial endometrium cells to study cell-cell interactions in the uterus [251, 252]. MacKintosh et al. [253] used electrospun PGA scaffolds seeded with primary bovine endometrial cells. They reported that the structural integrity of the bioengineered tissue was not ideal for in vivo use because of the choice of the material used. Park et al. [254] used primary endometrial cells in a hydrogel mixed with COL-1 to study the migration of uterine cells. A study by Wang et al. used primary human cells from the endometrium embedded in a human plasma-derived fibrin-agarose matrix to investigate the implantation of trophoblast spheroids, which demonstrated the formation of glandular structures of the uterus [255]. In addition to the endometrium of the uterus, myometrium was included in a few studies using human cells. Decellularized myometrium was used to incorporate neo-myometrium [256] or a smooth muscle layer to derive myometrium [257] to recapitulate the structural architecture of the uterus.

Sections of uterine tissue have been bioengineered for therapeutic purposes and tested in animal models. Synthetic materials such as polyetherurethane (PU/PLLA) and polytetrafluoroethylene (PTFE) were also tested to bioengineer partial uterine walls [227]. In another approach, Campbell et al. used the peritoneal cavity as a bioreactor to bioengineer hollow organs with smooth muscle-layered walls such as the uterus. They have implanted boiled blood clots in the shape of a tube and allowed myofibroblasts to infiltrate and populate the tubular structure transforming it into a functional muscular tube [258]. COL scaffolds in different forms have been used to bioengineer uterine tissue. Hu et al. showed successful restoration of uterine function in several studies [259–262]. Li et al. functionalized a collagen scaffold by incorporating bFGF to restore a functional uterus in a rat model [260].

Even though the use of natural materials such as COL has several advantages, including biocompatibility, they mostly failed to mimic the complexity of the native matrix. The use of decellularized ECM scaffolds offers the full structural complexity of the uterine tissue. It might serve as a better biomaterial framework to recapitulate uterine tissue that structurally and functionally resembles the native tissue. Santos et al. investigated three decellularization methods to bioengineer segments of rat uterine horn in vitro [263], which resulted in better structural integrity compared with studies using natural materials such as SIS as reported by Taveau et al. [264]. Full-length uterine tube reconstruction has also been attempted in a few studies using decellularized scaffolds. Miyazaki et al. [265] used decellularized uterine tissue to reconstruct the tissue by recellularization in vitro. They investigated its function in vivo by excision/replacement method, which yielded a 75% success rate for pregnancy. Similarly, Hellstrom et al. [266, 267] developed a decellularization process to obtain scaffolds that were completely devoid of MHC complexes on which GFP-incorporated BM-MSCs were injected to regenerate uterine tissue. The bioengineered uterus, when transplanted, supported full-term pregnancy in rats [266]. Previous work on regeneration of the uterus has proved successful only for small defects in rodent models, as they possess an inherent regenerative capacity. Furthermore, rodent models do not have the critical size needed for clinical translation. Attempts at regenerating bigger uterine defects in larger animal models, such as rabbits, have failed. Taveau et al. used porcine small intestine submucosa (SIS) to reconstruct the uterine wall in rabbits; however, it was noted that grafts 1 cm or longer collapsed, resulting in total architectural disruption [264].

Our group recently demonstrated that a PGA scaffold seeded with autologous cells, when transplanted into the rabbit uterus with a critical size defect in an excision/replacement method successfully regenerated, achieved normal tissue development and supported embryo implantation and fetal growth to full-term pregnancy and birth [248]. Animals implanted with the scaffold alone did not show normal uterine development. The study showed that both biodegradable scaffolds and autologous uterine cells are essential for the achievement of normal tissue. The bioengineering of functional uterine tissue for clinical applications is still in development.

Fallopian tube

The occlusion of the fallopian tube can occur due to inflammation, damage caused by disease, or trauma caused by an ectopic pregnancy. 3D models have been developed for investigating various physiological processes of the tissue. In one such study, ASCs of the fallopian tube or differentiated iPSCs were used to recreate the complex architecture of the fallopian tube in a 3D organoid model. In this model, hollow spheres of cells were bioengineered to mimic the structural architecture of the fallopian tube. The artificial model included stem cells, ciliated cells, and secretory cells of the native tissue. These artificial fallopian tube tissues have been reported to respond to hormones and nutrients [268]. These 3D fallopian tube organoids could be used for diagnostic and investigative purposes.

Cervical and vaginal tissue

In a normal pregnancy, maintenance of the anatomical architecture of the cervix is crucial during the development of the fetus and the growth of the uterus [22]. Cervical aplasia is one condition for which regenerative medicine approaches are being developed. Alborzi et al. [269] developed an approach that used a peritoneal graft to treat cervical aplasia. Initially, a plastic stent covered with a peritoneal graft was placed between the uterine cavity and upper vaginal region. Using this approach, at least four patients were successfully treated for cervical aplasia. Similarly, using an amniotic membrane and a decellularized porcine SIS have also been reported for such cervical reconstructions [270, 271]. House et al. bioengineered a cervix-like tissue construct for investigative purposes using cervical cells from two premenopausal women undergoing hysterectomy. In this bioengineered model, the cervical cells proliferated and produced ECM with a similar biochemical profile to native tissue [272]. Several other studies used bioengineered constructs in rabbit, rat, and mouse models [66–68, 269–286].

One in 5000 women suffers from a congenital disorder of the vagina called vaginal agenesis. The vagina does not develop properly and results in either an incomplete or partial development of the uterus [284–286]. The condition is also referred to as Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. Some of the standard corrective procedures for vaginal defects include surgery and/or transplantation of synthetic or biological meshes, which eventually leads to fibrosis, poor vascularization, or graft rejection [287].

Despite the multiple uses of stem cells in other tissues of the female tract, stem cells utilized in the repair of vaginal tissue have been poorly investigated. A few studies have been reported to use stem cells in vaginal regeneration. Muscle-derived stem cells (MDSCs), when seeded on SIS scaffolds and transplanted into the vaginal stump region, have been shown to reduce fibrosis and enhance epithelial lining formation [278]. Similarly, MSCs have shown to aid in the recovery of urinary continence when administered after vaginal distension in rats [288]. They have been shown to increase the number of stem cells at the injury site [289]. However, no promising human studies have been reported to date. In one study, bone marrow-derived MSCs were first induced to express the phenotype of vaginal epithelial cells, combined with SIS, and then transplanted in place of the native vagina and demonstrated the incorporation of the neovagina [66].

Additional pre-clinical approaches have also been reported using biomaterials for vaginal reconstruction, such as dermal matrix [280–283, 285, 286], human amniotic membrane [274], and decellularized SIS [66, 270, 275–279]. Several cell sources have been explored in the bioengineering vaginal tissue. Biomaterials have been also used with cells for the engineering of vaginal tissue. Our groups showed that vaginal epithelial and smooth muscle cells obtained from rabbits could be expanded and seeded on PGA scaffolds. When the bioengineered scaffolds were subcutaneously implanted in nude mice, they showed assembly of multilayered tissue architecture with functional contractility in response to electrical stimulus [273]. Using a similar strategy, engineered vaginal tissue was implanted to achieve partial vaginal replacement in a rabbit model [273], and then a full replacement of the vaginal organ, also in a rabbit model [290, 291]. These studies eventually led to a human clinical experience in female patients with the MRKH Syndrome who had vaginal aplasia. A small biopsy of vaginal tissue was obtained from the rudimentary structure, the patient's cells were isolated, expanded, and seeded onto biodegradable scaffolds, and then used to create a neovaginal organ [66]. The study was published with up to a 5-year follow-up. The engineered vaginal organs showed a trilayered arrangement of the epithelium, matrix, and smooth muscle similar to native tissue. In addition to structural restoration, functional restoration was also confirmed in women transplanted with these bioengineered vaginal tissues [67]. This technology is currently being developed for further clinical use.

Organ-On-Chip technologies

Organ-on-chip (OOC) is a microfluidics-based technology that serves as a novel 3D cell culture device to investigate the interactions between tissues of various organ systems in an in vitro setup. This organoid-based research is currently being utilized as an investigative tool to better understand the physiological and pharmacological interactions of tissues and organs [292]. This recent-developed technology has been applied in various organ systems, including cancer models, also used in female reproductive tissue research. The OOC technology is ideal for studying tissue interactions and could

serve as a great tool to design customized drug screening for a particular patient since it uses functional unit organoids of each tissue instead of the whole tissue. The OOC platform evolved along with the advancement in microfluidics. Several groups have recently reported the development of 3D micro-engineered devices to recapitulate the complexity of the female reproductive system. The studies using OOC of the female reproductive system include the modeling of the human placenta [293, 294], uterus [295], and developing a model of menstrual cycle physiology on a chip [296].

Blundell et al. [294] have developed the "placenta-on-a-chip" model where primary human placental villous endothelial cells (HPVECs) obtained from term placenta and BeWo b30 human trophoblast cell lines were used. The HPVECs were made to attach on the lower side of a porous membrane in a hanging down fashion in the bottom chamber of a two-layered microfluidic device, and in the top chamber, the BeWo b30 cells were enclosed. The two cell types in this chip device formed a placental-barrier model to study the function of the placenta and interaction of fetal-maternal cells. Other similar models were utilized to study the complex physiology of the placenta in a miniature OOC model where human trophoblasts (JEG-3) and human umbilical vein endothelial cells (HUVECs) [293]. Li et al. [295] developed the "uterus-on-a-chip" to replicate the functions of the uterus and to study the IVF-embryo transplantation (IVF-ET) process. The uterus-on-a-chip device was composed of two PDMS layers separated by a porous polycarbonate (PC) membrane to support the endometrial cell culture. The device's top layer was designed with a zigzag-shaped channel containing a series of interlaced micro-sieves to capture the oocytes. The bottom chamber included four parallel perfusion channels resembling the microcapillary bed to support the porous membrane. The study offers a viable alternative for conventional IVF-ET methods and demonstrates the beneficial aspects of co-culturing the embryo with endometrial cells and facilitate embryonic development. Although the device was developed to study the uterine function in a mouse model, it has great potential in applying for human studies. Woodruff and colleagues developed an organ-on-a-chip system to investigate the functional complexity of the 28-day menstrual cycle of humans, which was tested with mouse cells [296]. Organoids of various female reproductive tissues were first created and interconnected in a micro-engineered device. When the interconnected organoids were exposed to hormones, mimicking the menstrual cycle, the organoids functioned similarly to in vivo. Hence this organ-on-a-chip that recapitulates the interactive functions of different tissues of the female reproductive system provides another avenue to investigating diseases of female reproductive tract such as cervical cancer and infertility.

Conclusion and Future Prospective

Infertility and hormonal deprivation are the two significant outcomes of the female reproductive system's dysfunctional organs and tissues. While infertility affects a notable percentage of women in their reproductive age, hormone deprivation is inevitable to all women as they lose their ovarian functions in one way or other due to natural and unnatural causes. Regenerative medicine offers a viable alternative with biological and physiological outcomes compared to the current treatment options. Many regenerative technologies have been developed to bioengineer female reproductive tissues by taking the complexity of the organs and tissues into careful consideration. Bioengineering reproductive tissues provide therapeutic solutions to treat the various dysfunctions and add diagnostic modalities, including drug screening and investigative values, such as research in understanding the pathophysiology of the organ systems.

Simple approaches such as stem cell-based technologies have been shown to rectify a number of dysfunctions, repair the deformities, or regenerate and restore the tissue function. Researchers have developed regenerative medicine technologies to offer solutions for the two critical dysfunctions of ovaries, non-oogenetic infertility, and hormonal imbalance. Even though certain stem cells, including the postnatal oogonia stem cells, have been debatable, the concept of using adult OSCs opens new avenues in treating infertility for women with premature ovarian failure in their reproductive age. In vitro oogenesis from stem cells or from pre-existing immature oocytes has been improved and is believed to be ready for clinical application in the near future. Similarly, to correct hormonal imbalances due to ovarian failure, approaches such cell-based hormone replacement therapy would offer a natural remedy. This emerging technology with a thorough investigation would benefit women in the near future.

Although various bioengineering technologies have been developed to address clinical problems, the practical applicability in the clinic is of prime importance. In that aspect, among the strategies to address the disorders of female reproductive tissues including infertility, the use of autologous adult stem cells that are native to the tissue such as ovarian stem cells holds several merits over others. For example, in vitro oogenesis from adult ovarian stem cells seems to be a better and safer options compared to others. Existence of PGCs or OSCs in adult ovary that are gradually accepted with some skepticisms has provided a potential and viable option to treat infertility caused by ovarian dysfunction. Cells native to the tissue are more physiologically compatible and less deviated from the original cells. Since the in vitro oogenesis is completely an ex vivo process, it provides more control in monitoring and regulating the maturation process. The oocytes thus generated will be utilized for immediate or future use in ART such as IVF. Unlike in transplanting cryopreserved- or bioengineered-ovarian tissue into the recipient to resume reproductive function, the threat

of reintroducing any potential carcinogenic cell is minimized in this approach. In addition, several clinical steps that are routinely employed in ART including ovarian stimulation, egg retrieval and preparation of eggs for IVF could be bypassed in this approach as the end product itself is a fertilizable egg. Apart from deriving oocytes from ovarian stem cells, these adult ovarian stem cells have the potentials to differentiate into other supporting cells of ovarian follicles and therefore could be utilized for cell-based hormone therapies to rectify hormonal imbalance caused by ovarian failure.

When it comes to strategies to rectify any structure deformities, regenerative medicine provides options through either autologous stem cell therapy or corrective procedures using bioengineered tissues to reconstruct the female reproductive tract tissues. Although various scaffolds have produced some desirable outcomes, bioengineering tissues by combining stem cells with natural or synthetic biomaterials have successfully repaired tissue abnormalities and restored physiological functions. Many advancing technologies, such as bio-printing of structurally complex reproductive tissues, have been actively investigated to expedite the translation into the clinic. The emerging technology of organ-on-chip with bioengineered tissues would be a powerful tool to device customized medicine, drug screening, and investigate the complex interaction between various tissues in an *in vitro* setup. Despite the advanced technologies developed in regenerative medicine, many scientific, technological, and regulatory challenges must be identified and addressed to deliver safe and effective therapies to patients.

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