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Revolutionizing Female Reproductive Health: Cutting-Edge Regenerative Medicine Approaches

Swati Priya[1] and Gautam Kumar[2]

[1]Assistant Professor, Parul Institute of Applied Science, Parul University, Post Limda, Waghodia, Gujarat

[2]International Center for Genetic Engineering and Biotechnology, New Delhi, India

Abstract:

Disorders affecting female reproductive tissues often result in infertility or hormonal imbalances, with current treatment options often falling short in restoring tissue function. In search of alternative therapies, regenerative medicine has emerged as a promising solution, leveraging bioengineering techniques to repair and regenerate these tissues. This review concentrates on cutting-edge technologies stem cells, biomaterial scaffolds, bio-printing, and tissue/organoid bio-fabrication to potentially restore functional female reproductive tissues and combat infertility. The review also illustrates recent advancements in bioengineering approaches, with a particular focus on ovarian models. Innovative strategies like cell-based hormone replacement therapy offer a more natural method for restoring normal ovarian function, while the bioengineering of reproductive tissues holds promise for correcting developmental anomalies. While some of these technologies have already reached clinical application, others are still in the pipeline, reflecting the dynamic progress in biomaterials, bioprinting, and novel techniques. The ongoing development of these approaches, coupled with emerging ideas, points to a hopeful future in the treatment of disorders and dysfunctions in the female reproductive system.

Keywords: Female infertility, Reproductive tract dysfunction, Regenerative medicine, Stem cells, Bioengineering.

Introduction:

Despite ongoing medical advancements, a growing number of individuals face chronic disorders affecting various organs. However, research has predominantly focused on dysfunctions of vital organs, often overshadowing the study of reproductive system disorders. To date, most research has concentrated on vital organ dysfunctions, with comparatively less emphasis on understanding disorders within the reproductive system. The intricate nature of the female reproductive system amplifies the potential for dysfunction across multiple organs and tissues, manifesting as infertility or hormonal imbalances, areas that necessitates increased investigative focus within the medical field. These physiological dysfunctions significantly influence the mental well-being and socio-economic standing of those affected. Therefore, there is an urgent need to develop new medical strategies that can restore normal

Corresponding Author : Swati Priya
E-mail : swathi.bio@gmail.com

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physiological function and enhance the quality of life for affected women. Traditionally, hormonal and surgical treatments have been the primary approaches for managing reproductive system disorders. These interventions aim to manage conditions affecting fertility, hormonal imbalances, and various reproductive dysfunctions. In this era, regenerative medicine has become a promising concept, paving the way for innovative therapies across all fields of medicine, including reproductive health (Fuchs *et al.*, 2001).

Normal Function and Dysfunction of the Female Reproductive Tract :

A properly developed and functional reproductive tract is crucial for natural offspring production. The female reproductive system includes the ovaries, fallopian tubes, uterus, vagina, and external genitalia (Fig. 1), with most of these structures arising from the mesoderm-derived Mullerian duct (Ye *et al.*, 2011). The ovaries are dual-functioning organs, responsible for producing oocytes essential for reproduction and secreting hormones that regulate various physiological processes, particularly reproductive maturation. Early theories in ovarian biology, dating back to the 1950s, suggested that female mammals are endowed with a finite number of eggs during embryonic development, referred to as the "egg reserve" or primordial follicles (Borum *et al.,* 1961; Faddy *et al.,* 1976; Green *et al.,* 1951; Green *et al.,* 1954; Pan *et al.,* 2019). These follicles house meiotically arrested primary oocytes encircled by cuboidal cells, which transform into functional granulosa cells during the reproductive phase. 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 primordial follicles develop into ovarian follicles by differentiating the cuboidal cells into granulosa cells and recruit theca cells from the stroma of the ovary. Ovarian follicles act as the pivotal units in the ovary, generating the sex hormones estradiol (E2) and progesterone (P4) essential for preparing the uterus for potential implantation post-fertilization. Throughout the first half of the reproductive cycle, the endometrium, the uterus's innermost layer, undergoes thickening in response to E2, gearing up for potential implantation before ovulation. Following ovulation, the corpus luteum, the transformed structure from the follicle post-ovulation, produces P4, sustaining the endometrium in a secretory phase, conducive to potential embryo implantation. Beyond their roles in the reproductive cycle, ovarian sex steroids E2 and P4 exert significant influence on diverse physiological functions, including mammary tissue development, bone health, and various aspects of female sexual functions (Messinis *et.al.,* 2014; Mihm *et.al.,* 2011 ; Baerwald *et.al.,* 2012). The internal female reproductive tract consists of the ovaries, fallopian tubes, uterus and vagina. The figure also outlines some common disorders and dysfunctions affecting these organs (fig. 1) adapted from Zhao *et al.,* 2019.

Menopause is characterized by a gradual decline in ovarian function and a substantial reduction in sex

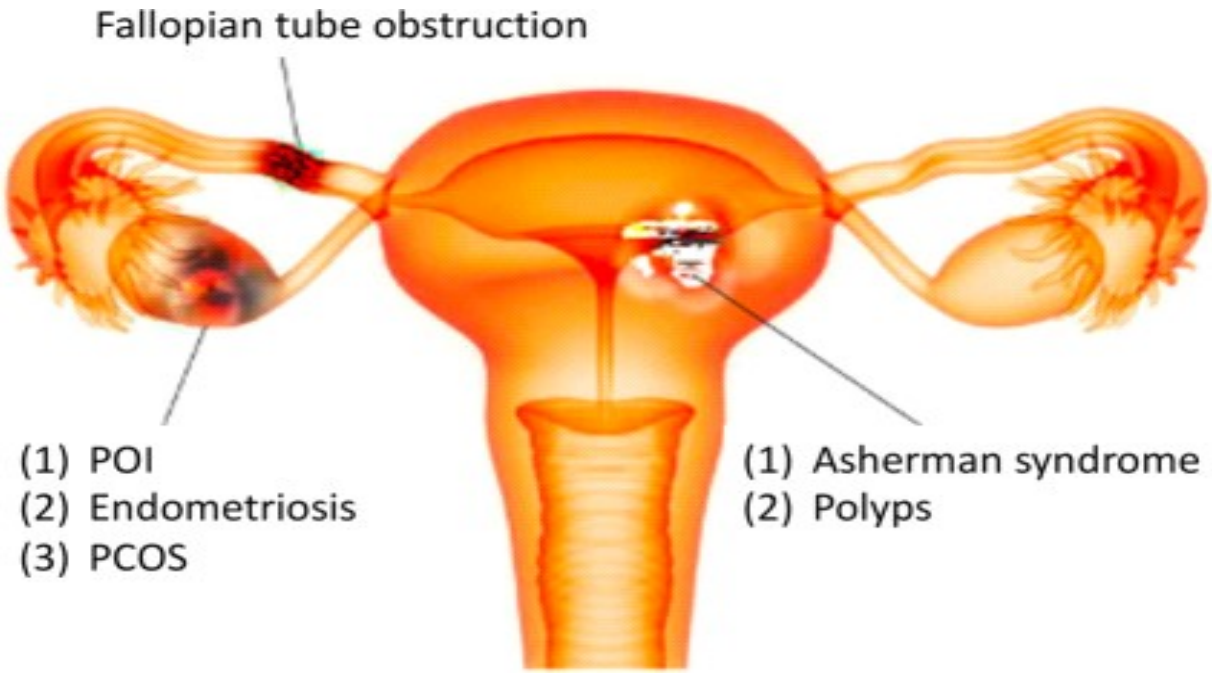

Fig: 1 disorder in internal female reproductive system

hormone production, especially estrogen (E2). This typically occurs in women aged 45–60 and leads to the cessation of menstruation (Hewlett *et al.,* 2015; Qin *et al.,* 2015;Suzuki *et al.,* 2015).

In addition to natural menopause associated with aging, younger women may experience induced menopause due to conditions such as premature ovarian insufficiency (POI) or as a result of surgical interventions for cancer treatment. POI, which affects women under the age of 40, occurs when the ovaries fail to produce normal hormone levels (E2) or release eggs, leading to infertility (Goswami *et al.,* 2005). Ovarian cancer (OC) is the 5th leading cause of cancer-related deaths among women (Momenimovahed *et al.,* 2019; Arora *et al.,* 2018). Chemotherapy can have gonadotoxic effects, increasing the risk of ovarian damage ranging from subfertility to POI. Women who undergo oophorectomy face heightened risks of cardiovascular disease and osteoporosis due to premature menopause (Takahashi *et al.,* 2015). Female reproductive dysfunction can arise from various factors, including ovarian insufficiency (whether natural or treatment-induced), polycystic ovarian syndrome (PCOS), endometriosis, fallopian tube occlusion, Asherman syndrome (AS) and other less common anomalies, as detailed in Table 1 and Fig. 1 (Roy *et al.,* 2011; Soderberg *et al.,* 1986). Additionally, causative factors encompass therapeutic alkylating agents, metabolic and autoimmune disorders, viral infections, and genetic alterations (Anderson *et al.,* 2011 ; Donnez *et al.,* 2013; Sadri *et al.,* 2015; Wallace *et.al.,* 2005 Yalcinkaya *et al.,* 2014).

Asherman's Syndrome (AS) arises from conditions such as endometriosis or aggressive removal of

Table 1 : Common Female Reproductive Tract Disorders, their Definitions, and Etiologies

Disorder/Dysfunction	Definition	Etiologies
Premature Ovarian Insufficiency (POI)	Cessation of ovarian function before the typical age of menopause.	Genetic disorders, autoimmune diseases, radiation, chemotherapy, infections
Polycystic Ovarian Syndrome (PCOS)	A multifactorial disorder linked to infertility, hirsutism, obesity and menstrual irregularities.	Maternal PCOS, intrauterine hyperandrogenism, inflammatory adipokines, diet
Resistant Ovary Syndrome (ROS)	Ovarian cells show resistance to gonadotropins, leading to symptoms similar to POI.	FSH receptor block, antibodies to FSH and LH, post receptor signaling defects.
Endometriosis	Growth and inflammation of endometrial tissue outside the uterus.	Oxidative stress, inflammatory genetics, epigenetic factors
Fallopian Tubal Occlusion	Blockage of the fallopian tube, often due to inflammation.	Neoplasms, infections, tubo ovarian abscesses
Uterine Fibroids	Benign tumors that develop in the uterus.	Elevated estrogen levels
Asherman Syndrome	Absence of a normal opening from the fallopian tubes to the vaginal canal.	Trauma, infection, low estrogen levels, aggressive curettage

uterine tissue, resulting in blockage or damage to the uterine cavity and destruction of the endometrial lining. In AS, intrauterine adhesions obliterate the uterine cavity, leading to an absence of a functional endometrial lining. This dysfunction results in infertility and often presents clinically as recurrent pregnancy loss or irregular menstruation (Yu *et al.,* 2008).

Heinrich Fritsch's publication in 1894 marked the first documented case of intrauterine adhesion, but it wasn't until 54 years later that Israeli gynecologist Joseph Asherman provided a comprehensive description of what came to be known as Asherman syndrome (AS). It is characterized by variable scarring inside the uterine cavity and it is also cause of menstrual disturbances, infertility and placental abnormalities. In his pioneering research, Asherman first identified this condition in 29 women presenting with amenorrhea and stenosis of the internal cervical os (Asherman *et al.,* 1948). He hypothesized that these symptoms could result from trauma to the endometrium. Two years later, Asherman published another case series that focused on intrauterine adhesions within the uterine cavity, revealing significant filling defects during hysterography (Asherman *et al.,* 1950). Intrauterine adhesions can cause partial or complete dysfunction of the endometrium, leading to impaired fertility and abnormal menstrual patterns, including amenorrhea and hypomenorrhea. When adhesions are confined to the lower uterine tract while a functional endometrium remains, the syndrome can also result in severe pelvic pain and retrograde menstruation. The pregnant or early pregnant uterus is particularly vulnerable to developing uterine scarring subsequent

to curettage procedures. However, any form of uterine insult or trauma, even stemming from minimally invasive surgical procedures, carries the potential to induce the development of intrauterine adhesions. The susceptibility to scarring and adhesion formation underscores the importance of cautious and precise surgical techniques, particularly in reproductive-aged women or those seeking to preserve their fertility.The impact of the AS on pregnancy is well documented with a high rate of infertility, miscarriage, poor implantation following in vitro fertilization and abnormal placentation (Yu *et al.,* 2008(b) ; Deans *et al.,* 2010). Numerous cases documented in the literature indicate that intrauterine adhesions (IUA) may not always present with symptoms. This observation has led some researchers to suggest avoiding the term Asherman Syndrome (AS) in such asymptomatic cases. AS is more accurately defined by the presence of adhesions within the uterine cavity and/or endocervix, which result in clinical manifestations such as amenorrhea, hypomenorrhea, recurrent pregnancy loss, infertility, or abnormal placentation. The condition has been reported most frequently in Israel, Greece, South America, and various European countries, although this geographic distribution does not appear to correlate with specific regional factors (Schenker *et al.,* 1982). The introduction of hysteroscopy has revealed a higher prevalence of IUA than previously understood, providing new insights into the condition. Additionally, the incidence of this pathology appears to be influenced significantly by the number of abortions performed, the prevalence of genital tuberculosis in certain regions, and the variability in criteria used to detect intrauterine adhesions.

Epidemiology and Aetiology :

The literature lacks extensive data on the pathophysiology of Asherman Syndrome (AS) and Intrauterine Adhesions (IUA). An electron microscopic study of endometrial glandular cells in women with severe AS revealed significant sub-cellular changes. These included the loss of ribosomes, mitochondrial swelling, closure of blood vessels, and alterations suggestive of cellular hypoxia (Chen *et al.,* 2013). These findings suggest substantial cellular modifications within affected tissues but require further investigation to fully grasp the underlying mechanisms of both conditions.

The fallopian tubes, crucial for fertilization and embryo transfer, can face occlusion due to conditions like neoplasms, inflammations, or tubo-ovarian abscesses, often diagnosed clinically (Cunha *et al.,* 2019). Meanwhile, the vaginal canal, where sperm is deposited during intercourse, experiences cyclic changes associated with ovarian physiology (Poonia *et al.,* 2006; Sjoberg *et al.,* 1988). Vaginal disorders encompass infections, MRKH Syndrome, vaginal fistula, prolapse, and trauma/surgical scars, all affecting this region differently (Soderberg *et.al.,* 1986).

Contemporary Infertility Treatments and their Therapies and Limitations

Current infertility treatments include hormonal stimulation, intrauterine insemination (IUI), in vitro fertilization (IVF), intracytoplasmic sperm injection

(ICSI), egg donation, and uterine surrogacy, each addressing different reproductive issues (Vassena *et al.,* 2015; Schlegel *et al.,* 2009). Factors such as genetic disorders and therapies like chemotherapy and radiation can lead to reduced egg reserves. Hormone replacement therapy (HRT) is used to restore estrogen (E2) and/or progesterone (P4) levels in post-menopausal women. However, prolonged use of HRT is debated due to potential increased risks of heart disease, stroke, and cancer (Rossouw *et al.,* 2002; Anderson *et al.,* 2004). Although recent research indicates that the risks associated with hormone replacement therapy (HRT) may be lower than previously thought, both physicians and patients remain cautious about its use for managing menopausal symptoms (Beshay *et al.,* 2015; Lobo *et al.,* 2017). Additionally, recent data suggest that HRT is effective only within a limited timeframe (Hodis *et al.,* 2013). While surgical corrections, tissue transplants, and uterus transplantation offer promising solutions, they come with significant health and ethical challenges, including the risk of graft rejection and moral issues related to surrogacy (Tulandi *et al.,* 2012; Gellert *et al.,* 2018). While some approaches, like allogeneic uterus transplants, have shown success, they're not universally suitable and may entail lifelong medical concerns or ethical complexities for patients and families (Brannstrom *et al.,* 2016). However, these approaches may not be suitable for all patients and can introduce a range of health and ethical issues. For example, tissue transplantation from donors can lead to graft rejection by the recipient's immune system, necessitating lifelong immunosuppressive therapy to prevent rejection. This ongoing treatment carries risks of infections and other complications due to the suppression of the immune system.

In cases of uterine defects or deformities, the use of a surrogate mother offers a potential solution but raises significant moral and ethical concerns. For the biological parents, there may be emotional and ethical considerations regarding the choice of a surrogate and the implications for the child. For the surrogate, the process involves complex psychological and physical challenges, and there may be ethical concerns about the commodification of reproduction and the rights of the surrogate. These issues highlight the need for careful consideration and ethical oversight in the use of these advanced reproductive technologies.

Regenerative Medicine potential for Advancing Reproductive Disorder Treatments:

Regenerative medicine offers a promising solution for treating conditions that currently have limited effective treatments and often come with significant side effects. By bioengineering female reproductive tissues through various regenerative medicine and tissue engineering approaches, we have the potential to transform the management of female reproductive dysfunction. These cutting-edge technologies not only aim to repair, regenerate, or replace damaged tissues but also hold promise for diagnostic and investigative applications. This could lead to a deeper understanding of the underlying mechanisms of female reproductive physiology and pave the way for more effective treatments. Bioengineering

strategies can be classified into two main categories: organ transplantation involving fresh or cryopreserved organs, and tissue engineering methods that employ a blend of cells, growth factors, and biomaterials, harnessing the body's natural regenerative capacity to repair and restore reproductive organs. While whole organ transplantation has demonstrated some success the source of the organ and the immunogenic effects of allografts remain challenging. Tissue engineering strategies through regenerative medicine largely avoid these issues (Brannstrom *et.al.,* 2016).

Transformative Potential of Regenerative Medicine :

Regenerative medicine emerges as a promising solution for conditions that lack effective treatments and are often accompanied by severe side effects. It offers a hopeful alternative for addressing disorders where existing therapies are inadequate and where severe side effects present significant challenges.. Utilizing diverse regenerative medicine and tissue engineering techniques, the bioengineering of female reproductive tissues holds the potential to transform the treatment landscape for female reproductive dysfunction significantly. 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. Bioengineering strategies can be broadly classified into two categories: (a) the transplantation of fresh or cryopreserved organs and (b) tissue engineering approaches that combine cells, growth factors, and biomaterials to harness the body's inherent ability to regenerate and repair reproductive organs. While whole organ transplantation has achieved notable success (Brannstrom *et al.,* 2016), it faces challenges related to organ sourcing and immune responses to allografts. Tissue engineering strategies within regenerative medicine offer promising alternatives by addressing these issues more effectively.

Regenerative medicine encompasses a range of technologies, including cell therapies, tissue engineering, and cloning. Tissue engineering specifically employs cells, biomaterials, bioactive factors, and bioengineering techniques to generate new tissue or organs. Each of these innovative approaches holds potential for new treatments for reproductive disorders. With thorough characterization and evaluation, bioengineered tissues show promise for significantly improving the quality of life for patients with reproductive tract defects. The rapid advancements in this field highlight its ongoing progress and future potential.

Innovative Bioengineering Models for Ovarian Research and Treatment:

The ovary, as the female gonad, houses various developmental stages of follicles, which serve as its functional units. Each follicle consists of an oocyte, the central germ cell, surrounded by somatic cells (Oatley *et al.,* 2012; Horan *et al.,* 2017). The ovarian follicle pool is typically established before birth and is considered non-renewable, potentially impacted by diseases, medications, or environmental exposures (Goswami *et.al.,* 2005). Compromising

follicle quantity or quality can heighten risks of premature ovarian insufficiency, hormonal imbalances, and infertility. In vitro cultivation of ovarian tissues or follicles has extensive applications in female reproductive science, aiding in understanding ovarian biology, fertility preservation after cancer therapy, and ovarian toxicity screening. Traditional methods using flat plastics or glass for follicle culture often disrupt the connections between oocytes and somatic cells, resulting in the loss of the 3D architecture and eventual follicle degradation. Modern bioengineering techniques seek to cultivate ovarian tissues or follicles within a 3D environment to better preserve these connections. One such promising approach is hydrogel encapsulation, which has gained significant attention for its ability to support and maintain the structural and functional integrity of ovarian follicles. Hydrogels, transitioning from liquid to solid states upon crosslinking, mimic native extracellular matrices (ECMs) and provide suitable scaffolds that maintain 3D architecture. They exhibit tissue-like elastic properties and facilitate the exchange of nutrients and waste, offering an environment akin to natural cellular settings.

Hydrogel encapsulation :

Hydrogel encapsulation has emerged as a promising bioengineering technique for facilitating 3D in vitro cell or tissue cultures. This method involves liquid precursor solutions that transform into solid hydrogels through crosslinking with curing agents, a process known as gelation. These resulting hydrogels are water-swellable and possess tissue-like elastic properties, enabling the diffusion of nutrients, wastes, and oxygen to the encapsulated cells or tissues. Such characteristics render hydrogels as optimal scaffolds that mimic native extracellular matrices (ECMs), offering adaptable stiffness akin to the in vivo cellular environment while preserving the 3D architecture of the encapsulated cells or tissues (Tibbitt *et.al.,* 2009; Orive *et.al.,* 2003). Several research teams have successfully utilized hydrogels, such as alginate, collagen and fibrin, to culture ovarian tissues or follicles in vitro, achieving notable advancements in the field. A pioneering example is Dr. Woodruff's work starting in 2003, where her team employed alginate hydrogel, a polysaccharide derived from brown algae, to encapsulate and culture mouse ovarian follicles in a process known as encapsulated in vitro follicle growth (eIVFG) (Pangas *et al.,* 2003). Their research demonstrated that eIVFG maintained the 3D architecture of the follicular complex, allowing granulosa cells to proliferate and oocytes to expand in volume and acquire characteristics of mature oocytes. These included the development of the zona pellucida, formation of gap junctions between granulosa cells and oocytes, and resumption of meiosis upon stimulation with human chorionic gonadotropin (hCG) (Pangas *et al.,* 2003). Subsequent studies confirmed that eIVFG replicates key events of mouse folliculogenesis and oogenesis in vivo, such as follicle development from preantral to antral stages, differentiation of mural granulosa and cumulus cells, development of the theca cell layer, ovarian hormone synthesis and secretion, oocyte maturation, ovulation, and luteinization. Notably, oocytes from eIVFG were capable of being

fertilized through in vitro fertilization (IVF) and led to live births following embryo transfer (Xu *et al.,* 2006(a); Kreeger *et al.,* 2005). eIVFG has proven to be a valuable research model, allowing the investigation of complex ovarian biology that is difficult to study in vivo. For instance, it has provided insights into follicular communication and collaborative development when multiple primary mouse follicles were encapsulated and cultured within a single alginate bead (Hornick *et al.,* 2013). The conditioned culture medium from eIVFG has potential for identifying interfollicular factors, such as miRNAs, exosomes, or bioactive compounds, although the mechanisms are not yet fully understood. Additionally, studies have shown that modifying the rigidity of alginate hydrogel affects follicle outcomes, with lower stiffness promoting favorable conditions for follicle development, while a rigid matrix increased androgen production and impaired oocyte maturation (West *et al.,* 2007; Xu *et al.,* 2006(a)).

These results are consistent with the phenotypes of aging or cystic ovaries, which have more fibrotic ovarian stroma tissues, compromised follicle/oocyte quality, and high androgen production. Such results suggest that the ovarian physical environment plays an essential role in determining the quality of follicles and oocytes. Research findings from mice were translated to humans by developing a two-step culture method for human preantral follicles. This approach offered a rigid growth environment during the preantral stage and a conducive setting for antral stage growth. Through this dynamic regimen, human MII oocytes were successfully generated using eVIFG, marking a significant advancement (Xiao *et.al.,* 2015). In conclusion, these studies underscore hydrogel encapsulation as a powerful bioengineering model for cultivating and maturing ovarian follicles in vitro. This method shows significant promise for various applications in reproductive biology and medicine, offering new avenues for research and potential treatments.

Decellularized Extracellular Matrix (ECM) Scaffolds

Decellularization involves utilizing physical, chemical, or enzymatic techniques to eliminate cellular components while retaining the intact extracellular matrix (ECM) scaffold of the original tissue (Crapo *et al.,* 2011). The ECM serves not only as a natural physical environment for cells but also performs crucial biochemical functions, regulating cellular activities like adhesion, migration, proliferation, and differentiation. Following injecting the decellularized ECM scaffold with targeting cells (e.g. stem cells, primary cells, or cell lines), the recellularized ECM scaffold has been increasingly researched for regenerative medicine and organ transplantation. The use of decellularization in creating artificial ovaries presents a potential fertility preservation avenue for young female cancer patients. This method, unlike conventional ovarian tissue transplantation, enables cell-free recellularization and transplantation on decellularized ECM scaffolds, reducing the risk of reintroducing cancer cells to survivors seeded primary murine ovarian cells onto decellularized bovine ECM scaffolds, successfully reconstructing mouse ovaries. These recellularized scaffolds produced estradiol in vitro and induced puberty in

ovariectomized mice after renal grafting (Frantz *et al.,* 2010) . Moreover, the same research group further used bovine ovary ECM powders to fabricate ovarian tissue pieces, which were termed ovarian tissue papers (OTPs) (Jakus *et.al.,* 2017). OTPs were employed to reconstruct human ovaries through co-culturing with human ovarian cortical strips, sustaining follicle viability and hormone production. However, further research is needed to ascertain their capability in supporting advanced folliculogenesis and oogenesis. Oktay et al. conducted transplantation of human decellularized ECM scaffolds seeded with ovarian cortical tissues in two POI patients post-cancer treatments. The ECM scaffolds facilitated vascularization in grafted ovarian tissues, with follicles producing estradiol and progressing to antral stages in both patients. Notably, both patients achieved pregnancy post-IVF, and one successfully delivered a healthy baby (Oktay *et.al.,* 2016; Oktay *et.al.,* 2018). Recently, another research group also used the decellularized human ovary ECM scaffold and isolated human preantral follicles to reconstruct human ovaries (Pors *et.al.,* 2019). Following subcutaneous grafting of reconstructed human ovaries onto immunodeficient mice for 3 weeks, approximately 40% of preantral follicles progressed to the antral stage. This outcome suggests that the decellularized ovarian scaffold presents a promising environment conducive to fostering follicle growth and development. Altogether, these studies illustrate that using decellularized ECM scaffolds presents a promising technique for reconstructing ovaries and reinstating ovarian functions. This method holds potential for restoring both female fertility and endocrine functions.

3D printed scaffold :

3D printing is an innovative and emerging tissue engineering technique with significant potential in regenerative medicine and tissue transplantation. Unlike decellularized ECM scaffolds, which rely on the natural tissue structure, 3D printing uses biomaterials to construct tissue scaffolds with precise control over attributes such as shape, size, geometry, porosity, and other physical and biochemical properties. This precision allows for customization to meet specific research and medical needs. Researchers have employed various biomaterials and 3D printing techniques to create functional live tissues and organs, including the heart, blood vessels, aortic valves, skin, and bone and cartilage (Lee *et al.,* 2019; Papaioannou *et al.,* 2019; Duan *et al.,* 2013; Lee *et al.,* 2014; Markstedt *et al.,* 2015).Researchers like Laronda et al. used 3D printing to create microporous gelatin scaffolds for bioprosthetic ovaries, discovering that pore geometry significantly affected follicle development (Laronda *et.al.,* 2017). It was found that the pore geometry of the printed scaffolds influenced the seeded follicle reproductive outcomes. The 30° and 60° with underlying struts better supported follicle development and survival than the 90° without underlying struts. Post in vivo transplantation into ovariectomized female mice, the reconstructed ovaries displayed robust vascularization, fully reinstating the animals' ovarian functions and enabling the delivery of live offspring through natural mating.

Additionally, apart from gelatin hydrogel, researchers utilized a blend of poly (epsilon caprolactone) (PCL), a biodegradable polyester and gelatin in scaffold fabrication. This mixture aimed to reduce hydrophobicity and enhance the biocompatibility of the 3D printed scaffold (Raffe *et.al.,* l201; Liverani *et.al.,* 2019).

Findings indicate that incorporating fibers within scaffold macropores significantly improves the attachment, infiltration, and development of seeded porcine ovarian follicles. An emerging technique in tissue engineering, known as bioprinting, extends beyond traditional 3D printing by using both biomaterials and encapsulated cells referred to as bioink to create tissue constructs (Gungor *et al.,* 2018). Bioink can be stabilized through crosslinking during or immediately after bioprinting, allowing for the fabrication of engineered tissues or organs with precise size, shape, and architecture. In summary, both 3D printing and bioprinting show substantial potential in reconstructing and fabricating artificial, functional, and implantable human ovaries. These advancements hold significant promise for restoring fertility and endocrine functions in prepubertal girls and young adult women.

Microfluidic Technologies:

Microfluidics, a cutting-edge bioengineering technique, simulates 3D cell and tissue culture within small channels under fluidic flow, creating 'organ on a chip' models that replicate organ-level functions. Unlike static cultures, this approach enhances nutrient delivery, waste removal, and mechanical stimulation. Our collaboration developed a microfluidic platform for mouse follicles and ovarian explants, successfully mimicking follicle development and hormone production akin to natural menstrual cycles. This platform supported long-term culture of ovarian tissues, overcoming previous oxygen and nutrient diffusion limitations. Similar success in large mammalian species, like cats and dogs, suggests potential for human 'ovary on a chip' models.

Conclusion and Future Perspectives :

Studying female reproduction presents significant challenges due to the intricate interactions among cells, organs, hormones, and organ systems. The integration of bioengineering into reproductive research has provided valuable insights into various aspects of female reproductive health, including the ovary, fallopian tubes, uterus, embryo implantation, and reproductive disorders. Advanced bioengineering techniques, such as hydrogel encapsulation, decellularized ECM scaffolds, 3D printing, and microfluidic platforms, have markedly improved our understanding of reproductive biology, fertility treatments, and toxicity screening. These technologies show considerable promise in developing functional reproductive organs and advancing both basic research and clinical applications in female reproductive health. Technologies like hydrogel encapsulation, decellularized ECM scaffolds, 3D printing, microfluidic platforms, and others exhibit promise in crafting functional reproductive organs. These innovations offer potential solutions for fertility restoration in prepubertal girls and young women, alongside aiding preterm neonates through artificial

womb systems. Additionally, these platforms facilitate advancements in gynecological cancer studies, drug screening, and pathology. While significant strides have been made in bioengineering for female reproductive health, further research is essential to ensure that bioengineered methods accurately replicate female reproduction at molecular, genetic, and epigenetic levels. Fine-tuning microfluidic systems and conducting thorough safety assessments of biomaterials are crucial steps in developing effective reproductive organ engineering techniques. Despite these challenges, the integration of bioengineering with female reproductive biology offers substantial promise. It holds the potential to enhance our understanding of female health across various dimensions, including fertility, genetics, medications, environmental factors, aging, nutrition, and disease.

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