International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

Vol. 31 No. 2 March 2022, pp. 21-42

3D-Bioengineering of Reproductive Organoids

Review

Maged Naser[1], Mohamed M. Naser[2], Lamia H. Shehata[3]

[1]Mazahmiya Hospital, Ministry of Health, Kingdom of Saudi Arabia, Department of Ob/Gyn,
[2]King Fahd Hospital, Ministry of Health, Kingdom of Saudi, Department of Surgery, ConsultantEndoscopic Surgery,
[3]Department of Radiology Care National Hospital, KSA

Abstract

Engineered male and female biomimetic reproductive tissues are being created as self-supporting in vitro units or as incorporated multi-organ in vitro constructions to help germ cell and embryo function, and to show characteristic endocrine phenotypic patterns, for example, the 28-days human ovulatory cycle. In this Review, we sum up how engineered reproductive tissues work with research in reproductive science, and outline strategies for making engineered reproductive tissues that may sometimes permit the rebuilding of reproductive potential in patients.

Individuals can confront reproductive or endocrine failure due to hereditary inclination, age, iatrogenic impacts of treatment or infection. More than a hundred years of progress that started with headways in reproductive tissue and reproductive organ transplantation, trailed by innovative improvements at the connection point of reproductive science, materials science, bioengineering and advanced manufacturing, has brought about engineered reproductive tissues that can restore and support normal organ function [1-20] (Table 1).

Corresponding Author: Maged Naser

Table 1. Major technological advancements in reproductive science and medicine

Year	Advancement	References
1895	First ovarian graft transplantation	1
1931	First human uterus transplantation	2
1936	First modern penile implant	3
1941	First testis prosthesis	4
1978	First human birth from IVF First human testis transplantation	5 6
1984	Human birth from frozen embryo	7
1986	Successful cryopreservation of human oocytes	8
1992	IVF by intracytoplasmic sperm injection	9
2004	Human birth from ovarian tissue transplantation Human whole ovary transplantation	10 11
2006	Cryopreservation of whole human ovary Oncofertility field established Live animal birth from follicles grown in vitro within a biomaterial	12 13 14
2014	Engineered vagina transplant	15
2015	Human metaphase-II oocyte in vitro Human birth from uterus transplantation	16 17
2017	EVATAR Biobag Functional 3D-printed ovarian bioprosthetic	18 19 20

Table 1 displays current trends in engineering reproductive tissues evolved from scientific and technological developments aimed to improve reproductive-health outcomes.

Current engineered reproductive tissues and culture structures are empowering an expanding number of physiological in vitro modelling of homeostasis, development, disease, pregnancy and aging. Engineered reproductive tissues are utilized for the proficient screening of new pharmacologic agents (for both therapeutic efficacy and toxicity), or are transplanted to restore damaged or diseased reproductive tissue.

In this Review, we spotlight current advancement in the development of engineering systems utilized in reproductive science and medicine, with a point of convergence on biomaterials and microfluidic approaches that permits the generation of functional builds at the tissue and organ levels for use in research and in clinical applications.

Keywords – 3D-Bioengineering, Reproductive Organoids.

I. INTRODUCTION

Approaches at the cellular, molecular and genetic levels, for example, the utilization of induced pluripotent stem cells [21,22], the controlled transport of drugs and different bioactive agents [23-27], and the utilization of microfluidic devices in assisted reproductive technology [28,29].

The female mammalian reproductive system comprises of the ovaries, fallopian tubes (likewise alluded to as oviducts in non-primate species), uterus, cervix and vagina. fertilization, implantation and maintenance of pregnancy to term all rely upon the unique intuitive physiology of the reproductive tract organs, what work in light of hormonal instructions created by utilizing the ovaries, the pituitary organ and the hypothalamus throughout the ovulatory cycle [30].

The essential parts of the mammalian reproductive tract in men comprise of the testicles, epididymis, seminal vesicle, vas deferens, prostate gland and penis. In differentiation to the female ovulatory cycle, the reproductive endocrine pattern of the male is portrayed by a daily increase and fall of testosterone, for the most part created through the Leydig cells of the testicles in response of pituitary and hypothalamic signals [30].

Until this point, data concerning these tissues comes from research using animal models and two-layered (2D) cell culture; notwithstanding, these procedures are far from ideal. In the first place, however there are numerous likenesses in mammalian reproductive biology all through species, there are likewise an amount of contrasts in expressions of each reproductive physiology and pathophysiology. For instance, including physiology, there are interspecies contrasts in receptor expression [31], metabolism [32], ovulation rate and cycle length [33], going from 4 days in the mouse to 28 days in the human female. As for pathophysiology, there are an assortment of conditions, like endometriosis or cervical malignant growth, for which there is no equivalent animal model. Second, in 2D culture, cells from reproductive tissues regularly lose a portion of their native properties, blocking the speculation of the aftereffects of in vitro experiments to the in vivo circumstance. For instance, the epithelium of the woman reproductive tract, as most epithelia, de-differentiates when cultured in vitro, transforming into basal-like, and the cell-cell interactions responsible for ovarian follicle function are lost in 2D culture [30]. Essentially, Sertoli cells, the somatic epithelial cells that make a commitment to germ cell development and differentiation in the testis, likewise lose key expression markers and secretory profiles when cultured in 2D, a task that has been hard to survive, even with the utilization of genetically engineered Sertoli cells or different feeder cell types [34]. Third, every male and woman reproductive organs and tissues produce and keep hormonal cycles that are quint essential for controlling various physiological processes inside and past the reproductive tract. In the female, the uterus is dependent on the rise and fall of estrogen and progesterone created by means of the ovary to drive endometrial proliferation and ensuing shedding. Cycling ovarian hormones moreover impact the function of the fallopian tube; for instance, progesterone diminishes the beat frequency of fallopian tube cilia [35]. In the male, testosterone is expected for the normal maintenance of epithelial cells in the prostate [36-38]. Testosterone actuates liquid production in the seminal vesicles and is expected for normal spermatogenesis[39]. Organs outside of the reproductive tract, like the heart and bone, additionally respond to steroid hormones and, thusly, the reproductive tissues and organs respond to hormones delivered through far away organs like the thyroid, pituitary and pancreas. For instance, the testis-bone-pancreas axis, which involves motioning through osteocalcin and insulin-like [3], stays inadequately understood [40-42]. Consequently, in vitro models that lack these hormonal factors don't totally catch the complexity of in vivo endocrine loops. Moreover, attributable to the complexity of the endocrine loops that occur for the length of the female ovulatory cycle, biomedical research has customarily designated on the 'a simpler' male cycle [43]. Taken together, state of the art strategies that depend on 2D in vitro monoculture and in vivo animal designs to concentrate on the human reproductive tract have routinely missed the mark regarding clarifying mechanistic responses and human-relevant findings. Creating advanced engineered choices for reproductive science and medicine that reiterate both the tissue and organ conditions as appropriately as endocrine components of the reproductive tract makes it suitable to once again introduce factors that have as needs be far been missing. These choices will work with in vitro drug screening and toxicity testing, tissue

transplantation and ex vivo fetal development, and fill information gaps by giving enormous designs to the investigation of sex differences in health and infection.

II. DESIGNED MODELS OF REPRODUCTIVE ORGANOIDS

To perceive the mechanisms of advancement, disease and normal function in reproductive systems, techniques utilizing biomaterials, tissue engineering and microfluidics have prompted the approach of in vitro forms of individual reproductive tissues and integrated tissue systems. Table (2) outlines the most recent bioengineered structures reiterating components of the male or female reproductive science that are referenced in this Review.

Table2 Overview of recent literature in engineered reproductive tissues

	Tissue	**Engineered system**	**References**
Female	Ovary	Hydrogels	14,16,46,47,48,49,50,51,52,53,133,142,143,144,145,146,147,148,149,150,151
		Decellularized or recellularized	63,64,65,66,67,68,81,88
		3D printing	20
		Microfluidic	18
	Fallopian tube	Hydrogels	61
		3D co-culture	116,117
		Microfluidic	18,118
	Uterus	Hydrogels	62,135
		Decellularized or recellularized	69,70,71,72,73,74
		3D printing	98
		Scaffold-free	110
		Microfluidic	18,122
	Cervix	Decellularized or recellularized	168
		Scaffold-free	102,103
		Microfluidic	18
	Vagina	Decellularized or recellularized	15,169,170,171,172,173
Male	Testis	Hydrogels	58,59,60,165
		Decellularized or	75,76,77,78,83,84,85,86,89

		recellularized	
		Scaffold-free	104,105,106,107,108,109,111,112
		3D printing	95
		Microfluidic	119,120,121
Peripheral	Placenta	Decellularized or recellularized	79,80
		3D bioprinting	96,97
		Microfluidic	123,124,125,126,127,128,129,130,131

2.1 Hydrogel

Maybe the simplest of recent attributes is the encapsulation of reproductive cells and tissues within hydrogels for 3D in vitro culture. Hydrogels have an extreme level of primary similitude to the native extracellular matrix (ECM) and have been comprehensively used to encapsulate cells and tissues for tissue-engineering applications [44-46]. Hydrogels are made out of more prominent than 90% water, permit efficient dispersion of nutrients, waste, supply physical aid and critical physical prompts to encapsulated cells and tissues. The functional unit of the ovary, the ovarian follicle, is an illustration of a reproductive tract component whose function is observably dependent on physical signals from its microenvironment to keep its cellular design. An ovarian follicle comprises of a central oocyte encompassed by layers of hormonal producing somatic cells. Without this 3D engineering, the oocyte-somatic cells connections are lost, and the follicle separates and passes on. Follicles have been encapsulated for in vitro 3D architecture in natural hydrogels, (for example, collagen [47], alginate [14,16,48,49,50], fibrin [51], hyaluronic acid [52]), synthetic hydrogels, (for example, poly (ethylene glycol) [53]), and mixes there [54,55]. This work mounted that when the 3D construction is kept up with in culture, follicles can survive and function autonomously, supporting hormonal production, oocyte development and ovulation (independently of the hypothalamic-pituitary-ovarian axis) [56,57].

The hydrogel encapsulation approach has likewise been utilized to create in vitro models of the testis utilizing Matrigel [58], agarose [59] or solubilized decellularized ECM [60]. Specifically, a model was once assembled the utilization of a three-layer gradient framework made out of a layer of murine testicular-cell-loaded Matrigel encompassed by utilizing two layers of cell-free Matrigel [58]. The testicular cells moved inside the Matrigel, forming coordinated testicular organoids with proliferating germ cells,

A functional blood-testis barrier and a physiological reaction to retinoic acid, tumour necrosis factor, and retinoic acid inhibitors, therefore fostering an additional physiological in vitro model than normal 2D culture techniques. Matrigel encapsulation has also been utilized for the period of human fallopian tube organoids [61], what's more human umbilical vein endothelial cells, human endometrial stromal cells and trophoblast spheroids have been implanted inside photo cross linked gelatine methacrylate hydrogels to study decasualization and placentation, which are key peculiarities of uterine physiology and early pregnancy [62]. The improvement of hydrogel encapsulation techniques and their application to reproductive tissue culture have empowered the presentation of all the more physiologically significant in vitro designs that replicate in vivo architecture vital for normal physiology.

2.2 Decellularized ECM scaffolds

In decellularization-generally utilized in reproductive medicine physical, enzymatic or chemical treatments are utilized to get rid of cellular material while the biochemical and structural features of the ECM (and accordingly its signalling roles) are saved. Dissimilar to cell surface markers, the biochemical components of the ECM are to a great extent monitored among people and

between species, in this way lessening the risk of a genuine immune reaction when decellularized ECMs are transplanted [63]. Strategies for the decellularization of tissue have been used to produce engineered ovarian [64-68], uterine [69-74], testis [75-78] and placental tissue [79,80]. Non-tissue-specific, 'universal' decellularized ECMs, like the human amniotic membrane, have furthermore been utilized for the culture of murine ovarian follicles [81].

Recellularized frameworks have been transplanted to improve or restore tissue function in preclinical [70] and clinical studies [82], and have likewise been utilized to study the molecular mechanisms of reproductive physiology. For instance, a human decellularized endometrial platform used to be repopulated with primary epithelial and stromal cells to establish long term cultures that respond to a 28-days ovulatory cycle [72]. In conventional 2D culture, endometrial cells will be more often to lose polarity and have changed gene expression and function with respect to the in vivo condition. Inside the bioactive decellularized platform, notwithstanding, the endometrial cells multiply, endure all through a drawn out subculture length and protect their characteristic morphology and hormone responsiveness. In some other model, human testis organoids were shaped inside a human testis decellularized scaffold [83-85]. These organoids had spermatogonia (undifferentiated male germ cells) and discharged testosterone and inhibin B, two significant markers of somatic cell function; in any case, it is indistinct whether the tissue-explicit decellularized platform outfitted a benefit (when contrasted with various ECMs) as far as testicular tissue arrangement. For instance, porcine spermatogonial stem cells have been accurately refined on ECM platforms from a various organ sources, proposing that laminin, as an option than testis-explicit factors, is the main environmental protein for undifferentiated germ cell expansion [86]. Human Sertoli cells have additionally been refined on decellularized porcine testis ECM, proposing that it very well might be feasible to utilize decellularized pig testis, or other sources of testis ECM, as a framework for future human testicular engineering [57,77].

A drawback of decellularized tissue frameworks is that their microarchitecture is 'locked in', which routinely makes it trying to repopulate the scaffold with cells [87]. In any case, with perceive to conventional or injection based framework seeding, the cultivating of a human decellularized ovarian scaffold was improved through the utilization of a rotational seeding technique (a spinner flask) [68]. To moderate this issue, decellularized ECM from various organ sources, along with cow-like ovary and uterus, has been processed into a powder and suspended in a biocompatible polymer matrix (made of poly (lactic-co-glycolic acid)), creating 'tissue papers' that can be cut, collapsed and sutured [88]. The ovary tissue paper is able to support murine ovarian follicle adhesion and viability and to work in vitro and maintain the practicality and function of non-human primate and human ovarian tissue for up to eight weeks [88]. One more technique involves processing decellularized tissue with the guide of utilizing an acidic pepsin arrangement into tissue-explicit hydrogels, which can then be utilized for cell encapsulation [60] or freeze-dried and chemically cross-linked preceding being cultivated with a cell suspension [89]. The Leydig cells in porcine testicular organoids endure better in decellularized testis-derived hydrogels than in collagen gels, demonstrating the upkeep of growth factors necessary for the maintenance of the testicular niche [60]. Decellularized ECM-derived materials are probably going to take on a greater job in reproductive tissue engineering, each as biomaterials in their own right and as formats for the sketch of progressively informative and biomimetic synthetic materials. Indeed, investigations of the arrangement of decellularized ECM, as exemplified by utilizing proteomic analysis of decellularized porcine [90] and human [91] ovarian tissue, will permit the generation of synthetic matrices with bioinspired composition.

2.3 3D-printed scaffolds and bioprinting

With 3D printing, materials can be created with specific precise of bulk geometry and of their inside pore design, likewise taking into consideration cutting edge biomimicry and personalization. 3D printing of biological frameworks refers to the printing of a cell free platform that might be a direct result of this seeded with cells, though 3D bioprinting portrays the most common way of depositing cell-loaded 'bioinks' in precise 3D areas. 3D-printing advances, materials and cell selection have been adequately discussed [92,93]. One example of 3D printing of reproductive tissue is the bioprosthetic ovary [20]. Murine follicles seeded into 3D-printed gelatin platforms with a tortuous (rather than grid like) network of pores kept up with the 3D architecture, survival and function (explicitly, hormonal production) of the follicles. A comparable meshwork of interconnected pores-for this situation, created through electrospinning of polycaprolactone (PCL)- was furthermore ready to maintain the 3D construction of porcine follicles [94]. For the testis, 3D-printed alginate platforms have been investigated for organoid generation; nonetheless, a biomimetic morphology like the native testis used to be currently not observed [95]. Another occasion is an in vitro model of the placenta [96,97], a transient organ that exchange nutrients, waste and gas between the mother and the developing fetus and that secretes hormones that help pregnancy used to explain the systems of preeclampsia, a disease of poor placental development. By

the utilization of extrusion based 3D bioprinting, human trophoblast-loaded gelatin methacrylate hydrogel bioinks can be printed close by the cell-free bioinks to learn about trophoblast migration, a significant stage in placental development. Additionally, human mesenchymal undifferentiated cells got from human endometrial biopsies have been bioprinted on top of conventional PCL meshes utilized in the treatment of pelvic organ prolapse. In contrast with cell-free meshes, the addition of the bioprinted endometrial stem cells brought about improved tissue integration and in the maintenance of an anti-inflammatory macrophage phenotype. The advancement of 3D printing and bioprinting for making personalised frameworks with uniquely customized cell-specific niches will help the improvement of clinical choices for patients and the study of in vivo physiology [98].

III. SCAFFOLD- FREE METHODOLOGIES

The traditional tissue-engineering involves cells, signals and a platform. However, because of the platform substances can affect cell behaviour, framework free procedures have arisen as another option. By depending on the self-get together of cells, framework free procedures create 3D multicellular totals that emit their own matrices [99-101]. Several physiological models of cervical epithelium, (for example, primary human fibroblasts secreting primarily collagen matrix to help epithelial differentiation [102] as well as cancerous and normal cervical models that utilization cell-line cultures of human dermis from neonatal foreskin [103] have utilized framework free strategies. These models regularly show of epithelial separation, yet the standard epithelial thickness is diminished and the morphology of the epithelial cells look like a neoplastic state, maybe attributable to sex mismatch between the cell source (male neonatal foreskin) and the target tissue (female cervix). These foreskins derived models have been utilized to learn about cervical infection, hormone regulation, epithelial-stromal interactions, neoplasia and malignant growth, yet they don't fittingly imply cervical biology and ignore any results of sex on disease progression.

In vitro models of human testicular organoids that perform testis-explicit function have likewise been made by means of framework free approaches [104]. Notwithstanding, the morphology of these organoids does now not look like testicular tissue. In vitro testis tissues have additionally been made through the utilization of fish, murine and non-human-primate (marmoset) cells in suspension-based (non-adherent) 3D culture [105-107]. Suspension culture models better mimic testicular design as they take into account the expansion of germ cells and the incorporation of somatic cells. In any case, nonhuman-primate tests have not confirmed spermatogenesis development, just spermatogonial development, maybe proposing that additional variables (specifically, extra physiological microenvironments) are required. Past their utilization as a platform, soluble human testis ECM has been utilized as a media added substance for culturing human testicular organoids as a method to mimic the prompts of the in vivo testis microenvironment aside from introducing a structural framework on which to foster all over again tissues [108].

Made by putting drop culture, these organoids contain all major testis cell types, increased in size over three weeks of culture and showed an upregulation of post-meiotic germ cell gene record over the culture period. This organoid framework has been utilized to learn about the steadiness of the Zika infection inside the particular cell types of the testis [109]. Moreover, cell-adhesion resistance microwell exhibits have been utilized to prompt assembly of organoids with reproducible and controllable diameters to create models of endometrial [110] and testicular [111,112] tissues with in-vivo-like 3D organization.

IV. MICROFLUIDIC, CO-CULTURE AND MULTI-TISSUE CULTURE FRAMEWORKS

No reproductive tissue exists in isolation in vivo; this is exceptionally real of the reproductive tract tissues, which are discernibly endocrine-dynamic. These tissues, most notably the ovary and testis, secrete factors that impact the growth, differentiation and function of different tissues in the tract and in other organ frameworks. To comprehend the crosstalk between reproductive tissues, co-culture methods, alongside microfluidic structures, have been utilized. Microfluidic culture frameworks take into account advantageous co-culture and for the expansion or deduction of media factors, like pituitary or sex hormones, to replicate dynamic hormone cycles, (for example, the female ovulatory cycle) that are expected for downstream tissue function. Microfluidic culture structures for the recapitulation of the physiology of tissue frameworks, regularly alluded to as 'organs-on-chips', additionally supply the upsides of prompt oxygen and nutrient delivery as well as waste end and permit likewise mechanical contribution from liquid flow (counting shear force, bulk flow and peristalsis-like contraction [113-115].

To more readily comprehend the microenvironment of the fallopian tube and oviduct for the span of fertilization and embryo development, 3D co-culture methods that maintain epithelial polarity and differentiation are utilized. Human fallopian tube epithelium has been cultured on a Transwell at the fluid air interface, with hormonal signals provided through microfluidically related murine ovarian follicles hormonally coordinated to mimic the human reproductive cycle [116]. In both cases, the scientists distinguished beating cilia and secreted factors in the culture medium, mirroring in vivo oviduct fluid. Fallopian tube epithelium

that used to be co-cultured with hormonal secreting ovarian follicles showed cyclic varieties in secreting factors and a thicker epithelium. Curiously, the expansion of fallopian tube epithelial cells to ovarian follicle cultures appeared to enhance ovarian function, as confirmed by utilizing the increased levels of progesterone secreted by means of the corpus luteum following ovulation, as a result showing the importance of crosstalk between reproductive organs in reproductive cycles. Co-cultures of bovine oviductal epithelium and embryos have unrevealed that crosstalk may likewise include signalling intervened by bone morphogenetic proteins (BMPs), a subfamily of growth factors in the transforming factors factor-β superfamily [117]. The presentation of bovine sperm and oocytes in an oviduct-on-a-chip system successful of aiding treatment, created to study about the oviduct microenvironment inside a microfluidic culture framework, showed the assistance of oocyte infiltration as well as the avoidance of polyspermy and parthenogenic activation, which are common events in current in vitro preparation (IVF) systems [118]. These advances have expanded the enthusiasm for the microenvironment of the fallopian tube at some stage in treatment and of early embryo development, and may likewise permit the creation of more noteworthy physiologically exact circumstances for IVF and embryo culture.

Microfluidic technology has additionally been used to the male's reproductive biology, for event in the development of a microfluidic framework for the culture of testis fragments from mice [119]. This system includes a testis culture chamber isolated from dynamic media drift via a microporous membrane, to mimic the microcirculation-tissue relationship in the in vivo microenvironment of the testis. While ordinary interphase culture procedures empower for tissue support for extended time terms (as long as 139 days), the utilization of microfluidic culture strategies empowers functional maintenance for as long as 180 days, with testosterone production in response to stimulation by the luteinizing hormone and production of sperm that brought about the live birth of healthy mice after intracytoplasmic sperm injection [119-121]. This system utilized hydrostatic pressure to make constant microfluidic flow for the duration of the culture, doing without the need for pumps and power sources. Albeit in its current day shape the throughput of the system is restricted, its advancement might upgrade the find out about of testis function and should stimulate the incorporation of male endocrinal function into other in vitro systems.

The male endocrine cycle happens on a large amount more limited time scale (one day) than that of the female's, which is portrayed through continuously adjusting phases of estradiol and progesterone across a 28-days cycle. The incorporation of microfluidic innovative skill into the culture of integrated woman reproductive tissues has prompted systems that extra definitively replicate the in vivo microenvironment and recreate the complex female endocrine cycle. The co-culture of human endometrial stromal cells and endothelial cells inside a microfluidic environment has empowered the study of the crosstalk between the two uterine cell types [122]. In this system, estradiol and progesterone had been supplemented in agreement to an idealized ovulatory cycle while just the endothelial cells had been quickly uncovered to the shear stress of the flow of media, subsequently imitating perivascular blood flow. The cultures have been kept up with for 28 days, all through which decidualization used to be observed in the stromal cell population. Likewise, the endothelial cells responded positively to shear stress exposure, as confirmed by utilizing cytoskeletal arrangement and the development of tight intersections. In a dynamic culture system (named EVATAR) comprising of an assortment of fluidically related wells containing microphysiological cultures of ovary, fallopian tube, endometrium, cervix and liver tissues [18], ovulation occurred after supplementing a base 'universal media' with changing levels of the pituitary hormones follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG). This furthermore prompted the creation, through the ovary, of follicular and luteal phase estradiol-and-progesterone profiles as indicated by an idealized 28-day human ovulatory cycle. By means of the microfluidic dissemination of media, the cyclic ovarian hormone profile additionally informed tissue function in the system's fallopian tube, endometrium, cervix and liver tissues. Such an integrated tissue culture system provided an alternative method for finding out about the endocrine loops of the reproductive tract in vitro.

Past the demonstrating of 'typical' reproductive biology, microfluidic systems can be utilized to model non-ordinary and disease states. For instance, all through pregnancy, the endocrine milieu is modified when as opposed to the normal ovulatory cycle all through gestation, attributable to the continued maintainenace of the progesterone-producing corpus luteum and the development of the placenta. There are many of microfluidic models of the placenta—the major endocrine organ accountable for maintaining pregnancy. These models normally consist of a membrane-separated co-culture of endothelial cells and placental trophoblasts as a simple recreation of the maternal–foetal interface [123-125]. Such placenta-on-a-chip models have been used to study the transport of caffeine [126], anti-depressants [127,128] nanoparticles [129] and the Zika virus [130] all through the placental barrier. Likewise, a microfluidic invasion assay permitted the study about of the migration of primary human

trophoblasts, a fundamental part to placentation that, if irregular or inhibited, can prompt gestational disorders, for example, pre-eclampsia [131].

The integration of fluidic forces with in vitro placental culture was a stage forward in the creating of better placental models. Specifically, as opposed to conventional static culture, these microfluidic models include a shear pressure part that is vital for the stress component of the placenta in vivo. In the EVATAR system, the primer endocrine state of pregnancy was reproduced by maintaining levels of HCG in the media throughout of the luteal stage. This brought about both the maintenance of the corpus luteum of the ovulated follicle and the resulting supported production of progesterone. Furthermore, in a microfluidic model of ovarian cancer in the peritoneal cavity during metastasis [132], ovarian malignant growth spheroids had been co-cultured in channels coated with human peritoneal mesothelial cells and revealed to shear stress, which has before been demonstrated to induce functional reactions in ovarian malignant growth. Such microfluidic models, created frameworks that hold onto complex tissue-tissue interactions, will produce cutting edge disease models that address the shortcomings of current in vitro and animal models of reproductive disease for problems like endometriosis, polycystic ovary disorder and hypogonadism.

V. Applications of Engineered Tissues, Organs and Organ Frameworks

Engineered tissues, organs and organ system styles have been utilized for a scope of utilizations, in exact as in vitro molds for toxicology and drug discovery studies, as transplanted tissues to substitute or restore damaged unhealthy organs, and as devices to help ex vivo fetal development.

5.1 In vitro toxicology testing and drug discovery

Past the conventional utilization of engineered in vitro models for research purposes, the models can also be utilized in toxicology studies. For example, epitomized follicle cultures have been utilized to predict reproductive toxicity in vitro, as exemplified with the guide of the utilization of murine ovarian follicles encapsulated in a fibrin-alginate composite system for the high-throughput toxicity testing doxorubicin [133]. Other studies have additionally demonstrated that doxorubicin has a dose dependent toxicity on alginate-encapsulated murine ovarian follicles [134], and human testis organoids respond in a dose dependendant fashion to four commonly utilized antimitotic chemotherapeutic drugs [108]. These testis organoid cultures affirmed IC50 values that had been impressively more prominent than these saw in 2D cultures, which could limit the quantity of false worthwhile outcomes. The testis organoid model was furthermore manageable to cryopreservation via slow freezing and verification, which will likely be important in the banking of organoids for use in large scale high-throughput toxicity testing [108].

Beyond toxicology, in vitro tissue models can furthermore be utilized to get the systems of mechanisms of drugs, or to screen for potential therapeutic agents. For instance, 3D human endometrial tissue models have been utilized to comprehend the aftereffects of the two often utilized fertility drugs levonorgestrel and mifepristone [135]. This model system ought to furthermore be utilized each to study drug mechanism and to find new agents for fertility control. Engineered in vitro reproductive tissue models are likewise liable to take on a greater role in toxicology testing and drug safety in pregnant women.

Furthermore, induced pluripotent stem cell (iPSC) advances, which are transforming into more accessible, will plausible be incorporated into engineered reproductive tissue models for personalized medicine [21,136].

5.2 Ovarian organoids

Ovarian tissue engineering is a promising system to manage each idiopathic and iatrogenic female infertility coming about because of exposure to gonadotoxic chemotherapy and radiation therapy [137]. Regardless of various attempts to protect fertility in patients with cancer, the cryopreservation of ovarian tissue with resulting transplantation is the only fertility preservation option accessible to pre-pubertal patients and to patients who can't delay cancer treatment [138-140]. Despite the fact that there have been more prominent than one hundred thirty live births expressed after the transplantation of cryopreserved ovarian tissue [141], the strategy is contraindicated for patients with cancer that have a moderate-to-high risk of ovarian metastasis because of the risk of the reintroducing malignant cells prompting disease recurrence. The various biomaterial-engineering strategies portrayed previously could likewise prompt new fertility restoration choices that can avoid the need for the transplantation of intact tissue and that may consequently moderate this risk.

Engineered ovarian tissue has been transplanted in mice in many structures: as encapsulated follicles, as recellularized ovarian ECM frameworks and as 3D-printed 'bioprostheses'. Transplanted encapsulated follicles develop and develop in vivo inside Matrigel, collagen, fibrin, alginate (a natural hydrogel got from algae) and poly (ethylene glycol) hydrogels [142-146]. Primordial follicles are even ready to develop into antral follicles and produce steroid hormones when seeded into macroporous alginate platforms with affinity bound BMP-4 [147]. Besides, artificial follicles-multi-layered made out of an internal centre of granulosa cell and bone-marrow-derived mesenchymal-stem cell-loaded alginate encompassed through a sheath of theca-cell-loaded alginate-re-establish estradiol secretion for at least ninety days in ovariectomized mice and improve estrogen-deficiency induced uterine atrophy without influencing endometrial hyperplasia, a precursor to cancer [148]. To actually look at the preclinical safety of this system for re-establishing fertility in survivors of childhood cancer, donor follicles have been confined from a mouse with breast malignant growth, encapsulated in fibrin matrices (with or without vascular endothelial blast factor (VEGF)) and transplanted into ovariectomized mice [143]. All mice getting transfers continued cycling, yet live birth was exclusively accomplished in mice who got VEGF-containing fibrin matrices. This find out with regards to affirmed the possibility of bringing down the opportunity of recurrent metastatic breast malignant growth by means of isolating and transplanting follicles, as an option than intact ovaries, from cancer loaded donor mice.

One more strategy to restrict the risk of tumour recurrence while involving cryopreserved ovarian tissue for auto-transplantation is to encapsulate the tissue in a biomaterial that can go about as a barrier to impede the movement of any residual cancer cells out of the transplanted tissue and into the body. Since the biomaterial forms a barrier between the transplanted ovarian tissue and the rest of the body, this technique can't be utilized to re-establish natural fertility; however, it very well may be utilized to rebuilding physiologic endocrine function [149].

Alginate is a promising competitor biomaterial for use as a tissue hindrance since it can't be debased by mammalian cells. As a hydrogel, alginate would permit the inactive dispersion of supplements to the relocated tissue and the departure of chemicals from the unite while halting the movement of growth cells from the transfer (and invulnerable cells toward it). TheraCyte, a FDA-supported poly(tetrafluroethylene) film, has additionally been utilized to guard relocated ovarian tissue from insusceptible responses [150]. Relocated follicles typified in both alginate and TheraCyte re-established endocrine trademark with a lesson in FSH stages in ovariectomized mice. Albeit these boundary gadgets forestall the send-off of mature oocytes, they are equipped for supporting in vivo follicle development and are all around coordinated with IVF. Another invulnerable separation approach involves the utilization of poly-L-ornithine and alginate to diminish the risk of immune responses against unfamiliar granulosa and theca cells (the chemical creating cells of the ovary). Multi-facet hydrogel dots (two alginate layers, containing both granulosa or theca cells, isolated by means of a Poly-L-ornithine layer to supply additional resistant segregation) have been made to control chemical trade solution for post-menopausal female and malignant growth victims after openness to gonadotoxic treatments [151]. These designed develops conveyed consistent scopes of chemicals for over of 90 days that had been more than adequate for the support of the mineral density of normal bone and of a healthy body composition in a murine model of menopause. Albeit comparative biomaterials have been utilized in individuals for the transplantation of pancreatic islets, correspondingly review are wished to test the safety and effectiveness of such biomaterial impediments in humans [152-154].

Recellularized ovarian ECM scaffolds have been transplanted into pre-pubertal ovariectomized mice, which mimic the physiology of young cancer survivors with premature ovarian failure. Following transplantation, the mice began puberty, with increasing levels of estrogen and inhibin A20. First pregnancies and livebirths following IVF have been suggested for female who had acquired minimally invasive transplantation of in the previously cryopreserved ovarian tissue with a commercially available decellularized ECM scaffold facilitated from human body skin [82] (trade name, Alloderm). In addition to live births, ovarian function was persevering for up to two years after transplantation, which recommends that ECM from non-tissue-specific sources may supply adequate assistance for a functional transplant. The 3D-printed ovarian bioprosthetic additionally restored fertility and endocrine function in ovariectomized mice. Following transplantation, the follicle-seeded 3D-printed scaffolds created to end up being outstandingly vascularized, bringing about live births through normal mating and in maintained maternal lactation, which suggests that the 3D-printed bioprosthetic restored each physiological fertility (via ovulation through the porous scaffolds) and endocrine function [20]. Recellularization of decellularized ovarian tissue and 3D-printed structures can in like manner thus be useful methodology to restore endocrine function after gonadotoxic treatment, therefore hindering sequelae of premature ovarian failure, such as, osteopenia and cardiovascular disease. Yet extra research and improvement is required sooner than these

strategies can end up in standard practice in individuals, the new animal work and the small pilot research in humans guarantee that there will be new open doors for restoring ovarian function in patients [82].

5.3 Testicular organoids

In like manner with pre-pubertal females, there is a necessity for additional fertility preservation choices for pre-pubertal males. Before the start of pubescence, the testis doesn't make haploid, fertility competent sperm for cryopreservation [30]. In like manner, cryopreservation of immature testicular tissue, but still an investigational technique, is the really open choice for fertility preservation in this patient population [155]. In 1978, the first transplantation of an intact human testis finished in identical twins (one of whom was born without gonads) resulted in a live birth [6]. This find out with respect to exhibited that testicular transplantation should be a practical methodology to restore fertility. Unfortunately, present shows can't cryopreserve intact testes; taking everything into account, only testicular tissue fragments are cryopreserved. On a basic level, areas of cryopreserved immature testicular tissue can be likewise thawed out and transplanted to restore fertility; yet this way has not yet been finished in humans. Preclinical studies have achieved live offspring in autografted and xenografted testis fragments from rodents, pigs and non-human primates [156-158]. In any case, xenotransplantation of human testicular tissue or cells into rodent models often fails to keep spermatogonia, and few studies in higher-warm blooded mammal testicular transplant have chosen the improvement of fully mature spermatocytes [156,159,160-162]. The principal challenges of human immature testicular tissue transplantation (ITT) are hypoxia and reperfusion injury, the assurance of early spermatogonial populations and poor or late neovascularization of the testicular graft [163]. In like manner, an enormous number of the indistinct fundamental worries of ovarian tissue transplantation moreover apply to ITT, including the reintroduction of malignant cells. A study of the in vitro production of haploid germ cells inside cultured human immature testicular tissue suggested that in vitro maturation before transplantation may likewise improve ITT [164]. The improvement of productive shows for ITT, which will most likely contain biomaterial scaffolds for the provision plan of a suitable area of interest for testicular cells, immune-isolation and ex vivo tissue maturation inside microfluidic systems, will be critical for this patient population.

Until this specific moment, the use of bioengineered biomaterials to additionally improve transplant results has been unexplored for testis transplantation (appeared differently in relation to ovarian transplantation). Two studies have encapsulated testicular cells inside Matrigel to promote vascularization [158,165]. As in going before studies by which testicular cell pellets had been transferred without a matrix [166], the Matrigel-encapsulated testicular cells self-assembled into denovo seminiferous tubules; however, few germ cells had been seen, suggesting that the technique can't restore fertility. One study used hydrogels (alginate or fibrin) loaded with VEGF nanoparticles to encapsulate testicular tissue for transplantation as a mechanism to progress vascularization23. Following 5 days, grafts containing the VEGF nanoparticles showed accelerated vascularity rather than the uncovered hydrogel and to encapsulated grafts; however, this gain disappeared 21 days post-transplantation.

As with ovarian bioprotheses, decellularized ECMs and scaffold fabrication technologies, for instance, 3D-printing ought to be used for design of a testis bioprosthesis. Such engineered biomaterials may likewise diminish the troubles related with ITT through promoting angiogenesis and preserving spermatogonia. Moreover, as in vitro grown testicular tissues become more common, testicular organoids may provide a choice to native tissue for transplantation. Moreover, biomaterials could improve spermatogonial stem cell transplantation into recipient seminiferous tubules, a promising fertility -preserving strategy that has seen accomplishment in several mammalian research species [157,167].

5.4 Uterine organoids

With advances in assisted reproductive technologies, for instance, IVF, many women who struggle with infertility can get pregnant. Nevertheless, absolute uterine factor infertility (owing to a missing uterus or to a non-functional uterus), gestational surrogacy has been the only decision. The use of tissue-engineered uterine forms for researching and treating absolute uterine factor infertility and other reproductive syndromes that impact uterine function has been examined. For example, decellularized uterine ECM was transplanted into murine uteri with artificially induced defects [69]. Uterine epithelial cells migrated into the decellularized ECM, molding an intact epithelial within a week. Stromal cell and myometrial cell migration and regeneration followed from that point, exhibiting that the use of a decellularized matrix is a potential procedure for the repair and regeneration of uterine tissue close to defects regions. In a rat model, recellularized uterine ECM scaffolds had been used to repair defects in native uterine tissue in vivo and to support healthy pregnancy[70]. Collagen scaffolds, loaded with human umbilical cord mesenchymal stem cells, have thusly been used to restore endometrial tissue structure and fertility in a murine uterine defect

model [168]. Additionally, the primary complete reproductive organ of a large animal to bear decellularization used to be a porcine uterus. The decellularized organ used to be then recellularized with primary human endometrial cells, giving affirmation of-thought verification that porcine scaffolds can help human endometrial regeneration and that recellularized ECM scaffolds could moreover be a promising solution for uterine factors infertility [71].

5.5 Cervico-vaginal organoids

Although rare, women without functional cervix or vagina at birth (known as cervical or vaginal aplasia), which can be caused by Mayer-Rokitansky-Küster-Hauser syndrome and different issues, experience infertility. In a clinical examination of 53 patients with this syndrome that used decellularized dermal ECM (a commonly used and commercially accessible 'universal' decellularized ECM) to reconstruct the vagina provided near normal sexual function to all patients and improved their body image perception [169]. In another clinical audit, decellularized porcine small intestinal submucosa (another reliably used and commercially accessible 'universal' decellularized ECM) was used to reconstruct the cervix and vagina in women with missing or malformed anatomy [170,171]. All patients acquired menstruation, and the engineered cervix and vagina remained patent. To speed up tissue regeneration further, the scaffolds were developed with human bone marrow mesenchymal stem cells (started to have a vaginal epithelial phenotype) [172] or with autologous cells from a vulvar biopsy [125] before transplantation. In the latter case, isolated epithelial and muscle cells expanded in culture, seeded onto the scaffolds and matured in an incubator before transplantation. For up to 8 years following surgical transplantation, yearly biopsies revealed that the vaginal implants had normal structure and function. Decellularized ECM scaffolds have besides been used to reconstruct human cervico-vaginal tissue in clinical trials [15], yet further work is understanding the factors that accelerate tissue regeneration and support long term function. For example, a case record depicted the use of a tilapia skin stage for vaginal reconstruction with the formation of a stratified squamous epithelium when assessed by means of histology at 180 days' post transplantation [173].

5.6 Ex vivo fetal new development

An artificial uterus that is fit to carry out uterine and placental functions ex vivo [19], perceived as the 'biobag', maintained fetal sheep for about a month without organ failure. The biobag involved a pumpless arteriovenous circuit inside a shut fluid environment with continuous fluid exchange, with blood flow driven by means of the fetal heart and got to through umbilical vasculature. Another pumpless, perfusion-driven microfluidic device used to be basic into a lung assist device to improve oxygenation pervasiveness in preterm neonates [174]. Though the factors causing premature birth (like cervical incompetence) are not completely understood, biobags and microfluidic lung help devices could give better outcomes in cases of premature birth.

VI. VIEWPOINT

Reproductive tissues can be engineered to serve as in vitro models for research and to replace or regenerate damaged tissues to restore reproductive part (fertility and endocrine function). Moreover, the usage of biomaterials and advanced culture systems to create or model functional reproductive tissues ex vivo has achieved significant progresses in engineering reproductive phenomena at the cellular and molecular levels. Regardless, one aspect of new biomaterials that is often overlooked is the pragmatic for adverse effects on reproductive health, similar to gamete development and quality. Biomaterials reliably contain plastics or leachates that can in like manner show up at be biocompatible with non- reproductive tissues yet are accordingly found to show reproductive toxicity [175]. It is thus necessary to check that tissue constructs, regardless of whether such a great amount for reproductive or non- reproductive tissues, are studied for their true biocompatibility, particularly with the reproductive system. What's more, with nearly 2% of Americans born through assisted reproductive technology since 1976 [176], medical intervention to assist men and women with infertility is a reality. In future, the assistance of endocrine function in people living and working in space may be required, and animal reproductive capacity may in addition require the same interventions that are under thought for human applications.

To be sure, even as new advances make, there should be close consideration about the ethics related with interventions that challenge traditional thoughts of reproductive abilities [177-179]. New concerns will occur as gametes and niches in which gametes develop are made, and the existential questions related with self and personhood will be debated. Since what will really be developmentally achievable in a laboratory setting and in the human context can't be expected, reproductive health ethics, law and religious beliefs should be considered (as happened in the region of oncofertility [178]. Tough transplantable reproductive tissues will conceivable require a combination of the techniques depicted in this Review: customized biomaterials, native matrix cues and sophisticated fabricate applied sciences successful of making personalized bioprostheses. They will likewise require the

relationship of clinical ethicists and legal experts to ensure that future interventions are made in a manner consistent with human values and necessities. Furthermore, the settled on decisions need to eliminate social disparities [180-182] and ensure that the science is in addition instructed by including people in whose interest the advances are being made [177,183-185].

The development of fundamental new knowledge from the engineered strategies depicted here will be hopefully enable the endocrine and fertility needs of current patients and the people of tomorrow. Taking everything into account, reproductive health is essential to human persistence as a species.

VII. Conflict of Interest

All authors declare no conflicts of interest.

VIII. Authors Contribution

Authors have equally participated and shared every item of the work.

References

[1] Morris, R. T. "The overian graft. New-York. med." *Journ. Oct* (1895): 221.

[2] Favre-Inhofer, A., et al. "Uterine transplantation: review in human research." *Journal of Gynecology Obstetrics and Human Reproduction* 47.6 (2018): 213-221.

[3] Rodriguez, Katherine M., and Alexander W. Pastuszak. "A history of penile implants." *Translational andrology and urology*6.Suppl 5 (2017): S851.

[4] Girsdansky, Joseph, and Herbert F. Newman. "Use of a vitallium testicular implant." *The American Journal of Surgery* 53.3 (1941): 514.

[5] Steptoe, Patrick C., and Robert G. Edwards. "Birth after the reimplantation of a human embryo." *The Lancet* 312.8085 (1978): 366.

[6] Silber, Sherman J. "Transplantation of a human testis for anorchia." *Fertility and Sterility* 30.2 (1978): 181-187.

[7] Zeilmaker, Gerard H., et al. "Two pregnancies following transfer of intact frozen-thawed embryos." *Fertility and sterility* 42.2 (1984): 293-296.

[8] Chen, Christopher. "Pregnancy after human oocyte cryopreservation." *The Lancet* 327.8486 (1986): 884-886.

[9] Palermo, Gianpiero, et al. "Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte." *The Lancet*340.8810 (1992): 17-18.

[10] Donnez, Jacques, et al. "Livebirth after orthotopic transplantation of cryopreserved ovarian tissue." *The lancet* 364.9443 (2004): 1405-1410.

[11] Hilders, Carina G., et al. "Successful human ovarian auto transplantation to the upper arm." *Cancer: Interdisciplinary International Journal of the American Cancer Society* 101.12 (2004): 2771-2778.

[12] Jadoul, Pascale, et al. "Laparoscopic ovariectomy for whole human ovary cryopreservation: technical aspects." *Fertility and sterility* 87.4 (2007): 971-975.

[13] Woodruff, Teresa K. "The emergence of a new interdiscipline: oncofertility." *Oncofertility fertility preservation for cancer survivors* (2007): 3-11.

[14] Xu, Min, et al. "Tissue-engineered follicles produce live, fertile offspring." *Tissue engineering* 12.10 (2006): 2739-2746.

[15] Raya-Rivera, Atlántida M., et al. "Tissue-engineered autologous vaginal organs in patients: a pilot cohort study." *The Lancet*384.9940 (2014): 329-336.

[16] Xiao, Shuo, et al. "In vitro follicle growth supports human oocyte meiotic maturation." *Scientific reports* 5.1 (2015): 1-5.

[17] Brännström, Mats, et al. "Livebirth after uterus transplantation." *The Lancet* 385.9968 (2015): 607-616.

[18] Xiao, Shuo, et al. "A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle." *Nature communications* 8.1 (2017): 1-13.

[19] Partridge, Emily A., et al. "An extra-uterine system to physiologically support the extreme premature lamb." *Nature communications* 8.1 (2017): 1-16.

[20] Laronda, Monica M., et al. "A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice." *Nature communications* 8.1 (2017): 1-10.

[21] Botman, Olivier, and Christine Wyns. "Induced pluripotent stem cell potential in medicine, specifically focused on reproductive medicine." *Frontiers in Surgery* 1 (2014): 5.

[22] Mahabadi, Javad Amini, et al. "Derivation of male germ cells from induced pluripotent stem cells by inducers: A review." *Cytotherapy* 20.3 (2018): 279-290.

[23] Poels, Jonathan, et al. "Transplantation of testicular tissue in alginate hydrogel loaded with VEGF nanoparticles improves spermatogonial recovery." *Journal of Controlled Release* 234 (2016): 79-89.

[24] Shikanov, Ariella, et al. "Fibrin encapsulation and vascular endothelial growth factor delivery promotes ovarian graft survival in mice." *Tissue engineering part A* 17.23-24 (2011): 3095-3104.

[25] Ensign, Laura M., Richard Cone, and Justin Hanes. "Nanoparticle-based drug delivery to the vagina: a review." *Journal of Controlled Release* 190 (2014): 500-514.

[26] Tanaka, Ayaka, et al. "Effect of sustained release of basic fibroblast growth factor using biodegradable gelatin hydrogels on frozen-thawed human ovarian tissue in a xenograft model." *Journal of Obstetrics and Gynaecology Research* 44.10 (2018): 1947-1955.

[27] Prakapenka, Alesia V., Heather A. Bimonte-Nelson, and Rachael W. Sirianni. "Engineering poly (lactic-co-glycolic acid) (PLGA) micro-and nano-carriers for controlled delivery of 17β-estradiol." *Annals of biomedical engineering* 45.7 (2017): 1697-1709.

[28] Kashaninejad, Navid, Muhammad Johirul Alam Shiddiky, and Nam-Trung Nguyen. "Advances in microfluidics-based assisted reproductive technology: From sperm sorter to reproductive system-on-a-chip." *Advanced Biosystems* 2.3 (2018): 1700197.

[29] Weng, Lindong, et al. "On-chip oocyte denudation from cumulus–oocyte complexes for assisted reproductive therapy." *Lab on a Chip* 18.24 (2018): 3892-3902.

[30] Khodr, Zeina G., et al. "Circulating sex hormones and terminal duct lobular unit involution of the normal breast." *Cancer Epidemiology and Prevention Biomarkers* 23.12 (2014): 2765-2773.

[31] Saunders, Philippa TK, et al. "Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates." *Molecular human reproduction* 7.3 (2001): 227-236.

[32] Toutain, Pierre-Louis, Aude Ferran, and Alain Bousquet-Mélou. "Species differences in pharmacokinetics and pharmacodynamics." *Comparative and veterinary pharmacology* (2010): 19-48.

[33] Sato, Junko, Masahiro Nasu, and Minoru Tsuchitani. "Comparative histopathology of the estrous or menstrual cycle in laboratory animals." *Journal of toxicologic pathology* 29.3 (2016): 155-162.

[34] Kanatsu-Shinohara, Mito, et al. "Reconstitution of mouse spermatogonial stem cell niches in culture." *Cell stem cell* 11.4 (2012): 567-578.

[35] Bylander, Anna, et al. "Rapid effects of progesterone on ciliary beat frequency in the mouse fallopian tube." *Reproductive Biology and Endocrinology* 8.1 (2010): 1-8.

[36] Cunha, Gerald R., et al. "The endocrinology and developmental biology of the prostate." *Endocrine reviews* 8.3 (1987): 338-362.

[37] Roberts, Rosebud O., et al. "Androgen receptor gene polymorphisms and increased risk of urologic measures of benign prostatic hyperplasia." *American journal of epidemiology*159.3 (2004): 269-276.

[38] Goldenberg, S. Larry, Anthony Koupparis, and Michael E. Robinson. "Differing levels of testosterone and the prostate: a physiological interplay." *Nature Reviews Urology* 8.7 (2011): 365-377.

[39] O'Shaughnessy, Peter J. "Hormonal control of germ cell development and spermatogenesis." *Seminars in cell & developmental biology*. Vol. 29. Academic Press, 2014.

[40] Davidyan, Arik, et al. "Maintenance of muscle mass in adult male mice is independent of testosterone." *PloS one* 16.3 (2021): e0240278.

[41] Karsenty, Gerard, and Franck Oury. "Regulation of male fertility by the bone-derived hormone osteocalcin." *Molecular and cellular endocrinology* 382.1 (2014): 521-526.

[42] McGrath, J. J., D. B. Savage, and I. R. Godwin. "The role and potential advantages of vitamin D metabolites in maintaining calcium status in high-producing dairy herds." *Animal Production Science* 55.9 (2015): 1081-1089.

[43] Kim, Alison M., Candace M. Tingen, and Teresa K. Woodruff. "Sex bias in trials and treatment must end." *Nature* 465.7299 (2010): 688-689.

[44] Nicodemus, Garret D., and Stephanie J. Bryant. "Cell encapsulation in biodegradable hydrogels for tissue engineering applications." *Tissue Engineering Part B: Reviews* 14.2 (2008): 149-165.

[45] Khatam, Sudhir, and Jason Burdick. "Cellular encapsulation in 3D hydrogels for tissue engineering." *JoVE (Journal of Visualized Experiments)* 32 (2009): e1590.

[46] West, Erin R., Lonnie D. Shea, and Teresa K. Woodruff. "Engineering the follicle microenvironment." *Seminars in reproductive medicine*. Vol. 25. No. 04. Copyright© 2007 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA., 2007.

[47] Joo, Sunyoung, et al. "The effect of collagen hydrogel on 3D culture of ovarian follicles." *Biomedical Materials* 11.6 (2016): 065009.

[48] Skory, Robin M., et al. "Engineering the ovarian cycle using in vitro follicle culture." *Human Reproduction* 30.6 (2015): 1386-1395.

[49] Amorim, Christiani A., et al. "Survival of human pre-antral follicles after cryopreservation of ovarian tissue, follicular isolation and in vitro culture in a calcium alginate matrix." *Human Reproduction* 24.1 (2009): 92-99.

[50] Pangas, Stephanie A., et al. "Novel approach for the three-dimensional culture of granulosa cell–oocyte complexes." *Tissue engineering* 9.5 (2003): 1013-1021.

[51] Xu, J., et al. "Fibrin promotes development and function of macaque primary follicles during encapsulated three-dimensional culture." *Human Reproduction* 28.8 (2013): 2187-2200.

[52] Desai, Nina, et al. "Three dimensional culture of fresh and vitrified mouse pre-antral follicles in a hyaluronan-based hydrogel: a preliminary investigation of a novel biomaterial for in vitro follicle maturation." *Reproductive Biology and Endocrinology* 10.1 (2012): 1-12.

[53] Shikanov, Ariella, et al. "Hydrogel network design using multifunctional macromers to coordinate tissue maturation in ovarian follicle culture." *Biomaterials* 32.10 (2011): 2524-2531.

[54] Shikanov, Ariella, et al. "Interpenetrating fibrin–alginate matrices for in vitro ovarian follicle development." *Biomaterials* 30.29 (2009): 5476-5485.

[55] Choi, Jung Kyu, et al. "The crucial role of mechanical heterogeneity in regulating follicle development and ovulation with engineered ovarian microtissue." *Biomaterials* 35.19 (2014): 5122-5128.

[56] Lebbe, M., and T. K. Woodruff. "Involvement of androgens in ovarian health and disease." *Molecular human reproduction*19.12 (2013): 828-837.

[57] West-Farrell, Erin R., et al. "The mouse follicle microenvironment regulates antrum formation and steroid production: alterations in gene expression profiles." *Biology of reproduction* 80.3 (2009): 432-439.

[58] Alves-Lopes, João Pedro, Olle Söder, and Jan-Bernd Stukenborg. "Testicular organoid generation by a novel in vitro three-layer gradient system." *Biomaterials* 130 (2017): 76-89.

[59] Gholami, Keykavos, et al. "Organ culture of seminiferous tubules using a modified soft agar culture system." *Stem cell research & therapy* 9.1 (2018): 1-8.

[60] Vermeulen, Maxime, et al. "Generation of organized porcine testicular organoids in solubilized hydrogels from decellularized extracellular matrix." *International journal of molecular sciences*20.21 (2019): 5476.

[61] Kessler, Mirjana, et al. "The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids." *Nature communications* 6.1 (2015): 1-11.

[62] Zambuto, Samantha G., Kathryn BH Clancy, and Brendan AC Harley. "A gelatin hydrogel to study endometrial angiogenesis and trophoblast invasion." *Interface Focus* 9.5 (2019): 20190016.

[63] Crapo, Peter M., Thomas W. Gilbert, and Stephen F. Badylak. "An overview of tissue and whole organ decellularization processes." *Biomaterials* 32.12 (2011): 3233-3243.

[64] Laronda, Monica M., et al. "Initiation of puberty in mice following decellularized ovary transplant." *Biomaterials* 50 (2015): 20-29.

[65] Eivazkhani, Farideh, et al. "Evaluating two ovarian decellularization methods in three species." *Materials Science and Engineering: C* 102 (2019): 670-682.

[66] Hassanpour, Ashraf, et al. "Decellularized human ovarian scaffold based on a sodium lauryl ester sulfate (SLES)-treated protocol, as a natural three-dimensional scaffold for construction of bioengineered ovaries." *Stem cell research & therapy* 9.1 (2018): 1-13.

[67] Alshaikh, Ahmed Baker, et al. "Decellularization of the mouse ovary: comparison of different scaffold generation protocols for future ovarian bioengineering." *Journal of ovarian research* 12.1 (2019): 1-9.

[68] Mirzaeian, Leila, et al. "Optimizing the cell seeding protocol to human decellularized ovarian scaffold: application of dynamic system for bio-engineering." *Cell Journal (Yakhteh)* 22.2 (2020): 227.

[69] Hiraoka, Takehiro, et al. "STAT3 accelerates uterine epithelial regeneration in a mouse model of decellularized uterine matrix transplantation." *JCI insight* 1.8 (2016).

[70] Hellström, Mats, et al. "Bioengineered uterine tissue supports pregnancy in a rat model." *Fertility and sterility* 106.2 (2016): 487-496.

[71] Campo, Hannes, et al. "De-and recellularization of the pig uterus: a bioengineering pilot study." *Biology of reproduction*96.1 (2017): 34-45.

[72] Olalekan, Susan A., et al. "Development of a novel human recellularized endometrium that responds to a 28-day hormone treatment." *Biology of reproduction* 96.5 (2017): 971-981.

[73] Miyazaki, Kaoru, and Tetsuo Maruyama. "Partial regeneration and reconstruction of the rat uterus through recellularization of a decellularized uterine matrix." *Biomaterials* 35.31 (2014): 8791-8800.

[74] Daryabari, Seyedeh Sima, et al. "Development of an efficient perfusion-based protocol for whole-organ decellularization of the ovine uterus as a human-sized model and in vivo application of the bioscaffolds." *Journal of assisted reproduction and genetics*36.6 (2019): 1211-1223.

[75] Gargus, Emma S., et al. "Engineered reproductive tissues." *Nature biomedical engineering* 4.4 (2020): 381-393.

[76] Vermeulen, Maxime, et al. "Development of a cytocompatible scaffold from pig immature testicular tissue allowing human sertoli cell attachment, proliferation and functionality." *International journal of molecular sciences* 19.1 (2018): 227.

[77] Kargar-Abarghouei, Elias, et al. "Characterization, recellularization, and transplantation of rat decellularized testis scaffold with bone marrow-derived mesenchymal stem cells." *Stem cell research & therapy* 9.1 (2018): 1-16.

[78] Akbarzadeh, Aram, et al. "Decellularised whole ovine testis as a potential bio-scaffold for tissue engineering." *Reproduction, Fertility and Development* 31.11 (2019): 1665-1673.

[79] Barreto, Rodrigo SN, et al. "Mouse placental scaffolds: a three-dimensional environment model for recellularization." *Journal of Tissue Engineering* 10 (2019): 2041731419867962.

[80] Favaron, Phelipe O., et al. "Establishment of 3-dimensional scaffolds from hemochorial placentas." *Placenta* 81 (2019): 32-41.

[81] Motamed, M., et al. "Tissue engineered human amniotic membrane application in mouse ovarian follicular culture." *Annals of biomedical engineering* 45.7 (2017): 1664-1675.

[82] Oktay, Kutluk, et al. "First pregnancies, live birth, and in vitro fertilization outcomes after transplantation of frozen-banked ovarian tissue with a human extracellular matrix scaffold using robot-assisted minimally invasive surgery." *American journal of obstetrics and gynecology* 214.1 (2016): 94-e1.

[83] Baert, Yoni, et al. "Derivation and characterization of a cytocompatible scaffold from human testis." *Human reproduction*30.2 (2015): 256-267.

[84] Baert, Yoni, et al. "Primary human testicular cells self-organize into organoids with testicular properties." *Stem Cell Reports* 8.1 (2017): 30-38.

[85] Baert, Yoni, Charlotte Rombaut, and Ellen Goossens. "Scaffold-based and scaffold-free testicular organoids from primary human testicular cells." *Organoids*. Humana, New York, NY, 2017. 283-290.

[86] Park, Min Hee, et al. "Effects of extracellular matrix protein-derived signalling on the maintenance of the undifferentiated state of Spermatogonial stem cells from porcine neonatal testis." *Asian-Australasian Journal of Animal Sciences* 29.10 (2016): 1398.

[87] Scarritt, Michelle E., Nicholas C. Pashos, and Bruce A. Bunnell. "A review of cellularization strategies for tissue engineering of whole organs." *Frontiers in bioengineering and biotechnology* 3 (2015): 43.

[88] Jakus, Adam E., et al. "“Tissue Papers” from organ-specific decellularized extracellular matrices." *Advanced functional materials* 27.34 (2017): 1700992.

[89] Topraggaleh, Tohid Rezaei, et al. "A testis-derived macroporous 3D scaffold as a platform for the generation of mouse testicular organoids." *Biomaterials science* 7.4 (2019): 1422-1436.

[90] Henning, Nathaniel F., et al. "Proteomic analyses of decellularized porcine ovaries identified new matrisome proteins and spatial differences across and within ovarian compartments." *Scientific reports* 9.1 (2019): 1-12.

[91] Ouni, Emna, et al. "A draft map of the human ovarian proteome for tissue engineering and clinical applications." *Molecular & Cellular Proteomics* 18 (2019): S159-S173.

[92] Murphy, Sean V., and Anthony Atala. "3D bioprinting of tissues and organs." *Nature biotechnology* 32.8 (2014): 773-785.

[93] Billiet, Thomas, et al. "A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering." *Biomaterials*33.26 (2012): 6020-6041.

[94] Raffel, Nathalie, et al. "Novel approach for the assessment of ovarian follicles infiltration in polymeric electrospun patterned scaffolds." *Plos one* 14.4 (2019): e0215985.

[95] Baert, Yoni, et al. "Mouse in vitro spermatogenesis on alginate-based 3D bioprinted scaffolds." *Biofabrication* 11.3 (2019): 035011.

[96] Kuo, Che-Ying, et al. "Development of a 3D printed, bioengineered placenta model to evaluate the role of trophoblast migration in preeclampsia." *ACS Biomaterials Science & Engineering* 2.10 (2016): 1817-1826.

[97] Kuo, Che-Ying, et al. "Trophoblast–endothelium signalling involves angiogenesis and apoptosis in a dynamic bioprinted placenta model." *Biotechnology and bioengineering* 116.1 (2019): 181-192.

[98] Paul, Kallyanashis, et al. "3D bioprinted endometrial stem cells on melt electrospun poly ε-caprolactone mesh for pelvic floor application promote anti-inflammatory responses in mice." *Acta biomaterialia* 97 (2019): 162-176.

[99] Ovsianikov, Aleksandr, Ali Khademhosseini, and Vladimir Mironov. "The synergy of scaffold-based and scaffold-free tissue engineering strategies." *Trends in biotechnology* 36.4 (2018): 348-357.

[100] Huch, Meritxell, and Bon-Kyoung Koo. "Modeling mouse and human development using organoid cultures." *Development*142.18 (2015): 3113-3125.

[101] Cui, X., Y. Hartanto, and H. Zhang. "Advances in multicellular spheroids formation " *Journal of the Royal Society Interface*14.127 (2017): 20160877.

[102] De Gregorio, Vincenza, et al. "An engineered cell-instructive stroma for the fabrication of a novel full thickness human cervix equivalent in vitro." *Advanced healthcare materials* 6.11 (2017): 1601199.

[103] Zuk, Anna Karolina, et al. "Modeling and validating three dimensional human normal cervix and cervical cancer tissues in vitro." *Journal of biomedical research* 31.3 (2017): 240.

[104] von Kopylow, Kathrein, et al. "Dynamics, ultrastructure and gene expression of human in vitro organized testis cells from testicular sperm extraction biopsies." *MHR: Basic science of reproductive medicine* 24.3 (2018): 123-134.

[105] Higaki, Shogo, et al. "In vitro differentiation of fertile sperm from cryopreserved spermatogonia of the endangered endemic cyprinid honmoroko (Gnathopogon caerulescens)." *Scientific reports* 7.1 (2017): 1-14.

[106] Azizi, Hossein, Thomas Skutella, and Abdolhossein Shahverdi. "Generation of mouse spermatogonial stem-cell-colonies in a non-adherent culture." *Cell Journal (Yakhteh)* 19.2 (2017): 238.

[107] Lin, Zachary Yu-Ching, et al. "Sphere-formation culture of testicular germ cells in the common marmoset, a small New World monkey." *Primates* 57.1 (2016): 129-135.

[108] Pendergraft, Samuel S., et al. "Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity in vitro." *Biology of Reproduction* 96.3 (2017): 720-732.

[109] Strange, Daniel P., et al. "Human testicular organoid system as a novel tool to study Zika virus pathogenesis." *Emerging microbes & infections* 7.1 (2018): 1-7.

[110] Murphy, Alina R., et al. "Generation of multicellular human primary endometrial organoids." *JoVE (Journal of Visualized Experiments)* 152 (2019): e60384.

[111] Sakib, Sadman, et al. "Generation of porcine testicular organoids with testis specific architecture using microwell culture." *JoVE (Journal of Visualized Experiments)* 152 (2019): e60387.

[112] Sakib, Sadman, et al. "Formation of organotypic testicular organoids in microwell culture." *Biology of reproduction* 100.6 (2019): 1648-1660.

[113] Zhang, Boyang, et al. "Advances in organ-on-a-chip engineering." *Nature Reviews Materials* 3.8 (2018): 257-278.

[114] Bhatia, Sangeeta N., and Donald E. Ingber. "Microfluidic organs-on-chips." *Nature biotechnology* 32.8 (2014): 760-772.

[115] Kim, Minseok S., et al. "A microfluidic in vitro cultivation system for mechanical stimulation of bovine embryos." *Electrophoresis*30.18 (2009): 3276-3282.

[116] Zhu, Jie, et al. "Human fallopian tube epithelium co-culture with murine ovarian follicles reveals crosstalk in the reproductive cycle." *MHR: Basic science of reproductive medicine* 22.11 (2016): 756-767.

[117] García, Elina V., et al. "Bovine embryo-oviduct interaction in vitro reveals an early cross talk mediated by BMP signalling." *Reproduction* 153.5 (2017): 631-643.

[118] Ferraz, Marcia AMM, et al. "Improved bovine embryo production in an oviduct-on-a-chip system: prevention of poly-spermic fertilization and parthenogenic activation." *Lab on a Chip* 17.5 (2017): 905-916.

[119] Komeya, Mitsuru, et al. "Long-term ex vivo maintenance of testis tissues producing fertile sperm in a microfluidic device." *Scientific reports* 6.1 (2016): 1-10.

[120] De Michele, Francesca, et al. "Preserved seminiferous tubule integrity with spermatogonial survival and induction of Sertoli and Leydig cell maturation after long-term organotypic culture of prepubertal human testicular tissue." *Human reproduction* 32.1 (2017): 32-45.

[121] Yamanaka, Hiroyuki, et al. "A monolayer microfluidic device supporting mouse spermatogenesis with improved visibility." *Biochemical and Biophysical Research Communications* 500.4 (2018): 885-891.

[122] Gnecco, Juan S., et al. "Compartmentalized culture of perivascular stroma and endothelial cells in a microfluidic model of the human endometrium." *Annals of biomedical engineering*45.7 (2017): 1758-1769.

[123] Lee, Ji Soo, et al. "Placenta-on-a-chip: a novel platform to study the biology of the human placenta." *The Journal of Maternal-Fetal & Neonatal Medicine* 29.7 (2016): 1046-1054.

[124] Blundell, Cassidy, et al. "A microphysiological model of the human placental barrier." *Lab on a Chip* 16.16 (2016): 3065-3073.

[125] Nishiguchi, Akihiro, et al. "In vitro placenta barrier model using primary human trophoblasts, underlying connective tissue and vascular endothelium." *Biomaterials* 192 (2019): 140-148.

[126] Pemathilaka, Rajeendra L., et al. "Placenta-on-a-Chip: In Vitro Study of Caffeine Transport across Placental Barrier Using Liquid Chromatography Mass Spectrometry." *Global Challenges*3.3 (2019): 1800112.

[127] Arumugasaamy, Navein, et al. "Assessing SSRIs' effects on fetal cardiomyocytes utilizing placenta-fetus model." *Acta biomaterialia* 99 (2019): 258-268.

[128] Arumugasaamy, Navein, et al. "Model placental barrier phenotypic response to fluoxetine and sertraline: a comparative study." *Advanced healthcare materials* 8.18 (2019): 1900476.

[129] Yin, Fangchao, et al. "A 3D human placenta-on-a-chip model to probe nanoparticle exposure at the placental barrier." *Toxicology in Vitro* 54 (2019): 105-113.

[130] Arumugasaamy, Navein, et al. "Biomimetic placenta-fetus model demonstrating maternal–fetal transmission and fetal neural toxicity of zika virus." *Annals of Biomedical Engineering* 46.12 (2018): 1963-1974.

[131] Abbas, Yassen, et al. "A microfluidics assay to study invasion of human placental trophoblast cells." *Journal of The Royal Society Interface* 14.130 (2017): 20170131.

[132] Li, Shan-Shan, et al. "Modeling ovarian cancer multicellular spheroid behavior in a dynamic 3D peritoneal microdevice." *JoVE (Journal of Visualized Experiments)* 120 (2017): e55337.

[133] Zhou, Hong, et al. "Hydrogel based 3-dimensional (3D) system for toxicity and high-throughput (HTP) analysis for cultured murine ovarian follicles." *PLoS One* 10.10 (2015): e0140205.

[134] Xiao, Shuo, et al. "Doxorubicin has dose-dependent toxicity on mouse ovarian follicle development, hormone secretion, and oocyte maturation." *Toxicological Sciences* 157.2 (2017): 320-329.

[135] Meng, Chun-Xia, et al. "Effect of levonorgestrel and mifepristone on endometrial receptivity markers in a three-dimensional human endometrial cell culture model." *Fertility and sterility* 91.1 (2009): 256-264.

[136] Laronda, Monica M., et al. "Recreating the female reproductive tract in vitro using iPSC technology in a linked microfluidics environment." *Stem Cell Research & Therapy* 4.1 (2013): 1-5.

[137] Cooper, Amber R., et al. "The time is now for a new approach to primary ovarian insufficiency." *Fertility and sterility* 95.6 (2011): 1890-1897.

[138] De Vos, Michel, Johan Smitz, and Teresa K. Woodruff. "Fertility preservation in women with cancer." *The Lancet* 384.9950 (2014): 1302-1310.

[139] Jeruss, Jacqueline S., and Teresa K. Woodruff. "Preservation of fertility in patients with cancer." *New England Journal of Medicine* 360.9 (2009): 902-911.

[140] Donnez, Jacques, and Marie-Madeleine Dolmans. "Fertility preservation in women." *New England Journal of Medicine*377.17 (2017): 1657-1665.

[141] Donnez, Jacques, et al. "Ovarian cortex transplantation: time to move on from experimental studies to open clinical application." *Fertility and sterility* 104.5 (2015): 1097-1098.

[142] Amorim, Christiani A., and Ariella Shikanov. "The artificial ovary: current status and future perspectives." *Future oncology* 12.19 (2016): 2323-2332.

[143] Kniazeva, E., et al. "Primordial follicle transplantation within designer biomaterial grafts produce live births in a mouse infertility model." *Scientific reports* 5.1 (2015): 1-11.

[144] Rios, Peter D., et al. "Retrievable hydrogels for ovarian follicle transplantation and oocyte collection." *Biotechnology and bioengineering* 115.8 (2018): 2075-2086.

[145] Vanacker, Julie, et al. "Transplantation of an alginate–matrigel matrix containing isolated ovarian cells: first step in developing a biodegradable scaffold to transplant isolated preantral follicles and ovarian cells." *Biomaterials* 33.26 (2012): 6079-6085.

[146] Day, James R., et al. "Immunoisolating poly (ethylene glycol) based capsules support ovarian tissue survival to restore endocrine function." *Journal of biomedical materials research Part A* 106.5 (2018): 1381-1389.

[147] Felder, Shani, et al. "Reconstruction of the ovary microenvironment utilizing macroporous scaffold with affinity-bound growth factors." *Biomaterials* 205 (2019): 11-22.

[148] Sittadjody, Sivanandane, et al. "Encapsulation of mesenchymal stem cells in 3D ovarian cell constructs promotes stable and long-term hormone secretion with improved physiological outcomes in a syngeneic rat model." *Annals of biomedical engineering* 48.3 (2020): 1058-1070.

[149] David, Anu, James Day, and Ariella Shikanov. "Immunoisolation to prevent tissue graft rejection: Current knowledge and future use." *Experimental Biology and Medicine* 241.9 (2016): 955-961.

[150] David, Anu, et al. "Restoring ovarian endocrine function with encapsulated ovarian allograft in immune competent mice." *Annals of biomedical engineering* 45.7 (2017): 1685-1696.

[151] Sittadjody, Sivanandane, et al. "In vivo transplantation of 3D encapsulated ovarian constructs in rats corrects abnormalities of ovarian failure." *Nature communications* 8.1 (2017): 1-13.

[152] Yakhnenko, Ilya, et al. "Cryopreservation of Human Insulin Expressing Cells Macro-Encapsulated in a Durable Therapeutic Immunoisolating Device Theracyte™." *CryoLetters* 33.6 (2012): 518-531.

[153] Veiseh, Omid, et al. "Size-and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates." *Nature materials* 14.6 (2015): 643-651.

[154] Calafiore, Riccardo, et al. "Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases." *Diabetes care* 29.1 (2006): 137-138.

[155] Woodruff, Teresa K., Divya K. Shah, and Wendy S. Vitek, eds. *Textbook of oncofertility research and practice: a multidisciplinary approach*. Springer, 2019.

[156] Kaneko, Hiroyuki, et al. "Generation of live piglets for the first time using sperm retrieved from immature testicular tissue cryopreserved and grafted into nude mice." *PloS one* 8.7 (2013): e70989.

[157] Hermann, BrianáP, et al. "Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm." *Cell stem cell* 11.5 (2012): 715-726.

[158] 158-Fayomi, Adetunji P., et al. "Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring." *Science* 363.6433 (2019): 1314-1319.

[159] Liu, Zhen, et al. "Generation of macaques with sperm derived from juvenile monkey testicular xenografts." *Cell research* 26.1 (2016): 139-142.

[160] Ohta, Hiroshi, and Teruhiko Wakayama. "Generation of normal progeny by intracytoplasmic sperm injection following grafting of testicular tissue from cloned mice that died postnatally." *Biology of reproduction* 73.3 (2005): 390-395.

[161] Poels, Jonathan, et al. "Vitrification preserves proliferation capacity in human spermatogonia." *Human Reproduction* 28.3 (2013): 578-589.

[162] Medrano, Jose V., et al. "Germ cell transplantation into mouse testes procedure." *Fertility and Sterility* 102.4 (2014): e11-e12.

[163] Poels, Jonathan, et al. "In search of better spermatogonial preservation by supplementation of cryopreserved human immature testicular tissue xenografts with N-acetylcysteine and testosterone." *Frontiers in surgery* 1 (2014): 47.

[164] De Michele, Francesca, et al. "Haploid germ cells generated in organotypic culture of testicular tissue from prepubertal boys." *Frontiers in Physiology* (2018): 1413.

[165] Lee, Kyung Hoon, et al. "Vitrified canine testicular cells allow the formation of spermatogonial stem cells and seminiferous tubules following their xenotransplantation into nude mice." *Scientific reports* 6.1 (2016): 1-11.

[166] Honaramooz, Ali, et al. "Building a testis: formation of functional testis tissue after transplantation of isolated porcine (Sus scrofa) testis cells." *Biology of reproduction* 76.1 (2007): 43-47.

[167] Brinster, Ralph L. "Germline stem cell transplantation and transgenesis." *Science* 296.5576 (2002): 2174-2176.

[168] Xin, Liaobing, et al. "A collagen scaffold loaded with human umbilical cord-derived mesenchymal stem cells facilitates endometrial regeneration and restores fertility." *Acta biomaterialia* 92 (2019): 160-171.

[169] Zhu, Lan, et al. "Anatomic and sexual outcomes after vaginoplasty using tissue-engineered biomaterial graft in patients with Mayer-Rokitansky-Küster-Hauser syndrome: A new minimally invasive and effective surgery." *The journal of sexual medicine* 10.6 (2013): 1652-1658.

[170] Ding J-X, Chen X-J, Zhang X.-y, Zhang Y & Hua K-Q Acellular porcine small intestinal submucosa graft for cervicovaginal reconstruction in eight patients with malformation of the

[171] uterine cervix. Hum. Reprod 29, 677–682 (2014). [PubMed: 24476994]

[172] Ding, Jing-Xin, et al. "Vaginoplasty using acellular porcine small intestinal submucosa graft in two patients with Meyer-von-Rokitansky-Küster-Hauser syndrome: a prospective new technique for vaginal reconstruction." *Gynecologic and obstetric investigation* 75.2 (2013): 93-96.

[173] Li, Yanan, et al. "Bone marrow mesenchymal stem cells could acquire the phenotypes of epithelial cells and accelerate vaginal reconstruction combined with small intestinal submucosa." *Cell biology international* 39.11 (2015): 1225-1233.

[174] Dias, Maria Tereza Pinto Medeiros, et al. "Tilapia fish skin as a new biologic graft for neovaginoplasty in Mayer-Rokitansky-Kuster-Hauser syndrome: a video case report." *Fertility and Sterility* 112.1 (2019): 174-176.

[175] Dabaghi, Mohammadhossein, et al. "An artificial placenta type microfluidic blood oxygenator with double-sided gas transfer microchannels and its integration as a neonatal lung assist device." *Biomicrofluidics* 12.4 (2018): 044101.

[176] Hunt, Patricia A., et al. "Bisphenol A exposure causes meiotic aneuploidy in the female mouse." *Current biology* 13.7 (2003): 546-553.

[177] Chambers, G., Kevin Doody, and Sara Crawford. "Reporting ART success rates." *Assisted Reproductive Technology Surveillance*. Cambridge University Press, Cambridge, UK, 2019. 37-46.

[178] Campo-Engelstein, Lisa, et al. "The ethics of fertility preservation for pediatric patients with differences (disorders) of sex development." *Journal of the Endocrine Society* 1.6 (2017): 638-645.

[179] Woodruff, Teresa K., et al., eds. *Oncofertility: ethical, legal, social, and medical perspectives*. Vol. 156. Springer Science & Business Media, 2010.

[180] Segers, Seppe, et al. "Balancing ethical pros and cons of stem cell derived gametes." *Annals of biomedical engineering* 45.7 (2017): 1620-1632.

[181] Basco, Daniel, Lisa Campo-Engelstein, and Sarah Rodriguez. "Insuring against infertility: expanding state infertility mandates to include fertility preservation technology for cancer patients." *Journal of Law, Medicine & Ethics* 38.4 (2010): 832-839.

[182] Fleetwood, Amanda, and Lisa Campo-Engelstein. "The impact of infertility: why ART should be a higher priority for women in the global South." *Oncofertility* (2010): 237-248.

[183] Campo-Engelstein, Lisa. "Consistency in insurance coverage for iatrogenic conditions resulting from cancer treatment including fertility preservation." *Journal of Clinical Oncology* 28.8 (2010): 1284.

[184] Chatzinikolaou, Nikolaos. "The ethics of assisted reproduction." *Journal of reproductive immunology* 85.1 (2010): 3-8.

[185] Brezina, Paul R., and Yulian Zhao. "The ethical, legal, and social issues impacted by modern assisted reproductive technologies." *Obstetrics and gynecology international* 2012 (2012).

[186] Campo-Engelstein, Lisa. "Offering testicular tissue cryopreservation to boys: the increasing importance of biological fatherhood." *The American Journal of Bioethics* 13.3 (2013): 39-40.