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REVIEW OPEN ACCESS

Developing Human Reproductive Organoids to Combat Infertility: A Literature Review

Ronit Mohapatra, BSc Student [1]*

[1] Department of Biochemistry, McGill University, Montreal, Quebec, Canada H3A 0G4

*Corresponding Author: ronit.mohapatra@mail.mcgill.ca

Abstract

Introduction: Infertility affects a significant portion of the population, up to 1 in 5 North American adults. The lack of accurate reproductive models has limited clinical research with 15% of infertility cases remaining untreatable. However, advances in stem cell technology have allowed for the development of organoids, artificial 3D organ systems in culture (also referred to as "organs-in-a-dish"), as accurate, human-specific research models. We propose that organoid systems are valuable tools to advance reproductive health research and aim to assess major progress and limitations of this technology relating to infertility.

Methods: A literature review was performed using the PubMed and Google Scholar databases. We identified 10 studies published from 2017 onwards that focused on the application of reproductive organoid systems in infertility treatments or the development of model systems for infertility research. These studies were compared and analyzed in terms of methodology, clinical applications, and potential limitations.

Results: Both female and male reproductive tracts (FRT and MRT) are complex systems with many potential causes for infertility. We identified the ovary, fallopian tubes and endometrium in the FRT and the prostate, epididymis and testes in the MRT as the most promising current organoid models. Organoid systems have been used in transplantation techniques to treat the infertility disorders of Asherman's syndrome and azoospermia. As well, organoids function as disease models for drug screening including chemotherapeutic compounds or as physiologic models to study fundamental mechanisms of fertility considering factors like ageing and environmental gonad toxicity.

Discussion: The various novel applications of reproductive organoids emphasize their potential in infertility research and the development of personalized medicine. However, lack of cross-organ communication and minimal microbiome modeling limit organoid-based research. Conversion from animal to human organoid models is also a major obstacle to be addressed for the advancement this technology in reproductive health science.

Conclusion: This review highlights the unique benefits of using organoids over traditional research models as well as the most critical research gaps in this field to guide future studies and accelerate the development of clinical techniques for human infertility treatment.

Keywords: organoids; stem cell technology; infertility; personalized medicine; research models; reproductive health

Introduction

Infertility is a reproductive disorder characterized by the prolonged inability to achieve pregnancy [1]. Despite the development of In Vitro Fertilization (IVF), 10-15% of infertility cases remain untreated [1,2]. IVF success rates remain below 40% and decrease with age, despite the global trend of delaying parenthood [3–5]. Therefore, improved characterization of human reproductive systems on both the physiological and molecular levels is needed to better address unresolved infertility cases.

Female and male reproductive tracts (FRT and MRT, respectively) are complex systems each containing a primary organ for gamete production, spermatozoa in the testis and oocytes in the ovary, which collectively function to achieve pregnancy [6]. Spermatozoa develop fertilization potential in the epididymis and are then combined with liquid produced by the prostate gland for ejaculation as semen [7]. In the FRT, mature oocytes travel from the ovaries through the fallopian tubes where male and female gametes combine to form a zygote (fertilized egg). The zygote then develops into a multicellular blastocyst before implanting on the endometrium for embryonic development [8]. Various factors such as cancer, genetics, hormone imbalance, or exposure to environmental toxins can disrupt this intricate process, leading to infertility [9]. Research and treatment of various infertility associated disorders has long been hindered by the lack of comprehensive biological models for either reproductive tract [10].

Currently, the most common biomedical research models used to assess cellular functions and biochemical

properties are 2D cell cultures which grow in plates as a monolayer [11]. However, these models have limitations. Primary cell cultures derived from live tissue samples have a limited lifespan and eventually reach replicative senescence. These cell cultures also fail to mimic the complex 3D microenvironment of human organs due to the absence of 3D cell-cell and cell-environment interactions [11,12]. Although model organisms like mice can be used for *in vivo* clinical testing and research, factors such as ethical concerns and inter-species differences complicate the translation of discoveries from animal models to humans [11,13]. For instance, a major physiological difference in reproductive health is that human FRTs undergo menstrual cycles, while female non-primate placental mammals follow an estrous cycle [7,8].

3D cell cultures which mimic live organs *in vitro* (i.e. organoids) are a potential complement or alternative to current research methodologies [11,12]. These models can be developed for various organs through the differentiation and proliferation of two classes of stem cells derived from live tissue samples (see Figure 1). Adult stem cells (ASCs) are multipotent and exist in most adult tissues with the potential to differentiate into a subset of cell types specific to a particular organ [11,14]. Alternatively, pluripotent stem cells have the potential to differentiate into any cell type and exist naturally as embryonic stem cells (ESCs). Though it is a more complicated methodology, human organoid cultures can also be established from differentiated adult cells by genetically reprogramming them into induced pluripotent stem cells (iPSCs) using ESC factors [11]. Cancerous tissues are also used to establish tumor models and advance oncological research [11,15,16].

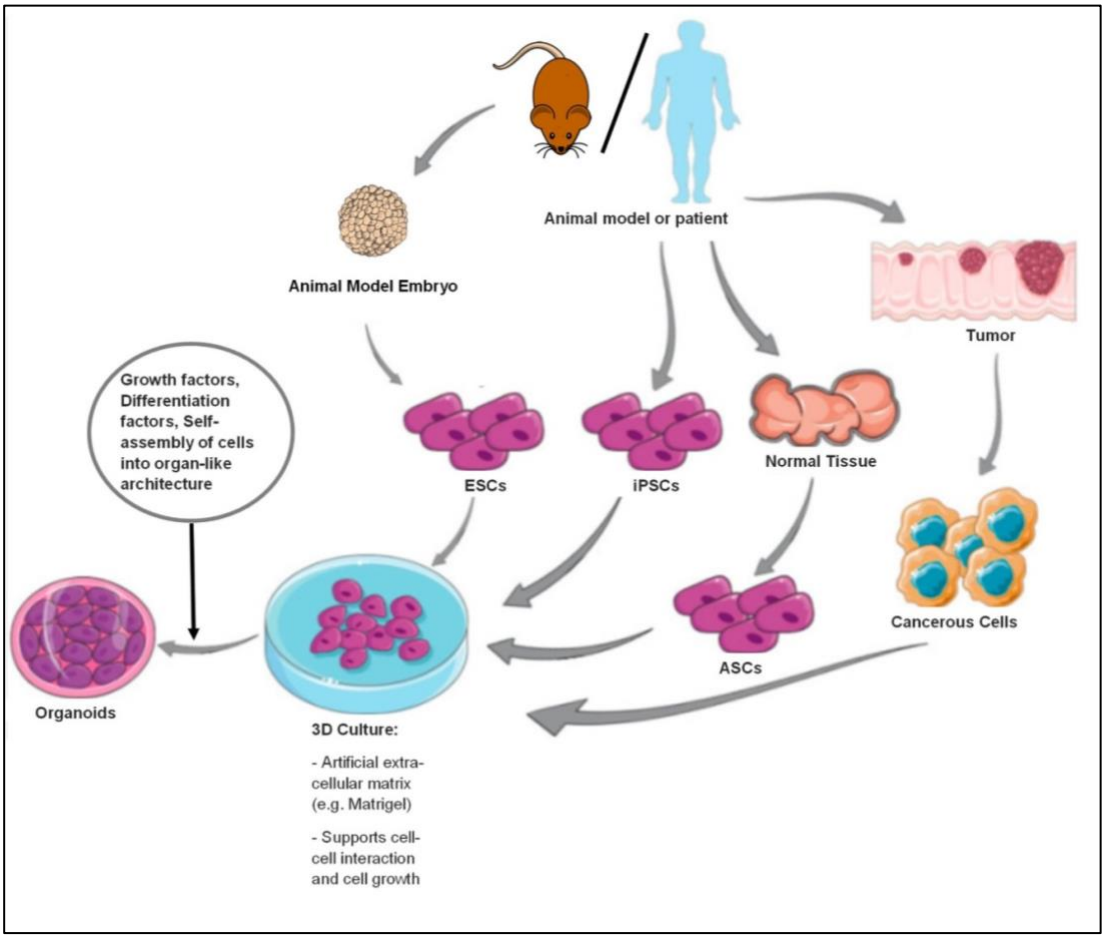

Figure 1. General procedures for the establishment of novel organoid systems. Multipotent adult stem cells (ASCs) can be isolated from normal tissue harvested from animal dissections or from patient samples. Cancerous cells may also be collected to induce tumor organoids. For pluripotent stem cells the options are either induced pluripotent stem cells (iPSCs, from genetically modified human or animal ASCs) or embryonic stem cells (ESCs, harvested from animal model embryos). In all cases, the original cells are seeded into a 3D culture medium to support their interactions and treated with growth and differentiation factors to induce the development of a specific organ type. Adapted from Azar et al. 2021 with permission from Dr. Al-Sayegh and Dr. Abou-Kheir from the International Journal of Molecular Sciences published by MDPI [14].

Stem cells are seeded into a culture which includes an extracellular matrix (ECM) to support cell-cell interactions and tissue growth. This can be achieved using a 3D biological matrix such as commercially available Matrigel derived from mouse sarcoma tissues or using recombinant human extracellular proteins like collagen and fibrin in an organic gel [17,18]. Differentiation factors, growth factors, and hormones are added to the 3D culture medium to induce the development of desired cell types and mimic the organ-specific microenvironment [19]. The combination of these molecular factors, along with a 3D biological matrix providing structural support to proliferating cells, simulates the process of natural organ development. On a microscopic level, resulting organoids closely resemble the living tissue upon which their artificial environment is based. This emerging model type is advantageous as it accurately represents live human organs and may be maintained long-term in culture [20]. Clinically, organoids may become more reliable and cost-effective alternative to animal testing and organotypic cultures may be developed for artificial transplantation [7,8].

Based on the findings of this review, we propose that reproductive organoids are essential tools for advancing research into the current major causes of human infertility. Organoids not only overcome the physical limitations of 2D cell cultures and animal models but also hold promise in developing patient-specific treatments and improving transplantation success [21]. Organoid models have already been established for various reproductive organs and are being applied in investigations related to infertility and reproductive mechanisms [6]. This study aims to assess the recent application of organoids on a case-specific basis, highlighting their vast potential and remaining challenges to their implementation in the field of reproductive health. Our focus is on reproductive tract organoids with direct applications to infertility research and treatments: ovary, fallopian tube and endometrium models in the FRT and the prostate, testis and epididymis in the MRT (see Figure 2).

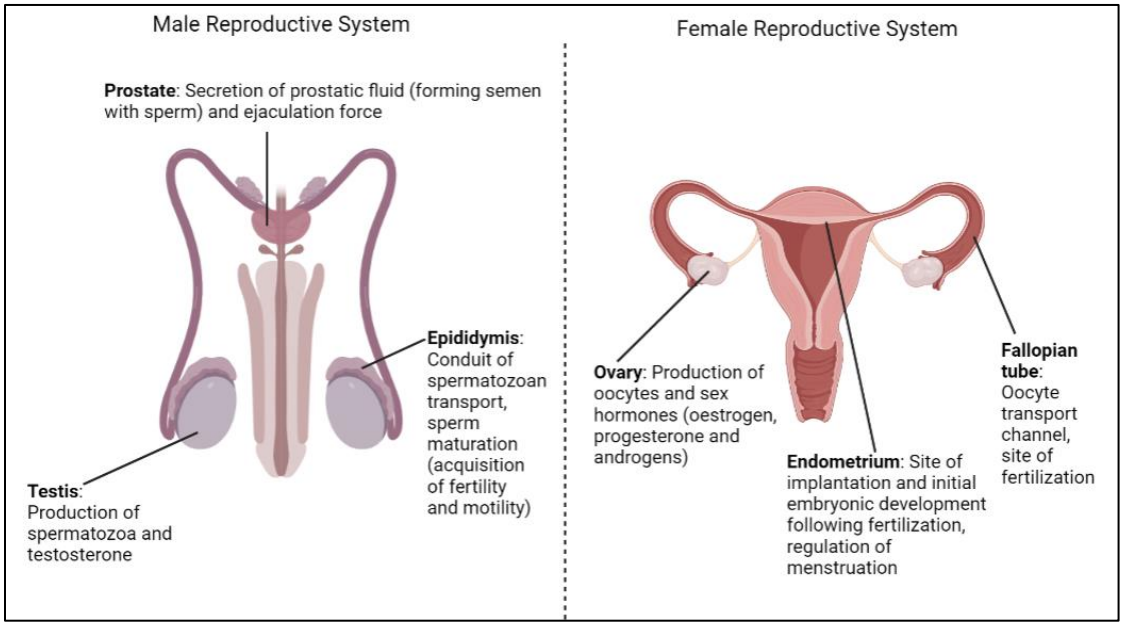

Figure 2. Targeted reproductive components for organoid-based reproductive health research. Graphical depiction of human male and female reproductive tracts. Organs which are primarily targeted for organoid development relating to infertility research and discussed in this review are identified with their respective functions in the reproductive process stated. Created with BioRender.

Methods

This study utilized Google Scholar and PubMed as the primary search engines to identify relevant sources. Continuous research was conducted throughout the writing process. Specific search terms, including "[organ of interest] organoids" and "[infertility disorder]," were combined to identify studies conducted within the last decade (2013-2023) that either developed reproductive organoids as models to study fertility or directly applied organoid technology to the treatment of infertility. The introduction and Figure 1 highlight the organs of interest, while the major infertility disorders include reproductive cancers, endometriosis, Asherman's syndrome, azoospermia, reproductive aging, and environmental toxicity. Ten organoid-related studies were selected as significantly relevant based on the outlined criteria. Their implications and major applications were analyzed and summarized in data tables, while their common limitations are later discussed (see Table 1 and Table 2).

Results

There are numerous potential mechanisms of infertility in both sexes. In this section we focus only on a few of the current major infertility associated disorders and explore specific studies which have aimed to use organoid technology to advance research and treatment of these disorders.

Organoids to Combat Female Infertility

Ovarian Organoids

The ovaries are crucial for female fertility, as they are responsible for oocyte production and hormone secretion [8]. Ovarian organoids have been developed and recently designed to model reproductive disorders and minimize the risk of infertility onset. Researchers have primarily focused on developing ovarian organoids using diseased tissue, particularly for the establishment of cancer models [22–24].

Ovarian cancer is the uncontrolled proliferation of ovarian epithelial cells and presents challenges in assessment and treatment due to its high heterogeneity, resulting in genetic and physiological variations among cancer cell populations [22]. Current treatments include chemotherapy which generally targets rapidly dividing tumor cells. For example, paclitaxel is a platinum/taxane drug which inhibits cell division and induces cell death by targeting microtubules, structures required for cellular partition. However, this action also inhibits gamete production thus reducing fertility, though a myriad of genetic factors will affect the intensity of each patient's drug response [22–24]. The side-effect of infertility highlights the necessity for the development of targeted, non-invasive treatment options for ovarian cancer.

In 2019, one study proposed the use of ovarian organoids as a potential solution for treating highly heterogeneous ovarian cancers [22]. Patient-derived tumor samples were utilized to establish 56 organoid cultures representing all major subtypes of ovarian carcinomas. These models faithfully recapitulated histological and molecular features of the original tumors including distinct drug sensitivities observed in clinical settings [22]. The ability of organoids to replicate patient-specific characteristics highlights their potential in personalized medicine, enabling the prediction of drug responses based on individual genetics. Furthermore, tumor organoids have been established from minimal tissue samples such as needle biopsies [25]. By combining non-invasive tissue harvesting techniques with the culture of patient-specific ovarian organoids, individualized disease models may be established for chemotherapeutic drug response testing. This approach could greatly optimize chemotherapy and improve ovarian cancer patient outcomes while preserving fertility [26].

Premature ovarian insufficiency (POI) is another significant cause of infertility, affecting 1 in 1000 women over 30 and is characterized by the decline of ovarian function during expected reproductive age [21]. The molecular mechanisms causing POI remain poorly understood though environmental toxins, oxidative damage and ageing are hypothesized to contribute to its onset [21].

To unravel the mechanism of POI, researchers in 2021 focused on the development of ovarian organoids which accurately mimic healthy ovarian function as opposed to disease models [27]. They established a novel method for generating functional ovarian organoids using female germline stem cells isolated from neonatal mice and cultured on Matrigel [27]. These organoids self-assembled into a complex multi-layered tissue structure similar to native ovaries. These models were confirmed to produce oocytes and the transplantation of organoid-generated oocytes into infertile mice restored fertility [27]. Additionally, toxicological surveys conducted on the ovarian organoids revealed decreased oocyte production in the presence of environmental toxicants which have been linked with POI onset such as bisphenol A [27]. Further research on these ovarian organoid models could contribute to identification of potential environmental triggers of POI and the development of novel transplantation treatments [21].

Fallopian Tube Organoids

The fallopian tubes (FTs) are the typical location of fertilization and facilitate the passage of oocytes from the ovaries to the uterus for implantation [8]. FT tissue architecture is crucial to its function as secretory and ciliated epithelial cells line the inner tube to support oocyte transport along with smooth muscle contractions [8]. Researchers have aimed to develop organoids using human ASCs and more recently iPSCs to mimic intricate FT tissue organization [28,29]. While there is limited progress in combining ovarian and FT organoids to simulate oocyte transport in vitro, FT organoids are valuable models for studying ectopic implantation (embryonic development outside of the uterus) and reproductive cancers relating to infertility [30,31].

Fallopian tube cancers share many clinical complications with ovarian cancers including harsh treatment leading to infertility, generating need for targeted, patient-specific therapies to improve treatment outcomes [8]. In 2019, a novel method for generating organoids was established using adult mouse FT epithelial cells scaffolded on Matrigel [32]. These organoid models were genetically comparable to their respective original donor cells [32]. Subsequently, healthy FT organoids were genetically engineered to express common mutations found in tubo-ovarian cancers [33]. This disease model platform later enabled researchers to preclinically evaluate the efficacy of various therapies and identify synergistic drug combinations which optimized tumor inhibition based on individual genetics [33]. While reproducing these results using human-patient derived organoid systems remains challenging, this research shows FT organoids could contribute to the development of less destructive cancer treatments which potentially preserve fertility.

Endometrial Organoids

The endometrium is the inner mucosal layer lining the uterine walls which goes through proliferative, secretory, and menstruation phases in response to ovarian hormones [8,34]. Endometrial organoids have been established from patient-derived ASCs which accurately represent the cellular composition and are responsive to hormonal signals to simulate all menstrual phases and early pregnancy [20]. To date, these organoids have been used to study endometrial regulation and disorders which are known to lead to infertility [35].

Endometriosis is one such major endometrial disorder in which endometrial tissue grows outside of the uterus triggered by genetics and hormonal disequilibrium [36]. Abnormal endometrial growth can obstruct fallopian tubes and cause severe pelvic inflammation, which increases the risk of infertility [36]. Due to the patient-specific nature of hormonal imbalances, there is a need to develop personalized disease models to optimize targeted, non-invasive hormonal therapies [37].

Patient-derived organoids have been recently established from endometrial biopsies using human ASCs scaffolded on a Matrigel-based medium [38]. These organoids capture the heterogeneity of endometrial diseases, exhibiting distinct molecular profiles and drug sensitivities among different patients, which highlighted the potential use of organoids for personalized therapies [38]. However, a 2020 study emphasized that such disease models lack key components of the organ microenvironment, such as the patient's microbiome, which may influence drug response *in vivo* [39].

Organoid technology also shows promise in the treatment of Asherman's Syndrome, a major cause of worldwide infertility. It is characterized by the formation of intrauterine adhesions (IUAs) which form as scar tissue in the uterus and impact endometrial function following trauma such as surgery [9]. Moderate to severe Asherman's Syndrome cases significantly reduce fertility even with modern surgical repairs and estrogen supplementation [9].

A 2022 study showed endometrial tissue harvested from mouse endometrial organoids scaffolded on Matrigel could be used in transplantation to improve reproductive outcomes in mice with induced IUAs [9]. The damaged endometria underwent enhanced repair following transplantation, returning to normal thickness with functional glands and blood vessels [9]. Successful endometrial organoid transplantation in animal models provides promise for further experiments in various species models and could represent a significant advancement in reproductive medicine if applied to humans.

Overall, FRT organoids are being applied both in disease modeling and in preclinical treatments like transplantations to preserve and restore fertility (see Table 1).

Table 1. Recent Applications of Organoids for Female Infertility Research

Organoid Model	Potential Cause of Infertility	Application	Reference No.
Ovary	Ovarian Cancer	Established method for culturing patient-derived organoids which accurately represent ovarian tumor heterogeneity and may be used to identify malignant states or perform personalized drug-screening.	[22]
	Premature Ovarian Insufficiency	Development of mouse female germline stem cell-derived ovarian organoids which developed follicles able to model molecular mechanisms and environmental factors leading to POI (e.g. oxidative stress).	[27]
Fallopian Tubes	Tubo-Ovarian Cancer	Development of combinatory chemotherapeutic approach based on drug screening and genotyping mouse fallopian tube-derived high grade serous tubo-ovarian cancer organoid models.	[33]
Endometrium	Asherman's Syndrome	Showed the transplantation of endometrial organoids improved reproductive rates in mice with severe intrauterine adhesions.	[9]
	Endometriosis	Developed patient-derived models of endometrial tissues for personalized drug screening and characterization of endometriosis.	[38]

Organoids to Combat Male Infertility

Prostate Organoids

The prostate gland is an integral part of the male reproductive system which produces prostatic fluid that supports sperm in ejaculation [7]. Prostate organoids have been derived from human prostate tumors, ASCs and iPSCs to model both normal and pathological prostate function [40–42].

Metabolic changes affecting the composition of prostatic secretions can cause male infertility. For instance, the onset of prostate cancer is associated with inhibited citrate secretion which negatively impacts sperm motility [42]. In 2022, researchers used ASC mouse and human organoids to study the truncated citric acid cycle pathway, responsible for prostatic citrate production, as a potential therapeutic target for prostate cancer [42]. Since organoids exist in a controlled environment while closely replicating in vivo physiological conditions, the researchers were able to precisely regulate metabolic inputs to the prostate tissue and measure prostatic secretions in isolation [40]. This approach allowed for the identification of metabolites which contribute to citrate production and provided a model to understand cancerous modifications to this process [42]. Inhibiting the cancer-specific citrate metabolism could serve as a significant clinical strategy to target prostate tumor cells and restore male fertility. The study emphasizes the unique advantages of organoid models in disease research [40,42].

Testicular Organoids

The testes are responsible for spermatozoa production in the MRT. Testicular organogenesis primarily requires the coordination of 3 cell types: spermatogonial stem cells (SSCs), which differentiate into sperm, as well as Sertoli cells and Leydig cells which release maturation factors and hormones like testosterone [43]. Testicular organoids aim to mimic the complex testicular tissue architecture and support normal spermatogenesis *in vitro* [7,44–46]. While murine and porcine ASC-derived organoids consistently satisfy both goals, current and future work aims to improve human-specific models [47,48].

Up to 25% of infertility cases are attributed to insufficient sperm quality [49]. Spermatogenesis is sensitive to environmental condition and increasing harmful toxin pollution may contribute to global trends of decreasing male fertility [50]. A major goal of testicular organoid development is to establish reproductive models for hazard assessment and identification of male gonadal toxins [51].

In 2017, Pendergraft et al. reported the use of human testicular organoids for toxicological assays, procuring testicular stem cells from brain-dead patients, along with human testis-specific ECM components, to generate organoid models producing testosterone and other molecular markers of testes tissue [51]. These organoids demonstrated low frequency *in vitro* spermatogenesis [51]. Assessing the impact of specific toxins, such as heavy metals, on sperm production using these organoid models could better reflect infertility risks for humans [51,52]. This concept has been demonstrated using rat SSCs scaffolded on Matrigel in a large-scale assay to examine how common antidepressants affect sperm production [53]. Utilizing the human organoid model from 2017 in similar assays could form a powerful platform for identifying novel gonadotoxic agents.

Age also has a significant effect on male fertility and semen quality, including reduced sperm count, viability, and motility [49]. Testicular organoids may be used to model long-term SSC pool degradation to develop reproductive treatment and management techniques to address this issue [54].

In 2022, testicular organoids mimicking *in vivo* structure and hormone responsiveness were derived from mouse testicular ASCs [55]. Instead of using an ECM component (e.g., Matrigel), isolated SSCs, Sertoli and Leydig cells were suspended in media droplets and self-assembled into a 3D structure which resembled testes [55]. Only 200 sample cells were required to establish each organoid, and cultures which genetically resembled their tissue of origin could be frozen and thawed, making this system relatively inexpensive and suitable for long-term personalized biobanks [55,56]. Individual samples could be stored at various points in life, thawed, and developed into organoids to examine the specific effect of ageing on spermatogenesis and propose personalized therapies to potentially correct declining fertility [55]. This study shows relatively non-complex organoid systems can offer meaningful models to understand the mechanisms of male fertility overtime.

Epididymis Organoids

Each testicle is connected to an epididymis, a tubule of epithelial cells responsible for sperm maturation, transport, and conservation [7,57]. Organoids are being used to model mammalian epididymis development as epididymis functionality significantly impacts male fertility, and like testicles, it is also affected by aging, leading to lower rates of sperm maturation overtime [7,57,58].

Beyond reduced sperm quality, azoospermia, a complete lack of spermatozoa in the ejaculate, is a more serious form of male infertility [59]. This disorder may be genetic or caused by trauma to the MRT. The primary treatment option for non-obstructive azoospermia, where no object or structure is physically blocking the MRT, is intracytoplasmic sperm injection using surgically retrieved sperm [60].

In 2022, Rahbar et al. devised a method for treating azoospermia in mice using a 3D culture system. Mouse SSCs were found to proliferate very efficiently in vitro when co-cultured with epididysomes, vesicles isolated from epididymis tissue, and scaffolded in three dimensions using a specialized testicular ECM hydrogel [61]. This system produced sufficient proliferated SSCs for successful transplantation into azoospermic mice to partially restore

fertility [61]. Application of this technique to humans could provide a promising alternative treatment for non-obstructive azoospermic patients without consistent surgical sperm retrieval surgeries.

In sum, MRT organoids have been repeatedly shown to be promising tools to study a variety of male reproductive disorders and maintain male fertility (see Table 2).

Table 2. Recent Applications of Organoids for Male Infertility Research

Organoid Model	Potential Cause of Infertility	Application	Reference No.
Prostate	Prostate Cancer	Modeled altered citrate secretion in prostate cancers using Mouse and Human *ex vivo* derived organoids for the development of novel therapies and improved understanding of the metabolism supporting male fertility.	[42]
Testis	Age-reduced Sperm Quality	Developed method to efficiently derive testicular organoids from primary testicular cells (mouse models) for personalized testicular biopsies and as experimental models to survey testicular function overtime.	[55]
	Gonadotoxic Exposure	1. Development of *ex vivo* human testicular organoid model for toxicological screening and modeling of sperm production under various conditions. 2. Rat SSC-derived testicular organoids used to test reproductive toxicity of antidepressants.	1. [51] 2. [53]
Epididymis	Non-obstructive Azoospermia	Use of 3D organoid system to scaffold spermatogonial stem cells for proliferation followed by transplantation into azoospermic mice as treatment.	[61]

Discussion

Reproductive organoid technology is rapidly progressing infertility research and treatment for both sexes. Organotypic cultures are the first *in vitro* models which represent both the architecture and many fundamental functions of live tissue [6–8,11]. Organoid systems have the prospective of being human-specific and are becoming less expensive to establish and maintain than mammalian research populations [11,56]. It is also morally advantageous to continue developing alternatives to live animal models. However, the development of gamete producing systems from human tissue raises also raises ethical concerns regarding informed consent and patient participation [62–64]. For this reason, human tissue samples and the use of human iPSCs are highly regulated. Most studies reviewed in this paper hence used more accessible animal models to establish their organoid systems. Converting from animal to human cell models will require significant resource investment to develop acceptable new methodologies for the consistent establishment of human organoids in reproductive research [11,65].

Examples highlighted in the results section demonstrated that, patient-derived organoids (ASCs in particular) genetically resemble their tissue of origin which opens the clinical possibility of personalized medicine based on individual patient genetics [22,27].This novel concept in regenerative medicine is rapidly becoming a reality to efficiently target reproductive disorders such as cancers while minimizing treatment side effects [22,33,38]. However, modern organoids lack multi-organ communication and microbiome interactions which limits their application.

The delivery of therapeutic treatments to diseased organs often depends on the interaction of multiple organ systems. For example, non-reproductive components of the endocrine system such as the thyroid may contribute to drug response [65]. Some studies have applied the co-culture of multiple tissue types in a 3D gel to mimic the interaction between different organs. For example, the co-culture of SSCs and epididysomes to observe how the epididymis enhances sperm production [62]. As individual organoid systems become more complex, more of these systems will need to be coupled to accurately represent certain aspects of cross-organ communication.

Microorganisms in both the MRT and FRT, also known as the reproductive microbiome, will interact with their main organism through the breakdown and secretion of metabolic products which will additionally influence fertility and drug response. Recent studies have linked female microbial signatures to endometriosis and gynecological cancers [65]. As well, there is evidence that common anticancer compounds such as 5-fluorouracil may be metabolized into more efficient forms by the microbiome to improve chemotherapeutic response [66]. Currently very limited progress has been made in

developing human organoid systems which accurately reflect the microbiome and its impact on organ function. This issue must be addressed to better model how reproductive systems may respond to various environmental and clinical exposures [39,57,66].

Despite the unique advantages of organoid models, crucial limitations remain in their application to infertility research and treatment. This technology may be optimized by focusing scientific efforts on developing human-specific organoid models with increasing complexity to simulate inter-organ interactions and microbial influences.

Conclusions

The development of organoids of the male and female reproductive tracts holds immense potential in infertility research. This study has highlighted recent progress made in reproductive organoid development for modeling personalized diseases, conducting pharmaceutical or toxicological assays, and contributing to the development of novel transplantation methods. A focus on resolving specific challenges identified here may accelerate advancements and establish organoids as beneficial alternatives to traditional cell culture or animal models in reproductive health science.

List of Abbreviations Used

IVF: in-vitro fertilization
ECM: extracellular matrix
FRT: female reproductive tract
MRT: male reproductive tract
ASC: adult stem cell
iPSC: induced pluripotent stem cell
ESC: embryonic stem cell
SSC: spermatogonial stem cell

Conflicts of Interest

The author declares that they have no conflicts of interests.

Ethics Approval and/or Participant Consent

This study conducted a literature review and thus did not require ethics approval nor participant consent as no new experimentation was performed.

Authors' Contributions

RM: Primary contributor to the initial conception and design of the study, performed research, collected of literary sources and data for analysis, drafted initial manuscript and performed edits and revisions suggested by mentor (see acknowledgements), final approval of the version to be published.

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