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Organoids of the male reproductive system: Challenges, opportunities, and their potential use in fertility research

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Abstract

Organoids are units of function of a given organ able to reproduce, in culture, a biological structure similar in architecture and function to its counterpart in vivo. Today, it is possible to develop an organoid from a fragment of tissue, a stem cell located in an adult organ, an embryonic stem cell, or an induced pluripotent stem cell. In the past decade, many organoids have been developed which mimic stomach, pancreas, liver and brain tissues, optic cups, among many others. Additionally, different male reproductive system organs have already been developed as organoids, including the prostate and testis. These 3D cultures may be of great importance for urological cancer research and have the potential to be used in fertility research for the study of spermatozoa production and maturation, germ cells–somatic cells interactions, and mechanisms of disease. They also provide an accurate preclinical pipeline for drug testing and discovery, as well as for the study of drug resistance. In this work, we revise the current knowledge on organoid technology and its use in healthcare and research, describe the male reproductive system organoids and other biomaterials already developed, and discuss their current application. Finally, we highlight the research gaps, challenges, and opportunities in the field and propose strategies to improve the use of organoids for the study of male infertility situations.

This article is categorized under:

Reproductive System Diseases > Stem Cells and Development

Reproductive System Diseases > Biomedical Engineering

KEYWORDS

biomaterials, male fertility, male reproductive system, organoid

1 | INTRODUCTION

Looking back on history, we observe how humans are fascinated to better understand the biology of their own body, how organs and organisms are formed, and the mechanisms involved in diseases since Aristotle (Federspil &

Sicolo, 1994). Cell lines have traditionally been the tools used for understanding the role of genetic and molecular alterations in many diseases and allowed the observation and manipulation of mammalian cells in vitro (Harrison et al., 1907). However, despite two-dimensional (2D) cultures remaining the most used platform for in vitro cell culture, this model has several limitations, including the genetic alterations caused by long-term culturing, lack of annotated clinical data, and the inability to mimic the growth environment of the organ in vivo (Choi et al., 2020; Wang et al., 2017). In fact, cultured cells only interact with neighboring cells in a small segment and most plasma membranes are exposed to the culture medium. The lack of interaction within cells and with the extracellular matrix (ECM), a structure composed of insoluble and soluble protein fibrils, polysaccharides, glycoproteins, and proteoglycan filaments that provides structural support to the cells and tissues, affects the intracellular signaling and differentiation, apoptosis, gene, and protein expression processes (Jensen & Teng, 2020; Wang et al., 2017; Weeber et al., 2017). Consequently, 2D cultures are not able to mimic and completely recapitulate the three-dimensional (3D) organization of cells and the ECM in tissues and organs. On the other hand, the use of organism models, such as *Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio*, and rodents further improved our knowledge of organism development, organ function, and diseases. This approach surpasses the limitation of 2D cultures, allowing the study of processes in a physiological context. However, due to ethical issues and considering that most of the observations in animal models cannot be always transposed to humans due to species-specific differences, there has been an increased tendency to develop alternative strategies to minimalize the number of animals used or to replace animal experiments.

To overcome these gaps, in vitro 3D culture techniques have been developed as organotypic or organoid cultures (Edington et al., 2018). The term "organoid" emerged in the 1950s to nominate structures formed by the reorganization capacity of primary dissociated cells in vitro (Moscana & Moscana, 1952; Weiss & Taylor, 1960). This simplistic definition led to the use of other terms such as organotypic culture, spheroid, enteroid, or assembloid, to define miniature simplified organs with 3D tissue structures in vivo, but lacking the characteristic functionality of organoids (Hu et al., 2018). The definition of organoids has changed in the past years and currently, organoids are defined as self-renewing and self-organizing 3D structures that mimic cell composition, architecture, and function of the organ of origin (Clevers, 2016). In 2019, Kratochvil and colleagues provided a more complete definition, considering an organoid "a micro physiological system that mimics a platform to model organs or tissues in vitro setting with two or more interacting cell types in contact with each other and embedded in a matrix or in a device which aims to partially mimic cellular interactions or functions" (Kratochvil et al., 2019). These 3D structures may derive from cell lines, primary tissues, and stem cells, mimicking in vivo cell morphology and functionality, cell–cell interactions, and its diffusion barriers (Ravi et al., 2015) and acquiring organ function over the time (Chinta et al., 2020; Figure 1). Stem cell organoids are currently the most used type of organoid since they are originated from undifferentiated cells able to proliferate, maintain and differentiate in several functional cell types. To originate these organoids, two main types of stem cells are usually used—embryonic stem cells (ESCs) and adult stem cells (ASCs; Cyr & Pinel, 2022). While ESCs are pluripotent stem cells isolated from the inner mass of blastocysts, ASCs are multipotent or unipotent stem cells from adult tissues (Cyr & Pinel, 2022). Induced pluripotent stem cells (iPSCs) are also used being adult cells reprogrammed back into an embryonic-like pluripotent state that enables the development of any type of human cell (Buskin et al., 2021).

Additionally, considering the importance of the ECM molecular network for the maintenance of tissue structural and mechanical integrity, and that ECM and cell interactions are crucial for homeostasis maintenance and rapid response to environmental changes (Lindahl et al., 2015), scaffold matrices have been developed to mimic the ECM (Howard et al., 2008; Moura et al., 2022; Figure 1). Besides they provide structural support to the cell culture, scaffold matrices ensure the delivery of growth factors, allowing the production of proper 3D cultures, with appropriate shape, size, architecture, and physical properties (Almany & Seliktar, 2005). These engineered matrices are made of 3D porous, fibrous, or permeable biomaterials that can have a natural source or be a synthetic polymer, or even a hybrid composition with mixed natural and synthetic resources (Nikolova & Chavali, 2019). The use of a synthetic ECM-like scaffold is advantageous to control biophysical parameters needed to modulate the cellular environment such as mechanical properties, permeability, or biomimetics of the matrix (Marchini & Gelain, 2021; Nicolas et al., 2020). The porosity of the biomaterial is highly important to release biomolecules such as cytokine, inhibitors, drugs, and/or antibiotics. To date, several scaffolds were used to develop 3D cultures, including hydrogels (El-Sherbiny & Yacoub, 2013; Sasano et al., 2020; Subramaniyan & Ravi, 2018; Varaa et al., 2018), microfluidic systems (Griffith & Swartz, 2006; Halldorsson et al., 2015; Jiang et al., 2014), air–liquid interfaces (Lamers et al., 2021), 3D bioprinting cultures (Jian et al., 2018; Taymour et al., 2021) and layer-by-layer (LBL) techniques (Decher & Hong, 1991; Keeney et al., 2015; U. N. Lee, Day, et al., 2020; Oliveira et al., 2016). Hydrogels, in particular, have been extensively used as a substrate for 3D organoid

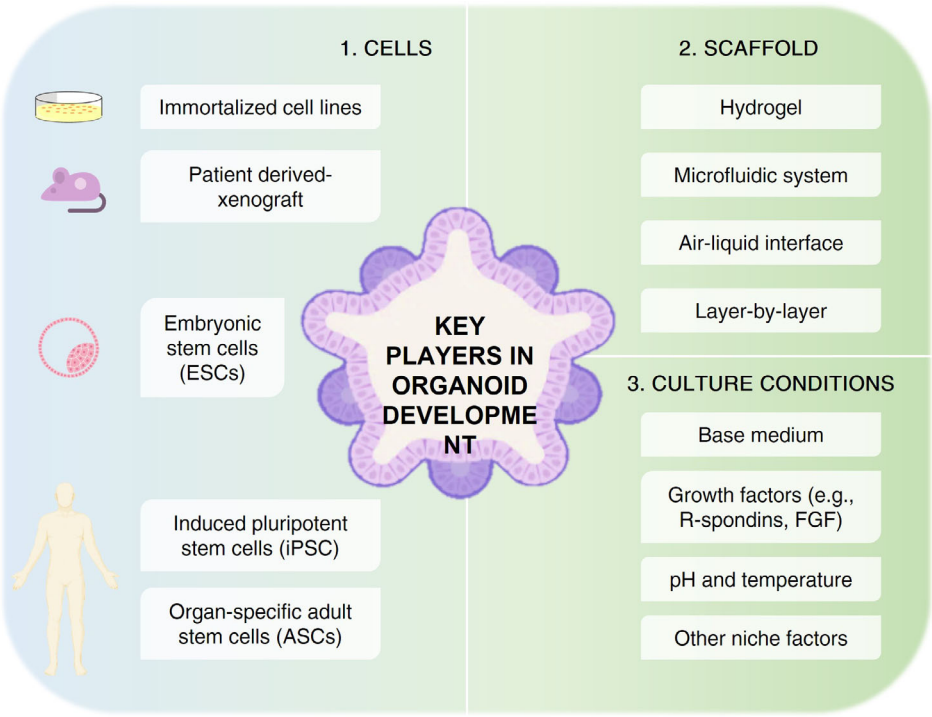

FIGURE 1 Organoids can be originated from tissue fragments (derived from patients or from patient-derived xenografts), immortalized cell lines, stem cells located in an adult organ, and embryonic or induced pluripotent stem cells derived from normal or diseased organs. They are usually developed under a biomaterial scaffold, such as hydrogel, microfluidic chambers, or bioprinting ink in culture until tri-dimensional structures are developed. The maintenance of specific culture conditions with appropriate niche factors that mimic the cellular environment is crucial for successful organoid development.

culture due to their framework for cellular proliferation and survival (Correia et al., 2020; Moura et al., 2022). Microfluidic systems, known as organ-on-a-chip (OOC), were designed to study physiological organ/tissue-specific cell behavior in microfluidic chambers, being able to mimic the cellular environment and its micro-architecture (Huh et al., 2010, 2011; Mi et al., 2018). Compared to conventional organotypic culture conditions, OOC allows a better cell and tissue characteristics representation not only for fundamental biological research but also for clinical diagnostics and treatment (Jiang et al., 2014). Recently, bioprinting has been used to develop LBL-based scaffolds in diverse shapes which were induced in hydrogels to construct small channels for tubular cell culture (Sousa et al., 2021). The development of suitable experimental models, including human reproductive organoids, will be valuable to investigating protein–protein interactions, cell signaling, gene and protein expression, and transcription factor control in conditions similar to physiological context, and represent excellent opportunities for identification of diseases biomarkers, drug screening, and tissue engineering.

2 | USE OF ORGANOIDS IN HEALTH CARE AND RESEARCH

To overcome the need to extrapolate results from animal models to humans the search for ex vivo human models has increased. The organoid technology appears to be a promising research model for human science advances as these 3D cellular structures can partially mimic the organs' morphology and function. This technology has been used to study several tissues and organs, including the brain (revised in Koo et al., 2019), pancreas (revised in Boj et al., 2016), liver (revised in Huch et al., 2015), kidney (Lawlor et al., 2021), prostate (Buskin et al., 2021), female (revised in Rawlings et al., 2021), and male reproductive tissues (revised in Alves-Lopes & Stukenborg, 2018). These 3D structures have been extensively used in basic research, to better understand the physiopathology of many organs. For instance, the advances in the development of brain organoids, their applications, and challenges have been well described across the last years (recently revised by Chiaradia & Lancaster, 2020; Koo et al., 2019; Sidhaye & Knoblich, 2021; Walters & Haigh, 2022).

Organoids have also been successfully used to study several disease mechanisms such as infectious and genetic diseases, cancer, and tumor modeling (recently revised by Shankaran et al., 2021).

Organoids typically contain multiple cells type from the ordinary tissue which facilitates their use to study infectious diseases and host–pathogen relations. Developing brain organoids has been used to study the infection and the teratogenic effect of Zika virus in the fetus (Lancaster et al., 2013; Mariani et al., 2015; Pasca et al., 2015) as well as to exploit the development of new drugs to prevent and treat the infection (Watanabe et al., 2017; M. Xu et al., 2016; T. Zhou et al., 2017). On the other hand, intestinal organoids were recurrently used as a host–pathogen model for enteric viruses, such as Rotavirus (Finkbeiner et al., 2012; Yin et al., 2015) and Noroviruses (Ettayebi et al., 2016) and to study primary response to bacterial infections (Bartfeld et al., 2015; Huang et al., 2015). Recently, the pandemic status worldwide due to SARS-CoV-2 spread has increased the need to understand the pathogenesis and molecular mechanisms of coronaviruses. Thus, several organoid models previously developed, such as lung-like organoids (Ebisudani et al., 2021; Lamers et al., 2021; Tindle et al., 2021), intestinal/colloidal organoids (Zang et al., 2020) and liver ductal organoids (Zhao et al., 2020), were used to understand the host–pathogen relationship of SARS-CoV-2, being a valuable tool to investigate COVID-19 progression and potential therapies.

Organoids also seem to retain the genetic specificity from the original tissue, being an exciting tool to study genetic diseases (J. Kim et al., 2020; Pollen et al., 2019). The use of organoids to study genetic diseases involves the introduction of the mutation in the wild-type organoid or the use of patient-derived organoids which already have the mutation. CRISPR technology allied with organoids appears to be a successful strategy to study the effect of lethal mutations during the development and when patient-derived biopsies are an obstacle. An example of the CRISPR–Cas9 genome-editing system is its use in patient-derived intestinal and colonic organoids to study the cystic fibrosis transmembrane conductor receptor (CFTR) and estimate a gene correction (Schwank et al., 2013). Human intestinal and rectal organoids from patients were also established to access CFTR functionality and evaluate the effect of drugs on cystic fibrosis disease (Dekkers et al., 2013, 2016; Vidović et al., 2016). Additionally, to identify the pathogenic context of nephrotic syndrome or polycystic kidney disease, kidney organoids-CRISPR were also developed (Cruz et al., 2017; Freedman et al., 2015; Ho et al., 2018). Moreover, organoids from two patients with microvillus inclusion disease, a disease characterized by life-threatening diarrhea in the first hours of life were developed to identify the role of the gene involved in the disease (Wiegerinck et al., 2014). In new-borns, organoids established from patient-derived cells helped to evaluate cerebral development and modulate diseases like microcephaly (Lancaster et al., 2013; W. Zhang et al., 2019).

In the cancer field, several successful cell lines were established from colorectal, pancreatic, liver, breast, prostate, brain, and bladder primary tumors and metastasis, which were then used to develop some tumor organoids. Organoids able to mimic the tumor microenvironment and used to study tumor biology, drug resistance, and new anti-cancer drugs, have already been accomplished in the brain (Bian et al., 2018; Boretto et al., 2019), intestine (Cristobal et al., 2017; Ganesh et al., 2019), lung (M. Kim et al., 2019; Sachs et al., 2019), liver (Broutier et al., 2017), kidney (Rosines et al., 2010), bladder (S. H. Lee et al., 2018), ovarian (Hill et al., 2018; Maenhoudt et al., 2020), breast (Bruno et al., 2017; Lancaster et al., 2013), esophagus (S. H. Lee et al., 2018), prostate (Gao et al., 2014), and endometrium (Boretto et al., 2019). Cristobal and colleagues used proteomics and transcriptomics to profile human colorectal tumors and healthy organoids derived from seven patients, which revealed that each patient possesses a distinct organoid signature at the proteomic level (Cristobal et al., 2017). These results may help to select the treatment approach based on the specific proteome of patient-specific organoid (Cristobal et al., 2017). Mice and human pancreatic cancer organoids were developed to investigate molecular and cellular mechanisms of neoplastic progression (Boj et al., 2015). The authors showed that the normal and neoplastic murine and human pancreatic tissues exhibited ductal- and disease stage-specific characteristics and, after orthotopic transplantation, recapitulate the full spectrum of tumor progression (Boj et al., 2016). This new platform can be used to understand tumor biology and identify potential biomarkers, therapeutics, and personalized medicine strategies for pancreatic tumors (Boj et al., 2016). Tumor organoids were used to identify the behavior of breast cancer cells in a healthy mammary environment or even the tumor response to new drugs (Mohan et al., 2021; C.-P. Sun et al., 2022). Regarding the female reproductive tract, its study has been a biological challenge. The lack of models to study ovarian cancer has been a handicap to understanding its pathobiology although organoids were already established from patient-derived ovarian cultures (Maenhoudt et al., 2020). Also, several female tract tissues have been widely developed under organoids technology to study endometrial and cervical carcinomas, endometriosis, infection and sexually transmitted diseases in the female tract tissues, such as *Chlamydia trachomatis*, *Treponema pallidum* (syphilis), *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, human papillomavirus (HPV), and herpes simplex virus (HSV; Heidari-Khoei et al., 2020; Rawlings et al., 2021). The impact of organoid technology in cancer

research has been well described in some works (Drost & Clevers, 2018; Tatullo et al., 2020). As the establishment of tumor organoids often involves the development of an organoid of the normal tissues, we can affirm the existence of human organoids from most of the human tissues/organs in the body.

Organoids have also been used to study eye physiology (Bennet et al., 2018; Susaimanickam et al., 2017) and ophthalmic diseases (Erbani et al., 2016), as well as to develop strategies for dental regeneration (Natsiou et al., 2017; Sano et al., 2020). Despite minimalists, cornea and retina chips are important in vitro tools to study drug effects, therapeutic approaches, and diseases in the eye (revised in Manafi et al., 2021). On the other hand, the ability of stem cells to differentiate into odontoblast-like cells has revolutionized the use of 3D modules and structures in dentistry (Jeong et al., 2020). Dental organoids have been applied to test the biocompatibility of some biomaterials (e.g, capping agents) and to evaluate the impact of drugs on dental pulp and tissue regeneration (X. Xu et al., 2022). The impact of organoid technology on human dental health has been highlighted in Rodriguez y Baena's work (Y Baena et al., 2022).

Organoid models have a huge potential to translate basic science into clinical research, as it allows the development of healthy and pathological tissues. Despite the relevant results obtained in clinical research, this technology remains in the early years of development and presents some challenges. The full functional mimic of organoid cultures is still one of the targets to reach and must involve the optimization and development of strategies to coculture different cell types using engineering approaches (J. Kim et al., 2020). The 3D reproducibility of the organoid can also be a challenge, especially from transitioning the scientific research for clinical applications or even for high throughput drug screening where the maintenance of the phenotype of several organoids at once is mandatory to evaluate the possible clinical effects (Correia et al., 2020). Nevertheless, organoids are a fast-evolving technology with robustness and diversity to revolutionize health research and patient-specific medicine.

3 | MALE REPRODUCTIVE SYSTEM ORGANOIDS AND THEIR CURRENT APPLICATION

The male reproductive system is composed of two testes, where the production of spermatozoa (spermatogenesis) and testosterone (steroidogenesis) occurs, a system of ducts responsible for spermatozoa transport and maturation (including epididymis), the accessory glands (seminal vesicles, prostate, Cowper and Littre glands) which release most of the semen fluids, and the penis, whose ultimate goal is the delivery of the male gamete into the female reproductive tract (Owen & Katz, 2005). The efficient and coordinated function of the male reproductive system is essential for reproduction, and any disturbance in this process may cause several disorders, such as infertility and cancer. The study of the male reproductive system in humans has been challenging due to the limited in vitro and in vivo tools available. Despite their limitations, primary cultures, cancer cell lines, tissue explants, and some organotypic cultures remain the most used in vitro models. Primary cells cannot be propagated indefinitely and frequently lose their phenotype; the cell lines available are derived from cancers or are immortalized, not representing their physiological state; and 2D cultures are deprived of stimuli from the ECM. Recent developments in 3D organoid technology that reproduce the distinct regions of the male reproductive tract include organoids of the prostate, testis, and urethra. These organoids can be derived from healthy or pathological tissues, constituting new avenues to investigate the normal biology and pathology of the male reproductive system, or to develop patient-tailored treatments (Figure 2). These models and the techniques used for their development are summarized in Tables 1 and 2.

3.1 | Testis

In testicular study, 2D co-cultures of primary and immortalized somatic cells (Sertoli, Leydig, or peritubular myoid cells) and germ cells have been used to evaluate the cell–cell and ECM–cell interaction (Hadley et al., 1985; Hofmann et al., 1992; Kierszenbaum et al., 1986; Richardson et al., 1995). Using Sertoli cells culture it was observed that the basement membrane is crucial to regulate the testicular microenvironment and that these cells can maintain the tight-junctions in this type of culture (Hadley et al., 1985). The co-culture of testicular cells has helped to characterize the effect of hormones (e.g., FSH; Saez et al., 1989; Tesarik et al., 1998), growth factors [e.g., fibroblast growth factor 2 and 9 (FGC2 and FGF9) and hepatocyte growth factor (HGF); El Ramy et al., 2005; van der Wee & Hofmann, 1999], reactive oxygen species (ROS) and other signaling molecules (Hung et al., 2016) in testicular somatic and germ cells, even though this strategy does not mimic the organ-specific structure and function. Also, these co-cultures helped to unravel

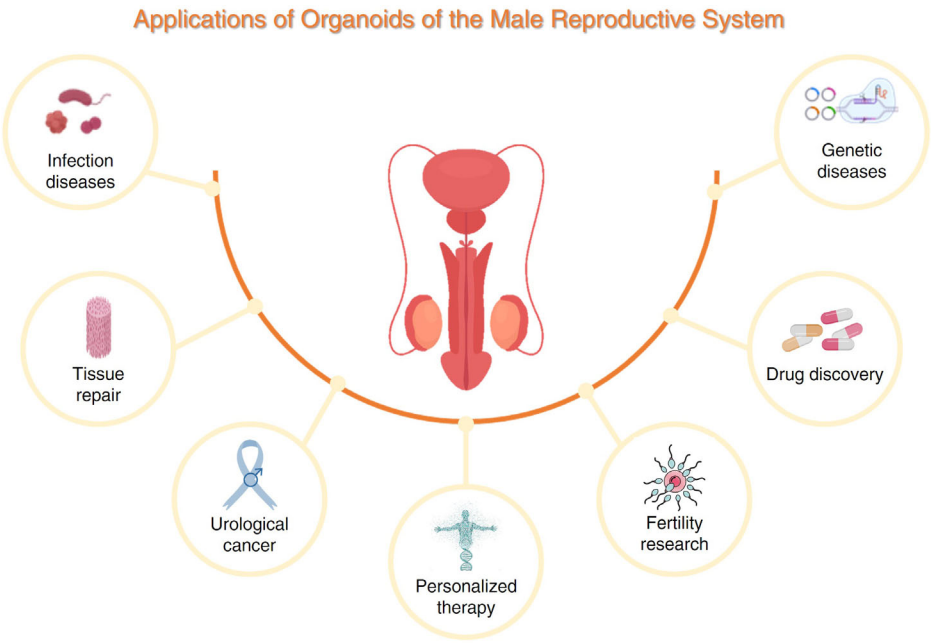

FIGURE 2 Main applications of male reproductive system organoids. The male organoids developed until today have proven to be helpful in many areas, from fertility and urological cancer research to the study of several infectious and genetic disease mechanisms. Moreover, organoids have been employed in tissue regeneration, used to repair ducts like the urethra. The generation of patient-derived organoids is extremely useful to develop and test new drugs and ultimately to personalize patients' treatment

some effects of environmental toxins, such as bisphenol A, revealing that this toxicant reduce spermatogonia stem cells viability and induce their apoptosis due to the over-expression of pro-apoptotic genes (Gong et al., 2017).

To address the lack of organ structure in the 2D culture and in order to study testes morphology, small rat testicular fragments were placed in culture (Steinberger et al., 1964). In this organotypic culture, spermatogonia and Sertoli cells survived after several weeks of maintenance, although early spermatids degradation was observed in the first days of culture (Steinberger et al., 1964). Later, organotypic cultures from human testicular fragments were maintained for 2 weeks, being then used for immunostaining, DNA fragmentation evaluation, and cell replication studies (Roulet et al., 2006). It was observed that the morphological, biochemical, and molecular parameters observed in this culture were similar to what was found in the testis in vivo. However, organotypic cultures do not allow the study of potential therapies since germ cells' differentiation is compromised and consequently, spermatogenesis is uncompleted (Roulet et al., 2006). Interestingly, Sato et al. reported that intracytoplasmic sperm injection using sperm produced in vitro by organoid culture resulted in the birth of healthy mice (Sato et al., 2011). Recently, some works reported the development of testicular organ cultures to study spermatogenesis in rodents (Pendergraft et al., 2017; Reda et al., 2016; Richer et al., 2020) and porcine (Cham, Chen, & Honaramooz, 2021; Goldsmith et al., 2020). Although the success in the testicular organ culture was reported, the search for an answer to study the human testis continued.

To date, only a few groups were able to develop testicular 3D organoids from testicular single cells (Baert, de Kock, et al., 2017; Oliver et al., 2021; Pendergraft et al., 2017; Strange et al., 2018), and even less were able to reproduce the testicular architecture (Table 1). In fact, Baert and colleagues developed human testicular organoids by seeding adult and teen primary testicular cells in agar blocks, resulting in compact structures with testis-specific functions (e.g., testosterone and inhibin B production, de novo matrix production, tight junctions formation, and germ cells renewal) but unable to reorganize into the typical testis histology during long-term culture (Baert, de Kock, et al., 2017; Baert, Rombaut, & Goossens, 2017). A few years later, the same authors developed mouse testicular organoids using alginate-based hydrogels and 3D bioprinting, to control scaffold design and cell deposition (Baert et al., 2019). Tubular-like structures were not formed but small-sized cellular aggregates formation and spermatogenesis completion were achieved (Baert et al., 2019). The development of a functional testicular system was also achieved through a co-culture of human spermatogonia stem-cell with immortalized Sertoli and Leydig cells in a hanging drop medium (Pendergraft et al., 2017). The histological organization was not reported in this study, but the maintenance and viability of compact testicular organoids and the production of testosterone in these structures were observed for 3 weeks of culture

TABLE 1 List of testicular and epididymal organoids developed

Study	Specie	Cell maturity		Culture conditions	Outcomes	Tissue-specific architecture
		Stage	Age			
Testicular organoids						
(Baert, de Kock, et al., 2017)	Human	Peripubertal	15 years old	Culture of testicular cells in agar blocks or decellularized adult testicular tissue slices.	Spermatogonia and somatic cells were functional for 1 month. Function of Sertoli and Leydig cells confirmed.	No
(Pendergraft et al., 2017)	Human	Adult	56–61 years old	Culture of germ cells together with immortalized Leydig and Sertoli cells onto a decellularized testicular tissue	Function of Leydig cells confirmed. Some germ cells were able to go from diploid to the haploid stage.	No
(Strange et al., 2018)	Human	Adult	–	Culture of germ cells together with primary Leydig and Sertoli cells onto a decellularized testicular tissue	Testicular organoids infected by the Zika virus had reduced viability as shown by low ATP production.	No
(Baert et al., 2019)	Mouse	Prepubertal Adult	<7 days old 6-month-old	Decellularized testicular cells cultures in alginate-based hydrogel by 3D bioprinting	Testicular tubular-like structures were observed. Postmeiotic germ cells were observed in the organoid including elongated spermatids.	No
(Topraggaleh et al., 2019)	Mouse Ram	Prepubertal Adult	3–5 days old 2–3 years	Testicular cell suspension cultures in hydrogel developed from decellularized testicular tissue.	Functionality of Sertoli and Leydig cells. Postmeiotic germ cells were observed inside the organoid only in mouse.	No
(Sakib, Voigt, et al., 2019)	Mouse Monkey Pig Human	Prepubertal	8–10 days old 2 years old 1-week-old 6 months to 5 years old	Centrifugation of testicular cells suspension in centrifugal plates before culture. Only on mice cells a Matrigel was added.	Expression of BTB proteins observed in all species. An inverted structure was observed from the inner and outer part in mouse, macaque, and human.	No
(Goldsmith et al., 2020)	Pig	Prepubertal	1 week old	Normal or knockdown for ODF2 and IFT88 Sertoli cells culture	A distinct membrane basement was observed in KO IFT88 organoids vs the control groups.	No
(Rajan et al., 2020)	Human	Adult	–	Microfluidic culture (organ-on-a-chip)		No
(Alves-Lopes et al., 2017)	Rat	Prepubertal Peripubertal Adult	5–8 days old 20 days old 60 days old	Culture of one drop with testicular cells in Matrigel®	Testicular cells in immature stage reform testicular organoid. Establishment of some BTB components.	Yes
(Vermeulen et al., 2019)	Pig	Prepubertal	<7 days old	Culture of testicular cells into drops of collagen or a solubilized of decellularized testicular tissue at the air–liquid interface.	A significant decrease of germ cells numbers. Function of Leydig and Sertoli cells maintained for 45 days.	Yes

(Continues)

TABLE 1 (Continued)

Study	Specie	Cell maturity		Culture conditions	Outcomes	Tissue-specific architecture
		Stage	Age			
(Edmonds & Woodruff, 2020)	Mouse Human	Prepubertal Peripubertal Adult Adult	5 days old 12 or 21 days old 8–16 weeks old 40–50 years old	Culture of testicular cells in agarose blocks for media diffusion.	Self-assembly only with immature testicular cells. Long-term endocrine function with gonadotropin responsiveness observed. Mature cells are able to self-assembly when co-cultured with immature testicular cells.	Yes
(Richer et al., 2021)	Mouse	Prepubertal	4–5 days old	Culture of germline cells in 3D printed scaffolds	Compartmentalized architecture for long survival of germ cells. Postmeiotic germline cells observed in the organoid.	Yes
(Cham, Ibtisham, et al., 2021)	Pig	Prepubertal	1 week old	Decellularized cells were cultured in U-bottom 96-well plates to spheroid culture and added to agarose gel for air–liquid interface	Self-reassemble testicular cells suspension able to form stable testis organoids. Sertoli and Leydig cells' function confirmed, and collagen fibers present in inter-tubular interstitial compartments. First time vascular structure reported developed by a single layer of endothelial cells	Yes
(Oliver et al., 2021)	Human	Embryonic gonads	First trimester	Cell suspensions into testis-like organoids in a three-layer gradient system	Cord-like structure of human embryonic testicular organoid. Functional Sertoli, Leydig, and peritubular myoid cells.	Yes
(AbuMadighem et al., 2022)	Mouse	Prepubertal	–	Microfluidic chip culture of dissociated cells	Sertoli and peritubular cell's function confirmed and able to form tubular structures. Premeiotic and postmeiotic germ cells observed.	Yes
Epididymal organoids						
(Pinel & Cyr, 2021)	Rat	Adult	48 days old	Epididymal epithelium single basal cell suspension in a 3D Matrigel	Epididymis basal cells can differentiate into principal cells in vitro. Epididymal cells self-reassembly was reported.	Yes
(Leir et al., 2020)	Human	Adult	<58 years old	2D and 3D epididymal from human epididymis epithelial cell in Matrigel	CFTR assay to study its function in epididymis.	Yes

TABLE 2 Prostate organoids developed and their application.

Study	Specie	Type of tissue	Organoid culture		Main goal
			Type of cells	Scaffold	
(Xin et al., 2007)	Mouse	Normal	Dissociated prostate epithelial cells from 6- to 10-week-old adult mice	Matrigel®	Investigate the ability to self-renew and differentiation of murine prostate stem cells in vitro.
(Crowell et al., 2019)	Mouse	Normal	Basal and luminal prostate epithelial cells	Matrix gel ring	Evaluate the intrinsic and extrinsic regulators of mouse prostate epithelial cells' differentiation capacity.
(Karthaus et al., 2014**)**	Mouse and human	Normal	Dissociated cells from anterior prostate, dorsolateral prostate, and ventral prostate epithelium	Matrigel®	Development of a 3D culture system that supports long-term expansion of primary mouse and human prostate organoids, composed of fully differentiated basal and luminal cells.
(Lang et al., 2001**)**	Human	Normal	Prostatic epithelial and stromal cells derived from human primary cultures	Matrigel®	Develop of a functional and morphologically correct prostate gland in vitro dependent of ECM, steroid hormones, and factors from stromal cells and serum.
(Tyson et al., 2007)	Human	Normal	RWPE-1, pRNS-1-1, PZ-HPV-7, PNT1A, BPH-1, and PrEC cell lines	Matrigel®	Determine the optimal culture conditions of prostate organoids for prostatic acinar morphogenesis in vitro and investigate the role of extracellular calcium in this process.
(H. Zhang et al., 2013)	Human	Normal	Normal human prostate epithelial cells (hPrECs) isolated from a healthy donor	Phenol red free Matrigel®	Investigate the role of Carcinoembryonic antigen-related cell adhesion molecule 20 (CEACAM20) and 1 (CEACAM1) in the lumen formation of normal prostate epithelium.
(Calderon-Gierszal & Prins, 2015**)**	Human	Normal	Human embryonic stem cells (hESC)	Matrigel®	Differentiate hESC into prostatic organoids in a spatial system with precise temporal control of growth factors and steroids to be used as an in vitro human prostate developmental model to study the effects of BPA.
(Dambal et al., 2017**)**	Human	Normal	Benign primary human prostatic epithelial cells established from fresh radical prostatectomy tissues	Matrigel®	Evaluate the effect of miR-183 family cluster overexpression in the regulation of zinc levels and carcinogenic pathways in prostate cells.
(Hepburn, Curry, et al., 2020**)**	Human	Normal	Induced pluripotent stem cells (iPSCs) from three patients	hESC-qualified Matrigel® coated plates	Establish a prostate organoid using iPSCs to overcome limitations in the primary culture of human prostate stem, luminal and neuroendocrine cells, as well as the stromal microenvironment.
(McCray et al., 2019)	Human	Normal	Human primary prostate epithelial cells	Matrix gel	Development of a protocol for 3D culturing, handling, and evaluation of human primary prostate organoids.
(Drost et al., 2016**)**	Mouse and human	Normal and neoplastic	Cells derived from mouse and human normal prostate and patients circulating tumor cells	Matrigel®	Develop a protocol to generate 3D prostate organoid cultures from healthy mouse and human prostate (either luminal and basal cells), metastatic PCa lesions, and circulating tumor cells.

(Continues)

TABLE 2 (Continued)

Study	Specie	Type of tissue	Organoid culture		Main goal
			Type of cells	Scaffold	
(Härmä et al., 2010)	Human	Normal and neoplastic	Normal epithelial cells and derivatives (PrEC, EP156T, RWPE-1, RWPE-2, RWPE-2/w99, WPE1-NB14, PWR-1 E, PZ-HPV-7 and CA-HPV-10)	Matrigel®	Develop a panel of 29 3D miniaturized prostate cell culture models, including nontransformed and classic PCa cell lines to analyze morphogenetic properties of PCa models in 3D, to compare phenotypes, gene expression and metabolism between 2D and 3D cultures, and to evaluate their potential application in preclinical drug discovery, disease modeling, and basic research.
(Chambers et al., 2014)	Human	Normal and neoplastic	Normal RWPE-1 cell line, RWPE-2 and LNCaP cancer cell lines	Polydimethylsiloxane (PDMS)	Develop a microwell platform and surface modification protocol to enable high throughput manufacture of 3D cancer aggregates.
(Chua et al., 2014)	Human	Normal and neoplastic	Castration-resistant Nkx3.1-expressing cells and normal prostate epithelium	Matrigel®	Investigate the ability of single luminal epithelial progenitors can generate prostate organoids in the absence of stroma.
(Fong et al., 2016)	Human	Neoplastic	Cells derived from MDA PCa 118b PDX	Thiolated HA: acrylate-PEG-GRGSD: acrylate-PEG-PQ-PEG-acrylate	Develop a 3D hydrogel system able to support the co-culture of PCa PDX cells and osteoblastic cells to recapitulate the PCa-osteoblast unit within the bone metastatic microenvironment in vitro.
(Park et al., 2016)	Human	Normal and neoplastic	Primary human prostate basal and luminal cells	Matrigel®	Unravel the cell of origin for PCa using an organoid transformation assay.
(Njoroge et al., 2017)	Human	Normal and Neoplastic	Benign human primary prostate epithelial cells, premalignant (RWPE-1), and malignant (LNCaP) cell lines	Matrigel®	Determine the effects of the SELECT (Selenium and Vitamin E Cancer Prevention Trial) supplements on benign (primary), premalignant (RWPE-1), and malignant (LNCaP) prostate epithelial organoids.
(Carneiro et al., 2018)	Human	Neoplastic	LNCaP cells	Matrigel®	Investigate the involvement of anaplastic lymphoma kinase (ALK) gene in small cell carcinoma of the prostate using organoids models.
(Mosaad et al., 2018)	Human	Normal and neoplastic	RWPE-1, RWPE-2, and LNCaP cell lines	Polydimethylsiloxane (PDMS)	Development of a microwell platform using a nylon mesh able to manufacture and retain ~150 3D micro-tumors per well in a 48-well plate (Microwell-mesh) and evaluate the response to anti-tumor drugs.
(Olson et al., 2019)	Mouse	Neoplastic	Cells isolated from primary prostate tissue collected from R26mTmG/+:Cdh1L/L: PB-Cre4 and R26mTmG/+:Cdh1L/ L littermates at 6 weeks of age	Matrigel®	Study the role of E-cadherin in maintaining prostatic epithelial integrity and organization using organoid culture approaches.

TABLE 2 (Continued)

Study	Specie	Type of tissue	Organoid culture		Main goal
			Type of cells	Scaffold	
(S. Lee, Burner, et al., 2020)	Mouse and human	Neoplastic	Cells derived from patient-derived xenograft PCa tissue and patient primary tumor tissue	Matrigel® or others	Establish a protocol for generation of 3D Organoids Derived from Patient PCa Bone Metastasis Specimens and their Xenografts
(Zhau et al., 1997)	Human	Neoplastic	Isolated human prostate fibroblasts cocultured with isolated human PCa cells (LNCaP)	Microcarrier beads	Establishment of a 3D human prostate organoid co-culture under microgravity-stimulated conditions.
(Gao et al., 2014)	Human	Neoplastic	Isolated metastatic PCa cells ($n = 32$) and circulating tumor cells ($n = 17$)	Growth factor reduced Matrigel®	Establishment of long-term organoid cultures of PCa from biopsy specimens and circulating tumor cells.
(Eder et al., 2016)	Human	Neoplastic	PCa cell line (LNCaP) and immortalized PCa-associated fibroblasts (CAFs)	Scaffold-free	Study the therapeutic effects of clinically used anti-androgens using a tissue-mimetic 3D in vitro model where PCa cells were cultured alone or with PCa-associated fibroblasts (CAFs)
(Eder & Eder, 2017)	Human	Neoplastic	PCa cell lines (e.g., LNCAP)	96-well hanging drop plate	Development of a 3D hanging drop system to be used to culture PCa organoids as tumor epithelial monocultures and as epithelial–stromal cocultures.
(Ma et al., 2017)	Human	Neoplastic	NCaP and C4-2B cell lines	Matrigel®	Development of organoids from human PCa cell lines LNCaP and C4-2B presenting glandular structures.
(Shenoy et al., 2017)	Human	Neoplastic	Patient-derived cancer cells from metastatic CRPC	Growth factor depleted Matrigel®	Study the role of CHD1 in PCa development and its clinical utility
(Aw Yong et al., 2017)	Human	Neoplastic	PC3 or DU145 PCa cells	Molded fluidic channel embedded in a collagen hydrogel	Development of a fluidic device for long-term 3D culture which recapitulated the tumor invasion front, allowing for the quantification of invasive potential and the molecular characterization of invasive leader cells.
(Beshiri et al., 2018)	Human	Neoplastic	20 models from the LuCaP mCRPC PDX cohort (adenocarcinoma and neuroendocrine lineages)	Growth factor reduced phenol red-free Matrigel®	Establishment of a representative, preclinical platform of PDX-derived organoids that is experimentally facile for high throughput and mechanistic analysis.
(Puca et al., 2018)	Human	Neoplastic	Tumor cells derived from patients with PCa with neuroendocrine differentiation	Growth factor reduced Matrigel®	Generate and characterize tumor organoids derived from needle biopsies of metastatic lesions from four patients.
(Spencer et al., 2018)	Human	Neoplastic	19I prostate stromal cells and the LNCaP prostate epithelial adenocarcinoma cells	12-mm Millicell-CM Cell Culture inserts (Millipore)	Investigate Zika virus cell tropism in human prostate cells.
(Linxweiler et al., 2019)	Human	Neoplastic	3D spheroid suspension cultures from radical prostatectomy	Scaffold-free	Development of patient-derived 3D spheroid cultures of PCa

(Continues)

TABLE 2 (Continued)

Study	Specie	Type of tissue	Organoid culture		Main goal
			Type of cells	Scaffold	
(Nath et al., 2019)	Human	Neoplastic	RWPE-1 cell line, MSK-PCa1, MSK-PCa2, and MSK-PCa3 metastatic human PCa cells and normal-26Nb human prostate cells	Matrigel® membrane matrix	Investigate the role of ABI1 in epithelial–mesenchymal transition, a key mechanism in progression to metastatic disease.
(Choo et al., 2021)	Human	Neoplastic	Cells obtained from patient-derived xenografts (PDXs)	Matrigel®	Development of a pipeline for automated seeding, treatment, and analysis of the drug responses of PCa organoids.
(Dhimolea et al., 2021)	Human	Neoplastic	Cancer cell lines (LNCaP) and immortalized nonmalignant cells lines (HS5, HS27A, HOBIT, hFOB, THLE3, and SVGP12)	Matrigel®	Development of hormone receptor-positive PCa patient-derived organoids in 3D extracellular matrices alone or together with bone marrow stromal cells to investigate the hormonal therapy resistance frequently developed within the complex metastatic microenvironment of the host organ.
(Van Hemelryk et al., 2021)	Human	Neoplastic	AR-negative (AR−) MSK-PCa1 and AR-positive (AR+) MSK-PCa2 organoid lines	Matrigel® or Noviogel	Investigate the impact of 3D organoid organization and the use of various 3D scaffolds in treatment efficacy.
(Heninger et al., 2021)	Human	Neoplastic	Patient-derived cancer spheres from patients with locally advanced PCa	BD Matrigel® GFR	Establishment of ex vivo primary patient-derived cancer organoid cultures from prostatectomy specimens of patients with locally advanced PC and characterization of the cellular composition using a novel approach for live-cell staining and direct imaging in the integrated microfluidic Stacks device.
(Servant et al., 2021)	Human	Neoplastic	Cell suspensions isolated from primary or metastatic PCa from 81 patients	Growth factor reduced Matrigel® domes	Development of PCa patient-derived organoids from a cohort of 81 specimens with diverse pathological and clinical features
(S. Lee et al., 2022)	Human	Neoplastic	PCa San Diego 1 tumor cells (PCSD1; castrate resistant bone metastatic PCa)	Growth factor reduced Matrigel® domes	Establishment of organoids from a PDX model of bone metastatic PCa to investigate castration resistance.
(Mosquera et al., 2022)	Human	Neoplastic	Cells derived from patient biopsies	PEG-4MAL macromer and cell cross-linker	Investigate the role of ECM in phenotypic, transcriptomic, and epigenetic underpinnings of CRPC-NEPCs using synthetic hydrogel-based organoids.

(Pendergraft et al., 2017). This testicular model was used to study Zika virus infection, along with some other organoids developed by the same group (Strange et al., 2018). Recently, a microwell centrifugal system was adopted to culture prepubertal porcine testicular cells (Sakib, Yu, et al., 2019). This approach allowed the generation of a testicular organoid model with an organized architecture similar to in vivo, with the cells aligned and separated by the basement membrane (Sakib, Yu, et al., 2019). Later, the same method was used for pig, mouse, macaque, and human (Sakib, Uchida, et al., 2019). In prepubertal pigs, the self-organization of testicular cells observed was abnormal when compared to other species, the inner and outer cells were on reversed sides when compared to the tissue in vivo architecture. In mice, macaques, and human prepubertal testicular cells testicular organoids were developed and the 3D organization of the mammalian testis was obtained (Sakib, Uchida, et al., 2019). The protocol from Sakib and colleagues was used to study the testicular tubules morphogenesis and the role of Hedgehog signaling in primary cilia loss and in vitro morphogenesis in pigs (Goldsmith et al., 2020).

Matrigel® testicular culture has been highly implemented in rats (Alves-Lopes et al., 2017; Yu et al., 2005) and mice (Sakib, Yu, et al., 2019). The first testicular 3D model with similar histological features to the in vivo organ was developed using a three-layer Matrigel gradient system (3-LGS) where rat Sertoli and germ cells were co-cultured (Alves-Lopes et al., 2017). The spherical-tubular structures developed presented a functional blood-testis barrier (BTB) after 7 days of culture and maintained undifferentiated germ cells proliferating up to 21 days of culture (Alves-Lopes et al., 2017). Due to the ability to mimic an intact BTB and maintain germ cell viability, this strategy might be truly valuable for the study of spermatogonia proliferation and differentiation, spermatogenesis, and investigation of the testicular toxicity of some chemicals that may affect male fertility. Another testicular organoid self-assembly in Matrigel® using murine cells was recently reported by Edmonds and Woodruff (Edmonds & Woodruff, 2020). The authors showed that the 3D organoid developed presented age-dependent endocrine function, which was inversely correlated with testis maturity across mouse puberty (Edmonds & Woodruff, 2020), confirming observations previously described (Alves-Lopes et al., 2017). For porcine, accurate organoid structures using collagen hydrogels and immature decellularized testicular tissues was also achieved where seminiferous tubule organization as in the native organ was observed (Vermeulen et al., 2019). To study the gonadal development during embryogenesis, Oliver et al., 2021 developed testis-like organoids by culturing human testicular cell suspensions in 3-LGS and showed that cells were able to reorganize into testis-like organoids with seminiferous-like cords after 7 days (Oliver et al., 2021). The authors included mesonephros in the model, involved in the testicular organogenesis in vivo, and observed organoid organization failure when compared to the testicular cells' suspension (Oliver et al., 2021). The successful establishment of this model will stimulate and facilitate the study of testicular organogenesis, a crucial process for male fertility, and the influence of maternal exposure to chemicals, pollutants, and other external factors in this event.

Other scaffolds have been employed for testicular organoid development, including printed scaffolds and microfluidic systems. Baert's group recently used 3D-printed scaffolds at the air–medium interface to developed testicular organoids from two different mouse strains and they showed that tubulogenesis was promoted, resulting in structurally compartmentalized organoids (Richer et al., 2021). In this work tubule-like structures and Leydig cell functionality were confirmed, leading to long-term survival and germ cell differentiation to the meiotic phase (Richer et al., 2021). Spheroid testis organoids from porcine cells were also developed using an agarose gel for air–liquid interface presenting tubular and interstitial compartments similar to testis in vivo (Cham, Ibtisham, et al., 2021). Besides the testis architecture, the authors were able to observe the inter-tubular interstitial compartments with Leydig cells and vascular structures (Cham, Ibtisham, et al., 2021). Organotypic cultures of mouse testis in microfluidic devices with a circulatory system have been employed and may lead to longer spermatogenesis improving the functionality of the organoids in these devices (Yamanaka et al., 2018). Premeiotic, meiotic and postmeiotic germ cells were reported in a testis-on-a-chip platform from isolated cells of immature mice, making this device a suitable tool for the study of spermatogenesis in vitro (AbuMadighem et al., 2022). A co-culture of liver and testis-organ chips with a liver spheroid-specific medium was used to study the liver–testis axis (Baert et al., 2020). The effect of cyclophosphamide was evaluated and upregulation of specific cytochromes in liver and germ cell loss was observed in testis organoids in the multi-organ-chip co-cultures when compared to single-testis culture (Baert et al., 2020). This tool has the potential to be used to study the reprotoxicity of other endocrine substances. Later, a multi-organoid body-on-a-chip with liver, cardiac, lung, endothelium, brain, and testis organoids was established and used to evaluate the effect of ifosfamide, an alkylating prodrug, and to confirm the multi-tissue intercommunication due to its neurotoxicity (Rajan et al., 2020).

Considering all the mentioned, it is plausible to assume that testicular cells need 3D support to rearrange themselves in testicular-like structures. The nature of the scaffolds seems to be important for the generation of testicular organ-like structures since not all testicular 3D models have allowed such reorganization (Table 1). For instance,

testicular cells cultured in a soft-agar culture system did not organize in a testicular-like structure and did not present some of the basal lamina components (Abu Elhija et al., 2012; Reda et al., 2014; Stukenborg et al., 2008), contrary to what usually happen when cultured using hydrogels/Matrigel (Alves-Lopes et al., 2017; Edmonds & Woodruff, 2020; Oliver et al., 2021). Laminin was evidenced as a factor to promote the reorganization of Sertoli cells into a tubular structure, since it is crucial for Sertoli cells and germ cells survival and differentiation in Matrigel® (Hadley et al., 1990; Legendre et al., 2010). Besides their use for the study of spermatogenesis and gonadal development during embryogenesis, testicular organoids had been implemented to study the toxic effect of chemicals such as phthalates (Harris et al., 2016) or bisphenol A (X. Zhang, Wang, et al., 2017). The development of testicular organoids can be a useful tool for disease modeling if organoid models of testicular malignancy are developed, such as spermatogenesis disfunction or Sertoli-cell only syndrome. Although some factors might influence the success of a testis organoid development, such as the scaffold used, these cultures seem to be a good tool for the study of spermatogenesis and for the study of male infertility mechanisms that can be used for male infertility prevention. These 3D culture models for testicular tissue/cell culture are also promising technologies to be applied in fertility preservation and treatment. The challenge now is to establish the best method for developing and maintaining a testis organoid, in order to make research in the area reproducible.

3.2 | Epididymis

The epididymis plays a crucial role in spermatozoa maturation, transport, protection, and storage, being divided into three main regions–caput, corpus, and cauda–in larger mammals, as humans and bovine (C. H. Yeung et al., 1991), and presenting an additional region–initial segment–in small animals like rodents (Pinel et al., 2019). It is a single convoluted tubule, with a pseudostratified epithelium (Breton et al., 2016). The epithelium, which regulates the composition of the lumen, contains principal cells, clear cells, basal cells, and halo cells (Pinel et al., 2019). Apical and narrow cells are present exclusively in the initial segment. Apical tight junctions between the cells that line the lumen of the epididymis form the blood-epididymis barrier (BEB) which regulates the content of the epididymal lumen by limiting the paracellular movement of ions between the blood and the lumen and protects maturing spermatozoa from the attack by the immune system (Gregory & Cyr, 2014). It is well known that spermatozoa are immature and immotile when leaving the testis after spermatogenesis and need the transit through the epididymis to acquire motility and the ability to fertilize the oocyte. In fact, it is clear the role of this duct in spermatozoa maturation, not only by its luminal fluid microenvironment but also because it provides molecules needed for motility acquisition (Sullivan & Belleannée, 2017).

The first attempts to generate epidydimal organoids resulted from the development of epididymis spheroid cultures from single cells in several species (Kristensen et al., 2010; Mandon et al., 2015; Moore et al., 1992). In humans, Kristensen et al. (2010) observed the dispersion of human epididymal cells in spheres under 2D culture conditions (Kristensen et al., 2010). Some years later, Mandon et al. (2015) reported the presence of acini in cultured rat epididymal basal cells (Mandon et al., 2015). The authors showed that the formation of acini was dependent on fibroblast growth factor (FGF) and dihydrotestosterone (DHT) (Mandon et al., 2015). Furthermore, they found that many cellular clusters expressed connexin 26, suggesting that within these clusters, basal cells could differentiate first into columnar cells and subsequently into principal cells, confirming the existence of stem cells within the epididymal epithelial cell population (Mandon et al., 2015). These 2D spherical structures presented a layer of basal cells on the outside and additional epithelial cells on the inside facing a luminal space, as observed in the epididymal lumen (Mandon et al., 2015).

The development of epididymal organoids (Table 1) was only recently reported by Leir et al., which used human epididymis epithelial cell organoids and polarized human epididymis epithelial cell cultures from caput epididymis to study the CFTR in the epididymis (Leir et al., 2020). To assess the ability of single basal cells to self-renew and differentiate in vitro and to generate organoids, basal cells from rat epididymis were isolated and 3D cultures established (Pinel & Cyr, 2021). After long-term culture, it was shown that epididymal basal cells were able to differentiate into cells expressing principal cell markers (aquaporin 9 and CFTR) and secrete Clusterin, a protein involved in spermatozoa maturation (Leir et al., 2020; Pinel & Cyr, 2021). Furthermore, these organoids were constituted by many polarized cell types presenting microvilli and able to form tight-junctions, crucial for the integrity of the BEB.

Despite the development of epididymal organoids is still very recent, and further studies are needed to optimize them, their potential is enormous. The establishment of a model of this nature will allow the study of epididymis structure and cellular regulation and differentiation under normal and pathological conditions, opening a new area of research in epididymal biology. Additionally, understanding physiological and pathological conditions may ultimately lead to the comprehension of how these alterations may lead to infertility.

3.3 | Prostate

The prostate gland is composed of a pseudostratified epithelium that contains three primary epithelial cell types (luminal cells, basal cells, and rare neuroendocrine cells), whose primary function is to produce the fluid that nourishes and transports sperm—prostatic fluid—composed of zinc, citric acid, prostate-specific antigen (PSA), and choline (Crowley & Shen, 2022). PSA, in particular, helps to liquefy the semen following ejaculation as it degrades Semenogelin I and II (Fardilha et al., 2015). Prostate organoids can be originated from nonmalignant prostate stem cells or cell lines mimicking the morphology of a nonmalignant prostate or can be derived from patients to study cancer (Table 2).

New experimental models of the prostate have been developed to study the most common malignant tumor in men—prostate cancer (PCa). The limited number of cell lines available, with reduced phenotypes of clinical disease, the limitations of the use of 2D cultures for drug screening, and the lack of surrounding microenvironment raised the need for alternative models (Vela & Chen, 2015). In vivo models of tumor propagation were developed to evaluate therapeutic efficacy (patient-derived xenograft, PDX; and genetically engineered models); however, the high costs and time required for tumor development, as well as the inability to screen a large number of compounds limits their use (Vela & Chen, 2015). In response to these constraints, several organoids of PCa have been successfully developed using the cell lines available (PC3, DU145, LNCaP, C4-2B; Aw Yong et al., 2017; Eder & Eder, 2017; Ma et al., 2017; Zhau et al., 1997), primary adult stem cells (Boj et al., 2015; Gao et al., 2014), embryonic (Calderon-Gierszal & Prins, 2015) and pluripotent stem cells (Hepburn, Sims, et al., 2020; Table 2). These 3D organoid models can mimic the tumor microenvironment in human bodies as they are able to demonstrate the interactions between cells and ECM, and are a promising tool for preclinical testing, an issue well revised (Gleave et al., 2020; Namekawa et al., 2019; Pamarthy & Sabaawy, 2021; Risbridger et al., 2018). In 2014, Gao et al. successfully established for the first time long-term 3D cultures of PCa derived from biopsy samples and circulating tumor cells using Matrigel® as a scaffold (Gao et al., 2014). They showed that the organoids developed recapitulated the histological and molecular signature of the PCa subtypes, including genetic alterations like *TMPRSS2-ERG* fusion, *SPOP*, *PIK3R1*, and *FOXA1* mutations, *SPINK1* overexpression, and *CHD1* loss (Gao et al., 2014). PCa organoids have been extensively used in the study of tumor development, progression, maintenance, and metastasis (Dambal et al., 2017; S. M. Kim et al., 2018; Nath et al., 2019; Njoroge et al., 2017; Olson et al., 2019; Shenoy et al., 2017). PCa organoids derived from patients were used to study the role of the chromatin remodeler CHD1 in the progression of local and metastatic PCa (Shenoy et al., 2017). Puca et al. developed rare PCa organoids and xenografts from patients and showed that *MYCN13*, *SOX216*, and *FOXA2* were overexpressed (Puca et al., 2018). Also, the physiological relevance of miR-183 family expression and its relevance in zinc levels regulation and carcinogenic pathways in prostate cells was demonstrated using prostate epithelial organoids (Dambal et al., 2017).

Since organoids can be generated from individual patients, it is also possible to study the individual cancer mutation and subtype, allowing the testing and selection of a personalized therapy (Conteduca et al., 2020; Jarchum, 2014; Pamarthy & Sabaawy, 2021; Servant et al., 2021). In fact, PCa organoids have been used in drug screening and personalized medicine to predict the effects and responses of therapies based on mutational profiles and analyzing drug resistance. For instance, castration-resistant PCa (CRPC)-derived organoids showed a high sensitivity for enzalutamide if harboring *AR* amplification, *PIK3R1* mutations, and *PTEN* loss (Elbadawy et al., 2020). Also, patient-derived-organoids with DNA/RNA helicase schlafen family member 11 (SLFN11) expression were subjected to *SLFN11* knockout by CRISPR-Cas9 and treated with platinum to assess changes in dose-response (Conteduca et al., 2020). Organoids expressing SLFN11 showed a reduced response to platinum therapy after knockout, suggesting that SLFN11 expression indicates a better response to platinum-chemotherapy (Conteduca et al., 2020). In 2021, Choo and colleagues developed a high-throughput imaging assay for analysis of the drug responses of PCa organoids (Choo et al., 2021). The authors established organoid cultures of PCa with diverse phenotypes, including castrate-sensitive and castrate-resistant disease, adenocarcinoma, and neuroendocrine tumor, and their analysis by live-cell imaging enabled in-depth assessment of morphological differences between patients and within organoid populations and the morphology and composition alterations after drug treatment (Choo et al., 2021). Organoids can also be useful to investigate the mechanisms underlying anti-cancer drug resistance. In PCa murine organoids, it was shown that dual loss of TP53 and PTEN resulted in complete resistance to next-generation anti-androgens (Elbadawy et al., 2020; Pappas et al., 2019). PCa organoids can also be used in the development and testing of new anti-cancer drugs, as a first approach to build preclinical data (Marzi et al., 2019). Tumor cells can be co-cultured in these models with other cellular components, such as peripheral blood lymphocytes (Dijkstra et al., 2018), allowing the study of the effect of the microenvironment on tumor growth

and drug response. PCa organoids co-cultured with bone marrow stem cells (BMSCs) were used to study tumor metastatic and hormone therapy resistance, revealing that IL-6 secreted by BMSCs plays a pivot role in bone metastasis through the activation of JAK/STAR pathway (Dhimolea et al., 2021). The authors demonstrated that metastasis-mimicking prostate organoids showed attenuated sensitivity to enzalutamide, which was restored after the treatment with IL-6 and IL-6R neutralizing antibodies or ruxolitinib (Dhimolea et al., 2021). Nevertheless, several cellular components are still missing in most organoids, such as stromal cells, immune cells, and vascular endothelial cells, which may hinder the efficiency of drug screening. Thus, co-cultures combining tumor, stromal, immune cells, and fibroblast should be established to effectively recapitulate the epithelial–stromal communication as well as cell–cell and cell–matrix interactions.

Interestingly, a well-characterized preclinical platform of PDX-derived organoids was developed, with the potential to be available to the public and be used for high throughput and mechanistic analysis in PCa translational research (Beshiri et al., 2018; Weeber et al., 2017). However, despite organoid-based studies have revolutionized in vitro preclinical research and represent a great promise for the cancer research field, attention should be paid to the experimental variability in organoid drug testing which complicates reproducibility. In fact, organoid organization, size, and the scaffold used may affect the treatment efficacy and explain why some PCa organoid models showed less response to some anti-cancer drugs compared to corresponding single cells before organoid assembly (Van Hemelryk et al., 2021). On the other hand, the optimization of culture media is required to avoid potential effects on tumor drug response (Servant et al., 2021). Furthermore, the fact that most PCa organoids derived from biopsy samples represent the major limitation of this technology, since biopsied tissues may be heterogeneous and not representative of the entire variety of clones derived from the primary tumor, which may result in treatment bias toward specific clones. However, this limitation is not restricted to just organoids, also happening in cell lines and PDXs. The optimization of these technical aspects will certainly lead to even better PCa models and personalized treatment resulting in enhanced clinical outcomes, less morbidity due to ineffective therapies, and improved quality of life in men with advanced PCa.

3.4 | Penis and urethra

Complex urethral problems can occur due to trauma, infection, inflammation, diseases, or congenital defects resulting in the narrowing or blockage of the urethral canal; however, the treatment options are limited. The field of stem cell biology and tissue engineering has been evolving, and it is expected that 3D bio-printed urethras would soon be available. This new biomaterial could be extremely useful in pediatrics regenerative medicine, to correct for instance congenital malformations (Sharma & Gupta, 2019). Atala and colleagues described for the first time a tissue-engineered graft composed of epithelial cells cultured in scaffolds that could be used for urethroplasty (Atala et al., 1999). Later, Raya-Rivera developed a tissue-engineered urethra by culturing smooth muscle and urothelial cells isolated from bladder tissue onto a synthetic tubular polyglycolic acid:poly(lactide-co-glycolide acid) scaffolds (Raya-Rivera et al., 2011). These tabularized urethras were used for urethral reconstruction in 5 boys (10–14 years old) with urethral defects, presenting normal appearing architecture 3 months after implantation (Raya-Rivera et al., 2011). Aiming to evaluate the efficacy, feasibility, and safety of in vivo and in vitro approaches for urethral regeneration using a human decellularized urethral scaffold, Kajbafzadeh and colleagues showed that the cell sheet approach was more effective in the recellularization process than perfusion-based methods for in vitro urethral regeneration (Kajbafzadeh et al., 2017). Recently, 3D bioprinting was used to replace traditional tissue engineering. Bladder epithelial cells and smooth muscle cells were cultured in fibrin hydrogel, maintaining cell viability and proliferation (K. Zhang, Fu, et al., 2017). Since hydrogel is highly porous, it mimics the natural urethral base-membrane and facilitates the contact between the printed epithelial cells and smooth muscle cells on both sides of the scaffold (K. Zhang, Fu, et al., 2017). However, decreased mechanical strength, poor tissue integration, and reduced biocompatibility of constructs when implanted in vivo make the use of 3D bioprinting limited in the clinic.

As already mentioned, urethral reconstruction is performed in patients with urethral strictures or for the correction of congenital disorders, and 3D cultured structures may enhance urethral reconstruction. Nevertheless, since corpus spongiosum supports urethra function, tissue engineering of the urethra should be combined with reconstruction of corpus spongiosum. The development of urethral organoids should be complemented with a 3D culture of this important structure of support, which will certainly have a lot of value and application in clinics.

4 | RESEARCH GAPS, CHALLENGES, AND OPPORTUNITIES IN THE USE OF ORGANOIDS FOR FERTILITY RESEARCH

Organoids have been important models to study organogenesis, organ function, disease, and drug response, including toxicity in a plethora of tissues that we cannot have direct access to from the human body (Pendergraft et al., 2017; Roulet et al., 2006). In male fertility research, the male reproductive system deterioration has a social concern, as it has an impact on families and communities. Infertility is considered a disease of the reproductive system affecting almost 15% of men in reproductive age (Barratt et al., 2017). It may result from abnormal sperm production or transport, ejaculation disorders, congenital or genetic diseases, infections, or even alterations in molecular components of sperm that may prevent fertilization; nevertheless, a huge number of cases remains unexplained (Barratt et al., 2017). The development of new in vitro strategies to study male infertility and/or testicular functions has opened up new areas in complex organ analysis, reprotoxicology, and regenerative technologies, with the arrival of the testicular organoid concept (Alves-Lopes & Stukenborg, 2018; Baert, de Kock, et al., 2017).

Several efforts have been made in the past decades to reproduce spermatogenesis in vitro, aiming to promote the differentiation of germ cells into fertilization-competent spermatozoa used to treat infertility associated with the male factor (J. H. Lee et al., 2006; M. Sun et al., 2018) or to restore fertility using cryopreserved prepubertal testicular tissue (de Michele et al., 2017; Kanbar et al., 2021). The absence of an adequate arrangement of testicular cells in 2D cultures at least partially explained the failure of in vitro maturation, which was surpassed by the use of 3D cell culture models, able to mimic the in vivo cell configuration required for a proper cellular response. Organotypic cultures have been the most successful technique in spermatogenesis reproduction ex vivo, although the outcomes are different depending on the species used. In mice, it was reported the birth of healthy fertile offspring with sperm obtained from cultures of 1–3 mm^3 immature testicular tissue fragments (Komeya et al., 2016; Sato et al., 2011; Yokonishi et al., 2014), while in humans few studies reported the reproduction of the meiotic process with poor efficiency, not allowing the full characterization of the haploid cells generated (de Michele et al., 2018; Yuan et al., 2020). The development of testicular organoids provided in vitro reproducible models to understand the complex niche of mammalian germ cells and the mechanisms involved in spermatogenesis regulation. Especially in nonrodent species, the study of spermatogenesis remains challenging; thus, the use of organoid cultures to study the complex factors and signal pathways involved will be more similar to in vivo process when compared with 2D cultures. The use of scaffolds-based approaches, such as hydrogels, have been increased (Hadley et al., 1985; Vermeulen et al., 2019) and human round spermatids able to fertilize mice oocyte were generated in a Matrigel®-based culture system (M. Sun et al., 2018). Despite some promising results, the spermatogenic efficiency of testicular 3D cultures remains minimal. Also, the limited recapitulation of testicular architecture, especially when using cells from adults presenting testicular failure, makes the possibility of sperm production in these systems still remote. This limitation can be surpassed by co-culturing immature and adult testicular cell suspensions, as already shown in mice (Edmonds & Woodruff, 2020). Further research should focus on the establishment of testicular organoids with a structure more approximate to the in vivo, which will facilitate the production of fertilizing spermatozoa. Furthermore, the development of testis organoids can improve the knowledge of several reproductive diseases, since testis disease-specific organoids can be developed using cells from patients with infertility scenarios (e.g., Klinefelter syndrome or testicular cancer) and the mechanisms involved in pathogenesis studied. On the other hand, testicular organoids are important tools to study molecular mechanisms involved in testicular dysfunction, to investigate drug toxicity (Pendergraft et al., 2017), and evaluate the impact of environmental toxicants (Sakib, Voigt, et al., 2019) and virus (Strange et al., 2018). Organoids are capable to respond to drugs and toxins similarly to their counterpart organ, becoming a perfect tool to test new drugs and reduce the slow process of drug approval by the competent agencies [e.g., U.S. Food & Drug Administration (FDA) and European Medicines Agency (EMA); Skardal et al., 2020]. Considering its diverse applications, this 3D culture model represents a promising alternative to animal models, especially for pharmaceutical drug development and testing.

In the male fertility field, epididymis has a high impact due to its important role in spermatozoa maturation and motility acquisition. The correct maturation is ensured by the luminal ion concentration, and the needed environment is ensured by several processes, highlighting the absorptive and secretory activities of the epithelial cell in the epididymal duct and ensuring the spermatozoa cells' survival and protection (Bedford, 1967; Orgebin-Crist, 1967; W. Zhou et al., 2018). However, its role in spermatozoa maturation, transport, protection, and storage is still poorly understood. Until now, only two 3D organoids of epididymis were developed (Pinel & Cyr, 2021). The main challenge in the development of this model is the need to generate an intact BEB, crucial for its use in drug testing and toxicity tests. The ability to mimic the different portions of the epididymis also represents a huge challenge. The generation of an epididymal

organoid presenting the four distinct segments of the epididymis or, at least, organoids derived from each portion, would be extremely helpful in the study of sperm transport, maturation, and motility acquisition and to investigate the underlying molecular mechanisms. Furthermore, considering the low rate of primary tumors in the epididymis (Yeung et al., 2012), it would be interesting to study the reason, if any exists, for this interesting feature. Indeed, although it is hypothesized that epididymis is prevented from malignant tumors due to the mechanisms involved in quiescence and immunologically silence of spermatozoa throughout the epididymal journey (Yeung et al., 2012), little remains known.

Additionally, the different male reproductive system organoids already developed and generated in the future, including prostatic, testicular, and epididymal organoids and, if possible, seminal vesicle organoids, can be joined in a single device such as multi-organ-on-a-chip. The development of this valuable platform may allow in the future the test of different drugs in the male reproductive system, and, considering that these organs are responsible for the secretion of all semen components (sperm cells, epididymal, prostatic, and seminal vesical secretions) can be used to investigate cellular and molecular mechanisms involved in semen quality deterioration.

The organoids of the male reproductive system developed have the potential to improve our knowledge of the mechanisms regulating their morphogenesis, physiology, and pathophysiology. They might represent useful tools to understand the complex mechanisms involved in infertility, as well as to study therapeutic efficacy and drug toxicity. Thus, it is crucial the optimization of culture media and its components, the development of standardized protocols to ensure the reproducibility and comparability of future research data. The opportunity to combine organoid technology with new bioengineering techniques, such as bioprinting or microfluidics, opens new doors for the development of interesting study models of male infertility.

5 | CONCLUSION

The tridimensionality of organoids provides advantages over conventional 2D culture conditions as it modulates cell–cell and cell–ECM interactions and mimics organs' function. Organoids have proven to be helpful in the in vitro study of tissues or even diseases, providing means to study their molecular and cellular mechanisms and possibly disclosing the ability to identify a patient-specific therapeutic approach. These in vitro models can mimic a situation closer to the in vivo representing a great resource for health care research and a promising tool to be used in personalized medicine. Nevertheless, this technology still presents some challenges, such as the need for optimization of culture conditions and organoid reproducibility.

The access to organs for the study of human reproduction is a challenge, especially in humans. Recently, different male reproductive system organoids have been developed, including prostate and testicular organoids. The 3D models of the prostate have been intensively used for the study of many types of PCa and represents a huge opportunity to study tumor progression and test anticancer drugs. Testicular organoids have been used in fertility research for the study of spermatozoa production, germ cells–somatic cells interactions, and mechanisms of disease. Although recent, the development of epididymal organoids opens a new area of research in epididymal biology. These new models have the potential to improve our knowledge of the mechanisms regulating their morphogenesis, physiology, and pathophysiology and might represent useful tools to (i) identify and characterize the complex mechanisms involved in infertility, (ii) study therapeutic efficacy, (iii) access drug toxicity, and (iv) develop strategies for fertility preservation and restoration.

The combination of organoid technology with new bioengineering techniques, such as bioprinting or microfluidics, allows the development of new models of male infertility. Additionally, 3D cultures can be applied to tissue regeneration and engineering, as already reached in the urological field for urethral reconstruction. In the future, biomaterial researchers and tissue engineers will undoubtedly find novel ways to repair and replace more tissues and organs within the human body, which will certainly have a lot of value and application in clinics.

AUTHOR CONTRIBUTIONS

Daniela Patrício: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Joana Santiago:** Data curation (equal); investigation (equal); writing – original draft (equal); writing – review and editing (equal). **João F. Mano:** Funding acquisition (equal); writing – review and editing (equal). **Margarida Fardilha:** Funding acquisition (equal); supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Abu Elhija, M., Lunenfeld, E., Schlatt, S., & Huleihel, M. (2012). Differentiation of murine male germ cells to spermatozoa in a soft agar culture system. *Asian Journal of Andrology*, *14*(2), 285–293. https://doi.org/10.1038/AJA.2011.112

AbuMadighem, A., Shuchat, S., Lunenfeld, E., Yossifon, G., & Huleihel, M. (2022). Testis on a chip—A microfluidic 3-dimensional culture system for the development of spermatogenesis in-vitro. *Biofabrication*, *14*, 035004. https://doi.org/10.1088/1758-5090/ac6126

Almany, L., & Seliktar, D. (2005). Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures. *Biomaterials*, *26*(15), 2467–2477. https://doi.org/10.1016/j.biomaterials.2004.06.047

Alves-Lopes, J. P., Söder, O., & Stukenborg, J.-B. (2017). Testicular organoid generation by a novel in vitro three-layer gradient system. *Biomaterials*, *130*, 76–89. https://doi.org/10.1016/j.biomaterials.2017.03.025

Alves-Lopes, J. P., & Stukenborg, J.-B. (2018). Testicular organoids: A new model to study the testicular microenvironment in vitro? *Human Reproduction Update*, *24*(2), 176–191. https://doi.org/10.1093/humupd/dmx036

Atala, A., Guzman, L., & Retik, A. B. (1999). A novel inert collagen matrix for hypospadias repair. *Journal of Urology*, *162*(3 Part 2), 1148–1150. https://doi.org/10.1016/S0022-5347(01)68105-9

Aw Yong, K. M., Li, Z., Merajver, S. D., & Fu, J. (2017). Tracking the tumor invasion front using long-term fluidic tumoroid culture. *Scientific Reports*, *7*(1), 10784. https://doi.org/10.1038/s41598-017-10874-1

Baert, Y., de Kock, J., Alves-Lopes, J. P., Söder, O., Stukenborg, J., & Goossens, E. (2017). Primary human testicular cells self-organize into organoids with testicular properties. *Stem Cell Reports*, *8*(1), 30–38. https://doi.org/10.1016/j.stemcr.2016.11.012

Baert, Y., Dvorakova-Hortova, K., Margaryan, H., & Goossens, E. (2019). Mouse in vitro spermatogenesis on alginate-based 3D bioprinted scaffolds. *Biofabrication*, *11*(3), 035011. https://doi.org/10.1088/1758-5090/ab1452

Baert, Y., Rombaut, C., & Goossens, E. (2017). Scaffold-based and scaffold-free testicular organoids from primary human testicular cells. *Methods in Molecular Biology*, *1576*, 283–290. https://doi.org/10.1007/7651_2017_48

Baert, Y., Ruetschle, I., Cools, W., Oehme, A., Lorenz, A., Marx, U., Goossens, E., & Maschmeyer, I. (2020). A multi-organ-chip co-culture of liver and testis equivalents: A first step toward a systemic male reprotoxicity model. *Human Reproduction*, *35*(5), 1029–1044. https://doi.org/10.1093/humrep/deaa057

Barratt, C. L. R., Björndahl, L., de Jonge, C. J., Lamb, D. J., Osorio Martini, F., McLachlan, R., Oates, R. D., van der Poel, S., St John, B., Sigman, M., Sokol, R., & Tournaye, H. (2017). The diagnosis of male infertility: An analysis of the evidence to support the development of global WHO guidance—Challenges and future research opportunities. *Human Reproduction Update*, *23*(6), 660–680. https://doi.org/10.1093/humupd/dmx021

Bartfeld, S., Bayram, T., van de Wetering, M., Huch, M., Begthel, H., Kujala, P., Vries, R., Peters, P. J., & Clevers, H. (2015). In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology*, *148*(1), 126–136.e6. https://doi.org/10.1053/J.GASTRO.2014.09.042

Bedford, J. M. (1967). Effects of duct ligation on the fertilzing ability of spermatozoa from different regions of the rabbit epididymis. *Journal of Experimental Zoology*, *166*(2), 271–281. https://doi.org/10.1002/jez.1401660210

Bennet, D., Estlack, Z., Reid, T., & Kim, J. (2018). A microengineered human corneal epithelium-on-a-chip for eye drops mass transport evaluation. *Lab on a Chip*, *18*(11), 1539–1551. https://doi.org/10.1039/C8LC00158H

Beshiri, M. L., Tice, C. M., Tran, C., Nguyen, H. M., Sowalsky, A. G., Agarwal, S., Jansson, K. H., Yang, Q., McGowen, K. M., Yin, J., Alilin, A. N., Karzai, F. H., Dahut, W. L., Corey, E., & Kelly, K. (2018). A PDX/organoid biobank of advanced prostate cancers captures genomic and phenotypic heterogeneity for disease modeling and therapeutic screening. *Clinical Cancer Research*, *24*(17), 4332–4345. https://doi.org/10.1158/1078-0432.CCR-18-0409

Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., Bagley, J. A., Krauditsch, C., & Knoblich, J. A. (2018). Genetically engineered cerebral organoids model brain tumor formation. *Nature Methods*, *15*(8), 631–639. https://doi.org/10.1038/s41592-018-0070-7

Boj, S. F., Hwang, C. I., Baker, L. A., Chio, I. I. C., Engle, D. D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M. S., Gracanin, A., Oni, T., Yu, K. H., van Boxtel, R., Huch, M., Rivera, K. D., Wilson, J. P., Feigin, M. E., Öhlund, D., … Tuveson, D. A. (2015). Organoid models of human and mouse ductal pancreatic cancer. *Cell*, *160*, 324–338. https://doi.org/10.1016/j.cell.2014.12.021

Boj, S. F., Hwang, C.-I., Baker, L. A., Engle, D. D., Tuveson, D. A., & Clevers, H. (2016). Model organoids provide new research opportunities for ductal pancreatic cancer. *Molecular & Cellular Oncology*, *3*(1), e1014757. https://doi.org/10.1080/23723556.2015.1014757

Boretto, M., Maenhoudt, N., Luo, X., Hennes, A., Boeckx, B., Bui, B., Heremans, R., Perneel, L., Kobayashi, H., van Zundert, I., Brems, H., Cox, B., Ferrante, M., Uji-I, H., Koh, K. P., D'Hooghe, T., Vanhie, A., Vergote, I., Meuleman, C., … Vankelecom, H. (2019). Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nature Cell Biology*, *21*(8), 1041–1051. https://doi.org/10.1038/s41556-019-0360-z

Breton, S., Ruan, Y. C., Park, Y.-J. J., & Kim, B. (2016). Regulation of epithelial function, differentiation, and remodeling in the epididymis. *Asian Journal of Andrology*, *18*(1), 3–9. https://doi.org/10.4103/1008-682X.165946

Broutier, L., Mastrogiovanni, G., Verstegen, M. M., Francies, H. E., Gavarró, L. M., Bradshaw, C. R., Allen, G. E., Arnes-Benito, R., Sidorova, O., Gaspersz, M. P., Georgakopoulos, N., Koo, B. K., Dietmann, S., Davies, S. E., Praseedom, R. K., Lieshout, R., IJzermans, J. N. M., Wigmore, S. J., Saeb-Parsy, K., … Huch, M. (2017). Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. *Nature Medicine*, *23*(12), 1424–1435. https://doi.org/10.1038/nm.4438

Bruno, R. D., Fleming, J. M., George, A. L., Boulanger, C. A., Schedin, P., & Smith, G. H. (2017). Mammary extracellular matrix directs differentiation of testicular and embryonic stem cells to form functional mammary glands in vivo. *Scientific Reports*, *7*(1), 40196. https://doi.org/10.1038/srep40196

Buskin, A., Singh, P., Lorenz, O., Robson, C., Strand, D. W., & Heer, R. (2021). A review of prostate organogenesis and a role for iPSC-derived prostate organoids to study prostate development and disease. *International Journal of Molecular Sciences*, *22*(23), 13097. https://doi.org/10.3390/ijms222313097

Calderon-Gierszal, E. L., & Prins, G. S. (2015). Directed differentiation of human embryonic stem cells into prostate organoids In vitro and its perturbation by low-dose Bisphenol a exposure. *PLoS One*, *10*(7), e0133238. https://doi.org/10.1371/journal.pone.0133238

Carneiro, B. A., Pamarthy, S., Shah, A. N., Sagar, V., Unno, K., Han, H., Yang, X. J., Costa, R. B., Nagy, R. J., Lanman, R. B., Kuzel, T. M., Ross, J. S., Gay, L., Elvin, J. A., Ali, S. M., Cristofanilli, M., Chae, Y. K., Giles, F. J., & Abdulkadir, S. A. (2018). Anaplastic lymphoma kinase mutation (ALK F1174C) in small cell carcinoma of the prostate and molecular response to Alectinib. *Clinical Cancer Research*, *24*(12), 2732–2739. https://doi.org/10.1158/1078-0432.CCR-18-0332

Cham, T.-C., Chen, X., & Honaramooz, A. (2021). Current progress, challenges, and future prospects of testis organoids. *Biology of Reproduction*, *104*(5), 942–961. https://doi.org/10.1093/biolre/ioab014

Cham, T.-C., Ibtisham, F., Fayaz, M. A., & Honaramooz, A. (2021). Generation of a highly biomimetic organoid, including vasculature, resembling the native immature testis tissue. *Cell*, *10*(7), 1696. https://doi.org/10.3390/cells10071696

Chambers, K. F., Mosaad, E. M. O., Russell, P. J., Clements, J. A., & Doran, M. R. (2014). 3D cultures of prostate cancer cells cultured in a novel high-throughput culture platform are more resistant to chemotherapeutics compared to cells cultured in monolayer. *PLoS One*, *9*(11), e111029. https://doi.org/10.1371/journal.pone.0111029

Chiaradia, I., & Lancaster, M. A. (2020). Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. *Nature Neuroscience*, *23*(12), 1496–1508. https://doi.org/10.1038/S41593-020-00730-3

Chinta, M. S., Desjardins-Park, H. E., Wan, D. C., & Longaker, M. T. (2020). "Tissues in a dish": A review of organoids in plastic surgery. *Plastic and Reconstructive Surgery*, *8*(4), e2787. https://doi.org/10.1097/GOX.0000000000002787

Choi, K.-Y. G., Wu, B. C., Lee, A. H.-Y., Baquir, B., & Hancock, R. E. W. (2020). Utilizing organoid and air-liquid interface models as a screening method in the development of new host defense peptides. *Frontiers in Cellular and Infection Microbiology*, *10*, 228. https://doi.org/10.3389/fcimb.2020.00228

Choo, N., Ramm, S., Luu, J., Winter, J. M., Selth, L. A., Dwyer, A. R., Frydenberg, M., Grummet, J., Sandhu, S., Hickey, T. E., Tilley, W. D., Taylor, R. A., Risbridger, G. P., Lawrence, M. G., & Simpson, K. J. (2021). High-throughput imaging assay for drug screening of 3D prostate cancer organoids. *SLAS Discovery*, *26*(9), 1107–1124. https://doi.org/10.1177/24725552211020668

Chua, C. W., Shibata, M., Lei, M., Toivanen, R., Barlow, L. J., Bergren, S. K., Badani, K. K., McKiernan, J. M., Benson, M. C., Hibshoosh, H., & Shen, M. M. (2014). Single luminal epithelial progenitors can generate prostate organoids in culture. *Nature Cell Biology*, *16*(10), 951–961. https://doi.org/10.1038/ncb3047

Clevers, H. (2016). Modeling development and disease with organoids. *Cell*, *165*(7), 1586–1597. https://doi.org/10.1016/J.CELL.2016.05.082

Conteduca, V., Ku, S.-Y., Puca, L., Slade, M., Fernandez, L., Hess, J., Bareja, R., Vlachostergios, P. J., Sigouros, M., Mosquera, J. M., Sboner, A., Nanus, D. M., Elemento, O., Dittamore, R., Tagawa, S. T., & Beltran, H. (2020). SLFN11 expression in advanced prostate cancer and response to platinum-based chemotherapy. *Molecular Cancer Therapeutics*, *19*(5), 1157–1164. https://doi.org/10.1158/1535-7163.MCT-19-0926

Correia, C. R., Nadine, S., & Mano, J. F. (2020). Cell encapsulation systems toward modular tissue regeneration: From Immunoisolation to multifunctional devices. *Advanced Functional Materials*, *30*(26), 1908061. https://doi.org/10.1002/adfm.201908061

Cristobal, A., van den Toorn, H. W. P., van de Wetering, M., Clevers, H., Heck, A. J. R., & Mohammed, S. (2017). Personalized proteome profiles of healthy and tumor human colon organoids reveal both individual diversity and basic features of colorectal cancer. *Cell Reports*, *18*, 263–274. https://doi.org/10.1016/j.celrep.2016.12.016

Crowell, P. D., Giafaglione, J. M., Hashimoto, T., Diaz, J. A., & Goldstein, A. S. (2019). Evaluating the differentiation capacity of mouse prostate epithelial cells using organoid culture. *Journal of Visualized Experiments*, *176*(153), 139–148. https://doi.org/10.3791/60223

Crowley, L., & Shen, M. M. (2022). Heterogeneity and complexity of the prostate epithelium: New findings from single-cell RNA sequencing studies. *Cancer Letters*, *525*(July), 108–114. https://doi.org/10.1016/j.canlet.2021.10.035

Cruz, N. M., Song, X., Czerniecki, S. M., Gulieva, R. E., Churchill, A. J., Kim, Y. K., Winston, K., Tran, L. M., Diaz, M. A., Fu, H., Finn, L. S., Pei, Y., Himmelfarb, J., & Freedman, B. S. (2017). Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. *Nature Materials*, *16*(11), 1112–1119. https://doi.org/10.1038/nmat4994

Cyr, D. G., & Pinel, L. (2022). Emerging organoid models to study the epididymis in male reproductive toxicology. *Reproductive Toxicology*, *112*, 88–99. https://doi.org/10.1016/j.reprotox.2022.07.001

Dambal, S., Baumann, B., McCray, T., Williams, L., Richards, Z., Deaton, R., Prins, G. S., & Nonn, L. (2017). The miR-183 family cluster alters zinc homeostasis in benign prostate cells, organoids and prostate cancer xenografts. *Scientific Reports*, *7*(1), 7704. https://doi.org/10.1038/s41598-017-07979-y

de Michele, F., Poels, J., Vermeulen, M., Ambroise, J., Gruson, D., Guiot, Y., & Wyns, C. (2018). Haploid germ cells generated in organotypic culture of testicular tissue from prepubertal boys. *Frontiers in Physiology*, *9*, 1413. https://doi.org/10.3389/fphys.2018.01413

de Michele, F., Vermeulen, M., & Wyns, C. (2017). Fertility restoration with spermatogonial stem cells. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *24*(6), 424–431. https://doi.org/10.1097/MED.0000000000000370

Decher, G., & Hong, J.-D. (1991). Buildup of ultrathin multilayer films by a self-assembly process, 1 consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces. *Makromolekulare Chemie, Macromolecular Symposia*, *46*(1), 321–327. https://doi.org/10.1002/masy.19910460145

Dekkers, J. F., Berkers, G., Kruisselbrink, E., Vonk, A., de Jonge, H. R., Janssens, H. M., Bronsveld, I., van de Graaf, E. A., Nieuwenhuis, E. E., Houwen, R. H., Vleggaar, F. P., Escher, J. C., de Rijke, Y. B., Majoor, C. J., Heijerman, H. G., de Winter-de Groot, K. M., Clevers, H., van der Ent, C. K., & Beekman, J. M. (2016). Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Science Translational Medicine*, *8*(344), 344ra84. https://doi.org/10.1126/scitranslmed.aad8278

Dekkers, J. F., Wiegerinck, C. L., de Jonge, H. R., Bronsveld, I., Janssens, H. M., Bronsveld, I., van de Graaf, E. A., Nieuwenhuis, E. E., Houwen, R. H., Vleggaar, F. P., Escher, J. C., de Rijke, Y. B., Majoor, C. J., Heijerman, H. G., de Winter-de Groot, K. M., Clevers, H., van der Ent, C. K., & Beekman, J. M. (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nature Medicine*, *19*(7), 939–945. https://doi.org/10.1038/nm.3201

Dhimolea, E., de Matos Simoes, R., Kansara, D., Weng, X., Sharma, S., Awate, P., Liu, Z., Gao, D., Mitsiades, N., Schwab, J. H., Chen, Y., Jeselsohn, R., Culhane, A. C., Brown, M., Georgakoudi, I., & Mitsiades, C. S. (2021). Pleiotropic mechanisms drive endocrine resistance in the three-dimensional bone microenvironment. *Cancer Research*, *81*(2), 371–383. https://doi.org/10.1158/0008-5472.CAN-20-0571

Dijkstra, K. K., Cattaneo, C. M., Weeber, F., Chalabi, M., van de Haar, J., Fanchi, L. F., Slagter, M., van der Velden, D. L., Kaing, S., Kelderman, S., van Rooij, N., van Leerdam, M. E., Depla, A., Smit, E. F., Hartemink, K. J., de Groot, R., Wolkers, M. C., Sachs, N., Snaebjornsson, P., ... Voest, E. E. (2018). Generation of tumor-reactive T cells by Co-culture of peripheral blood lymphocytes and tumor organoids. *Cell*, *174*(6), 1586–1598.e12. https://doi.org/10.1016/j.cell.2018.07.009

Drost, J., & Clevers, H. (2018). Organoids in cancer research. *Nature Reviews Cancer*, *18*(7), 407–418. https://doi.org/10.1038/s41568-018-0007-6

Drost, J., Karthaus, W. R., Gao, D., Driehuis, E., Sawyers, C. L., Chen, Y., & Clevers, H. (2016). Organoid culture systems for prostate epithelial and cancer tissue. *Nature Protocols*, *11*(2), 347–358. https://doi.org/10.1038/nprot.2016.006

Ebisudani, T., Sugimoto, S., Haga, K., Mitsuishi, A., Takai-Todaka, R., Fujii, M., Toshimitsu, K., Hamamoto, J., Sugihara, K., Hishida, T., Asamura, H., Fukunaga, K., Yasuda, H., Katayama, K., & Sato, T. (2021). Direct derivation of human alveolospheres for SARS-CoV-2 infection modeling and drug screening. *Cell Reports*, *35*(10), 109218. https://doi.org/10.1016/J.CELREP.2021.109218

Eder, T., & Eder, I. E. (2017). 3D hanging drop culture to establish prostate cancer organoids. *Methods in Molecular Biology*, *1612*, 167–175. https://doi.org/10.1007/978-1-4939-7021-6_12

Eder, T., Weber, A., Neuwirt, H., Grünbacher, G., Ploner, C., Klocker, H., Sampson, N., & Eder, I. (2016). Cancer-associated fibroblasts modify the response of prostate cancer cells to androgen and anti-androgens in three-dimensional spheroid culture. *International Journal of Molecular Sciences*, *17*(9), 1458. https://doi.org/10.3390/ijms17091458

Edington, C. D., Chen, W. L. K., Geishecker, E., Kassis, T., Soenksen, L. R., Bhushan, B. M., Freake, D., Kirschner, J., Maass, C., Tsamandouras, N., Valdez, J., Cook, C. D., Parent, T., Snyder, S., Yu, J., Suter, E., Shockley, M., Velazquez, J., Velazquez, J. J., ... Griffith, L. G. (2018). Interconnected microphysiological systems for quantitative biology and pharmacology studies. *Scientific Reports*, *8*(1), 4530. https://doi.org/10.1038/s41598-018-22749-0

Edmonds, M. E., & Woodruff, T. K. (2020). Testicular organoid formation is a property of immature somatic cells, which self-assemble and exhibit long-term hormone-responsive endocrine function. *Biofabrication*, *12*(4), 045002. https://doi.org/10.1088/1758-5090/ab9907

el Ramy, R., Verot, A., Mazaud, S., Odet, F., Magre, S., & Le Magueresse-Battistoni, B. (2005). Fibroblast growth factor (FGF) 2 and FGF9 mediate mesenchymal–epithelial interactions of peritubular and Sertoli cells in the rat testis. *Journal of Endocrinology*, *187*(1), 135–147. https://doi.org/10.1677/joe.1.06146

Elbadawy, M., Abugomaa, A., Yamawaki, H., Usui, T., & Sasaki, K. (2020). Development of prostate cancer organoid culture models in basic medicine and translational research. *Cancers*, *12*(4), 777. https://doi.org/10.3390/cancers12040777

El-Sherbiny, I. M., & Yacoub, M. H. (2013). Hydrogel scaffolds for tissue engineering: Progress and challenges. *Global Cardiology Science and Practice*, *2013*(3), 338–342. https://doi.org/10.5339/gcsp.2013.38

Erbani, J., Aberdam, D., Larghero, J., & Vanneaux, V. (2016). Pluripotent stem cells and other innovative strategies for the treatment of ocular surface diseases. *Stem Cell Reviews and Reports*, *12*(2), 171–178. https://doi.org/10.1007/s12015-016-9643-y

Ettayebi, K., Crawford, S. E., Murakami, K., Broughman, J. R., Karandikar, U., Tenge, V. R., Neill, F. H., Blutt, S. E., Zeng, X. L., Qu, L., Kou, B., Opekun, A. R., Burrin, D., Graham, D. Y., Ramani, S., Atmar, R. L., & Estes, M. K. (2016). Replication of human noroviruses in stem cell-derived human enteroids. *Science (New York, N.Y.)*, *353*(6306), 1387–1393. https://doi.org/10.1126/SCIENCE.AAF5211

Fardilha, M., Silva, J. V., & Conde, M. (2015). *Reprodução humana masculina—Princípios fundamentais* (1st ed.). ARC Publishing.

Federspil, G., & Sicolo, N. (1994). The nature of life in the history of medical and philosophic thinking. *American Journal of Nephrology*, *14*(4–6), 337–343. https://doi.org/10.1159/000168745

Finkbeiner, S. R., Zeng, X. L., Utama, B., Atmar, R. L., Shroyer, N. F., & Estesa, M. K. (2012). Stem cell-derived human intestinal organoids as an infection model for rotaviruses. *MBio*, *3*(4), e00159–12. https://doi.org/10.1128/MBIO.00159-12

Fong, E. L. S., Wan, X., Yang, J., Morgado, M., Mikos, A. G., Harrington, D. A., Navone, N. M., & Farach-Carson, M. C. (2016). A 3D in vitro model of patient-derived prostate cancer xenograft for controlled interrogation of in vivo tumor-stromal interactions. *Biomaterials*, *77*, 164–172. https://doi.org/10.1016/j.biomaterials.2015.10.059

Freedman, B. S., Brooks, C. R., Lam, A. Q., Fu, H., Morizane, R., Agrawal, V., Saad, A. F., Li, M. K., Hughes, M. R., Werff, R. V., Peters, D. T., Lu, J., Baccei, A., Siedlecki, A. M., Valerius, M. T., Musunuru, K., McNagny, K. M., Steinman, T. I., Zhou, J., ... Bonventre, J. V. (2015). Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nature Communications*, *6*(1), 8715. https://doi.org/10.1038/ncomms9715

Ganesh, K., Wu, C., O'Rourke, K. P., Szeglin, B. C., Zheng, Y., Sauvé, C.-E. G., Adileh, M., Wasserman, I., Marco, M. R., Kim, A. S., Shady, M., Sanchez-Vega, F., Karthaus, W. R., Won, H. H., Choi, S. H., Pelossof, R., Barlas, A., Ntiamoah, P., Pappou, E., ... Smith, J. J. (2019). A rectal cancer organoid platform to study individual responses to chemoradiation. *Nature Medicine*, *25*(10), 1607–1614. https://doi.org/10.1038/s41591-019-0584-2

Gao, D., Vela, I., Sboner, A., Iaquinta, P. J., Karthaus, W. R., Gopalan, A., Dowling, C., Wanjala, J. N., Undvall, E. A., Arora, V. K., Wongvipat, J., Kossai, M., Ramazanoglu, S., Barboza, L. P., Di, W., Cao, Z., Zhang, Q. F., Sirota, I., Ran, L., ... Chen, Y. (2014). Organoid cultures derived from patients with advanced prostate cancer. *Cell*, *159*(1), 176–187. https://doi.org/10.1016/j.cell.2014.08.016

Gleave, A. M., Ci, X., Lin, D., & Wang, Y. (2020). A synopsis of prostate organoid methodologies, applications, and limitations. *The Prostate*, *80*(6), 518–526. https://doi.org/10.1002/pros.23966

Goldsmith, T. M., Sakib, S., Webster, D., Carlson, D. F., van der Hoorn, F., & Dobrinski, I. (2020). A reduction of primary cilia but not hedgehog signaling disrupts morphogenesis in testicular organoids. *Cell and Tissue Research*, *380*(1), 191–200. https://doi.org/10.1007/s00441-019-03121-8

Gong, X., Xie, H., Li, X., Wu, J., & Lin, Y. (2017). Bisphenol a induced apoptosis and transcriptome differences of spermatogonial stem cells in vitro. *Acta Biochimica et Biophysica Sinica*, *49*(9), 780–791. https://doi.org/10.1093/abbs/gmx075

Gregory, M., & Cyr, D. G. (2014). The blood-epididymis barrier and inflammation. *Spermatogenesis*, *4*(2), e979619. https://doi.org/10.4161/21565562.2014.979619

Griffith, L. G., & Swartz, M. A. (2006). Capturing complex 3D tissue physiology in vitro. *Nature Reviews Molecular Cell Biology*, *7*(3), 211–224. https://doi.org/10.1038/nrm1858

Hadley, M. A., Byers, S. W., Suàrez-Quian, C. A., Kleinman, H. K., & Dym, M. (1985). Extracellular matrix regulates sertoli cell differentiation, testicular cord formation, and germ cell development in vitro. *Journal of Cell Biology*, *101*(4), 1511–1522. https://doi.org/10.1083/jcb.101.4.1511

Hadley, M. A., Weeks, B. S., Kleinman, H. K., & Dym, M. (1990). Laminin promotes formation of cord-like structures by sertoli cells in vitro. *Developmental Biology*, *140*(2), 318–327. https://doi.org/10.1016/0012-1606(90)90082-T

Halldorsson, S., Lucumi, E., Gómez-Sjöberg, R., & Fleming, R. M. T. (2015, January). Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. *Biosensors and Bioelectronics, 63*, 218–231. https://doi.org/10.1016/j.bios.2014.07.029

Härmä, V., Virtanen, J., Mäkelä, R., Happonen, A., Mpindi, J.-P., Knuuttila, M., Kohonen, P., Lötjönen, J., Kallioniemi, O., & Nees, M. (2010). A comprehensive panel of three-dimensional models for studies of prostate cancer growth, invasion and drug responses. *PLoS One*, *5*(5), e10431. https://doi.org/10.1371/journal.pone.0010431

Harris, S., Shubin, S. P., Wegner, S., van Ness, K., Green, F., Hong, S. W., & Faustman, E. M. (2016). The presence of macrophages and inflammatory responses in an in vitro testicular co-culture model of male reproductive development enhance relevance to in vivo conditions. *Toxicology in Vitro: An International Journal Published in Association with BIBRA*, *36*, 210–215. https://doi.org/10.1016/J.TIV.2016.08.003

Harrison, R. G., Greenman, M. J., Mall, F. P., & Jackson, C. M. (1907). Observations of the living developing nerve fiber. *The Anatomical Record*, *1*(5), 116–128. https://doi.org/10.1002/ar.1090010503

Heidari-Khoei, H., Esfandiari, F., Hajari, M. A., Ghorbaninejad, Z., Piryaei, A., & Baharvand, H. (2020). Organoid technology in female reproductive biomedicine. *Reproductive Biology and Endocrinology*, *18*(1), 64. https://doi.org/10.1186/s12958-020-00621-z

Heninger, E., Kosoff, D., Rodems, T. S., Sethakorn, N., Singh, A., Gungurthi, H., Carlson, K. N., Yang, B., Gilsdorf, C., Pasch, C. A., Deming, D. A., Ellis, L., Beebe, D. J., Jarrard, D. F., & Lang, J. M. (2021). Live cell molecular analysis of primary prostate cancer organoids identifies persistent androgen receptor signaling. *Medical Oncology*, *38*(11), 135. https://doi.org/10.1007/s12032-021-01582-y

Hepburn, A. C., Curry, E. L., Moad, M., Steele, R. E., Franco, O. E., Wilson, L., Singh, P., Buskin, A., Crawford, S. E., Gaughan, L., Mills, I. G., Hayward, S. W., Robson, C. N., & Heer, R. (2020). Propagation of human prostate tissue from induced pluripotent stem cells. *Stem Cells Translational Medicine*, *9*(7), 734–745. https://doi.org/10.1002/sctm.19-0286

Hepburn, A. C., Sims, C. H. C., Buskin, A., & Heer, R. (2020). Engineering prostate cancer from induced pluripotent stem cells—New opportunities to develop preclinical tools in prostate and prostate cancer studies. *International Journal of Molecular Sciences*, *21*(3), 905. https://doi.org/10.3390/ijms21030905

Hill, S. J., Decker, B., Roberts, E. A., Horowitz, N. S., Muto, M. G., Worley, M. J., Feltmate, C. M., Nucci, M. R., Swisher, E. M., Nguyen, H., Yang, C., Morizane, R., Kochupurakkal, B. S., Do, K. T., Konstantinopoulos, P. A., Liu, J. F., Bonventre, J. V., Matulonis, U. A., Shapiro, G. I., ... D'Andrea, A. D. (2018). Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. *Cancer Discovery*, *8*(11), 1404–1421. https://doi.org/10.1158/2159-8290.CD-18-0474

Ho, B., Pek, N., & Soh, B.-S. (2018). Disease modeling using 3D organoids derived from human induced pluripotent stem cells. *International Journal of Molecular Sciences*, *19*(4), 936. https://doi.org/10.3390/ijms19040936

Hofmann, M. C., Narisawa, S., Hess, R. A., & Millán, J. L. (1992). Immortalization of germ cells and somatic testicular cells using the SV40 large T antigen. *Experimental Cell Research*, *201*(2), 417–435. https://doi.org/10.1016/0014-4827(92)90291-F

Howard, D., Buttery, L. D., Shakesheff, K. M., & Roberts, S. J. (2008). Tissue engineering: Strategies, stem cells and scaffolds. *Journal of Anatomy*, *213*(1), 66–72. https://doi.org/10.1111/j.1469-7580.2008.00878.x

Hu, J. L., Todhunter, M. E., LaBarge, M. A., & Gartner, Z. J. (2018). Opportunities for organoids as new models of aging. *Journal of Cell Biology*, *217*(1), 39–50. https://doi.org/10.1083/JCB.201709054

Huang, J. Y., Sweeney, E. G., Sigal, M., Zhang, H. C., Remington, S. J., Cantrell, M. A., Kuo, C. J., Guillemin, K., & Amieva, M. R. (2015). Chemodetection and destruction of host urea allows helicobacter pylori to locate the epithelium. *Cell Host & Microbe*, *18*(2), 147–156. https://doi.org/10.1016/J.CHOM.2015.07.002

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M. M. A., Ellis, E., van Wenum, M., Fuchs, S. A., de Ligt, J., van de Wetering, M., Sasaki, N., Boers, S. J., Kemperman, H., de Jonge, J., Ijzermans, J. N. M., Nieuwenhuis, E. E. S., Hoekstra, R., Strom, S., ... Clevers, H. (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell*, *160*, 299–312. https://doi.org/10.1016/j.cell.2014.11.050

Huh, D., Hamilton, G. A., & Ingber, D. E. (2011). From 3D cell culture to organs-on-chips. *Trends in Cell Biology*, *21*(12), 745–754. https://doi.org/10.1016/j.tcb.2011.09.005

Huh, D., Matthews, B. D., Mammoto, A., Montoya-Zavala, M., Yuan Hsin, H., & Ingber, D. E. (2010). Reconstituting organ-level lung functions on a chip. *Science*, *328*(5986), 1662–1668. https://doi.org/10.1126/science.1188302

Hung, J. H., Chen, C. Y., Omar, H. A., Huang, K. Y., Tsao, C. C., Chiu, C. C., Chen, Y. L., Chen, P. H., & Teng, Y. N. (2016). Reactive oxygen species mediate Terbufos-induced apoptosis in mouse testicular cell lines via the modulation of cell cycle and pro-apoptotic proteins. *Environmental Toxicology*, *31*(12), 1888–1898. https://doi.org/10.1002/tox.22190

Jarchum, I. (2014). Prostate cancer in 3D. *Nature Methods*, *11*(11), 1096. https://doi.org/10.1038/nmeth.3167

Jensen, C., & Teng, Y. (2020). Is it time to start transitioning from 2D to 3D cell culture? *Frontiers in Molecular Biosciences*, *7*(33), 1–15. https://doi.org/10.3389/fmolb.2020.00033

Jeong, S. Y., Lee, S., Choi, W. H., Jee, J. H., Kim, H.-R., & Yoo, J. (2020). Fabrication of dentin-pulp-like organoids using dental-pulp stem cells. *Cell*, *9*(3), 642. https://doi.org/10.3390/cells9030642

Jian, H., Wang, M., Wang, S., Wang, A., & Bai, S. (2018). 3D bioprinting for cell culture and tissue fabrication. *Bio-Design and Manufacturing*, *1*(1), 45–61. https://doi.org/10.1007/s42242-018-0006-1

Jiang, B., Zheng, W., Zhang, W., & Jiang, X. (2014). Organs on microfluidic chips: A mini review. *Science China Chemistry*, *57*(3), 356–364. https://doi.org/10.1007/s11426-013-4971-0

Kajbafzadeh, A.-M., Abbasioun, R., Sabetkish, S., Sabetkish, N., Rahmani, P., Tavakkolitabassi, K., & Arshadi, H. (2017). Future prospects for human tissue engineered urethra transplantation: Decellularization and Recellularization-based urethra regeneration. *Annals of Biomedical Engineering*, *45*(7), 1795–1806. https://doi.org/10.1007/s10439-017-1857-x

Kanbar, M., Vermeulen, M., & Wyns, C. (2021). Organoids as tools to investigate the molecular mechanisms of male infertility and its treatments. *Reproduction*, *161*(5), R103–R112. https://doi.org/10.1530/REP-20-0499

Karthaus, W. R., Iaquinta, P. J., Drost, J., Gracanin, A., van Boxtel, R., Wongvipat, J., Dowling, C. M., Gao, D., Begthel, H., Sachs, N., Vries, R. G. J., Cuppen, E., Chen, Y., Sawyers, C. L., & Clevers, H. C. (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*, *159*(1), 163–175. https://doi.org/10.1016/j.cell.2014.08.017

Keeney, M., Jiang, X. Y., Yamane, M., Lee, M., Goodman, S., & Yang, F. (2015). Nanocoating for biomolecule delivery using layer-by-layer self-assembly. *Journal of Materials Chemistry B. Royal Society of Chemistry.*, *3*, 8757–8770. https://doi.org/10.1039/c5tb00450k

Kierszenbaum, A. L., Crowell, J. A., Shabanowitz, R. B., DePhilip, R. M., & Tres, L. L. (1986). Protein secretory patterns of rat Sertoli and peritubular cells are influenced by culture conditions. *Biology of Reproduction*, *35*(1), 239–251. https://doi.org/10.1095/biolreprod35.1.239

Kim, J., Koo, B. K., & Knoblich, J. A. (2020). Human organoids: Model systems for human biology and medicine. *Nature Reviews Molecular Cell Biology*, *21*(10), 571–584. https://doi.org/10.1038/s41580-020-0259-3

Kim, M., Mun, H., Sung, C. O., Cho, E. J., Jeon, H.-J., Chun, S.-M., Jung, D. J., Shin, T. H., Jeong, G. S., Kim, D. K., Choi, E. K., Jeong, S. Y., Taylor, A. M., Jain, S., Meyerson, M., & Jang, S. J. (2019). Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. *Nature Communications*, *10*(1), 3991. https://doi.org/10.1038/s41467-019-11867-6

Kim, S. M., Nguyen, T. T., Ravi, A., Kubiniok, P., Finicle, B. T., Jayashankar, V., Malacrida, L., Hou, J., Robertson, J., Gao, D., Chernoff, J., Digman, M. A., Potma, E. O., Tromberg, B. J., Thibault, P., & Edinger, A. L. (2018). PTEN deficiency and AMPK activation promote nutrient scavenging and anabolism in prostate cancer cells. *Cancer Discovery*, *8*(7), 866–883. https://doi.org/10.1158/2159-8290.CD-17-1215

Komeya, M., Kimura, H., Nakamura, H., Yokonishi, T., Sato, T., Kojima, K., Hayashi, K., Katagiri, K., Yamanaka, H., Sanjo, H., Yao, M., Kamimura, S., Inoue, K., Ogonuki, N., Ogura, A., Fujii, T., & Ogawa, T. (2016). Long-term ex vivo maintenance of testis tissues producing fertile sperm in a microfluidic device. *Scientific Reports*, *6*(1), 21472. https://doi.org/10.1038/srep21472

Koo, B., Choi, B., Park, H., & Yoon, K.-J. (2019). Past, present, and future of brain organoid technology. *Molecules and Cells*, *42*(9), 617–627. https://doi.org/10.14348/molcells.2019.0162

Kratochvil, M. J., Seymour, A. J., Li, T. L., Paşca, S. P., Kuo, C. J., & Heilshorn, S. C. (2019). Engineered materials for organoid systems. *Nature Reviews Materials*, *4*(9), 606–622. https://doi.org/10.1038/s41578-019-0129-9

Kristensen, D. M., Nielsen, J. E., Kalisz, M., Dalgaard, M. D., Audouze, K., Larsen, M. E., Jacobsen, G. K., Horn, T., Brunak, S., Skakkebaek, N. E., & Leffers, H. (2010). OCT4 and downstream factors are expressed in human somatic urogenital epithelia and in culture of epididymal spheres. *Molecular Human Reproduction*, *16*(11), 835–845. https://doi.org/10.1093/molehr/gaq008

Lamers, M. M., Vaart, J., Knoops, K., Riesebosch, S., Breugem, T. I., Mykytyn, A. Z., Beumer, J., Schipper, D., Bezstarosti, K., Koopman, C. D., Groen, N., Ravelli, R. B. G., Duimel, H. Q., Demmers, J. A. A., Verjans, G. M. G. M., Koopmans, M. P. G., Muraro, M. J., Peters, P. J., Clevers, H., & Haagmans, B. L. (2021). An organoid-derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type II-like cells. *The EMBO Journal*, *40*(5), e105912. https://doi.org/10.15252/EMBJ.2020105912

Lancaster, M. A., Renner, M., Martin, C. A., Wenzel, D., Bicknell, L. S., Hurles, M. E., Homfray, T., Penninger, J. M., Jackson, A. P., & Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature*, *501*(7467), 373–379. https://doi.org/10.1038/NATURE12517

Lang, S. H., Stark, M., Collins, A., Paul, A. B., Stower, M. J., & Maitland, N. J. (2001). Experimental prostate epithelial morphogenesis in response to stroma and three-dimensional matrigel culture. *Cell Growth & Differentiation: The Molecular Biology Journal of the American Association for Cancer Research*, *12*(12), 631–640.

Lawlor, K. T., Vanslambrouck, J. M., Higgins, J. W., Chambon, A., Bishard, K., Arndt, D., Er, P. X., Wilson, S. B., Howden, S. E., Tan, K. S., Li, F., Hale, L. J., Shepherd, B., Pentoney, S., Presnell, S. C., Chen, A. E., & Little, M. H. (2021). Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. *Nature Materials*, *20*(2), 260–271. https://doi.org/10.1038/s41563-020-00853-9

Lee, J. H., Kim, H. J., Kim, H., Lee, S. J., & Gye, M. C. (2006). In vitro spermatogenesis by three-dimensional culture of rat testicular cells in collagen gel matrix. *Biomaterials*, *27*(14), 2845–2853. https://doi.org/10.1016/j.biomaterials.2005.12.028

Lee, S., Burner, D. N., Mendoza, T. R., Muldong, M. T., Arreola, C., Wu, C. N., Cacalano, N. A., Kulidjian, A. A., Kane, C. J., & Jamieson, C. A. M. (2020). Establishment and analysis of three-dimensional (3D) organoids derived from patient prostate cancer bone metastasis specimens and their xenografts. *Journal of Visualized Experiments*, *2020*(156), 1–8. https://doi.org/10.3791/60367

Lee, S., Mendoza, T. R., Burner, D. N., Muldong, M. T., Wu, C. C. N., Arreola-Villanueva, C., Zuniga, A., Greenburg, O., Zhu, W. Y., Murtadha, J., Koutouan, E., Pineda, N., Pham, H., Kang, S. G., Kim, H. T., Pineda, G., Lennon, K. M., Cacalano, N. A., Jamieson, C. H. M., … Jamieson, C. A. M. (2022). Novel dormancy mechanism of castration resistance in bone metastatic prostate cancer organoids. *International Journal of Molecular Sciences*, *23*(6), 3203. https://doi.org/10.3390/ijms23063203

Lee, S. H., Hu, W., Matulay, J. T., Silva, M. V., Owczarek, T. B., Kim, K., Chua, C. W., Barlow, L. M. J., Kandoth, C., Williams, A. B., Bergren, S. K., Pietzak, E. J., Anderson, C. B., Benson, M. C., Coleman, J. A., Taylor, B. S., Abate-Shen, C., McKiernan, J. M., al-Ahmadie, H., … Shen, M. M. (2018). Tumor evolution and drug response in patient-derived organoid models of bladder cancer. *Cell*, *173*(2), 515–528.e17. https://doi.org/10.1016/j.cell.2018.03.017

Lee, U. N., Day, J. H., Haack, A. J., Bretherton, R. C., Lu, W., DeForest, C. A., Theberge, A. B., & Berthier, E. (2020). Layer-by-layer fabrication of 3D hydrogel structures using open microfluidics. *Lab on a Chip*, *20*(3), 525–536. https://doi.org/10.1039/C9LC00621D

Legendre, A., Froment, P., Desmots, S., Lecomte, A., Habert, R., & Lemazurier, E. (2010). An engineered 3D blood-testis barrier model for the assessment of reproductive toxicity potential. *Biomaterials*, *31*(16), 4492–4505. https://doi.org/10.1016/J.BIOMATERIALS.2010.02.029

Leir, S.-H. H., Yin, S., Kerschner, J. L., Xia, S., Ahmadi, S., Bear, C., & Harris, A. (2020). An organoid model to assay the role of CFTR in the human epididymis epithelium. *Cell and Tissue Research*, *381*(2), 327–336. https://doi.org/10.1007/s00441-020-03208-7

Lindahl, U., Couchman, J., Kimata, K., & Esko, J. D. (2015). Proteoglycans and sulfated glycosaminoglycans. In *Essentials of Glycobiology*. Cold Spring Harbor Laboratory Press.

Linxweiler, J., Hammer, M., Muhs, S., Kohn, M., Pryalukhin, A., Veith, C., Bohle, R. M., Stöckle, M., Junker, K., & Saar, M. (2019). Patient-derived, three-dimensional spheroid cultures provide a versatile translational model for the study of organ-confined prostate cancer. *Journal of Cancer Research and Clinical Oncology*, *145*(3), 551–559. https://doi.org/10.1007/s00432-018-2803-5

Ma, L., Li, J., Nie, Q., Zhang, Q., Liu, S., Ge, D., & You, Z. (2017). Organoid culture of human prostate cancer cell lines LNCaP and C4-2B. *American Journal of Clinical and Experimental Urology*, *5*(3), 25–33.

Maenhoudt, N., Defraye, C., Boretto, M., Jan, Z., Heremans, R., Boeckx, B., Hermans, F., Arijs, I., Cox, B., van Nieuwenhuysen, E., Vergote, I., van Rompuy, A. S., Lambrechts, D., Timmerman, D., & Vankelecom, H. (2020). Developing organoids from ovarian cancer as experimental and preclinical models. *Stem Cell Reports*, *14*(4), 717–729. https://doi.org/10.1016/J.STEMCR.2020.03.004

Manafi, N., Shokri, F., Achberger, K., Hirayama, M., Mohammadi, M. H., Noorizadeh, F., Hong, J., Liebau, S., Tsuji, T., Quinn, P. M. J., & Mashaghi, A. (2021). Organoids and organ chips in ophthalmology. *The Ocular Surface*, *19*, 1–15. https://doi.org/10.1016/J.JTOS.2020.11.004

Mandon, M., Hermo, L., & Cyr, D. G. (2015). Isolated rat epididymal basal cells share common properties with adult stem cells. *Biology of Reproduction*, *93*(5), 1–15. https://doi.org/10.1095/biolreprod.115.133967

Marchini, A., & Gelain, F. (2021). Synthetic scaffolds for 3D cell cultures and organoids: Applications in regenerative medicine. *Critical Reviews in Biotechnology*, *42*(3), 468–486. https://doi.org/10.1080/07388551.2021.1932716

Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., Amenduni, M., Szekely, A., Palejev, D., Wilson, M., Gerstein, M., Grigorenko, E. L., Chawarska, K., Pelphrey, K. A., Howe, J. R., & Vaccarino, F. M. (2015). FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism Spectrum disorders. *Cell*, *162*(2), 375–390. https://doi.org/10.1016/J.CELL.2015.06.034

Marzi, L., Szabova, L., Gordon, M., Weaver Ohler, Z., Sharan, S. K., Beshiri, M. L., Etemadi, M., Murai, J., Kelly, K., & Pommier, Y. (2019). The Indenoisoquinoline TOP1 inhibitors selectively target homologous recombination-deficient and Schlafen 11-positive cancer cells and synergize with Olaparib. *Clinical Cancer Research*, *25*(20), 6206–6216. https://doi.org/10.1158/1078-0432.CCR-19-0419

McCray, T., Richards, Z., Marsili, J., Prins, G. S., & Nonn, L. (2019). Handling and assessment of human primary prostate organoid culture. *Journal of Visualized Experiments*, *176*(143), 139–148. https://doi.org/10.3791/59051

Mi, S., Yi, X., Du, Z., Xu, Y., & Sun, W. (2018). Construction of a liver sinusoid based on the laminar flow on chip and self-assembly of endothelial cells. *Biofabrication*, *10*(2), 025010. https://doi.org/10.1088/1758-5090/aaa97e

Mohan, S. C., Lee, T. Y., Giuliano, A. E., & Cui, X. (2021). Current status of breast organoid models. *Frontiers in Bioengineering and Biotechnology*, *9*, 1–7. https://doi.org/10.3389/FBIOE.2021.745943

Moore, H. D. M., Curry, M. R., Penfold, L. M., & Pryor, J. P. (1992). The culture of human epididymal epithelium and in vitro maturation of epididymal spermatozoa. *Fertility and Sterility*, *58*(4), 776–783. https://doi.org/10.1016/S0015-0282(16)55327-8

Mosaad, E. O., Chambers, K. F., Futrega, K., Clements, J. A., & Doran, M. R. (2018). The microwell-mesh: A high-throughput 3D prostate cancer spheroid and drug-testing platform. *Scientific Reports*, *8*(1), 253. https://doi.org/10.1038/s41598-017-18050-1

Moscana, A., & Moscana, H. (1952). The dissociation and aggregation of cells from organ rudiments of the early chick embryo. *Journal of Anatomy*, *86*(3), 287–301.

Mosquera, M. J., Kim, S., Bareja, R., Fang, Z., Cai, S., Pan, H., Asad, M., Martin, M. L., Sigouros, M., Rowdo, F. M., Ackermann, S., Capuano, J., Bernheim, J., Cheung, C., Doane, A., Brady, N., Singh, R., Rickman, D. S., Prabhu, V., ... Singh, A. (2022). Extracellular matrix in synthetic hydrogel-based prostate cancer organoids regulate therapeutic response to EZH2 and DRD2 inhibitors. *Advanced Materials*, *34*(2), 2100096. https://doi.org/10.1002/adma.202100096

Moura, B. S., Monteiro, M. V., Ferreira, L. P., Lavrador, P., Gaspar, V. M., & Mano, J. F. (2022). Advancing tissue Decellularized hydrogels for engineering human organoids. *Advanced Functional Materials*, *32*, 2202825. https://doi.org/10.1002/adfm.202202825

Namekawa, T., Ikeda, K., Horie-Inoue, K., & Inoue, S. (2019). Application of prostate cancer models for preclinical study: Advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. *Cell*, *8*(1), 74. https://doi.org/10.3390/cells8010074

Nath, D., Li, X., Mondragon, C., Post, D., Chen, M., White, J. R., Hryniewicz-Jankowska, A., Caza, T., Kuznetsov, V. A., Hehnly, H., Jamaspishvili, T., Berman, D. M., Zhang, F., Kung, S. H. Y., Fazli, L., Gleave, M. E., Bratslavsky, G., Pandolfi, P. P., & Kotula, L. (2019). Abi1 loss drives prostate tumorigenesis through activation of EMT and non-canonical WNT signaling. *Cell Communication and Signaling*, *17*(1), 120. https://doi.org/10.1186/s12964-019-0410-y

Natsiou, D., Granchi, Z., Mitsiadis, T. A., & Jimenez-Rojo, L. (2017). Generation of spheres from dental epithelial stem cells. *Frontiers in Physiology*, *8*, 1–7. https://doi.org/10.3389/fphys.2017.00007

Nicolas, J., Magli, S., Rabbachin, L., Sampaolesi, S., Nicotra, F., & Russo, L. (2020). 3D extracellular matrix mimics: Fundamental concepts and role of materials chemistry to influence stem cell fate. *Biomacromolecules*, *21*(6), 1968–1994. https://doi.org/10.1021/acs.biomac.0c00045

Nikolova, M. P., & Chavali, M. S. (2019). Recent advances in biomaterials for 3D scaffolds: A review. *Bioactive Materials*, *4*, 271–292. https://doi.org/10.1016/J.BIOACTMAT.2019.10.005

Njoroge, R. N., Unno, K., Zhao, J. C., Naseem, A. F., Anker, J. F., McGee, W. A., Nonn, L., & Abdulkadir, S. A. (2017). Organoids model distinct vitamin E effects at different stages of prostate cancer evolution. *Scientific Reports*, *7*(1), 16285. https://doi.org/10.1038/s41598-017-16459-2

Oliveira, M. B., Hatami, J., & Mano, J. F. (2016). Coating strategies using layer-by-layer deposition for cell encapsulation. *Chemistry, an Asian Journal*, *11*, 1753–1764. https://doi.org/10.1002/asia.201600145

Oliver, E., Alves-Lopes, J. P., Harteveld, F., Mitchell, R. T., Åkesson, E., Söder, O., & Stukenborg, J.-B. (2021). Self-organising human gonads generated by a Matrigel-based gradient system. *BMC Biology*, *19*(1), 212. https://doi.org/10.1186/s12915-021-01149-3

Olson, A., le, V., Aldahl, J., Yu, E.-J., Hooker, E., He, Y., Lee, D. H., Kim, W. K., Cardiff, R. D., Geradts, J., & Sun, Z. (2019). The comprehensive role of E-cadherin in maintaining prostatic epithelial integrity during oncogenic transformation and tumor progression. *PLoS Genetics*, *15*(10), e1008451. https://doi.org/10.1371/journal.pgen.1008451

Orgebin-Crist, M. C. (1967). Sperm maturation in rabbit epididymis. *Nature*, *216*(5117), 816–818.

Owen, D. H., & Katz, D. F. (2005). A review of the physical and chemical properties of human semen and the formulation of a semen simulant. *Journal of Andrology*, *26*(4), 459–469. https://doi.org/10.2164/JANDROL.04104

Pamarthy, S., & Sabaawy, H. E. (2021). Patient derived organoids in prostate cancer: Improving therapeutic efficacy in precision medicine. *Molecular Cancer*, *20*(1), 125. https://doi.org/10.1186/s12943-021-01426-3

Pappas, K. J., Choi, D., Sawyers, C. L., & Karthaus, W. R. (2019). Prostate organoid cultures as tools to translate genotypes and mutational profiles to pharmacological responses. *Journal of Visualized Experiments*, *176*(152), 139–148. https://doi.org/10.3791/60346

Park, J. W., Lee, J. K., Phillips, J. W., Huang, P., Cheng, D., Huang, J., & Witte, O. N. (2016). Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(16), 4482–4487. https://doi.org/10.1073/pnas.1603645113

Paşca, A. M., Sloan, S. A., Clarke, L. E., Tian, Y., Makinson, C. D., Huber, N., Kim, C. H., Park, J. Y., O'Rourke, N. A., Nguyen, K. D., Smith, S. J., Huguenard, J. R., Geschwind, D. H., Barres, B. A., & Paşca, S. P. (2015). Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nature Methods*, *12*(7), 671–678. https://doi.org/10.1038/NMETH.3415

Pendergraft, S. S., Sadri-Ardekani, H., Atala, A., & Bishop, C. E. (2017). Three-dimensional testicular organoid: A novel tool for the study of human spermatogenesis and gonadotoxicity in vitro. *Biology of Reproduction*, *96*(3), 720–732. https://doi.org/10.1095/biolreprod.116.143446

Pinel, L., & Cyr, D. G. (2021). Self-renewal and differentiation of rat epididymal basal cells using a novel in vitro organoid model. *Biology of Reproduction*, *105*(4), 987–1001. https://doi.org/10.1093/biolre/ioab113

Pinel, L., Mandon, M., & Cyr, D. G. (2019). Tissue regeneration and the epididymal stem cell. *Andrology*, *7*(5), 618–630. https://doi.org/10.1111/andr.12635

Pollen, A. A., Bhaduri, A., Andrews, M. G., Nowakowski, T. J., Meyerson, O. S., Mostajo-Radji, M. A., di Lullo, E., Alvarado, B., Bedolli, M., Dougherty, M. L., Fiddes, I. T., Kronenberg, Z. N., Shuga, J., Leyrat, A. A., West, J. A., Bershteyn, M., Lowe, C. B., Pavlovic, B. J., Salama, S. R., … Kriegstein, A. R. (2019). Establishing cerebral organoids as models of human-specific brain evolution. *Cell*, *176*(4), 743–756.e17. https://doi.org/10.1016/J.CELL.2019.01.017

Puca, L., Bareja, R., Prandi, D., Shaw, R., Benelli, M., Karthaus, W. R., Hess, J., Sigouros, M., Donoghue, A., Kossai, M., Gao, D., Cyrta, J., Sailer, V., Vosoughi, A., Pauli, C., Churakova, Y., Cheung, C., Deonarine, L. D., McNary, T. J., … Beltran, H. (2018). Patient derived organoids to model rare prostate cancer phenotypes. *Nature Communications*, *9*(1), 2404. https://doi.org/10.1038/s41467-018-04495-z

Rajan, S. A. P., Aleman, J., Wan, M., Pourhabibi Zarandi, N., Nzou, G., Murphy, S., Bishop, C. E., Sadri-Ardekani, H., Shupe, T., Atala, A., Hall, A. R., & Skardal, A. (2020). Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform. *Acta Biomaterialia*, *106*, 124–135. https://doi.org/10.1016/j.actbio.2020.02.015

Ravi, M., Paramesh, V., Kaviya, S. R., Anuradha, E., & Solomon, F. D. P. (2015). 3D cell culture systems: Advantages and applications. *Journal of Cellular Physiology*, *230*(1), 16–26. https://doi.org/10.1002/jcp.24683

Rawlings, T. M., Makwana, K., Tryfonos, M., & Lucas, E. S. (2021). Organoids to model the endometrium: Implantation and beyond. *Reproduction & Fertility*, *2*(3), R85. https://doi.org/10.1530/RAF-21-0023

Raya-Rivera, A., Esquiliano, D. R., Yoo, J. J., Lopez-Bayghen, E., Soker, S., & Atala, A. (2011). Tissue-engineered autologous urethras for patients who need reconstruction: An observational study. *The Lancet*, *377*(9772), 1175–1182. https://doi.org/10.1016/S0140-6736(10)62354-9

Reda, A., Hou, M., Landreh, L., Kjartansdóttir, K. R., Svechnikov, K., Söder, O., & Stukenborg, J. B. (2014). In vitro spermatogenesis—Optimal culture conditions for testicular cell survival, germ cell differentiation, and steroidogenesis in rats. *Frontiers in Endocrinology*, *5*(FEB), 21. https://doi.org/10.3389/FENDO.2014.00021

Reda, A., Hou, M., Winton, T. R., Chapin, R. E., Söder, O., & Stukenborg, J. B. (2016). In vitro differentiation of rat spermatogonia into round spermatids in tissue culture. *Molecular Human Reproduction*, *22*(9), 601–612. https://doi.org/10.1093/MOLEHR/GAW047

Richardson, L. L., Kleinman, H. K., & Dym, M. (1995). Basement membrane gene expression by Sertoli and peritubular Myoid cells in vitro in the Rat1. *Biology of Reproduction*, *52*(2), 320–330. https://doi.org/10.1095/biolreprod52.2.320

Richer, G., Baert, Y., & Goossens, E. (2020). In-vitro spermatogenesis through testis modelling: Toward the generation of testicular organoids. *Andrology*, *8*(4), 879–891. https://doi.org/10.1111/andr.12741

Richer, G., Hobbs, R. M., Loveland, K. L., Goossens, E., & Baert, Y. (2021). Long-term maintenance and meiotic entry of early germ cells in murine testicular organoids functionalized by 3D printed scaffolds and air-medium Interface cultivation. *Frontiers in Physiology*, *12*-(December), 757565. https://doi.org/10.3389/fphys.2021.757565

Risbridger, G. P., Toivanen, R., & Taylor, R. A. (2018). Preclinical models of prostate cancer: Patient-derived xenografts, organoids, and other explant models. *Cold Spring Harbor Perspectives in Medicine*, *8*(8), a030536. https://doi.org/10.1101/cshperspect.a030536

Rosines, E., Johkura, K., Zhang, X., Schmidt, H. J., Decambre, M., Bush, K. T., & Nigam, S. K. (2010). Constructing kidney-like tissues from cells based on programs for organ development: Toward a method of in vitro tissue engineering of the kidney. *Tissue Engineering. Part A*, *16*(8), 2441–2455. https://doi.org/10.1089/TEN.TEA.2009.0548

Roulet, V., Denis, H., Staub, C., le Tortorec, A., Delaleu, B., Satie, A. P., Patard, J. J., Jégou, B., & Dejucq-Rainsford, N. (2006). Human testis in organotypic culture: Application for basic or clinical research. *Human Reproduction*, *21*(6), 1564–1575. https://doi.org/10.1093/humrep/del018

Sachs, N., Papaspyropoulos, A., Zomer-van Ommen, D. D., Heo, I., Böttinger, L., Klay, D., Weeber, F., Huelsz-Prince, G., Iakobachvili, N., Amatngalim, G. D., Ligt, J., Hoeck, A., Proost, N., Viveen, M. C., Lyubimova, A., Teeven, L., Derakhshan, S., Korving, J., Begthel, H., … Clevers, H. (2019). Long-term expanding human airway organoids for disease modeling. *The EMBO Journal*, *38*(4), e100300. https://doi.org/10.15252/embj.2018100300

Saez, J. M., Sanchez, P., Berthelon, M. C., & Avallet, O. (1989). Regulation of pig Leydig cell aromatase activity by gonadotropins and Sertoli Cells. *Biology of Reproduction*, *41*(5), 813–820. https://doi.org/10.1095/biolreprod41.5.813

Sakib, S., Uchida, A., Valenzuela-Leon, P., Yu, Y., Valli-Pulaski, H., Orwig, K., Ungrin, M., & Dobrinski, I. (2019). Formation of organotypic testicular organoids in microwell culture. *Biology of Reproduction*, *100*(6), 1648–1660. https://doi.org/10.1093/biolre/ioz053

Sakib, S., Voigt, A., Goldsmith, T., & Dobrinski, I. (2019). Three-dimensional testicular organoids as novel in vitro models of testicular biology and toxicology. *Environmental Epigenetics*, *5*(3), 1–8. https://doi.org/10.1093/eep/dvz011

Sakib, S., Yu, Y., Voigt, A., Ungrin, M., & Dobrinski, I. (2019). Generation of porcine testicular organoids with testis specific architecture using microwell culture. *Journal of Visualized Experiments*, *2019*(152), 2–6. https://doi.org/10.3791/60387

Sano, K., Usui, M., Moritani, Y., Nakazawa, K., Hanatani, T., Kondo, H., Nakatomi, M., Onizuka, S., Iwata, T., Sato, T., Togari, A., Ariyoshi, W., Nishihara, T., & Nakashima, K. (2020). Co-cultured spheroids of human periodontal ligament mesenchymal stem cells and vascular endothelial cells enhance periodontal tissue regeneration. *Regenerative Therapy*, *14*, 59–71. https://doi.org/10.1016/j.reth.2019.12.008

Sasano, Y., Fukumoto, K., Tsukamoto, Y., Akagi, T., & Akashi, M. (2020). Construction of 3D cardiac tissue with synchronous powerful beating using human cardiomyocytes from human iPS cells prepared by a convenient differentiation method. *Journal of Bioscience and Bioengineering*, *129*(6), 749–755. https://doi.org/10.1016/j.jbiosc.2020.01.001

Sato, T., Katagiri, K., Gohbara, A., Inoue, K., Ogonuki, N., Ogura, A., Kubota, Y., & Ogawa, T. (2011). In vitro production of functional sperm in cultured neonatal mouse testes. *Nature*, *471*(7339), 504–507. https://doi.org/10.1038/nature09850

Schwank, G., Koo, B.-K., Sasselli, V., Dekkers, J. F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C. K., Nieuwenhuis, E. E. S., Beekman, J. M., & Clevers, H. (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell*, *13*(6), 653–658. https://doi.org/10.1016/j.stem.2013.11.002

Servant, R., Garioni, M., Vlajnic, T., Blind, M., Pueschel, H., Müller, D. C., Zellweger, T., Templeton, A. J., Garofoli, A., Maletti, S., Piscuoglio, S., Rubin, M. A., Seifert, H., Rentsch, C. A., Bubendorf, L., & le Magnen, C. (2021). Prostate cancer patient-derived organoids: Detailed outcome from a prospective cohort of 81 clinical specimens. *The Journal of Pathology*, *254*(5), 543–555. https://doi.org/10.1002/path.5698

Shankaran, A., Prasad, K., Chaudhari, S., Brand, A., & Satyamoorthy, K. (2021). Advances in development and application of human organoids. *3. Biotech*, *11*(6), 257. https://doi.org/10.1007/s13205-021-02815-7

Sharma, S., & Gupta, D. (2019). Tissue engineering and stem cell therapy in pediatric urology. *Journal of Indian Association of Pediatric Surgeons*, *24*(4), 237. https://doi.org/10.4103/jiaps.JIAPS_77_18

Shenoy, T. R., Boysen, G., Wang, M. Y., Xu, Q. Z., Guo, W., Koh, F. M., Wang, C., Zhang, L. Z., Wang, Y., Gil, V., Aziz, S., Christova, R., Rodrigues, D. N., Crespo, M., Rescigno, P., Tunariu, N., Riisnaes, R., Zafeiriou, Z., Flohr, P., ... Wu, H. (2017). CHD1 loss sensitizes prostate cancer to DNA damaging therapy by promoting error-prone double-strand break repair. *Annals of Oncology*, *28*(7), 1495–1507. https://doi.org/10.1093/annonc/mdx165

Sidhaye, J., & Knoblich, J. A. (2021). Brain organoids: An ensemble of bioassays to investigate human neurodevelopment and disease. *Cell Death and Differentiation*, *28*(1), 52–67. https://doi.org/10.1038/s41418-020-0566-4

Skardal, A., Aleman, J., Forsythe, S., Rajan, S., Murphy, S., Devarasetty, M., Pourhabibi Zarandi, N., Nzou, G., Wicks, R., Sadri-Ardekani, H., Bishop, C., Soker, S., Hall, A., Shupe, T., & Atala, A. (2020). Drug compound screening in single and integrated multi-organoid body-on-a-chip systems. *Biofabrication*, *12*(2), 025017. https://doi.org/10.1088/1758-5090/ab6d36

Sousa, C. F. V., Saraiva, C. A., Correia, T. R., Pesqueira, T., Patrício, S. G., Rial-Hermida, M. I., Borges, J., & Mano, J. F. (2021). Bioinstructive layer-by-layer-coated customizable 3d printed perfusable microchannels embedded in photocrosslinkable hydrogels for vascular tissue engineering. *Biomolecules*, *11*(6), 1–16. https://doi.org/10.3390/BIOM11060863/S1

Spencer, J. L., Lahon, A., Tran, L. L., Arya, R. P., Kneubehl, A. R., Vogt, M. B., Xavier, D., Rowley, D. R., Kimata, J. T., & Rico-Hesse, R. R. (2018). Replication of Zika virus in human prostate cells: A potential source of sexually transmitted virus. *The Journal of Infectious Diseases*, *217*(4), 538–547. https://doi.org/10.1093/infdis/jix436

Steinberger, A., Steinberger, E., & Perloff, W. H. (1964). Mammalian testes in organ culture. *Experimental Cell Research*, *36*(1), 19–27. https://doi.org/10.1016/0014-4827(64)90156-9

Strange, D. P., Zarandi, N. P., Trivedi, G., Atala, A., Bishop, C. E., Sadri-Ardekani, H., & Verma, S. (2018). Human testicular organoid system as a novel tool to study Zika virus pathogenesis. *Emerging Microbes & Infections*, *7*(1), 1–7. https://doi.org/10.1038/s41426-018-0080-7

Stukenborg, J.-B., Wistuba, J., Luetjens, C. M., Elhija, M. A., Huleihel, M., Lunenfeld, E., Gromoll, J., Nieschlag, E., & Schlatt, S. (2008). Coculture of Spermatogonia with somatic cells in a novel three-dimensional soft-agar-culture-system. *Journal of Andrology*, *29*(3), 312–329. https://doi.org/10.2164/jandrol.107.002857

Subramaniyan, A., & Ravi, M. (2018). Agarose hydrogel induced MCF-7 and BMG-1 cell line progressive 3D and 3D revert cultures. *Journal of Cellular Physiology*, *233*(4), 2768–2772. https://doi.org/10.1002/jcp.25965

Sullivan, R., & Belleannée, C. (2017). Role of the epididymis in sperm. In C. J. de Jonge & C. L. R. Barratt (Eds.), *The sperm cell: Production, maturation, fertilization, regeneration* (pp. 73–86). Cambridge University Press.

Sun, C.-P., Lan, H.-R., Fang, X.-L., Yang, X.-Y., & Jin, K.-T. (2022). Organoid models for precision cancer immunotherapy. *Frontiers in Immunology*, *13*, 465. https://doi.org/10.3389/FIMMU.2022.770465

Sun, M., Yuan, Q., Niu, M., Wang, H., Wen, L., Yao, C., Hou, J., Chen, Z., Fu, H., Zhou, F., Li, C., Gao, S., Gao, W. Q., Li, Z., & He, Z. (2018). Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system. *Cell Death and Differentiation*, *25*(4), 749–766. https://doi.org/10.1038/s41418-017-0015-1

Susaimanickam, P. J., Maddileti, S., Kumar, V., Boyinpally, S. R., Naik, R. R., Naik, M. N., Reddy, G. B., Sangwan, V. S., & Mariappan, I. (2017). Generating minicorneal organoids from human induced pluripotent stem cells. *Development*, *144*, 2338–2351. https://doi.org/10.1242/dev.143040

Tatullo, M., Marrelli, B., Benincasa, C., Aiello, E., Makeeva, I., Zavan, B., Ballini, A., de Vito, D., & Spagnuolo, G. (2020). Organoids in translational oncology. *Journal of Clinical Medicine*, *9*(9), 2774. https://doi.org/10.3390/jcm9092774

Taymour, R., Kilian, D., Ahlfeld, T., Gelinsky, M., & Lode, A. (2021). 3D bioprinting of hepatocytes: Core–shell structured co-cultures with fibroblasts for enhanced functionality. *Scientific Reports*, *11*(1), 5130. https://doi.org/10.1038/s41598-021-84384-6

Tesarik, J., Guido, M., Mendoza, C., & Greco, E. (1998). Human spermatogenesis in vitro: Respective effects of follicle-stimulating hormone and testosterone on meiosis, Spermiogenesis, and Sertoli cell apoptosis. *The Journal of Clinical Endocrinology & Metabolism*, *83*(12), 4467–4473. https://doi.org/10.1210/jcem.83.12.5304

Tindle, C., Fuller, M., Fonseca, A., Taheri, S., Ibeawuchi, S. R., Beutler, N., Katkar, G. D., Claire, A., Castillo, V., Hernandez, M., Russo, H., Duran, J., Crotty Alexander, L. E., Tipps, A., Lin, G., Thistlethwaite, P. A., Chattopadhyay, R., Rogers, T. F., Sahoo, D., … das, S. (2021). Adult stem cell-derived complete lung organoid models emulate lung disease in COVID-19. *eLife*, *10*, e66417. https://doi.org/10.7554/ELIFE.66417

Topraggaleh, T. R., Valojerdi, M. R., Montazeri, L., & Baharvand, H. (2019). A testis-derived macroporous 3D scaffold as a platform for the generation of mouse testicular organoids. *Biomaterials Science*, *7*(4), 1422–1436. https://doi.org/10.1039/C8BM01001C

Tyson, D. R., Inokuchi, J., Tsunoda, T., Lau, A., & Ornstein, D. K. (2007). Culture requirements of prostatic epithelial cell lines for acinar morphogenesis and lumen formation in vitro: Role of extracellular calcium. *The Prostate*, *67*(15), 1601–1613. https://doi.org/10.1002/pros.20628

Van Der Wee, K., & Hofmann, M. C. (1999). An in vitro tubule assay identifies HGF as a morphogen for the formation of seminiferous tubules in the postnatal mouse testis. *Experimental Cell Research*, *252*(1), 175–185. https://doi.org/10.1006/excr.1999.4630

Van Hemelryk, A., Mout, L., Erkens-Schulze, S., French, P. J., van Weerden, W. M., & van Royen, M. E. (2021). Modeling prostate cancer treatment responses in the organoid era: 3D environment impacts drug testing. *Biomolecules*, *11*(11), 1572. https://doi.org/10.3390/biom11111572

Varaa, N., Azandeh, S., Khorsandi, L., Bijan Nejad, D., Bayati, V., & Bahreini, A. (2018). Ameliorating effect of encapsulated hepatocyte-like cells derived from umbilical cord in high mannuronic alginate scaffolds on acute liver failure in rats. *Iranian Journal of Basic Medical Sciences*, *21*(9), 928–935. https://doi.org/10.22038/IJBMS.2018.27928.6847

Vela, I., & Chen, Y. (2015). Prostate cancer organoids: A potential new tool for testing drug sensitivity. *Expert Review of Anticancer Therapy*, *15*(3), 261–263. https://doi.org/10.1586/14737140.2015.1003046

Vermeulen, M., del Vento, F., Kanbar, M., Pyr dit Ruys, S., Vertommen, D., Poels, J., & Wyns, C. (2019). Generation of organized porcine testicular organoids in solubilized hydrogels from Decellularized extracellular matrix. *International Journal of Molecular Sciences*, *20*(21), 5476. https://doi.org/10.3390/ijms20215476

Vidović, D., Carlon, M. S., da Cunha, M. F., Dekkers, J. F., Hollenhorst, M. I., Bijvelds, M. J. C., Ramalho, A. S., van den Haute, C., Ferrante, M., Baekelandt, V., Janssens, H. M., de Boeck, K., Sermet-Gaudelus, I., de Jonge, H. R., Gijsbers, R., Beekman, J. M., Edelman, A., & Debyser, Z. (2016). rAAV-CFTRΔR rescues the cystic fibrosis phenotype in human intestinal organoids and cystic fibrosis mice. *American Journal of Respiratory and Critical Care Medicine*, *193*(3), 288–298. https://doi.org/10.1164/rccm.201505-0914OC

Walters, R. O., & Haigh, C. L. (2022). Organoids for modeling prion diseases. *Cell and Tissue Research*. https://doi.org/10.1007/S00441-022-03589-X. Online ahead of print.

Wang, S., Gao, D., & Chen, Y. (2017). The potential of organoids in urological cancer research. *Nature Reviews Urology*, *14*(7), 401–414. https://doi.org/10.1038/nrurol.2017.65

Watanabe, M., Buth, J. E., Vishlaghi, N., de la Torre-Ubieta, L., Taxidis, J., Khakh, B. S., Coppola, G., Pearson, C. A., Yamauchi, K., Gong, D., Dai, X., Damoiseaux, R., Aliyari, R., Liebscher, S., Schenke-Layland, K., Caneda, C., Huang, E. J., Zhang, Y., Cheng, G., … Novitch, B. G. (2017). Self-organized cerebral organoids with human-specific features predict effective drugs to combat Zika virus infection. *Cell Reports*, *21*(2), 517–532. https://doi.org/10.1016/J.CELREP.2017.09.047

Weeber, F., Ooft, S. N., Dijkstra, K. K., & Voest, E. E. (2017). Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chemical Biology*, *24*(9), 1092–1100. https://doi.org/10.1016/j.chembiol.2017.06.012

Weiss, P., & Taylor, A. C. (1960). Reconstruction of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. *Proceedings of the National Academy of Sciences*, *46*(9), 1177–1185. https://doi.org/10.1073/pnas.46.9.1177

Wiegerinck, C. L., Janecke, A. R., Schneeberger, K., Vogel, G. F., van Haaften-Visser, D. Y., Escher, J. C., Adam, R., Thöni, C. E., Pfaller, K., Jordan, A. J., Weis, C.–. A., Nijman, I. J., Monroe, G. R., van Hasselt, P. M., Cutz, E., Klumperman, J., Clevers, H., Nieuwenhuis, E. E. S., Houwen, R. H. J., … Middendorp, S. (2014). Loss of Syntaxin 3 causes variant microvillus inclusion disease. *Gastroenterology*, *147*(1), 65–68.e10. https://doi.org/10.1053/j.gastro.2014.04.002

Xin, L., Lukacs, R. U., Lawson, D. A., Cheng, D., & Witte, O. N. (2007). Self-renewal and multilineage differentiation In vitro from murine prostate stem cells. *Stem Cells*, *25*(11), 2760–2769. https://doi.org/10.1634/stemcells.2007-0355

Xu, M., Lee, E. M., Wen, Z., Cheng, Y., Huang, W. K., Qian, X., Julia, T. C. W., Kouznetsova, J., Ogden, S. C., Hammack, C., Jacob, F., Nguyen, H. N., Itkin, M., Hanna, C., Shinn, P., Allen, C., Michael, S. G., Simeonov, A., Huang, W., … Tang, H. (2016). Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nature Medicine*, *22*(10), 1101–1107. https://doi.org/10.1038/NM.4184

Xu, X., Li, Z., Ai, X., Tang, Y., Yang, D., & Dou, L. (2022). Human three-dimensional dental pulp organoid model for toxicity screening of dental materials on dental pulp cells and tissue. *International Endodontic Journal*, *55*(1), 79–88. https://doi.org/10.1111/iej.13641

Y Baena, A. R., Casasco, A., & Monti, M. (2022). Hypes and hopes of stem cell therapies in dentistry: A review. *Stem Cell Reviews and Reports*, *18*(4), 1294–1308. https://doi.org/10.1007/S12015-021-10326-4

Yamanaka, H., Komeya, M., Nakamura, H., Sanjo, H., Sato, T., Yao, M., Kimura, H., Fujii, T., & Ogawa, T. (2018). A monolayer microfluidic device supporting mouse spermatogenesis with improved visibility. *Biochemical and Biophysical Research Communications*, *500*(4), 885–891. https://doi.org/10.1016/J.BBRC.2018.04.180

Yeung, C. H., Cooper, T. G., Bergmann, M., & Schulze, H. (1991). Organization of tubules in the human caput epididymidis and the ultrastructure of their epithelia. *The American Journal of Anatomy*, *191*(3), 261–279. https://doi.org/10.1002/aja.1001910306

Yeung, C. H., Wang, K., & Cooper, T. G. (2012, May). Why are epididymal tumours so rare? *Asian Journal of Andrology*, *14*(3), 465–475. https://doi.org/10.1038/aja.2012.20

Yin, Y., Bijvelds, M., Dang, W., Xu, L., van der Eijk, A. A., Knipping, K., Tuysuz, N., Dekkers, J. F., Wang, Y., de Jonge, J., Sprengers, D., van der Laan, L. J. W., Beekman, J. M., ten Berge, D., Metselaar, H. J., de Jonge, H., Koopmans, M. P. G., Peppelenbosch, M. P., & Pan, Q. (2015). Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. *Antiviral Research*, *123*, 120–131. https://doi.org/10.1016/J.ANTIVIRAL.2015.09.010

Yokonishi, T., Sato, T., Komeya, M., Katagiri, K., Kubota, Y., Nakabayashi, K., Hata, K., Inoue, K., Ogonuki, N., Ogura, A., & Ogawa, T. (2014). Offspring production with sperm grown in vitro from cryopreserved testis tissues. *Nature Communications*, *5*(1), 4320. https://doi.org/10.1038/ncomms5320

Yu, X., Sidhu, J. S., Hong, S., & Faustman, E. M. (2005). Essential role of extracellular matrix (ECM) overlay in establishing the functional integrity of primary neonatal rat sertoli cell/gonocyte co-cultures: An improved in vitro model for assessment of male reproductive toxicity. *Toxicological Sciences*, *84*(2), 378–393. https://doi.org/10.1093/toxsci/kfi085

Yuan, Y., Li, L., Cheng, Q., Diao, F., Zeng, Q., Yang, X., Wu, Y., Zhang, H., Huang, M., Chen, J., Zhou, Q., Zhu, Y., Hua, R., Tian, J., Wang, X., Zhou, Z., Hao, J., Yu, J., Hua, D., … Sha, J. (2020). In vitro testicular organogenesis from human fetal gonads produces fertilization-competent spermatids. *Cell Research*, *30*(3), 244–255. https://doi.org/10.1038/s41422-020-0283-z

Zang, R., Castro, M. F. G., McCune, B. T., Zeng, Q., Rothlauf, P. W., Sonnek, N. M., Liu, Z., Brulois, K. F., Wang, X., Greenberg, H. B., Diamond, M. S., Ciorba, M. A., Whelan, S. P. J., & Ding, S. (2020). TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Science Immunology*, *5*(47), eabc3582. https://doi.org/10.1126/sciimmunol.abc3582

Zhang, H., Eisenried, A., Zimmermann, W., & Shively, J. E. (2013). Role of CEACAM1 and CEACAM20 in an In vitro model of prostate morphogenesis. *PLoS One*, *8*(1), e53359. https://doi.org/10.1371/journal.pone.0053359

Zhang, K., Fu, Q., Yoo, J., Chen, X., Chandra, P., Mo, X., Song, L., Atala, A., & Zhao, W. (2017). 3D bioprinting of urethra with PCL/PLCL blend and dual autologous cells in fibrin hydrogel: An in vitro evaluation of biomimetic mechanical property and cell growth environment. *Acta Biomaterialia*, *50*, 154–164. https://doi.org/10.1016/j.actbio.2016.12.008

Zhang, W., Yang, S. L., Yang, M., Herrlinger, S., Shao, Q., Collar, J. L., Fierro, E., Shi, Y., Liu, A., Lu, H., Herring, B. E., Guo, M. L., Buch, S., Zhao, Z., Xu, J., Lu, Z., & Chen, J. F. (2019). Modeling microcephaly with cerebral organoids reveals a WDR62–CEP170–KIF2A pathway promoting cilium disassembly in neural progenitors. *Nature Communications*, *10*(1), 1–14. https://doi.org/10.1038/s41467-019-10497-2

Zhang, X., Wang, L., Zhang, X., Ren, L., Shi, W., Tian, Y., Zhu, J., & Zhang, T. (2017). The use of KnockOut serum replacement (KSR) in three dimensional rat testicular cells co-culture model: An improved male reproductive toxicity testing system. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *106*(Pt A), 487–495. https://doi.org/10.1016/J.FCT.2017.05.001

Zhao, B., Ni, C., Gao, R., Wang, Y., Yang, L., Wei, J., Lv, T., Liang, J., Zhang, Q., Xu, W., Xie, Y., Wang, X., Yuan, Z., Liang, J., Zhang, R., & Lin, X. (2020). Recapitulation of SARS-CoV-2 infection and cholangiocyte damage with human liver ductal organoids. *Protein & Cell*, *11*(10), 771–775. https://doi.org/10.1007/S13238-020-00718-6

Zhau, H. E., Goodwin, T. J., Chang, S.-M., Baker, T. L., & Chung, L. W. K. (1997). Establishment of a three-dimensional human prostate organoid coculture under microgravity-simulated conditions: Evaluation of androgen-induced growth and psa expression. *In Vitro Cellular & Developmental Biology. Animal*, *33*(5), 375–380. https://doi.org/10.1007/s11626-997-0008-3

Zhou, T., Tan, L., Cederquist, G. Y., Fan, Y., Hartley, B. J., Mukherjee, S., Tomishima, M., Brennand, K. J., Zhang, Q., Schwartz, R. E., Evans, T., Studer, L., & Chen, S. (2017). High-content screening in hPSC-neural progenitors identifies drug candidates that inhibit Zika virus infection in fetal-like organoids and adult brain. *Cell Stem Cell*, *21*(2), 274–283.e5. https://doi.org/10.1016/J.STEM.2017.06.017

Zhou, W., de Iuliis, G. N., Dun, M. D., & Nixon, B. (2018). Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. *Frontiers in Endocrinology*, *9*(FEB), 59. https://doi.org/10.3389/FENDO.2018.00059

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