ABSTRACT

Increasing rates of male infertility have led to a greater need for relevant model systems to gain further insight into male fertility and its failings. Spermatogenesis and hormone production occur within distinct regions of the testis. Defined by specialized architecture and a diverse population of cell types, it is no surprise that disruption of this highly organized microenvironment can lead to infertility. To date, no robust in vitro system has facilitated full spermatogenesis resulting in the production of fertilization-competent human spermatozoa. Here, we review a selection of current in vitro systems available for modelling the human testis microenvironment with focus on the progression of spermatogenesis and recapitulation of the testis microenvironment.

THE NEED FOR IN VITRO MODELS

Infertility is estimated to affect approximately 7% of the male population, with the most recent cross-population study suggesting a prevalence closer to 10% (Krausz, 2011; Datta, et al., 2016). Male infertility can result from defects in both sperm production and sperm delivery associated with a multitude of underlying factors including genetic disease, obstruction of the urogenital tract and trauma (Krausz, 2011). The recently reported decline in sperm counts (more than 50%) over the last 50 years has further raised concern about the effect of modern environmental influences on fertility (Levine, et al., 2017). Collectively, these studies highlight the urgent need for relevant model systems to gain further insight into male fertility and its shortcomings.

Cancer treatments are a further recognized disruptor of fertility. Studies have reported a 46% prevalence of infertility in male childhood cancer survivors compared to 18% in siblings (Wasilewski-Masker, et al., 2014; Jahnukainen, et al., 2015). Therapeutic treatments can both deplete germ cell numbers directly or indirectly damage the somatic components, which are then unable to support spermatogenesis (Anderson, et al., 2015; Stukenborg, et al., 2018). Advances in treatment have increased the 5-year survival rate for childhood cancers to approximately 80% (Ward, et al., 2014). With increasing numbers of long-term survivors, the preservation of fertility and endocrine function has now become an important focus. While sperm cryopreservation is standard practice for adult males, options for those unable to produce spermatozoa (e.g. prepubertal boys) are limited (Picton, et al., 2015; Stukenborg, et al., 2018). As it stands, the current strategy comprises cryopreservation of testicular tissue or cells containing spermatogonial stem cells (SSCs) for future use in reproductive technologies. Subsequent experimental methodologies can be broadly divided into two approaches: (i) autotransplantation of the tissue or SSCs back to the testes on completion of treatment and (ii) in vitro generation of spermatozoa from tissue fragments or single cells for subsequent use in assisted reproduction (e.g. in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI)).

Autotransplantation of testicular tissue is an attractive option and has resulted in the generation of non-human primate offspring (Fayomi, et al., 2019), when translated to the clinic; however, the approach poses the risk of reintroducing tumour cells back to the patient. Xenografting, which comprises the grafting of immature testicular tissue under the back skin of immune-deficient mice, could overcome this. Despite having resulted in the generation of live offspring (as demonstrated in mice, pigs and
monkeys) (Shinohara, et al., 2002; Schlatt, et al., 2003; Kaneko, et al., 2013; Liu, et al., 2016), the risk of infection by murine retroviruses must be considered. An alternative approach comprises the implantation of dissociated testis cells back into the testis. This has led to de novo organization of seminiferous tubules and full spermatogenesis in the monkey (Shetty, et al., 2018). As this technology allows for prior cell sorting, the risk of reintroducing tumour cells could be reduced with particular relevance for prepubertal cancer patients.

While SSC transplantation restores spermatogenesis in non-human primates (Hermann, et al., 2012), the only reported clinical trial in humans has proven unsuccessful (Radford, et al., 1999; Hermann, et al., 2012). If cancer treatment has led to damage of the somatic environment within the seminiferous tubule; however, SSC or testicular tissue transplantation may not be sufficient to restore fertility. In this case, co-transplantation of SSCs with functional niche cells, as mentioned above (Shetty, et al., 2018), or factors which have a supporting role may improve the efficiency of fertility restoration. An additional limitation of SSC transplantation comes with the relatively small numbers of SSCs present in the human testis and the limited scope of human SSC propagation in vitro (Sadri-Ardekani, et al., 2009; Nickkhohl, et al., 2014; Yokonishi & Ogawa, 2016). A further avenue of investigation lies in the in vitro production of gametes from reprogrammed stem cells. While significant progress has been made in the differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) into the male germ line, including haploid, round spermatid-like cells, further progression through spermatogenesis has yet to be achieved (Eguizabal, et al., 2011; Panula, et al., 2011; Easley, et al., 2012). In vitro production of functional spermatozoa from prepubertal tests could overcome all these limitations. In 2011, Sato and colleagues reported complete in vitro spermatogenesis generating fertilization-competent spermatozoa using culture of intact immature mouse testicular tissue. To date, however, the generation of functional spermatozoa using this approach has not been replicated in primates.

It is clear novel model systems are required to further elucidate the molecular mechanisms of spermatogenesis, with emphasis on recreation of the somatic microenvironment, to enable propagation and maturation of male germ cells in vitro. These would benefit both investigations into the causes of male infertility as well as the development of future strategies to restore fertility. Here, we will discuss current technologies available for modelling the human testis in vitro with a focus on the progression of spermatogenesis and recapitulation of the tests microenvironment.

THE TESTICULAR MICROENVIRONMENT IN VIVO

Spermatogenesis is a complex process by which SSCs self-renew and differentiate into haploid spermatids within the highly specialized architecture of the testis microenvironment. The testses are organized as two structurally discrete compartments, the seminiferous tubule, within which the germ cells differentiate into mature spermatozoa, and the surrounding interstitial space, hosting testosterone producing Leydig cells as well as blood and lymph vessels, nerve fibres, connective tissue and various immune cell populations (Fig. 1) (Oatley & Brinster, 2012). Within the seminiferous tubules, the SSCs reside in the seminiferous epithelium on the basement membrane in distinct locations known as the SSC niche. The niche provides the cues necessary to regulate the fine balance between self-renewal and differentiation of the SSC population that is required to maintain spermatogenesis throughout the reproductive lifespan (Li & Xie, 2005). The SSC niche is species specific, characterized by distinct organization of the seminiferous epithelium and pattern of spermatogonial development (Fayomi & Orwig, 2018; Hermann, et al., 2018). This is important to consider when selecting an appropriate system in which to model male germ cell differentiation and testicular function in vitro.

The somatic Sertoli cells are generally regarded a fundamental component of the niche given both their location within the seminiferous tubule and direct association with the SSCs. Anchored to the basement membrane, they are in intimate association with germ cells at various stages of maturation. Here, they secrete important growth factors critical for the proliferation and renewal of SSCs including glial cell line-derived neurotropic factor (GDNF), in addition to fibroblast growth factor 2 (FGF2) and colony-stimulating factor 1 (CSF-1) (De Rooij, 2009). GDNF is the primary factor regulating SSC self-renewal both in vivo and in vitro. In the tests of mice overexpressing GDNF, increased numbers of undifferentiated spermatogonia are observed, whereas germ cell numbers become depleted in GDNF-deficient mice, indicating an inability of undifferentiated spermatogonia to sustain the germline (Meng et al., 2000). FGF2

![Figure 1](image-url)
immunomodulatory functions (Mayerhofer et al., 2007). Macrophages are a further cell subset, a unique SSC gene expression signature still remains elusive (Li et al., 2017; Guo et al., 2018; Hermann et al., 2018; Wang et al., 2018; Sohni et al., 2019). Studies are further limited by the low number of SSCs in the testis, which range from 0.01% to 1% of all testicular cells (Tegelenbosch & De Rooij, 1993). While stable proliferation over several months has been achieved in various mammalian species (Kanatsu-Shinohara et al., 2003; Hamra et al., 2005), a long-term expansion system has yet to be established in humans, with culture and propagation in vitro currently reported up to 28 weeks (Sadri-Ardekani et al., 2009). Nevertheless, Yang et al. report retinoic acid (RA) and SCF stimulated generation of haploid spermatids from isolated human SSCs in conventional single cell culture (Yang et al., 2014).

Progression of spermatogenesis from post-spermatogonial developmental stages has additionally been described. Co-culture with Vero cells or Sertoli cells as a cell feeder layer allows for generation of elongating fertilization-competent spermatids from round spermatids with increased efficacy observed with follicle stimulating hormone (FSH) supplementation (Tesarik et al., 1998; Tesarik et al., 1998; Cremades et al., 1999; Sousa et al., 2002). These findings were subsequently extended using only conditioned medium from Vero cells (Cremades et al., 2001), while Tanaka et al. later demonstrated completion of meiosis describing the generation of round spermatids from primary spermatocytes in culture with Vero cells (Tanaka et al., 2003). Further to supporting germ cell development, somatic cell interactions are necessary for establishing testicular structural organization in culture. Two-dimensional co-culture of rodent Sertoli cells with peritubular myoid cells promotes Sertoli cell reorganization, generating seminiferous tubule-like structures in vitro (Tung & Fritz, 1980), while Sertoli cells from rats grown on ECM deposited by Sertoli-myoid cell co-cultures establish polarized monolayers on which germ cells can be maintained for

2D IN VITRO MODELS

Numerous 2D culture models have been employed to explore the conditions required for complete spermatogenesis (Fig. 2). A popular approach has been the culture of SSCs; however, such attempts are limited by an inability to isolate pure populations of SSCs due to a lack of defined markers. While a recent spate of single-cell transcriptome studies has led to the identification of four undifferentiated human spermatogonial cell clusters, allowing for purification of the most primitive (and likely SSC-enriched) cell subset, a unique SSC gene expression signature still supports spermatogenesis in vitro (Li et al., 2017; Guo et al., 2018; Hermann et al., 2018; Wang et al., 2018; Sohni et al., 2019). Studies are further limited by the low number of SSCs in the testis, which range from 0.01% to 1% of all testicular cells (Tegelenbosch & De Rooij, 1993). While stable proliferation over several months has been achieved in various mammalian species (Kanatsu-Shinohara et al., 2003; Hamra et al., 2005), a long-term expansion system has yet to be established in humans, with culture and propagation in vitro currently reported up to 28 weeks (Sadri-Ardekani et al., 2009). Nevertheless, Yang et al. report retinoic acid (RA) and SCF stimulated generation of haploid spermatids from isolated human SSCs in conventional single cell culture (Yang et al., 2014).

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up to 5 weeks in contrast to the squamous monolayer formed when cultured on plastic alone (Hadley et al., 1985). Tubular reorganization in these cultures is driven by fibronectin, a component of the basement membrane synthesized by myoid cells, which promotes the migration of Sertoli cells (Tung & Fritz, 1986; Richardson et al., 1995). Despite achieving basic structural reorganization, these studies result in limited germ cell development, suggesting a full complement of somatic testicular cell types is required for the progression of spermatogenesis in culture. A more recent approach describes the culture of single cell digested adult testicular biopsies on uncoated culture dishes in a 2D system (Mincheva et al., 2018; Von Kopylow et al., 2018).

While permitting cultivation of various somatic testicular cells over 12 weeks, the reorganization of seminiferous cord-like structures was limited and insufficient to support the maintenance of germ cells which were often located outside the cell clusters (Von Kopylow et al., 2018). Dedifferentiation of somatic cell types was speculated to underlie the failure of germ cells to incorporate into the cellular aggregates (Von Kopylow et al., 2018).

Two-dimensional in vitro culture systems therefore fail to replicate the complete process of spermatogenesis, providing an insufficient niche for long-term germ cell maintenance, propagation and differentiation. Despite this, many of the culture conditions employed are necessary to recapitulate aspects of the testis microenvironment and are common to a number of the models covered in this review (Table 1). In most mammals, including humans, spermatogenesis requires a temperature 2–3°C below core body temperature (Newman & Wilhelm, 1950). Given this, cultures are routinely maintained at 33–35°C, with greater temperatures having a deleterious effect on the viability of spermatogonia and Sertoli cells (Medrano et al., 2018). Supplementation of culture medium with pituitary gonadotrophins luteinizing hormone (LH) or FSH promotes Sertoli cell survival and maturation of spermatogonia up to meiotic initiation (Tesarik et al., 1998; Medrano et al., 2018), while various other factors are necessary to maintain the balance between SSC renewal (GDNF) and differentiation (RA) (De Rooij, 2009; Yang et al., 2014).

### 3D IN VITRO MODELS

Culture of intact testes fragments exploits the native organization of the tissue conserving structural support and cellular interactions which cannot be achieved in a 2D system (Fig. 2). The pioneering studies of testes tissue culture were performed by Steinberger who described the culture of rat (Steinberger et al., 1964; Steinberger & Steinberger, 1965), and later, human testicular tissue (Steinberger, 1967) using an air–liquid interface method. Adapted from Trowell, the system comprises the suspension of tissue fragments that are partially immersed in
culture medium on a semi-solid support, balancing the delivery of nutrients from the culture medium with efficient gas exchange (Trowell, 1954). Applying the same principle, Sato and colleagues achieved complete spermatogenesis in vitro nearly 50 years later, generating fertilization-competent spermatozoa from immature mouse testis fragments (Sato et al., 2011). While the success of the system is likely underpinned by maintaining the native testis microenvironment, the complete maturation of spermatozoa appears dependent on the medium component knockout serum replacement (KSR). Using the same system, subsequent studies from our group revealed that a minimum concentration of 10% KSR is required to induce both full spermatogenesis and testosterone production (Reda et al., 2017). The latter of which may be explained by the lipid-rich cholesterol component of KSR which is the precursor for testosterone synthesis (Garcia-Gonzalo & Izipisua Belmonte, 2008). While the exact factors required for germ cell differentiation remain undefined, studies suggest testosterone is necessary to sustain both the meiotic process and germ cell survival (especially in the later stages of differentiation) (Erkkila et al., 1997; Tesarik et al., 1998). Sato and colleagues, however, further noted maturation of the somatic Sertoli cells and peritubular myoid cells within the culture system, as illustrated by the onset of androgen receptor expression, demonstrating their maturity to support spermatogenesis (Sato et al., 2011). This suggests that the system may in fact be enabling spontaneous spermatogenesis by providing germ cells with an appropriate niche instead of simply supplying the growth factors necessary for differentiation.

Despite the subsequent success in other species, for example rats (Reda et al., 2016), the air–liquid interface system has yet to been refined for human application, although partial spermatogenesis has been described. Roulet et al. report differentiation of primary spermatocytes from the preleptotene to pachytene stage in cultured adult testis obtained from prostate cancer patients, however, observed a progressive loss of meiotic and post-miotic cells within the 16-day culture (Roulet et al., 2006). Using

**Table 1.** Experimental details of select in vitro human testis studies and resulting progression of spermatogenesis

| Culture model          | Age of tissue donor | Progression of spermatogenesis | Medium       | Serum   | Additional factors | Gonadotrophins | Scaffold | Temperature/CO₂ | Selected references                  |
|------------------------|---------------------|--------------------------------|--------------|---------|--------------------|----------------|----------|-----------------|---------------------------------------|
| 2D – Enriched SSCs     | Adult               | SSC – rSpt                      | DMEM/F12     | 10% FBS | RA (2 µg)          | –              | –        | 34°C            | Yang et al. (2014)                    |
| 2D – Single-cell tissue digest | Adult               | Presence of eSpt                | DMEM         | 15% KSR | SCF (20–150 ng/ml) 2-Mercaptoethanol (0.1 µm) L-Glutamine (2 µm) 100 × MEM non-essential amino acids (0.1 µm) EGF (40 ng/ml) FGFα (20 ng/ml) FGFβ (20 ng/ml) FGF9 (20 ng/ml) GDNF (100 ng/ml) IG (10 ng/ml) | –              | Matrigel | 34°C            | Von Koplyov et al. (2018)             |
| 3D – Gel matrix Peripubertal – adult | SSC – rSpt     | DMEM/F12                        | 10% KSR      | RA (2 µg) SCF (100 ng/ml) BMP4 100 ng/ml Testosterone (10⁻⁸ M) L-Glutamine (0.35 mg/ml) Retinol (10⁻⁶ M) Vitamin C (0.05 mg/ml) Pyruvate (0.0025 µ) Triiodothyronine (T3) (5 pmol/L) GDNF (10 ng/ml) 22(R)-hydroxycholesterol (20 µmol/L) Prolactin (5 ng/ml) | –              | Matrigel | 34°C            | Sun et al. (2018)                     |
| 3D – Air-liquid interphase Prepubertal interphase | Diploid to haploid germ cells | DMEM/F12 | 10% KSR | FSH (5 UI/L) hCG (1 UI/L) | –              | –        | 34°C            | de Michele et al. (2018)              |
| 3D – Hanging drop Adult | Diploid to haploid germ cells | StemPro-34 | – | RA (2 µg) SCF (100 ng/ml) FSH (2.5 × 10⁻³ IU) HU (2.5 × 10⁻⁵ IU) Prolactin (5 µg/100 ml) | –              | –        | 34°C            | Pendergraft et al. (2017)             |
| 3D – Bioreactor Adult | Generation of morphologically mature spermatozoa | DMEM/F12 | – | NaHCO₃ (1.2 g/L) Insulin (10 µg/ml) Transferrin (10 µg/ml) Vitamin C (10⁻⁶ M) Vitamin E (10 µg/ml) RA (3.3 × 10⁻⁷) Retinoid (3.3 × 10⁻⁷) Pyruvate (10⁻³) Testosterone (10⁻₇) | FSH (1 ng/ml) | –        | 33°C            | Perrard et al. (2018)                 |

BMP, bone morphogenetic protein; DMEM, Dulbecco’s modified Eagle’s medium; EGF, epidermal growth factor; eSpt, elongating spermatid; FBS, fetal bovine serum; FGF, fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; IGF, insulin-like growth factor; KSR, KnockOut serum replacement; RA, retinoic acid; rSpt, round spermatid; SCF, stem cell factor; SSC, spermatogonial stem cell.
the same system, de Michele and colleagues describe preserved seminiferous tubule structure and somatic cell function capable of maintaining spermatogonial proliferation for up to 139 days in prepubertal testis tissue (De Michele et al., 2017). Leydig cell functionality was demonstrated by testosterone production which peaked at 10 days in culture while the observed decrease in anti-Müllerian hormone expression suggests Sertoli cell maturation. While the approach preserves paracrine interactions facilitating maturation of the somatic cell niche, germ cell differentiation was not achieved. These findings were recently extended with reports of haploid cells generated from immature testis tissue (De Michele et al., 2018). Inclusion of factors required for both SSC renewal (e.g. GDNF) and differentiation (e.g. retinoic acid) in the differentiation medium allowed for accelerated maturation of the SSC niche supporting germ cell differentiation. Whether these cell types are capable of differentiating further into fertilization-competent spermatozoa is yet to be defined.

Three-dimensional culture systems are an alternative strategy used to re-engineer the in vivo testis microenvironment. Such approaches are broadly classified as scaffold-free or scaffold-based and can comprise natural or synthetic materials. Scaffold-free systems primarily consist of the formation of multi-cellular aggregates, an example of which is the hanging drop system. Based on studies described in 1907 by Harrison, the method has often been used for the generation of embryoid bodies from immature testis tissue (De Michele et al., 2018). Inclusion of factors required for both SSC renewal (e.g. GDNF) and differentiation (e.g. retinoic acid) in the differentiation medium allowed for accelerated maturation of the SSC niche supporting germ cell differentiation. Whether these cell types are capable of differentiating further into fertilization-competent spermatozoa is yet to be defined.

Precise topological organization of the testis is crucial for compartmentalizing signalling cues which drive cell fate decisions (Lord et al., 2018). While supporting partial human spermatogenesis, the described 3D systems fail to fully replicate the specialized architecture of the testicular microenvironment. Scaffold-based culture strategies offer an alternative approach. One such example is the use of a biocompatible decellularized testicular matrix. In an effort to induce de novo formation of testicular architecture, Baert and colleagues combined isolated cell suspensions from either adult or prepubertal donors with a decellularized human cadaveric testicular matrix (Baert et al., 2015, 2017). While retaining native 3D tissue structure and testis-specific ECM components, such as collagen, laminin and fibronectin, the scaffold was unable to offer a more favourable environment for tissue reorganization (Baert et al., 2015, 2017). Despite this, the primary testicular cells from adult and pubertal tissue demonstrated self-assembly into spheroid structures. While lacking testis-specific topography, the resulting organoids supported the maintenance and propagation of germ cells throughout culture and retained somatic cell functionality, as demonstrated by testosterone and inhibin B production (Baert et al., 2017). Additional scaffold approaches have been used to explore the de novo arrangement of testis structure. Collagen sponges, which offer a porous 3D structure with an ECM like surface, allow for partial reorganization of tubule-like structures following colonization by dissociated rat testicular cells (Reuter et al., 2014). While topological cues in the form of silicon-based nanostructures can direct cord-like formation by aligning peritubular cell and Sertoli cell bodies in the direction of nanogratings (Pan et al., 2013). Synthetic scaffolds such as silicon also offer a defined chemical composition allowing for reproducibility however lack sites for cellular adhesion and often require a coating of ECM proteins in an attempt to mimic the nice in which cells reside naturally (Knight & Przyborski, 2015). Despite the reported lack of germ cell differentiation, such approaches are relevant for better understanding the mechanisms of testicular cord formation.

Although 3D culture systems favour progression of spermatogenesis and testicular reorganization, it appears combination with ECM components provides a more desirable environment. Encapsulation of dissociated testicular cells from azoospermic adult patients in collagen gel matrix results in cellular aggregation sufficient for the differentiation of spermatids from spermatocytes (Lee et al., 2007). The same system potentiates the meiotic progression and post-meiotic differentiation of rodent germ cells
In a recent study, Sun and colleagues report the generation of post-meiotic cells from SSCs isolated from azoospermic patients using a 3D approach (Sun et al., 2018). Comprised of purified SSCs co-cultured with Sertoli cells in a Matrigel matrix, the system supports germ cell differentiation producing functional haploid spermatids with the capacity to fertilize mouse oocytes. Inclusion of factors essential for SSC renewal and differentiation including RA, SCF, BMP4 and testosterone, in addition to KSR (10%), allowed for increased differentiation efficiency, 10-fold greater than previously reported by Sato et al. (Sato et al., 2011). Studies therefore suggest the effect of ECM is further pronounced when cells are embedded within a gel matrix, such as collagen or Matrigel (Sun et al., 2018), as oppose to simply occupying cavities of a scaffold (Pan et al., 2013; Reuter et al., 2014; Baert et al., 2017). In addition to ECM however, other matrices (e.g. agarose, methylcellulose) have been used with similar success, highlighting the principle need of an encapsulating 3D structure to initiate male germ cell maturation in vitro (Stukenborg et al., 2008, 2009; Abu Elhija et al., 2012).

In a novel approach to 3D culture, we have recently detailed a multilayer model termed the three-layer gradient system (3LGS) to generate rat testicular organoids in vitro (Alves-Lopes et al., 2017, 2018). Using a multilayer approach, whereby testicular cell suspensions are embedded in a layer of Matrigel situated between two cell-free layers, we report the reorganization of seminiferous tubule-like structures with a functional blood–testis barrier supporting germ cell maintenance and proliferation up to 21 days in culture. Uniquely, the system generates a whole organ structure, comprising multiple tubule-like structures situated within an interstitial environment as oppose to individual clusters as described in other studies (Baert et al., 2017; Pendergraft et al., 2017; Sakib et al., 2019). We propose that the success of system centres on the generation of two concentration gradients formed by the layered structure – the inflow of factors from the Matrigel (to be consumed by the cells) and the subsequent outflow of cellular metabolites. Supporting this hypothesis, tubule-like structures do not reorganize in a single layer of Matrigel using the same volume and cell concentration as used in the 3LGS (Alves-Lopes et al., 2017).

Alternative dynamic culture approaches have been described in an attempt to promote germ cell differentiation in vitro. Using a bioreactor system, Perrard and colleagues report differentiation of morphologically mature spermatozoa from spermatogonia in human seminiferous tubule fragments enclosed in porous chitosan hydrogel tubes immersed in culture medium (Perrard et al., 2016). Whether the resulting cell types are fertilization-competent has yet to be defined. Building on the success of the air–liquid interface method (Sato et al., 2011), Komeya et al describe the development of a microfluidic device (Komeya et al., 2016). Composed of a continuously flowing medium channel separated from a tissue chamber by a nutrient permeable membrane, the authors report maintenance of murine testis fragments and complete spermatogenesis for up to 6 months. The success of the described systems over the previous static organ-culture approach is likely underpinned by the dynamic exchange of nutrients, waste and gas. Normally regulated by the microvasculature, both systems allow for locally established homoeostasis through molecular diffusion similar to the blood plasma-interstitial tissue relationship in vivo. Dynamic culture may also confer a gradient of paracrine and autocrine factors formed in the basal, adluminal and luminal compartments of the seminiferous tubes. While we recapitulated this to some extent with our 3LGS, the approach is limited in regard to tissue size (resulting in the formation of central necrotic areas) and duration of culture, perhaps due to the saturation of supporting Matrigel. Lack of vascularization is a limitation of organoid technologies, particularly when considering the generation of tissue for clinical transplantation. Given the success in other fields, one approach may be the supplementation of cultures with an exogenous source of endothelial and mesenchymal progenitor cells in order to induce tissue vascularization (Takebe et al., 2015). Further studies will be required to fully explore the 3LGS system as a model for the testsis.

CONCLUSIONS

A functioning somatic microenvironment is crucial for the process of spermatogenesis. When considering in vitro modelling of the human testis with the intent of generating mature spermatozoa, this must be a primary consideration. Studies that have made the most progress have either exploited the intrinsic microenvironment using testsis tissue fragments or encapsulated dissociated cells within a supportive matrix to generate a 3D structure. In the effort to replicate human spermatogenesis in vitro, future focus should be on maintaining the functional niche or, in the case of immature tissue, maturation of the niche to support spontaneous spermatogenesis.

ACKNOWLEDGEMENTS

The authors E.O and J.B.S acknowledge support from the Frimurare Barnhuset i Stockholm Foundation and the Swedish Childhood Cancer Foundation (TJ2016-0093), respectively.

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