Original Article

Mini Bioreactor Can Support *In Vitro* Spermatogenesis of Mouse Testicular Tissue

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Abstract

Objective: It was in the early 20th century when the quest for *in vitro* spermatogenesis started. *In vitro* spermatogenesis is critical for male cancer patients undergoing gonadotoxic treatment. Dynamic culture system creates *in vivo*-like conditions. In this study, it was intended to evaluate the progression of spermatogenesis after testicular tissue culture in mini-perfusion bioreactor.

Materials and Methods: In this experimental study, 12 six-day postpartum neonatal mouse testes were removed and fragmented, placed on an agarose gel in parallel to bioreactor culture, and incubated for 8 weeks. Histological, molecular and immunohistochemical evaluations were carried out after 8 weeks.

Results: Histological analysis suggested successful maintenance of spermatogenesis in tissues grown in the *bioreactor but not on* agarose gel, possibly because the central region did not receive sufficient oxygen and nutrients, which led to necrotic or degenerative changes. Molecular analysis indicated that Plzf, Tekt1 and *Tnp1* were expressed and that their expression did not differ significantly between the bioreactor and agarose gel. Immunohistochemical evaluation of testis fragments showed that PLZF, SCP3 and ACRBP proteins were expressed in spermatogonial cells, spermatocytes and spermatozoa. PLZF expression after 8 weeks was significantly lower ($P<0.05$) in tissues incubated on agarose gel than in the bioreactor, but there was no significant difference between SCP3 and ACRBP expression among the bioreactor and agarose gel culture systems.

Conclusion: This three-dimensional (3D) dynamic culture system can provide somewhat similar conditions to the physiological environment of the testis. Our findings suggest that the perfusion *bioreactor* supports induction of spermatogenesis for generation of haploid cells. Further studies will be needed to address the fertility of the sperm generated in the bioreactor system.

***Keywords**:* Agarose Gel, Mouse, Perfusion Bioreactor, Spermatogenesis, Tissue Culture

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Introduction

Investigations into the progress of spermatogenesis *in vitro* began early last century (1), although the differentiation of spermatogonial stem cells (SSCs) into sperm cells remained a challenge. In the 1960s and 1970s, testis tissue culture was used to evaluate the process of spermatogenesis. In those experiments, spermatogenesis progressed as far as meiosis but haploid cells were never formed (2). In the 1980s, cell culture was used instead of tissue culture, but the development of fertile sperm cells remained problematic (3). Although there are many ways of culturing tissue fragments, the gold standard is the interphase method in which specimens are positioned at the interphase between the culture medium and a gas layer (4). By isolating seminiferous tubules acquired from immature mice, Sato et al. (5) generated fertile sperm using *ex vivo* culture. However, the overall duration and efficiency of spermatogenesis were not close to those reported *in vivo*.

Capillaries around a tissue provide oxygen and nutrients, as well as removing waste effectively, thereby supporting tissue homeostasis. Because it lacks such a microcirculatory network, the interphase method cannot provide the appropriate *in vivo*-like conditions. Researchers have tried innovative circulatory mechanisms to improve their culture systems (6, 7). To maintain physiological functions more efficient than conventional methods, microfluidic devices and bioreactors have been developed recently to culture testis tissue pieces (8-11). Different types of perfusion bioreactors have also been developed and have yielded more favourable results compared with static culture (12-14). In this study, a mini-perfusion bioreactor was designed that was capable of successfully sustaining spermatogenesis from immature mouse testis tissue fragments incubated for 8 weeks. This device provided sufficient nutrients and oxygen for tissue culture for having an *in vitro* model to study spermatogenesis progression during maturation of neonatal testicular tissue. The fertility of sperm generated in the bioreactor system will be addressed in future studies. Finally, the dynamic culture method described here must

be assessed in human testicular tissue culture to determine its potential utility in addressing male infertility.

Materials and Methods

Design of the mini-perfusion bioreactor system

In this experimental study, the mini-perfusion bioreactor system is composed of three polydimethylsiloxane (PDMS) layers (Sylgard 184, Dow Corning, Germany)-upper, middle and lower annular rings-with a central cylindrical cavity and a porous polyvinylidene fluoride membrane filter (pores size 0.22 μm, Millipore, Germany). The lower layer is composed of a medium flow chamber (5 mm wide and 5 mm high) and a channel for continuously supplying culture medium from a perfusion pump to an outlet. The middle layer comprises a tissue chamber of the same size. The culture medium was drawn through the inlet by a syringe pump at a rate of 15, 27, 50 and 100 μL/hour. The porous membrane was placed between the flow channel and the tissue chamber to separate sample tissues from the flowing medium. The upper layer was a waste material chamber. The thickness of the PDMS layers was 5 mm each (15).

Agarose support gel preparation for tissue culture

The method described by Yokonishi et al. (16) was used to prepare the agarose support gel. In brief, 1.5% w/v agarose solution (Carl Roth, Germany) was prepared and sterilised. Segments measuring 10×10×5 mm^3 were arranged using a scalpel blade under sterile conditions. The segments were then placed in a six-well plate containing alpha-minimum essential medium (αMEM; Bio-Ideal, Iran) supplemented by10% Knockout Serum Replacement (KSR, Gibco, UK), 60 ng/mL progesterone (Invitrogen, UK), 30 ng/mL beta-estradiol (PeproTech, Germany), 20 ng/mL epithelial growth factor (EGF, PeproTech), 10 ng/mL human basic fibroblast growth factor (bFGF, PeproTech), 10 ng/mL human glial cell line-derived neurotropic factor (GDNF, PeproTech) and 10 ng/mL leukemia inhibitory factor (LIF, Royan Institute, Iran) as the culture medium.

Animals

Six-day-old NMRI neonatal male mice provided by the Pasteur Institute of Iran were used as the source of testis tissue. The mice were maintained at an ambient temperature of 22°C and a 12/12 hours light/dark cycle. This study was approved by the Ethics Committee of Tarbiat Modares University, Tehran, Iran (IR.TMU.REC.1395.522).

Culture of testis tissues

Two groups of neonatal mouse testis were created for this study: tissue cultivated in the perfusion bioreactor and on agarose gel. For each group, 12 mouse pups were euthanised and the testes were removed, decapsulated, fragmented (1 mm^3 in size) and randomly allocated to either the bioreactor or the agarose gel. In the bioreactor group, the tissue was placed in the middle chamber. Assessments were performed after 2 and 8 weeks of culture. For static cultures, testis fragments were placed on agarose stands in a six-well culture plate, and medium was added to one-half to four-fifths the height of the agarose gel. The medium was changed twice a week. The culture incubator conditions were maintained under 5% CO_2 and 34°C temperature (5).

Viability test of the tissue during the culture

For checking the cytotoxic effects of the bioreactor components and accessories, the cell suspension was exposed to PTFE tubes, PDMS and bioreactor accessories for 72 hours under normal culture conditions. Cell viability was assessed at the beginning of culture and after 72 hours by Trypan blue staining. Moreover, after 8 weeks of culture by capturing multiple images, the viability of the cells in the tissue was assessed based on morphology.

Histology, morphology and functional examinations

Specimens were fixed with Bouin's fixative and embedded in paraffin wax. Sections were cut and stained with hematoxylin and eosin (H&E) or Weigert's hematoxylin and periodic acid-schiff (PAS, Merck, Germany). All sections were examined using a light microscope (Zeiss, Germany) (17). After 8 weeks of culture, the tissues were mechanically dissociated using needles to release the spermatid and sperm for evaluation on an inverted microscope (Zeiss Axiovert 40 CFL) (9).

Papanicolaou staining was performed to assess the sperm-like cell morphology (18). A Diff-Quick staining kit (Faradid Pardaz Pars Inc., Iran) was utilized to assess sperm morphology. Smears were firstly stained with Diff-Quick staining solutions I and II for 25 seconds. Afterwards, they were washed in distilled water. In the Diff-Quick smears, acrosomes stain pink or light purple, and the sperm nucleus, midpiece and tail stain dark purple (19). Double staining was performed to assess the acrosome reaction. Briefly, the smears were fixed with 3% glutaraldehyde for 30 minute, and the slides were stained with Bismarck brown (0.8% in deionised water, pH=1.8) for 10 minute and then with Rose Bengal (0.8% in 0.1 M Tris buffer, pH=5.3) for 25 minutes. Sperm-like cells with acrosomes that stained bright brown were considered to be sperm-like cells with an intact acrosome region (20).

Quantitative reverse transcription polymerase chain reaction analysis: gene expression quantitative analysis

Expression of promyelocytic leukaemia zinc finger (*Plzf*), *Tekt1* and *Tnp1* genes in testicular tissue fragments were evaluated after 2 and 8 weeks. Total RNA was extracted from the tissue fragments from both groups using RNX-Plus™ (CinnaGen, Iran) following the manufacturer's recommendation. The RNA concentration was then determined using an ultraviolet

spectrophotometer (Eppendorf Company, Germany). cDNA synthesis was performed using a RevertAid™ First Strand cDNA Synthesis kit (Fermentas, Germany) and oligo (dT) primers. For the polymerase chain reaction (PCR) reactions, primers for *Plzf*, *Tekt1* and *Tnp1* genes were designed. Designed primers were blasted using the NCBI website (https://www.ncbi.nlm.nih.gov/) (21) and were synthesised by a commercial source (CinnaGen, Iran) (Table S1, See Supplementary Online Information at www.celljournal.org). PCR was performed using Master Mix and SYBR Green I (Fluka, Switzerland) in an Applied Biosystems StepOne™ instrument (Applied Biosystems, UK). Melting curve analyses were used to confirm the quality of the PCR reactions. A standard curve was used to determine the efficiency for each gene (logarithmic dilution series of cDNA from the testes). The reference gene β-*actin* and the target genes were amplified in the same run. This process was repeated and duplicated three times for all target and reference genes. The reference genes were relatively equal, and the target gene expression levels were normalised to that of the reference gene.

Immunohistochemistry

The identity of SSCs, spermatocytes and sperm-like cells was verified by tracking the promyelocytic leukaemia zinc finger protein (PLZF), synaptonemal complex protein 3 (SCP3) and acrosin binding protein (ACRBP) (22-24). These markers were detected after 8 weeks of culture. For immunohistochemistry, primary antibody, mouse monoclonal anti-mouse antibody against PLZF, SCP3 or ACRBP (1:100, Santa Cruz Biotechnology, Germany) was added and the samples were incubated at 4°C overnight. The secondary antibody Alexa 488-conjugated anti-mouse IgG (1:200, Sigma, Germany) was added for 2 hours at 37°C in the dark. For nuclear staining 4′,6-diamidino-2-phenylindole (DAPI, 1:200, Sigma, Germany) was applied for 1 minute. The specimens were observed with a fluorescence microscope (Olympus, type CH2, Japan). To quantify the results, germ cells were defined as cells that stained positive for PLZF, SCP3 and ACRBP. The results are reported as the percentage of germ cells that were positive for the protein of interest relative to the entire population. From each sample, 5 sections were randomly selected and after high-magnification photography (magnification: x400), 5 fields from each section were analyzed by image-j software.

Statistical analysis

The data was analysed using one-way analysis of variance followed by Tukey's post hoc test and are shown as mean ± standard deviation (SD). Calculations were performed using SPSS (Version 15.0, SPSS Inc., USA). Each data point represents the average of three separate experiments, and five repeats were performed for each experiment. A $P \leq 0.05$ was considered to be significant.

Results

Organ culture

Neonatal mouse testicular tissue was cultured on agarose gel and in a mini-perfusion bioreactor (Fig.1A, B). The tissue samples were positioned on agarose gel and in the tissue chamber of the bioreactor (Fig.1C-F). In the agarose cultures, we observed necrotic changes, a hallmark of degenerative changes in the tissue, as darkened regions in the central parts (Fig.2B, (f) white arrow). In the bioreactor, the tissue samples were positioned in the tissue chamber. For the best flow rate, histological analyses were done (Fig.S1, See Supplementary Online Information at www.celljournal.org) and the best flow rate (27 µl/hour) was chosen for the tissue culture in the bioreactor, the central areas of the testicular tissue remained viable, which suggests that this tissue received vital ingredients from the medium.

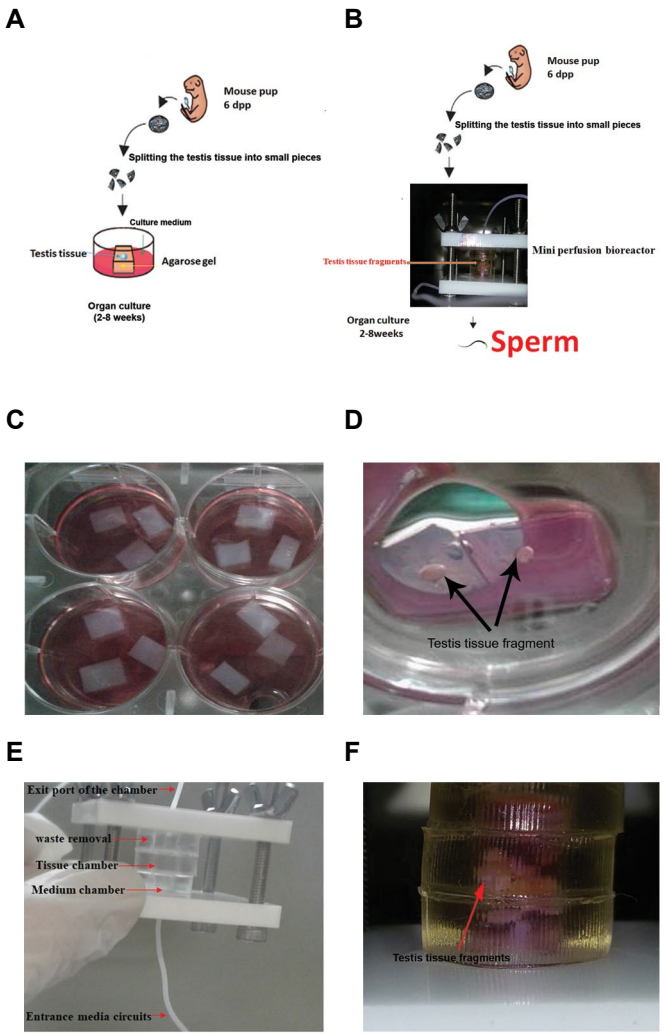

Fig.1: Schematic diagram of the neonatal mouse testicular tissue organ culture. **A.** Testicular tissue pieces placed on agarose gel, **B.** In the bioreactor. **C.** Agarose gel hexahedrons stand transferred to 6-well culture plates. **D.** Testicular tissue was cut to small pieces and placed on agarose gel (black arrows). **E.** Mini-perfusion bioreactor device. **F.** Testicular tissue fragments in the tissue chamber of the perfusion bioreactor system (red arrow).

Viability assessment of cells and tissues

Sertoli cells and spermatogonial cells were cultured with PTFE, PDMS and bioreactor accessories. After 72 hours, viability assay was done by Trypan blue staining, there was no cytotoxic reaction monitored after this co-culture (Fig.2A). Progression of spermatogenesis in organ culture was assessed by bright field and H&E staining for 8 weeks (Fig.2B). In the bioreactor, the central regions of the testicular tissue remained viable, and sperms were distinguished (Fig.2Bg, black arrows). Meanwhile in agarose culture, the central region was degenerated (Fig.2Bf, white arrow) and there was no evidence of existing sperm cells.

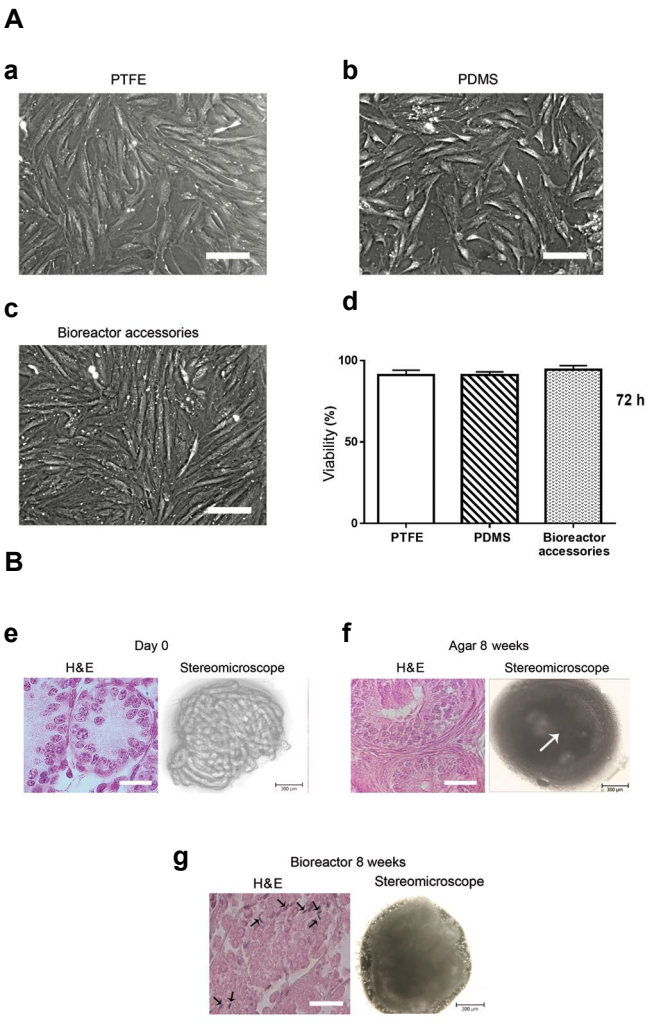

Fig.2: Results of viability assessment of cells, H&E staining and photomicrograph of the testicular sections. **A.** Cell suspension 3 days after culture with PTFE (a), PDMS (b), and bioreactor accessories (c). Viability assay graph of cells after 72 hours of this co-culture (d). **B.** H&E staining and photomicrographs of testicular tissue fragments day 0, 8 weeks of agarose gel and bioreactor culture (e-g). White arrow; Degenerative regions, Black arrows; Sperm cells. H&E images (scale bars e-g: 10 µm, magnification: x1000). PTFE; Polytetrafluoroethylene and PDMS; Polydimethylsiloxane.

Histology, morphology, and functional examinations

Spermatogenesis was maintained in the peripheral areas of the tissue samples cultured on agarose gel (Fig.3A, a, B, b). Histology showed seminiferous tubules exhibiting spermatogenesis in all tissue areas in the tissue cultured in the bioreactor. Different stages of spermatogenesis were seen in different regions of the tissue. Bioreactor cultures showed sperm-like cells after 8 weeks of culture (Fig.3C, c, D, d, Fig.4a). It is noteworthy that tissue integrity was preserved in both groups. In the tissue cultured on agarose gel, tubules were not observed centrally, probably because of the hypoxic conditions and limited access to nutrients. In the agarose gel cultures, no sperm cells were observed in the suspension produced by tissue dissociation (Fig.4b). By contrast, after removal of tissue from the bioreactor and mechanical dissociation, sperm-like cells were observed (Fig.4c), and staining showed that they appeared as normal sperm (Fig.4d-f).

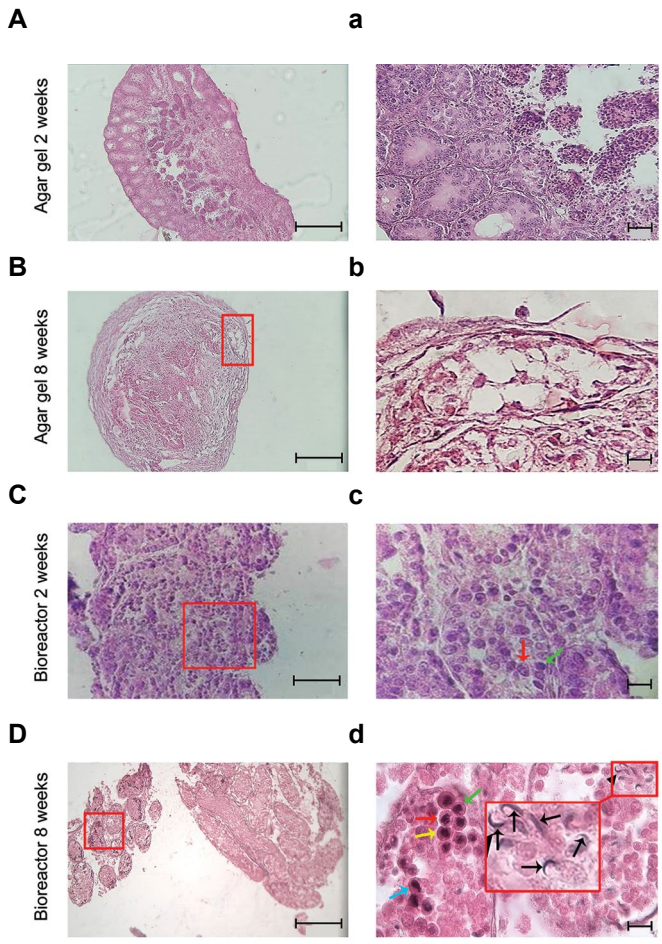

Fig.3: Progression of spermatogenesis in organ culture. **A, B.** H&E staining after 2 and 8 weeks of agarose gel culture, respectively. Higher magnification images of A and B (a, b). **C, D.** H&E staining after 2 and 8 weeks of bioreactor culture, respectively. Higher magnification images of C and D (c, d). Green arrow; Spermatogonia, Red arrow; Spermatocyte (c). Green arrow; Spermatogonia, Red arrow; Primary spermatocyte, Yellow arrow; Secondary spermatocyte, Blue arrow; Spermatid, Arrow head and black arrows; Long spermatids or sperm-like cells (d) [scale bars: 200 µm, magnification: x100 (A, B, D), 30 µm, magnification: x400 (C), 10 µm, magnification: x1000 (a-d)].

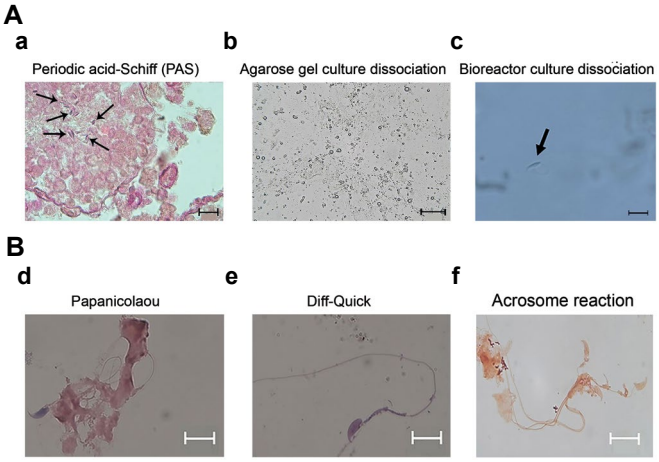

Fig.4: Staining to assess the morphology and function of sperm. After removal of testicular tissue from the bioreactor and mechanical dissociation, Papanicolaou and Diff-Quick staining were used to assess sperm morphology. Sperms were stained by a double staining protocol for acrosome reaction. **A.** Periodic acid-schiff (PAS) staining after 8 weeks of bioreactor culture (a). Black arrows in (a); Sperm cells. Agarose gel culture dissociation (b). Arrow indicates a sperm cell with flagella found in dissociated tissue in the bioreactor group (c). **B.** Papanicolaou staining (d). Diff-Quick staining (e). Acrosome reaction (f) (scale bars: 10 µm, magnification: x1000).

Molecular assessment

2 and 8 weeks after 3D culture, bioreactor and agarose gel groups were compared with neonatal testis. *Plzf* expression was significantly lower (P≤0.05) in both the bioreactor and agarose gel groups than in fresh neonatal testis. However, *Plzf* expression did not differ significantly between the bioreactor and agarose gel groups (Fig.5A, B). *Tekt1* and *Tnp1* expression also did not differ significantly between the groups after the 2-week culture period (Fig.5A). *Tekt1* and *Tnp1* expression after the 8-week culture was significantly higher (P≤0.05) in all groups compared with fresh neonatal testis (Fig.5B). 8 weeks after 3D culture, the bioreactor and agarose gel groups were compared with adult testis. Additionally, *Plzf* gene expression indicated that there was no significant difference between the groups. *Tekt1* expression was significantly lower in both culture groups than in adult mice testicular tissue but did not differ significantly between the two groups. *Tnp1* expression was significantly lower in the agarose group than in the adult mice testicular tissue after 8 weeks but did not differ significantly between the bioreactor culture and adult mouse testicular tissue (Fig.5C).

Immunohistochemistry

PLZF protein was expressed in the tissues that showed spermatogonial cells. SCP3 protein, was detected in the spermatocytes and in ACRBP-positive cells, indicating the existence of sperm-like cells (Fig.6). Quantification of the immunohistochemical staining indicated that PLZF expression was significantly lower (P<0.05) in adult tissue and both the bioreactor and agarose groups than in neonatal tissue. However, there was no difference at 8 weeks between the bioreactor culture and adult expression levels. At 8 weeks, PLZF expression was significantly reduced in the agarose gel group compared with that of the adult tissue and bioreactor groups. At 8 weeks, the SCP3 and ACRBP expression levels were significantly lower in both the agarose and bioreactor groups than in adult tissue but did not differ significantly between the bioreactor and agarose gel groups (Fig.6).

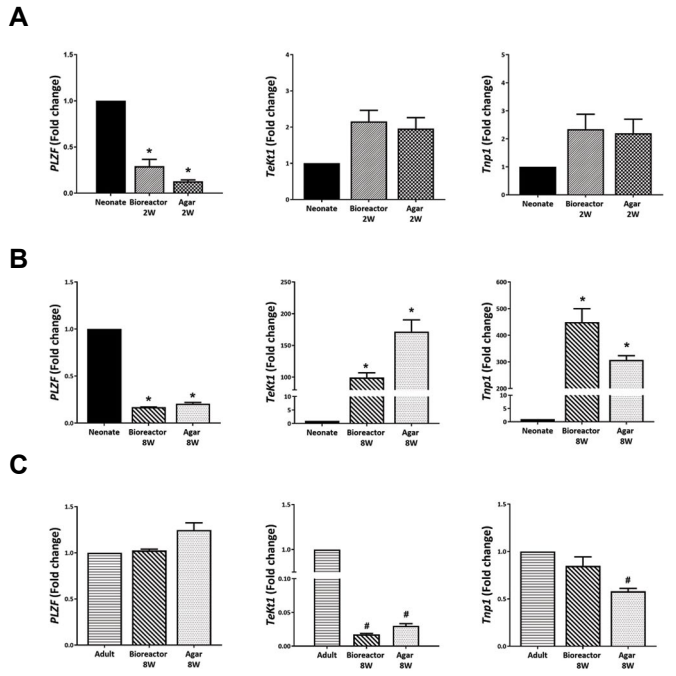

Fig.5: Gene expression in testicular tissue fragments in experimental groups. Expression of *Plzf*, *Tekt1* and *Tnp1* gene was assessed in tissues cultured in the bioreactor and on agarose gel for 2 and 8 weeks. **A-C.** Expression level was normalised to that of β-actin and is represented as mean ± SD after three repeats of the experiments. *; P<0.05 versus neonate and #; P<0.05 versus adult. Similar symbols indicate no significant differences between those groups.

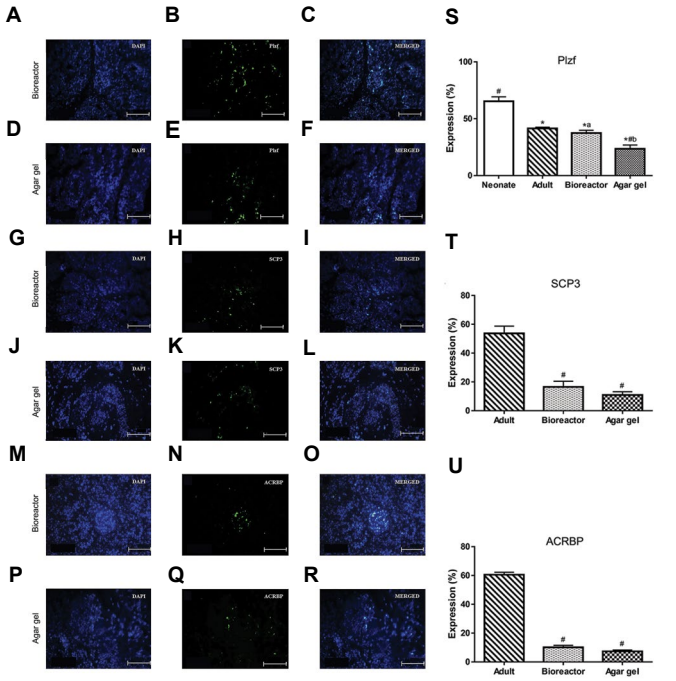

Fig.6: Immunohistochemistry of neonatal mouse testicular tissue after 8 weeks of organ culture. **B, H, N.** Expression of specific protein of spermatogonial cells (PLZF), spermatocytes (SCP3) and spermatozoa (ACRBP) in the bioreactor and **E, K, Q.** On agarose gel, respectively. **A-P.** Nuclei were stained by DAPI. **C-R.** The merged images. **S-U.** Expression of specific protein in experimental groups. Data are represented as mean ± SD of experiments performed in triplicate. #; P<0.05 versus adult and *; P<0.05 versus neonate. a and b indicate P<0.05 versus the respective between the bioreactor and agarose gel (scale bars: 10 µm).

Discussion

3D tissue culture is an effective technique for the preservation and development of various tissues, including germinal tissues. In this study, we developed a mini-scale perfusion bioreactor and compared it with agarose gel for 3D culture of immature mouse testicular tissue. Inside the body, capillaries around a tissue provide oxygen and nutrients and clear waste effectively, thereby supporting tissue homeostasis (9).

SSCs and A-pair/A-aligned spermatogonia have been suggested to be limited to the surrounding vasculature and interstitial tissue around the seminiferous tubules (25). A number of factors are responsible for diffusion within culture, such as cell density, tissue thickness and concentration of the materials on the tissue surface (26). In 3D cultures, static flow might lead to the formation of passive gradation of materials that will be equilibrated over the long term. Therefore, a forced but controlled culture medium perfusion is indispensable (27).

In the method using agarose gel, extensive induction of spermatogenesis did not happen because seminiferous tubules on the agarose gel merged to create a dome-shaped structure even if initially extended flat. As a result, the supply of nutrients and oxygen to the central region was insufficient, which led to necrotic and degenerative changes in the tissue (9). Thus, for the lack of microcirculatory system, the agarose gel method cannot provide conditions similar to those *in vivo*. Given the failure of the static culture methods, dynamic culture methods seem to be more effective in increasing spermatogenesis and producing haploid cells in a more effective 3D culture system that provides optimal conditions that simulate the physiological environment of the body. These conditions include temperature, oxygen and carbon dioxide content, mechanical, chemical and electrical stimulation, and improved access to nutrients and elimination of waste. Together, these factors should help to prevent necrosis in the central region of the tissue.

In this study, it was shown that the mini-perfusion bioreactor could induce more efficient spermatogenesis than the agarose gel method when loaded with mouse testis tissue and that this effect was consistent for 8 weeks. We had hypothesised that a dynamic culture system would adequately supply the tissue with oxygen and nutrients through the effective exchange of molecules in culture medium streaming across the tissue surface (28). Throughout the 8 weeks of culture on agarose gel, spermatogenesis was maintained only in the peripheral parts of the tissue. We suggest that, apart from the effective transfer of molecules between testis tissue and the culture medium, the tissues inside the mini-perfusion bioreactor were supplied with a greater amount of oxygen through the PDMS. If so, this would reduce oxygen toxicity in comparison with direct exposure (29-31).

It is also possible that the tissue chamber of the mini-perfusion bioreactor might also help to replicate the chemical environment of the body. For example, the porous membrane that disconnects the tissue chamber from the streaming medium, should increase the retention of the secreted molecules in the chamber. It is important to maintain efficient exchange and balance of molecules between the tissue and the medium (32). A dynamic system will achieve such a balance more easily than a static system, which is why it was suggested that the mini-perfusion bioreactor we used better fulfills this requirement compared with the agarose gel method.

The findings suggest that the mini-perfusion bioreactor can promote differentiation up to the stage of post-meiotic spermatozoa. It was concluded that the mini-perfusion bioreactor may be useful for developing a dynamic culture system for the maturation of premeiotic mouse germ cells to post-meiotic levels as well as morphologically normal spermatozoa. Such findings are consistent with those of Komeya et al. (9, 33).

Molecular changes in germ cells are useful for stimulating spermatogenesis. Our real-time PCR analysis of specific markers (*Plzf*, *Tekt1* and *Tnp1*) after 8 weeks of culture in the bioreactor and agarose gel revealed the presence of premeiotic, meiotic and post-meiotic cells, respectively. *Tnp1* expression after 8 weeks was significantly lower in the agarose group than in the adult mouse testicular tissue but did not differ significantly between the bioreactor culture and adult mouse testicular tissue. The mini-perfusion bioreactor provided sufficient nutrients and oxygen for tissue culture. In the tissue cultured on agarose gel, tubules were not observed centrally, probably because of the hypoxic conditions and limited access to nutrients. In the agarose cultures, we observed necrotic and degenerative changes in the central parts of tissue. Therefore, expression was lower in the agarose group after 8 weeks of culture compared to the bioreactor and adult groups. Yokonishi et al. (34) reported the presence of spermatid cells and sperm, which resulted in the formation of embryos. Aflatoonian et al. (35) reported the successful *in vitro* production of post-meiotic spermatid cells. Alrahel et al. (36) reported the expression of the post-meiotic gene, *Tnp1*, but only at the molecular scale and not beyond meiosis.

Immunohistochemical analyses have shown that epithelial cells express PLZF, SCP3, and ACRBP proteins, which are exclusive to SSCs, spermatocytes and spermatozoa, respectively. Our immunofluorescence analysis of the tissues after 8 weeks of culture in the bioreactor and agarose gel on these specific markers (PLZF, SCP3, ACRBP) showed the presence of premeiotic, meiotic, and post-meiotic cells.

Our results are in line with those of Mohaqiq et al. (37). Immunohistochemical studies of Rahmani et al. (38) showed the expression of the premeiotic marker PLZF in SSCs and undifferentiated spermatogonia. The immunohistochemical analysis of Gharenaz et al. (39) verified that PLZF-positive cells (spermatogonial stem cells) and SYCP3-positive cells (spermatocytes) exist

in the seminiferous tubules. Also in the agarose gel cultures, no sperm were observed in the suspension produced by tissue dissociation. By contrast, after removal of tissue from the bioreactor and mechanical dissociation, sperm- like cells were observed, suggesting that maturation to elongated spermatids is stopped on the agarose gel culture.

We were able to improve the culture conditions for testis tissues using our bioreactor for tissue culture. Bioreactor systems may prove valuable for preventing ischemia and facilitating the long-term culture of testis tissue. However, further research is needed to investigate the effects of enriching the culture medium with different supplements and growth factors. In the bioreactor system we are able to culture the tissue pieces (size 1 mm^3) but in the microscale dynamic culture systems only the seminiferous tubules can be cultured in the microchannels. Also at the end of the culture period, tissue removal from the middle chamber of the bioreactor is easily performed, but the extraction of the seminiferous tubules from the microchannels of other devices seems more complicated and difficult (40). Our bioreactor device is very simple, easy to use or user friendly, and more economically feasible than existing ones. Bioreactors can be optimised further by adjusting parameters including tissue chamber dimensions (particularly height), PDMS wall thickness, medium flow speed, and membrane porosity, and pore size. Such optimizations and other improvements of the culture medium could pave the way for developing new organ culture methods in the future.

Conclusion

The culture of testis tissues was improved by using a mini-perfusion bioreactor. Future studies are needed to determine the optimal culture conditions, for example the speed of flow of the medium and size of the tissue chamber. Optimisation of the culture conditions, including the culture medium may help to improve the methods for organ cultivation.

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Authors' Contributions

Z.A.; Performed all laboratory procedures, data collection, evaluation, statistical analysis, and drafted the manuscript. M.M.; Participated in the study design, revised the manuscript, contributed extensively in interpretation of the data and the conclusion. N.B.; Performed advising also performed editing the final version of this paper. A.Gh.; Designed the bioreactor, contributed to data and statistical analysis, and interpretation of data. All authors read and approved the final manuscript.

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