
بسم الله الرحمن الرحيم

Spermatogonial stem cell In Vitro Transplantation as a Suitable Tool for In Vitro spermatogenesis

MANSOUREH MOVAHEDIN, PH.D.

PROFESSOR OF ANATOMICAL SCIENCES DEPT.

MEDICAL SCIENCES FACULTY

TARBIAT MODARES UNIVERSITY

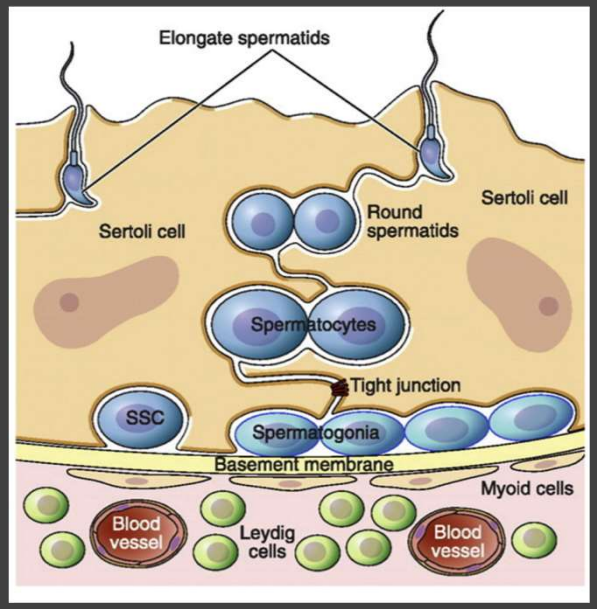
TEHRAN, IRAN

Male Infertility

Since the birth of the first child conceived via in vitro fertilization 40 years ago, fertility treatments and assisted reproductive technology have allowed many couples to reach their reproductive goals.

As of yet, no fertility options are available for men who cannot produce functional sperm, but many experimental therapies have demonstrated promising results in animal models.

SPERMATOGONIAL STEM CELLS AND SPERMATOGENESIS



Pelzman et al., Fertility and Sterility, 2020

In Vitro Spermatogenesis

The study of culturing spermatogonial stem cells (SSCs) dates back to the 1950s. However, regeneration of complete spermatogenesis process in vitro is still a greater challenge.

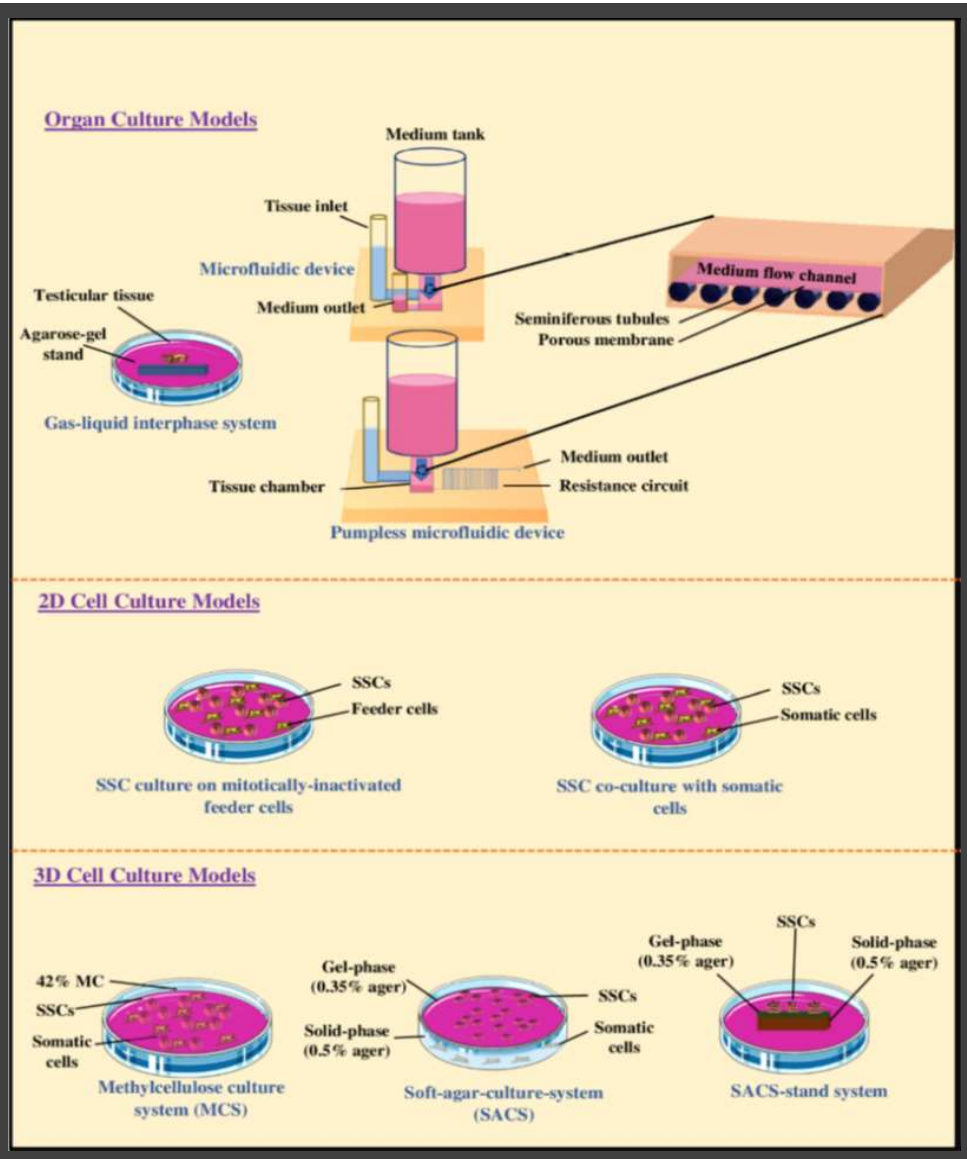
Studying spermatogenesis in vitro is significant in elucidating germ cell biology, and the knowledge may be useful for genetic manipulations of defective germ cells or producing transgenic animals, fertility preservation, and treatment of infertility.

In Vitro Models of SSC Differentiation

A- Organotypic Culture of Testicular Tissue Fragments

B- Two-Dimensional Culture of Testis Cell Suspensions

C- Three- -Dimensional Culture of Testis Cell Suspensions


Organ Culture Models
Medium tank
Tissue inlet
Microfluidic device
Medium outlet
Testicular tissue
Agarose-gel stand
Gas-liquid interphase system
Medium flow channel
Seminiferous tubules
Porous membrane
Medium outlet
Tissue chamber
Resistance circuit
Pumpless microfluidic device
2D Cell Culture Models
SSCs
Feeder cells
SSC culture on mitotically-inactivated feeder cells
SSCs
Somatic cells
SSC co-culture with somatic cells
3D Cell Culture Models
42% MC
SSCs
Somatic cells
Methylcellulose culture system (MCS)
Gel-phase (0.35% ager)
Solid-phase (0.5% ager)
SSCs
Somatic cells
Soft-agar-culture-system (SACS)
SSCs
Gel-phase (0.35% ager)
Solid-phase (0.5% ager)
SACS-stand system

Journal List › Iran Biomed J › v.22(4); 2018 Jul › PMC5949128

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Study of *Tnp1*, *Tekt1*, and *Plzf* Genes Expression During an *in vitro* Three-Dimensional Neonatal Male Mice Testis Culture

Ahmad Alrahel,[1] Mansoureh Movahedin,[1,*] Zohre Mazaheri,[1] and Fardin Amidi[2]

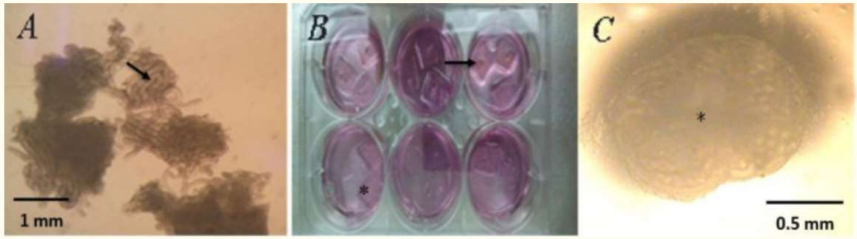

Fig. 1

Preparation of mouse neonatal testis. (A) Dissociation of the testis tissue into fragments (they were approximately 1 mm^3 in size when compacted) suitable for tissue culture. Arrow shows the seminiferous tubules. (B) Testes tissue pieces put on the agarose gel in three-dimensional organ culture method; agarose gel is shown by *, and fragment tissue is indicated by arrow. (C) Photomicrograph of tissue sections following a 12-week culture; the dark part in the center of tissue section represents limited access to nutrition (*).

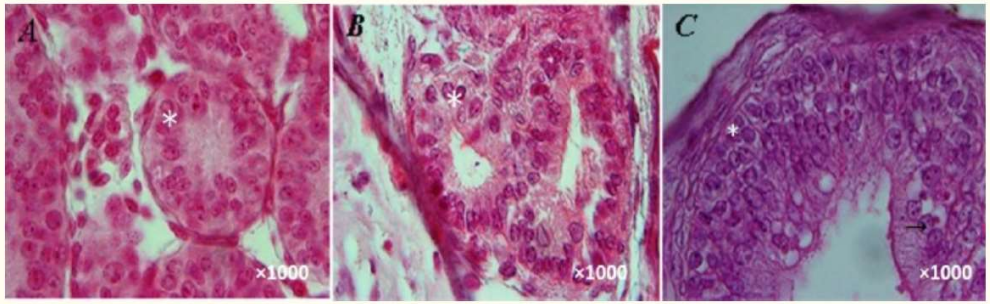

Fig. 2

Histological section of the testis tissue fragment cultured for (A) 8 weeks, (B) 10 weeks, and (C) 12 weeks. Stars and the arrow demonstrate spermatogonial cell and spermatocyte cell during culture, respectively.

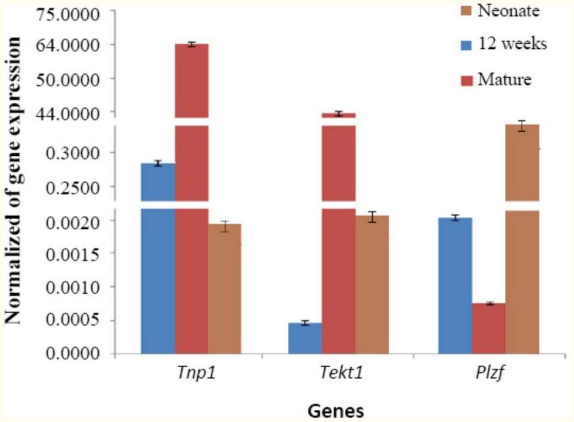

Fig. 3

Expression mitotic and meiotic genes *Tnp1*, *Tekt1*, and *Plzf*. 12 weeks, testis tissue fragment that cultured for 12 weeks; mature, testis tissue from mature mouse. Data were normalized to β-actin and represented mean ± SE after three times repeats. There are significant differences between the groups in each gene ($p < 0.05$).

Health Biotechnology and Biopharma (2018), 2(3): 24-35

Original Research Article

Investigation of slow freezing of human testicular tissue on proliferation, colonization and viability of human spermatogonial stem cells

Mahdi Mohaqiq[1,5], Mansoureh Movahedin[2]*, Zohreh Mazaheri[3], Naser Amirjannati[4], Ruhollah Roien[1,5]

[1]Department of Paraclinic, Faculty of Medicine, Kateb University, Kabul, Afghanistan
[2]Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[3]Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[4]Department of Andrology and Embryology, Reproductive Biotechnology Research Center, Avicenna Research Institute, Tehran, Iran
[5]Medical Research Center, Kateb University, Kabul, Afghanistan

***Corresponding author:** Mansoureh Movahedin, Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Email: movahed.m@modares.ac.ir*
DOI: 10.22034/HBB.2018.24

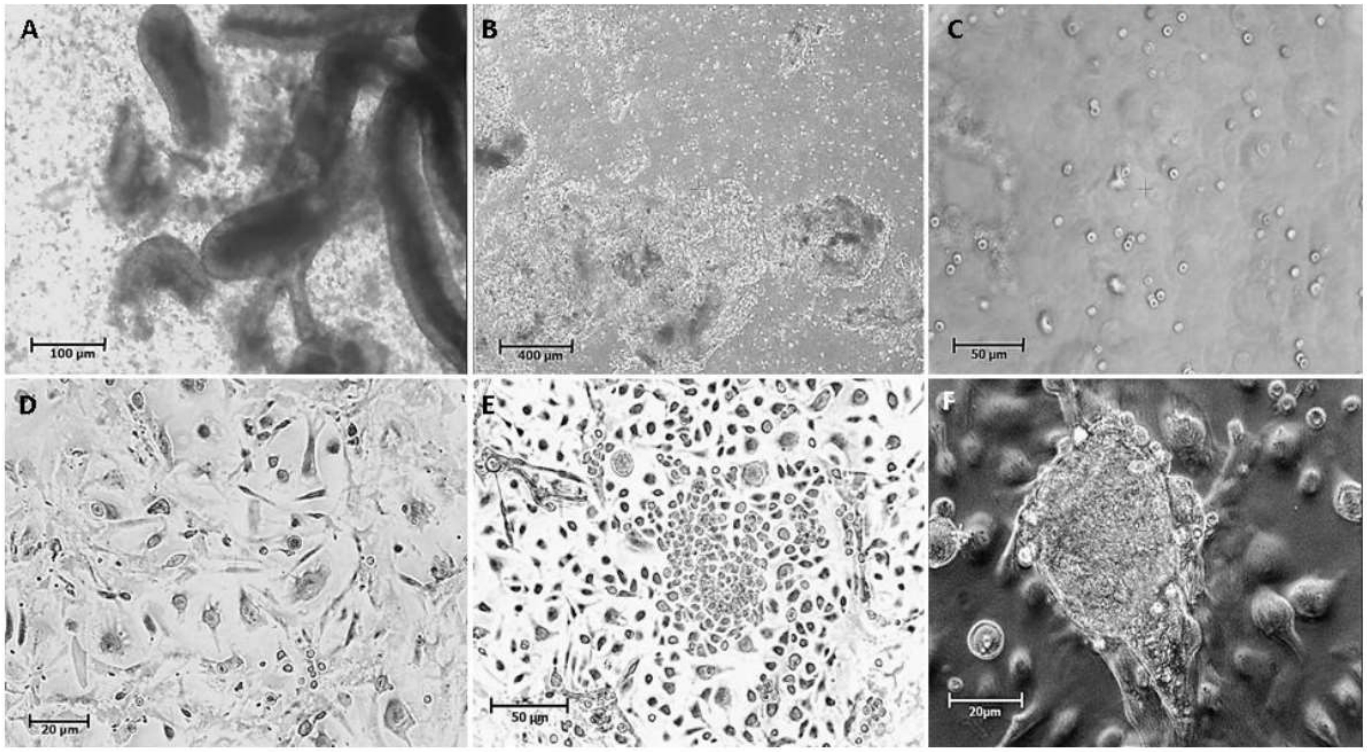

Figure 1. Human spermatogonial cell isolation process: interstitial tissue digestion [A], basal membrane digestion and residual interstitial tissues [B], cell suspension containing spermatogonial cell and sertoli cells obtained after two digestive step [C] progression cell division and cloning formation: spermatogonial and sertoli cells in co-culture conditions [D]. The spermatogonial cells make mitotic divisions close to each other [E]. Typical colony with a specific border [F].

Movahedin et al. *Slow freezing of testicular tissue*

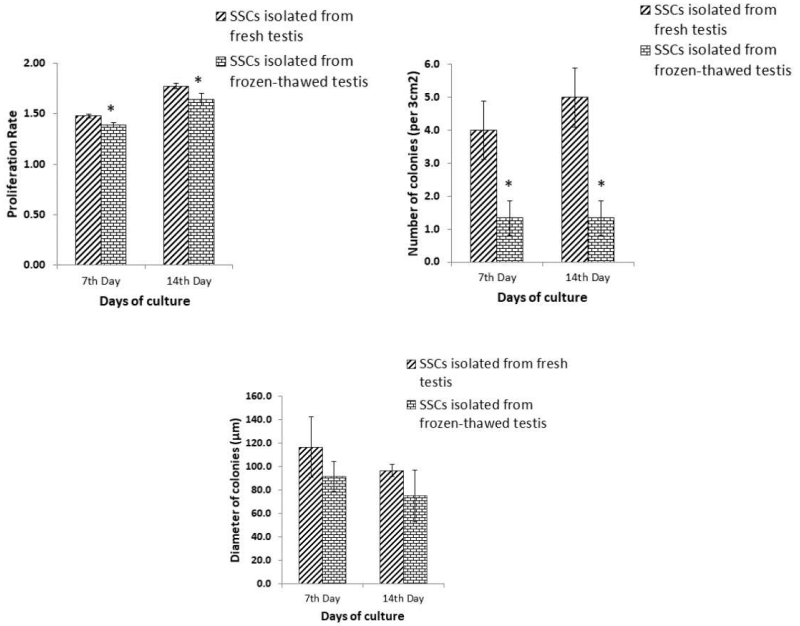

Figure 3. Comparison of proliferation rates on the 7th and 14th days of cell culture in different groups. Results are reported as mean ± standard deviation. Experiments were repeated at least 3 times for each group and comparison the diameter of colonies on the 7th and 14th day of cell culture in different groups Results are reported as mean ± standard deviation. Experiments were repeated at least 3 times for each group.

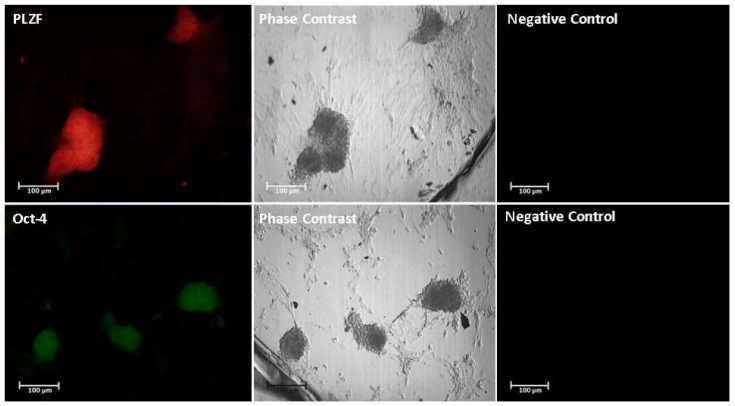

Figure 2. Confirmation of the nature of SSCs isolated from human testicular tissue. Expression of *PLZF* and *Oct-4* proteins in colonies derived from spermatogonial cells culture. Negative control is without primary antibodies.

Biological Research

BMC

Biol Res. 2019; 52: 16.

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PMCID: PMC6438003

PMID: 30917866

In vitro transplantation of spermatogonial stem cells isolated from human frozen–thawed testis tissue can induce spermatogenesis under 3-dimensional tissue culture conditions

Mahdi Mohaqiq,[1,4] Mansoureh Movahedin,[1] Zohreh Mazaheri,[2] and Naser Amirjannati[3]

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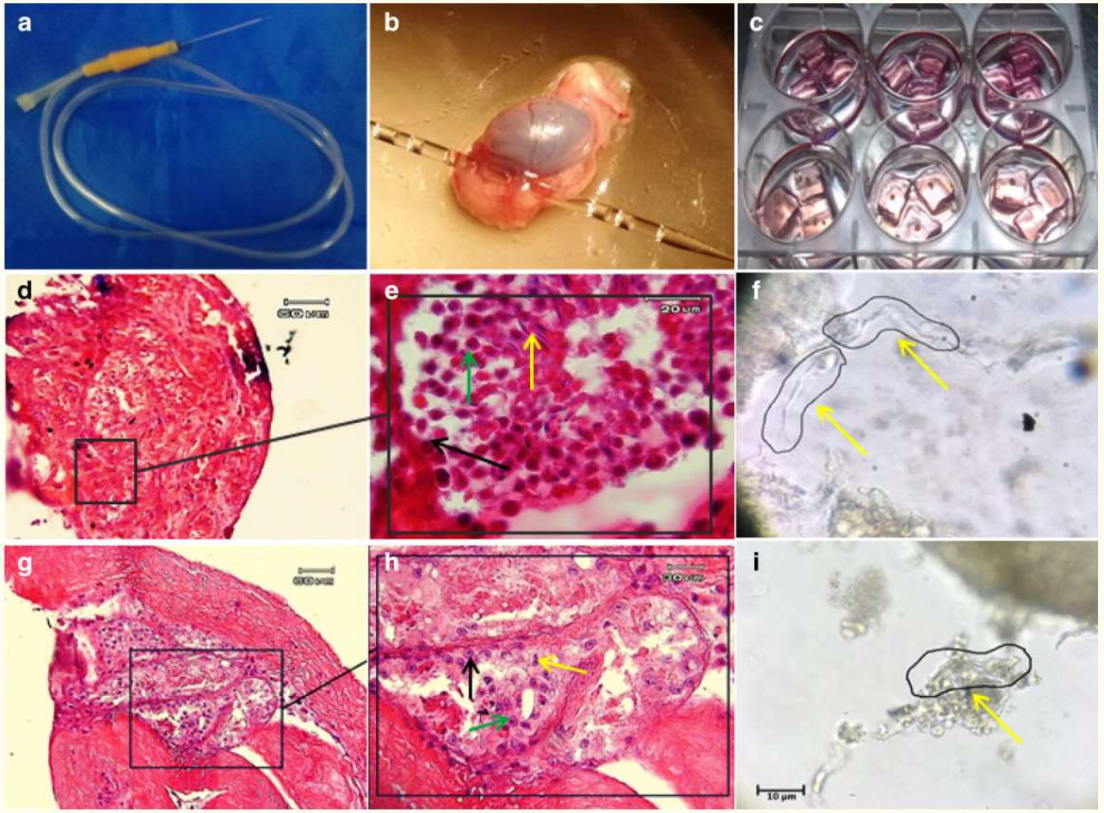

Fig. 2

Transplantation of SSCs to host testes and following in organ culture results. IVT of SSs to host testis and organ culture (**a**–**c**). H&E staining of tissue sections IVT group (**d**, **e**) and control group (**g**, **h**). Dynamic dissection of testis fragments after 8 weeks in IVT group (**f**) and control group (**i**). Black arrow: SCs, green arrow: spermatocyte and yellow arrow: long spermatid or sperm like cells

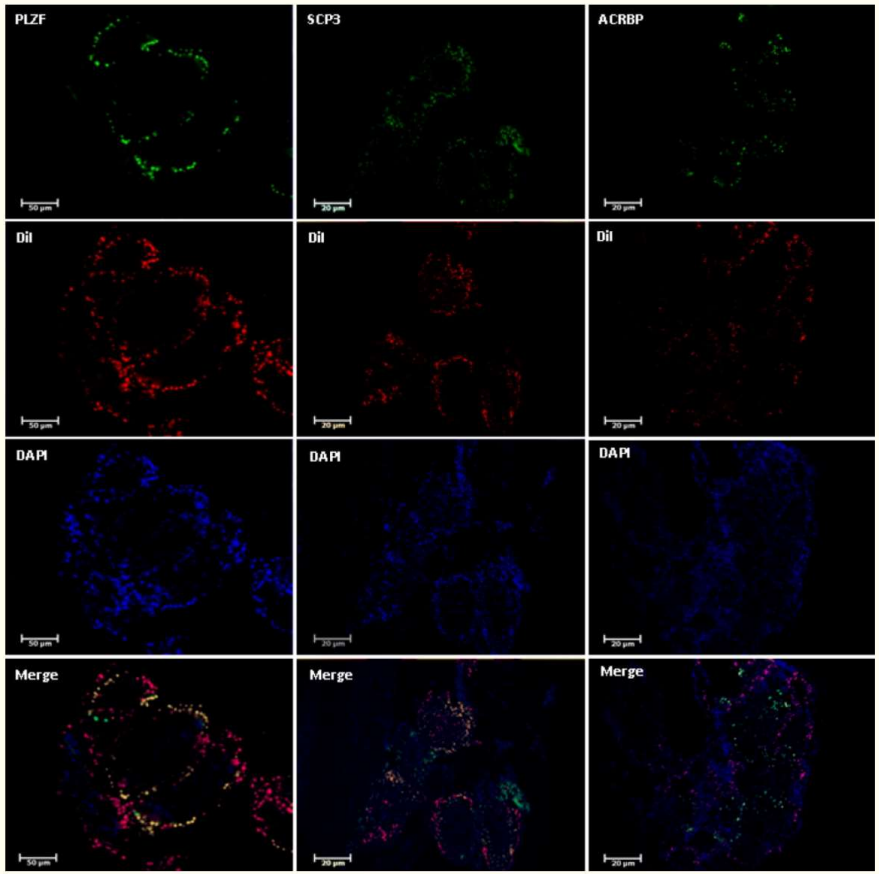

Fig. 3

Immunohistochemistry of host testes after transplantation and organ culture. Expression of specific proteins of spermatogonial cells (PLZF), spermatocytes (SCP3) and spermatozoa (ACRBP) and detection of DiI in host testes after 8 weeks of tissue culture

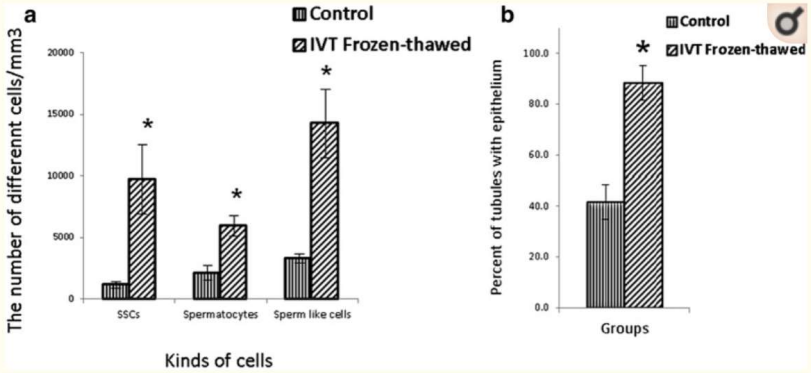

Chart 1

Results of histomorphometric studies in host testes. Number of different types of cells of seminiferous tubule epithelium (**a**) and percentage of seminiferous tubules containing epithelium (**b**) after 8 weeks of tissue culture in different groups. *Significant different with control group ($P < 0.05$)

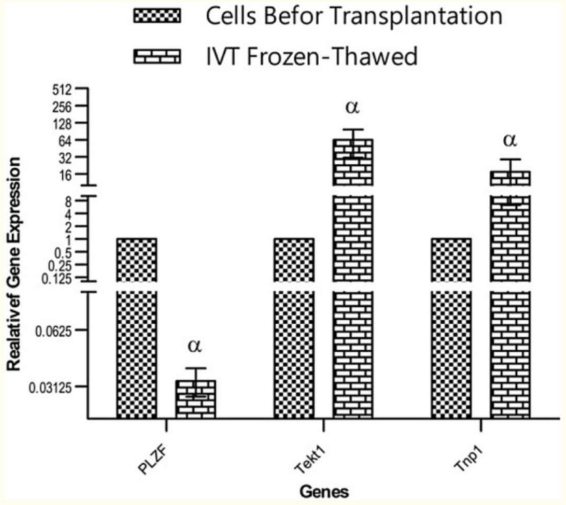

Chart 2

The relative expression of human specific SSCs gene in the host testes after 8 weeks of tissue culture.
$^{\alpha}$Significant different with other group in same gene ($P < 0.05$)

International Journal of Reproductive BioMedicine IJRM

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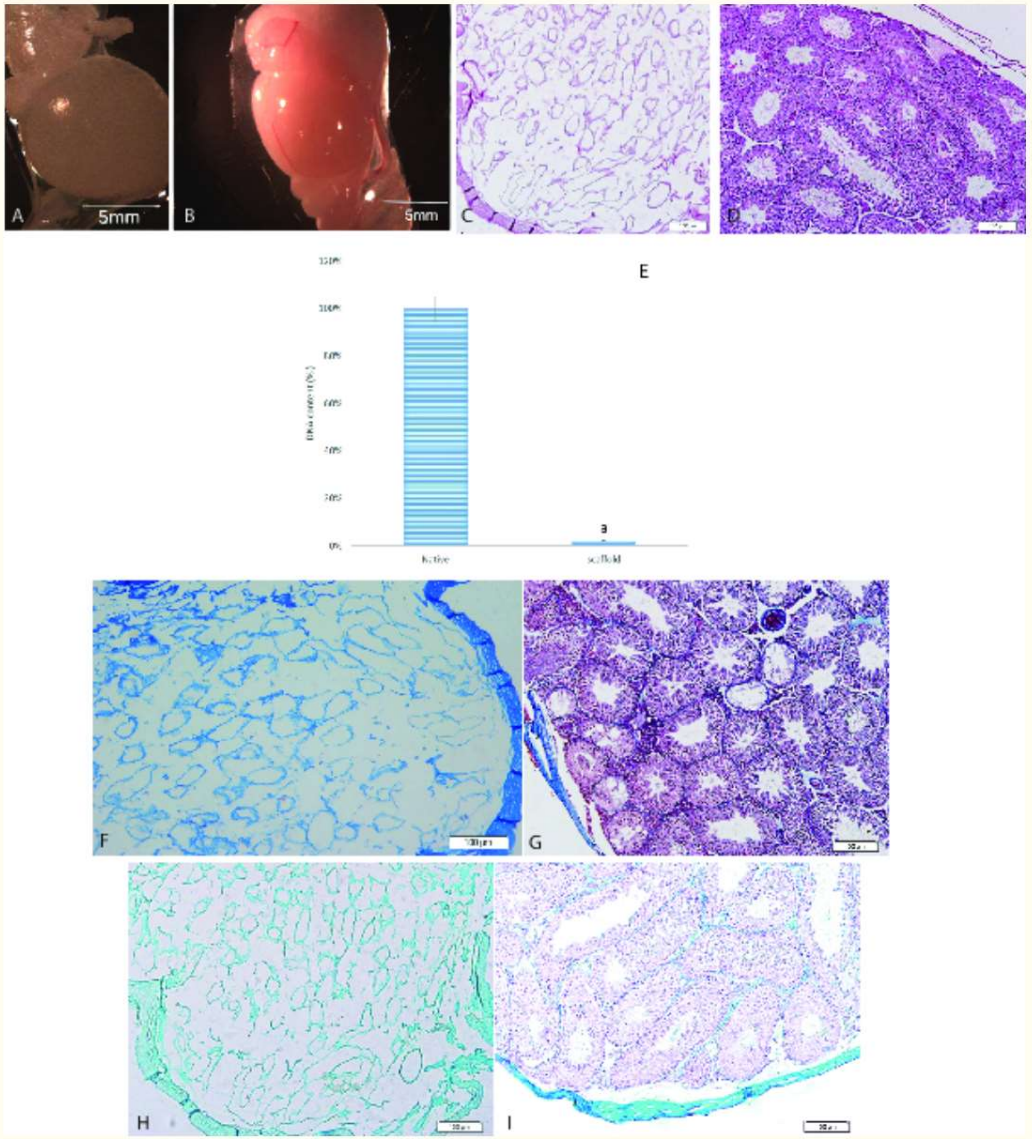
PMID: 33997591

Comparison of two methods for prolong storage of decellularized mouse whole testis for tissue engineering application: An experimental study

Nasrin Majidi Gharenaz, Ph.D., [1] Mansoureh Movahedin, Ph.D., [1] and Zohreh Mazaheri, Ph.D. [2]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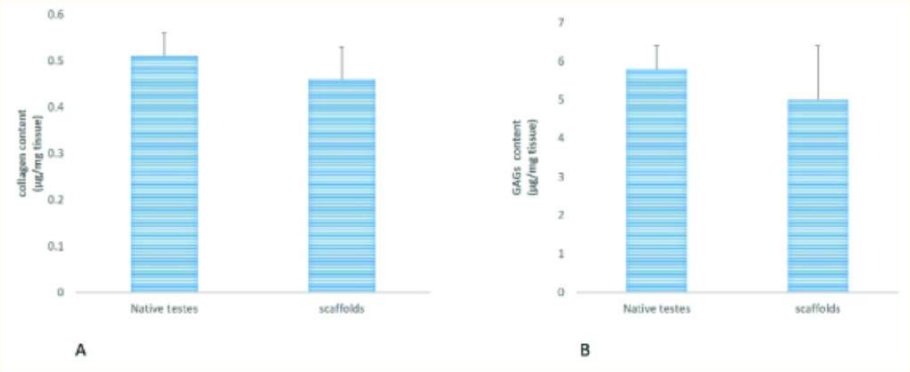


Figure 2

Quantification of collagen and GAGs content in native and scaffolds. Collagen in native and scaffolds was quantified using the Sircol assay (A). Quantification of GAGs in native and scaffolds using the Blyscan assay (B). Results are presented as Means ± SD ($p < 0.05$).

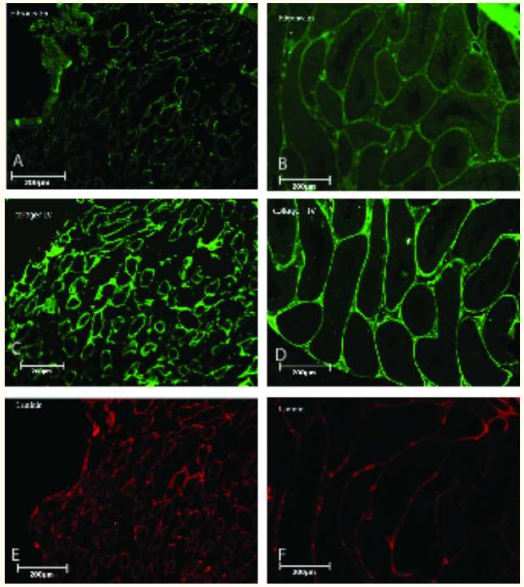


Figure 3

Immunohistochemical and ultrastructural analyses of testicular scaffolds and native testes. Representative images of fibronectin expression in scaffolds (A) and native testis (B), collagen IV expression in scaffolds (C) and native testis (D), and laminin expression in scaffolds (E) and native testis (F). Original magnification 100 ×.

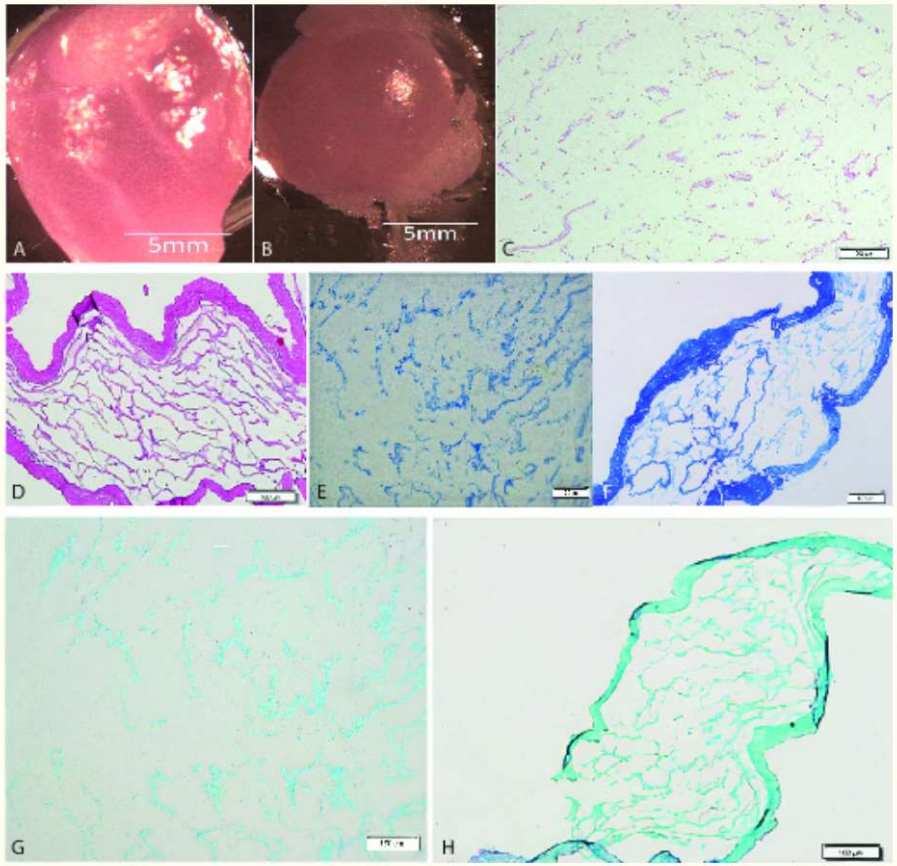
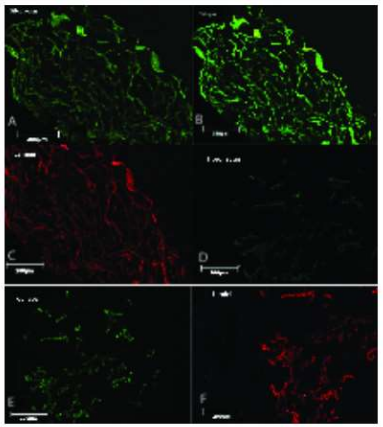


Figure 5

Characterization of testicular scaffolds after storage. Representative image of macroscopic appearance (A&B), H&E staining (C&D), Masson's trichrome staining (E&F), Alcian blue staining (G&H) of scaffolds after storage at 4°C or cryopreservation by slow freezing method, respectively. The images confirmed the superiority of the slow freezing method in the preservation of the ECM component. Original magnification 100 ×.



Immunohistochemical analyses of frozen-thawed scaffolds. Representative images of fibronectin, collagen IV, and laminin expression in frozen-thawed (A-C) and 4°C-stored scaffolds (D-F). Original magnification 100×.

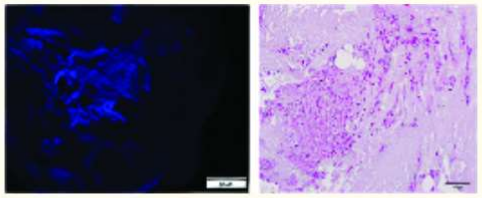


Figure 8

Evaluation of cell attachment to the frozen-thawed scaffold. Spermatogonial cells were seeded directly on the frozen-thawed scaffolds to study cell attachment. Cell culture was carried out for up to seven days. Spermatogonial-cell attachment and infiltration as seen after seven days by DAPI staining (A) and H&E staining (B). Original magnification 200×.

Journal List › Cell J › v.21(4); Winter 2020 › PMC6722448

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PMID: 31376322

Three-Dimensional Culture of Mouse Spermatogonial Stem Cells Using A Decellularised Testicular Scaffold

Nasrin Majidi Gharenaz, Ph.D.,[1] Mansoureh Movahedin, Ph.D.,[1,*] and Zohreh Mazaheri, Ph.D[2]

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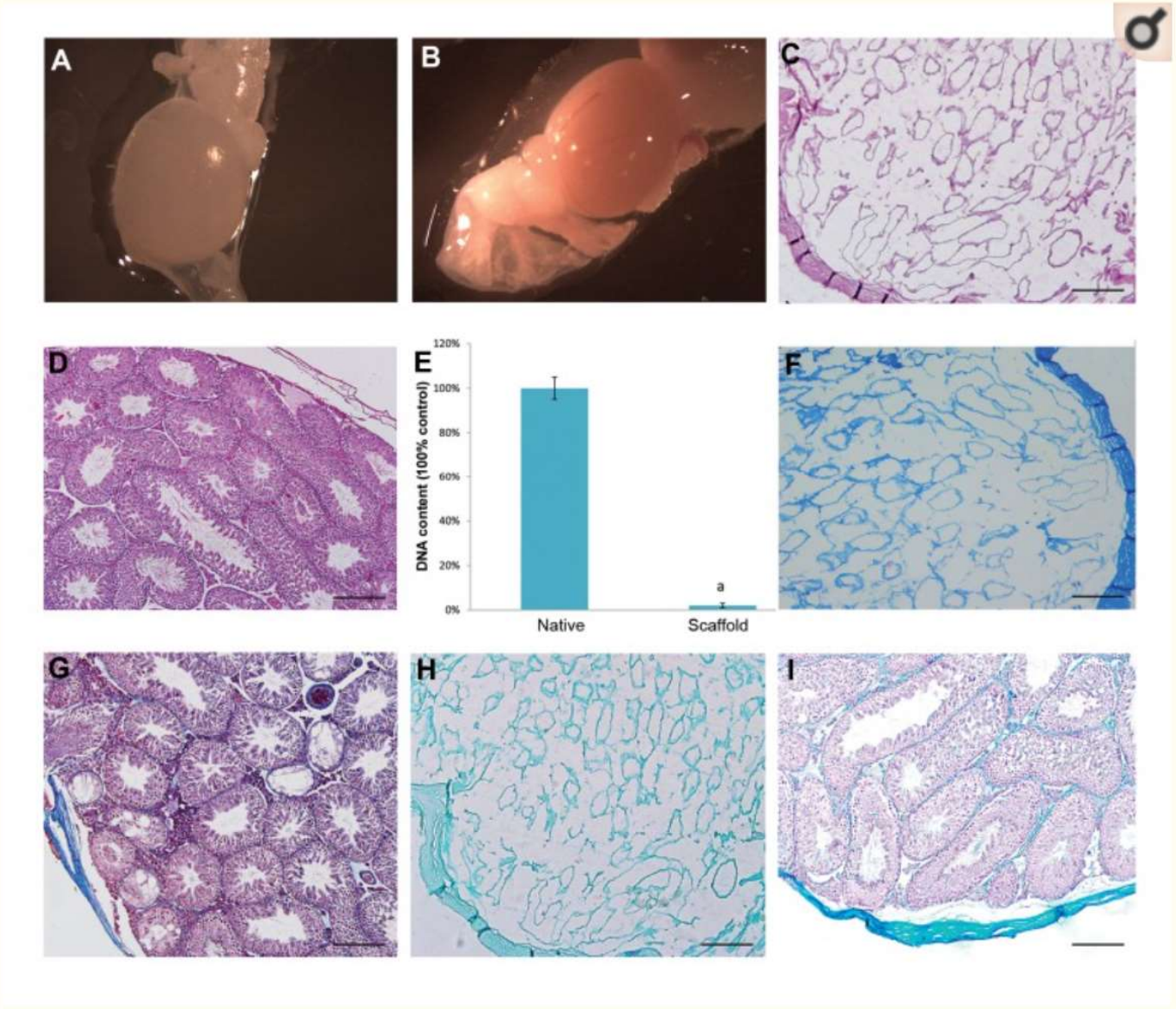
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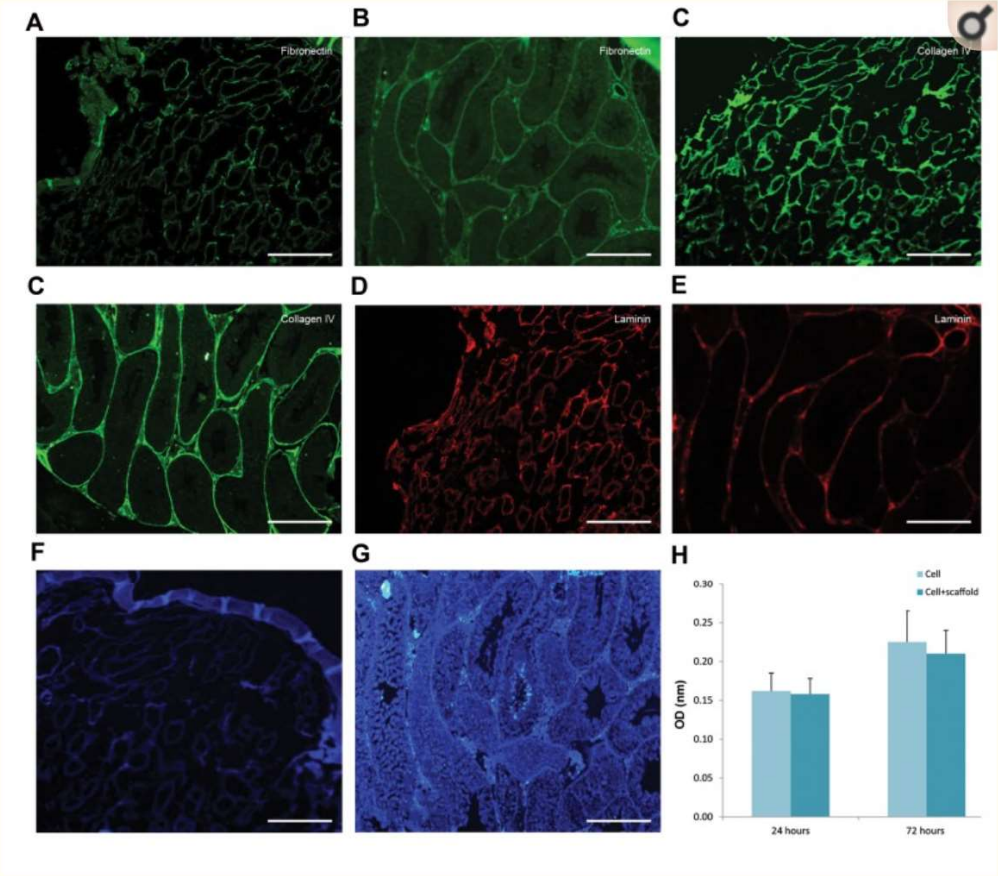


Fig.2

Protein and nucleic acid analyses of the decellularised scaffolds and intact testes. **A.** Representative images of fibronectin expression in decellularised scaffolds, **B.** Intact testis, **C.** Collagen IV expression in decellularised scaffolds, **D.** Intact testis, **E.** Laminin expression in decellularised scaffolds, **F.** Intact testis, **G.** DAPI staining of decellularised scaffolds, **H.** Intact testis, and **I.** Evaluation of scaffold cytocompatibility using MTT test did not show any significant difference in the optical density (OD) values, meaning that the cells proliferated at a rate similar to that of the controls (scale bar: 100 μm).

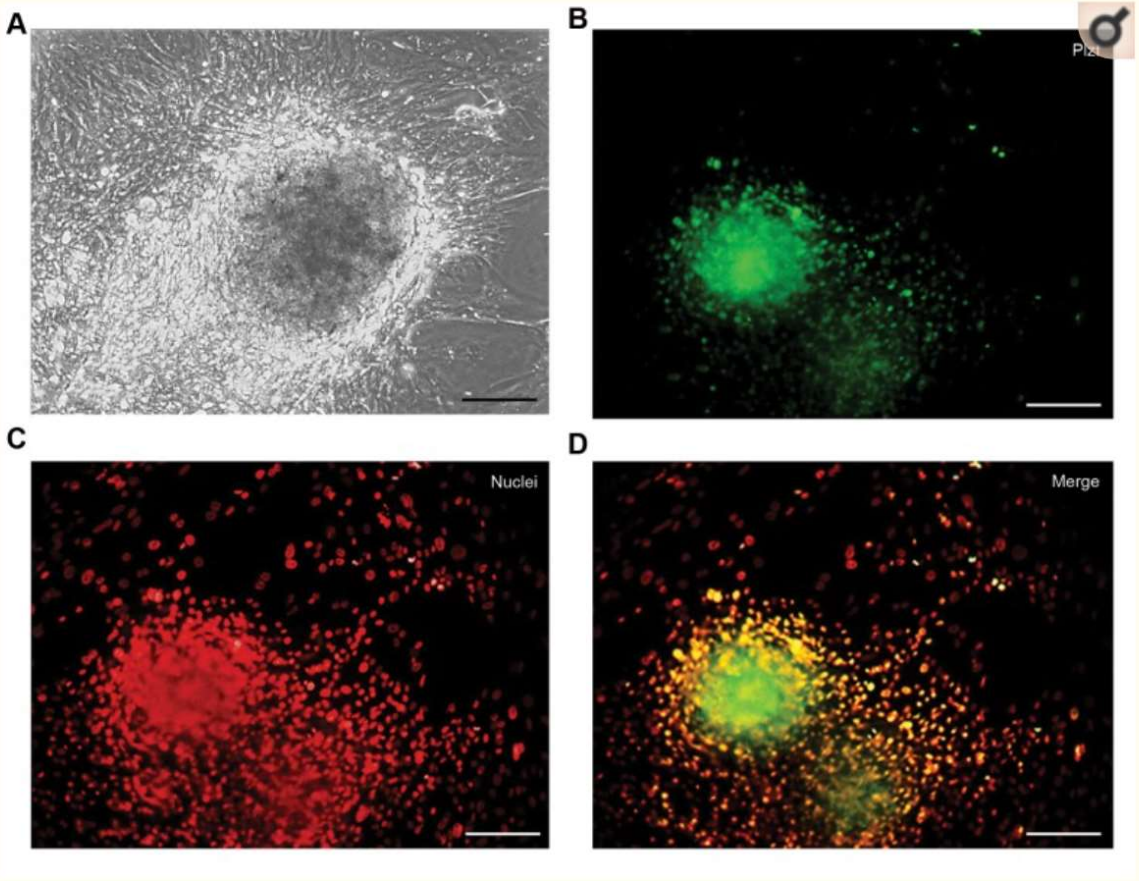


Fig.3

Characterization of spermatogonial stem cells harvested from neonatal mouse testes. **A.** Phase contrast images of spermatogonial stem cell colonies after two weeks of culture, and **B-D.** IHC staining of spermatogonial stem cell colonies with PLZF marker. Cell nuclei were stained by propidium iodide (PI) (scale bar: 30 µm).

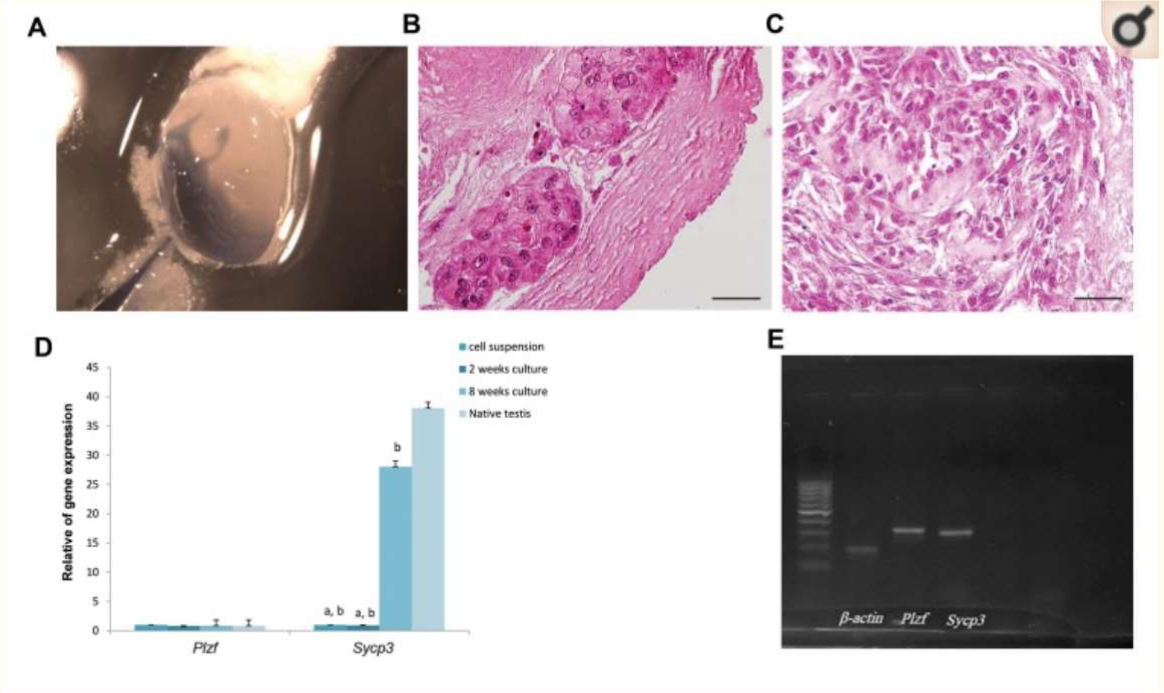


Fig.4

Characterization of cell injected scaffolds. **A.** Gross image of repopulated testicular scaffolds using in vitro transplantation (IVT) of spermatogonial stem cells, **B.** Haematoxylin-eosin images of the recellularized scaffolds after two weeks (scale bar: 20 µm), **C.** Eight weeks of culturing. Representative image of decellularised scaffolds without IVT after eight weeks in culture (scale bar: 20 µm), **D.** Relative gene expression of recellularized scaffolds after two and eight weeks of culture, and **E.** Bands of *Plzf* and *Sycp3* genes, and *β-actin* gene as the housekeeping control were obtained by real-time polymerase chain reaction (PCR). a; Indicated significant difference with samples cultured for eight weeks and b; Indicated significant difference with intact testis.

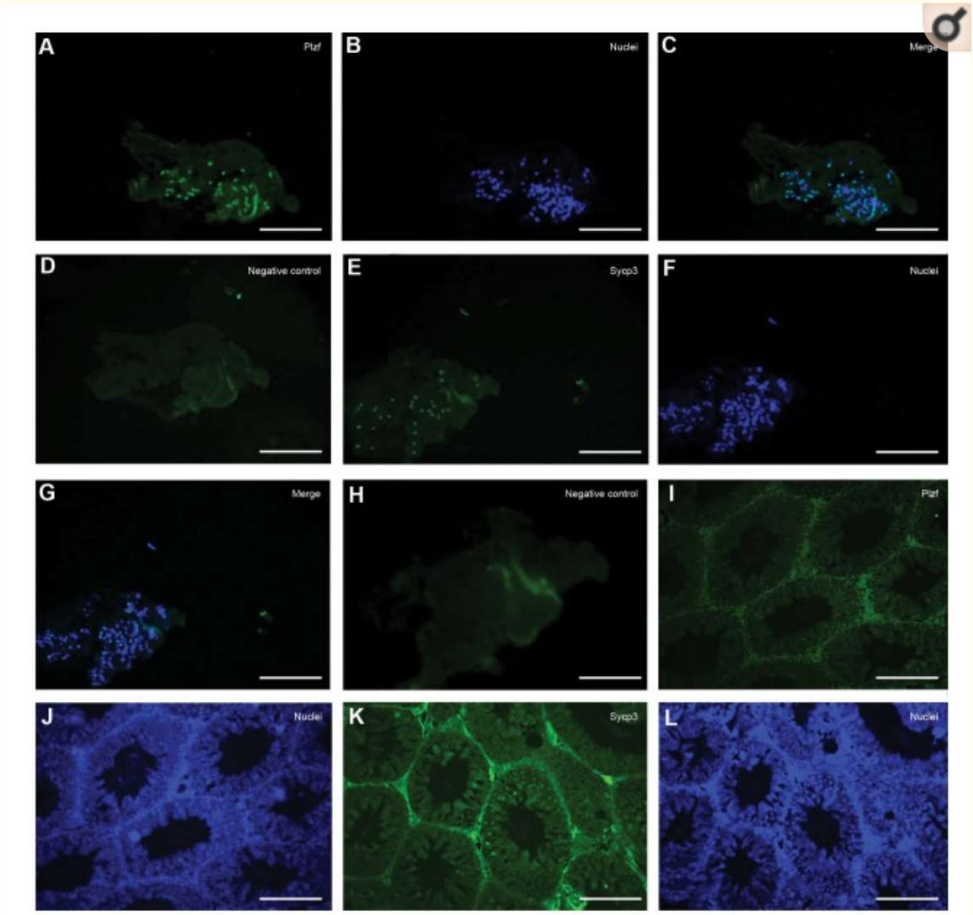
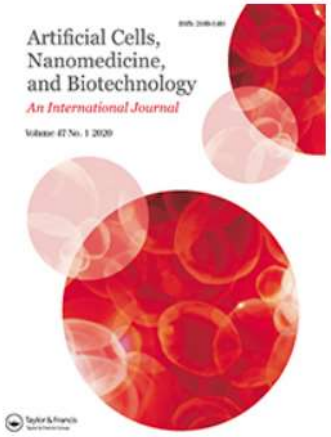


Fig.5

Immunohistochemistry (IHC) images of the cell-injected scaffolds and intact testes. **A-C.** IHC staining showed PLZF-positive cells in scaffolds cultured for eight weeks, **D.** Negative control of PLZF, **E-G.** SYCP3-positive cells in scaffolds cultured for eight weeks, **H.** Negative control of SYCP3, I, J. Positive control of PLZF, **K,** and **L.** SYCP3 in adult testis (scale bar: 50 μm).



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Transplantation of mouse iPSCs into testis of azoospermic mouse model: *in vivo* and *in vitro* study

Forouzan Rahmani, Mansoureh Movahedin, Zohreh Mazaheri & Masoud Soleimani

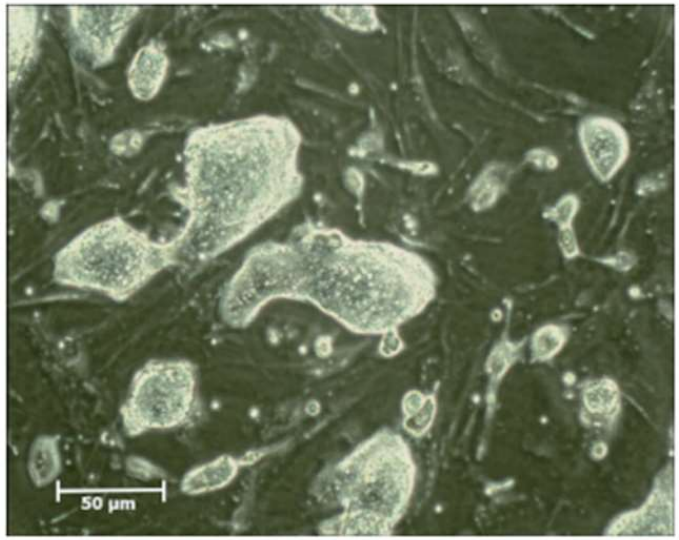


Figure 1. Mouse iPSCs colonies. Morphology of mouse iPSCs colonies after 2 days of culture on a layer of inactivated MEFs.

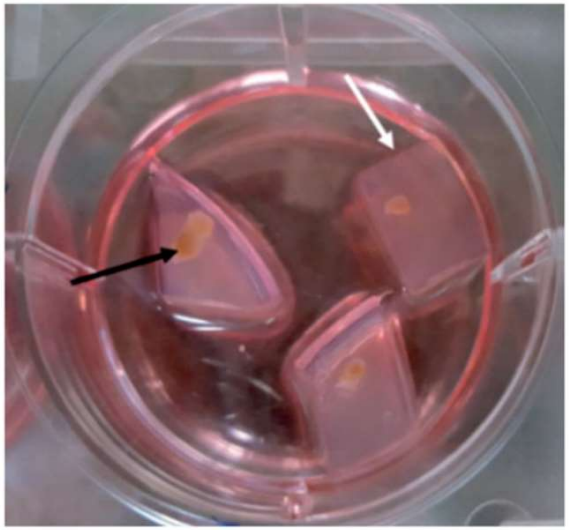


Figure 3. Preparation of agarose gel stand and 3D organ culture. The prepared gel agarose was cut into pieces of 10 mm × 10 mm × 5 mm in size. Three or four pieces of agarose gel were placed in each well of 6 wells plate and were soaked with the medium. The testis was fragmented into ~1 cm × 1 cm × 1 cm pieces to be appropriate for organ culture. Testes fragments were put on the agarose gel in the three-dimensional organ culture method. Agarose gel is shown by the white arrow, and fragmented testis is pointed by the black arrow.

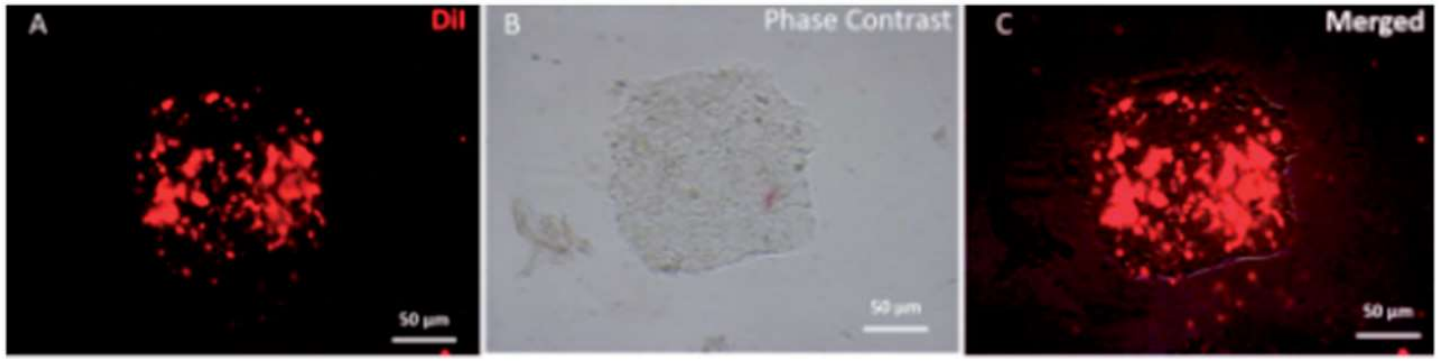


Figure 2. DiI labelled iPSCs colony Fluorescence image of iPSCs colony that labelled by DiI (A) Phase-contrast microscopy of iPSCs colony morphology. (B) Merged image of iPSCs and DiI labelled colony (C).

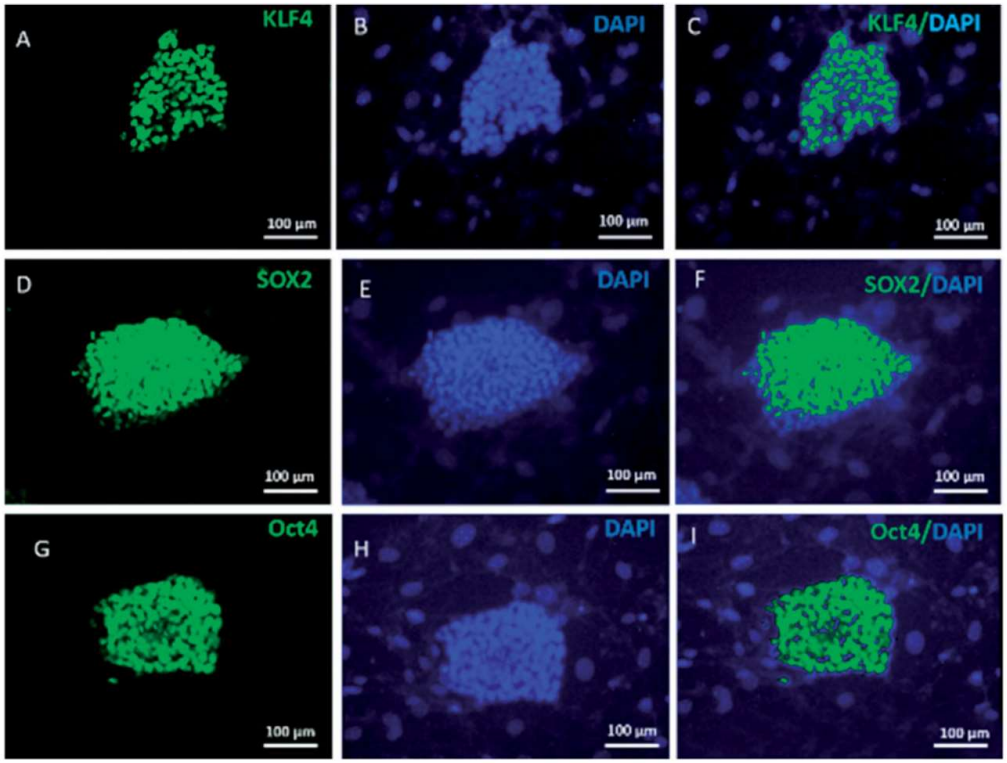


Figure 5. Mouse iPSCs Characterization. Mouse iPSCs were characterized by immunofluorescence staining and specific pluripotent antibodies including Oct4, Sox2 and Klf4. iPSCs were strongly positive for pluripotent stem cell markers Oct-4, Sox2 and Klf4 (green) (A, D, G). Nuclei were stained blue with DAPI. (B, E, H). The merged picture of iPSCs colony with positive pluripotent stem cell markers and DAPI staining (C, F, I).

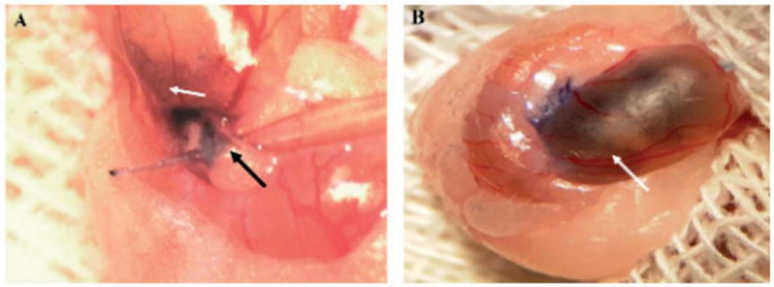


Figure 4. Cells transplantation into seminiferous tubules. The cell suspension was injected into seminiferous tubules through the efferent duct using a fine micro-injection needle (A). ~70–90% of seminiferous tubules were filled in each recipient testis. (B) Seminiferous tubules are shown by the white arrow, and the efferent duct is indicated by black arrow.

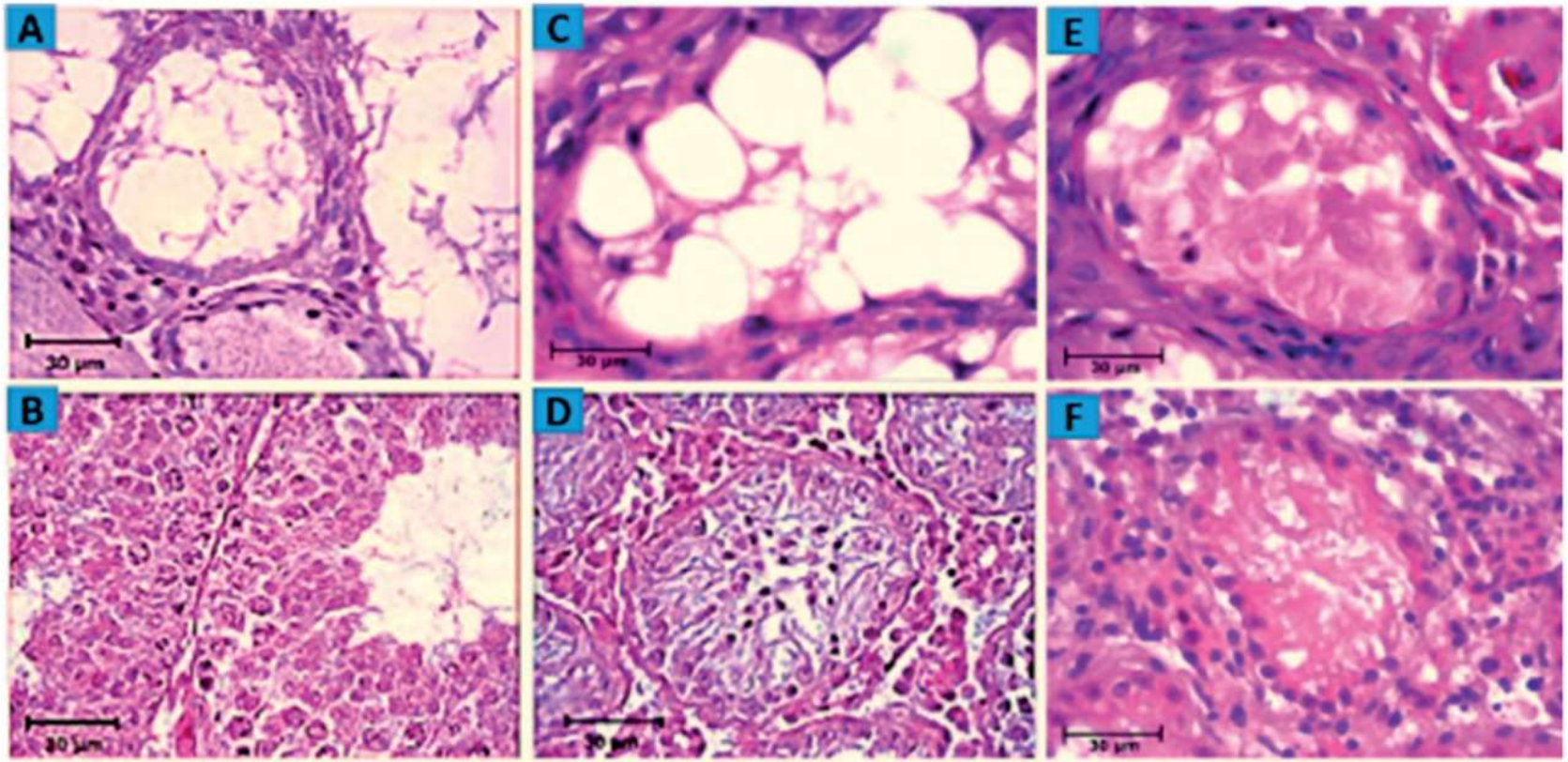


Figure 7. Assessment of morphological changes. H&E staining showing the morphology of mouse testes after 4 weeks of 40 mg/kg busulfan treatment most seminiferous tubules are depleted and no spermatogenetic activity. (A) Wild type mouse testis without busulfan treatment, seminiferous tubules contain spermatogonia, spermatid and spermatozoa. (B) Host testis 2 weeks after iPSCs transplantation and morphological changes from *in vivo* studies. Restoration of spermatogenesis in seminiferous tubule following iPSCs transplantation is illustrated (C) compared to the control group (D). Histological section of testis fragment cultured for 2 weeks following iPSCs transplantation and 3D organ culture. Due to the cutting of seminiferous tubules, they are not seen regularly in the tissue section. The initiation of spermatogenesis process is visible in the seminiferous tubule of host testis fragments (E) in comparison to control testis (F).

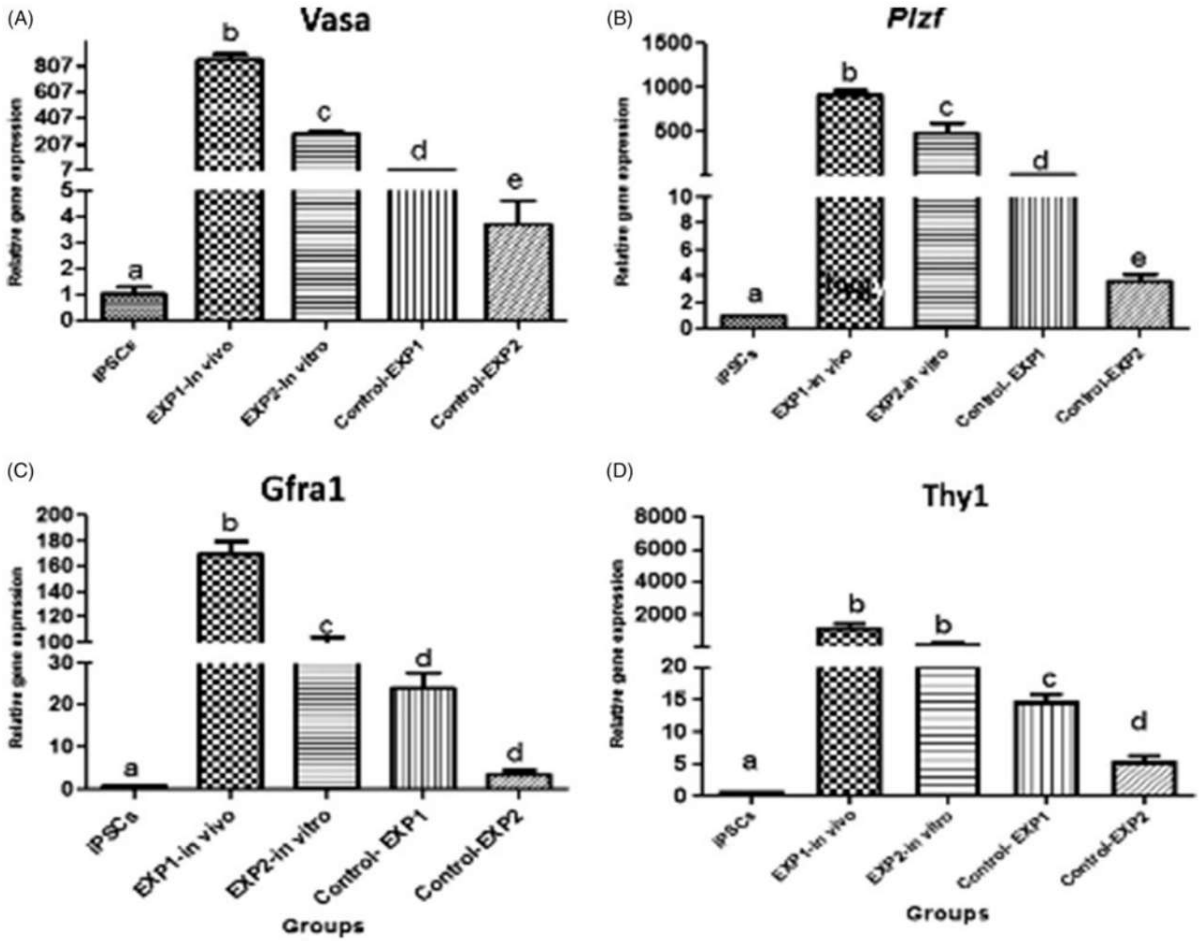


Figure 8. Relative gene expression profile of pre-meiotic *Plzf*, *Vasa*, *Thy1* and *Gfra1* by qRT-PCR. Gene expression of target genes in iPSCs before and 2 weeks after cells transplantation in experimental groups were evaluated by Real Time PCR (qPCR). Data were normalized againt β-actin and represented by mean ± SE after three times repeats. Target gene expressions in experimental groups were investigated in relation to genes expression in iPSCs before transplantation. There are Different letters indicating statistically significant differences between groups ($p < .05$).

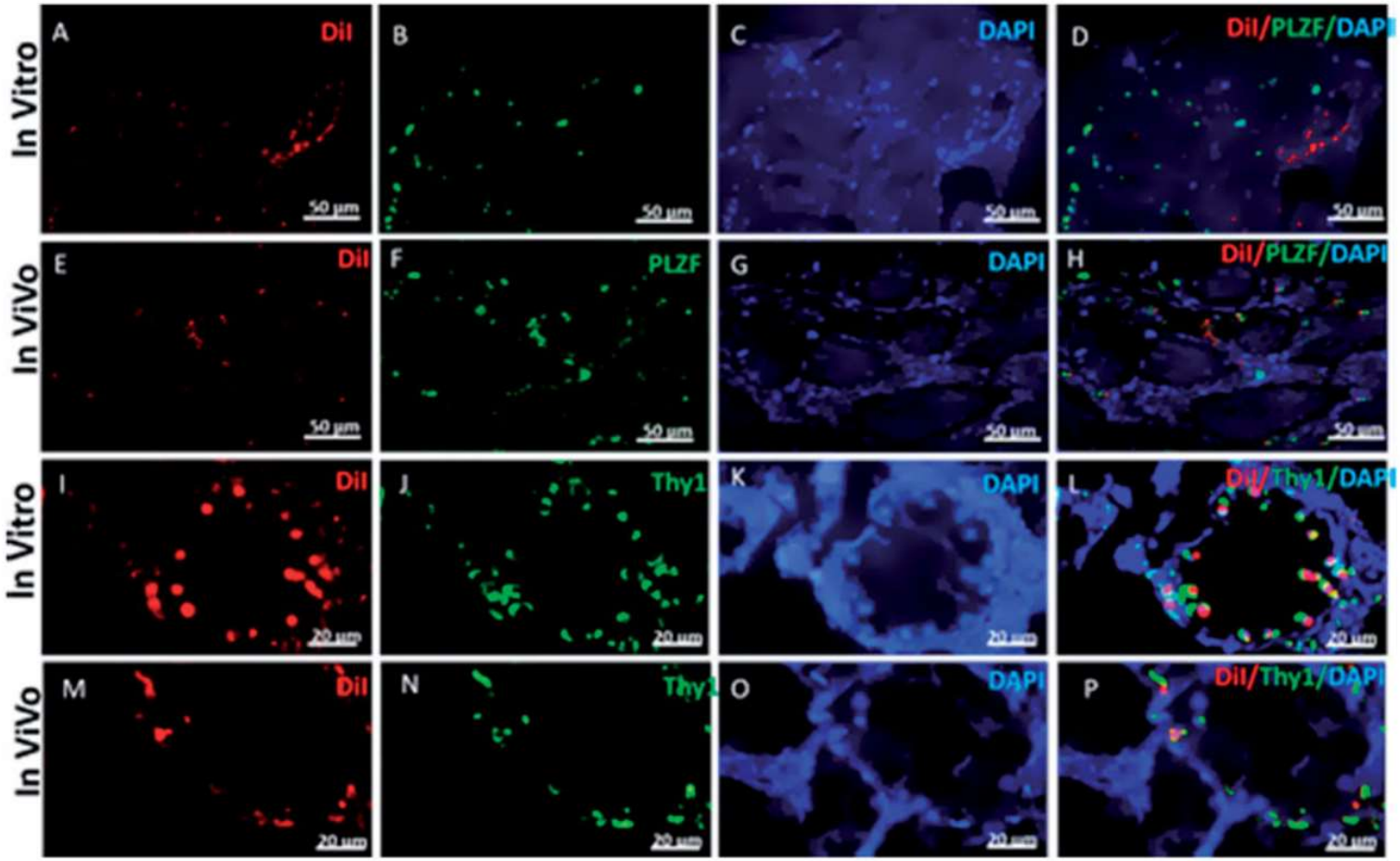
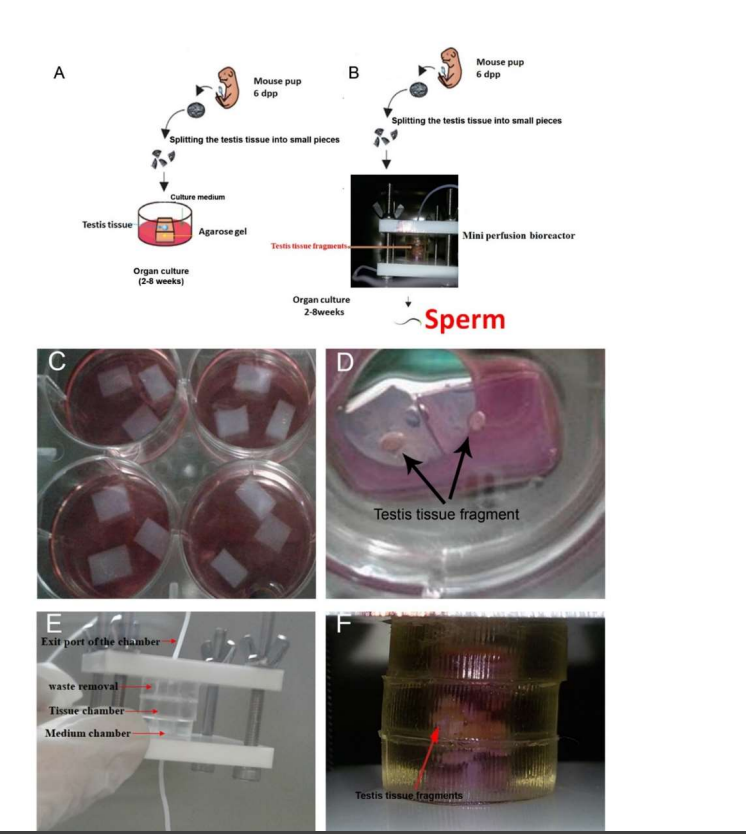
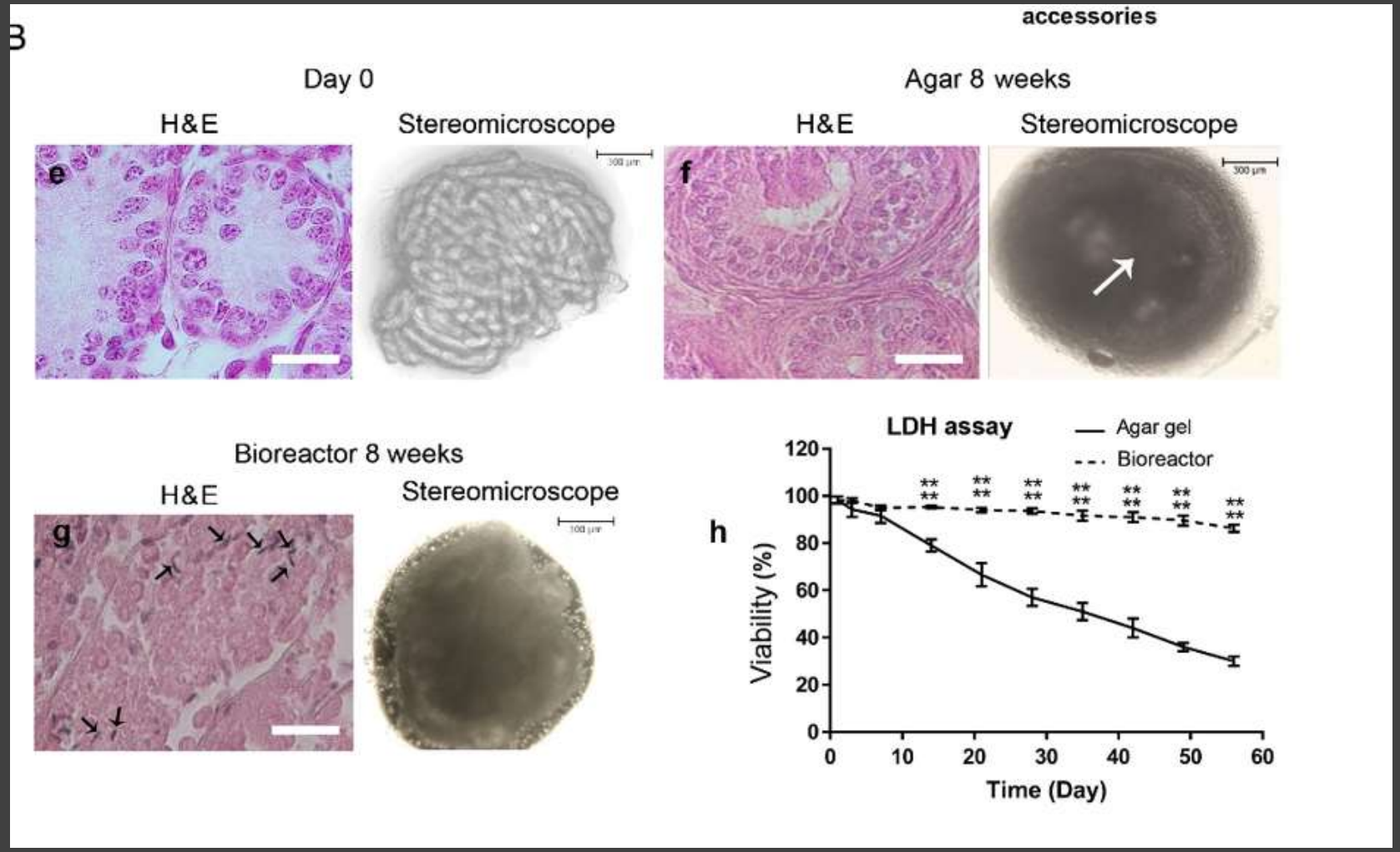


Figure 9. Representative immunohistostaining of SSCs markers in testis section 2 weeks after *in vivo* cells transplantation and 3D organ culture DiI fluorescent-labelled cells were traced in the recipient testes by fluorescent red light. Images (A, E, I and M) show that the DiI positive cells are localized at the base of seminiferous tubules and have gained homing. The host testis sections were immunostained by the antibodies for *Plzf* and *Thy1* (green) (B, F, J and N). DAPI was used for nuclei staining. (C, G, K and O). The co-localization *Plzf* and *Thy1*with DiI labelled iPSCs (D, H, L and P) around seminiferous tubules in recipient testes transplanted is shown by fluorescent microscope.

Mini bioreactor can support in vitro spermatogenesis of mouse testicular tissue

Zahra Amirkhani, Mansoureh Movahedin, Nafiseh Baheiraei, Ali Ghiaseddin.




B
accessories
Day 0
H&E
Stereomicroscope
e
Agar 8 weeks
H&E
Stereomicroscope
f
Bioreactor 8 weeks
H&E
Stereomicroscope
g
h
LDH assay
Agar gel
Bioreactor
Viability (%)
Time (Day)
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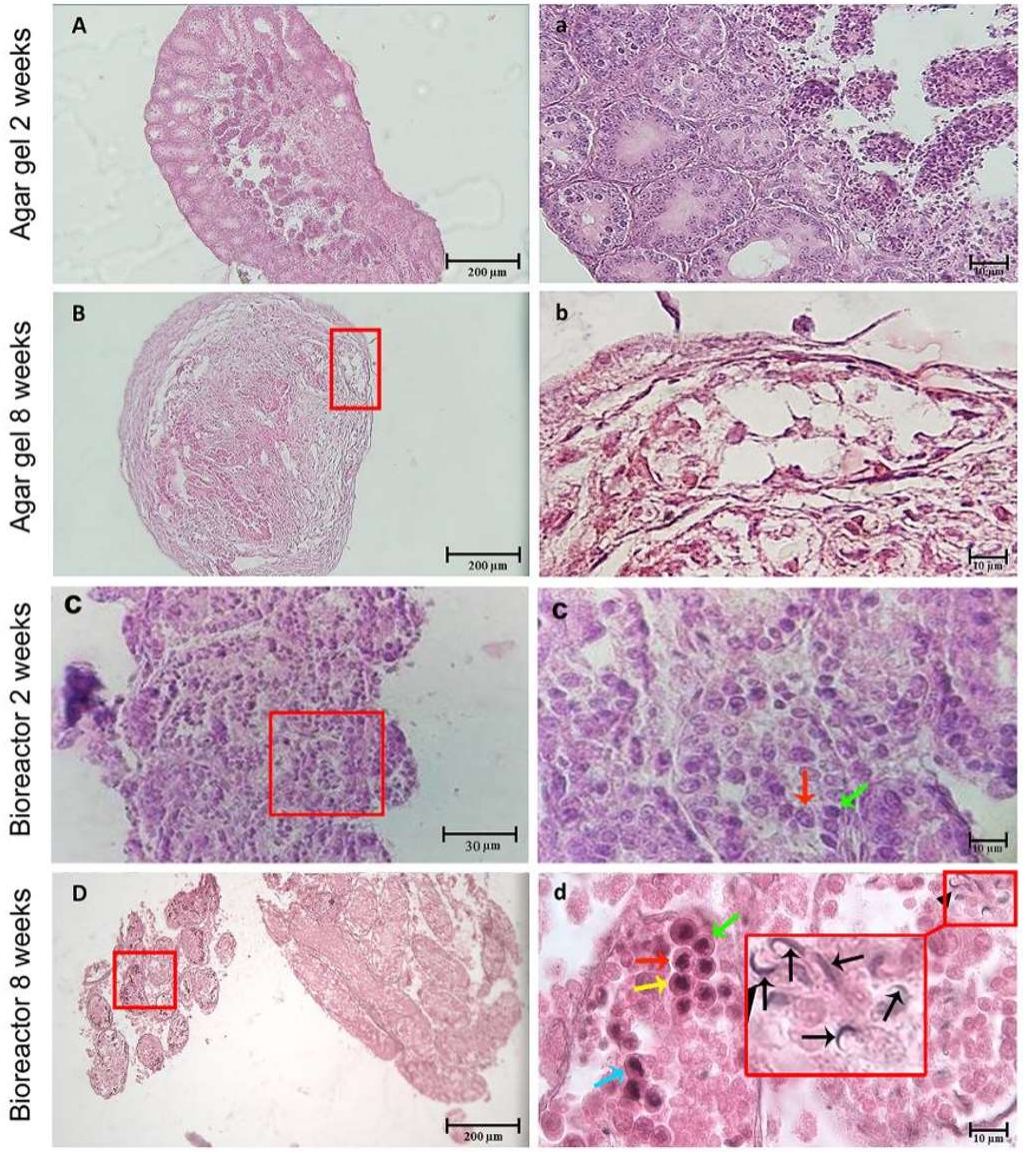
20
40
60
80
100
120
0
10
20
30
40
50
60


Agar gel 2 weeks
Agar gel 8 weeks
Bioreactor 2 weeks
Bioreactor 8 weeks
A
a
B
b
C
C
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d
200 µm
200 µm
30 µm
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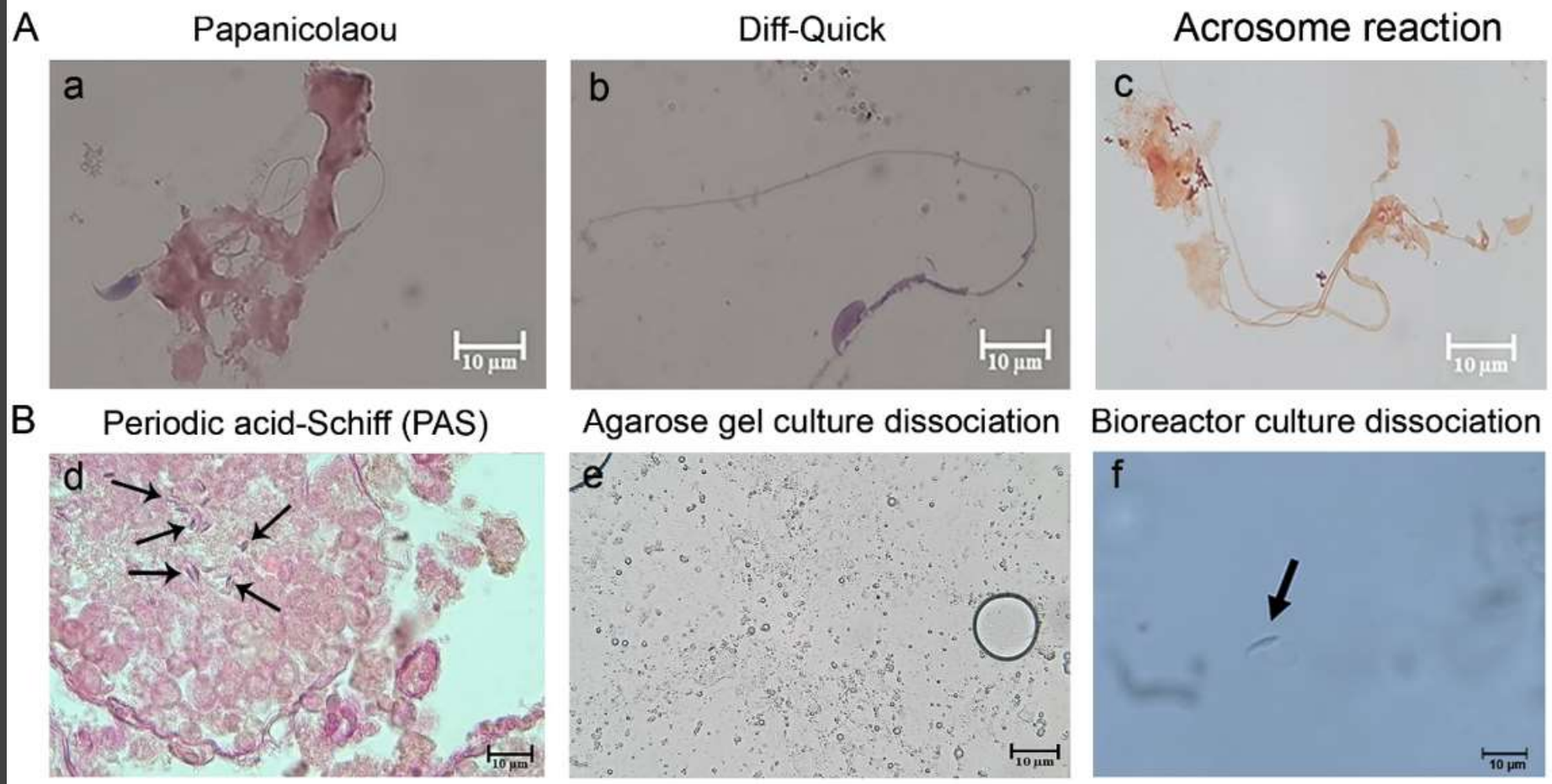
10 µm
10 µm
10 µm


A
Papanicolaou
Diff-Quick
Acrosome reaction
a
b
c
10 µm
10 µm
10 µm
B
Periodic acid-Schiff (PAS)
Agarose gel culture dissociation
Bioreactor culture dissociation
d
e
f
10 µm
10 µm
10 µm

Spermatogenesis of ex vivo transplantation of mouse spermatogonial stem cells to the harvested testis of azoospermia mouse model in mini-Perfusion bioreactor

Zahra Amirkhani, Mansoureh Movahedin, Nafiseh Baheiraei, Ali Ghiaseddin, Neda Dadgar.

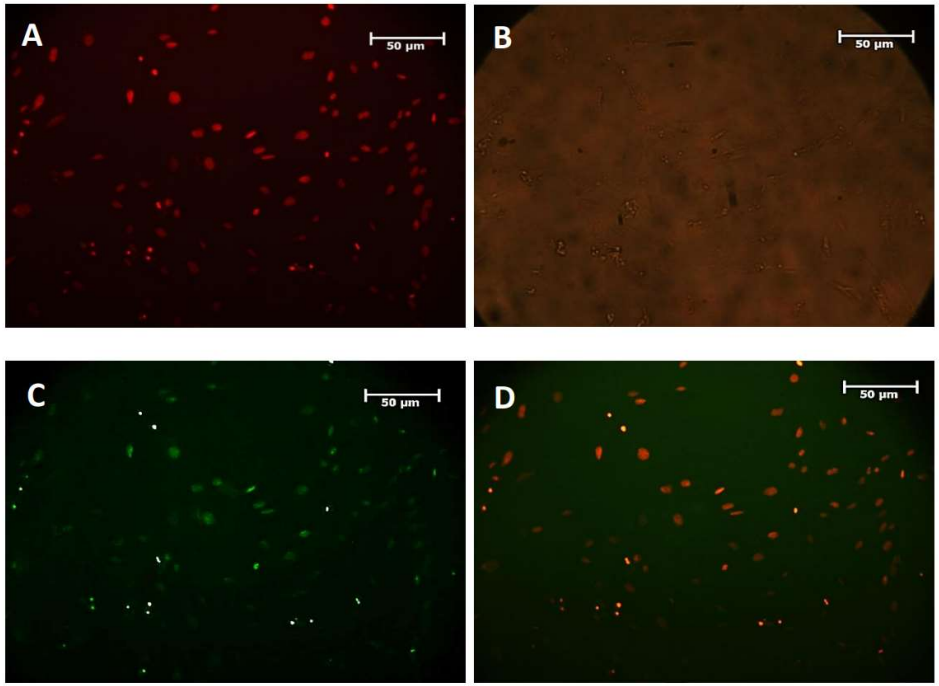
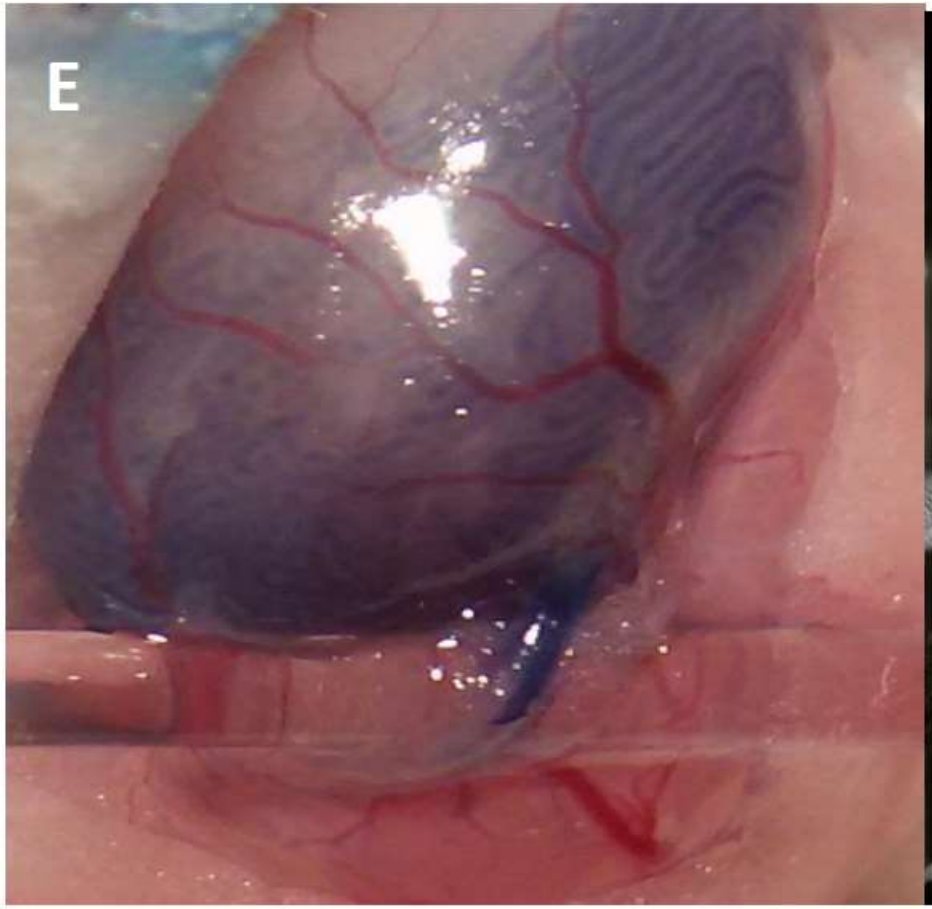
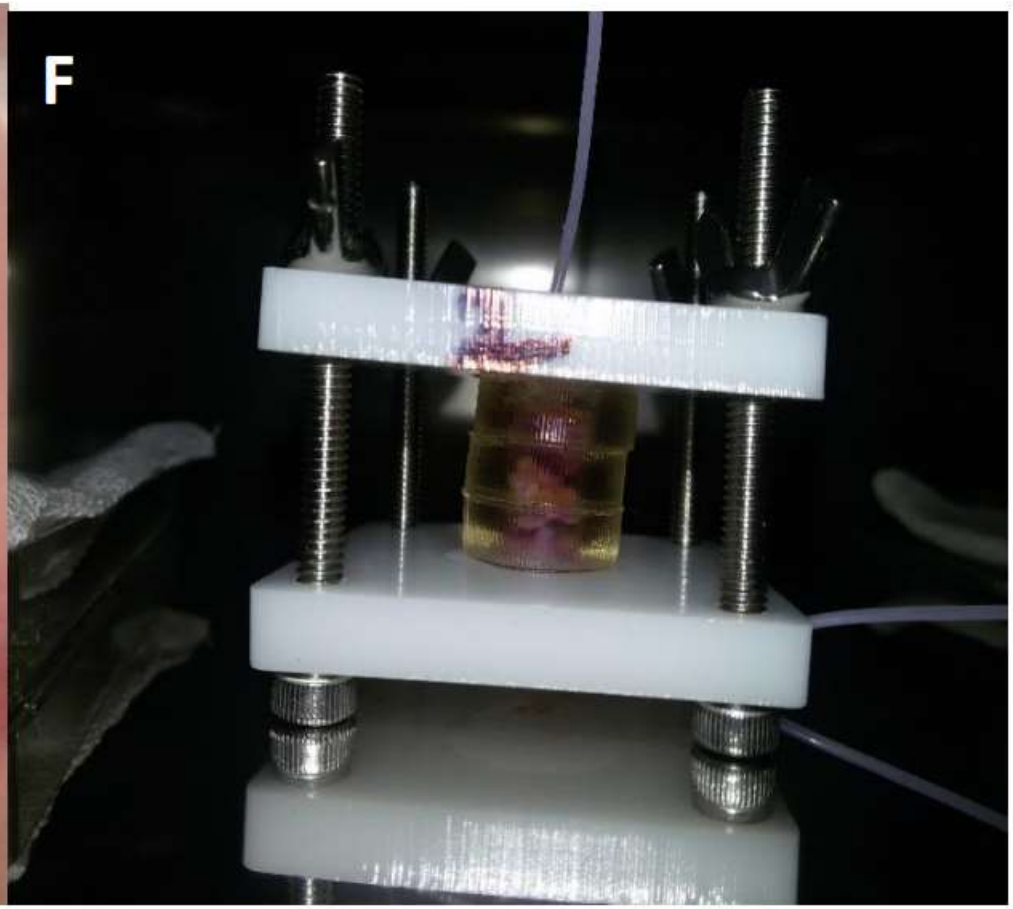


Fig 1. Confirmation of the nature of spermatogonia stem cells isolated from mouse testicular tissue. The nuclei of the cells are stained with PI dye (A). Phase contrast image (B). Expression of PLZF protein in spermatogonia cells (C). The nuclei images and the marker expression examined in Figure D are merged together.


E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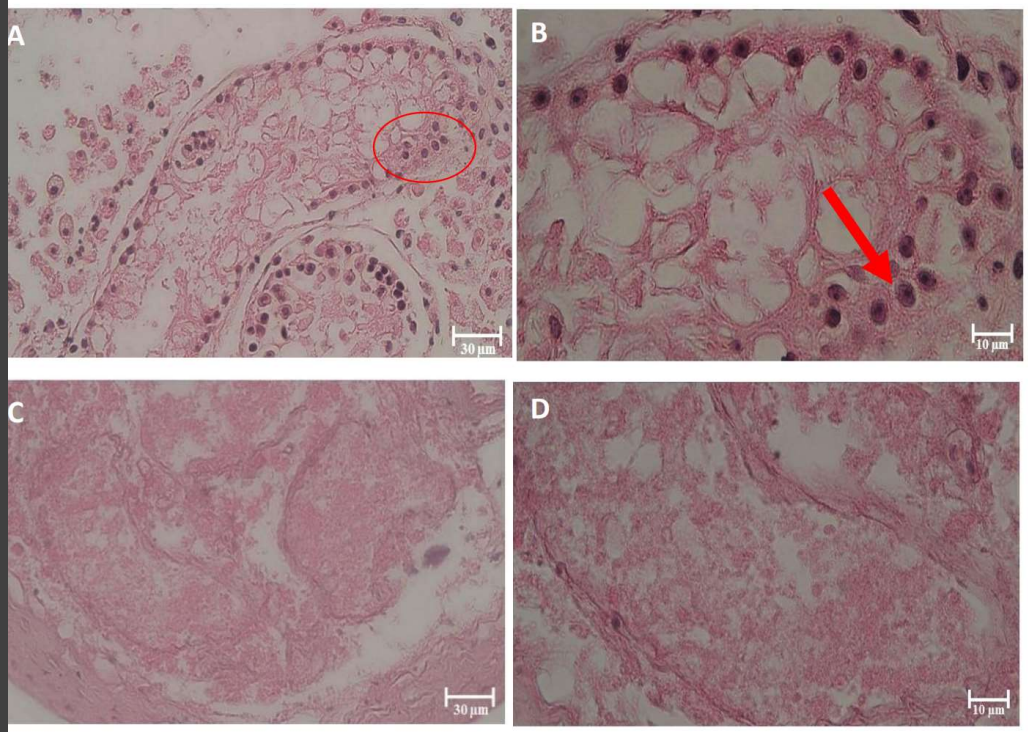


Fig 3. H&E staining of tissue sections IVT group. Tissue sections of the recipient testis 2 weeks after transplantation in IVT groups from fresh testicular cells (A, B) and non-transplanted (control group) (C, D) in perfusion bioreactor. Red arrow: Spermatogonia cells settled on the basement membrane.

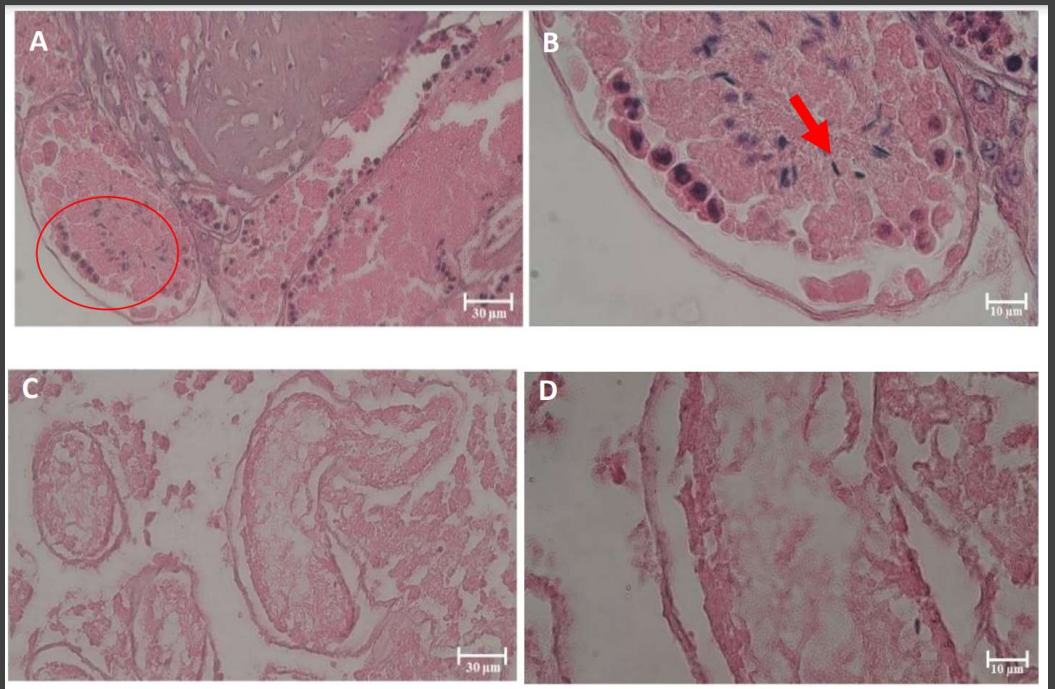
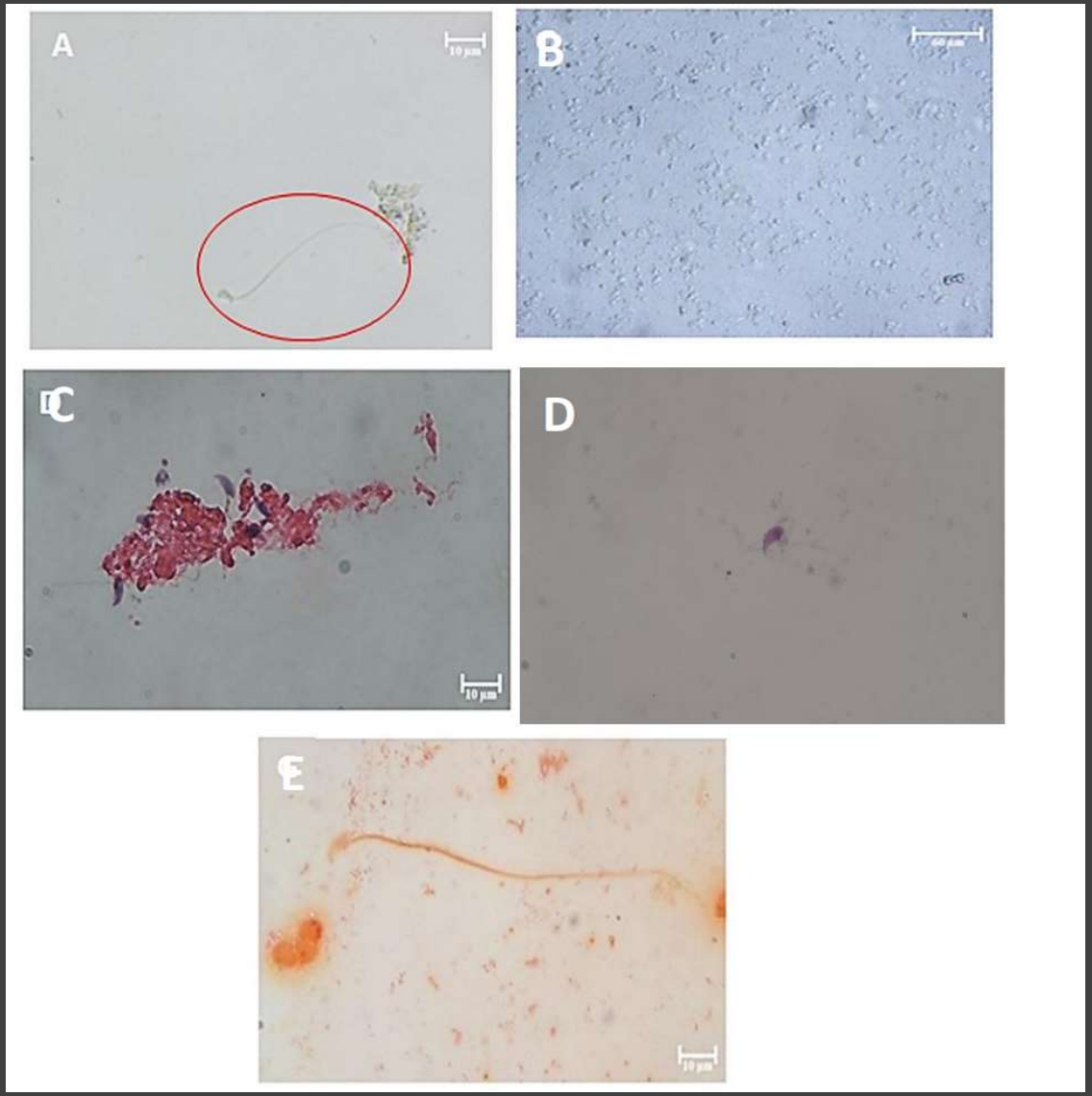


Fig 4. H&E staining of tissue sections IVT group. Tissue sections of the recipient testis 8 weeks after transplantation in IVT groups from fresh testicular cells (A, B) and non-transplanted (control group) (C, D) in perfusion bioreactor. Red arrow: Sperm cells.


A
B
C
D
E

Conclusion

- 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 testis 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

Conclusion

- **In vitro spermatogenesis** has some inherent advantages in humans, namely that it does not portend a risk for malignant reintroduction or retroviral infection (as in testicular tissue grafting and xenografting, respectively).
- Although complete in vitro spermatogenesis from SSC to spermatozoa has been shown in mice, these results have not established in primates or humans.
- Haploid cells at the round spermatid stage have been observed using in vitro cultures of immature human testicular tissue, but the **epigenetic/genetic normality** and **fertilization capability** of these cells has not yet been established. If the ***safety profile*** is established, it may be possible to use in vitro–derived spermatids or sperm for fertilization and embryo production.
- Better characterization of these postmeiotic germ cells and assessment of their fertility potential in primates will be needed before entry into the clinical realm.

Any
Question?