



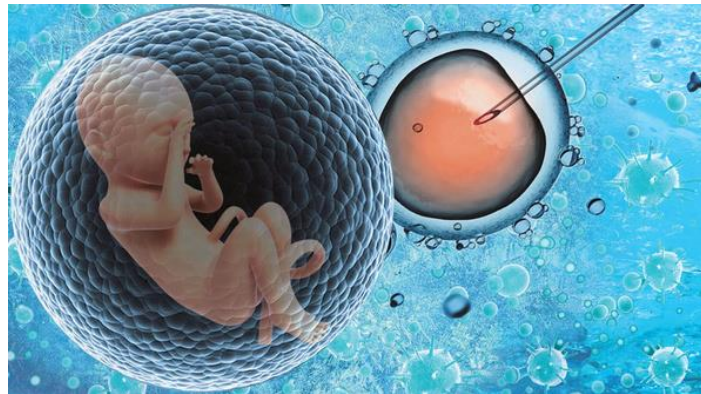
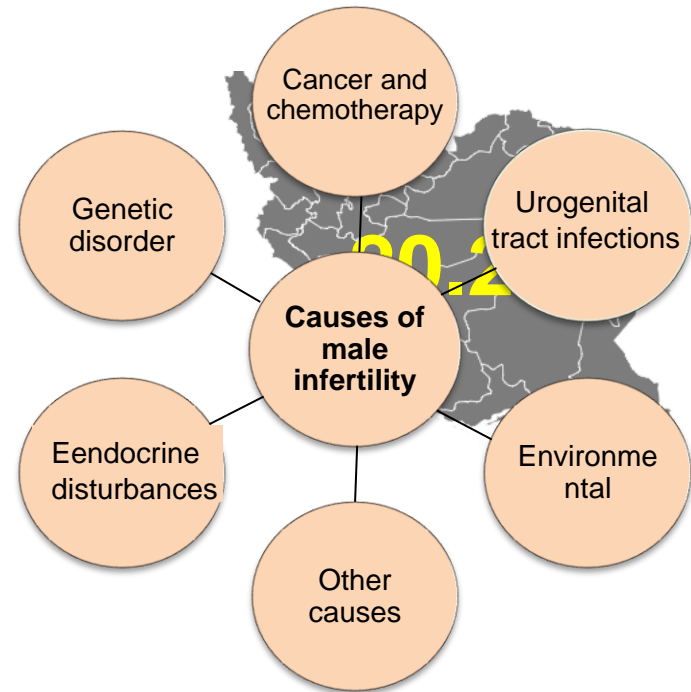
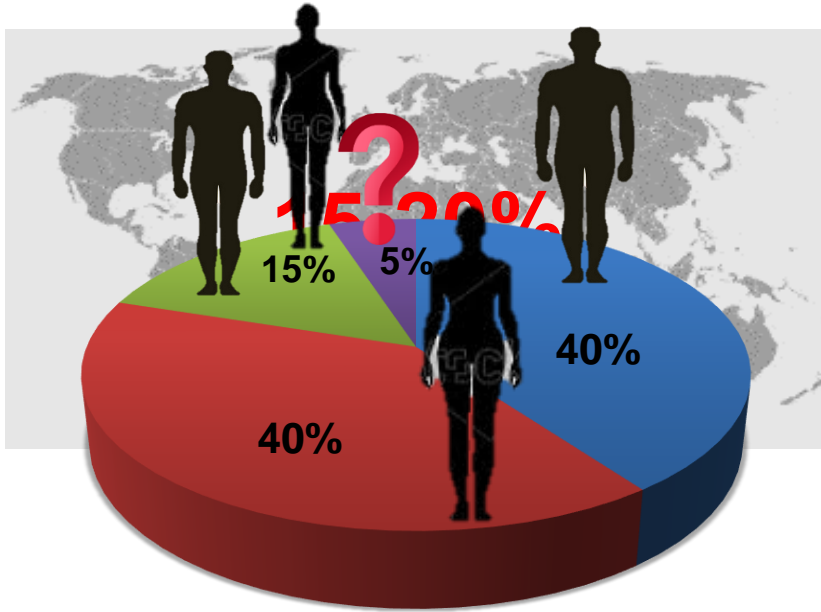


The study of differentiation of mouse embryonic stem cell into male germ cells using gelatin nanofibrous 3D scaffold and co-culture with Sertoli cells

Mina Vardiani, Ph.D
Clinical embryologist
Avicenna fertility center



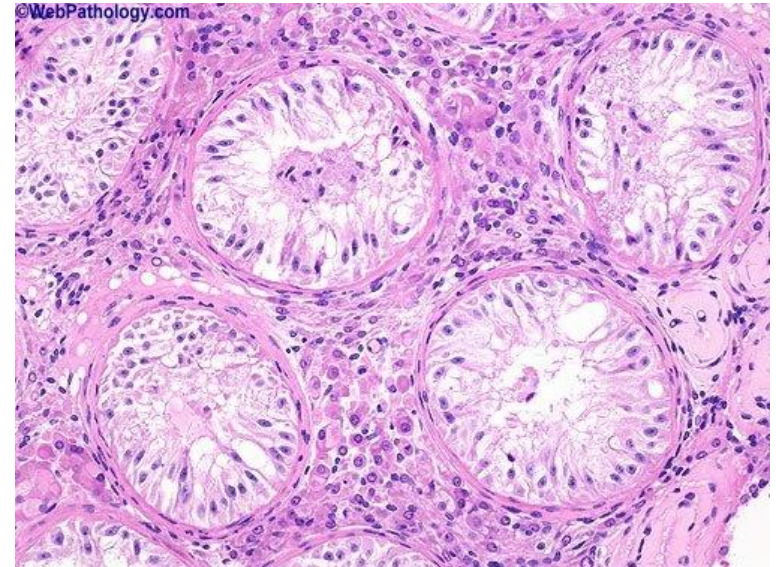
Male infertility





Male infertility

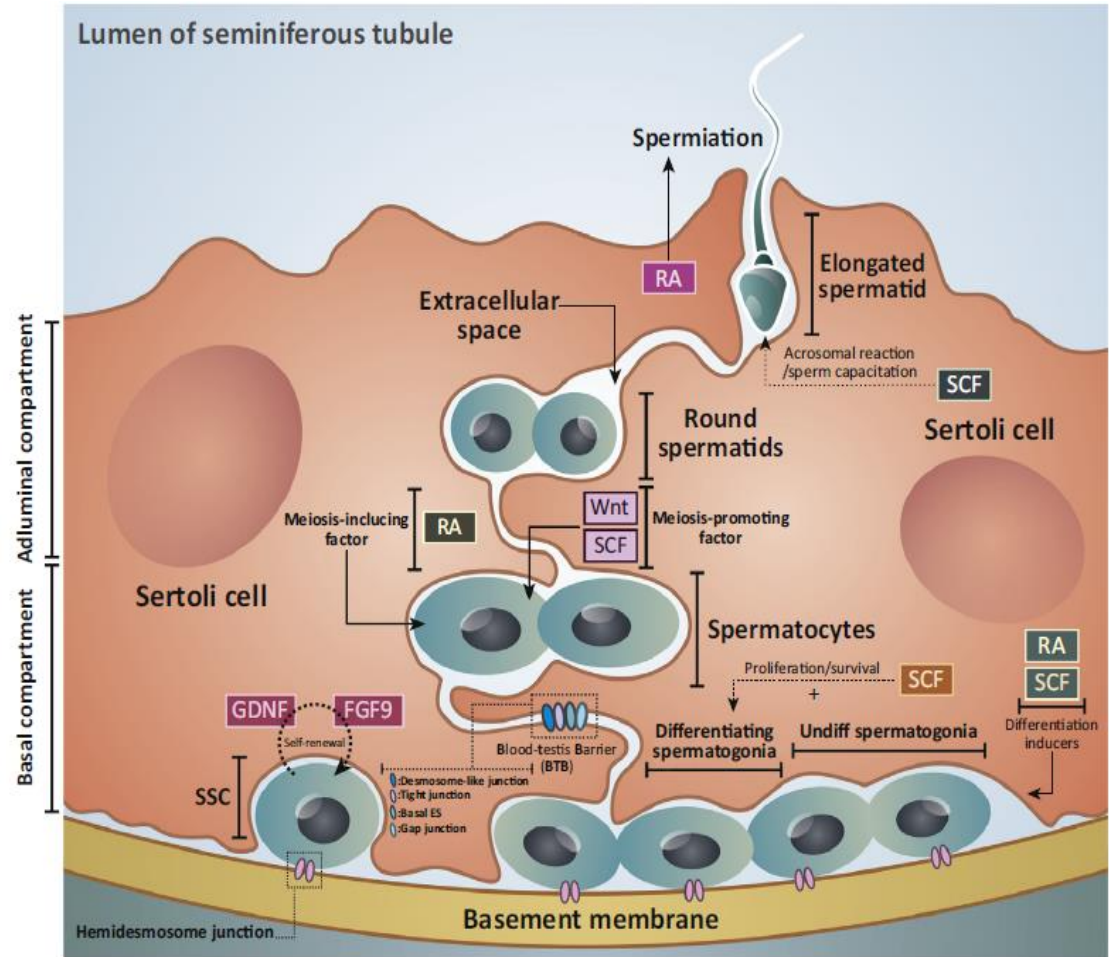
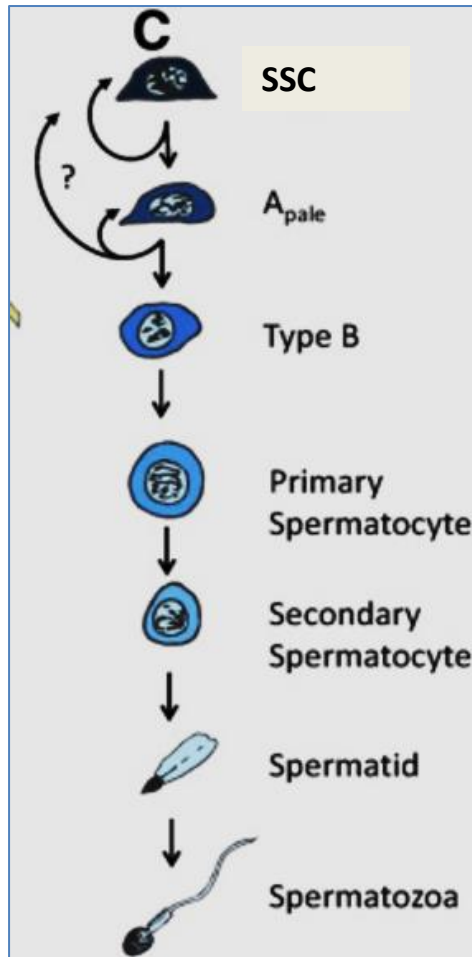
- **Non-obstructive Azoospermia**
 - Sertoli-cell-only syndrome (SCOS)
 - Maturation arrest
- **Klinefelter syndrome**
- **Gonadotoxic treatments**



Artificial Sperm Is Closer to Being a Reality



Spermatogenesis





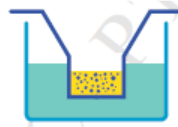
In vitro culture systems for spermatogenesis

Testicular 2D cell culture



Co-culture with Sertoli cells

Testicular 3D cell culture



Soft-Agar-Culture-System,

Culture of testicular tissue fragments



Hanging drop



Agar method (gas-liquid interphase)

Advantage

Feasible

Try to resemble the spatial environment

Maintain the spatial structure of the seminiferous tubule

Preserve Native niche

Multicellular Cell-cell and cell-matrix interaction

Disadvantage

Do not maintain the spatial structure

Do not maintain the natural structure

Disruption of blood supply hampers nutrient and oxygen supplies

Disruption of blood supply hampers nutrient and oxygen supplies

Iwanami et al.,
Theriogenology, 2006

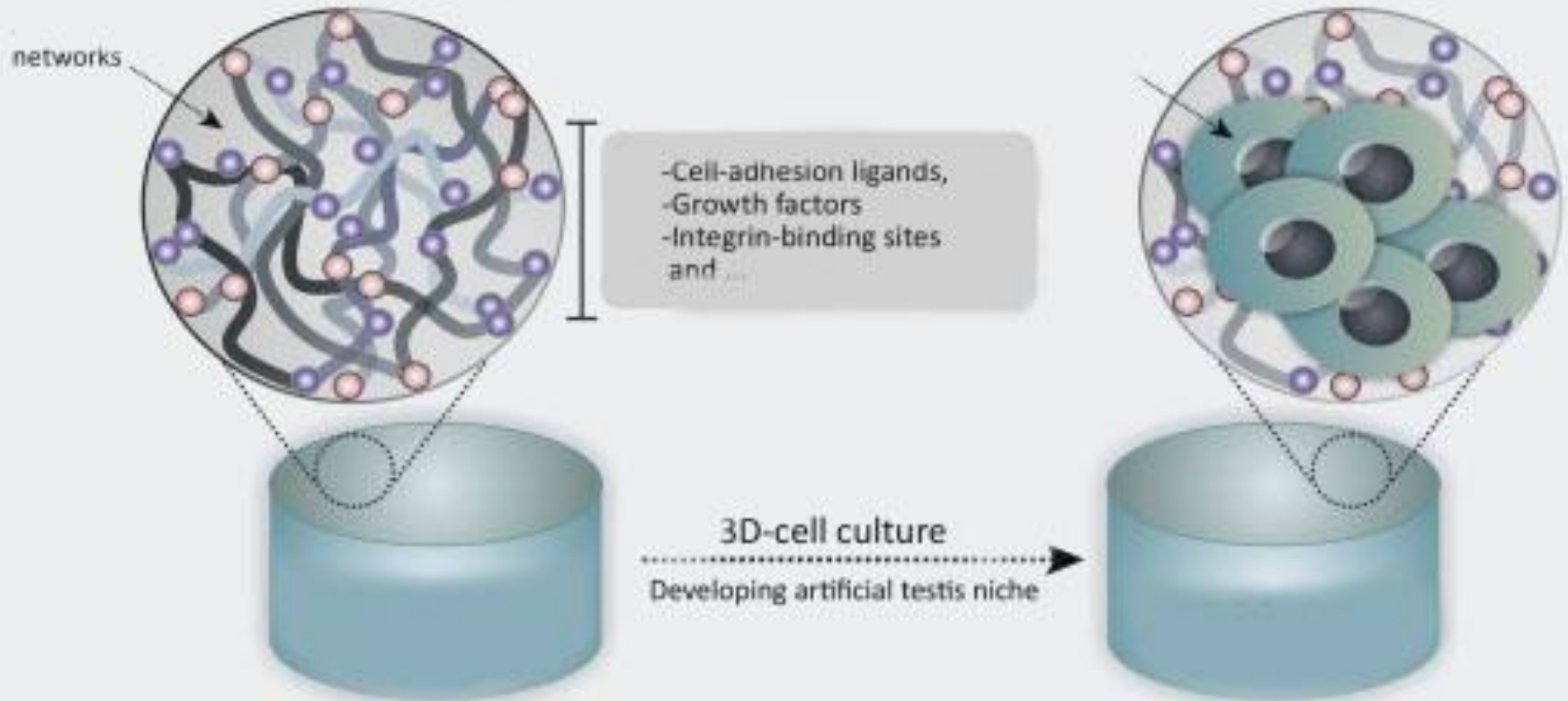
Elhija et al., Asian
J Androl, 2011

Jorgensen et al.,
Br J Cancer, 2014

Sato et al.,
Nature, 2011

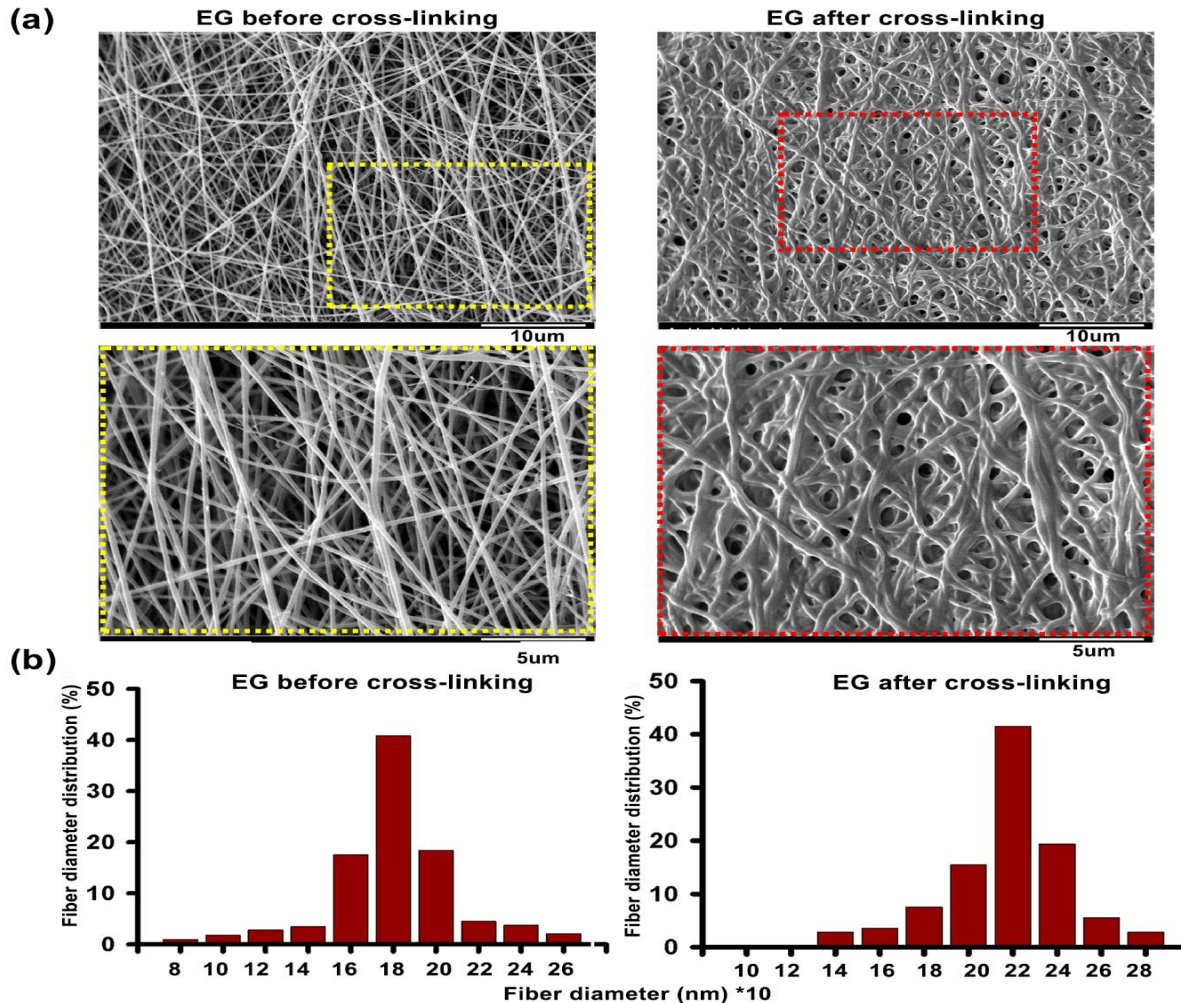


Scaffolds





The morphology of the scaffolds

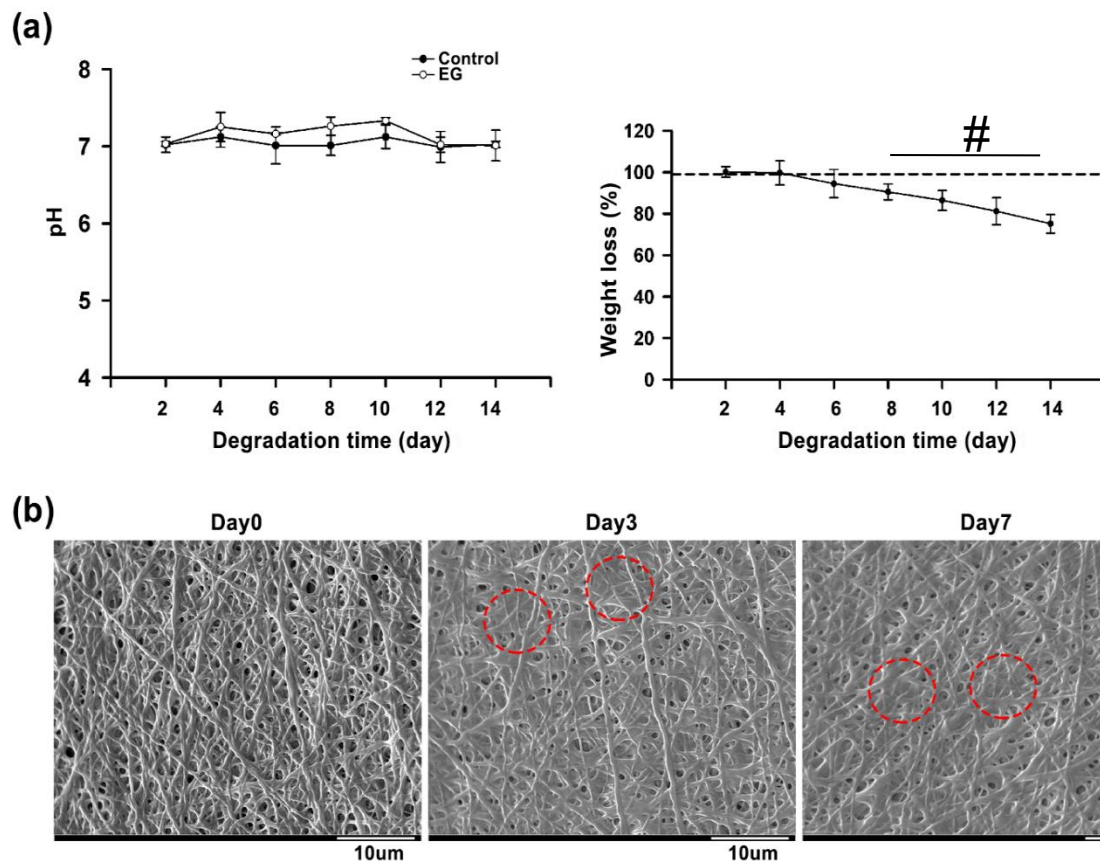


A, Morphology of electrospun gelatin scaffold before and after crosslinking with glutaraldehyde under the scanning electron microscope.

B, Distribution of fiber's diameter before and after crosslinking



Biodegradability

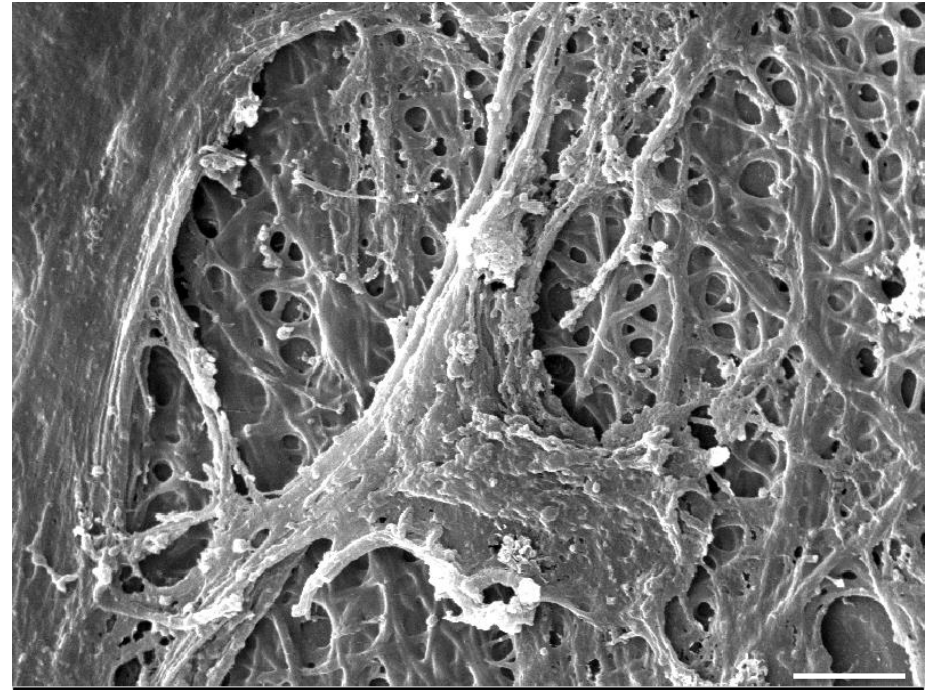
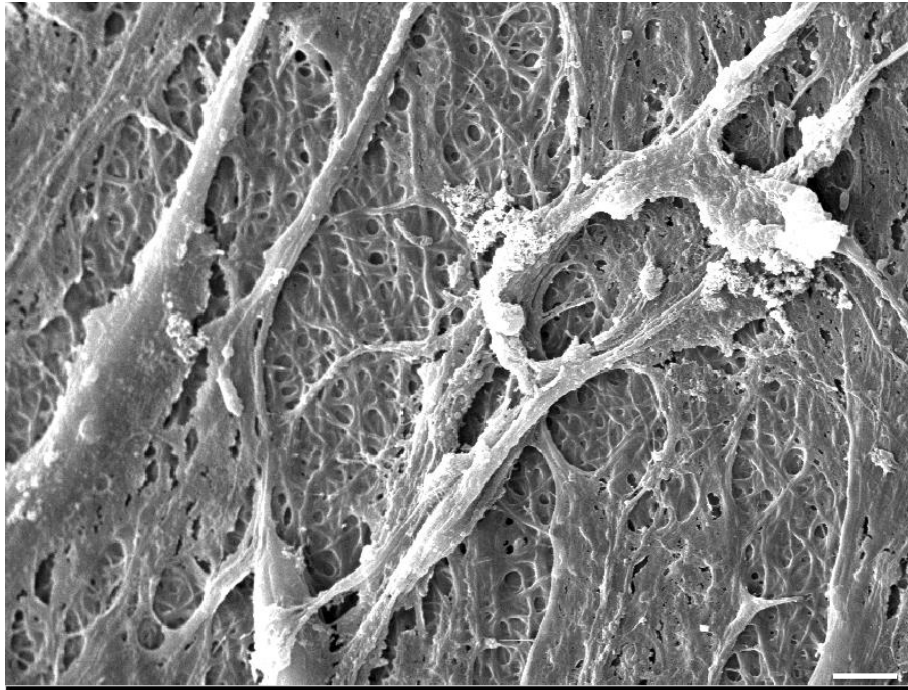


A, The pH value changes and weight loss (percentage) at days 2, 4, 6, 8, 10, 12, and 14 postdegradation time. Electrospun gelatin (EG) scaffold showed $24.8\% \pm 4.5\%$ weight loss after 14 days with no changes in pH value compared with control (PBS).

B, SEM micrographs of EG scaffold at days 0, 7, and 14 postdegradation times. Red circles indicate the changes in the morphology of fibers. PBS, phosphate-buffered saline; SEM, scanning electron microscope



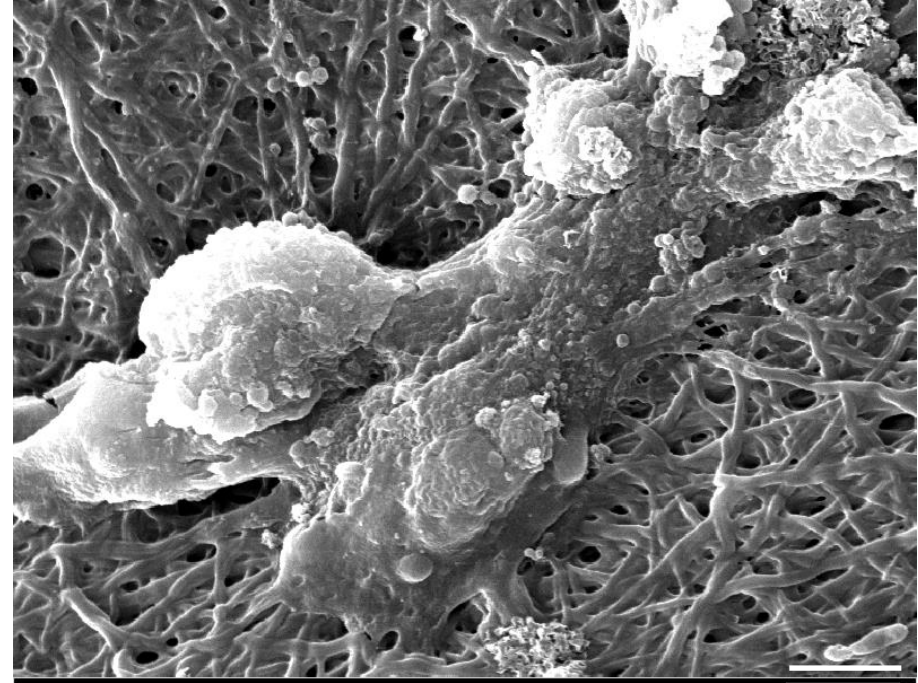
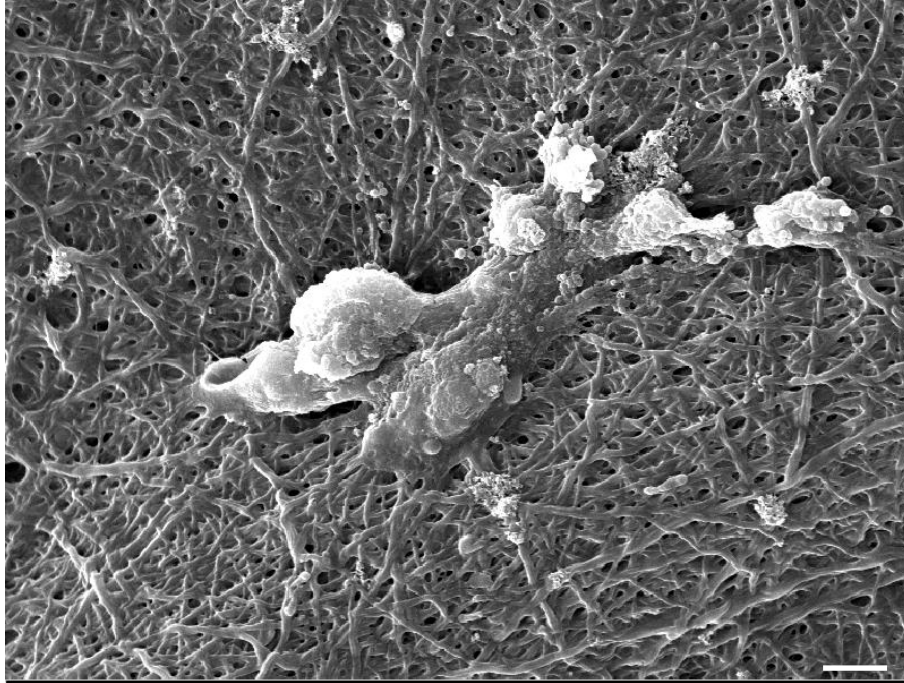
Cell adhesion properties of scaffold



Scanning electron microscope (SEM) image showing the Sertoli cells seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm .



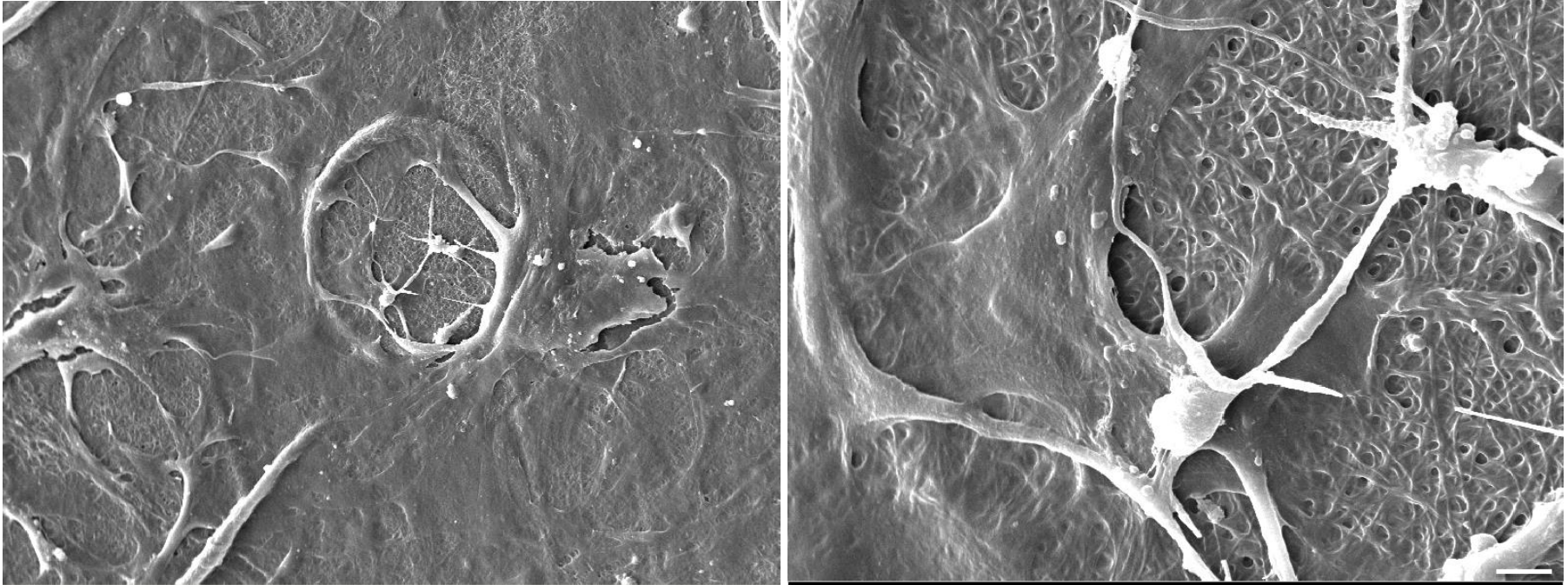
Cell adhesion properties of scaffold



Scanning electron microscope (SEM) image showing the embryonic stem cells (ESCs) seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm .



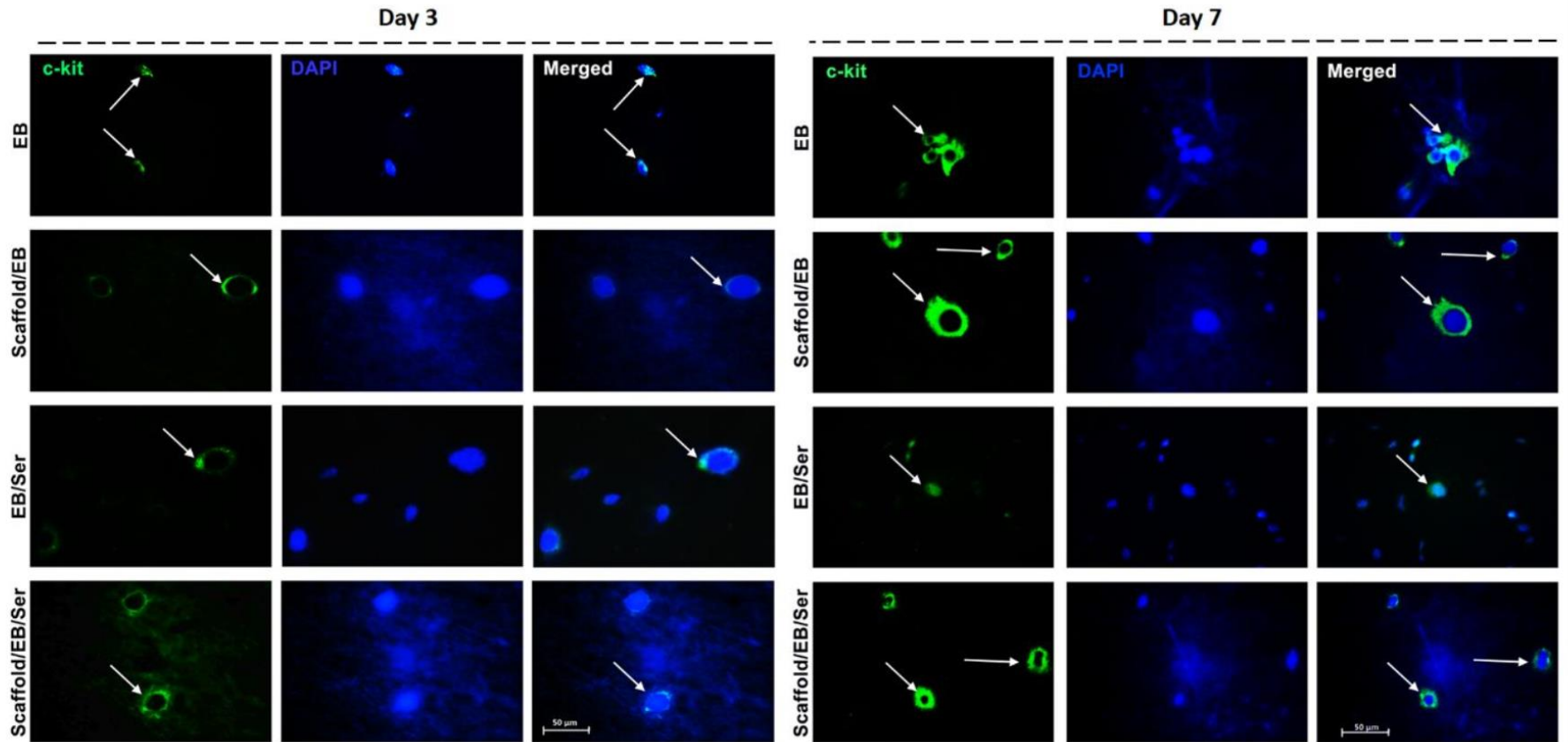
Cell adhesion properties of scaffold



Scanning electron microscope (SEM) image showing the Sertoli cells and ESCs seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm .



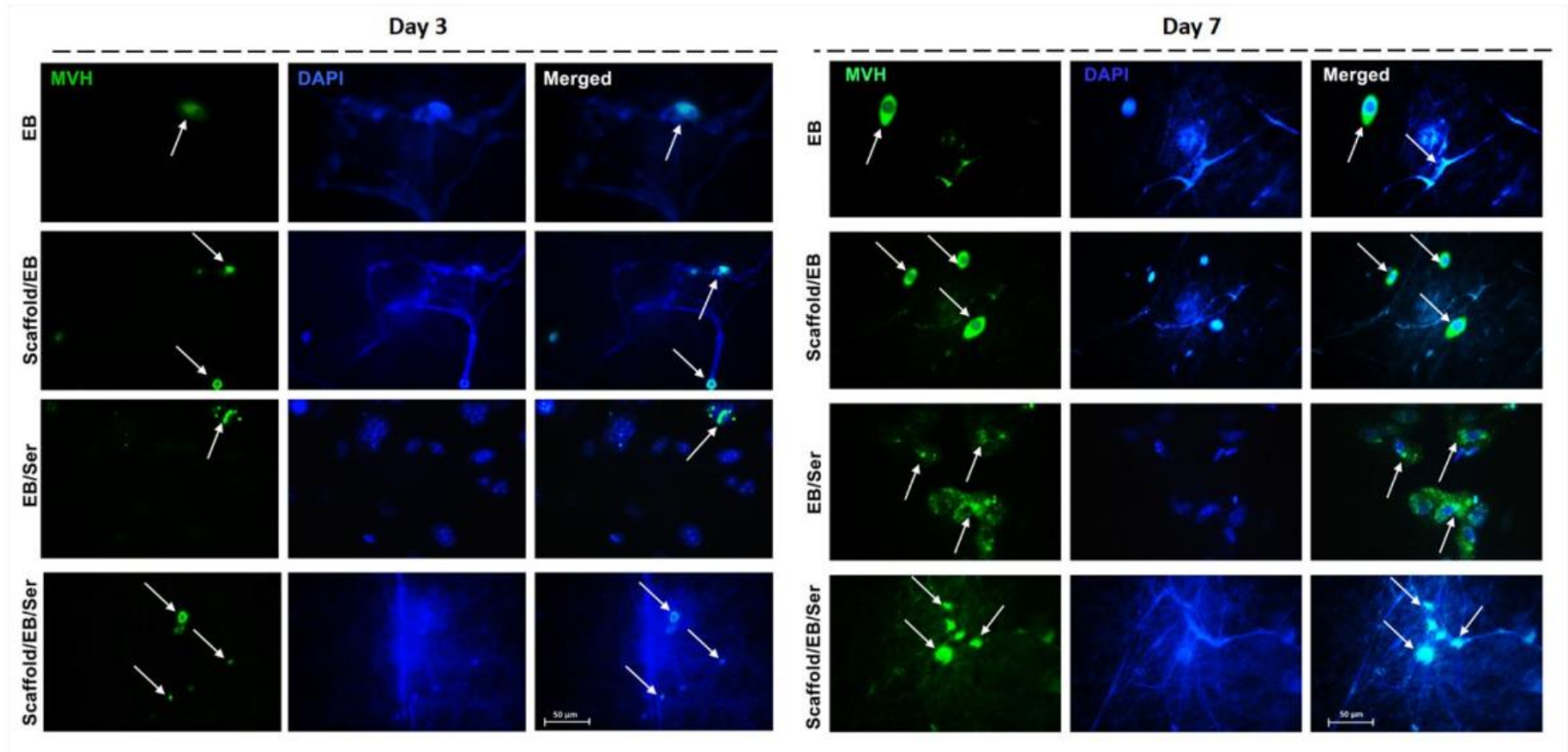
stem cells differentiation toward germ cells and sperm producing cells



Immunostaining for c-KIT at day 3 (a) and 7 (b) of differentiation induction



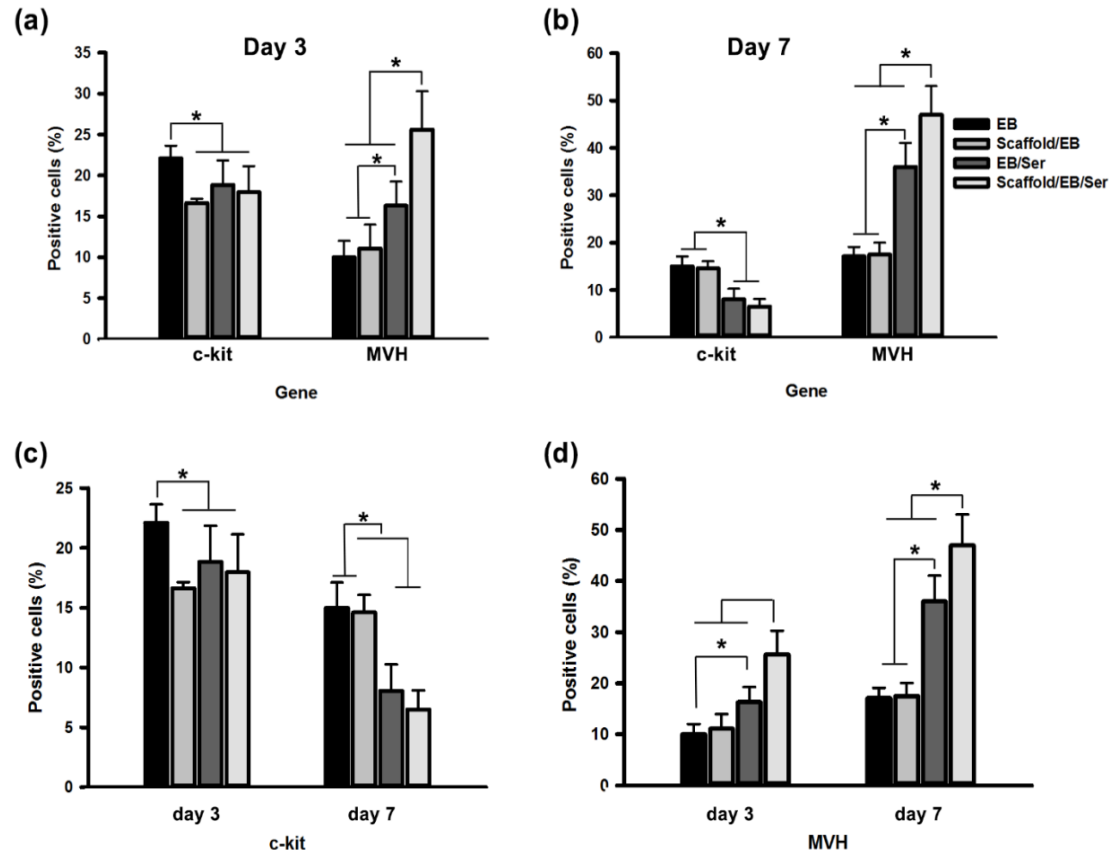
stem cells differentiation toward germ cells and sperm producing cells



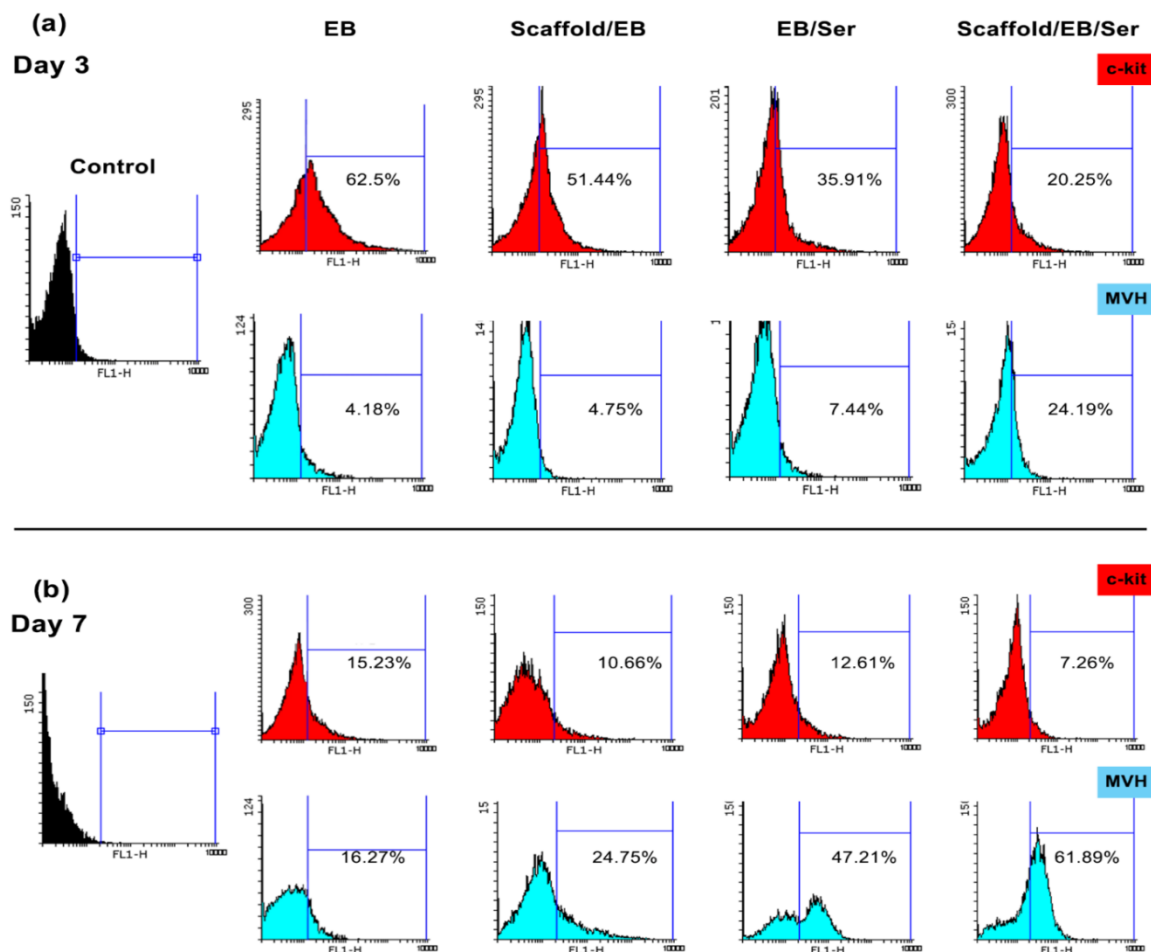
Immunostaining for MVH at day 3 (a) and (b) of differentiation induction



stem cells differentiation toward germ cells and sperm producing cells



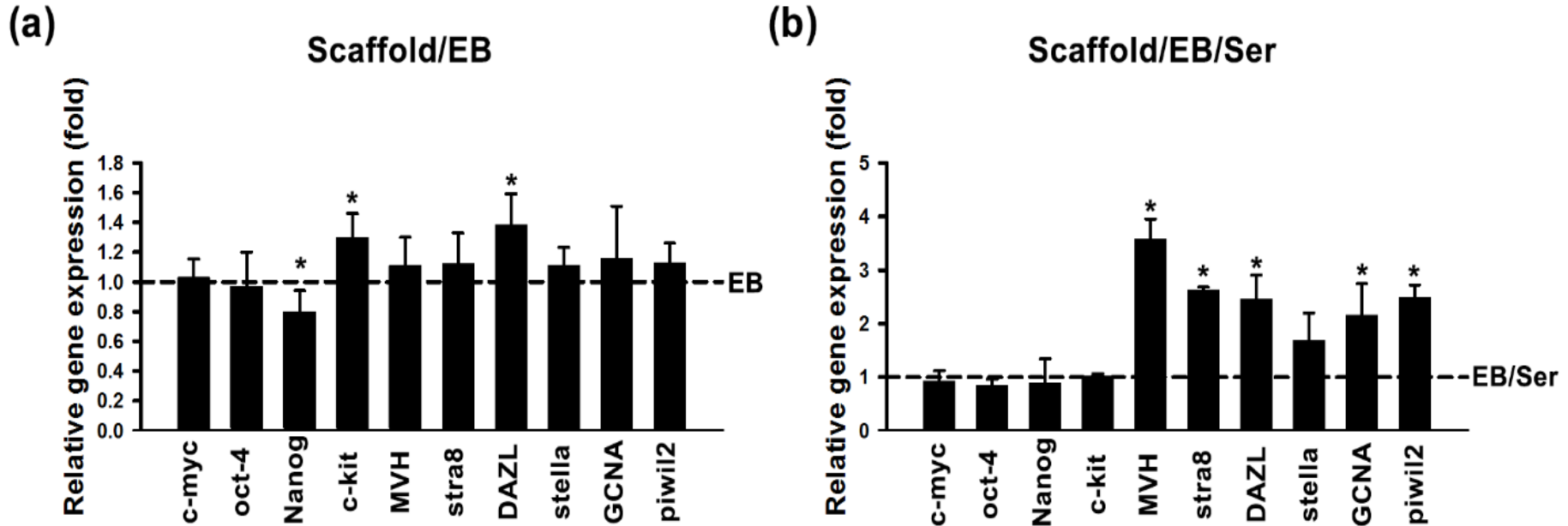
Immunocytochemistry staining. Comparison of the percentage of cells expressing c-KIT and MVH at days 3 and 7 of differentiation



Flow cytometry analysis for phenotypic determination of the cells.



stem cells differentiation toward germ cells and sperm producing cells



Comparison of the relative expression (fold) of the pluripotency genes (Oct4, c-Kit, Nanog, c-Myc) and germline genes (GCNA, Stella, MVH, Stra8, Piwil2, DAZL) at day 7 of differentiation between the experimental groups.



Summary

- Our findings revealed that the EG scaffold can provide an excellent substrate bio-mimicking the micro/nanostructure of native seminiferous tubules, and a platform for Sertoli cells-Ebs communication required for growth and differentiation of ESCs into germline cells.

