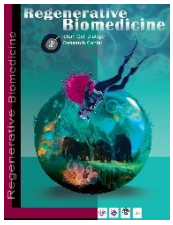

Regenerative Biomedicine

Production and Hosting: Shahid Sadoughi University of Medical Sciences

Journal homepage: jrb.ssu.ac.ir

Mini-Review Article

Tissue Engineering Approaches for Polycystic Ovary Syndrome

Fatemeh Kuchakzade*

Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

***Corresponding Author:**
Kuchakzade, Fatemeh

Email:
fatemehkuchakzade@gmail.com

Received:
2024-02-26
Revised:
2025-02-01
Accepted:
2025-02-11

Volume:1
Issue no.2

Editor-in-Chief:
Behrouz Aflatoonian Ph.D.

Abstract

Regenerative medicine and tissue engineering have opened new possibilities for repairing damaged tissues and organs. Key components of tissue engineering include cells, scaffolds, and biochemical markers, which play essential roles in regeneration. Polycystic ovary syndrome (PCOS) is a prevalent metabolic and endocrine disorder affecting 4–18% of reproductive-age women worldwide. It manifests through symptoms such as hyperandrogenism, menstrual irregularities, infertility, and anovulation. Despite its prevalence, the exact causes of PCOS remain unclear, complicating the understanding of its long-term consequences.

Current treatments, including ovulation stimulants, insulin sensitizers, and nonsteroidal antiandrogens, help manage symptoms but often lead to adverse effects such as obesity, cardiovascular disease, and gastrointestinal issues. Given the increasing impact of PCOS on women's fertility, alternative therapies are necessary.

Tissue engineering has been explored in addressing various women's health issues, including urethral defects, sex reassignment surgery, and hormonal disorders. Engineered ovarian tissue structures have shown promise in enhancing follicle survival and growth. Regenerative approaches leveraging tissue engineering may provide a novel, side-effect-free treatment for PCOS by promoting tissue repair and hormonal balance. However, the application of tissue engineering in PCOS remains largely unexplored. This study aims to investigate the potential of tissue-engineered constructs as an innovative therapeutic strategy for PCOS treatment.

Keywords: Biomaterials, Polycystic Ovary Syndrome (PCOS), Regenerative Medicine, Scaffold Design, Tissue Engineering

How to cite this article:
Kuchakzade, F. Tissue Engineering Approaches for Polycystic Ovary Syndrome. *Regenerative Biomedicine*, 2025; 1(2): 139-147.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder. The National Institutes of Health (NIH) states that the prevalence of PCOS has increased dramatically in the past 25 years (1). Studies have shown that in recent years, 4 to 18% of women of reproductive age worldwide suffer from this condition (2). PCOS can be identified by symptoms such as hyperandrogenism, menstrual irregularities, impaired folliculogenesis, multiple small cystic follicles, infertility, chronic anovulation, etc. In the last decade, PCOS has also become one of the major global public health concerns, such that its management and prevention have become extremely important (1, 3, 4). Tissue engineering is the science that provides a variety of three-dimensional scaffolds to restore function, repair, or replace damaged tissues or organs. These three-dimensional scaffolds can contain cells, drugs, growth factors, etc. Currently, tissue engineering-based regenerative therapy can be used in PCOS treatments, which utilizes the body's own innate conditions to initiate tissue repair and hormonal balance without any side effects. The role of tissue engineering is still under investigation as a new therapeutic area and has not been extensively studied in PCOS (5-9). Although it has been proven in numerous articles that the ovary can be regenerated using 3D scaffolds, limited animal studies were found in this field and no clinical studies were found in the field of PCOS treatment through tissue engineering (10-12). The aim of this study was to investigate the role of tissue engineering in the treatment of PCOS.

PCOS

PCOS is a common endocrine and metabolic disorder (1) that occurs in approximately 4% to 18% of women of reproductive age worldwide (2). The National Institutes of Health (NIH) estimates that PCOS cases have tripled in the past 25 years. One in ten women worldwide shows PCOS symptoms, including impaired folliculogenesis, hyperandrogenism, menstrual irregularities, large numbers of small cystic follicles, infertility, and chronic anovulation. In addition to reproductive abnormalities, PCOS is strongly associated with many other disorders, including dyslipidemia, insulin resistance, type 2 diabetes, cardiovascular disease, obesity, chronic low-grade inflammation, alopecia, hirsutism, acne, and psychiatric disorders. As a result, PCOS can be considered a growing global public health concern, and its prevention and management should be addressed. Abnormal hormonal responses by ovarian granulosa cells (GCs) during follicular development are considered to be the main cause of PCOS. However, all the factors involved in the pathophysiology of PCOS are not yet fully understood (1).

Currently, different treatments are used for PCOS such as weight management, drugs targeting insulin resistance, or hormonal drugs. In particular, inositol therapy used as an adjunct to clomiphene citrate to improve ovulation has been shown to be effective and beneficial. However, according to the reports, using enough myo-inositol in PCOS patients who experience ovulation induction is impossible (7). treatment for anovulatory women with PCOS, leads to tissue adhesions and other complications. Therefore, considering such limitations, there is a need

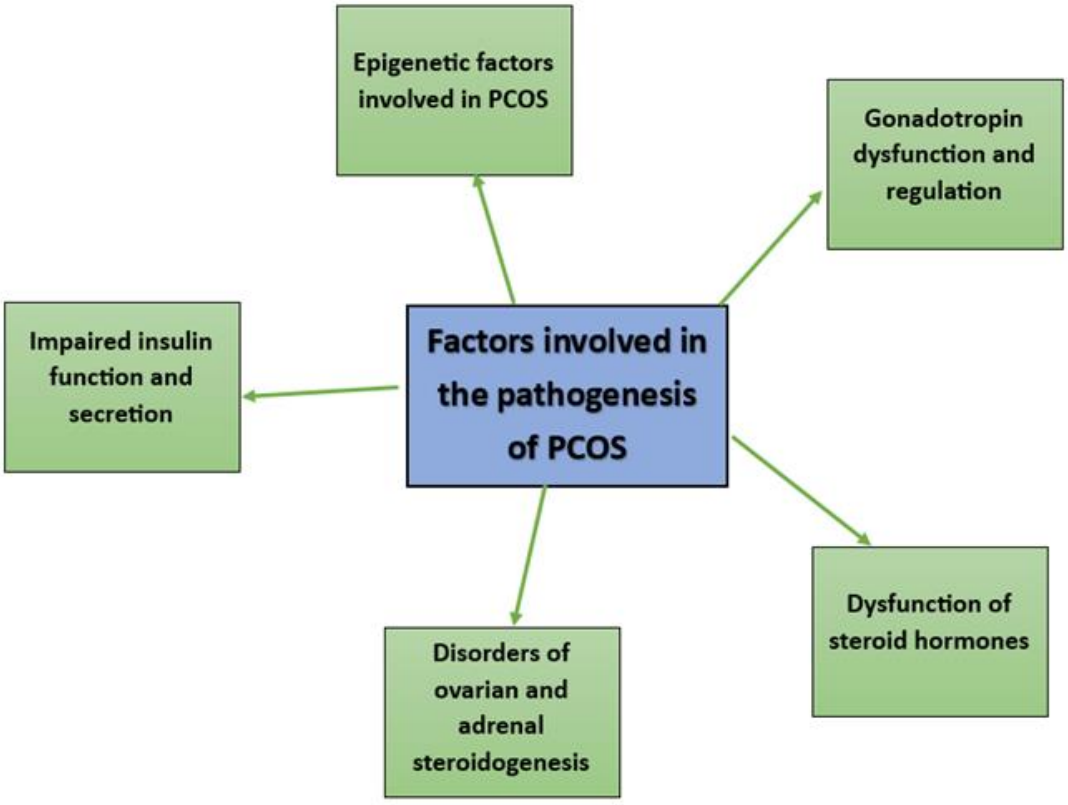

Figure 1. Summary of factors involved in PCOS (4).

Tissue Engineering

AF-MSCs and their clinical products, including EVs (AF-MSC-EVs) and exosomes The most important components involved in tissue engineering constructs are cells, biomaterials, and biochemical markers. The cells used in these constructs can be of endogenous origin (i.e. stem cells derived from the individual's own tissues) or exogenous (i.e. allogeneic cells harvested from donors).

What can be inferred from the literature in recent years is that all tissues of the human body, except the cornea and cartilage, can be regenerated by tissue engineering constructs due to their limited self-healing properties (9, 14-17). The main basis of tissue engineering constructs are various three-dimensional scaffolds that can contain cells, drugs, growth factors, etc. In recent years, these tissue engineering constructs have been used and implanted in the body to restore function or replace damaged tissue or organs. In various studies, various tissue engineering constructs have been proposed to improve the function and regeneration of the ovary.

These constructs provide a 3D system for resuming folliculogenesis, stimulating follicle survival and growth. The effects of these 3D constructs on ovarian follicles and cells depend largely on the biomaterial used to construct these constructs. By encapsulating ovarian cells and follicles in a suitable biomaterial, it is even possible to construct a

3D engineered ovary (5-8). Biomaterials are usually biocompatible and biodegradable. Biomaterials can play a role in the exchange of nutrients and waste by forming a porous structure. On the other hand, biomaterials used in ovarian tissue engineering must provide an appropriate balance between stiffness and elasticity to maintain the spherical shape of the follicle and provide a substrate for its radial growth. In fact, as the diameter of follicles increases, they receive compressive forces from the surrounding biological material. The intensity of the compressive forces depends on the elasticity of the surrounding biological material. Therefore, simulating the mechanical properties of the natural ovary is very important when creating a suitable scaffold for the survival and growth of follicles (8, 18, 19).

Cell

In recent decades, cell-based therapy (especially stem cells) has been proven to be a major revolution in the world of science and medicine today. The mainstay of regenerative medicine is cell-based therapy. Regenerative medicine and cell therapy have opened up endless avenues for the treatment of the most deadly human diseases. Regenerative medicine and cell therapy are a new, expanding and successful approach. The main goal of this approach is the efficient replacement and repair of damaged cells, tissues and organs, which ultimately leads to improved function of damaged cells, tissues and organs (20). Stem cells have attracted more attention than other cells due to their fundamental properties, anti-inflammatory activity, secretion of active factors and differentiation into various types of somatic cells, and have become the focus of clinical treatment research. Stem cells can secrete many active factors such as vascular endothelial growth factor (VEGF), TNF-α and IL-10, which are anti-fibrotic, anti-inflammatory and angiogenic. Therefore, it can be beneficial for lung, liver, kidney, endometrial, and ovarian injuries (10, 13).

Biomaterials

“Biomaterials” are biocompatible molecules or materials that have low toxicity and high compatibility with body functions and are currently widely used in regenerative medicine and tissue engineering. Biomaterials restore biological functions with high compatibility properties and mechanical strength. However, their use in women is very limited. To this end, this article highlights the importance of biomaterials such as fibrin, collagen, and alginate-based hydrogels used in 3D scaffolds for female genital reconstruction. Their role in tissue engineering is still under investigation as a new therapeutic area and has not been studied in detail in PCOS. (9, 21, 22).

Treatment of PCOS by means of tissue engineering

Biomaterials have played an important role in tissue engineering and regenerative medicine in gynecological issues such as gender reassignment surgery, urethral defects, and hormonal diseases, namely PCOS. Given the complex and interconnected pathways of PCOS pathogenesis, it is very difficult to find the root cause for treatment. Currently, standard drugs for the treatment of polycystic ovary syndrome are ovulation inducers, insulin sensitizers, and nonsteroidal antiandrogens, which are used to manage the

disease. These drugs have adverse effects such as obesity, cardiovascular disease, or gastrointestinal problems. Therefore, regenerative therapy based on biomaterials and stem cells can be used in the treatment of polycystic ovary syndrome. This type of treatment causes tissue recovery and hormonal balance without any complications.

For ovarian tissue engineering, when choosing a biomaterial, it is very important to combine biological properties (such as the degree of cell attachment, migration, differentiation, regeneration, etc.) and mechanical properties similar to a natural organ or tissue. While the biological materials used for ovarian tissue engineering must have a certain amount of elasticity (storage modulus between 1 and 2.5 kPa), during follicular growth the granulosa tissue expands and the spherical shape of the follicles is maintained (8). The most abundant type of protein in the human body and the first natural matrix used for grafting isolated preantral follicles is collagen (8, 23, 24).

Recently, cell survival rate, follicular growth rate, hormone production, and oocyte maturation have been investigated in rat ovarian follicles enclosed in type I collagen hydrogels as a three-dimensional culture system. The results showed that changing the density and elasticity of collagen hydrogel can significantly affect the growth of follicles in terms of phenotype, hormone secretion, and maturation (25). In another study, it was shown that the long-term restoration of ovarian function and fertility in mice after ovarian damage caused by triterygium glycosides (Tripterygium glycosides (TGTs) are widely used in clinical practice for the treatment of rheumatoid arthritis and other autoimmune diseases, but they mainly have cytotoxic effects on ovarian tissue) can be done by transplanting adipose-derived stem cells on collagen scaffolds (26).

Fibrin is a biocompatible and biodegradable material that has been shown to have a positive effect on cell proliferation, scaffold-mediated cell interaction, and angiogenesis. Fibrin has also been investigated as a cell and drug carrier (8). In one study, isolated preantral follicles from mice were encapsulated in fibrin and used for autografts. This resulted in follicle survival and growth to the antral stage (27). In another study, fibrin hydrogel was used to enhance mouse follicle growth, which resulted in favorable results such as improved survival and growth of secondary follicles compared to primary follicles. In addition, angiogenesis was enhanced in secondary follicles (28, 29). To increase the biological properties, bioactive agents can be combined with fibrin. For example, fibrin with VEGF was used to graft mouse preantral follicles, which led to the resumption of the estrous cycle in mice (30).

In one study, fibrin hydrogel containing platelet lysate was used to transplant preantral follicles in mice. This treatment resulted in an increased rate of follicle recovery (31). In another study, transplantation of umbilical cord mesenchymal stem cells encapsulated in fibrin hydrogel was used to improve the symptoms of PCOS in a rat model. This treatment had a positive effect on ovarian function and serum levels of progesterone (P), estradiol (E2), gonadotropins (LH/FSH), testosterone (T), and transforming growth factor-β1 (TGF-β1). In this study, it also improved ovarian weight and size, granulosa

cell count, number of estrous cycle follicles, and immature cystic follicles (10).

In recent years, alginate has become one of the most widely used polymers for in vitro culture of preantral follicles. It has been used as a tool to study folliculogenesis, to test the effects of cryopreservation and chemotherapy on follicular population, and to promote follicle growth in vitro (8). In one study, a positive relationship between follicle growth and their density in alginate beads was demonstrated by encapsulating rat follicles in alginate beads (32).

Also, mouse preantral follicles and ovarian cells are encapsulated in alginate. The survival and development of follicles and the viability and proliferation of ovarian cells after one week indicated the success of transplantation and the effective performance of alginate (33). In addition, several studies reported alginate as a suitable polymer for encapsulating ovarian follicles (33, 34). Decellularized extracellular matrix (DECM) from ovarian tissue has shown promising results in isolated follicle transplantation for ovarian tissue engineering. DECM may be an ideal scaffold for the construction of bioengineered ovaries due to its preservation of ovarian microstructure, bioactivity, high survival of ovarian primary cells, and regeneration of primary or follicle-like structures. DECM may also play a positive role in the production of the hormone estradiol. Plasma clot is a growth factor-rich, highly biocompatible, and autologous material. Plasma clot has been used successfully for the culture of various cell types and for follicle encapsulation. The presence of platelets in plasma clots makes it stiffer than pure fibrinogen, and plasma clots are therefore used to increase matrix stiffness (8). In a study, after transplanting mouse ovarian follicles and cells into plasma clots, the animals were able to ovulate and give birth to normal children (35, 36).

In another study, endometrial stem cells were encapsulated in an injectable alginate/gelatin hydrogel. This construct was used to treat rats with PCOS. Treatment with endometrial stem cells in an injectable alginate/gelatin hydrogel significantly improved PCOS parameters including ovarian and body weight, serum testosterone, luteinizing hormone (LH), anti-Müllerian hormone (AMH), progesterone, follicle-stimulating hormone (FSH), inflammatory markers, immature follicles, corpus luteum, ovarian cysts, and granulosa cell count (37).

Discussion

There are significant challenges in female reproductive tissue engineering, such as the unique architecture of the follicle, the selection of appropriate biomaterials for ovarian tissue, and the construction of tissue vessels, which have caused the limitations of tissue engineering in the field of female reproductive diseases such as PCOS (8). Currently, there are several articles on the treatment of PCOS through cell therapy. Bone marrow mesenchymal stem cells (BM-hMSCs) by secreting IL-10 provide a regulatory pathway for the treatment of PCOS (38). Bone morphogenetic proteins (BMPs) are among the many growth factors secreted by BM-hMSCs, and several studies have shown a decrease in BMP levels in patients with PCOS (39, 40). The findings indicate that BMP-2, BMP-4, BMP-6, and BMP-7 suppress androgen secretion in bovine theca cells (40). In fact, BMP-2 is a key molecule that regulates the steroidogenesis of theca

cells in PCOS, and BMP-2 may be a suitable option for the treatment of PCOS (38).

Umbilical cord mesenchymal stem cells (hUC-MSC) reduced the inflammatory response in PCOS mice by inhibiting Th1, Th17, and β cells (producing pro-inflammatory factors) and inducing Treg differentiation. Therefore, treatment with hUC-MSC can inhibit Inflammatory responses and effectively improve pathological changes, function of ovaries, and uterus in PCOS rats. hUC-MSC can inhibit the expression of pro-inflammatory factors (IFN-γ, TNF-α, and IL-1β) and increase the expression of anti-inflammatory factor (IL-10) in ovarian and uterine tissue. In mice with PCOS, hUC-MSC transplantation was able to change the pro-inflammatory state to an anti-inflammatory state and reduce the pathological changes of ovarian and uterine tissues in mice with PCOS (41). Adipose mesenchymal stem cells (hAD-MSCs) secrete cytokines and growth factors, hematopoietic, angiogenic and anti-apoptotic factors in the PCOS model. Also, hAD-MSCs improve the number of granulosa cells (42). The findings show that hAD-MSCs injection increases the secretion of growth factors, induces angiogenesis, and improves the number of ovarian follicles and corpus luteum in the body (43).

Although there are many articles on the treatment of polycystic ovary syndrome through cell therapy, very limited studies have been conducted on the treatment of PCOS with tissue engineering. On the other hand, many studies have been conducted on the improvement of ovarian and uterine diseases through tissue engineering. Therefore, this study suggests that, given the high potential of tissue engineering and cell therapy for the treatment of PCOS, more tissue engineering constructs through cell therapy and biomaterials should be investigated and presented for the treatment of PCOS in the future.

Acknowledgements

We would like to thank all the friends and professors who helped us write and publish this article.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Vezza T, Rocha M, Víctor VM. PGK1-AR axis: Benefits of a novel actor in PCOS pathology. EBioMedicine. 2020 Dec;62:103110. doi: 10.1016/j.ebiom.2020.103110. Epub 2020 Nov 4.
2. Li X, Feng Y, Lin JF, Billig H, Shao R. Endometrial progesterone resistance and PCOS. J, Biomed Sci. 2014 Jan 9;21(1):2. doi: 10.1186/1423-0127-21-2.
3. Rosenfield RL. Curr Opin Pediatr. Current concepts of polycystic ovary syndrome pathogenesis. 2020 Oct;32(5):698-706.
4. Khan MJ, Ullah A, Basit S. Genetic Basis of Polycystic Ovary Syndrome (PCOS): Current Perspectives. Appl Clin Genet. 2019 Dec 24;12:249-260. .
5. K. Das et al., Epidermal growth factor in human follicular fluid stimulates mouse oocyte maturation in vitro Fertil. Steril., (1992).
6. J.J. Eppig et al., Conditions that affect acquisition of developmental competence by mouse oocytes in vitro: FSH, insulin, glucose and ascorbic acid. Mol. Cell. Endocrinol., (2000).
7. A. Pessoa et al., Effect of morphological integrity, period, and type of culture system on the in vitro development of isolated caprine preantral follicles, Theriogenology, (2014).
8. Arezoo Dadashzadeh, Saeid Moghassemi, Amin Shavandi, Christiani A. Amorim, A review on biomaterials for ovarian tissue engineering, Volume 135, November 2021, Pages 48-63.

9. Nidhi Chauhan, Anjali Peter, Shringika Soni, Rachna Rawal, Utkarsh Jain, Biomaterials as regenerative medicine in Poly Cystic Ovarian Syndrome (PCOS) treatment, Volume 187, November 2022, 108649.

10. Yuanyuan Li, Jia Guo, Shoulong Deng, Zili Gao, Yixun Liu, Qi Gu. Fibrin Facilitates Mesenchymal Stem Cells to Ameliorate Rats with Polycystic Ovary Syndrome .Appl. Sci. 2020, 10(10), 3598.

11]. C.A. Amorim, A. Van Langendonckt, A. David, M.-M. Dolmans, J. Donnez, Survival of human pre-antral follicles after cryopreservation of ovarian tissue, follicular isolation and in vitro culture in a calcium alginate matrix, Human Reproduction 24(1) (2009) 92-99.

12. T.K. Woodruff, L.D. Shea, The role of the extracellular matrix in ovarian follicle development, Reproductive sciences 14(8_suppl) (2007) 6-10.

13. Maas K, Mirabal S, Penzias A, Sweetnam PM, Eggan KC, Sakkas D.Hippo signaling in the ovary and polycystic ovarian syndrome. J Assist Reprod Genet. 2018 Oct;35(10):1763-1771. doi: 10.1007/s10815-018-1235-0. Epub 2018 Aug 17.

14. S. Patel, Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy, J. Steroid Biochem. Mol. Biol. (2018).

15. R. Azziz, Introduction: determinants of polycystic ovary syndrome, Fertil. Steril. (2016).

16. Y. Yang et al., Applications of nanotechnology for regenerative medicine; healing tissues at the nanoscale, 2019.

17. E. West et al. Physical properties of alginate hydrogels and their effects on in vitro follicle development. Biomaterials, (2007).

18. W. Zhang et al., Vascularization of hollow channel-modified porous silk scaffolds with endothelial cells for tissue regeneration Biomaterials, (2015).

19. S.Z. Sadr et al., Mouse preantral follicle development in two-dimensional and three-dimensional culture systems after ovarian tissue vitrification, Eur. J. Obstet. Gynecol. Reprod. Biol. (2015).

20. Riham Mohamed Alycorresponding, Current state of stem cell-based therapies: an overview, Published online 2020 May 15. doi: 10.21037/sci-2020-001.

21. K.A. Walters et al. New perspectives on the pathogenesis of PCOS: neuroendocrine origins Trends Endocrinol. Metab. (2018).

22. R. Naamneh Elzenaty et al., Basics of androgen synthesis and action, Best. Pract. Res. Clin. Endocrinol. Metab. (2022).

23. C. Torrance , E. Telfer , R. Gosden , Quantitative study of the development of isolated mouse pre-antral follicles in collagen gel culture, Reproduction 87 (1) (1989) 367–374.

24. E. Telfer , C. Torrance , R. Gosden , Morphological study of cultured preantral ovarian follicles of mice after transplantation under the kidney capsule, Re- production 89 (2) (1990) 565–571.

25. S. Joo , S.-H. Oh , S. Sittadjody , E.C. Opara , J.D. Jackson , S.J. Lee , J.J. Yoo , A. Atala , The effect of collagen hydrogel on 3D culture of ovarian follicles, Biomed. Mater. 11 (6) (2016) 065009.

26. D. Mondal , M. Griffith , S.S. Venkatraman , Polycaprolactone-based biomaterials for tissue engineering and drug delivery: Current scenario and challenges, Int. J. Polym. Mater. Polym. Biomater. 65 (5) (2016) 255–265.

27. V. Luyckx , M.M. Dolmans , J. Vanacker , C. Legat , C.F. Moya , J. Donnez , C.A. Amorim , A new step toward the artificial ovary: survival and prolifer- ation of isolated murine follicles after autologous transplantation in a fibrin scaffold, Fertil. Steril. 101 (4) (2014) 1149–1156.

28. M.C. Chiti , M.M. Dolmans , C. Lucci , F. Paulini , J. Donnez , C. Amorim , Further insights into the impact of mouse follicle stage on graft outcome in an artifi- cial ovary environment, MHR Basic Sci. Reprod. Med. 23 (6) (2017) 381–392.

29. R.M. Smith , A. Shikanov , E. Kniazeva , D. Ramadurai , T.K. Woodruff, L.D. Shea , Fibrin-mediated delivery of an ovarian follicle pool in a mouse model of in- fertility, Tissue Eng. Part A 20 (21-22) (2014) 3021–3030.

30. E. Kniazeva , A. Hardy , S. Boukaidi , T. Woodruff, J. Jeruss , L. Shea , Primordial follicle transplantation within designer biomaterial grafts produce live births in a mouse infertility model, Sci. Rep. 5 (2015) 17709.

31. A. Rajabzadeh , F. Jahanpeyma , A. Talebi , F. Moradi , H. Eimani , Fibrin scaf- fold incorporating platelet lysate enhance follicle survival and angiogenesis in cryopreserved preantral follicle transplantation, Galen Med. J. 9 (2020) 1558.

32. J.E. Hornick , F.E. Duncan , L. Shea , T.K. Woodruff, Multiple follicle culture sup- ports primary follicle growth through paracrine-acting signals, Reproduction 145 (1) (2013) Cambridge, England.

33. J. Vanacker , M.M. Dolmans , V. Luyckx , J. Donnez , C.A. Amorim , First trans- plantation of isolated murine follicles in alginate, Regen. Med. 9 (5) (2014) 609–619.

34. P.K. Kreeger , N.N. Fernandes , T.K. Woodruff, L.D. Shea , Regulation of mouse follicle development by follicle-stimulating hormone in a three-dimensional in vitro culture system is dependent on follicle stage and dose, Biol. Reprod. 73 (5) (2005) 942–950.

35. R. Gosden , Restitution of fertility in sterilized mice by transferring primordial ovarian follicles, Hum. Reprod. 5 (2) (1990) 117–122.

36. J. Carroll , R.G. Gosden , Transplantation of frozen-thawed mouse primordial follicles, Hum. Reprod. 8 (8) (1993) 1163–1167.

37. Kouchakzadeh, F., Ebrahimi-Barough, S., Aflatoonian, B., Ai, J., Mazaheri, F., Montazeri, F., Hajizadeh-Tafti, F., Golzadeh, J., Naser, R., Sepehri, M., & Kalantar, S. M. (2024). Therapeutic potential of endometrial stem cells encapsulated in alginate/gelatin hydrogel to treat of polycystic ovary syndrome. Regenerative therapy, 26, 693–707.

38. Rishi Man Chugh , Hang-soo Park , Sahar Esfandyari , Amro Elsharoud , Mara Ulin and Ayman Al-Hendy, Int. Mesenchymal Stem Cell-Conditioned Media Regulate Steroidogenesis and Inhibit Androgen Secretion in a PCOS Cell Modelvia BMP-2, J. Mol. Sci. 2021.

39. Dilogo, I.H.; Fiolin, J.; Aprianto, P. Osteogenic Potency of Secretome Bone Marrow Derived Mesenchymal Stem Cells: A Literature Review. Adv. Sci. Lett. 2018, 24, 6206–6208.

40. Glister, C.; Satchell, L.; Bathgate, R.A.D.; Wade, J.D.; Dai, Y.; Ivell, R.; Anand-Ivell, R.; Rodgers, R.J.; Knight, P.G. Functional link between bone morphogenetic proteins and insulin-like peptide 3 signaling in modulating ovarian androgen production. Proc. Natl. Acad. Sci. USA 2013, 110, E1426–E1435.

41. Dash B C, Xu Z , Lin L , Koo A, Ndon S, Berthiaume F, Dardik A and Hsia H. (2018): Stem Cells and Engineered Scaffolds for Regenerative Wound Healing Bioengineering,. 5 (23): 1-12.

42. J. Tadros, Dina H. Mohamed, Marwa M. S.Ahmed and Yasser Hefney Ahmed Hussein. Effect of Adipose-derived stem cells versus clomiphen on treatment of experimental polycystic ovary in rats: Histological and Immunohistochemical study Soad, 20 July 2018, Accepted: 09 August 2018.

43. Li H, Xu Y, Fu Q and Li C. (2012): Effects of multiple agents on epithelial differentiation of rabbit adipose-derived stem cells in 3D culture. Tissue Engineering; 18(17): 1760–70.