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Clinicopathological effects of induced pluripotent stem cells and hormonal therapy on premature ovarian failure

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ABSTRACT

This experiment was designed to investigate the roles of induced pluripotent stem cells and hormonal therapy on experimentally-induced ovarian ablation (OA) by evaluating of hormonal (E2, FSH, LH), molecular (Oct4, Runx2, Stra8), histopathological (H&E) and immunohistochemical (PCNA) examinations. Fifty female rats were classified into five main groups. Group (I): 10 rats as control normal group. Group II (OA):10 rats infused with (3 mg/kg) of DOX and (50 mg) CYP dissolved in clean physiological saline I/P once a week for 5 weeks. Group (III):10 rats go through chemoablation, after that, rats were injected with 5 IU of PMSG single subcutaneous (S/C) injection. Group (IV): 10 rats undergo chemoablation, after that induced pluripotent stem cells (iPSc) were injected intravenous (I/V) single injection for 2 months. Group (V): 10 rats undergo chemoablation then iPSc were injected I/V combined with single S/C injection of 5 IU PMSG. Results revealed that rats injected with doxorubicin and cyclophosphamide showed a significant increase in FSH, LH levels while a significant decrease in E2 level, PCNA, Oct4,Stra8 and Runx2 when compared with control (-ve) group. On the other hand, rats treated with PMSG or IPSc only or PMSG and IPSc revealed that there were increase inE2 level, PCNA, OCT4, Stra8 and Runx2 while there is decrease in FSH, LH levels when compared with their chemo-ablated control group. Also, ovarian sections of chemo-ablated group showed degenerated surface epithelia, loss of secondary and antral follicles as well as degenerated cortex and medulla. PMSG treated group revealing development of follicles in normal sequence, but in rudimentary number. Also, group was treated with iPSc revealing ovary with normal architecture harboring a high number of anular follicles. In contrast ovarian sections of group treated with PMSG and iPSc showed well observed primordial, primary, and secondary follicles.

1. INTRODUCTION

Premature ovarian failure (POF) is characterized by estrogen deficiency, reduced follicles, and elevated gonadotropin (Ana et al., 2013). The ovary is the most sensitive organ to chemotherapeutic agents (Oktem and Oktay, 2007). Previous examinations had shown the apoptotic changes in the pre-granulosa cells which results in follicular harm, injury to veins and central ovarian cortical fibrosis following cyclophosphamide (Meirow et al., 2007). Cyclophosphamide exposure prevents folliculogenesis by causing anovulation and results in infertility (Ray and Potu, 2010). Doxorubicin has been demonstrated to cause apoptosis in mature ovulated murine oocytes (Jurisicova et al., 2006). DOX caused DNA damage and apoptotic cell death in both oocytes and granulosa cells of human primordial follicles in vitro (Soleimani et al., 2011).Injection of mice with doxorubicin leads to a significant reduction in the population of secondary follicles (Ben-Aharon et al., 2010). Recently the most efficient treatment of POF is hormonal therapy. However, hormone therapy may have side effects as high cancer risk. Scientists have attempted to develop alternative therapeutics using stem cell-based strategies (Sullivan et al., 2016). Stem cells have the ability to self-renew and differentiate into specific tissues according to the surrounding environment and signals. iPSCs are artificial stem cells made by reprogramming specialized cells such as fibroblasts with limited potency (Volarevic et al., 2014).When IPSCs were transplanted into POF mice caused growth in ovarian tissues, expression of ovarian granulosa cell markers, increase inE2 hormone and reduction of atretic follicles numbers (Liu et al., 2016).

Our study was designed to evaluate the effects of iPSc on experimentally-induced ovarian ablation (OA) by evaluating FSH, LH and E2 levels, Molecular (Oct_4, $Stra_8$ and $Runx_2$) and histopathological examination, and immune-histochemical (PCNA)

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2. MATERIAL AND METHODS

2.1. Experimental design

In this study, 50 adult Wister rats were divided equally into five groups (10 rat /cage) in room temperature as following:
Group (I):10 rats as control normal group.
Group II (OA): 10 rats infused with (3 mg/kg) of DOX according to Jagetia and Lalnuntluangi (2016) and (50 mg) CYP dissolved in clean physiological saline I/P once week for 5 weeks according to Alison et al., (2010).
Group (III): 10 rats go through chemoablasion, after that, rats were injected with 5 IU of PMSG single subcutaneous (S/C) injection.
Group (IV): 10 rats undergo chemoablasion, after that induced pluripotent stem cells (iPSc) were injected intravenous (I/V) single injection for 2 months.
Group (Group V): 10 rats undergo chemoablasion then iPSc were injected I/V combined with single S/C injection of 5 IU PMSG.

2.2. Sampling

2.2.1. Serum samples

Blood was collected in plain clean well dried centrifuge tubes for separation of serum to be used for evaluation of biochemical parameters. Serum samples were obtained by centrifugation at 3000 rpm / 15 minutes. Sera were transferred into clean dry labeled Eppendorf tubes and kept in deep freezer till examination.

2.2.2. Tissue specimens

Ovary specimens were collected from all groups after scarification then preserved in neutral buffered formalin solution (10%) for histopathological and immune-histochemical examinations. 5 micron tissue paraffin sections were routinely prepared and stained with H&E after Bancroft and Stevens.(1990) Proliferating cell nuclear antigen immunoreactivity (PCNA-ir) was performed according to Tousson et al. (2011).

2.2.3. Assessment of biochemical parameters

FSH level was determined according toFSH Rodent ELISA Kits manufactured by Abnova Company (Cat No: KA2330). E2 level were measured by using Rat E2 ELISA Kits manufactured by Elabscience Company. (Cat No: E-EL-0152) and LH hormone according to Rat Luteinizing Hormone ELIS Kits manufactured by Novus Biologicals a-biotechne brand (NBP2-61257).

2.2.4. Determination of RT-PCR Detection of OCT4, Stra8 and Runx2 gene expression:

It was determined according to the RNA extraction kit was provided by Thermo Fisher Scientific Inc. Germany (Gene JET, Kit, #K0732). RT- PCR kit was provided by Bioline, UK (SensiFAST™ SYBR® Hi-ROX One-Steb Kit).

2.3. Statistical analysis

Statistical analysis was performed using the statistical software package SPSS for windows (Version 16.0; SPSS Inc., Chicago, I11). One-way ANOVA was used to determine significant differences among four experimental groups. Results are expressed as the mean & standard error (SE). LSD post a hoc at $p \leq 0.05$.

3. RESULTS

3.1. Serum biochemical findings

3.1.1. Ovarian Hormones levels:

Serum FSH, LH and E2 levels were illustrated in Table (1)

3.1.1.1. Estradiol hormone (E2) level:

Dox and CYP injected rats (OA group) showed a significant decrease in serum E2 level when compared with control (-ve) group. On the other hand, rats treated with PMSG or IPSc only or PMSG and IPSc revealed a significant increase in E2 level when compared with their OA control group.

3.1.1.2. Luteinizing Hormone (LH) and Follicular stimulating hormone (FSH) levels:

There was a significant increase in LH and FSH levels in OA group compared with control group. While, rats treated with PMSG or iPSc only or PMSG and iPSc revealed a significant decrease in LH and FSH levels compared with their OA control group.

Table 1 Effect of pluripotent stem cells treatment on FSH, LH and E2 serum levels in ovarian failure induced experimentally in female rats

Groups	FSH(ng/ml)	LH(mIU/ml)	E2(mg/ml)
Group I	1.27± 0.05^{a}	55.83±2.3^{a}	4.42±0.11^{a}
Group II	5.65±0.15^{b}	156.67±12.01^{b}	0.8±0.14^{b}
Group III	2.48±0.11^{c}	72.5±3.8^{c}	2.27±0.8^{c}
Group IV	2.23±0.10^{c}	79.17±3.9^{c}	2.28±0.8^{c}
Group V	1.48±0.07^{d}	60±3.1^{d,a}	4.25±0.14^{d,a}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript numbers in the same column are significantly different at (P≤0.05).

3.2. Molecular Results

Oct4, Runx2 and Stra8 were illustrated in table (2)

Data demonstrating that there was a significant decrease of Oct4, Runx2 and Stra8 in OA group compared with control normal group. While, there was a significant increase ofOct4, Runx2 and Stra8 in group treated with PMSG or IPSc only or IPSc and PMSG compared with their control (OA) group.

Table 2 Effect of pluripotent stem cells treatment onOct4, Stra8 and Runx-2 gene expression in ovarian failure induced experimentally in female rats.

Groups	Oct4	Stra8	Runx2
Group I	1.044±0.06^{a}	3.39± 0.09^{a}	3.68±0.08^{a}
Group II	0.81±0.01^{b}	1.09±0.03^{b}	1.15±0.04^{b}
Group III	1.33±0.05^{c}	2.29±0.09^{c}	2.29±0.05^{c}
Group IV	2.11±0.11^{d}	2.32±0.07^{c}	2.12±0.05^{c}
Group V	2.09±0.05^{d}	3.35±0.08^{a}	3.4±0.03^{d}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

3.3. Histopathological and Immunihistochemical results

3.3.1. Histopathological result

The ovary of normal group revealing normal histological architecture, the cortex contained primordial, primary, secondary, and tertiary follicles. While ovarian sections of chemoablated group showed degenerative surface epithelia, loss of secondary and antral follicles as well as degenerated cortex and medulla.PMSG treated group revealed development of follicles in normal sequence, but in low number. Also, the group treated with IPSc showed ovary with normal architecture harboring a high number of anular follicles. In contrast ovarian sections obtained from the group treated with PMSG and iPSc showed well observed primordial, primary, secondary follicles. The obtained histopathological alterations of ovary obtained from different treated groups were presented in figure 1.

3.3.2. Immunohistochemical results (PCNA)
Ovarian sections of OA group illustrated a significant decrease in PCNA compared with their control normal group. While rats treated with PMSG, iPSc, PMSG and iPSc revealed a significant increase in PCNA compared with control OA group as shown in figure 2.

4. DISCUSSION

POF is the incomplete or complete loss of regenerative and hormonal capacity of the ovaries as a result of follicular degeneration or early loss of eggs (Martin et al., 2017).Management of POF involves hormone replacement and infertility treatment, however, HRT does not restore normal ovarian functions and may increase the risk of breast cancer (Edessy et al., 2016).Transplantation of stem cells into animal of POF restores ovarian function and generates immature oocytes (Agung et al., (2006) and Liu et al., (2016). According to our biochemical results, Dox and CYP injected rats revealed decrease in E2 level, increase in LH and FSH levels. These results agree with Amira (2017), Ayat et al (2017), Abdeljabar et al and Lei et al (2018).

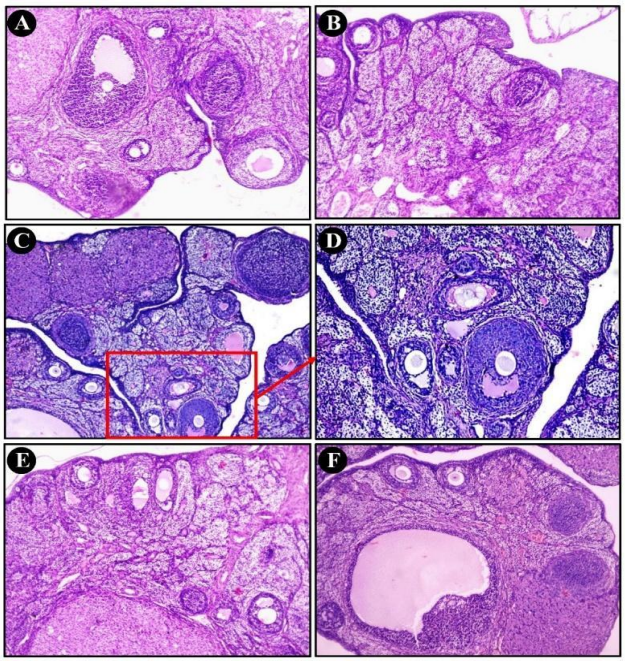

Figure 1 Photomicrograph showed H &E stained ovaries sections A) control (-ve) showing primordial (short arrow), primary (P), secondary (s) and Graafian (long arrow) follicles. (B): ovary of rats of OA group revealing absence of primary, secondary and tertiary follicles, corpus luteum is almost degenerated (long arrow). (C&D):ovary of rats treated with hormones and IPSc showing follicles of different types (primary (p), secondary (S), tertiary (T), Graafian and corpus luteum (C). (E):ovary of rats of hormonal groups revealing reactivation of folliculogenesis indicated by reappearance of primordial (arrow), primary (p) and secondary follicles (S), medulla contained congested pre follicular blood vessels. (F): ovary of rats of treated with IPSc revealing reappearance and follicular development of primary (P), secondary (S) and antral (A) follicles(H&E x 200).

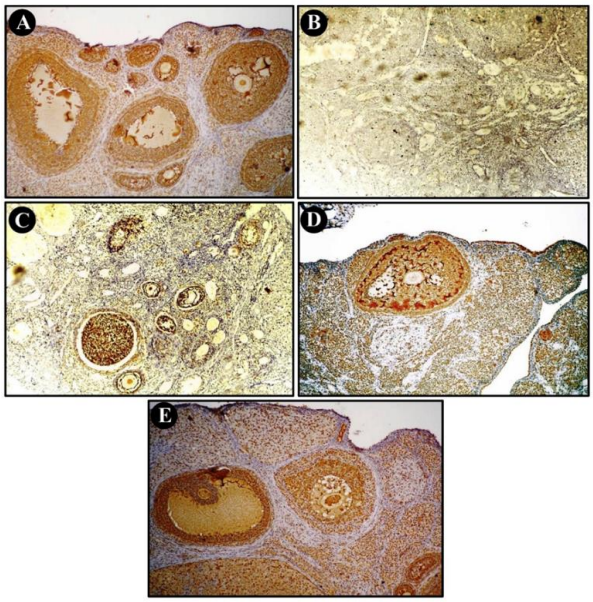

Figure 2 illustrate PCNA immunostaining in different groups. (A): ovarian section of rats of the control group showing (+) PCNA in some follicular cells (f) and (-) immunostaining in the surface epithelial cells. (B): ovarian section of rats of the (OA) group showing negative PCNA in the surface epithelial cells, follicular cells and stromal cells (S). (C): ovarian section of rats of PMSG group showing (-ve) PCNA in epithelial surface cells, some follicular cells and stromal cells. (D): ovarian section of rats of the iPSc group showing (+) PCNA in epithelial cells, follicular cells and stromal cells. (E): ovarian section of rats of the IPSc and PMSG group exhibiting negative reaction in some epithelial cells, follicular cells and positive reaction in stromal cells (PCNA immunostaining X 200).

These biochemical results confirmed by the histopathological changes in ovary of rat administered Dox and CYP as the ovary showed degenerated surface epithelium, loss of primordial, secondary, and tertiary follicles, high collagen fibers deposition in the stroma as well as degenerated cortical areas. These findings were agreed with (Fabbri et al (2016) and Fathy et al (2011). As indicated by the biochemical outcome, there was an increase in E2, decrease LH and FSH levels after treatment with stem cells. This result agree with Liu et al., (2014),Zhang et al., (2015) and Song et al., (2016) who reported that Stem cells relocate into harmed ovarian tissue and differentiated into ovarian tissue-like cells, especially into granulosa cells. Also, iPSCs might be separated into steroidogenic ovarian cells which produce chemicals as estrogen and progesterone. Concerning to Oct4, Stra8 and Runx2gene expression, there was a significant decrease in Oct4, Stra8, Runx2 in OA treated group.These data agree with Meirow et al., (2007) and Salama et al., (2013), Titus and Oktay, (2014). They stated that lower Oct4 Stra8 and Runx2 expression at both mRNA and protein levels were associated with massive apoptosis and reduction of follicles of different growth stages. Chemotherapy affects ovarian functions by rapid decrease of the oocyte reserve. On the other side, there was a significant increase of Oct4, Stra8 and Runx2 in PMSG,iPSc and PMSG and iPSc treated groups. This result agree with Zou et al(2009), Shi and Jin, (2010), Abdel Aziz et al (2011) and Hanan et al (2016) who stated that increased gene expression is related to neo-oogenesis induction in young female.

The results of the current study showed that, the treatment with chemotherapy decrease PCNA, this outcome confirmed byXu et al (2011) andAref et al (2019).Meanwhile, rats treated with PMSG, iPSc, PMSG and IPSc revealed a significant increase in PCNA compared with control group. This result was matched withChuai et al (2012), Boxian et al (2019)and Jeeyoon and Gi (2020).They detailed that implantation of IPSc have different capacities (folliculogenesis and fertilizable egg creation) in the chemotherapy-harmed ovaries. PCNA has been viewed as a proliferative marker in the granulosa cells that considered as an essential marker of growing ovarian follicles.

5. CONCLUSION

We could conclude that there were improvements in ovarian functions (hormones as FSH, LH and E2) and OCT4, Stra8 and Runx2, immunohistochemical and histopathological changes after treatment with stem cells or stem cells and PMSG compared with DOX and CYP with superiority of stem cells and PMSG together.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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6. REFERENCES

1. Abdel Aziz, M. T.; Mostafa, T.; Atta, H.; Assad, S.; Fouad, H.; Mohsen, G.; Rashed, L.; Sabry, D. and Abbas, M. (2011): In Vitro and in Vivo Lineage Conversion of Bone Marrow Stem Cells Into Germ Cells in Experimental Azoospermia in Rat. Stem Cell Studies.1:e15.
2. Agung, M.; Ochi, M.; Yanada, S.; Adachi, N.; Izuta, Y.; Yamasaki, T. and Toda, K. (2006): Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. Knee Surg Sports Traumatol Arthrosc, 14: 1307-1314.
3. Amira, F. (2017):Histological and Immunohistochemical study on the possible protective effect of hesperidin on the ovaries of adult female albino rats treated with cyclophosphamide. Journal of American Science, 13(9):31-42 Aref, D.; Masoud, D.; Ali, A. T.; Ali, M. B.; Vahid, N.; Hamed, S.; Majid, B,.; Hossein, K.; Majid, S.; Amin, A. and Mahdi, M. (2019): Upregulation of FSHR and PCNA by administration ofcoenzyme Q10 on cyclophosphamide-induced premature ovarian failure in a mouse model. J Biochem Mol Toxicol. 33:e22398. doi: 10.1002/jbt.22398
4. Ayat, A. S.; Sahar, E. M.; Mona, A. E.; Dina, S.; Aliaa, A.; Aishaa, M.; Fatma, Y. M. and Khaled, A. (2017): Exosomes Derived from Bone Marrow Mesenchymal Stem Cells Restore Cisplatin Induced Ovarian Damage by Promoting Stem Cell Survival, Meiotic, and Apoptotic Markers. Global Advanced Research Journal of Medicine and Medical Sciences. 6 (6). 116-130.
5. Bancroft JD, Stevens A. (1990) Theory and Practice of Histological Technique. 3rd Ed. Churchill Livingstone. Edinburgh, London
6. Ben-Aharon, I.; Bar-Joseph, H.; Tzarfaty, G.; Kuchinsky, L.; Rizel, S.; Stemmer, S. M. and Shalgi, R. (2010): Doxorubicin-induced ovarian toxicity. Reprod Biol Endocrinol. doi: 10.1186/ 1477-7827-8-20. doi: 10.1186/1477-7827-8-20.
7. Blumenfeld, Z. (2002): Preservation of fertility and ovarian function and minimalization of chemotherapy associated gonadotoxicity and premature ovarian failure: The role of inhibin-A and -B as markers. Molecular and Cellular Endocrinology 187 (1-2): 93-105.
8. Boxian, H.; Chunfeng, Q.;Chenyue, D.; Qingxia, M.; Qinyan, Z. and Hong, L. (2019) Fetal liver mesenchymal stem cells restore ovarian function in premature ovarian insufficiency by targeting MT1.Huang et al. Stem Cell Research and Therapy, 10:362. doi: 10.1186/s13287-019-1490-8
9. Chuai, Y.; Xu, X.; Wang, A (2012): Preservation of fertility in females treated for cancer. Int. J. Biol. Sci. 8, 1005–1012.
10. Edessy, M.; Hosni, H. N.; Shady, Y.; Waf, Y.; Bakr, S. and Kamel, M. (2016): Autologous stem cells therapy, the first baby of idiopathic premature ovarian failure. Acta Med Int 3: 19-23.
11. Fabbri, R.; Macciocca, M.; Vicenti, R.; Paradisi, R.; Klinger, F. G. and Pasquinelli, G. (2016): Doxorubicin and cisplatin induce apoptosis in ovarian stromal cells obtained from cryopreserved human ovarian tissue. Future Oncol. 12:1699-1711.
12. Fathy, Z. H.; Dorreia, A. Z.; Rasha, I. A. and Mona H. T. (2011): Effect of Cyclophosphamide on the Postnatal Development of the Ovary of the Albino Rat. Egyptian Journal of Anatomy, ~~Jul.~~ 34(2):69-84.
13. Hanan, F.; Dina, S.; Khaled, E. and Naglaa, F. (2016): Therapeutic efficacy of amniotic membrane stem cells and adipose tissue stem cells in rats with chemically induced ovarian failure. J Adv Res. 7(2): 233–241.
14. Jeeyoon, N. and Gi, J. K. (2020): Recent trends in stem cell therapy for premature ovarian insufficiency and its therapeutic potential: a review.Na and Kim Journal of Ovarian Research. 13: 74.
15. Jurisicova, A.; Lee, H. J.; Estaing, S. G.; Tilly, J. and Perez, G. (2006): Molecular requirements for doxorubicin-mediated death in murine oocytes.Cell Death Differ. 13 (9): 1466-74.
16. Lei, C.; Shilei, G.; Cui, W.; Honglan, L.; Haiya, W. and Yan, X. (2018): Effect of stem cell transplantation of premature ovarian failure in animal models and patients: A meta-analysis and case report.Experimental and Therapeutic Medicine 15: 4105-4118.
17. Liu, J.; Zhang, H.; Zhang, Y.; Li, N.; Wen, Y.; Cao, F.; Ai, H. and Xue, X. (2014): Homing and restorative effects of bone

marrow-derived mesenchymal stem cells on cisplatin injured ovaries in rats. Molecules and cells. 37(12): 865-872.

18. Liu, T.; Li, Q.; Wang, S.; Chen, C. and Zheng, J. (2016): Transplantation of ovarian granulosa like cells derived from human induced pluripotent stem cells for the treatment of murine premature ovarian failure, Mol. Med. Rep. 13 (6): 5053–5058.
19. Martin, L. A.; Porter, A. G.; Pelligrini, V. A.; Schnatz, P. F.; Jiang, X and Kleinstreuer, N. (2017): A design thinking approach to primary ovarian insufficiency. Panminerva Medica, 59 (1): 15–32. doi:10.23736/S0031-0808.16.03259-6. PMID 27827529.
20. McLaren, J. and Bates, G. (2012): Fertility preservation in women of reproductive age with cancer. Am J Obstet Gynecol, 207 (6): 455-62.
21. Meirow, D.; Dor, J.; Kaufman, B.;Shrim, A.; Rabinovici, J.; Schiff, E.; Raanani, H.; Levron, J. and Fridman, E. (2007): Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms on ovarian injury. Hum Reprod. 22: 1626–1633.
22. Morgan. S.; Anderson, R. A.; Gourley, C.; Wallace, W.H. and Spears. N. (2012): How do chemotherapeutic agents damage the ovary?Human Reproduction Update. 18(5):525-35
23. Oktem, O. and Oktay, K. (2007): Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer, 110: 2222-2229.
24. Ray, B. and Potu, B. K. (2010): Ovarian folliculogenesis Detrimental effect of prenatal exposure to cyclophosphamide: a preliminary study. Bratislava Medical Journal, 111 (7): 369-372.
25. Ana, R.; Miguel, A. C.; Maria, P. and Ramo´n, C. (2013): Medical alternatives to oocyte donation in women with premature ovarian failure: a systematic review.Gynecol Endocrinol, 29 (7): 632–637.
26. Salama, M.; Winkler, K.; Murach, K. F.; Seeber, B.; Ziehr, S. C. and Wildt, L. (2013): Female fertility loss and preservation: Threats and opportunities .Ann. Oncol, 24:598-608.
27. Shi, G. and Jin. Y. (2010): Role of Oct4 in maintaining and regaining stem cell pluripotency. Stem Cell Res Ther. 14;1(5):39.
28. Soleimani, R.; Heytens, E.; Darzynkiewicz, Z. and Oktay, K. (2011): Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise. Aging, 3: 1-12.
29. Song, D.; Zhong, Y.; Qian, C.; Zou, Q.; Ou, J.; Shi, Y.; Gao, L.; Wang, G.; Liu, Z. and Li, H. (2016): Human umbilical cord mesenchymal stem cells therapy in cyclophosphamide-induced premature ovarian failure rat model. Biomed Res Int. (6):1-13
30. Sullivan, S. D.; Sarrel, P. M. and Nelson, L. M. (2016): Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. Fertil Steril.106 (7):1588–99.
31. Titus, S. and Oktay, K. (2014): Mechanisms of chemotherapy-induced primordial follicle death in human. Presented at: Society for the Study of Reproduction 2014. Grand Rapids, MI, USA, 19–23.
32. Tousson E, Ali EM, Ibrahim W, Mansour MA. (2011) Proliferating Cell Nuclear Antigen as a Molecular Biomarker for Spermatogenesis in PTU-Induced Hypothyroidism of Rats. Reproductive Sciences 18(7): 679-686
33. Volarevic, V.; Sanja, B.; Jasmin, N.; Ana, V.; Biljana, L.; Nebojsa, A.; Majlinda, L. and Miodrag, S. (2014): Stem Cells as New Agents for the Treatment of Infertility: Current and Future Perspectives and Challenges. Biomed Res Int: DOI: 10.1155/2014/507234
34. Xu, B.; Hua, J.; Zhang, Y.; Jiang, X.; Zhang, H.; Ma, T.; Zheng, W.; Sun, R.; Shen, W.; Sha, J.; Cooke, H. J. and Shi, Q. (2011): Proliferating cell nuclear antigen (PCNA) regulates primordial follicle assembly by promoting apoptosis of oocytes in fetal and neonatal mouse ovaries.PLOS One. 6. 1–15.
35. Zhang, Q.; Xu, M.; Yao, X.; Li, T.; Wang, Q. and Lai, D. (2015): Human amniotic epithelial cells inhibit granulosa cell apoptosis induced by chemotherapy and restore the fertility. Stem Cell Res Ther 6:152. doi: 10.1186/s13287-015-0148-4
36. Zou, K.; Yuan, Z.; Yang, Z.; Luo, H.; Sun, K. and Zhou, L. (2009): Production of offspringfrom a germline stem cell line derived from neonatal ovaries. Nat Cell Biol. 11:631–6.