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Case Report

Autologous ovarian in vitro activation with ultrasound-guided orthotopic re-transplantation

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Abstract: Reduction of the number of primordial follicles, associated with cellular and molecular damage of the ovarian tissue, leads to failure of ovarian function and, ultimately, to menopause. It has been proven that the ovarian fragmentation leads to the interruption of Hippo signaling pathway, thereby leading to promotion of follicular growth. It has been shown that AKT (protein kinase B) stimulator [PTEN (phosphatase with tensin homology deleted in chromosome 10) inhibitor and phosphatidyinositol-3-kinase (PI3 kinase) stimulator] can activate dormant primordial follicles. Exposure of ovarian tissue to autologous concentrated growth factors results in interrupting Hippo signaling and stimulating AKT pathway, restoring and improving reproductive function of the ovary. Here we show that it is possible to improve reproductive function by modifying ovarian cell genetic control using autologous growth factors. A 39-year-old patient with amenorrhea and FSH 42.3 mIU/ml was diagnosed with premature ovarian insufficiency (POI). Monthly bleeding was HRT induced and she was treated for insulin resistance and hyperthyroidism. Laparoscopic biopsy of the cortical tissue of the ovary was done during cystectomy, with vitrification in April 2014 when the patient was 42 years old. For vitrification, the Kitazato protocol was used. Fragmentation of ovarian tissue was done after thawing (blockage of HIPPO signaling) in August 2015. Blood was taken for PLRP (platelet-leukocyte rich plasma) procedure. Next, autologous PLRP incubation of the obtained ovarian tissue was done (activation of AKT signaling). After 48 hrs, the processed tissue was installed subcortically in both ovaries, under the color Doppler ultrasound control. Three months after the orthotopic retransplantation of activated ovarian tissue, in November 2015, there was growth of a follicle in the spontaneous cycle, and the mature MII cell was obtained under ultrasonic aspiration. After intracytoplasmatic sperm injection (ICSI), fertilization and cleavage occurred, and the embryo was vitrified on day two, on patient's request. The authors discuss the possibilities of genetic treatment of the ovarian tissue in order to restore both reproductive and endocrine functions of the ovary. To our knowledge, this is the first case of human embryo obtained after autologous PLRP in vitro activation of ovaries by interrupting Hippo signaling and PLRP stimulating AKT pathway with ultrasound-guided orthotropic re-transplantation.

Keywords: Ovarian activation, in vitro, HIPPO, AKT, PRP, genetic treatment, transplantation, ultrasound, human embryo, in vitro fertilization

Introduction

Oogenesis is a complex process of the formation of egg cells from immature forms. This process depends on a number of factors, including the existence of immature primordial oogonia, as well as energy resources, expressed through number, structure, and function of mitochondria.

For decades it was believed that the woman's reproductive potential was entirely dependent on the size of the stock (pool) of primordial follicles in the ovary [1, 2]. The paradigm that has prevailed in the scientific world about the existence of a consistent number of primordial follicles, established during embryonic and fetal periods, was in many ways changed by works done by Tilly's group, demonstrating the existence of germline or oogonial stem cells [3].

Ovarian function is realized by creation of the egg. Primordial follicles enter further development by process of follicular activation, and

then undergo a series of developmental changes before reaching maturity. During this process, a large number of follicles enter atresia, a programmed apoptosis. Some follicles survive by making the cell cycle slower. Their dormant status is characterized by communication with surrounding granulosa cells, and numerous mechanical and chemical factors controlling the progression of their cell cycle. These factors control signaling activation of the pathways included in the primordial follicle dormant status regulation, such as Hippo and AKT signaling.

Reduction of the number of primordial follicles leads to failure of ovarian function and, ultimately, to menopause and it is associated with cellular and molecular damage of the ovarian tissue, which gradually reduces the possibility of tissue to perform its functions. Removal or correction of these defects by biotechnological regenerative processes may lead to recovery of ovarian function.

At the same time, cryopreservation of ovarian tissue with re-transplantation has long been used for the purpose of preservation of fertility in patients who must undergo gonadotoxic therapy. During the process, the ovarian cortex is routinely fragmented for more efficient vitrification and grafting [4-7].

Wedged resection has been used for a century as a treatment of PCOS (polycystic ovarian syndrome) in order to induce follicle growth. A newer version of this therapy is ovarian drilling, by diathermy or laser. Other authors suggest transvaginal ovarian trauma puncture in order to restitute the ovary function. Those reports indicate potential change of conditions of folliculogenesis by using physical methods [8].

Case report

The patient was a 42-year-old G0P0 who presented to our fertility center seeking fertility treatment. The POI diagnosis was made at the age of 39, when she was amenorrheic for 3 months and FSH before the therapy was 42.3 mIU/ml and AMH was 0.0 ng/ml. Her medical history included hyperthyroidism and insulin resistance. She had no known allergies. Medications included Estradiol/Norgestrel, Propylthiouracil, Metformin, Ubiquinone, Melatonin, and Prasterone. She denied any history of sexually transmitted infections and had a normal hysterosalpingogram and saline sonohysterogram.

In April 2014, the 42-year-old patient underwent hysteroscopy with polypectomy, and laparoscopy with bilateral ovarian cystectomy and biopsies of the peritoneum and omentum. The histopathological finding was fibroglandular polyp and bilateral endometrioses.

At the time of surgery, a part of the cortical tissue of the ovary was cryopreserved using the Kitazato protocol, and the partner's semen was frozen. Kitazato Vitrification media (Basic solution - BS, Equilibration solution - ES and Vitrification solution - VS; Kitazato BioPharma Co.) was used for ovarian tissue cryopreservation. We used a modified protocol for oocyte vitrification. Briefly, finely chopped ovarian tissue was plated at center well dish and washed in BS for 5-10 min at room temperature. After washing, tissue samples were transferred in ES and incubated for 45 min at 4 C followed by 10 min of incubation in VS. Finally, samples were collected in the vitrification straws, and then stored in liquid nitrogen at -196 C.

In the next 18 months the patient had follicle monitoring in spontaneous IVF cycles. On one occasion, a mild stimulation was used with 75 IU FSH/LH, daily, from the follicle size of 12 mm. The growth of the follicle has not been registered during the time monitored.

Procedure-ovarian in vitro activation

In August of 2015, 16 months after the cryopreservation of the ovarian cortical tissue, the in vitro autologous platelet/leucocyte rich plasma (PLRP) activation of thawed ovarian tissue was performed, followed by orthotopic retransplantation under sonographic guidance into both ovaries. The procedure was approved by the institutional IRB No 63/295/2015.

To thaw vitrified ovarian tissue, the Kitazato thawing media kit was used (Kitazato Biopharma Co., Japan). It contains Thawing solution - TS, Diluent solution - DS and Washing solution - WS. Protocol used for tissue thawing was modified protocol for thawing vitrified oocytes and embryos: pieces of vitrified tissue were immersed in warmed (37 C) TS for 3 min. After that, they were transferred and incubated

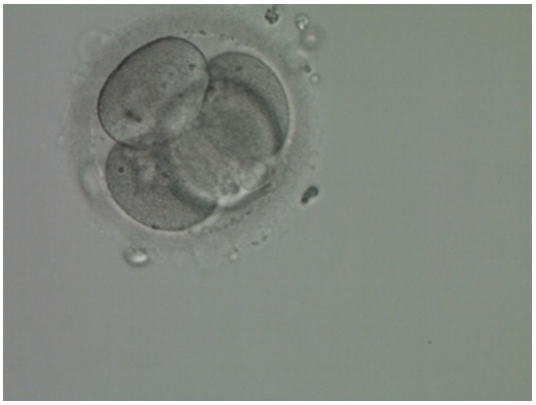

Figure 1. The picture of 2 cell embryo obtained after In vitro ovarian tissue autologous PLRP activation, graded 4A1.

in DS for 9 min and then washed twice in WS (15 and 3 minutes). When the thawing procedure was completed, pieces of ovarian tisue were transferred in a fresh culture medium and left in an incubator for 3 h for complete recovery.

For Platelet/leucocyte rich plasma (PLRP) preparation process and laboratory analyses Wholeblood was withdrawn from the cubital vain and was anticoagulated with acid citrate dextrose formula A (ACD-a) in a 7:1 ratio. Two 60 mL syringes were taken and filled up with blood up to 60 mL, making a total volume of 104 mL of whole blood and 16 mL of ACD-a. After withdrawal, the whole blood was processed using the Angel whole blood separation system, (Arthrex, USA). Separation of blood is fully automated and did not require any assistance during process. After separation, 24 mL of red blood cells (RBC), 3 mL of platelet rich plasma (PRP) and 30 mL of platelet poor plasma (PPP), as end products were obtained. After the platelet count, PRP with PPP up to 5 mL was diluted. This gave s 4.19 × platelets baseline and 0.48 × leukocytes baseline with the same percentage of monocytes. Autologous thrombin is produced from 12 mL of autologous PPP, using Arthrex ActivAT set (Arthrex, USA). The end product was 6 mL of autologous thrombin. Activation was performed in order to release growth factors from the platelets.

The volume of PRP (5 mL) was mixed with thawed ovarian fragments. Just before application, PRP was activated by autologous thrombin in ratio 10:1. Maximal time interval from activation to the end of instillation in ovarium was 45 seconds. Activation was done for each ovary separately. Amount of PRP for each ovary was calculated according to the ovarian volume. We injected 2.1 mL and 2.3 mL of activated PRP in the left and right ovary, respectively. Remained volume (0.6 mL) was discarded. The previously thawed ovarian tissue was fragmented to 1 × 1 × 1 mm. PLRP was activated with previously tested thrombin. The fragmented ovarian tissue was incubated in PLRP for 2 hours.

Mapping of the ovarian vessels was done by the 3D color ultrasound GE Voluson 730 Pro. Ultrasound guided transvaginal puncture of both ovaries was performed in general anesthesia, with the 16 G needle, 30 cm long. Fragmented ovarian tissue with PLRP was injected into the into the subcortical ovarian region of the right and left ovary (2.3 ml and 2.1 ml, respectively).

During September 2015, follicular activity was noticed, and in October the patient had the puncture with an empty follicle - no oocyte was obtained. In November 2015 (three months after the in vitro activation), during the spontaneous cycle, follicle growth was observed. On November 22nd, day 12, the endometrium was 11 mm, with 15 mm follicle on the left ovary. Hormonal levels were: E2 - 297.3 pg/ml, LH - 7.75 mIU/ml, Pg - 0.6 ng/ml. On November 23rd, at midnight, 250 micrograms choriogonadotropin alfa (Ovitrell, Merck Serono) were administered. Oocyte pick up was performed on November 25th, at 10.40 AM and one MII oocyte was obtained. Intracytoplasmatic sperm injection (ICSI) was performed. Normal fertilization and cleavage occurred. The embryo was graded 4A1, vitrified on day 2, on patient's demand. (**Figure 1**). During next three months in 2016, the patient did not have follicle growth.

Discussion

Here we present a case of an early menopausal patient for whom ovarian cortex was frozen, thawed, treated with platelet-leukocyte rich plasma (PLRP) isolated from the same patient, and then transplanted into her menopausal ovaries. Two months after the procedure, follicule formation was noted and egg retrieved resulting in a single embryo.

To our knowledge this is the first report that oocyte originating in human ovarian tissue

after receiving in vitro follicule activation, was capable of producing an embryo.

Recent studies have shown that female germline stem cells still exist in mammalian ovaries after birth. In 2004, animal studies showed that the ovaries possess rare female germline or oogonial stem cells (OSCs), which could generate oocytes and offspring [3]. Moreover, cases were reported of women who had regained their fertility after losing it due to cancer therapy. It had been reported that women who had received bone marrow transplants, and who had months to years of suffering from infertility and postmenopausal symptoms, went on to spontaneously begin cycling again. Additionally, several cases of spontaneous pregnancies were reported. Where were the eggs coming from? There were two possibilities. One was that factors in the bone marrow transplants were contributing growth factors and other molecules that were resuscitating inactivated follicles, allowing them to release a supply of egg cells that were secretly lying dormant within the ovaries. The other more exciting possibility, is that the transplanted cells were homing in to the follicles and regenerating their capacity to produce completely new egg cells. The OSC lie dormant in aging women, waiting to be revitalized with the right combination of cells or signaling factors. This would be expected to extend reproductive lifespan and postpone or reverse menopausal symptoms, so long as the body can continue to generate new OSC.

In the past few years there have been many insights about intra-cellular communication between oocytes and granulosa cells, as well as extra-follicular cells and other developing follicles, all necessary for oogenesis.

Our hypothesis has been that in vitro interruption of Hippo signaling, coupled with activation of Act signaling, would restore germ cell recruitment in autologously transplanted menopausal ovarian cortex.

Our rationale is based on studies in genetically modified mice [9]. that explored the Pi3K/AKT signaling, after the identification of downstream transcription factors of forkhead box O3a (Foxo3a) as the main regulator and suppressor of the activation of primordial follicles [10]. The signals induce pyruvate dehydrogenase kinase isoenzyme 1 (Pdk1)/AKT/ribosomal protein S6 kinase polypeptide 1 (S6K1)/ribosomal protein S6 (rpS6) signaling with the primordial oocytes follicle. Pdk1 is a Pi3K-dependent protein kinase that binds PI3K-generated 3, 4, 5-triphosphate (Pip3) in order to co-activate AKT phosphorylation of T308 residue [11].

Biotechnological methods of tissue engineering are trying to replace organs or tissue, or modify them. For example, it was shown that the ovarian fragmentation leads to the interruption of Hippo signaling pathway, which leads to promotion of follicular growth [12, 13]. The Hippo signaling contains several negative growth regulators, which operate on a kinase cascade, ultimately phosphorylating and inactivating key Hippo signaling effectors, Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ).

When there is an interruption of Hippo signaling, reduction of YAP phosphorylation enhances its nuclear levels. YAP works with TEAD (Transcriptional enhancer factor TEF-1 or TEA domain family member) by means of transcription factors to increase CCN (cysteine-rich angiogenic protein 61 or CCN1, CTGF connective tissue growth factor, or CCN2, and NOV nephroblastoma overexpressed or CCN3) growth factors and BIRC (baculoviral inhibitor of apoptosis repeat containing). CCN proteins stimulate cell growth, survival, and proliferation [14]. Ovarian fragmentation leads to increased actin polymerization, reduction of phospho-YAP (pYAP) levels, increase of YAP nuclear content, and increased expression of CCN growth factors and BIRC apoptosis inhibitors. These effects may be partially blocked by CCN2 antibodies and verteporfin, (blocks YAP interaction with TEAD transcriptional factors [15].

It has been shown that AKT (protein kinase B) stimulator [PTEN (phosphatase with tensin homology deleted in chromosome 10) inhibitor and phosphatidyinositol-3-kinase (PI3 kinase) stimulator] can activate dormant primordial follicles [16]. The PI3K/AKT signaling pathway has a key role in initiation of follicle growth and hence maintenance of the oocyte pool, as oocytes deficient in FOXO3 (substrate for AKT and inducing cell cycle arrest) and PTEN (inhibiting PI3K/AKT pathway) both result in global primordial follicle activation.

Our study design and methods also rely on Kawamura's group report showing that fragmentation of mouse ovaries leads to actin polymerization and interruption of Hippo signal [12]. Disruption of this signaling pathway leads to the increased expression of downstream growth factors, promotion of follicle growth, and maturation of oocytes. This group reported on 27 premature ovarian failure (POF) patients. After laparoscopic ovariectomy, parts of the ovarian cortex were vitrified. After thawing, in the framework of preparation for autografting into mesosalpinx, the fragmentation and exposure of AKT by simulating substances (PTEN [phosphatase with TENsin homology deleted in chromosome 10) inhibitor and phosphatidyinositol-3-kinase (PI3 kinase) stimulator] was done over the course of two days [17].

Laparoscopic auto-transplantation of activated tissue in mesosalpinx was done in this study. Follicular growth was observed in eight patients in whom there were residual follicles in the ovarian tissue. Aspiration was used to obtain egg cells, ICSI fertilized embryos, and vitrified embryos. After the transfer three pregnancies occurred, one of which ended in miscarriage, while the two children born with normal outcome, by 2015 [17]. The application of PLRP in ovarian rejuvenation and the return of the biological clock, reversion of the menopause, was published in 2016 [18]. Plasma is the liquid component of blood which normally contains thrombocytes thrombocytes and lymphocytes, clotting factors, dissolved proteins and electrolytes. Thrombocytes have no cell nucleus, but contain around one thousand small granule (alpha, lambda, delta) signal proteins. In 50 to 80 alpha granules per thrombocyte, there are almost 30 types of growth factors (FR) connected with hemostasis, wound healing, and repairing.

In PRP the thrombocyte concentration is 5 to 8 times higher than in physiological plasma. Thrombocyte response is important for the initiation of wound repair. Besides the function in hemostasis, thrombocytes are an important source of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b) 1 and 2, and vascular endothelial growth factor (VEGF). Adding calcium chloride and thrombin to PRP automatically activates alfa granules. This activation leads to the release of the above mentioned biological growth factors, PDGF, TGF-b, VEGF, insulin-like growth factor I, epidermal growth factor (EGF) and epithelial cell growth factor. A number of studies have shown the important role of leukocytes in PRP. On one hand, they are important for fighting infection, and on the other, for immunoregulation. Leukocytes produce large amounts of VEGF. Considering that thrombocytes are rich in VEGF stimulators, additional VEGF of leukocyte origin can be crucial for angiogenesis.

Therapy with growth factors, obtained by activation of autologous platelets and leukocytes, has been used increasingly over the last decade in various fields of medicine [19].

Most of the growth factors contained in PLRP, such as, Platelet-derived growth factor (PDGF), transforming growth factor (TGF)-beta, platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), insulin-like growth factor (IGF-1), and platelet factor 4 (PF-4), vascular endothelial growth factors (VEGF), and endothelial growth factors (EGF) stimulate cell proliferation, matrix remodeling and angiogenesis [20-25].

The PDGF family, consisting of hetero or homodimers of A, B, C and D chains, activates receptors α and β tyrosine kinase. Intracellular signaling pathways include Ras-MAPK, PI3K, and PLCγ. Receptor activation leads to initiation of the cell cycle, DNA synthesis and cell division [26-28].

This signaling pathway is involved in many cellular processes, including cell growth, differentiation, and apoptosis, among others. TGFβ ligands bind to the receptor II which recruits and phosphorylates a receptor type I that subsequently phosphorylates receptor-regulated SMADs (R-SMADs, which bind coSMAD with SMAD4. These complexes accumulate in the nucleus, where they act as transcription factors that regulate the expression of target genes.

Keeping in mind the important biomechanical HIPPO control, F-actin polymerization changes can be seen in a short period of time. This subject is well covered in a review article by Cheng et al, emphasizing the possibility of treatment with actin polymerization factors (sphingosine-1-phosphate), instead of physical tissue fragmentation. It is supposed that PLRP factors, as mitogen stimulators, could

contribute to HIPPO blocking. It is proven that EGF, IGF, serum and LPA, inhibit Hippo-pathway signaling. These factors lead to nuclear accumulation of Yorkie/YAP, which then activates growth-related genes. Yorkie/YAP is required for growth-factor-induced proliferation. This suggests that inhibition of Hippo pathway signaling is a crucial component of mitogenic signaling.

There are other mechanisms that mediate the regulation of Hippo-YAP signaling. EGF, serum and LPA, stimulating the nuclear accumulation of YAP, lead to a novel downstream effect of PI 3-kinase (PI3K) and PDPK1 (PDK1) on the Hippo pathway. This action is independent of AKT signaling. PDK1 interacts with the core Hippo kinase complex, and stimulation of PI3K by EGF causes the complex to dissociate. This dissociation impairs the ability of Lats to phosphorylate YAP [30].

There is a positive effect of the cell to cell contact inhibition on the Hippo-YAP signaling. This elucidates the spatial control of mitogenic signaling by growth factors. In the case of a lower degree of cell to cell contact shifts, a lower concentration of growth factors is required to elicit the same response (proliferation) [31].

The relationship between PLRP factors and AKT signalization has been the subject of several papers. Bone marrow stem cells have been analyzed with platelet-rich clot releasate (PRCR). Positive effect of PRCR on reduction of apoptosis in the increased oxidative stress and nutrients deficiency conditions. The stimulation of PDGFR)/PI3K/AKT/NF-κB signalization was evidenced, and the positive effect of PRCR preconditioned BM-MSC by way of of increased transplantation, epithelialization, and blood vessel regeneration. The AKT signaling pathway was stimulated with PRCP. [32] The influence of PRP on Adipose-Derived Mesenchymal Stem Cells (ASC) was the subject of recent Atashi's paper. The expansion of the ASC was proved after 10 days of 20% autologous PRP). The presence of PDGF-AB, FGF, TGFβ, VEGF, as well as MIF in PRP. Activation of AKT, ERK and Smad2 was proven [33].

To our knowledge, this is the first case of human embryo obtained by Autologous PLRP in Vitro activation of ovarian tissue by interrupting Hippo signaling and PLRP stimulating AKT pathway with ultrasound-guided orthotropic retransplantation. We are encouraged to continue this line of research to establish the origin of the oocyte, achieve greater number of follicles and ultimately treat more patients.

Declaration of conflict of interest

None.

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References

[1] Richards JS and Pangas SA. The ovary: basic biology and clinical implications. J Clin Invest 2010; 120: 963-972.

[2] Richards JS and Pangas SA. New insights into ovarian function. Handb Exp Pharmacol 2010; 198: 3-27.

[3] Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428: 145-50.

[4] von Wolff M, Donnez J, Hovatta O, Keros V, Maltaris T, Montag M, Salle B, Sonmezer M, Andersen CY. Cryopreservation and auto transplantation of human ovarian tissue prior to cytotoxic therapy—a technique in its infancy but already successful in fertility preservation. Eur J Cancer 2009; 45: 1547-1553.

[5] Gamzatova Z, Komlichenko E, Kostareva A, Galagudza M, Ulrikh E, Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E. Differentiation factor-9. Mol Endocrinol 1995; 9: 131-136.

[6] Zubareva T, Sheveleva T, Nezhentseva E, Kalinina E. Autotransplantation of cryopreserved ovarian tissue—effective method of fertility preservation in cancer patients. Gynecol Endocrinol 2014; 30: 43-47.

[7] Donnez J, Dolmans MM. Cryopreservation and transplantation of ovarian tissue. Clin Obstet Gynecol 2010; 53: 787-796.

[8] Siristatidis C, Vogiatzi P, Bettocchi S, Basios G, Mastorakos G, Vrachnis N. Transvaginal ovarian trauma, poor responders and improvement of success rates in IVF: anecdotal data and a hypothesis. Medical Hypotheses 2014; 83: 227-231.

[9] Sobinoff AP, Sutherland JM, Mclaughlin EA. Intracellular signalling during female gametogenesis. Mol Hum Reprod 2013; 19: 265-278.

[10] Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle ac-

tivation in mice by the transcription factor Foxo3a. Science 2003; 301: 215-218
[11] Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006; 7: 606-619.
[12] Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, Ho CH, Kawamura N, Tamura M, Hashimoto S, Sugishita Y, Morimoto Y, Hosoi Y, Yoshioka N, Ishizuka B, Hsueh AJ. Hippo signaling disruption and AKT stimulation of ovarian follicles for infertility treatment. Proc Natl Acad Sci U S A 2013; 110: 17474-9.
[13] Halder G, Johnson RL. Hippo signaling: growth control and beyond. Development 2011; 138: 9-22.
[14] Holbourn KP, Acharya KR, Perbal B. The CCN family of proteins: structure-function relationships. Trends Biochem Sci 2008; 33: 461-473.
[15] Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, Liu JO, Pan D. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev 2012; 26: 1300-1305.
[16] Li J, Kawamura K, Cheng Y, Liu S, Klein C, Liu S, Duan EK, Hsueh AJ. Activation of dormant ovarian follicles to generate mature eggs. Proc Natl Acad Sci U S A 2010; 107: 10280-10284.
[17] Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, Morimoto Y, Kawamura K. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. Hum Reproduct 2015; 30: 608-615.
[18] Hamzelou J. Reversing the menopause. New Scientist 2016; 3083: 8-9.
[19] Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 1997; 55: 1294-1299
[20] Mannaioni PF, Di Bello MG, Masini E. Platelets and inflammation: role of platelet-derived growth factor, adhesion molecules and histamine. Inflamm Res 1997; 46: 4-18.
[21] van den Dolder J, Mooren R, Vloon AP, Stoelinga PJ, Jansen JA. Platelet-rich plasma: quanti cation of growth factor levels and the effect on growth and differentiation of rat bone marrow cells. Tissue Eng 2006; 11: 3067-3073.
[22] Sharif PS, Abdollahi M. The role of platelets in bone remodeling. Inflamm Allergy Drug Targets. 2010; 9: 393-399.
[23] Bowen-Pope DF, Raines EW. History of discovery: platelet-derived growth factor. Arterioscler Thromb Vasc Biol 2011; 31: 2397-2401.
[24] Pintucci G, Froum S, Pinnell J, Mignatti P, Rafii S, Green D. Trophic effects of platelets on cultured endothelial cells are mediated by platelet-associated fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF). Thromb Haemost 2002; 88: 834-842.
[25] Salgado R, Benoy I, Bogers J, Weytjens R, Vermeulen P, Dirix L, Van Marck E. Platelets and vascular endothelial growth factor (VEGF): a morphological and functional study. Angiogenesis 2001; 4: 37-43.
[26] Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev 2008; 22: 1276-312.
[27] Heldin CH, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999; 79: 1283-316.
[28] Deuel TF, Senior RM, Huang JS, Griffin GL. Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. J Clin Invest 1982; 69: 1046-9.
[29] Cheng Y, Feng Y, Jansson L, Sato Y, Deguchi M, Kawamura K, Hsueh AJ. Actin polymerization-enhancing drugs promote ovarian follicle growth mediated by the hippo signaling effector YAP. FASEB J 2015; 29: 2423-30.
[30] Gumbiner BM, Kim NG. The Hippo-YAP signaling pathway and contact inhibition of growth. J Cell Sci 2014; 127: 709-17.
[31] Kim SH, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. J Endocrinol 2011; 209: 139-151.
[32] Peng Y, Huang S, Wu Y, Cheng B, Nie X, Liu H, Ma K, Zhou J, Gao D, Feng C, Yang S, Fu X. Platelet rich plasma clot releasate preconditioning induced PI3K/AKT/NFκB signaling enhances survival and regenerative function of rat bone marrow mesenchymal stem cells in hostile microenvironments. Stem Cells Dev 2013; 22: 3236-51.
[33] Atashi F, Serre-Beinier V, Nayernia Z, Pittet-Cuénod B, Modarressi A. Platelet rich plasma promotes proliferation of adipose derived mesenchymal stem cells via activation of AKT and smad2 signaling pathways. J Stem Cell Res Ther 2015; 5: 301.