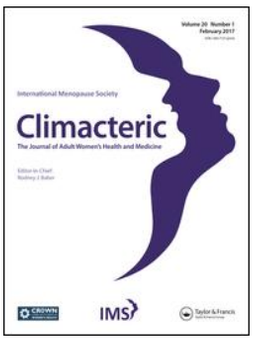

Climacteric

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icmt20

Novel approaches to fertility restoration in women with premature ovarian insufficiency

R. Rosario & R. A. Anderson

To cite this article: R. Rosario & R. A. Anderson (2021): Novel approaches to fertility restoration in women with premature ovarian insufficiency, Climacteric, DOI: 10.1080/13697137.2020.1856806

To link to this article: https://doi.org/10.1080/13697137.2020.1856806

Published online: 11 Jan 2021.

CLIMACTERIC
https://doi.org/10.1080/13697137.2020.1856806

Taylor & Francis
Taylor & Francis Group

REVIEW

Novel approaches to fertility restoration in women with premature ovarian insufficiency

R. Rosario and R. A. Anderson

MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

ABSTRACT

The diagnosis of premature ovarian insufficiency (POI) brings with it the loss of fertility, an immediate concern for many affected women, and a future one for many others. While there is a low natural conception rate, for most the choice is between oocyte donation and alternative methods of family building such as adoption. There is, however, a lot of research into novel methods for increasing or restoring the fertility of women with POI, which are discussed in this review. Many approaches involve the use of mesenchymal stem cells, from a variety of sources including bone marrow, placenta and umbilical cord, and menstrual blood. These seem to have efficacy in animal models of POI, although through unclear mechanisms. Activation of remaining primordial follicles is also being explored, through physical or chemical manipulation of key regulatory pathways, notably the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and Hippo pathways. Much of the clinical data are uncontrolled, and mostly in women with a reduced ovarian reserve rather than POI, as are the results thus far for administration of platelet-rich plasma. Clinical studies with appropriate controls are needed to substantiate the preliminary claims of effectiveness of these approaches.

ARTICLE HISTORY
Received 2 November 2020
Revised 13 November 2020
Accepted 22 November 2020
Published online 11 January 2021

KEYWORDS
Premature ovarian insufficiency; treatment; mesenchymal stem cell; platelet-rich plasma; primordial follicle activation

Introduction

Loss of fertility can be the main presenting issue for women with premature ovarian insufficiency (POI), or in younger women will be one of their main concerns for the future. Other than oocyte donation, there are no established treatments to increase the chance of conception, although many approaches have been investigated[1–3]. Some women, especially those in the early stages of the condition, will continue to ovulate erratically and thus there is the possibility of natural conception: estimates for the chance of this vary widely in the literature but the more controlled studies indicate that only about 5% of women with POI will conceive naturally[2,4]. Reflecting the nature of the condition with a critical loss of follicles at all stages, most of these conceptions will occur within a year of diagnosis, but pregnancies have been reported many years later[5]. This indicates the remaining presence of some primordial follicles, at least in some patients, which may be supported to develop right through to ovulation, and this has been confirmed histologically[6]. In this review, we discuss emerging approaches that may provide new opportunities for women with POI in the future, mostly based on the concept of activation of residual primordial follicles (summarized in Figure 1). The alternative possibility, that follicles can be formed in postnatal and adult life following neo-oogenesis, has been proposed[7] but this concept, and the existence of germline stem cells within the ovary, remains very controversial: a recent review[8] summarizes current data and this is not covered further here.

Mesenchymal stem cell-based therapeutics

Several avenues that are being explored as alternative treatment options for women with POI are in the field of stem cell-based therapeutics. Stem cells are unique as they have the ability to self-renew and differentiate into specific tissues according to their surrounding environment and signals, and many studies are investigating their applicability in the treatment of reproductive diseases. This is often combined with administration of platelet-rich plasma, as a potential rich source of growth factors, although its undefined composition can make interpretation difficult. Embryonic stem cells are the most pluripotent of all stem cells and possess the ability to differentiate into any type of human cell needed for therapeutic purposes; however, the requirement for their isolation from the inner cell mass of the blastocyst is limiting for both research and, potentially, treatment. This is circumnavigated in scientific research with the use of induced pluripotent stem cells and mesenchymal stem cells (MSCs). Induced pluripotent stem cells are artificial stem cells derived from adult cells that have been reprogrammed back into an embryonic-like pluripotent state; therefore, these cells are the most comparable to embryonic stem cells with regards to their self-renewal and differentiation potential. However,

CONTACT R. A. Anderson Richard.Anderson@ed.ac.uk MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

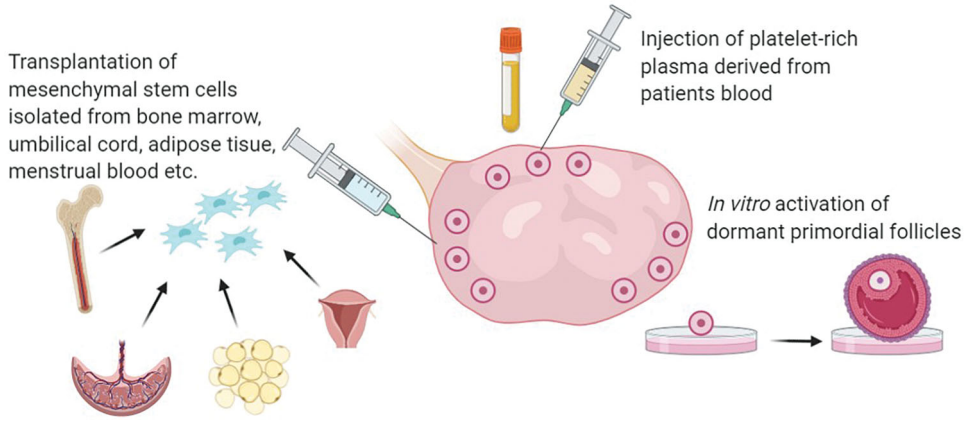

Figure 1. Summary of emerging approaches that may provide new opportunities for women with premature ovarian insufficiency in the future.

their genomic stability is still questionable[9]. MSCs are adult stem cells and can be derived from numerous adult tissues including the bone marrow, adipose tissue, peripheral blood mononuclear cells, amniotic fluid, placenta, and, most recently, menstrual blood, which has been used in multiple studies because of its relative convenience as well as potential for autologous use. Although multipotent rather than pluripotent, MSCs are advantageous as they are obtained by minimally invasive procedures, do not raise major ethical concerns, have low immunogenicity and limited immunomodulatory function, display homing to sites of damaged tissue for repair, and can be expanded *in vitro* using standard cell culture protocols with exceptional genomic stability; MSCs thus have great potential in regenerative medicine[10].

Numerous studies have verified the restorative potential of MSC transplantation/infusion in animal models of POI. In these investigations, human MSCs have been derived from various adult tissues and tested in rodent models of POI that are chemically induced with administration of busulfan and cyclophosphamide, thus mimicking the effects of chemotherapy treatment in women. Naturally aged and knockout models are also used, the latter being particularly useful for studying known or proposed genetic causes of POI. In these models, the therapeutic effect of MSCs is assessed via a range of aspects of ovarian function including follicle development, granulosa cell apoptosis, neoangiogenesis, serum hormone levels, and, most importantly of course, pregnancy rates. Indeed, folliculogenesis and recovery of serum hormone levels (follicle stimulating hormone, anti-Müllerian hormone, and estradiol) have been achieved in chemically induced models of POI following treatment with MSCs extracted from bone marrow[11], peripheral blood mononuclear cells[12], adipose tissue[13], umbilical cord[14,15], and amniotic fluid[16]. MSCs have been found to secrete growth factors including vascular endothelial growth factor, insulin-like growth factor-1, and hepatocyte growth factor into culture medium[17], which could enhance folliculogenesis through improvements in the microenvironment[18]. Similarly, through upregulation of the anti-apoptotic gene B-cell lymphoma-2 (Bcl-2)[18], these cytokines may inhibit granulosa cell and stromal cell apoptosis. This has also been found following administration of human placental[19] and menstrual blood[20] MSCs, via modulation of the stress inositol-requiring enzyme-1α (IRE1α) and extracellular matrix-dependent FAK–AKT signaling pathways, respectively. In a recent article, exosomes (extracellular vesicles that carry cargo composed of proteins, DNA, and RNA species such as microRNAs) isolated from MSCs improved the proliferation and suppressed the apoptosis of cyclophosphamide-damaged human granulosa cells that had been transplanted into mouse ovaries[21]. This was shown to be mediated by miR-17-5p, which was overexpressed in MSC-derived exosomes and is a critical regulator of the G1/S phase cell cycle transition. While experiments have determined roles of the MSC secretome (which includes secreted growth factors and exosomes as already described), conditioned media from *in vitro* cultured MSCs is less efficient at restoring ovarian function in animal models of POI[16,22], highlighting the importance of the stem cells themselves. However, the underlying mechanisms by which MSC transplantation promotes ovarian function are unclear and need further investigation.

Despite the overwhelmingly positive evidence gained from exploring the restorative potential of human-derived MSCs in animal POI models, there has been little published in the way of human studies and robust clinical trials. In fact, the majority of work in this area has been largely preliminary, observational, and uncontrolled; thus, with the well-established possibility of unexpected ovulation and pregnancy, many such studies require extreme caution in interpretation. In one such study, investigators assessed the therapeutic potential of autologous MSC transplantation by laparoscopically injecting stem cells derived from bone marrow into the ovaries of 10 women with POI[23]. Study participants were followed up monthly for 1 year, within which two women resumed menstruation 3 months after MSC transplantation, and one became pregnant and delivered a healthy baby at term. The use of collagen has been explored to increase the long-term retention of MSCs delivered to compromised ovaries, as collagen scaffolds support cell attachment, proliferation, and differentiation of stem cells, and have been shown to improve folliculogenesis and fertility in POI rodent models following transplantation of adipose tissue-derived MSCs[24]. Women with POI were randomized into two treatment groups: one group received transplantation of umbilical cord-derived MSCs directly into the ovary via intraovarian injection ($n = 6$, two patients in this group

dropped out), whilst the second group received the same stem cell transplant combined with transplantation of collagen scaffolds ($n = 8$)[25]; there was thus no control group. The stem cells were injected directly into the ovary under transvaginal ultrasonographic guidance, as this has been shown to minimize cellular diffusion to other organs[26]. Three months after the transplantation, one of the six patients in the MSC group and five of the eight patients in the MSC/collagen group showed signs of follicle-like structures by ultrasound and one woman from each group conceived naturally within 1 year of treatment. Whilst the addition of collagen did not appear to have any benefits in this small study, complementary studies carried out by this group in mice demonstrate that collagen/umbilical cord-derived MSCs facilitated primordial follicle activation via phosphorylation of Forkhead box O protein-3A (FOXO3A) and FOXO1 transcription factors, thus alluding to a mechanism of action of umbilical cord MSCs and a premise for use of collagen in the future. The restorative potential of bone marrow-derived MSCs in women with POI was also studied in a robustly controlled study by Herraiz *et al*. As a proof-of-concept study, human ovarian tissue from women identified as poor responders in previous *in vitro* fertilization (IVF) cycles (i.e. not women with POI) was xenografted into immune-deficient mice[27]. Although not clear on the route of delivery, these mice subsequently received infusions of human bone marrow-derived MSCs and their $CD133^+$ fraction, the most immature of the hematopoietic progenitor cells that can promote cell proliferation and neoangiogenesis[28]. Engraftment of these cells close to follicles promoted follicular growth to the secondary stage and increased estrogen secretion. The infusion also increased ovarian stroma proliferation and blood vessel formation, while decreasing apoptosis, raising the possibility that promoting ovarian angiogenesis by bone marrow-derived MSC infusion could be an alternative approach to improve follicular development in women with impaired ovarian function[27]. On the basis of these findings, Herraiz *et al*. conducted a prospective pilot study (registered clinical trial NCT0220342) where MSCs derived from the patient's own bone marrow were delivered directly into the ovarian artery in one ovary via an intra-arterial catheter, whilst the contralateral ovary served as a control[29]. Improved ovarian function was observed in 81.3% of patients as measured by antral follicle counts and/or serum anti-Müllerian hormone, and this benefit was observed within 4 weeks of treatment. During controlled ovarian stimulation, autologous transfer of MSCs increased the number of antral follicles and of oocytes collected, but the embryo euploidy rate was low (16.1%). However, using this approach, in the 17 study participants who were diagnosed as poor responders according to European Society of Human Reproduction and Embryology (ESHRE) criteria, five pregnancies were achieved, three of which were natural conceptions, and three healthy babies were born after MSC administration. While promising, it is important to note that the women in these studies did not have established POI so extrapolation to this condition cannot be made.

Although initial findings from these studies appear promising, there remain ethical concerns and technical issues surrounding MSC use in humans. Despite the therapeutic effects of MSCs having been shown by various studies in animal models, it is also difficult to extrapolate these findings to human patients. In particular, differences in the immune system can affect the immunogenicity of transplanted cells, which may elicit autoimmune responses not evident in animals. Similarly, POI is induced in animals via various chemicals that may not accurately recapitulate the human condition in many women with POI, and chemically induced damage (which generally does not result in complete loss of follicles) may spontaneously resolve depending on the timing of cessation of chemical exposure in relation to the experimental time scales. Other areas of concern include the invasiveness of direct transplantation of MSCs into the patient, the route and methodology used for MSC administration, and what constitutes a therapeutic dose of MSCs. Such issues should be evaluated in further *in vivo* studies and clinical trials, and care should be taken to ensure such studies are robustly controlled, so that valid conclusions can be made from their findings.

Platelet-rich plasma

Platelet-rich plasma is a preparation of autologous human plasma with an increased platelet concentration produced by centrifuging a large volume of a patient's own blood. This process also concentrates the multitude of growth factors and cytokines produced by platelets, and can thus potentially augment the healing process at a site of injury; this has been utilized in a range of medical conditions including musculoskeletal injury[30] and cosmetic dermatology[31]. Co-transplantation of adipose-derived MSCs and platelet-rich plasma into a chemotherapy-induced POI rodent model improved follicle counts by 63% compared to the control group; however, no difference was observed after platelet-rich plasma alone[13]. Furthermore, use of platelet-rich plasma improved the growth and survival rates of preantral follicles isolated from fresh and vitrified ovarian tissue cultured in a three-dimensional culture system[32]. The first report describing its clinical application treated women identified as poor responders during IVF with an intraovarian platelet-rich plasma injection[33]. Restoration of the menstrual cycle was noted 1–3 months post treatment, and natural IVF cycles yielded one to five oocytes per retrieval. This work was followed by a much larger pilot study[34] where groups of 30 women with either poor ovarian response, POI, perimenopause, or menopause were given intraovarian platelet-rich plasma to assess its effectiveness at improving ovarian function. Eighteen out of the 30 women with POI showed some restoration of menstrual cycles, along with a significant improvement in serum levels of anti-Müllerian hormone and follicle stimulating hormone, and improved antral follicle counts. Thus far, however, only one live birth has been reported following ovarian administration of platelet-rich plasma, in a 37-year-old woman with a very short duration of POI[35]. There is a clear need for controlled clinical studies in order to determine whether it does indeed have any efficacy and, if so, in which patient groups.

Primordial follicle activation

The menopause and POI do not mean the complete absence of primordial follicles in the ovary, and indeed it is believed that, on average, approximately 1000 such follicles remain in the ovary at the natural menopause[36,37]. Activation of these apparently dormant follicles is therefore a promising avenue with therapeutic potential. Multiple activators and suppressors have been reported to be related to primordial follicle development (reviewed in reference 38) yet, despite this knowledge, the molecular mechanisms underlying maintenance and activation of dormant follicles is far from completely understood. Recent focus, however, has been on the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and Hippo signaling pathways, which have the potential to be manipulated *in vitro* and thus enable primordial follicle activation. Primordial follicle activation protocols using these approaches have been used in human patients, with a small number of live births reported[39].

In the mammalian ovary, the PI3K/AKT/mTOR pathway plays an essential role in the regulation of dormancy of the primordial follicle pool, and of initial follicle activation. Phosphatase and tensin homolog (PTEN) is a negative regulator of this signaling pathway; oocyte-specific depletion of PTEN in mice causes global primordial follicle activation, with all primordial follicles being depleted by early adulthood, resulting in POI[40]. This is similar to the phenotype of FOXO3a-null female mice[41], although both the PTEN and FOXO3a-knockout mice are initially fertile. FOXO subclasses are transcription factors that have important roles in cell cycle arrest, apoptosis, and stress response *in vitro*, and shuttling of FOXO3a from the oocyte nucleus during primordial follicle assembly to the cytoplasm upon follicle activation may be important to its suppressive role[42]. This shuttling is caused by phosphorylation of FOXO3a by AKT, a prominent kinase within the ovary found in both oocytes and granulosa cells of human follicles[43,44]. The PI3K/AKT/mTOR signaling pathway is also implicated in the pathogenesis of many cancers, and thus numerous inhibitors and activators directed against individual or multiple components of this pathway have been developed (Figure 2), although activation of this signaling pathway can induce malignancy[45]. Rapamycin is an mTOR inhibitor that can inhibit the excessive activation of primordial follicles in PTEN-knockout ovaries and maintain the ovarian reserve[46]; conversely, the PTEN inhibitor bpV(HOpic) increases follicle activation and has been used to generate mature oocytes from neonatal mouse ovaries[47]. In clinical studies, two live births from women with POI following chemotherapy have been achieved using an *in vitro* activation technique in which primordial follicle activation was triggered via AKT stimulation (using 740YP) and PTEN inhibition (using bpV(HOpic)) in ovarian cortical fragments prior to auto-transplantation[6,48]. This has been achieved using both cryopreserved and fresh ovarian tissue[49]; however, the use of PTEN inhibition in these *in vitro* protocols has been shown to affect human follicular survival[44,50] and, in a bovine model, interfere with DNA repair[51], thus warranting further investigation before such a technique is used routinely in a clinical setting.

An important aspect of the *in vitro* activation technique described is the fragmentation of ovarian cortical tissue. This alters the intercellular tension and disrupts actin polymerization, which has downstream consequences for specific growth factors and apoptosis inhibitors, and ultimately interferes with the Hippo signaling pathway, causing increased primordial follicle activation (Figure 3)[52]. The Hippo signaling pathway is a highly conserved pathway that controls organ size by strictly regulating cell growth, differentiation, and apoptosis; therefore, dysregulation of Hippo signaling can lead to cellular overgrowth, dysfunction, and tumorigenesis[53]. In *Drosophila*, Hippo promotes the proliferation and maintenance of somatic stem cells, enables germline differentiation, and supports subsequent follicular maturation and oogenesis[54,55]. In humans, the role of Hippo signaling is less clear; however, this pathway has been implicated in follicular activation and maturation, with downstream Hippo signaling genes (YAP, TAZ, MST1/2, SAV1, and LATS1/2) being

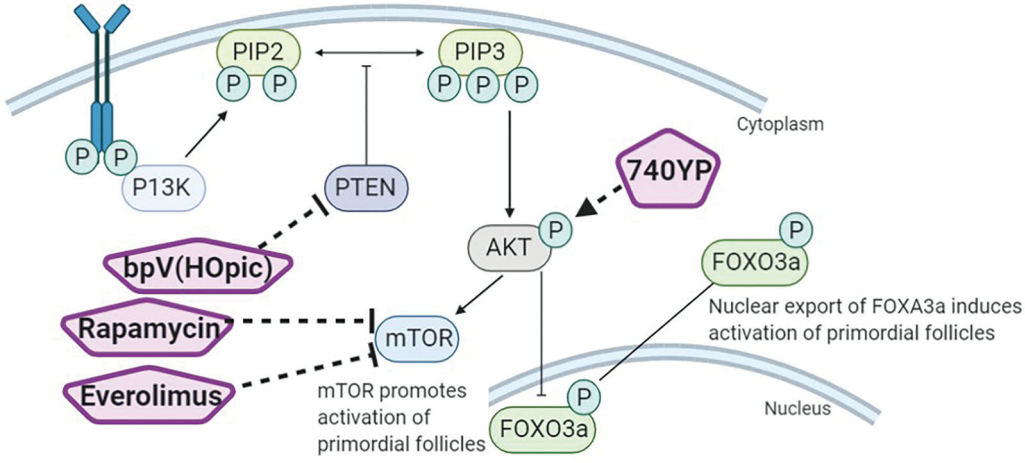

Figure 2. The phosphoinositide 3-kinase (P13K) signaling pathway, highlighting points that have been manipulated in current *in vitro* activation protocols. FOXO3A, Forkhead box O protein-3A; mTOR, mammalian target of rapamycin; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog.

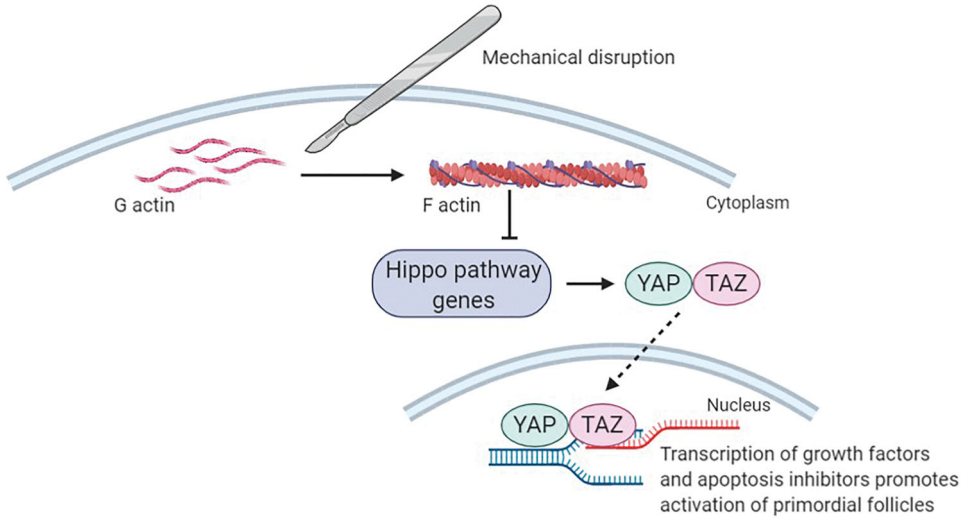

Figure 3. The Hippo signaling pathway.

Table 1. Summary of clinical research aimed at improving the fertility of women with premature ovarian insufficiency (POI).

Study	Intervention	Number of patients	Inclusion criteria	Intervention description	Control	Pregnancy (method)	Live birth
Edessy *et al.*[23]	Autologous MSC transplant	10	Post menarche <40 yo FSH ≥20 mIU/ml	Autologous transplant of bone marrow-derived MSCs	N/A	1 (unclear)	1
Ding *et al.*[25]	Autologous MSC transplant	14	POI, 18–39 yo Amenorrhea >1 year FSH ≥40 mIU/ml	Group 1 ($n = 6$): autologous transplant of umbilical cord-derived MSCs Group 2 ($n = 8$): autologous transplant of umbilical cord-derived MSCs + collagen	N/A	2 (natural, one from each group)	1
Herraiz *et al.*[29]	Autologous MSC transplant	17	Poor responders according to ESHRE guidelines	Autologous transplant of bone marrow-derived MSCs	Contralateral ovary	3 (natural) 2 (IVF)	3
Sfakianoudis *et al.*[34]	Infusion of platelet-rich plasma	30	POI, <40 yo Amenorrhea >1 year FSH ≥25 mIU/ml	Intraovarian infusion of platelet-rich plasma	N/A	3 (natural)	0
Hsu *et al.*[35]	Infusion of platelet-rich plasma	1 (case report)	37 yo Amenorrheic with FSH 43.5 mIU/ml and AMH <0.02 ng/ml	Intraovarian infusion of platelet-rich plasma	N/A	1 (ICSI)	1
Kawamura *et al.*[6]	*In vitro* activation	27	POI, <40 yo Amenorrhea >1 year FSH ≥40 mIU/ml	Ovarian cryopreservation + thaw, fragmentation, *in vitro* culture with bpV(HOpic) and 740YP	N/A	2 (ICSI)	1
Suzuki *et al.*[48]	*In vitro* activation	10	POI, <40 yo Amenorrhea >4 months FSH ≥35 mIU/ml	Ovarian cryopreservation + thaw, fragmentation, *in vitro* culture with bpV(HOpic) and 740YP	N/A	1 (ICSI)	1
Zhai *et al.*[49]	*In vitro* activation	14	POI, <40 yo Amenorrhea >1 year FSH ≥35 mIU/ml	Ovarian fragmentation, *in vitro* culture with bpV(HOpic) and 740YP	N/A	1 (IVF)	1
Lunding *et al.*[59]	*In vitro* activation (without pharmacological intervention)	20	Indication for IVF/ICSI 25–39 yo Serum AMH <0.7 ng/ml	Ovarian biopsy, fragmentation, and auto-transplantation to a peritoneal pocket	Contralateral ovary	3 (natural) 9 (IVF/ICSI)	10
Fabregues *et al.*[60]	*In vitro* activation (without pharmacological intervention)	1 (case report)	30 yo Amenorrheic with FSH 89.9 mIU/ml and AMH 0.02 ng/ml	Ovarian biopsy, fragmentation, and auto-transplantation to a peritoneal pocket	N/A	1 (IVF)	1

Note that some studies include women with poor ovarian reserve rather than POI. AMH, anti-Müllerian hormone; ESHRE, European Society of Human Reproduction and Embryology; FSH, follicle stimulating hormone; ICSI, intra-cytoplasmic sperm injection; IVF, in vitro fertilization; MSC, mesenchymal stem cell; N/A, not applicable; yo, years old.

expressed in follicles at different stages in mouse and human ovaries[6]. Unlike many signaling pathways, the Hippo pathway does not have dedicated extracellular ligands and receptors, but is instead controlled mainly by a network of components involved in regulating cell adhesion, shape, and polarity[56]; this is how fragmentation of ovarian tissue disrupts Hippo signaling and why it precedes P13K/AKT signaling manipulation in *in vitro* activation protocols[6,48]. Interestingly, a recent study in mouse has shown that removal of the ovary from its physiological environment is as

effective as fragmentation for disruption of Hippo signaling[57]. Furthermore, this work showed that both P13K/AKT/mTOR and Hippo pathways were involved in follicular activation in postnatal day 3 mouse ovaries cultured for 48 h following exposure to 4-hydroperoxy cyclophosphamide (4HC) chemotherapy; the use of the mTOR complex 1 (mTORC1) inhibitor everolimus was able to prevent this activation via both these pathways, suggesting cross-talk between them. Indeed, Hippo activity has been found to be mediated via AKT[58], thus highlighting new potential targets for future drug development to enhance *in vitro* activation protocols. The value of ovarian fragmentation without pharmacological treatment has been assessed in a carefully designed study of 20 women with decreased ovarian reserve (thus not POI), involving laparoscopic biopsy, fragmentation, and replacement of one ovary and using the other as a control[59]. Detailed analysis, however, showed minimal hormonal effects, and no increase in the number of recruitable follicles on ovarian stimulation for IVF/intracytoplasmic sperm injection. A case report also describes how ovarian fragmentation without the use of pharmacological intervention resulted in a pregnancy[60]. Here, fresh ovarian tissue was removed from one ovary, fragmented, and transplanted into the contralateral ovary. Following surgery, ovarian stimulation resulted in the growth of three pre-ovulatory follicles and the retrieval of two mature eggs, which were fertilized with IVF and transferred to result in a singleton pregnancy.

Conclusion

In conclusion, there is substantial research activity in relation to improving the fertility of women with POI (summarized in Table 1). Much work surrounds the potential value of stem cells, potentially in combination with other poorly defined treatments such as injection of platelet-rich plasma, and in investigating the applicability of *in vitro* activation protocols, both physical and chemical. While the use of animal models shows some evidence of efficacy, the clinical data on such treatments are largely very preliminary and often from poorly or uncontrolled observations. Given the potential for benefit and the importance of this topic to the patients involved, it is essential that robustly performed trials are performed before claims of effectiveness are made, as emphasized by the relevant societies[1,3], and before these interventions are used outside a research setting.

Potential conflict of interest R.R. declares no conflict of interest. R.A.A. has participated in advisory boards and received speaker's or consultancy fees from Roche Diagnostics, Ferring Pharmaceuticals, IBSA, Merck Serono, KaNDy Therapeutics, and Sojournix Inc. outside the submitted work.

Source of funding The authors' work in this field is support by grants from the Medical Research Council [G1100357 to R.A.A.], [MR/N022556/1 to the MRC Centre for Reproductive Health]; Wellbeing of Women [PRF005 to R.R.].

References

1. Webber L, Davies M, Anderson R, *et al.* ESHRE guideline: management of women with premature ovarian insufficiency. *Hum Reprod* 2016;31:926–37
2. Fraison E, Crawford G, Casper G, *et al.* Pregnancy following diagnosis of premature ovarian insufficiency: a systematic review. *Reprod Biomed Online* 2019;39:467–76
3. Panay N, Anderson RA, Nappi RE, *et al.* Premature ovarian insufficiency: an international menopause society white paper. *Climacteric* 2020;23:426–46
4. Bidet M, Bachelot A, Bissauge E, *et al.* Resumption of ovarian function and pregnancies in 358 patients with premature ovarian failure. *J Clin Endocrinol Metab* 2011;96:3864–72
5. Sauer MV. Spontaneous pregnancy in women awaiting oocyte donation. *J Reprod Med* 1995;40:630–2
6. Kawamura K, Cheng Y, Suzuki N, *et al.* Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 2013;110:17474–9
7. Johnson J, Canning J, Kaneko T, *et al.* Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature* 2004;428:145–50
8. Telfer EE, Anderson RA. The existence and potential of germline stem cells in the adult mammalian ovary. *Climacteric* 2019;22:22–6
9. Attwood SW, Edel MJ. iPS-cell technology and the problem of genetic instability-can it ever be safe for clinical use? *J Clin Med* 2019;8:288
10. Pittenger MF, Discher DE, Péault BM, *et al.* Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med* 2019;4:22
11. Lee HJ, Selesniemi K, Niikura Y, *et al.* Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. *J Clin Oncol* 2007;25:3198–204
12. Huang Q, Liu B, Jiang R, *et al.* G-CSF-mobilized peripheral blood mononuclear cells combined with platelet-rich plasma accelerate restoration of ovarian function in cyclophosphamide-induced POI rats. *Biol Reprod* 2019;101:91–101
13. Vural B, Duruksu G, Vural F, *et al.* Effects of VEGF + Mesenchymal Stem Cells and Platelet-Rich Plasma on Inbred Rat Ovarian Functions in Cyclophosphamide-Induced Premature Ovarian Insufficiency Model. *Stem Cell Rev Rep* 2019;15:558–73
14. Song D, Zhong Y, Qian C, *et al.* Human umbilical cord mesenchymal stem cells therapy in cyclophosphamide-induced premature ovarian failure rat model. *Biomed Res Int* 2016;2016:2517514
15. Mohamed SA, Shalaby S, Brakta S, *et al.* Umbilical cord blood mesenchymal stem cells as an infertility treatment for chemotherapy induced premature ovarian insufficiency. *Biomedicines* 2019;7:7
16. Ling L, Feng X, Wei T, *et al.* Human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation improves ovarian function in rats with premature ovarian insufficiency (POI) at least partly through a paracrine mechanism. *Stem Cell Res Ther* 2019;10:46
17. Elfayomy AK, Almasry SM, El-Tarhouny SA, *et al.* Human umbilical cord blood-mesenchymal stem cells transplantation renovates the ovarian surface epithelium in a rat model of premature ovarian failure: possible direct and indirect effects. *Tissue Cell* 2016;48:370–82
18. Fu X, He Y, Xie C, *et al.* Bone marrow mesenchymal stem cell transplantation improves ovarian function and structure in rats with chemotherapy-induced ovarian damage. *Cytotherapy* 2008;10:353–63
19. Li H, Zhao W, Wang L, *et al.* Human placenta-derived mesenchymal stem cells inhibit apoptosis of granulosa cells induced by IRE1α pathway in autoimmune POF mice. *Cell Biol Int* 2019;43:899–909
20. Feng P, Li P, Tan J. Human menstrual blood-derived stromal cells promote recovery of premature ovarian insufficiency via regulating the ECM-dependent FAK/AKT signaling. *Stem Cell Rev Rep* 2019;15:241–55

21. Ding C, Zhu L, Shen H, *et al.* Exosomal miRNA-17-5p derived from human umbilical cord mesenchymal stem cells improves ovarian function in premature ovarian insufficiency by regulating SIRT7. *Stem Cells* 2020;38:1137–48
22. Huang B, Lu J, Ding C, *et al.* Exosomes derived from human adipose mesenchymal stem cells improve ovary function of premature ovarian insufficiency by targeting SMAD. *Stem Cell Res Ther* 2018;9:216
23. Edessy M, Hosni H, Shady Y, *et al.* Autologous stem cells therapy, The first baby of idiopathic premature ovarian failure. *Acta Med Int* 2016;3:19–23
24. Su J, Ding L, Cheng J, *et al.* Transplantation of adipose-derived stem cells combined with collagen scaffolds restores ovarian function in a rat model of premature ovarian insufficiency. *Hum Reprod* 2016;31:1075–86
25. Ding L, Yan G, Wang B, *et al.* Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF patients with long history of infertility. *Sci China Life Sci* 2018;61:1554–65
26. Terraciano P, Garcez T, Ayres L, *et al.* Cell therapy for chemically induced ovarian failure in mice. *Stem Cells Int* 2014;2014:720753
27. Herraiz S, Buigues A, Díaz-García C, *et al.* Fertility rescue and ovarian follicle growth promotion by bone marrow stem cell infusion. *Fertil Steril* 2018;109:908–18.e2
28. Cervelló I, Gil-Sanchis C, Santamaría X, *et al.* Human CD133(+) bone marrow-derived stem cells promote endometrial proliferation in a murine model of Asherman syndrome. *Fertil Steril* 2015;104:1552–60.e1-3
29. Herraiz S, Romeu M, Buigues A, *et al.* Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders. *Fertil Steril* 2018;110:496–505.e1
30. Martínez-Martínez A, Ruiz-Santiago F, García-Espinosa J. Platelet-rich plasma: myth or reality? *Radiologia* 2018;60:465–75
31. Emer J. Platelet-rich plasma (PRP): current applications in dermatology. *Skin Therapy Lett* 2019;24:1–6
32. Hosseini L, Shirazi A, Naderi MM, *et al.* Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage. *Reprod Biomed Online* 2017;35:343–50
33. Sfakianoudis K, Simopoulou M, Nitsos N, *et al.* Autologous platelet-rich plasma treatment enables pregnancy for a woman in premature menopause. *J Clin Med* 2018;8:1
34. Sfakianoudis K, Simopoulou M, Grigoriadis S, *et al.* Reactivating ovarian function through autologous platelet-rich plasma intraovarian infusion: pilot data on premature ovarian insufficiency, perimenopausal, menopausal, and poor responder women. *J Clin Med* 2020;9:1809
35. Hsu CC, Hsu L, Hsu I, *et al.* Live birth in woman with premature ovarian insufficiency receiving ovarian administration of platelet-rich plasma (PRP) in combination with gonadotropin: a case report. *Front Endocrinol* 2020;11:50
36. Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. *Hum Reprod* 1996;11:1484–6
37. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. *PLoS One* 2010;5:e8772
38. Ford EA, Beckett EL, Roman SD, *et al.* Advances in human primordial follicle activation and premature ovarian insufficiency. *Reproduction* 2020;159:R15–R29
39. Lee HN, Chang EM. Primordial follicle activation as new treatment for primary ovarian insufficiency. *Clin Exp Reprod Med* 2019;46:43–9
40. Reddy P, Liu L, Adhikari D, *et al.* Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science* 2008;319:611–13
41. Castrillon DH, Miao L, Kollipara R, *et al.* Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 2003;301:215–18
42. Hosaka T, Biggs WH, 3rd, Tieu D, *et al.* Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proc Natl Acad Sci U S A* 2004;101:2975–80
43. Goto M, Iwase A, Ando H, *et al.* PTEN and Akt expression during growth of human ovarian follicles. *J Assist Reprod Genet* 2007;24:541–6
44. McLaughlin M, Kinnell HL, Anderson RA, *et al.* Inhibition of phosphatase and tensin homologue (PTEN) in human ovary *in vitro* results in increased activation of primordial follicles but compromises development of growing follicles. *Mol Hum Reprod* 2014;20:736–44
45. Matsuda S, Nakanishi A, Wada Y, *et al.* Roles of PI3K/AKT/PTEN pathway as a target for pharmaceutical therapy. *Open Med Chem J* 2013;7:23–9
46. Guo Y, Kwiatkowski DJ. Equivalent benefit of rapamycin and a potent mTOR ATP-competitive inhibitor, MLN0128 (INK128), in a mouse model of tuberous sclerosis. *Mol Cancer Res* 2013;11:467–73
47. Adhikari D, Gorre N, Risal S, *et al.* The safe use of a PTEN inhibitor for the activation of dormant mouse primordial follicles and generation of fertilizable eggs. *PLoS One* 2012;7:e39034
48. Suzuki N, Yoshioka N, Takae S, *et al.* Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015;30:608–15
49. Zhai J, Yao G, Dong F, *et al. In vitro* activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. *J Clin Endocrinol Metab* 2016;101:4405–12
50. Lerer-Serfaty G, Samara N, Fisch B, *et al.* Attempted application of bioengineered/biosynthetic supporting matrices with phosphatidylinositol-trisphosphate-enhancing substances to organ culture of human primordial follicles. *J Assist Reprod Genet* 2013;30:1279–88
51. Maidarti M, Clarkson YL, McLaughlin M, *et al.* Inhibition of PTEN activates bovine non-growing follicles *in vitro* but increases DNA damage and reduces DNA repair response. *Hum Reprod* 2019;34:297–307
52. Grosbois J, Demeestere I. Dynamics of PI3K and Hippo signaling pathways during *in vitro* human follicle activation. *Hum Reprod* 2018;33:1705–14
53. Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nat Cell Biol* 2011;13:877–83
54. Huang J, Reilein A, Kalderon D. Yorkie and Hedgehog independently restrict BMP production in escort cells to permit germline differentiation in the Drosophila ovary. *Development* 2017;144:2584–94
55. Polesello C, Tapon N. Salvador-warts-hippo signaling promotes Drosophila posterior follicle cell maturation downstream of notch. *Curr Biol* 2007;17:1864–70
56. Johnson R, Halder G. The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment. *Nat Rev Drug Discov* 2014;13:63–79
57. Devos M, Grosbois J, Demeestere I. Interaction between PI3K/AKT and Hippo pathways during in vitro follicular activation and response to fragmentation and chemotherapy exposure using a mouse immature ovary model. *Biol Reprod* 2020;102:717–29
58. Hu LL, Su T, Luo RC, *et al.* Hippo pathway functions as a downstream effector of AKT signaling to regulate the activation of primordial follicles in mice. *J Cell Physiol* 2019;234:1578–87
59. Lunding SA, Pors SE, Kristensen SG, *et al.* Biopsying, fragmentation and autotransplantation of fresh ovarian cortical tissue in infertile women with diminished ovarian reserve. *Hum Reprod* 2019;34:1924–36
60. Fabregues F, Ferreri J, Calafell JM, *et al.* Pregnancy after drug-free in vitro activation of follicles and fresh tissue autotransplantation in primary ovarian insufficiency patient: a case report and literature review. *J Ovarian Res* 2018;11:76