RBM ONLINE

ELSEVIER

Article

Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage

Laleh Hosseini [a,b], Abolfazl Shirazi [a,b,*], Mohammad Mehdi Naderi [a], Sara Borjian Boroujeni [a], Ali Sarvari [a], Samaneh Sadeghnia [a], Bahareh Behzadi [a], Mohammad Mehdi Akhondi [a]

[a] *Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
[b] *Research Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran*

Laleh Hosseini received her BSc in Biology and MS in Zoology in 1996 and 2003, respectively. She worked in the Embryology Department of the Royan Institute in Tehran from 2007 to 2010. She has been studying female human fertility preservation as a PhD student since 2013 at the Avicenna Research Institute in Tehran.

KEY MESSAGE
Growth and survival of human primordial follicles isolated from fresh and vitrified-warmed ovarian tissue in a three-dimensional in-vitro culture were improved by supplementation of the culture media with platelet-rich plasma. This culture system has the potential to treat infertility in cancer patients when there is a risk of introducing malignant cells.

ABSTRACT

This study aimed to assess the effects of platelet-rich plasma (PRP) on growth and survival of isolated early human follicles in a three-dimensional culture system. After fresh and vitrified-warmed ovarian tissue was digested, isolated early preantral follicles and ovarian cells were separately encapsulated in 1% alginate (w/v). The encapsulated follicles and ovarian cells were cultured together in a medium supplemented with foetal bovine serum (FBS), PRP, PRP + FBS, or human serum albumin (HSA) for 10 days. Growth and survival of the follicles were assessed by measurement of diameter and staining with trypan blue. Follicular integrity was assessed by histological analysis. After culturing, all follicles increased in size, but growth rate was greater in follicles isolated from fresh samples than those from vitrified-warmed ones ($P < 0.001$). Similarly, follicular viability of fresh samples after culturing was higher than that of vitrified-warmed ones. The growth and survival rates of follicles from both fresh and vitrified groups cultured in PRP supplemented media were significantly higher than those of other groups (growth $P < 0.001$ and survival $P < 0.05$, in both groups). In conclusion, media supplementation with PRP can better support viability and growth of isolated human early preantral follicles *in vitro*.

© 2017 Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.

* Corresponding author.
E-mail addresses: a.shirazi@ari.ir, shiraziabbas@yahoo.com (A Shirazi).

http://dx.doi.org/10.1016/j.rbmo.2017.04.007
1472-6483/© 2017 Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.

UNCORRECTED PROOF

ARTICLE IN PRESS

Introduction

Common cancer treatments, including chemotherapy and radiation therapy, are often effective and life-preserving but may have a profound negative impact on a woman's reproductive health by damaging the reserve of ovarian follicles, which can ultimately lead to premature ovarian failure (Wo and Viswanathan, 2009). Standard methods for preserving fertility include cryopreservation of both oocytes and embryos (Bedoschi and Oktay, 2013; Cobo et al., 2010). To do so, the ovaries must be stimulated to retrieve mature oocytes. Such strategies are not recommended for patients with aggressive cancers because delaying treatment is not advisable. Moreover, ovarian stimulation is not applicable in prepubertal girls. An alternative for these patients is ovarian tissue cryopreservation and transplantation, a promising technique that has been shown to restore ovarian function and has resulted in more than 60 live births (Donnez and Dolmans, 2015; Loren et al., 2013). Despite these positive results, some studies have demonstrated a large percentage of follicle loss in grafted tissues as a result of post-grafting ischaemia (Demeestere et al., 2009). Moreover, transplantation of cryopreserved ovarian tissue is not advised in cases of blood-borne malignancies and ovarian cancers because of the risk of reintroduction of malignant cells (Dolmans et al., 2010; Rosendahl et al., 2010).

In-vitro culture of preantral follicles is an alternative fertility preservation approach for both reproductive-age women and prepubertal girls without hormonal stimulation or risk of reintroducing cancer cells. This procedure has produced successful results in several species, including sheep, goats, cows and non-human primates (Arunakumari et al., 2010; Saraiva et al., 2010; Sun and Li, 2013; Xu et al., 2013). However, attempts in humans have been less successful (Abir et al., 1999; Telfer et al., 2008). This could be due to the lack of growth factors and hormones necessary for the survival and development of human preantral follicles. However, supplementing media with numerous growth factors is not practical or affordable. Moreover, their addition in the appropriate culture periods and required concentrations can be very challenging.

Platelet-rich plasma (PRP), a blood by-product containing a significant concentration of platelets suspended in a small volume of plasma, can successfully replace foetal bovine serum, supporting the growth and viability of different cell types (Hemeda et al., 2014; Rauch et al., 2011). The positive effect of PRP is probably due to the high concentration of growth factors stored within platelet α-granules, which include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor (FGF) and insulin-like growth factor (IGF) (Wang and Avila, 2007). Although PRP has never been used for in-vitro culture of isolated preantral follicles, evidence indicates that the factors in PRP have a positive effect on survival and growth of follicles (Danforth et al., 2003; Gutierrez et al., 2000; Matos et al., 2007; Nilsson et al., 2006; Zhou and Zhang, 2005). Therefore, the goal of this study was to evaluate the possible effects of PRP supplementation on survival and development of isolated human early preantral follicles *in vitro*.

Materials and methods

All experimental procedures were reviewed and approved by the Research Ethics Committee of the Avicenna Research Institute on 20 September 2011 (reference number 90/270) and by the Iranian National Committee of Ethics in Medical Research on 19 November 2011. All chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA), unless otherwise indicated in the text.

Experimental design

In our experiments, in order to clarify the effect of media supplementation on follicle development we first applied FBS, PRP and FBS + PRP in fresh samples. Because in practice we have to preserve the samples for future applications, we considered more experimental groups for vitrified samples, which were comprised of FBS, PRP, FBS + PRP and HSA groups. Each experiment included three replicates.

Collection of ovarian tissue

Ovarian tissues were obtained from three females under 35 years of age whose ovaries were donated after brain death following written informed consent. Ovaries were removed and transported to the laboratory at 4°C in a medium consisting of α-MEM + 1% FBS (Gibco) +100 μg/ml penicillin and 50 μg/ml streptomycin. The ovarian medulla was removed using a scalpel, and the cortex was cut into small cubes (1 × 1 × 1 mm) with a McIlwain Tissue Chopper.

Ovarian tissue vitrification and warming

Vitrification of ovarian cortical strips was performed as previously described (Amorim et al., 2012). First, ovarian tissues were exposed to 6.5% ethylene glycol (EG) and 2.5% DMSO in α-MEM supplemented with 20 mg/ml BSA as a base medium (equilibration solution) for 5 min. The samples were then transferred into vitrification solution 1 containing 13% EG and 5% DMSO in base medium; kept for 5 min; transferred into vitrification solution 2 with 26% EG, 10% DMSO, 2.5% polyvinylpyrrolidone (PVP, MW 10,000), and 1 mol/l sucrose in base medium; and kept for 1 min. All samples were maintained at 4°C. The tissue pieces were then transferred into a cryovial with a minimum volume of medium and immersed in liquid nitrogen.

For warming, the vitrified ovarian cortical pieces were transferred into warming solution 1 containing 1 mol/l sucrose in base medium for <15 s and then transferred to different concentrations of sucrose (0.5 mmol/l and 0.25 mmol/l, respectively) for 5 min each at 37°C. After each step, the samples were rinsed with the base medium.

Ovarian follicles and cell isolation

Follicles were isolated from fresh and vitrified-warmed ovarian cortical tissues as previously described (Rice et al., 2008). The tissue was enzymatically digested in Krebs ringer bicarbonate buffer supplemented with 1 mg/ml collagenase IA and 1 mg/ml DNase (Roche) in a shaking water bath at 37°C for 50–60 min. Digestion was terminated by adding an equal volume of bicarbonate tissue culture medium (BTCM) supplemented with 10% FBS at 4°C. The resulting suspension was centrifuged at 50*g* for 10 min at 4°C. The supernatant was discarded, 5 ml of warm HEPES tissue culture medium (HTCM) containing 4 mg/ml BSA and 3 mg/ml polyvinyl alcohol was added, and the suspension was pipetted to mechanically disrupt the digested tissue then filtered through a 70-μm filter. The isolated follicles were picked up under a stereomicroscope and washed three times with α-MEM containing 10% serum. Next, the rest of the cell suspension was filtered through a sterilized 40-μm filter. The filtered solution was centrifuged at 260*g* for 5 min, and the pellet was resuspended in

Please cite this article in press as: Laleh Hosseini, et al., Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.04.007

Download English Version:

https://daneshyari.com/en/article/5696571

Download Persian Version:

https://daneshyari.com/article/5696571

Daneshyari.com