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ORIGINAL ARTICLE

Comparing the growth and the development of mouse pre-antral follicle in medium with PL (Platelet Layset) and with FBS

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Platelet Layset (PL);
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Pre-antral follicle;
Fetal Bovine Serum (FBS)

Abstract *Introduction:* In recent years a great deal of effort has been made to improve the culture medium which can be used for different cell culturing and Folliculogenesis. PL (Platelet Layset) with a high percentage of growth factors and also microelements can be effective on the growth of follicle and oocyte. *Material and method:* Preantral follicles were collected from 12 to 14 days female NMRI mice and cultured in culture medium with a different supplement as the serum for 12 days. The different serums were, 5% and 10% FBS as the control groups and the experimental groups were enriched by 5% PL, 10% PL and a combination of 5% PL and 5% FBS. The growth and development of follicle and oocyte was monitored and also the amount of Estradiol (E2) and progesterone (P4) was detected in different mediums on days 9 and 12 of culturing. *Result:* Oocytes had a significant growth in all medium compared to day zero and reached to the size of a mature oocyte ($p < 0.05$). Although survival rate after one day had significant decrease in all medium compared to 5% FBS ($p < 0.05$), after 12 days of culturing, the best survival rate belonged to 5% PL. Progesterone secretion increased in experimental groups significantly ($p < 0.05$) while Estradiol secretion in 5% and 10% PL decreased in half of control groups. *Discussion:* This result

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demonstrates that PL can be a considerable supplement that can be added to the culture medium or a good replacement for FBS as serum used in Folliculogenesis.

1. Introduction

The growth and development of preantral follicle has become an effective method in obtaining mature and fertilizable oocytes intended to be used in ART (Assist Reproductive Technology) (1). This method along with freezing methods plays a crucial role in restoring fertility in the human and other mammalians (2). In Vitro Folliculogenesis technique needs to be better improved because immature oocytes are potentially tentative to mature by In Vitro Folliculogenesis and reach MII phase. However, future development and growth cannot be promised. Culture medium can play an important role in the field of culturing. In recent years numerous studies have focused on improving culture medium (3–5).

In general, culture medium has three important parts including serum, additive supplement and growth factors. In appropriate conditions, preantral follicles with meiotic potential oocytes will be able to reach their final size and release mature oocytes (6). In cell culturing we need to use serum for enriching culture medium and the common serum between laboratories is Fetal Bovine Serum (FBS) (7). Using FBS has some advantages in cell culturing such as having stimulatory factors as well as low concentration of immunoglobulin, however, there are some inevitable disadvantages to it including its high cost, difficulty in purifying of the product and complication of this method on one side, and on the other side using unborn calves is an ethically questionable source (8). In recent years researchers have been trying to improve culture media to use in cell culturing and lots of research have focused on find an alternative supplement to overcome problems associated with the use of FBS (9).

PL (Platelet Layset) with high percentage of growth factors and microelements can be used as a suitable replacement for FBS in cell culturing and Folliculogenesis. Platelet is present in blood and its important role is to aggregate at the injury site and after clotting it starts the heeling process of the wound by releasing growth factors and other elements (10,11). For the first time, in 1970 a research showed that platelet is effective in restoring the wound and is reaching the source of growth factor such as PDGF, TGFβ, Fibronectin (12–14). In recent years, some other studies have been carried out to use PL as a good replacement for FBS, such as the study by Anna Alden et al., in 2007. They used PL for culturing hamster ovary cell and the result was impressive (15). In 2014 Pazoki et al. cultured preantral follicles in a medium enriched by PL for the first time and the survival rate and oocyte growth were acceptable (16).

The purpose of this study was evaluating the components of a platelet lysate medium to demonstrate its potential as a growth-promoting supplement in comparison with the Fetal Bovine Serum in Folliculogenesis.

2. Material and method

2.1. Production of platelet lysate

Platelet Layset (PL) was prepared from cord blood in Royan Institute in Tehran, Iran. Cord blood was transferred to laboratory at 2–8 °C during transferring. First, cord blood was centrifuged at 300*g* to get PRP (Plasma Riched Platelet) and after that PRP centrifuged at 3000*g* for separating platelet in high concentration from PPP (Plasma Poor Platelet). High concentration platelet was frozen at −70 °C. At the end, PRP was warmed at 37 °C to get PL and after freezing/warming platelet membrane was broken and platelet extraction (PL) was obtained.

2.2. Follicle collection

Preantral follicles were collected from prepubertal NMRI mice (aged 12–14 days). All mice were housed and bred according to national legislation for animal care. Animals were killed by cervical dislocated and then ovary were removed aseptically and placed on prewarmed isolation medium consisting α-MEM (sigma, USA) supplemented with 10% Fetal Bovine Serum (Gibco, Germany) and 100 IU/ml penicillin + 100 mg/ml streptomycin (sigma, USA). The ovaries were dissected mechanically by fine hypodermic needles (p-med china). Follicles with average size of 100–130 μm with two layers of granulosa cells and oocytes in normal condition were selected. The average number of collected preantral follicle was ∼30 in averages per ovary and 480 follicles were collected in total.

2.3. Follicle culture

Selected follicles were washed in isolation medium and then cultured individually in 20 μl droplets of culture medium overlaid with mineral oil (sigma, USA) in Petri dishes (60 × 15 Falcon, Germany), 16 droplets per dish. Culture medium consisted of α-MEM (sigma, **USA**), ITS (5 mg/ml Insulin, 5 mg/ml Transferrin, 5 mg/ml Selenium; (Gibco), 100 mIU/ml follicle-stimulating hormone (FSH)(Gibco) and 10 mIU/ml luteinizing hormone (LH)(Gibco) but the mediums were enriched by different amount of Fetal Bovine Serum (FBS)(Gibco) or platelet lysate (PL) as listed; first and second mediums were enriched by 5% and 10% FBS as control groups, third medium was enriched by 5% FBS and 5% PL together, and in last two mediums just 5% and 10% PL were added as experimental groups and without any serum. All fol-