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The potentiality of two-dimensional preantral follicle culture as an in vitro model in predicting premature ovarian failure

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ABSTRACT

The purpose of this study is to identify the potential of a two-dimensional preantral follicle culture as an in vitro model of predicting premature ovarian failure. The two-dimensional preantral follicles culture method was established by cultivating preantral follicles collected from ICR F1 hybrids (aged 12–14 days) for 12 days. The preantral follicles were incubated with 0.54 mg/ml cyclophosphamide, 0.5 mg/ml busulfan, 0.12 mg/ml cisplatin, 3.12 mg/ml 4-vinylcyclohexene diepoxide, 5 mg/ml D (+) galactose, and 0.5 mg/ml hydrocortisone for 24 h at culture days 2, 6 and 11. The diameter of follicles, the cumulus-oocyte complex number and the maturity of oocytes were recorded as the parameters to detect follicular maturation induced by the culture agents. The results indicated that, except for busulfan, D (+) galactose, and hydrocortisone, such test articles could significantly decrease follicular growth ($p < 0.05$ or $p < 0.01$), and induce oocyte degeneration and follicle atresia when the follicles were treated at day 2. With the exception of hydrocortisone, such agents also gradually decreased follicular development ($p < 0.05$ or $p < 0.01$) when the follicles were treated at day 6. All of the test articles but hydrocortisone can interfere with the ovulation, the cumulus-oocyte complex discharge and oocyte maturation of follicles when treated at days 2, 6 and 11. It is suggested that two-dimensional preantral follicle culture could be utilized as a potential in vitro system to mimic the POF model. It may also be employed in screening potential ovarian toxic agents, reducing laboratory animal use and promoting animal welfare.

1. Introduction

Premature ovarian failure (POF) is the cessation of ovarian function before the age of 40 years, and currently affects approximately 1% of all women. It is characterized by amenorrhea, either primary or secondary, and symptoms of hypoestrogenism, with elevated serum gonadotropin concentrations (Mashchak et al., 1981; Coulam et al., 1986; Nelson, 2009; Ikeme et al., 2011). The causes of premature ovarian failure are varied and complex, and include abnormal autoimmune reactions, psychological factors, exogenous substances, infections, chemotherapy treatment, and surgical interventions like bilateral oophorectomy (Alper and Garner, 1985; Hoek et al., 1997; Sklar, 2005).

It is only recently that researchers have gained knowledge about premature ovarian failure. However, several different types of POF animal models have now been established as methods to explore POF prevention (Hoyer et al., 2001; Hoyer and Sipes, 2007; Ben-Aharon et al., 2010; Kappeler and Hoyer, 2012; Li et al., 2013a, 2013b; Chen et al., 2014; Zhang et al., 2016). POF models are mainly conducted by in vivo methods, and ovotoxic effects are induced by some agents such as cyclophosphamide (Jiang et al., 2013), busulfan (Liu et al., 2014), cisplatin (Ai et al., 2007; Li et al., 2013a, 2013b), 4-vinylcyclohexene diepoxide (Haas et al., 2007), and D (+) galactose (Bandyopadhyay et al., 2003a, 2003b), but not including hydrocortisone. It was reported that prenatal exposure to corticosteroids altered the hypothalamus-pituitary-gonadal axis, at least partially, resulting in the damages observed in adult life. However, hydrocortisone neither endangers the neonate nor results in damage to the gonadal axis (Piffer and Pereira, 2004). Theoretically, success in the establishment of an animal model is measured by factors such as abnormal ovarian follicle development, a lack of good quality mature oocytes and elevated serum gonadotropin concentrations, as well as clinical evidences. However, it has been suggested that current animal models of perimenopause fail to ade-

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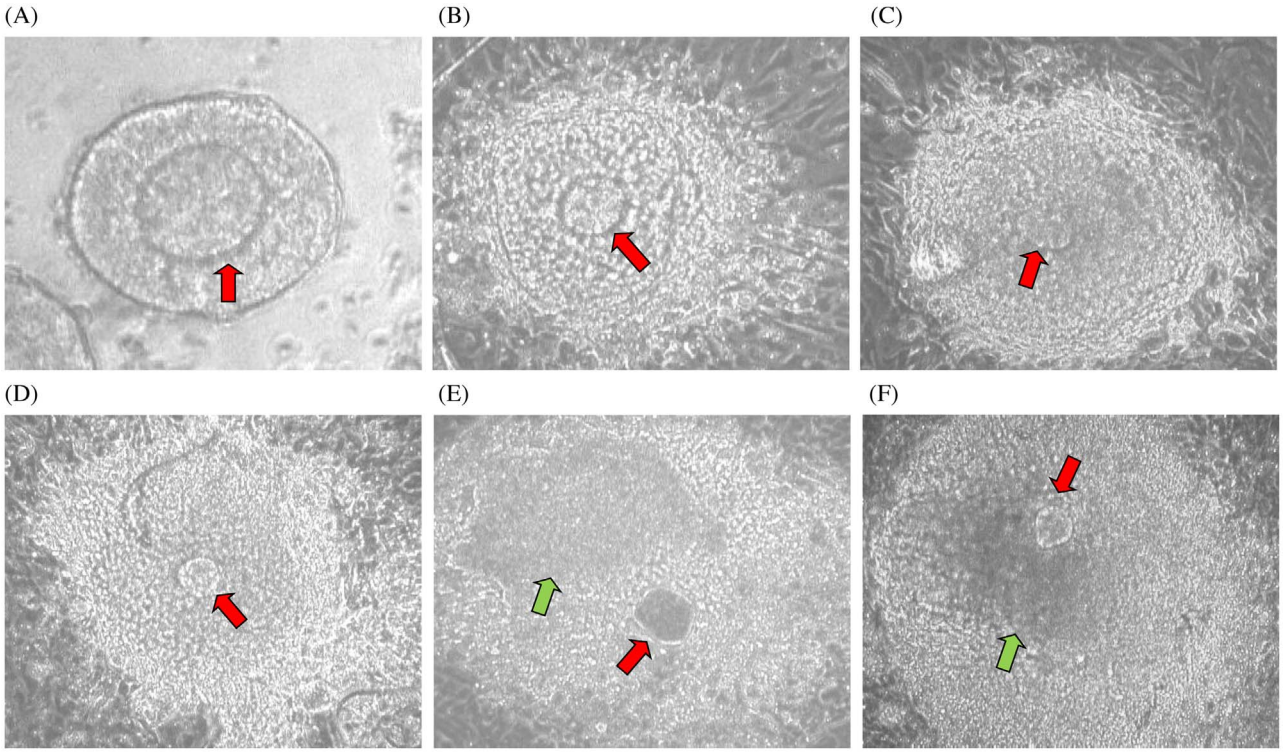

Fig. 1. 1Photomicrographs of cultured preantral follicle development in the validation study. (A) Separated ovarian preantral follicle in mouse (Day 0: primordial follicle) (B) Cultured follicle at day 2: primordial/primary follicles (C) Cultured follicle at day 4: primary/secondary follicles (D) Cultured follicle at day 6: primary/secondary/preantral follicles) (E) Cultured follicle at day 8: antral follicles (F) Cultured follicle at day 12: antral follicles with an antrum-like cavity. Red arrow refers to oocyte and green arrow refers to antrum-like cavity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quately replicate this particular stage in female life (Liu et al., 2015). Therefore, the establishment of suitable POF models in vivo and in vitro still needs further investigation.

During the last two decades, cultures of preantral follicles have provided a large number of oocytes for the manipulation and production of embryos, and have been widely applied in animal reproduction (Gupta and Nandi, 2012; Silva et al., 2016) and assisted reproductive technology (ART) to help female patients who have difficulty producing qualified oocytes for fertility treatments (Anastácio et al., 2013; Dong et al., 2014). In the past ten years, cultures of preantral follicles as a novel in vitro system have also provided the possibility of varying culture parameter, gaining insights into the mechanisms of chemical exposure (Sun et al., 2004). They are also considered a new in vitro culture system to predict reproductive toxicity of exogenous chemicals (Wan et al., 2010, 2011). The key characteristics of POF models include a lack of mature follicle, ovarian dysfunction and a lack of mature oocytes. Two-dimensional preantral follicle cultures may be used as an in vitro model to predict POF as the cultures mimic the process. They may also provide an alternative method of replacing and reducing laboratory animal use and, thus, promoting animal welfare (Davila et al., 1998; Jackson, 1998). However, the potential of a two-dimensional preantral follicle culture to predict POF is still in discussion. We selected several agents such as cyclophosphamide, busulfan, cisplatin, 4-vinylcyclohexene diepoxide, D (+) galactose, and hydrocortisone to be applied in the two-dimensional preantral follicle culture, and to validate the method in predicting POF.

2. Material and methods

2.1. The test articles

Cyclophosphamide (CTX, C7H17Cl2N2O3P, purity 99%) was provided by the Shanghai Jin Sui Biological Technology Co., Ltd (Shanghai, China). Busulfan (C6H14O6S2, purity 99%) was provided by Sigma-Aldrich Co., Ltd (USA). Cisplatin (Pt(NH3)2Cl2, purity 99%) was provided by Sigma-Aldrich Co., Ltd (USA). 4-vinylcyclohexene diepoxide (VCD, C8H12O, purity 99%) was purchased from Shanghai Aladdin Technology Co., Ltd (Shanghai, China). D(+) galactose was provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Hydrocortisone was purchased from Tianjin Kingyork group CO., LTD (Specification 100 mg: 20 ml). DMSO was provided by Sigma-Aldrich Co., Ltd (USA). The culture medium served as the control group. All other reagents were analytical grade. Water was purified by reverse osmosis.

2.2. Animal donor

All mice used in this study were female (ICR) F1 hybrids raised under standard laboratory conditions of room temperature (22 ± 2 °C) and relative humidity (50 ± 10%), with a 12 h light-dark cycle. Water and food were provided ad libitum. All of the ICR mice were purchased from BK Laboratory Animal Co., Ltd (Shanghai, China). This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Shanghai Institute of Planned Parenthood Research (Permit Number: 2013-22). All surgery was performed under sodium pentobarbital anesthesia, and every effort was made to minimize suffering.

2.3. Follicle collection for in vitro growth

ICR F1 hybrids (aged 12-14 days) were intraperitoneally anesthetized with 5 mg/kg pentobarbital sodium and then euthanized. Ovaries were aseptically removed and collected in an M2 medium (Gibco, Australia) supplemented with 20% heat-inactivated fetal calf serum (Gibco, Australia), 100 IU/ml penicillin, and 100 IU/ml streptomycin (Hyclone, China). The ovaries were mechanically dissected using fine