RBM ONLINE

ELSEVIER

Article

Leukaemia inhibitory factor in serum and follicular fluid of women with polycystic ovary syndrome and its correlation with IVF outcome

Zewu Li [a,b], ***Yueting Zhu*** [c], ***Hongchang Li*** [c], ***Wenjie Jiang*** [c], ***Hongbin Liu*** [c], ***Junhao Yan*** [c], ***Zi-Jiang Chen*** [a,b,c,*], ***Weiping Li*** [a,b,*]

[a] *Centre for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, China*

[b] *Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai 200135, China*

[c] *Centre for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University; National Research Centre for Assisted Reproductive Technology and Reproductive Genetics; The Key laboratory of Reproductive Endocrinology (Shandong University), Ministry of Education Shandong Provincial Key Laboratory of Reproductive Medicine, China*

Zewu Li received his doctorate from Qilu Hospital, Shandong University, China, in 2015. He undertook postdoctoral research at the Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, China. His research has focused on finding new diagnostic markers for ovulation disorder illness and embryo selection.

KEY MESSAGE

Serum and follicular fluid LIF concentrations were found to be lower in PCOS women compared with non-PCOS controls, suggesting that reduced LIF concentrations could be related to the disordered folliculogenesis seen in PCOS patients. LIF concentrations in embryo culture medium may be useful to predict pregnancy outcome following IVF.

A B S T R A C T

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, ovarian dysfunction and polycystic ovarian morphology. Leukaemia inhibitory factor (LIF) affects many reproductive activities, including follicular development, embryo implantation and growth. The aim of this study was to evaluate LIF concentrations in serum and follicular fluid of women with PCOS and controls who underwent IVF with embryo transfer (IVF–ET). Serum and follicular fluid LIF concentrations were lower in women with PCOS compared with controls. Oestradiol concentrations in follicular fluid were higher in PCOS subjects compared with controls. LIF concentrations in serum ($r = 0.6263$, $P < 0.05$) and follicular fluid ($r = 0.7093$, $P < 0.05$) were negatively correlated with oestradiol concentration in the PCOS group. LIF concentrations in follicular fluid showed no difference between women who conceived and women who did not in both PCOS and control groups. However, LIF concentrations in embryo culture medium were higher in women who conceived following IVF compared with women who did not, in combined PCOS and control groups. The findings indicate that low LIF concentrations in serum and follicular fluid may contribute to disordered folliculogenesis in PCOS. LIF concentrations in embryo culture medium may predict the outcome of IVF treatment.

* Corresponding author.
E-mail address: chenzijiang@hotmail.com (ZJ Chen); liweiping@renji.com (W Li).

https://doi.org/10.1016/j.rbmo.2017.12.020
1472-6483/

Please cite this article in press as: Zewu Li, et al., Leukaemia inhibitory factor in serum and follicular fluid of women with polycystic ovary syndrome and its correlation with IVF outcome, Reproductive BioMedicine Online (2018), doi: 10.1016/j.rbmo.2017.12.020

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous condition characterized by hyperandrogenism, ovarian dysfunction and polycystic ovarian morphology (Lebkowska et al., 2016). It is the most common hormonal disorder in young women, estimated to affect 5.0–13.9% of women during their reproductive years (Melo et al., 2010; Yu and Wang, 2016). The aetiology of PCOS is multifactorial and involves endocrine and metabolic components, as well as imbalance of local ovarian regulatory factors. The pathologic changes in PCOS are characterized by hyperandrogenism and endocrine disorders due to ovulatory dysfunction, which is one cause of female infertility. PCOS is not only a reproductive disorder, but also a syndrome with metabolic consequences that could affect a woman's health during different stages of reproductive and post-reproductive life (Dunaif and Fauser, 2013; Orio and Palomba, 2014). The prevalence of PCOS is related to both genetic and environmental factors (Crosignani and Nicolosi, 2001). However, the pathogenesis of PCOS has not been fully elucidated.

It has been demonstrated that PCOS is related to local regulatory factors (Sahin et al., 2014). Leukaemia inhibitory factor (LIF) is a local regulatory factor in the ovary (Hsieh et al., 2005; Ozörnek et al., 1999) belonging to the interleukin (IL)-6 family, and is a highly glycosylated, secreted protein composed of 180 amino acid residues. It has different biological activities in different tissues and cells. LIF can regulate the growth and differentiation of many kinds of cells, including embryonic stem cells, archaeocytes, liver cells and endothelial cells (Salleh and Giribabu, 2014). In recent years, it has been reported that LIF may affect reproductive processes including follicle growth, embryo growth and differentiation (Aghajanova, 2010; Dozio et al., 2009). Previous studies have demonstrated that LIF concentrations in the follicular fluid increase before ovulation, and that there is a positive correlation between LIF concentrations and quality of oocytes (Lédée-Bataille et al., 2001; Paiva et al., 2009). However, the concentrations of LIF in the serum of PCOS patients, and whether LIF can act as a biomarker for predicting the outcomes of IVF with embryo transfer (IVF–ET), remain unknown.

In the present study, the concentrations of LIF and oestradiol were examined in women with PCOS and controls, and the relationship between LIF and oestradiol concentrations in serum and follicular fluid was explored, with the aim of further investigating the pathogenesis of PCOS-related infertility. The correlation of LIF concentrations in embryo culture medium with IVF–ET outcomes was also investigated to evaluate the potential of LIF as a biomarker for predicting clinical pregnancy.

Materials and methods

Subjects

A total of 94 patients who underwent IVF–ET between August 2016 and January 2017 in the Centre for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, were recruited for this study. Forty women with PCOS and 40 weight-matched control subjects participated in the study because 14 patients were lost to follow-up. Diagnosis of PCOS was carried out according to the revised Rotterdam consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004). The women with PCOS were diagnosed based on olio-amenorrhoea and hyperandrogenaemia after excluding non-classic congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinaemia and thyroid disease. Polycystic ovaries were verified by ultrasound in all subjects with PCOS. The control group consisted of women who had regular menstrual cycles (28 ± 2 days, blood progesterone concentrations measured between the 18th and 21st days of the menstrual cycle, >10 ng/ml in two consecutive cycles) without clinical or biochemical hyperandrogenism or polycystic ovary and with no history of any drug intake for at least 3 months. Women in the control group had either Fallopian tube obstruction, or normal reproductive health but an infertile male partner. Sex hormone concentrations and routine biochemical examinations of control subjects were normal, and patients had no history of genitourinary diseases, or severe cardiovascular, liver or kidney disease. Additional exclusion criteria for both groups were smoking and alcohol consumption. All patients in the PCOS and control groups underwent IVF–ET. The data for all subjects were obtained from clinical and pathologic records including age and history of menstruation. Routine measurements of body weight and height, hair growth and distribution were recorded. All subjects provided written informed consent in accordance with Institutional Review Board guidelines for the protection of human subjects. The study received ethical approval from the Institutional Review Board on 6 January 2017 (reference number 16).

Ovarian stimulation and follicular fluid and embryo culture supernatant collection

All patients were stimulated by the long protocol. Ovarian follicular development was stimulated with recombinant human FSH (Merck Serono, Switzerland) at doses of 225–450 IU/day. Ovulation was triggered by human chorionic gonadotrophin (HCG 4000–10000 IU) (Livzon Pharmaceutical, China) when at least two follicles were 18 mm and half of the remainder were >15 mm. Oocytes were recovered transvaginally under ultrasound guidance approximately 34.5 h later. All monitoring of controlled ovarian hyperstimulation (COH) as well as egg retrievals and embryo transfers were performed by one of five physicians. Follicular fluid was preserved at oocyte retrieval, by collecting the liquid aspirated from the follicle into the suction tube, to avoid contamination by blood. Follicular fluid samples from each follicle were pooled for each patient for measurement of LIF concentrations. Pooled follicular fluid was centrifuged at 1500*g* for 10 min and the supernatant was stored at –20°C.

Collected oocytes were cultured in a four-well multi-dish with 600 ml of culture medium added with serum substitute supplement. Each well contained from one to four oocytes. An IVF technique was used for insemination. Oocyte fertilization was observed 16–18 h after insemination under an inverted microscope. Fertilized eggs were cultured to blastocysts. The same culture methodology and media were used for embryos in both groups. All embryo transfers were performed using a Wallace catheter under direct ultrasound guidance 120 h after egg retrieval. Embryos were cultured individually and LIF concentration in culture medium was only measured for the embryos that were transferred when comparing pregnant and non-pregnant groups. The original droplets of embryo culture supernatants collected for each embryo were centrifuged at 1000*g* for 10 min and stored at –20°C, then were tested for LIF concentration.

Please cite this article in press as: Zewu Li, et al., Leukaemia inhibitory factor in serum and follicular fluid of women with polycystic ovary syndrome and its correlation with IVF outcome, Reproductive BioMedicine Online (2018), doi: 10.1016/j.rbmo.2017.12.020