Luteal phase ovarian stimulation versus follicular phase ovarian stimulation results in different human cumulus cells gene expression: a pilot study

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Abstract

OBJECTIVE: Physiologic elevated levels of progesterone in luteal phase can impede early-onset LH surge. However, the impact of high levels of progesterone on the oocyte or cumulus cells (CCs) remains indistinct. Therefore, the aim of study was to investigate the CCs gene expression between luteal phase ovarian stimulation (LPOS) and follicular phase ovarian stimulation (FPOS) in poor ovarian responders (PORs) undergoing in vitro fertilization (IVF) cycles. MATERIALS AND METHODS: This was a prospective non-randomized trial (ClinicalTrials.gov Identifier: NCT03238833). A total of 36 PORs who conformed Bologna criteria and underwent IVF cycles were enrolled. 15 PORs were allocated to the LPOS group and 21 PORs were allocated to the FPOS group. Basic characteristics, cycle characteristics and pregnancy outcomes were compared between the two groups. Moreover, CCs genes regarding inflammation (CXCL1, CXCL3, TNF, PTGES), oxidative-phosphorylation (NDUFB7, NDUFA4L2, SLC25A27), apoptosis (DAPK3, BCL6B) and metabolism (PCK1, LDHC) were analyzed using real-time quantitative PCR between the two groups. RESULTS: Basic characteristics and IVF outcomes were similar between the two groups except significantly high progesterone level in the LPOS group. The mRNA expression of CXCL1 and PTGES were significantly lower in the LPOS group than in the FPOS group ($p < 0.05$). The LPOS group had significantly lower mRNA expression of NDUFB7 and NDUFA4L2 than the FPOS group ($p < 0.05$). DAPK3 and BCL6B mRNA expression were significantly higher in the LPOS group compared to FPOS group ($p < 0.05$). Increased expression of PCK1 and decreased expression of LDHC were observed in the LPOS group compared to the FPOS group. ($p < 0.05$). CONCLUSIONS: Compared to the FPOS, the LPOS seemed to reduce favorable inflammation and mitochondrial function, and induce apoptosis and abnormal glucose metabolism in CCs.

Introduction

Luteal phase ovarian stimulation (LPOS), referring to the initiation of ovarian stimulation from the luteal phase, has been regarded as a feasible protocol for in vitro fertilization (IVF) cycles (1) following the theory of multiple follicular recruitment waves in the same menstrual cycle had been proposed (2). LPOS was first applied in the fertility preservation of cancer patients (3, 4), and then used in the general infertile couples (5, 6). Studies showed that similar number of retrieved oocytes and mature oocytes and fertilization rate were noted between LPOS and follicular phase ovarian stimulation (FPOS) in the urgent fertility preservation (3, 4) or women with normal ovarian response (5, 6). In poor ovarian responders (PORs), some studies revealed that more competent oocytes and embryos could be obtained in the LPOS, compared to FPOS (7–9). The possible rationale was that physiologic high levels of progesterone in the luteal phase could effectively block premature luteinizing hormone (LH) surge which more frequently occurred in the PORs during ovarian stimulation. Our previous study demonstrated that the numbers of retrieved oocytes, metaphase II oocytes, fertilized oocytes, and day-3 embryos were significantly higher in the LPOS group than in the FPOS group (7). However, some studies had conflicting results (10, 11). Furthermore, there was lack of large-scare randomized controlled trials to bolster the consequences. Therefore, no solid evidence support that PORs could really get more benefit from LPOS until now.

Cumulus cells (CCs) are somatic cells surrounding the oocyte in cumulus-oocyte complexes (COCs). Bidirectional intercellular communication between CCs and the oocyte mediated by a network of specialized gap junctions is crucial for development of follicles (12). Oocyte-secreted factors, such as growth-differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), generated from the oocyte regulate proliferation, apoptosis, luteinization, metabolism and expansion of CCs (13). CCs protect and nurture the oocyte, playing an essential role in oocyte maturation, ovulation and fertilization (14). Therefore, the expression profiles of CCs have the potential to reflect oocyte competence and even serve as predictors to determine embryo quality, pregnancy and live birth outcomes (15–17).

Although progesterone can prevent premature luteinization effectively (18, 19), the influence of high levels of progesterone on oocytes or CCs were poorly understood. Therefore, the goal of this study was to investigate the differences of human CCs gene expression between LPOS and FPOS.

Materials And Methods

Study Population and design

This prospective cohort study was implemented at the Reproductive Medicine Center of the Kaohsiung Veterans General Hospital from August 2017 to December 2018. We enrolled PORs undergoing IVF cycles. The inclusion criteria for POR in this study were defined according to the Bologna Criteria (20), having at least two of the three following features: (i) advanced maternal age (≥ 40 years) or any other risk factor for POR; (ii) a previous POR (≤ 3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test. An abnormal ovarian reserve test was defined as antral follicle count (AFC) < 5 or anti-Müllerian hormone (AMH) < 1 ng/mL in this study. Furthermore, two episodes of a previous POR after maximal stimulation alone would be sufficient to define a patient as a POR. Patients were excluded if they had any of the following: (i) a diagnosis of primary ovarian insufficiency; (ii) a history of oophorectomy; (iii) a history of exposure to cytotoxic agents or pelvic irradiation for malignancy; (iv) a history of adjuvant supplementation or hormonal replacement therapy during the previous 3 months. The enrolled participants were then divided into two groups: follicular phase ovarian stimulation (FPOS) or luteal phase ovarian stimulation (LPOS). The choice of ovarian stimulation protocol was determined by physicians' consideration. Baseline and cycle characteristics and IVF outcomes were compared between the two groups.

Ethics Statement

This study was approved by the institutional review board of Kaohsiung Veterans General (VGHKS15-CT11-12) and Clinical Trial Register (ClinicalTrials.gov Identifier: NCT03238833). All participants were fully counselled and written informed consent was obtained. This study was performed adherence to approved guidelines and the Declaration of Helsinki.

Treatment protocol

In FPOS group, ovarian stimulation with a 300 IU daily dose of combined recombinant follicle stimulating hormone (rFSH) plus recombinant LH (rLH) (Pergoveris, Merck Serono, Aubonne, Switzerland) was commenced within 5 days of the menstrual cycle. In LPOS group, spontaneous ovulation was confirmed by transvaginal sonography and progesterone level from day 15 to day 18 of the menstrual cycle. After confirmation of spontaneous ovulation, the women with at least one follicle of less than 8 mm started to undergo ovarian stimulation with a 300 IU daily dose of rFSH plus rLH (Pergoveris, Merck Serono, Aubonne, Switzerland).

In both FPOS and LPOS groups, when the leading follicle reached 12 mm in diameter, the women received 0.25 mg of GnRH antagonist (Cetrotide; Merck Serono, Idron, France) daily until the day of oocyte trigger. Dual trigger, combination of recombinant human chorionic gonadotropin (rHCG) (Ovidrel, Merck Serono, Modugno, Italy) and GnRH agonist (Lupro, Nang Kuang Pharmaceutical Co., Ltd., Tainan, Taiwan), was administered when at least one dominant follicle reached the size of 17 mm. 36 hours after ovulation induction, oocyte retrieval was conducted by transvaginal ultrasound-guided needle aspiration.

Oocytes were inseminated by intracytoplasmic sperm injection (ICSI) for all women in order to diminish the possibility of fertilization failure. Oocytes were denuded and inseminated if maturation status was verified by the presence of the first polar body. Fertilization was evaluated 18 ~ 20 hours after insemination and was defined success by the presence of two pronuclei. Embryos development and quality were assessed based on the number, symmetry of the blastomeres and embryonic fragmentation according to the criteria established by the Istanbul consensus workshop (21). All embryos were cryopreserved by vitrification on the third day after oocyte retrieval. An artificial frozen embryo transfer protocol was used for all participants. Oral estradiol (Ediol 8 mg, Synmosa Biopharma Corporation, Hsinchu County, Taiwan) was initiated on the third day of the menstrual cycle and endometrial thickness was monitored by transvaginal ultrasonography. When the endometrial thickness exceeded 8 mm, luteal support with progesterone intravaginal gel (Crinone 8% gel 90 mg/day, Merck Serono, Hertfordshire, UK) plus oral dydrogesterone (Duphaston 40 mg, Abbott, Olst, The Newtherlands) was added. Transabdominal ultrasound-guided embryo transplantation was performed 4 days after commencement of luteal support. The women underwent a pregnancy test 15 days after embryo transfer. If the pregnancy test was positive, progesterone was continued until 8 ~ 10 weeks of gestation. Clinical pregnancy was defined by the presence of fetal cardiac activity in an intrauterine gestational sac by transvaginal ultrasound. Live birth was determined by delivery of a live fetus after 20 weeks of gestation.

Cumulus cells collection and genes expression

COCs were collected during oocyte aspiration and washed in the medium. CCs were removed mechanically using a sterile scalpel. CCs separated from the same patient's COCs were pooled together for study. Isolated CCs were then transferred immediately into a sterile tube, centrifuged at 200 g for 5 min at room temperature and stored at -80 °C for further study.

CCs were analyzed for the expression of genes related to inflammation (CXCL1, CXCL3, TNF, PTGES), oxidative-phosphorylation (NDUFB7, NDUFA4L2, SLC25A27), apoptosis (DAPK3, BCL6B) and metabolism

(PCK1, LDHC) using a real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

RNA extraction and real-time qRT-PCR

As previously described (22), total RNA was extracted from CCs with the use of the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Each RNA pool was reverse transcribed to cDNA. To detect mRNA expression, real-time qRT-PCR analysis was performed using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). PCR was performed using the SYBR Green PCR Core Reagents kit (Perkin-Elmer Applied Biosystems). Gene-specific qRT-PCR primers that were used are shown in supplemental Table S1. The thermal cycling conditions included an initial denaturation step at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Each set of qRT-PCR reactions was repeated three times. All of the samples with a coefficient of variation for Ct value > 1% were retested. The GAPDH served as an internal control to normalize the expression of target genes. Relative expression levels were calculated for each sample after normalization against GAPDH.

Statistical analysis

The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 22.0 (Chicago, IL, USA). The 2-tailed Student's t-test were used to compare quantitative variables. The categorical variables were compared using Chi-Square tests or Fisher's exact tests. The differences between groups were considered significant when the p value was less than 0.05.

Results

Comparison of basic characteristics between FPOS and LPOS groups

A total of 36 patients were recruited in this study and divided into FPOS (n = 21) and LPOS (n = 15) groups. The baseline characteristics in the two groups are presented in Table 1. The mean age (39.7 ± 3.8 years vs. 40.0 ± 3.4 years) and body mass index (21.7 ± 3.1 kg/m^2 vs. 23.6 ± 3.8 kg/m^2) of patients between the two groups were similar. Additionally, there were no statistically significant differences between groups in terms of infertility duration, previous IVF attempts, primary or secondary infertility, basal FSH, AFC and AMH.

Table 1
Basic characteristics of poor ovarian responders undergoing follicular phase ovarian stimulation (FPOS) or luteal phase ovarian stimulation (LPOS)

Parameters	FPOS (n = 21)	LPOS (n = 15)	p value
Age (years)	39.7 ± 3.8	40.0 ± 3.4	0.818
Body mass index (kg/m2)	21.7 ± 3.1	23.6 ± 3.8	0.128
Infertility duration (years)	5.1 ± 3.2	5.3 ± 6.6	0.882
Previous IVF attempts (n)	2.9 ± 2.9	2.1 ± 2.6	0.441
Types of infertility (%)			0.204
Primary infertility	52.4	73.3	
Secondary infertility	47.6	26.7	
Basal FSH (IU/l)	5.6 ± 4.0	5.4 ± 2.2	0.864
Antral follicle counts (n)	4.4 ± 1.4	4.9 ± 1.7	0.273
Anti-Müllerian hormone (ng/ml)	0.7 ± 0.4	0.7 ± 0.6	0.441
Data are presented as mean ± standard deviation or percentage.			
IVF, in vitro fertilization; FSH, follicle stimulation hormone			

Comparison of cycle characteristics and pregnancy outcome between FPOS and LPOS groups

The stimulation cycle outcomes of each group are shown in Table 2. No statistically significant difference existed in duration of stimulation, total dose of gonadotrophins, or peak serum estradiol level. However, the peak serum progesterone level was significantly higher in LPOS group than FPOS group (6.8 ± 6.8 vs. 0.5 ± 0.2, p = 0.004).

Table 2

Cycle characteristics and pregnancy outcome of poor ovarian responders undergoing follicular phase ovarian stimulation (FPOS) or luteal phase ovarian stimulation (LPOS)

Parameters	FPOS (n = 21)	LPOS (n = 15)	p value
Stimulation duration (days)	11.3 ± 2.2	12.1 ± 2.8	0.363
Gonadotropin dosage (IU)	2882.1 ± 690.1	2885.0 ± 744.0	0.991
Peak estradiol (pg/mL)	749.9 ± 553.2	756.7 ± 671.5	0.975
Progesterone (ng/ mL)	0.5 ± 0.2	6.8 ± 6.8	0.004
No. of oocytes retrieved (n)	3.0 ± 1.4	3.1 ± 1.6	0.713
No. of metaphase II oocytes (n)	2.2 ± 1.5	2.3 ± 1.3	0.847
Maturation rate (%)	69.1 ± 36.7	72.9 ± 28.5	0.742
No. of fertilized oocytes (n)	1.4 ± 1.1	2.0 ± 1.3	0.207
Fertilization rate (%)	61.7 ± 41.1	79.6 ± 32.2	0.170
No. of Day 3 embryos (n)	1.5 ± 1.2	1.9 ± 1.3	0.357
No. of top-quality Day 3 embryos (n)	0.4 ± 0.6	0.7 ± 1.0	0.249
Clinical pregnancy rate (%)	14.3	13.3	0.935
Live birth rate (%)	9.5	13.3	0.720
Data are presented as mean ± standard deviation or percentage.			

No difference was observed between FPOS and LPOS groups regarding the number of retrieved oocytes (3.0 ± 1.4 vs. 3.1 ± 1.6, p = 0.713), metaphase II oocytes (2.2 ± 1.5 vs. 2.3 ± 1.3, p = 0.847), fertilized oocytes (1.4 ± 1.1 vs. 2.0 ± 1.3, p = 0.207), embryos on Day 3 (1.5 ± 1.2 vs. 1.9 ± 1.3, p = 0.357) and top-quality embryos on Day 3 (0.4 ± 0.6 vs. 0.7 ± 1.0, p = 0.249). Moreover, the clinical pregnancy rate (14.3% vs. 13.3%, p = 0.935) and live birth rate (9.5% vs. 13.3%, p = 0.720) were similar between the two groups.

Cumulus cells gene expression between FPOS and LPOS groups

As shown in the Fig. 1, regarding inflammation related genes, CXCL1 (0.51 vs 1.00, p < 0.001) and PTGES (0.30 vs 1.00, p < 0.01) mRNA expression was significantly lower in the LPOS group than in the FPOS group. However, mRNA expression of CXCL3 and TNF were not significantly different between the two groups.

In the Fig. 2, regarding genes related to oxidative-phosphorylation, NDUFB7 (0.12 vs 1.00, $p < 0.001$) and NDUFA4L2 (0.33 vs 1.00, $p < 0.01$) were expressed at lower levels in the LPOS group than in the FPOS group. However, the mRNA expression of SLC25A27 was similar between the two groups. In terms of apoptosis related genes, DAPK3 (3.81 vs 1.00, $p < 0.05$) and BCL6B (2.59 vs 1.00, $p < 0.01$) were more highly expressed in the LPOS group, compared to the FPOS group.

In terms of metabolism related genes (Fig. 3), compared to the FPOS group, significant increased PCK1 mRNA expression (3.13 vs 1.00, $p < 0.001$) and decreased LDHC mRNA expression (0.12 vs 1.00, $p < 0.001$) were found in the LPOS group.

Discussion

To the best of our knowledge, this is the first study to investigate different mRNA expression in human CCs between LPOS and FPOS. This prospective study suggested that ovarian stimulation started from luteal phase or follicular phase could influence CCs mRNA expression which are related to inflammation, oxidative-phosphorylation, apoptosis and metabolism. However, in this study, the number of retrieved oocytes, metaphase II oocytes, embryos on Day 3, top-quality embryos on Day 3, clinical pregnancy rate and live birth rate were not significantly different between LPOS and FPOS, mainly due to small population.

This study demonstrated that lower mRNA levels of CXCL1 and PTGES was found in the LPOS group than in the FPOS group. CXCL1 (C-X-C motif chemokine ligand 1), also called interleukin 1 (IL-1), is a member of the CXC subfamily of chemokines. CXCL1 plays a role in inflammation and as a chemoattractant for neutrophils. A prospective study conducted by Zollnerand colleagues enrolled 256 couples undergoing the IVF/ICSI cycles, showing that high levels of IL-1 beta in the follicular fluid were positively associated with fertilization rates (23). Furthermore, a prospective study of Rehman et al. including a total of 323 patients opting for ICSI demonstrated that higher serum IL-l beta levels were observed in clinical pregnancy group than non-pregnant group or preclinical abortion group (24). PTGES (prostaglandin E synthase) has three known forms: PTGES1, PTGES2, and PTGES3 (25). PTGES is a key enzyme required for the synthesis of PGE2, specifically converting PGH2 to PGE2 (26). During the maturation process of bovine oocytes, PTGES, especially PTGES1, works in coordination with PTGS2 to stimulate PGE2 generation (27). During the process of oocyte maturation, PGE2 plays a vital role in cumulus expansion and oocyte meiosis resumption (28). In addition to oocyte maturation, PGE2 has been showed to be a critical mediator to promote successful fertilization, embryo development and early implantation (29).

The mRNA expression of NDUFB7 and NDUFA4L2 was significantly lower in the LPOS group compared with the FPOS group in this study. NDUFB7 (NADH:ubiquinone oxidoreductase subunit B7) and NDUFA4L2 (NDUFA4 mitochondrial complex associated like 2) encode the protein involved in the electron transport chain which is the main process of ATP production in the mitochondria. Numerous studies have indicated that mitochondrial dysfunction of oocytes has the negative impact on oocyte maturation,

fertilization, embryo development, and pregnancy (30–32). Additionally, this study showed that the LPOS group presented higher mRNA expression of DAPK3 and BCL6B than the FPOS group. DAPK3 (death-associated protein kinase 3) and BCL6B (BCL6B transcription repressor) both play a role in the induction of apoptosis. Increased apoptosis of CCs has been reported to be poorly associated with oocyte maturation, fertilization, embryo development, and pregnancy (33–35). Accordingly, this study seemed to reveal that LPOS may lead to mitochondrial dysfunction and increased apoptosis of CCs, causing adverse reproductive outcomes.

Moreover, in this study, increased mRNA expression of PCK1 and decreased mRNA expression of LDHC were observed in the LPOS group compared to the FPOS group. PCK1 (phosphoenolpyruvate carboxykinase 1) is a central regulator of gluconeogenesis and is regulated by Cited2. Fang et al. demonstrated that the high Cited2 protein levels in CCs significantly increased the expression of PCK1 mRNA and glucose in CCs. It was suggested that the high Cited2 level might impair oocyte quality via up-regulating PCK1 mRNA expression to result in abnormal glucose metabolism in CCs (36). Therefore, increased mRNA expression of PCK1 in the LPOS group seemed to have unfavorable influence on oocytes by disordering glucose metabolism. LDHC (lactate dehydrogenase C) catalyzes the conversion of L-lactate and NAD to pyruvate and NADH in the final step of anaerobic glycolysis. Enhanced glucose metabolism by increasing activity of glycolysis in oocytes was positively associated with oocyte maturation and embryo development (37–39). Hence, LPOS seemed to harm oocyte competence and embryo growth by decreasing LDHC mRNA expression.

Taken together, the present study showed that LPOS may diminish IL-1 and PGE2 production, reduced mitochondrial function and elevate apoptosis, increase gluconeogenesis and decrease glycolysis in the CCs, implying that LPOS might have detrimental effects on the CCs. Although it has been proposed that PORs may earn benefit from LPOS because physiologic elevated progesterone could prevent premature LH rise naturally in the luteal phase (7), there were no definite clinical evidence to support it. Some studies revealed that the LPOS increased the chance to gain more competent oocytes and embryos in PORs compared with the FPOS (7–9). However, these studies were not randomized controlled trials and had small numbers of patients. The only randomized controlled pilot trial performed by Kansal Kalra and colleagues revealed that IVF outcomes, including number of oocytes retrieved and embryos transferred, clinical pregnancy rate and live birth rate, between LPOS and FPOS were similar in PORs (10). Additionally, a retrospective study conducted by Wu et al. included 274 PORs, suggesting that there was no significant difference regarding mean number of retrieved oocytes and embryos, implantation and clinical pregnancy rates between LPOS and FPOS (11), the same results as the current study in spite of quite small population. Admittedly, progestins have been proven to be able to inhibit early-onset LH surge effectively, but the effects of high levels of progesterone on oocytes or CCs were still unclear. This study showed that LPOS might have harmful effects on CCs. However, large-scare randomized controlled trials are required to confirm the results from this study.

Several limitations of our study should be taken into account for data interpretation. First, this was a non-randomized trial and had small study population. Second, the participants enrolled based on Bologna

criteria may be heterogeneous. Third, limited CCs genes were analyzed in this study. However, the strength of this study was that all the IVF protocols were carried out by the same physician and all the laboratory procedures were executed by the same embryologist, which minimize the bias in performance.

In conclusion, this study showed LPOS might have disadvantageous influence on CCs via decreased expression of CXCL1, PTGES, NDUFB7, NDUFA4L2, LDHC and increased expression of DAPK3, BCL6B, PCK1, indicating that LPOS seemed to diminish beneficial inflammation and mitochondrial function, and augment apoptosis and abnormal glucose metabolism in CCs. However, further randomized controlled trials with large populations are needed to verify these results.

List Of Abbreviations

AFC: antral follicle count; AMH: anti-Müllerian hormone; BCL6B: BCL6B transcription repressor; BMP15: bone morphogenetic protein 15; CC: cumulus cell; COC: cumulus–oocyte–complex; CXCL1: C-X-C motif chemokine ligand 1; DAPK3: death-associated protein kinase 3; FPOS: follicular phase ovarian stimulation; FSH: follicle stimulation hormone; GDF9: growth-differentiation factor 9; ICSI: intracytoplasmic sperm injection; IL-1: interleukin 1; IVF: in vitro fertilization; LDHC: lactate dehydrogenase C; LH: luteinizing hormone; LPOS: Luteal phase ovarian stimulation; NDUFA4L2: NDUFA4 mitochondrial complex associated like 2; NDUFB7:NADH:ubiquinone oxidoreductase subunit B7; PCK1: phosphoenolpyruvate carboxykinase 1; POR: poor ovarian responder; PTGES: prostaglandin E synthase; qRT-PCR: real-time quantitative reverse-transcription polymerase chain reaction; rFSH: recombinant follicle stimulating hormone; rHCG: recombinant human chorionic gonadotropin

Declarations

Ethics approval and consent to participate

The study conformed to the “Declaration of Helsinki for Medical Research involving Human Subjects”. Additionally, approval was obtained from the institutional review board at Kaohsiung Veterans General Hospital, with the identifier VGHKS15-CT11-12. The study was performed in accordance with approved guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding disclosure

No funding.

Authors' contributions

PH and ZH contributed conception and design of the study; LT and CJ organized the database and performed the statistical analysis; CJ did the laboratory research; LT and JY wrote the first draft of the manuscript; LT, JY and KH wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Figures

Fig. 1

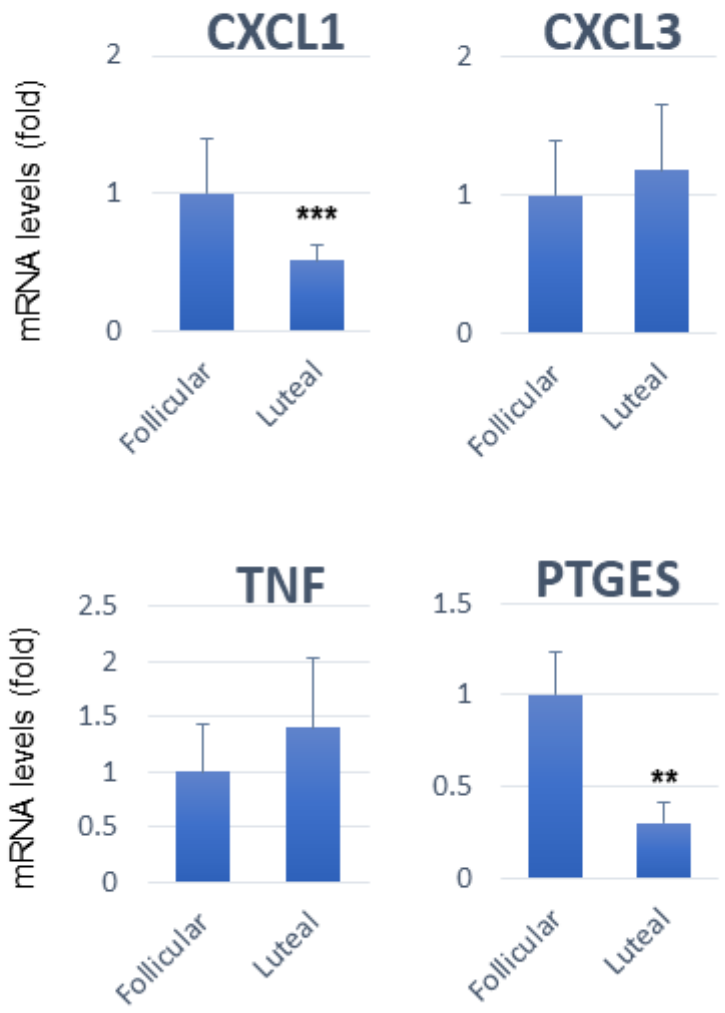

Figure 1

mRNA levels of cumulus cell genes regarding inflammation (CXCL1, CXCL3, TNF, PTGES) between follicular phase ovarian stimulation group (Follicular) and luteal phase ovarian stimulation group (Luteal)

Fig. 2

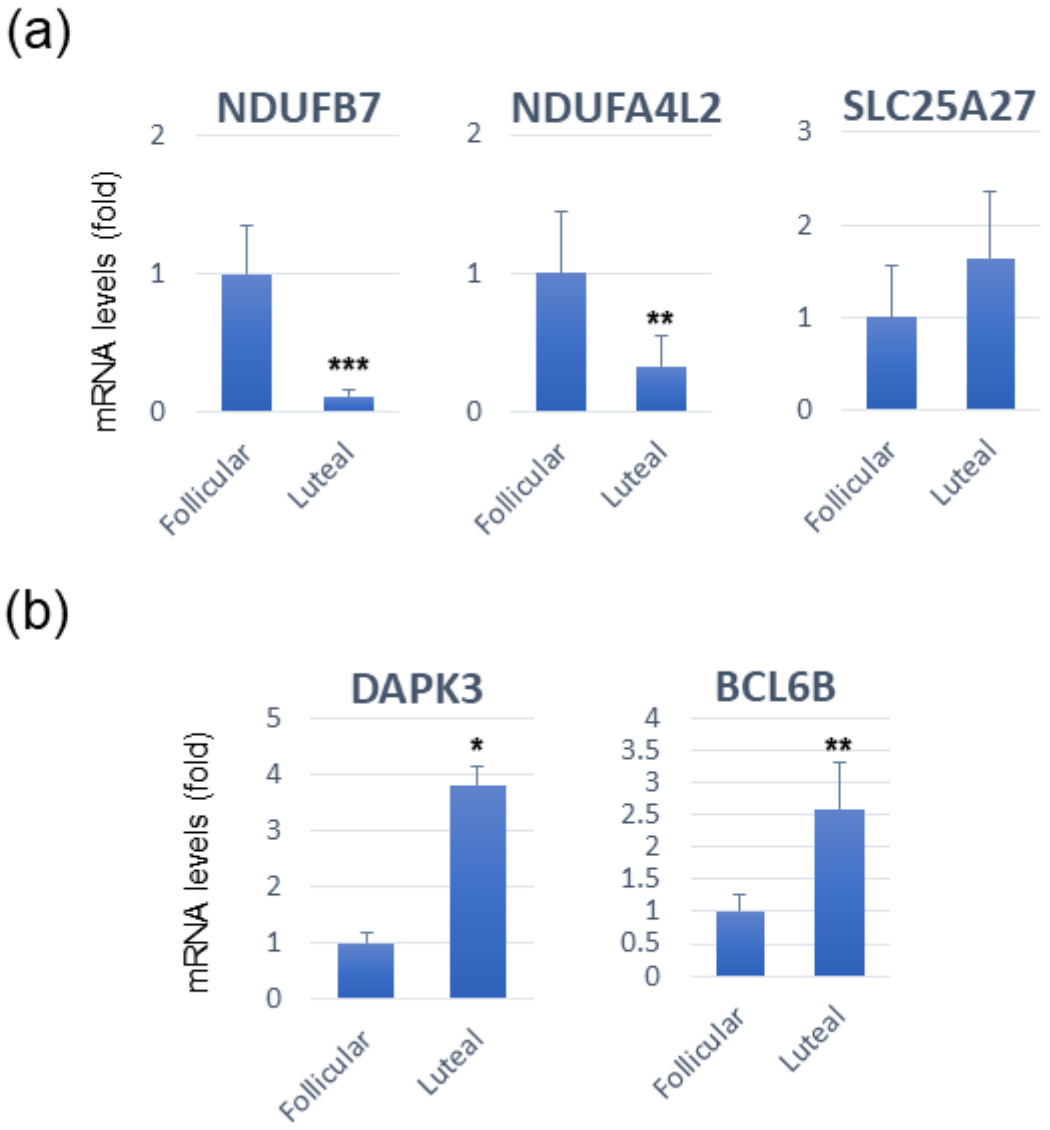

Figure 2

mRNA expression of cumulus cell genes regarding (a) oxidative phosphorylation (NDUFB7, NDUFA4L2, SLC25A27) and (b) apoptosis (DAPK3, BCL6B) between follicular phase ovarian stimulation group (Follicular) and luteal phase ovarian stimulation group (Luteal)

Fig. 3

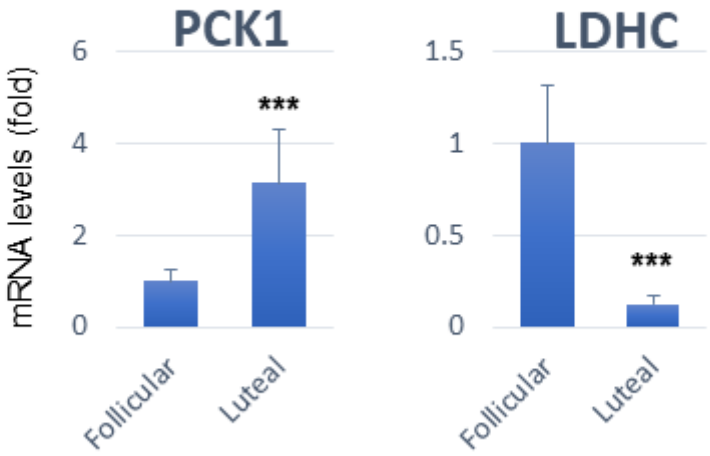

Figure 3

mRNA levels of cumulus cell genes regarding glucose metabolism (PCK1, LDHC) between follicular phase ovarian stimulation group (Follicular) and luteal phase ovarian stimulation group (Luteal)

Supplementary Files

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