Follicular Fluid Steroid Levels and Mitochondrial Function from Exosomes Predict Embryonic Development

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Research Article

Keywords: LH, FSH, Follicular fluid, Mitochondrial abnormalities, Oocyte competence

Posted Date: March 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1456333/v1

Abstract

Purpose

Human follicular fluid (FF) is a complex biological fluid that contributes to the microenvironment of oocyte development. The aim of this study was to evaluate the role of steroid levels and mitochonrial function in embryo development during in vitro fertilization cycles.

Methods

This was a cohort study of 138 women receiving IVF/ICSI, including 136 FF samples from 109 infertile women. FF steroid levels were tested by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and immunoassays. The mRNA expression levels of mitochondrial electron transport chain (ETC) complex genes from FF exosomes were detected by qPCR.

Results

Analysis of these individual FF concentrations revealed that LH and FSH concentrations were higher in follicles in which the oocyte developed into a top quality (TQ) blastocyst (LH: 9.44±2.32mIU/ml, FSH: 9.32±1.01mIU/ml) than those in which there was a failure of fertilization (LH: 5.30±0.84mIU/ml, FSH: 6.91±0.62mIU/ml). In contrast, follicular cortisone concentrations were the lower for oocytes that resulted in a TQ blastocyst (12.20±0.82mIU/ml). The receiver operating characteristic analysis showed that FF LH and FSH levels predicted TQ blastocyst with excellent AUC value of 0.711 and 0.747. Mitochondrial ETC complex I and III mRNA levels were increased in the FF exosomes of TQ blastocyst. Correlation analysis showed that mRNA levels of ETC complex I was positively correlated with LH and FSH levels in FF.

Conclusion

The levels of FF steroids from single follicle can predetermine subsequent embryo development to some extent. Furthermore, impaired exosome mitochondrial dysfunction is a potiential event that causes hormone change in embryo development.

Introduction

In the assisted reproductive technology (ART) cycle, only a few retrieved oocytes can develop into a fertilizable embryo [1]. Despite multiple methods of embryo selection, including morphological assessment, embryo imaging and preimplantation genetic testing, the ability to predetermine the developmental potential of oocytes is still a major obstacle that IVF needs to overcome to increase pregnancy rates [2]. In some cases, doctors choose to transfer multiple embryos per treatment cycle to increase pregnancy rates, which could increase the risk of complications for mother and offspring [3]. As a result, further understanding the mechanisms regulating the oocyte competence is important. In addition, the discovery of an accurate, non-invasive and cost-effective test for predicting the development potential of oocytes may have a significant impact on the field of assisted reproduction.

The follicular fluid (FF) microenvironment is known to regulate oocyte maturation, oocyte quality and subsequent embryonic development [4]. Previous studies have correlated follicular fluid steroid concentrations and embryonic outcomes to determine whether these can predict IVF outcomes [5, 6]. In our study, we use a multi-analyte LC-MS/MS method [7] for the profiling of 20 steroids and FF AMH, FSH, LH, PRL and hCG levels were measured by immunoassays. The main objective of our study was to interrogate the sex hormones in FF as potential biomarkers of successful development to a blastocyst stage embryo.

Exosomes are small membrane vesicles (30–200 nm in diameter) secreted by a variety of living cells under normal or pathophysiological conditions [8]. In ovarian follicles, bidirectional communication occurs between oocytes and somatic cells (granulosa cells and theca cells) in the follicular fluid, and exosomes are one of their communication carriers. In several previous studies of human follicular fluid [9, 10], it has been clearly established that extracellular vesicle miRNAs secreted by ovarian follicular cells play an important role in follicular growth and oocyte maturation.

Completion of oocyte cytoplasmic and meiotic maturation is necessary for oocyte development. The competence of oocytes is the result of the combined action of multiple cellular processes. Mitochondria are organelles required for energy production [11]. Emerging evidence demonstrates their role in oocyte development and reproduction. In immature eggs, mitochondria are silent in transcription and bioenergy [12], and ATP production is slow [13]. First, glycolysis is restricted during oocyte maturation and early preimplantation embryo development until the blastocyst stage [14]. In addition, mitochondria replication is suppressed and mtDNA count does not change from metaphase II oocytes to blastocyst stage [15, 16]. Mitochondria from the oocyte are the main source of ATP during preimplantation embryo development. At the same time, mitochondria play a key role in maintaining metabolic homeostasis. Alterations in mitochondrial functions are often related with peripheral insulin resistance and glucose intolerance[17] [18].

The present prospective study was tried to investigate the possible relationship between the hormone levels of follicle with the developmental potential of the oocyte from fertilization to develop into an embryo. We also investigated the relationship between the expression of mitochondria ETC complexes and hormone metabolic involved in the intrafollicular environment of oocyte development.

Materials And Methods

Subjects and inclusion/exclusion criteria

Samples were collected from January 2020 to May 2021. Patients have given their written informed consent. The study was approved by the Ethical Committee of the Zhongshan Hospital, Fudan University (Shanghai, China), and carried out in compliance with the Population and Family Planning Law of the People's Republic of China (The ethical approval number: B2021-665).

This study aims to evaluate the maternal contribution to embryo outcomes. Women who may have adverse effects on oocyte or embryo quality and implantation,such as PCOS and endometriosis were excluded. In addition, decreased fertilization rates and stunted blastocyst development due to male factors should be excluded [19]. Therefore, the exclusion criteria were as follows: age is over 38 years; poor ovarian response to gonadotropin stimulation with less than 3 retrieved oocytes; moderate or severe male factor (based on semen quality on the day of oocyte retrieval).

Clinical examination

The patients' BMI values, hormone levels, biochemical and ultrasound parameters were recorded. Patients were treated with a standard in vitro fertilization (IVF) antagonist stimulation protocol. Exogenous FSH and human menopausal gonadotropins were received at individualized doses and were treated with either human chorionic gonadotropin (hCG) and/or GnRH agonist. Patients underwent transvaginal ultrasound-guided follicle aspiration 36 hours later.

Follicular fluid collection

Aspirate each follicle independently and collect the FF into a separate tube to match it with a single COC obtained from the same follicle. After the first ovarian puncture, remove the needle to flush and aspirate air until the tube is empty. The collected FFs were checked for erythrocytes; FFs with erythrocytes were excluded from the study. FF samples were centrifuged at 10,000g for 10 min, and the supernatant was aliquoted and stored at -80°C for subsequent analysis.

Measurement of FF markers

20 steroids were measured in organic solvent extracts of 200 μl of follicular fluid samples using LC-MS/MS. Details of the LC- MS/MS methods are presented in the reference[7]. LH, FSH, PRL, AMH, hCG are measured by immunoassays.

Exosome isolation, RNA isolation and quantitative RT-PCR

The follicular fluid exosomes were isolated by ultracentrifugation. Transmission electron microscopy, nanoparticle tracking analysis and western blot analysis are used to characterize exosomes according to our previous study [20]. Total RNA was extracted using TRIzol (Invitrogen), and RNA was then reverse-transcribed using SuperScript First-Strand cDNA System (Takara) according to the manufacturer's instructions. Quantitative RT-PCR (qRT-PCR) was performed using the SYBR Green PCR master mix (Takara) and the StepOnePlus PCR system (Thermo Fisher Scientific) according to the manufacturer's instructions. The house- keeping gene GAPDH was used as an endogenous control. The primer sequences are shown in Table S1.

Statistical analysis

SPSS (Chicago, IL, USA) was used for statistical analysis. t-tests for normally distributed variables and Mann-Whitney U test for not-normally distributed variables were used to compare groups. The two-tailed unpaired t-test was used to evaluate the difference between different groups. Correlation analysis between FF steroid and other clinical and laboratory parameters were performed with the Pearson test for normally distributed variables and the Spearmen test for not normally distributed parameters. Variables were presented as means and SEM. For all comparisons, statistical significance was defined by $p < 0.05$.

Results

Study group and follicular fluid characteristics

Table 1 summaries the characteristics of the patients who completed the study. Out of 138 patients eligible for the study between January 2020 and May 2021, 112 patients were included. The flow of embryo development from oocytes with matched FF is presented in Fig. 1. On the third day of culture, embryos with eight symmetrical, non-fragmented blastomeres were defined as high-quality embryos (Fig. 2C).On the fifth day of the culture, at the blastocyst stage, high-quality embryos will show the development of the inner cell mass (ICM), the appearance of the trophectoderm (TE) and the expansion of the blastocyst (Fig. 2D). Embryos underwent embryo assessment according to 2011 Istanbul consensus [21]. Of the 136 oocytes with paired FF samples, 34 (25%) oocytes were not fertilized. The remaining 102 2PN embryos were cultured as single embryos on Day 3, while 42 embryos were cultured as single embryos on Day 5. The TQ embryo rate on the 3rd day and 5th day are 46.1% and 30.95% respectively.

Table 1
Patients and follicular fluid sample characteristics and embryological outcome of the oocytes with matched FF.

Patients clinical characteristics	
Age (year)	34.93 ± 0.481
BMI (kg/m^2)	22.70 ± 0.33
Basal serum LH (mIU/ml)	5.56 ± 0.24
Basal serum FSH (mIU/ml)	7.91 ± 0.30
Basal serum E2 (pmol/ml)	380.40 ± 89.87
Basal serum P (nmol/l)	1.14 ± 0.45
Basal serum T (nmol/l)	0.86 ± 0.06
Basal serum PRL (mIU/l)	395.17 ± 20.56
Number of oocytes retrieved	10.99 ± 0.75
Number of MII oocytes	8.49 ± 0.58
Follicular fluid sample characteristics	
Number of identified MII oocytes, n	136
Patients with FF samples from both ovaries, n	30
Patients with FF sample from one ovary, n	56
Embryological outcome of the oocytes with matched FF	
Fertilisation rate, n (%)	102/136(75.0)
TQ embryo on the 3rd day, n (%)	47/102(46.1)
TQ blastocyst on the 5th day, n (%)	13/42(30.95)
Abbreviations: BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; T, testosterone; P: progesterone; PRL, prolactin. Data are expressed as mean ± SEM.	

FF steroid profiles and embryological data in patients

As seen in Fig. 2 and Table 2, LH and FSH level was significantly higher in FF matched to oocytes that developed TQ blastocysts on the fifth day of culture, compared to those that were not fertilized after ICSI (9.44 ± 2.32 vs. 5.30 ± 0.84 mIU/ml, 9.32 ± 1.01 vs. 6.91 ± 0.62 mIU/ml)(Fig. 2E&2F). Furthermore, the 17-hydroxy pregnenolone (17-OHP), testosterone and cortisone level were lower in FF matched to oocytes of TQ blastocysts on the fifth day than the not fertilized group (993.15 ± 69.65 vs. 1316.57 ± 97.90 ng/ml,

0.49 ± 0.21 vs. 1.69 ± 0.52 ng/ml, 15.37 ± 0.87 vs. 12.20 ± 0.82 ng/ml) (Fig. 2G&2H&2I). FF DHT concentrations were significantly lower in follicles that resulted in fertilisation after ICSI than in follicles in which the oocyte failed to fertilize (0.32 ± 0.02 vs. 0.45 ± 0.03 ng/ml)(Fig. 2J).

Table 2
Distribution of steroid concentrations in FF in relation to the corresponding oocytes: fertilisation and subsequent embryo development.

	No fertilisation after IVF (n = 34)	Fertilisation after IVF (n = 102)	TQ embryo on the 3rd day (n = 47)	TQ blastocyst on the 5th day (n = 13)
AMH (mIU/ml)	2.96 ± 0.34	2.91 ± 0.25	2.80 ± 0.37	2.89 ± 0.32
LH (mIU/ml)	5.30 ± 0.84	7.51 ± 0.76	7.66 ± 1.30	9.44 ± 2.32*
FSH (mIU/ml)	6.91 ± 0.62	8.34 ± 0.42	8.36 ± 0.56	9.32 ± 1.01*
PRL(mIU/l)	1173.15 ± 112.70	1089.60 ± 66.29	1277.82 ± 137.72	1109.46 ± 149.47
hCG(mIU/l)	36.03 ± 14.10	25.56 ± 2.04	24.11 ± 2.97	27.71 ± 6.80
Pregnenolone(ng/ml)	176.92 ± 20.56	214.01 ± 13.14	233.06 ± 20.18	200.41 ± 27.09
Progesterone(ng/ml)	8108.99 ± 534.01	8621.52 ± 330.46	8360.66 ± 570.05	7496.51 ± 469.58
17-OH Pregnenolone(ng/ml)	69.37 ± 3.46	66.83 ± 2.00	67.18 ± 2.76	60.16 ± 3.53
17-OHP(ng/ml)	1316.57 ± 97.90	1257.82 ± 53.22	1227.39 ± 74.45	993.15 ± 69.65*
21-OHP(ng/ml)	13.75 ± 0.36	15.47 ± 0.44	15.20 ± 0.76	13.42 ± 0.54
DHEA(ng/ml)	24.74 ± 1.69	27.22 ± 1.66	24.60 ± 2.04	22.52 ± 3.24
Testosterone(ng/ml)	1.69 ± 0.52	0.76 ± 0.16#	0.96 ± 0.34	0.49 ± 0.21*
A4(ng/ml)	7.20 ± 1.13	5.96 ± 0.65	6.10 ± 1.11	5.94 ± 1.47
DHT(ng/ml)	0.45 ± 0.03	0.32 ± 0.02#	0.32 ± 0.04^	0.32 ± 0.06*
Estriol(ng/ml)	8.26 ± 0.67	9.09 ± 1.13	9.75 ± 2.83	7.31 ± 0.87
Estrone(ng/ml)	84.56 ± 12.09	90.07 ± 10.29	71.57 ± 10.19	74.49 ± 22.12
Estradiol(ng/ml)	624.52 ± 52.06	585.18 ± 29.41	545.50 ± 42.00	493.84 ± 58.56

Abbreviations: AMH: anti-Müllerian hormone; hCG—human chorionic gonadotropin; 17-OHP: 17-hydroxy pregnenolone; 21-OHP: 21-hydroxy pregnenolone; DHT: Dihydrotestosterone; DHEA, dehydroepiandrosterone; A4: Androstenedione. Data are expressed as mean ± SEM. Differences between groups were considered significant for $p \leq 0.05$.

#Student t-test $p < 0.05$ for no fertilisation vs. fertilisation;

^ Student t-test $p < 0.05$ for no fertilisation vs. TQ embryo on the 3rd day

∗Student t-test $p < 0.05$ for no fertilisation vs. TQ blastocyst on the 5th day

	No fertilisation after IVF (n = 34)	Fertilisation after IVF (n = 102)	TQ embryo on the 3rd day (n = 47)	TQ blastocyst on the 5th day (n = 13)
Corticosterone(ng/ml)	2.51 ± 0.32	2.50 ± 0.15	2.66 ± 0.30	3.36 ± 0.69
Cortisone(ng/ml)	15.37 ± 0.87	14.41 ± 0.59	14.56 ± 1.03	12.20 ± 0.82*
ALD(ng/ml)	0.058 ± 0.004	0.07 ± 0.004	0.07 ± 0.01	0.059 ± 0.01
Cortisol(ng/ml)	53.11 ± 2.89	51.16 ± 1.43	53.93 ± 2.52	60.04 ± 5.92
11-deoxycortisol (ng/ml)	2.15 ± 0.19	2.05 ± 0.17	2.05 ± 0.33	1.61 ± 0.22
Abbreviations: AMH: anti-Müllerian hormone; hCG—human chorionic gonadotropin; 17-OHP: 17-hydroxy pregnenolone; 21-OHP: 21-hydroxy pregnenolone; DHT: Dihydrotestosterone; DHEA, dehydroepiandrosterone; A4: Androstenedione. Data are expressed as mean ± SEM. Differences between groups were considered significant for $p \leq 0.05$.				
#Student t-test $p < 0.05$ for no fertilisation vs. fertilisation;				
^ Student t-test $p < 0.05$ for no fertilisation vs. TQ embryo on the 3rd day				
∗Student t-test $p < 0.05$ for no fertilisation vs. TQ blastocyst on the 5th day				

Correlation of follicular fluid FSH and LH levels with embryo quality on the fifth day

To evaluate the possibility of using key reproductive hormones as potential indicators of an oocytes developmental potential, we further divided the collected FF sample on the fifth day into two group. (1) Follicular fluid derived from oocytes fertilised but failed to reach blastocyst stage on the 5th day (Fig. 3A, n = 29). (2) Follicular fluid derived from oocytes fertilised and developed into a blastocyst on the 5th day (Fig. 3B, n = 13). Similarly, FF LH and FSH levels were significantly higher (LH: p = 0.037; FSH: p = 0.033) in the group 2 (LH: 9.45 ± 2.32 mIU/ml; FSH: 9.32 ± 1.01 mIU/ml) compared to the group 1 (LH: 5.63 ± 0.59 mg/ml; FSH: 6.86 ± 0.59 mIU/ml) (Fig. 3C&3D). There were no differences between the two groups in 17-OHP, testosterone, cortisone and DHT levels (Fig. 3E-3H).

Correlation of follicular fluid hormone levels with embryo quality

The ROC analysis showed that FF LH levels predicted the TQ embryos on the fifth day with high sensitivity (100%) and specificity (55.6%), while FF FSH predicted live birth with 88.9% sensitivity and 64.6% specificity. Our results showed that 100% of the oocytes that matured in FF with LH levels ≥ 5.75 ng/ml led to develop TQ blastocysts on the fifth day of culture, while 88.9% of the oocytes with FF FSH level ≥ 8.44 yielded the blastocysts on the fifth day of culture.The AUC for FF LH had a good value of 0.711, and that for FF FSH had a value of 0.744, implying that both are strong predictors of the TQ embryos on the fifth day (Table 3, Fig. 4A).

Table 3
Receiver operating characteristic curve analysis of LH, FSH and Cortisone levels in FF for top quality blastocysts on the 5th day from 136 matched embryos.

TQ blastocyst on the 5th day	Cut-off value	AUC	Sensitivity	Specificity	SE	95% CI		P
LH	5.75	0.711	1	0.556	0.055	0.602	0.820	0.037
FSH	8.44	0.747	0.889	0.646	0.071	0.608	0.887	0.014
Cortisone	12.659	0.707	0.778	0.626	0.078	0.555	0.859	0.04
Abbreviations: AUC—area under the ROC curve; SE—standard error; 95% CI—95% confidence interval;								

Table 4
Receiver operating characteristic curve analysis of DHT levels in FF for fertilised oocytes from 136 matched oocytes.

Fertilisation after ICSI	Cut-off value	AUC	Sensitivity	Specificity	SE	95% CI		P
DHT	0.432	0.756	0.817	0.654	0.049	0.66	0.851	< 0.01
Abbreviations: AUC—area under the ROC curve; SE—standard error; 95% CI—95% confidence interval;								

Furthermore, the cortisone level (AUC = 0.707) also had a fair significance as predictors of the blastocyst stage on the fifth day (Fig. 4A). Cortisone had high sensitivity and specificity for predicting live birth, with a cutoff value of 12.66 ng/ml. The ROC analysis of DHT showed that FF DHT level predicted the fertilization embryos with high sensitivity (81.7%) and specificity (65.4%), indicating that DHT is a predictor of fertilized embryos (Fig. 4B). 81.7% of the oocytes with FF DHT level ≤ 0.432 ng/ml led to fertilized.

Mitochondrial ETC mRNA expression in FF exosomes

Mammalian mitochondrial DNA (mtDNA) consists of a 16.5kb double-stranded circular DNA molecule. All 13 polypeptide genes in mtDNA are involved in the production of mitochondrial complex components. mtDNA encodes 7 of the 43 subunits of complex I (ND1, 2, 3, 4, 4L, 5, and 6), 1 of the 11 subunits of complex III (cytochrome b), 3 of complex III (COX1, COX2 and COX3), 13 subunits of complex IV and 2 of 17 subunits of complex V (ATPase 6 and ATPase 8) [22] (Fig. 5A). The mRNA expression levels of mitochondrial ETC genes from the FF exosomes were examined in our study, only ETC complex I sub1, ETC complex I sub2, ETC complex I sub4, ETC complex I sub6, ETC complex III CytB, ETC complex IV

COX1 and ETC complex IV COX2 can be detected in FF exosomes. The results showed significantly increased ETC complex I sub1(Fig. 5B) and ETC complex III CytB (Fig. 5F) mRNA expression in the group of oocyte fertilized and developed into a TQ blastocyst as on day 5 than the group of no fertilisation, whereas the other ETC complex mRNA expression were not affected ($p > 0.05$).

We further elucidated whether mRNA expression levels of mitochondrial ETC genes can predict the hormone in FF. Pearson correlation analysis showed that ETC complex I sub1 mRNA level was positively and significantly correlated with LH and FSH levels (Fig. 6A&6B). The increases in the ETC complex I sub1 expression levels of were accompanied by the changes in hormonal levels in FF. The lower ETC expression indicated the impaired mitochondria function observed in oocytes, which correlates with the ability of the human oocytes to be fertilized.

Discussion

As the oocyte microenvironment may be crucial for healthy development and maturation of oocytes, biochemical analysis of follicular fluid composition provide informative biomarkers of oocyte health and maturation. Most human oocytes retrieved during in vitro fertilization (IVF) are developmentally unable to form viable blastocysts[23, 24]. During oogenesis, understanding the developmental capacity of the oocyte, also known as oocyte quality, is a major factor in improving reproductive success rate.

In the present study, FF LH and FSH levels were lowest in FF matched to oocytes that were not fertilised after ICSI. Compared with the no blastocyst embryos and blastocyst embryos, LH and FSH are also lower in the no blastocyst group. FSH and LH are hormones secreted by the pituitary gland and are crucial for promoting follicular growth, differentiation and maturation[25]. LH binds the G protein–coupled receptors in mural granulosa cells which activates the cAMP system. FSH binds its GPR receptor (FSHR), at the same time, collaborating with Estrogen/ER signal pathway. S Marchiani et al.[26] found LH supplementation in low-responsive women modifies ovarian steroid production, mimicking physiological production better and may help improve ovarian response. With the addition of FSH, LH or HCG [27] to the IVM medium, immature oocytes rapidly undergo spontaneous oocyte meiotic maturation. Oocyte maturation begins with the conversion of germinal vesicle (GV) oocytes to MI oocytes then to MII oocytes. LH initiates oocyte meiotic maturation[28]. Funahashi et al. [29] exposed porcine oocytes to the cAMP analogue dbcAMP. Although the rate of oocyte maturation did not increase, there was an increase in oocyte quality. Blastocyst rates were higher in the treated group compared with the untreated group (21.5% vs. 9%). Our study indicated that FF-LH and FF-FSH levels are good predictors of embryo quality on the fifth day and the levels of LH and FSH are rise with the development of oocytes, which was consistent with previous research results. However, previous study[30] also found the excessive FSH of follicular fluid could interfere with oocyte meiosis, resulting in the formation of aneuploid gametes.

In our present study, cortisone level in FF is another predictor of embryo quality on the fifth day, A E Michael et al.[31] found concentrations of cortisone was significantly lower in FF samples obtained from patients that conceived by IVF-ET than in those obtained from nonconception cycles. S D Keay et al.[32]

showed the higher cortisol: cortisone ratios in conception cycles may be important for final oocyte maturation and embryo implantation. Sabina Lewicka et al.[33] found lower follicular cortisone and higher cortisol/cortisone ratio in both serum and FF are associate with the high clinical pregnancy. In our present study, cortisol: cortisone ratios also higher in the TQ blastocyst on the fifth day than the no fertilization group. All these results indicated cortisone level could predict the embryo quality to some extent. In our study, FF DHT level predicted unsuccessful fertilization with 81.7% sensitivity and 65.4% specificity. DHT, as one of the androgen, plays important roles in female reproduction in healthy and pathological states. Research on DHT in human embryos or follicular fluid is limited, and the existing research on DHT mainly focuses on mouse studies. Mouse study[34] has found long-term DHT treatment replicated a breadth of ovarian, endocrine, and metabolic features of human PCOS. Another research in mouse has the opposite conclusion that the high dose of DHT prompted normal follicular development in granulosa cells of the ovarian follicle[35]. We are currently expanding the sample size to obtain more reliable results.

Electron transport chain (ETC) composed of complexes I-IV, the activity of which regulates mitochondrial oxidative phosphorylation [36]. Mitochondrial ATP is the main source of energy for the FSH-dependent proliferation and differentiation of granulosa cells during follicular development [37] and is generated by mitochondrial oxidative phosphorylation in ETC complexes[38]. Our research showed significantly increased ETC complex I (Fig. 5B) and ETC complex III CytB (Fig. 5F) mRNA expression in the group of oocyte fertilised and developed into blastocyst on day 5. Furthermore, there was a strong positive correlation between FF FSH and LH and ETC complex I mRNA expression, while no correlation between FF DHT and cortisone and ETC complex I/III mRNA expression. A large number of researches have proved mitochondrial dysfunction causes a decrease in oocyte quality and interferes with embryonic development [39–41].

Follicles in development mainly depend on the surrounding granulosa cells for energy. FSH induced granulosa cell proliferation and differentiation, steroidogenesis, and LH receptor expression[42]. LH then acts on its receptors on the mural granulosa cells of preovulatory follicles, leading to oocyte maturation and ovulation[43]. In our study, the TQ blastocysts group, with increased LH and FSH levels, induced granulosa cell proliferation and follicular fluid exosomes which are mainly released by granulosa cells. Oocyte and embryo development require optimal energy production, the increased mitochondrial ETC mRNA expression on the TQ blastocysts group indicated the sufficient source of ATP during preimplantation embryonic development. Both the steroid and mitochondrial function promoted granulosa cell proliferation and lead to the oocyte and embryo development.

Our study is the first to correlate the changes of hormone levels in follicular fluid with the expression levels of mitochondrial complexes. The extraction of exosomes is time-consuming and laborious. By detecting the levels of LH and FSH levels in follicular fluid, the expression level of mitochondrial complexes can be reflected, which can predetermine subsequent embryonic developmental competence to some extent.

Conclusion

With the widespread use of assisted reproductive techniques, the effects of these techniques on oocyte quality and mitochondrial function have been extensively studied. Oocyte maturation, fertilization and embryonic development require optimal mitochondrial function. Regulation of mitochondrial-encoded subunits of respiratory chain complexes may play a key role in related mechanisms. Our study is a preliminary exploration of hormone levels in follicular fluid and exosomes mitochondrial expression. We still need verify these results in a larger sample size and more cytology and animal study to further prove these mechanisms.

Abbreviations

FF
Follicular fluid
LC-MS/MS
Liquid chromatography-tandem mass spectrometry
ETC
Electron transport chain
ART
Assisted reproductive technology
IVF
In vitro fertilization
hCG
Human chorionic gonadotropin
ICM
Inner cell mass
TE
Trophectoderm
EVs
Extracellular vesicles
qRT-PCR
Quantitative RT-PCR

Declarations

• Ethical Approval and Consent to participate
The study was approved by the Ethical Committee of the Zhongshan Hospital, Fudan University (Shanghai, China), and carried out in compliance with the Population and Family Planning Law of the People's Republic of China (The ethical approval number: B2021-665).

• Consent for publication

Not applicable.

• Availability of supporting data

The data that support the study are available upon reasonable request to the corresponding author.

• Competing interests

The authors declare that they have no competing interests.

• Funding

This work was supported by grants National Natural Science Foundation of China 81972000, 81772263, 82172348, Specialized Fund for the clinical researches of Zhongshan Hospital affiliated Fudan University 2018ZSLC05, Constructing project of clinical key disciplines in Shanghai shslczdzk03302, Shanghai Medical Key Specialty ZK2019B28, Key medical and health projects of Xiamen YDZX20193502000002 (to Wei Guo); National Science Foundation of China 81902139 and Specialized Fund for the clinical researches of Zhongshan Hospital affiliated Fudan University 2020ZSLC54(to Beili Wang); Youth Fund of Zhongshan Hospital affiliated Fudan University 2020ZSQN34 (to Li Yu); National Natural Science Foundation of China 81971345 (to Suying Liu); National Natural Science Foundation of China 82001545 (to Miao Liu).

• Authors' contributions

LY, ML, SX and XD collected samples and clinical data; SL and XD performed assisted reproductive technology; ZW tested the steroid of follicular fluid; JZ, TL, BP, BW and WG designed the study; LY analyzed the data and wrote the paper. The authors read and approved the final manuscript.

• Acknowledgements

The authors would like to thank the patients for their participation in this study.

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Figures

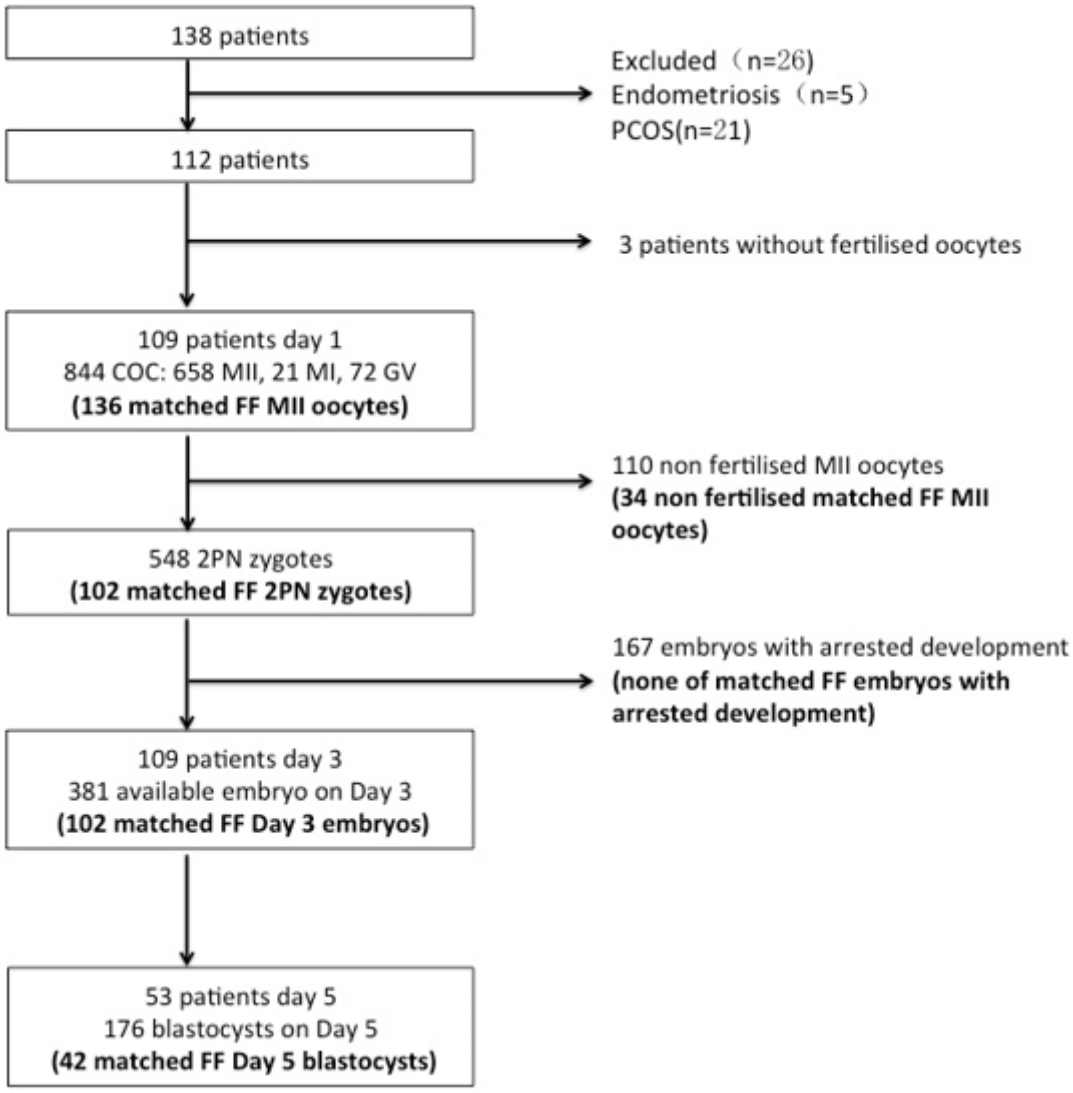

Figure 1

Flowchart of participants, oocytes, embryos and embryo transfer procedure in the study.

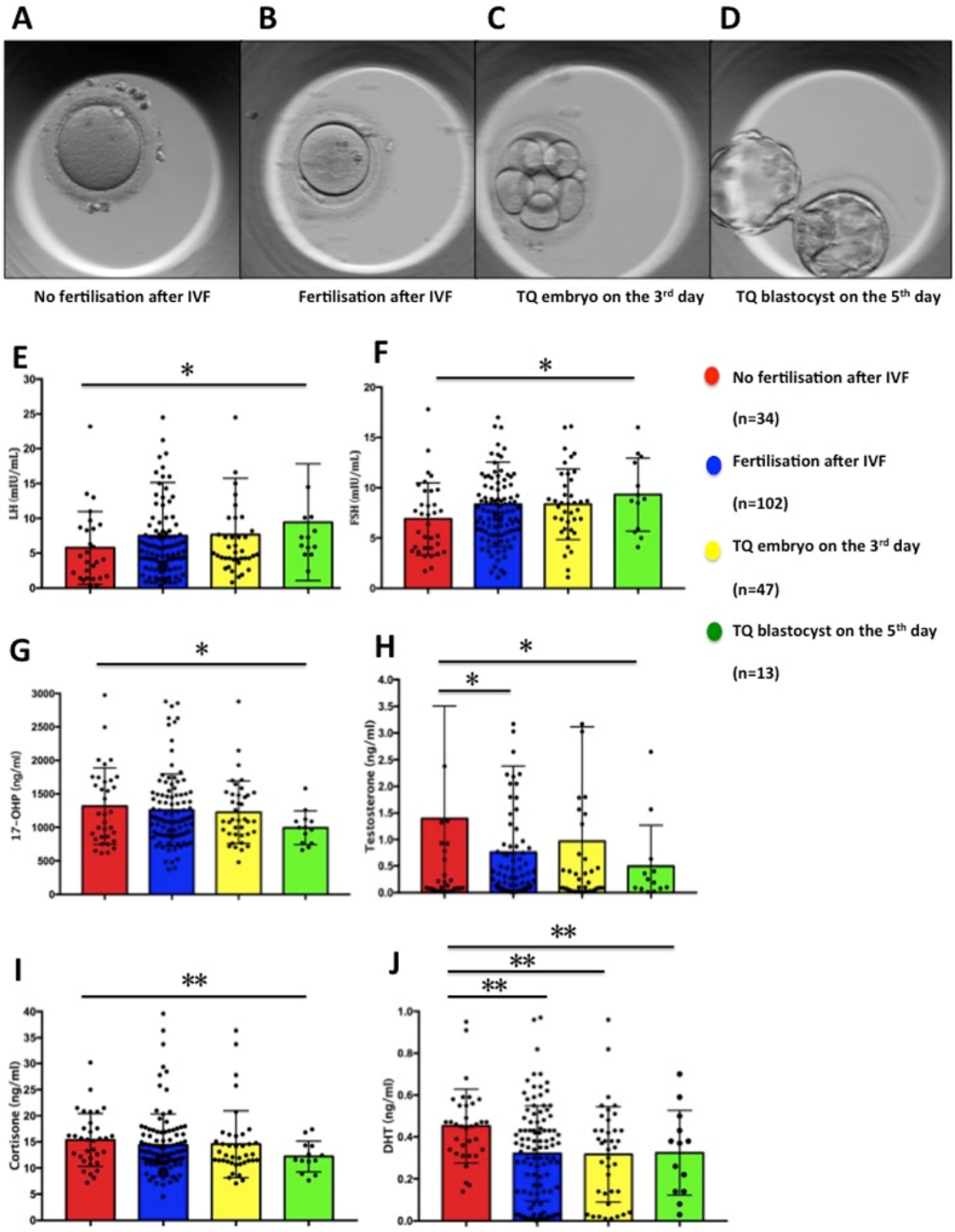

Figure 2

Figures of oocyte and embryo in different developmental states and distribution of steroid concentrations in follicular fluid in relation to the corresponding oocytes: fertilisation and subsequent embryo development.

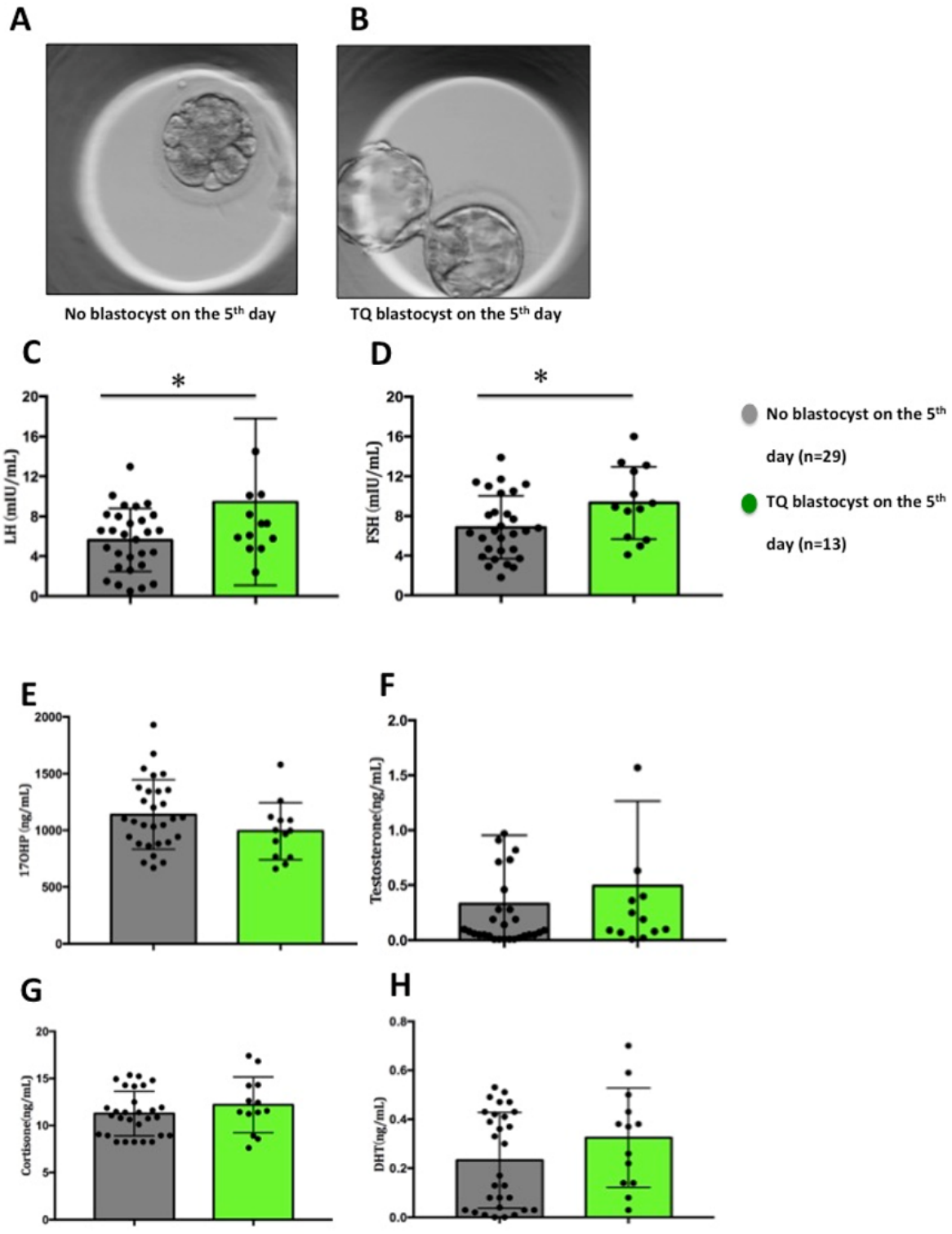

Figure 3

Figures of embryos and distribution of steroid concentrations in follicular fluid in relation to the embryo development on the 5th day.

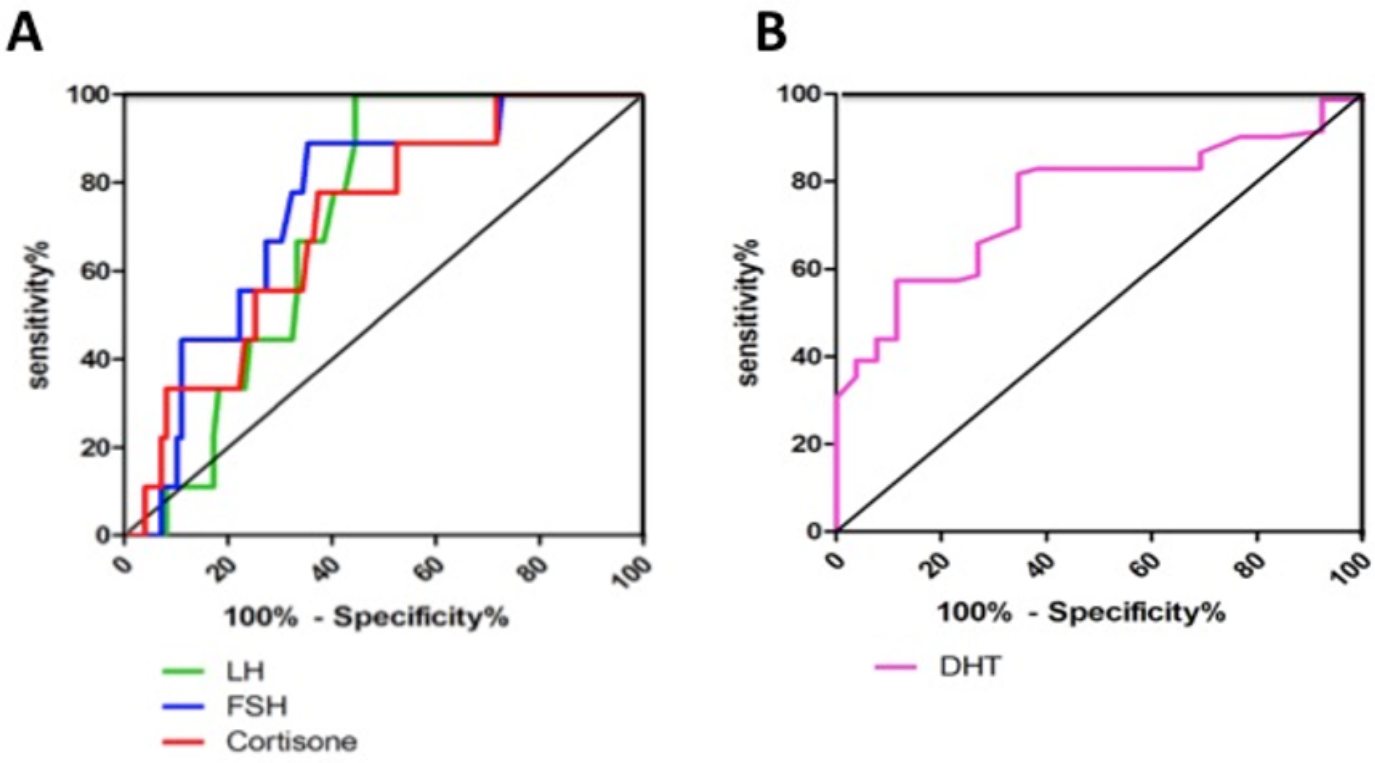

Figure 4

Receiver operating characteristic curve analysis of LH, FSH, cortisone and DHT levels in FF.

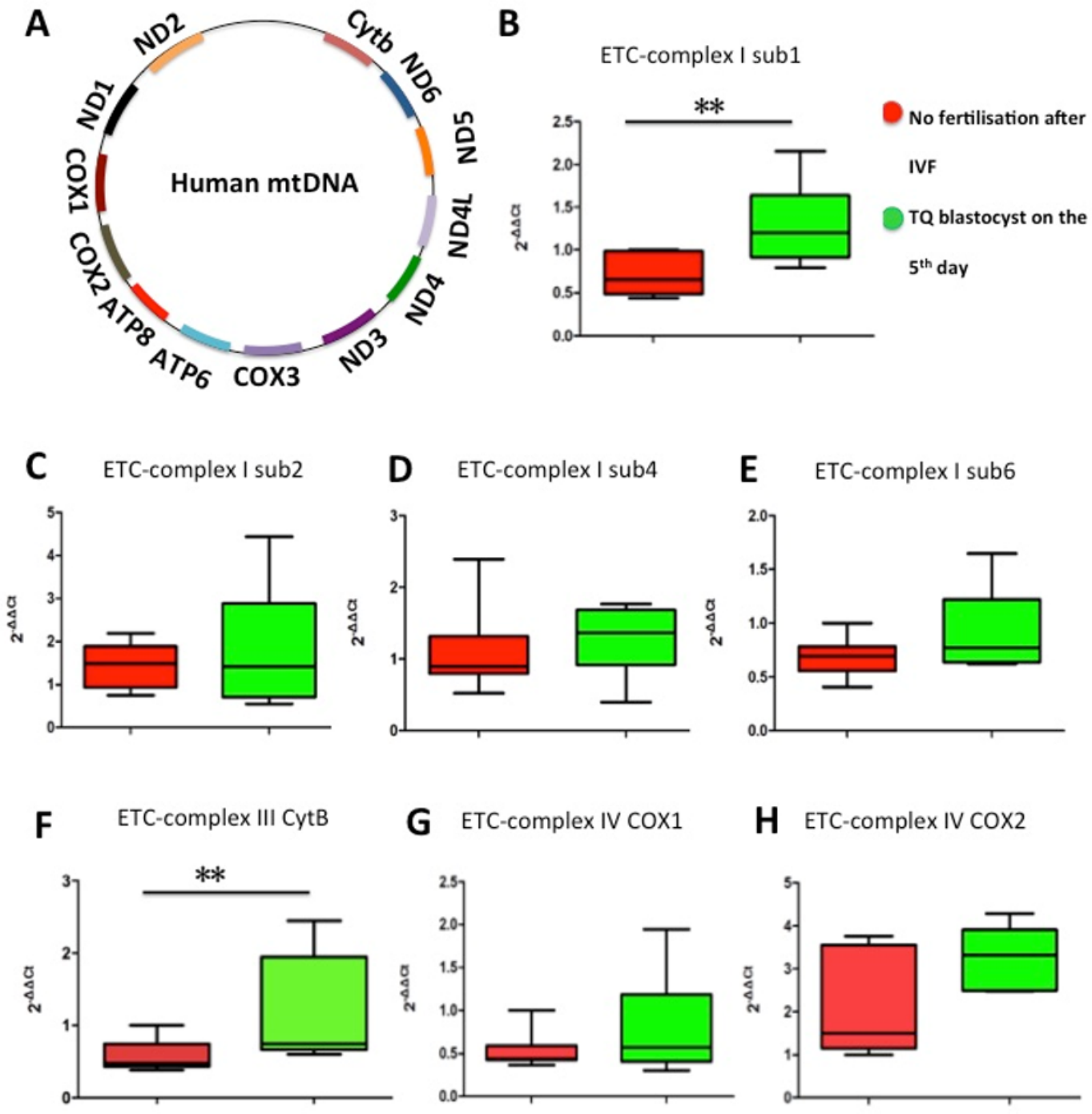

Figure 5

Representation of the mitochondrial ETC genes and mRNA expression levels of mitochondrial ETC genes in FF exosomes.

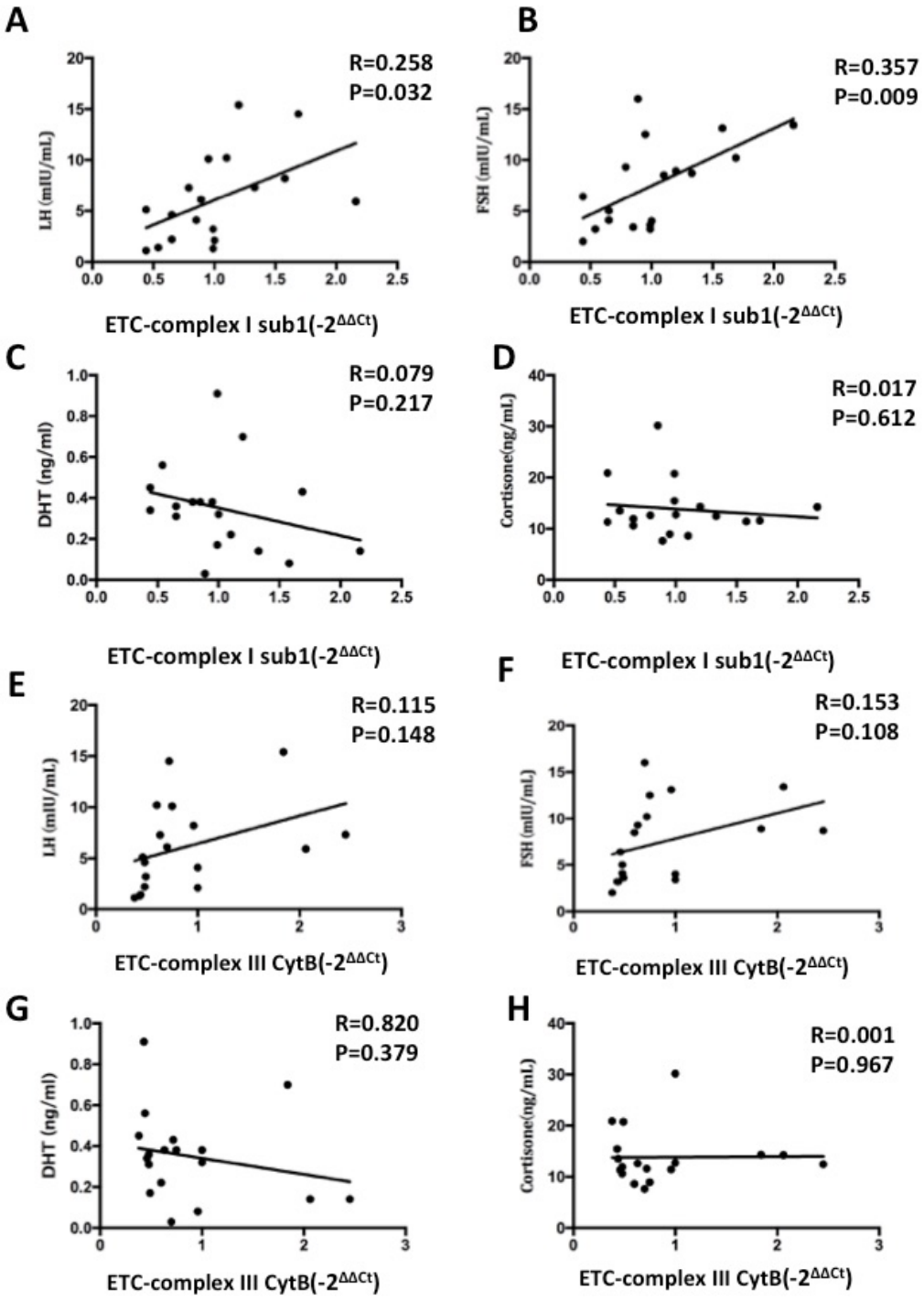

Figure 6

Correlations between the mRNA expression levels of mitochondrial ETC genes in FF exosomes with LH, FSH, DHT and cortisone levels.

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