Telomere length is short in PCOS and oral contraceptive does not affect the telomerase activity in granulosa cells of patients with PCOS

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Abstract

Purpose Our study aimed to investigate the association of telomerase activity (TA) and telomere length (TL) in granulosa cells (GCs) with IVF outcomes of polycystic ovary syndrome (PCOS) patients, and the effects of oral contraceptive pill (OCP) pretreatment on these two parameters.

Methods One hundred sixty-three infertile women were enrolled and divided into a PCOS group ($n = 65$) and a non-PCOS group ($n = 98$). The PCOS group was further divided into an OCP pretreatment group ($n = 35$) and a non-OCP pretreatment group ($n = 30$), a TA <0.070 group ($n = 34$) and a TA ≥ 0.070 group ($n = 31$), and a TL <1 group ($n = 41$) and a TL ≥ 1 group ($n = 24$), respectively.

Results No obvious differences were observed in TA between these groups. The TL was 0.971 in PCOS group and 1.118 in non-PCOS group ($P = 0.005$). The patients with TL ≥ 1

accounted for 36.9% in PCOS group and 54.1% in non-PCOS group ($P = 0.032$). The average duration of infertility for PCOS patients was 5 years in TA <0.070 group and 4 years in TA ≥ 0.070 group ($P = 0.038$), and 5 years in TL <1 group and 3 years in TL ≥ 1 group ($P = 0.006$), respectively. No obvious differences were observed in IVF outcomes between these groups. No obvious differences were observed in TA, TL, or IVF outcomes between OCP pretreatment group and non-OCP pretreatment group in PCOS patients.

Conclusions Shorter TL was found in PCOS patients. The TA levels did not change significantly in PCOS patients. PCOS patients with a lower TA level and shorter telomeres had an earlier onset of infertility symptoms. No predictive value was found for TA and TL in terms of embryo quality or IVF outcomes in PCOS patients, and no effect OCP pretreatment was observed on either TA and TL.

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Keywords Polycystic ovary syndrome · Granulosa cell · Telomerase activity · Telomere length · Oral contraceptive pill · In vitro fertilization-embryo transfer

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Introduction

Approximately 74% of polycystic ovary syndrome (PCOS) patients are infertile [1]. Some PCOS patients can be pregnant after in vitro fertilization (IVF) [2]. But some patients are insensitive to drugs because of the abnormal basal hormone levels such as the high androgen level, which can affect the growth status of granulosa cells (GCs) [3, 4]. Follicular development is associated with the growth status of GCs [5], which is determined by the telomerase activity (TA) and telomere length (TL) [6]. Telomerase is the critical enzyme for maintaining TL [6].

The TA in GCs is highest in the preantral follicle and gradually decreases during the subsequent developmental stages. A reduction of TA can result in a decrease in serum follicle-stimulating hormone (FSH) and follicular atresia. An abnormal TL in GCs may lead to low quality and a low number of oocytes [5–7]. Longer TLs were found in cumulus cells of mature oocytes and good quality embryos, indicating that TL might be related to follicle development and embryo quality [8]. Chen H et al. showed that the TA in GCs of non-PCOS patients was positively correlated with IVF pregnancy outcomes [9]. Several research reports on the relationship between PCOS and TL found that TLs were shorter in the peripheral blood lymphocytes of patients with PCOS [10]. TL in peripheral blood lymphocytes was negatively correlated with inflammatory markers in PCOS patients [11]. Oral contraceptive pills (OCP) could reduce the basal luteinizing hormone (LH) and androgen levels [12] in PCOS patients and improve IVF outcomes [13]. Townsley et al. illuminated that danazol treatment caused telomere elongation in those with telomere diseases [14].

However, it is unknown whether the abnormal growth status of GCs in PCOS patients was associated with the TA or TL. Studies on the relationship between TL and PCOS have mainly focused on the peripheral blood lymphocytes of PCOS patients. Is the TL shortened in GCs of PCOS patients? We were also interested in several additional questions: Is the TA lower in GCs of PCOS patients? Are the TA and TL in GCs of PCOS patients associated with the outcomes of IVF? Given that OCP pretreatment can reduce the levels of androgen and LH in PCOS patients and improve their pregnancy outcomes, would OCP pretreatment affect the TA or TL in GCs of PCOS patients? Our study aimed to investigate the association of the TA and TL in GCs of PCOS patients with their IVF outcomes and to explore the effects of OCP pretreatment on the TA and TL in the same individuals.

Materials and methods

Study design and setting

One hundred sixty-three infertile women for IVF at the Reproductive Medicine Center of Sun Yat-sen Memorial Hospital between March 2011 and July 2011 were divided into a PCOS group ($n = 65$) and a non-PCOS group ($n = 98$), and all patients in this study provided written informed consent for the use of their medical records. The research was approved by the Ethics Committee of Reproductive Medicine, Sun Yat-sen Memorial Hospital.

The inclusion criteria for PCOS group were the PCOS Rotterdam diagnosis criteria [15] recommended by the European Society of Human Reproduction and Embryology. The inclusion criteria for non-PCOS group were a regular

menstrual cycle (22–35 days) [16], normal ovulation, basal FSH <10 IU/L, and the main infertility factor was tubal factor infertility (obstruction of oviduct confirmed by hysterosalpingography or laparoscopy). The exclusion criteria were patients younger than 23 years old or older than 38 years old, endocrine diseases other than PCOS (adrenal gland disease, diabetes mellitus, thyroid disease, hyperprolactinemia, or endocrine diseases of other organs), other severe internal medicine or surgical diseases, chromosomal abnormalities in one or both spouses, congenital or acquired structural abnormalities of the reproductive tract, a history of benign or malignant ovarian cancer, ovarian endometriosis as indicated by B-type ultrasound or laparoscopy, and unexplained infertility. The PCOS group was further divided into an OCP pretreatment group ($n = 35$) and a non-OCP pretreatment group ($n = 30$).

OCP pretreatment

Before the IVF-ET treatment cycle, OCP pretreatment was performed for three cycles for the PCOS patients with hyperandrogenism and abnormal LH levels. One OCP was administered starting on days 3–5 of the menstrual cycle and 21 days was one cycle (Diane-35 Bayer, Leverkusen, Germany).

IVF treatment

All enrolled patients underwent the mid-luteal phase long protocol for IVF. Short-acting gonadotropin-releasing hormone agonist (GnRH-a) (Decapeptyl; Ferring GmbH, Oberhausen, Germany) was used for pituitary downregulation at 0.1 mg/day before recombinant human FSH (Gonal-F; Merck Serono, Geneva, Switzerland) and gonadotropin (Gn) stimulation. Oocyte retrieval was performed 34–36 h after 6000–10,000 IU human chorionic gonadotropin (HCG; Lizhu Medical Company, Zhuhai, China) injection when the diameter of three follicles was ≥ 16 mm, the diameter of two follicles was ≥ 17 mm, or the diameter of one follicle was ≥ 18 mm, and when the serum E2 level reached or exceeded the level that corresponded to the size and number of follicles. Pronuclear formation was monitored 16–18 h after insemination. On day 3, embryonic quality was assessed [17]. Embryo transfer was performed 72 h after the oocyte retrieval. Conventional luteal support was performed after the transfer. The presentation of the gestational sac and fetal heart under a B-ultrasound 5 weeks after the transfer indicated clinical pregnancy [18].

Hormone testing

Blood samples of all patients were collected on days 2–4 of the menstrual cycle and on HCG day. And basal hormone levels, including FSH, LH, estrogen (E2), and testosterone

(T) levels, as well as estrogen and FSH levels on HCG day, were determined by Beckman Coulter UniCel DxI 800 and the associated reagents (Beckman Coulter, CA, USA).

The baseline and clinical data of the patients we used to compare the results, including age, duration of infertility, body mass index (BMI), basal hormone levels including FSH, LH, E2, and T levels, were retrieved from the central database. The main outcome measures were the number of oocytes retrieved, metaphase II oocytes, available embryos, good quality embryos, pregnancy rate, and cumulative pregnancy rate.

Definitions

Mature oocytes: Corona radiata cells extended radially from the surroundings of the oocytes, with GCs extended into translucent uncertain bodies and visible first polar bodies.

Embryo assessment was performed according to the Istanbul consensus [17]. **Good quality embryos:** on day 2, the number of embryonic blastomeres ranged between two and four cells, with a uniform size and no. or <5% cell debris. On day 3, the number of embryonic blastomeres reached 7–9 cells, with a uniform size, <5–20% cell debris, and grade 1–2 embryos.

The pregnancy rate was the number of pregnant patients following fresh embryo transfer divided by the total number of patients undergoing fresh embryo transfer. The cumulative pregnancy rate was the total chances of being pregnant after one IVF cycle including fresh embryo transfer and frozen embryo transfer.

GC isolation and extract preparation

GC collection and extract preparation were performed according to the literature [19]. The follicular fluid obtained on oocyte retrieval day was centrifuged at 1500 rpm for 5 min. The precipitate was separated using lymphocyte separation medium (the interface between the two liquids was maintained) and then purified. GC samples with a live cell percentage <50% were excluded, and protein of GCs was determined [20].

Telomerase activity assay

The telomeric repeat amplification protocol enzyme-linked immunosorbent assay (TRAP-ELISA) was performed to determine TA using a commercial kit (*Telo TAGGG Telomerase PCR ELISA^{PLUS}*; Roche Diagnostics GmbH, Mannheim, Germany). Based on the non-isotopic method introduced by Wen [21], the experiment was performed according to the manufacturer's instructions. The values obtained for low control template should be in the range of 0.2–0.5 $A_{450}-A_{690nm}$ units after 10-min substrate reaction. Values for negative controls were <0.1. All samples were analyzed three times. The

TA was calculated as $\Delta A = A_{450} - A_{690nm}$ (A_{450} and A_{690nm} were absorbance values).

Telomere length assay

The DNA of GCs was extracted using the DNA extraction reagent in the kit (DNeasy Blood & Tissue kit; Qiagen, Hilden, Germany). The extracted samples were evaluated to determine the optical absorbance values at 260 and 280 nm using a spectrophotometer and using the A_{260}/A_{280} formula to calculate the DNA concentration and purity. Samples with a value between 1.8 and 2.0 were selected. The TL of the DNA samples was measured using real-time fluorescence quantitative PCR according to the literature [22]. Primer sequences were determined based on the literature [23] and were synthesized by TaKaRa (TaKaRa, Kyoto, Japan). The materials included 2.8 nmol/OD forward telomere primer 1 (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT-3'), 3.1 nmol/OD reverse telomere primer 2 (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3'), 4.3 nmol/OD 36B4 upstream forward primer (5'-CAG CAA GTG GGA AGG TGT AATCC-3'), and 4.15 nmol/OD 36B4 downstream reverse primer (5'-CCC ATT CTA TCA TCA ACG GGT ACAA-3'). TL was calculated according to the formula of Cawthon [22] for the telomere to single copy gene ratio (T/S): $[2^{C_t(\text{telomeres})}/2^{C_t(36B4)}]^{-1} = 2^{-\Delta C_t}$.

Statistical analyses

All continuous variables were examined using the Kolmogorov-Smirnov test. Data that followed a normal distribution were expressed as mean $\pm$ standard deviation and were examined using the *t* test or ANOVA. Data that did not follow a normal distribution were described as median (IQR) and examined using a non-parametric test. Comparison of the percentages between two groups was performed using the χ^2 test or the Fisher exact probability test. The correlations of TA and TL with age and basal hormone levels, including FSH, LH, E2, and T levels, were determined using the Spearman correlation analysis. The correlations of TA and TL with BMI, total Gn dose, number of retrieved oocytes, metaphase II oocytes, available embryos, and good quality embryos were determined using the Pearson correlation analysis. The associations between TA, TL, age, BMI, basal hormone levels, including FSH, LH, E2, and T levels, and the total Gn dose were analyzed using multiple linear regression analysis. Statistical analysis was performed using SPSS 22.0 software. $P < 0.05$ was considered statistically significant.

Results

Comparison of PCOS patients with and without OCP pretreatment

The TA and TL in the GCs of the PCOS patients did not significantly differ between the OCP pretreatment group and the non-OCP pretreatment group ($P > 0.05$). The percentages of PCOS patients with TA <0.070 and TA ≥ 0.070 did not significantly differ between these two groups ($P > 0.05$). No obvious differences were observed in the percentages of PCOS patients with TL <1 and TL ≥ 1 between these two groups ($P > 0.05$). In the OCP pretreatment group, the average duration of infertility for PCOS patients with TA <0.070 and TA ≥ 0.070 was 5.1 and 3.4 years, respectively ($P = 0.031$). And the average duration of infertility for PCOS patients with TL <1 and TL ≥ 1 was 5.1 and 3.1 years, respectively ($P = 0.008$). In non-OCP pretreatment group, the duration of infertility did not significantly differ in PCOS patients with TA <0.070 and TA ≥ 0.070 ($P > 0.05$). And no obvious differences were observed in the duration of infertility for PCOS patients with TL <1 and TL ≥ 1 ($P > 0.05$) (Fig. 1). Age did not significantly differ in PCOS patients with TA <0.070 and TA ≥ 0.070 ($P > 0.05$) or in PCOS patients with TL <1 and TL ≥ 1 ($P > 0.05$) between these groups (Fig. 2). The average basal serum testosterone levels in PCOS patients were 1.9 nmol/L in the OCP pretreatment group and 1.6 nmol/L in the non-OCP pretreatment group ($P = 0.045$). The age, BMI, basal hormone levels, total Gn dose, and IVF outcomes of PCOS patients did not significantly differ between these two groups ($P > 0.05$) (Table 1).

Although the basal testosterone level was different between the two groups, correlation analysis revealed that the basal testosterone level was not associated with the TA ($r = -0.011$, $P = 0.912$) or TL ($r = 0.160$, $P = 0.119$) of GCs in our study population. Therefore, the OCP pretreatment group was included when we compared the PCOS patients to non-PCOS patients.

Associations between TA, TL, and clinical characteristics and laboratory parameters

The age distribution of all participants is shown in Fig. 3. The age range of all participants was 23 to 38 years old. Multiple

linear regression analysis revealed that the relationship between TL and the total Gn dose (IU) was determined by TL (T:S ratio) = $-0.00017 \times \text{IU} + 1.462$ [$R^2 = 0.077$; multiple correlation coefficient (R) = 0.277; $P = 0.008$]. Age did not correlate with the TA ($r = -0.185$, $P > 0.05$) or TL ($r = -0.083$, $P > 0.05$). TA and TL did not correlate with other clinical characteristics and laboratory parameters ($P > 0.05$) (Table 2).

TA, TL, clinical characteristics, and IVF outcomes between the PCOS group and the non-PCOS group

No obvious differences were observed in TA between PCOS patients and non-PCOS patients ($P > 0.05$). The average TL was 0.971 in PCOS group and 1.118 in non-PCOS group ($P = 0.005$). The patients with TL ≥ 1 accounted for 36.9% in PCOS group (24/65) and 54.1% in non-PCOS group (53/98) ($P = 0.032$).

The mean age of patients was 30.3 years old in PCOS group and 31.7 years old in non-PCOS group ($P = 0.024$). The mean basal serum testosterone level was 1.9 nmol/L in PCOS group and 1.3 nmol/L in non-PCOS group ($P < 0.001$). The average FSH/LH ratio of patients was 1.18 in PCOS group and 2.43 in non-PCOS group ($P = 0.024$). The mean total dose of Gn for patients was 1817 IU in PCOS group and 2063 IU in non-PCOS group ($P = 0.045$). The mean FSH level on the HCG day was 11.3 ng/L for patients in PCOS group and 15.9 ng/L in non-PCOS group ($P < 0.001$). The BMI, duration of infertility, basal E2 levels, E2 levels on the HCG day, IVF laboratory indicators, and IVF outcomes did not differ significantly between these two groups ($P > 0.05$) (Table 3).

Comparison of PCOS patients in different TA levels

The PCOS group was further divided into a TA <0.070 group ($n = 34$) and a TA ≥ 0.070 group ($n = 31$). The average duration of infertility for PCOS patients was 4 years in the TA ≥ 0.070 group and 5 years in the TA <0.070 group ($P = 0.038$). The number of retrieved oocytes, number of metaphase II oocytes, number of available embryos, number of good quality embryos, and IVF outcomes of PCOS patients did not differ significantly between these two groups ($P > 0.05$). In the fresh embryo transfer cycle,

Fig 1 The duration of infertility for PCOS patients with different TA and TL levels in the OCP pretreatment group and non-OCP pretreatment group

Fig 2 The age of patients with different TA and TL levels in the OCP pretreatment group and non-OCP pretreatment group

although the TA in PCOS patients without pregnancy was higher than that in pregnant PCOS patients and PCOS

patients with persistent pregnancy, the difference was not significant ($P > 0.05$) (Table 4).

Table 1 TA, TL, clinical characteristics, and IVF laboratory parameters of PCOS patients between the OCP pretreatment group and non-OCP pretreatment group

Characteristics	OCP pretreatment group ($N = 35$)	Non-OCP pretreatment group ($N = 30$)	P^a
TA, OD $\times$ mm, median (IQR)	0.066 (0.059–0.087)	0.073 (0.059–0.096)	0.540
TA level			0.180
TA < 0.070, n (%)	21 (60.0)	13 (43.3)	
TA $\geq$ 0.070, n (%)	14 (40.0)	17 (56.7)	
TL, T:S ratio, median (IQR)	0.890 (0.785–1.149)	0.937 (0.793–1.131)	0.864
TL level			0.634
TL < 1, n (%)	23 (65.7)	18 (60.0)	
TL $\geq$ 1, n (%)	12 (34.3)	12 (40.0)	
Age, years, mean $\pm$ SD	30.1 $\pm$ 3.9	30.4 $\pm$ 4.7	0.746
BMI, kg/m ² , median (IQR)	21.7 (20.4–24.0)	21.4 (18.3–23.3)	0.216
Basal serum FSH level, IU/L, mean $\pm$ SD	6.6 $\pm$ 2.3	7.0 $\pm$ 1.5	0.443
Basal serum LH level, IU/L, median (IQR)	6.8 (4.2–11.7)	5.4 (4.0–7.8)	0.202
Basal serum testosterone level, nmol/L, median (IQR)	1.9 (1.5–2.4)	1.6 (1.0–1.9)	0.045*
FSH/LH ^b , median (IQR)	1.0 (0.6–1.5)	1.2 (0.8–1.8)	0.076
Retrieved oocytes, median (IQR)	14 (9–18)	16 (13–20)	0.090
Total Gn dose, IU, median (IQR)	1650 (1275–2250)	1500 (1219–2081)	0.470
Metaphase II oocytes, median (IQR)	13 (8–17)	14 (11–19)	0.168
Proportion of mature oocytes ^c , %, median (IQR)	92.3 (83.3–100)	92.3 (81.8–100)	0.846
Available embryos, median (IQR)	9 (4–13)	9 (6–12)	0.989
Good quality embryos ^d , median (IQR)	3 (1–5)	3 (1–5)	0.785
Rate of good quality embryos ^e , %, median (IQR)	33.3 (14.3–55.6)	32.1 (10.8–58.8)	0.884
Pregnancy rate ^f , n (%)	17 (63.0)	13 (54.2)	0.524
Miscarriage rate ^g , n (%)	1 (5.9)	1 (7.7)	1.000
Cumulative pregnancy rate ^h , n (%)	27 (79.4)	20 (69.0)	0.342
Cumulative rate of taking baby home ⁱ , n (%)	23 (67.6)	17 (58.6)	0.458
Cumulative miscarriage rate ^j , n (%)	4 (14.8)	3 (15.0)	1.000

TA telomerase activity, TL telomere length, PCOS polycystic ovary syndrome, OCP oral contraceptive pill, IQR interquartile range, SD standard deviation, BMI body mass index, FSH follicle-stimulating hormone, LH luteinizing hormone

* $P < 0.05$ was considered statistically significant

^a P values: t test for age and basal serum FSH level, Mann–Whitney U test for other continuous variables, chi-square or Fisher's exact test for categorical variables

^b FSH/LH = basal serum FSH level/basal serum LH level

^c Proportion of mature oocytes = metaphase II oocytes/retrieved oocytes

^d Good quality embryos were embryos identified as grade A (4 cells in day 2 or 7–8 cells in day 3) or grade B (4–5 cells in day 2 or 7–9 cells in day 3) [17]

^e Rate of good quality embryos = good quality embryos/available embryos

^f Pregnancy rate = the number of pregnant patients with fresh embryo transfer/the total number of patients with fresh embryo transfer

^g Miscarriage rate = the number of patients with spontaneous abortion/the total number of patients with fresh embryo transfer

^h Cumulative pregnancy rate was total chances of being pregnant after one IVF cycle including fresh embryo transfer and frozen embryo transfer [24]

ⁱ Cumulative rate of taking baby home was total chances of successfully giving birth to a healthy baby after one IVF cycle including fresh embryo transfer and frozen embryo transfer

^j Cumulative miscarriage rate was total chances of spontaneous abortion after one IVF cycle including fresh embryo transfer and frozen embryo transfer

Fig 3 Age distribution of all participants

Comparison of PCOS patients in different TL levels

The PCOS group was further divided into a TL <1 group ($n = 41$) and a TL ≥ 1 group ($n = 24$). The average duration of infertility was 5 years for PCOS patients in the TL <1 group and 3 years for PCOS patients in the TL ≥ 1 group ($P = 0.006$). In all cases with TL ≥ 1 , the duration of infertility was 3 years in PCOS patients and 5 years in non-PCOS patients ($P = 0.017$). The mean E_2 level of each mature oocyte on the HCG day in PCOS patients was 245.27 ng/L in the TL <1 group and 331.49 ng/L in the TL ≥ 1 group ($P = 0.024$). No obvious differences were observed in the age, BMI, basal hormone levels, total number of oocytes, number of metaphase II oocytes, number of available embryos, number of good quality embryos, and IVF outcomes between these two groups ($P > 0.05$) (Table 5).

Discussion

Patients less than 38 years old were enrolled because fertility of women significantly decreased after 38 years old [25]. In our study, TA and TL did not correlate with age after restricting the cohort age range. Previous research in fields outside gynecology has reported that androgen levels were associated with TA and TL. Androgens might reduce the TA through inhibiting hTERT expression in prostate cancer cells [26]. A recent study published in the *New England Journal of Medicine* showed that danazol treatment could increase TL in patients with telomere diseases [14]. In our study, neither TA nor TL was related to testosterone levels. No obvious differences were observed in the TA in GCs between PCOS patients and non-PCOS patients. Although the PCOS patients in our study were younger than the non-PCOS patients, the baseline levels were consistent in these two groups. Given that the TA in the patients of our study did not correlate with age and

testosterone levels, it was suggested that PCOS might not influence the TA in GCs. We hypothesized that the poor growth status of GCs in PCOS patients might not correlate with the TA in GCs but rather might be associated with the metabolic disorder and hormone abnormalities. Chen H et al. showed that the TA in GCs of non-PCOS patients was positively correlated with IVF pregnancy outcomes [9]. In PCOS patients, however, no predictive value was found for the TA in terms of embryo quality and IVF outcomes.

Several reports on the relationship between PCOS and TL found that TL was shorter in the peripheral blood lymphocytes of patients with PCOS [10]. TL in peripheral blood lymphocytes is negatively correlated with inflammatory markers in the peripheral blood of PCOS patients [11]. But these studies mainly focused on the peripheral blood lymphocytes of PCOS patients. In our study, PCOS patients had shorter telomeres in the GCs than non-PCOS patients. The total Gn dose was

Table 2 Correlation analysis of clinical characteristics and laboratory parameters with the TL and TA

	TL		TA	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	-0.083	0.416	-0.185	0.069
BMI	-0.159	0.130	0.032	0.761
Basal serum FSH level	0.022	0.832	-0.045	0.661
Basal serum LH level	0.129	0.207	0.011	0.917
Basal serum estrogen level	-0.054	0.601	-0.054	0.600
Basal serum testosterone level	0.160	0.119	-0.011	0.912
Total Gn dose	-0.250	0.013*	-0.029	0.780
Retrieved oocytes	0.020	0.847	0.048	0.641
Metaphase II oocytes	0.018	0.863	0.062	0.545
Available embryos	-0.052	0.611	0.085	0.408
Good quality embryos	0.152	0.136	0.105	0.302

* $P < 0.05$ was considered statistically significant

Table 3 Telomerase activity, telomere length, clinical characteristics, and IVF outcomes between the PCOS group and non-PCOS group

Characteristics	PCOS group (N = 65)	Non-PCOS group (N = 98)	P ^a
TA, OD × mm, mean ± SD	0.094 ± 0.085	0.107 ± 0.119	0.424
TA level			0.184
TA <0.070, n (%)	34 (52.3)	45 (45.9)	
TA ≥0.070, n (%)	31 (47.7)	53 (54.1)	
TL, T:S ratio, mean ± SD	0.971 ± 0.289	1.118 ± 0.369	0.005*
TL level			0.032*
TL <1, n (%)	41 (63.1)	45 (45.9)	
TL ≥1, n (%)	24 (36.9)	53 (54.1)	
Age, years, mean ± SD	30.3 ± 4.3	31.7 ± 3.8	0.024*
BMI, kg/m ² , mean ± SD	21.8 ± 3.3	21.3 ± 3.4	0.365
Duration of infertility, years, mean ± SD	4.7 ± 3.0	5.4 ± 3.7	0.180
Basal serum FSH level, IU/L, mean ± SD	6.8 ± 2.0	9.0 ± 9.9	0.069
Basal serum LH level, IU/L, mean ± SD	7.4 ± 4.8	7.3 ± 28.5	0.967
FSH/LH ^b , mean ± SD	1.2 ± 0.6	2.4 ± 4.4	0.024*
Basal serum testosterone level, nmol/L, mean ± SD	1.9 ± 0.8	1.3 ± 0.7	<0.001*
Basal serum estrogen level, ng/L, mean ± SD	45.6 ± 30.1	48.8 ± 59.4	0.692
Total Gn dose, IU, mean ± SD	1817 ± 911	2063 ± 636	0.045*
Serum FSH level on HCG day, ng/L, median (IQR)	11.3 (8.0–14.6)	15.9 (12.5–20.0)	<0.001*
Serum estrogen level on HCG day, ng/L, mean ± SD	3501 ± 1484	4206 ± 7745	0.498
Retrieved oocytes, mean ± SD	16 ± 8	17 ± 7	0.510
Metaphase II oocytes, mean ± SD	14 ± 6	14 ± 6	0.663
Proportion of mature oocytes ^c , %, mean ± SD	89.1 ± 12.1	86.0 ± 13.8	0.139
Available embryos, mean ± SD	9 ± 5	9 ± 5	0.793
Good quality embryos ^d , mean ± SD	3 ± 3	3 ± 3	0.974
Rate of good quality embryos ^e , %, mean ± SD	37.1 ± 30.7	35.5 ± 29.5	0.749
Fertilization rate, %, mean ± SD	82.7 ± 18.4	80.3 ± 19.9	0.437
Cleavage rate, %, mean ± SD	97.9 ± 5.4	95.5 ± 15.9	0.178
Pregnancy rate ^f , n (%)	30 (58.8)	38 (52.8)	0.506
Miscarriage rate ^g , n (%)	2 (6.7)	2 (5.3)	1.000
Cumulative pregnancy rate ^h , n (%)	47 (74.6)	69 (74.2)	0.954
Cumulative rate of taking baby home ⁱ , n (%)	40 (63.5)	64 (68.8)	0.489
Cumulative miscarriage rate ^j , n (%)	7 (14.9)	5 (7.2)	0.309

PCOS polycystic ovary syndrome, TA telomerase activity, SD standard deviation, TL telomere length, BMI body mass index, FSH follicle-stimulating hormone, LH luteinizing hormone, Gn gonadotropin, IQR interquartile range, HCG human chorionic gonadotropin

*P < 0.05 was considered statistically significant

^a P values: Mann–Whitney U test for serum FSH level on HCG day, t test for other continuous variables, chi-square or Fisher's exact test for categorical variables

^b FSH/LH = basal serum FSH level/basal serum LH level

^c Proportion of mature oocytes = metaphase II oocytes/retrieved oocytes

^d Good quality embryos were embryos identified as grade A (4 cells in day 2 or 7–8 cells in day 3) or grade B (4–5 cells in day 2 or 7–9 cells in day 3) [17]

^e Rate of good quality embryos = good quality embryos/available embryos

^f Pregnancy rate = the number of pregnant patients with fresh embryo transfer/the total number of patients with fresh embryo transfer

^g Miscarriage rate = the number of patients with spontaneous abortion/the total number of patients with fresh embryo transfer

^h Cumulative pregnancy rate was total chances of being pregnant after one IVF cycle including fresh embryo transfer and frozen embryo transfer [24]

ⁱ Cumulative rate of taking baby home was total chances of successfully giving birth to a healthy baby after one IVF cycle including fresh embryo transfer and frozen embryo transfer

^j Cumulative miscarriage rate was total chances of spontaneous abortion after one IVF cycle including fresh embryo transfer and frozen embryo transfer

Table 4 Clinical characteristics and IVF laboratory parameters of PCOS patients in different levels of TA

Characteristics	TA <0.070 group (N = 34)	TA ≥0.070 group (N = 31)	P ^a
Age, years, mean ± SD	30.3 ± 4.4	30.1 ± 4.2	0.880
BMI, kg/m ² , mean ± SD	22.1 ± 2.9	21.5 ± 3.7	0.466
Duration of infertility, y, median (IQR)	5 (3–7)	4 (2–4)	0.038*
Basal serum FSH level, IU/L, mean ± SD	7.0 ± 2.4	6.6 ± 1.4	0.425
Basal serum LH level, IU/L, median (IQR)	6.4 (4.3–11.3)	5.3 (4.1–8.3)	0.533
Basal serum testosterone level, nmol/L, mean ± SD	1.8 ± 0.8	1.9 ± 1.0	0.764
FSH/LH, mean ± SD	1.2 ± 0.6	1.2 ± 0.6	0.932
Retrieved oocytes, mean ± SD	16 ± 7	16 ± 8	0.676
Metaphase II oocytes, mean ± SD	14 ± 5	14 ± 6	0.992
Proportion of mature oocytes, %, median (IQR)	92.6 (86.6–100)	92.3 (78.9–100)	0.318
Available embryos, mean ± SD	9 ± 5	9 ± 5	0.856
Good quality embryos, median (IQR)	4 (1–5)	2 (1–4)	0.529
Rate of good quality embryos, %, median (IQR)	39.6 (13.2–58.8)	33.3 (11.1–53.3)	0.540
Pregnancy rate, n (%)	19 (67.9)	11 (47.8)	0.148
Miscarriage rate, n (%)	1 (5.3)	1 (9.1)	1.000
Cumulative pregnancy rate, n (%)	25 (73.5)	22 (75.9)	0.832
Cumulative rate of taking baby home, n (%)	21 (61.8)	19 (65.5)	0.758
Cumulative miscarriage rate, n (%)	4 (16.0)	3 (13.6)	1.000

IVF in vitro fertilization

*P < 0.05 was considered statistically significant

^a P values: Mann–Whitney U test for the duration of infertility, basal serum LH level, proportion of mature oocytes and rate of good quality embryos; t test for other continuous variables; chi-square or Fisher's exact test for categorical variables

negatively correlated with the TL. In addition, the total Gn dose was significantly lower in the PCOS group. Given that the TL of all subjects did not correlate with age and other clinical characteristics, suggesting that the abnormal growth status of GCs in PCOS patients might be associated with the decreased TL. Some studies indicated that telomerase could maintain the TL [7]. The cell growth status changes when the telomere shortens to a certain level. However, we showed that the TA in PCOS patients was not significantly different from that in non-PCOS patients, but TL was shorter in PCOS, suggesting that other factors such as insulin resistance might influence the TL [27]. Wang W et al. demonstrated that no predictive ability was found in the TL in GCs in terms of IVF pregnancy outcome in non-PCOS patients [19]. Keefe et al. indicated that the TL of oocytes was associated with the development of embryos, and shorter telomeres suggested a poorer IVF outcome [28]. We are interested in whether the TL in GCs of patients with PCOS is related to their outcomes of IVF. However, no predictive value was found in the TL in GCs of patients with PCOS in terms of their IVF outcomes in our study. While in PCOS patients, abnormal follicular development [29], genetic factors, hormone regulation, regulatory factors in the ovary, and cell apoptosis could also affect their

oocyte quality [30]. The TL in GCs in PCOS patients in our study might not be able to intuitively reflect the TL of oocyte cells and predict IVF outcomes.

In our study, PCOS patients with lower TA and shorter TL had a longer duration of infertility. The duration of infertility might also be influenced by age and infertile factors. Given that age did not differ between these groups in our study, and the main infertile factors of PCOS patients in our study were ovulation dysfunction and tubal obstruction, suggesting that the TA and TL in GCs may be associated with the duration of infertility in PCOS patients. PCOS patients with a lower TA and shorter TL might exhibit infertility symptoms earlier. Therefore, the TA and TL might play an important role in the maintenance of ovarian function. Reduction in ovarian function might be associated with a reduction in the TA in GCs [31].

Long-term estrogen replacement therapy can delay telomere shortening in somatic cells [32]. When the amount of estrogen synthesis decreases, the TA of large follicular GCs also decreases accordingly [33]. Another study showed that exogenous estrogen could significantly improve telomerase activity and TERT mRNA expression in the heart, liver, and brain cells in an ovariectomized menopausal mouse model

Table 5 Clinical characteristics and IVF laboratory parameters of PCOS patients in different levels of TL

Characteristics	TL <1 group (N = 41)	TL ≥1 group (N = 24)	P ^a
Age, years, mean ± SD	30.3 ± 3.6	30.1 ± 5.3	0.834
BMI, kg/m ² , median (IQR)	21.6 (20.2–23.7)	21.6 (18.7–23.9)	0.693
Duration of infertility, y, median (IQR)	5 (3–7)	3 (2–4)	0.006*
Basal serum FSH level, IU/L, mean ± SD	6.9 ± 2.3	6.6 ± 1.4	0.505
Basal serum LH level, IU/L, median (IQR)	6.9 (4.3–11.9)	5.2 (4.1–7.2)	0.073
FSH/LH, median (IQR)	1.0 (0.6–1.5)	1.2 (0.9–1.8)	0.082
Basal serum testosterone level, nmol/L, median (IQR)	1.8 (1.1–2.3)	1.8 (1.5–2.4)	0.605
Retrieved oocytes, median (IQR)	15 (12–22)	14 (10–17)	0.273
Metaphase II oocytes, mean ± SD	15 ± 6	13 ± 5	0.202
Proportion of mature oocytes, %, median (IQR)	92.9 (86.2–100)	91.3 (80.0–100)	0.589
Available embryos, mean ± SD	10 ± 5	8 ± 4	0.206
Good quality embryos, median (IQR)	3 (1–5)	3 (1–5)	0.848
Rate of good quality embryos, median (IQR)	33.3 (7.8–54.4)	33.3 (19.9–67.1)	0.309
Serum estrogen level on HCG day/retrieved oocytes, ng/L, mean ± SD	219 ± 93	290 ± 144	0.043*
Serum estrogen level on HCG day/metaphase II oocytes, ng/L, mean ± SD	245 ± 88	331 ± 159	0.024*
Pregnancy rate, n (%)	18 (60.0)	12 (57.1)	0.838
Miscarriage rate, n (%)	0 (0)	2 (16.7)	0.152
Cumulative pregnancy rate, n (%)	31 (77.5)	16 (69.6)	0.486
Cumulative rate of taking baby home, n (%)	27 (67.5)	13 (56.5)	0.384
Cumulative miscarriage rate, n (%)	4 (12.9)	3 (18.8)	0.676

* $P < 0.05$ was considered statistically significant

^a P values: t test for age, basal serum FSH level, metaphase II oocytes, available embryos, serum estrogen level on HCG day/retrieved oocytes and serum estrogen level on HCG day/metaphase II oocytes; Mann–Whitney U test for other continuous variables; chi-square or Fisher's exact test for categorical variables

[34]. Estrogen could increase TA through the MAPK pathway in human endometrial cancer cells [35]. Townsley DM et al. indicated the telomere elongation in patients with telomere diseases after danazol treatment [14]. However in PCOS patients of our study, no effect of OCP pretreatment on the TA or TL in GCs was found. OCP pretreatment did reduce androgen levels in PCOS patients to improve the ovarian microenvironment, reduce the number of antral follicles, and improve the IVF outcomes [13]. We also found that PCOS patients in our study with OCP pretreatment had higher levels of serum testosterone, but their basal FSH and LH levels, and IVF outcomes did not significantly differ from patients without OCP pretreatment. These results suggested that OCP pretreatment might only improve the microenvironment in the ovary of PCOS patients through hormone levels but not improve the growth status of GCs through the TA or TL levels.

As a retrospective study, our study had limitations on the enrollment of PCOS patients with OCP pretreatment. Only PCOS patients with hyperandrogenism and abnormal LH level were treated with OCP for three cycles. The sample size of PCOS patients was not large enough in our study. A better design would be a 1:1 paired study comparing PCOS and non-

PCOS groups to increase the power of the test. Whether extension of the OCP pretreatment time affects the TA and TL in GCs of PCOS patients still needs further exploration.

Conclusions

Our study indicated that telomeres were shortened in the GCs of PCOS patients compared with non-PCOS patients. PCOS patients with a lower TA level and shorter telomeres had an earlier onset of infertility symptoms. No predictive value was found for the TA and TL in terms of embryo quality and IVF outcomes in PCOS patients. No effect was found for the OCP pretreatment on the TA and TL in GCs of PCOS patients.

E2, estrogen; FSH, follicle-stimulating hormone; GCs, granulosa cells; Gn, gonadotropin; GnRH-a, gonadotropin-releasing hormone agonist; HCG, human chorionic gonadotropin; IVF, in vitro fertilization; LH, luteinizing hormone; OCP, oral contraceptive pills; PCOS, polycystic ovary syndrome; T, testosterone; TA, telomerase activity; TL, telomere length; TRAP-ELISA,

telomeric repeat amplification protocol enzyme-linked immunosorbent assay

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Compliance with ethical standards

Informed consent Informed consent was obtained from all the patients involved in the study.

Ethical approval All procedures performed were in accordance with the ethical standards of the Ethics Committees of Reproductive Medicine, Sun Yat-Sen Memorial Hospital, and with the 1964 Helsinki declaration and its subsequent amendments. This study was approved by the Ethics Committee of Reproductive Medicine, Sun Yat-sen Memorial Hospital.

Conflict of interest The authors declare that they have no conflict of interest.

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