Impact of metabolic disorders on endometrial receptivity in patients with polycystic ovary syndrome

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Abstract. The present study investigated the expression of endometrial receptivity-related molecules in patients with polycystic ovary syndrome (PCOS) and different androgen status, insulin resistance (IR) levels, and body mass indexes (BMI) to identify the mechanism underlying their effects on pregnancy outcomes. The present study recruited 43 participants from November 2020 to January 2021, which were classified into five groups: i) Hyperandrogenemia (HA) combined with impaired glucose tolerance group (n=8); ii) HA combined with diabetes mellitus group (n=8); iii) HA combined with non-IR (NIR) group (n=10); iv) non-HA (NHA) androgen combined with IR group (n=8); and v) NHA combined with NIR group (n=9). In addition, according to their BMIs, patients were sub-grouped into lean/normal (n=27), overweight (n=8) or obese (n=8) groups. The mRNA expression levels of endometrial receptivity-related molecules were detected using reverse transcription-quantitative PCR. In addition, flow cytometry was used to determine the phenotype and percentage of uterine natural killer cells (uNK). According to the results, patients with PCOS and IR status, HA and obesity (BMI ≥24 kg/m^2) demonstrated significantly decreased mRNA expression levels of adiponectin, adiponectin receptor (AdipoR)1, AdipoR2, adapter protein containing PH domain, PTB domain and leucine zipper motif 1, estrogen receptor (ER) α, ERβ, progesterone receptor (PR), IL-15, integrin β3 avβ3, and insulin-like growth factor binding protein-1, but increased mRNA expression levels of IL-6 and IL-8 compared with NHA + NIR group or lean/normal group, respectively. In addition, obese patients with PCOS demonstrated increased mRNA expression levels of PR compared with overweight patients. This suggested that insulin resistant status, HA, and obesity could alter the endometrial receptivity of patients with PCOS, which may explain poorer embryo implantation and pregnancy outcomes in clinics.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine and metabolic disease with a prevalence of 4-10% in reproductive-age women globally (1). The main manifestations of the disease are oligomenorrhea, chronic anovulation, polycystic ovarian ultrasonography changes and clinical or biochemical hyperandrogenism. Reportedly, 50-60% of patients with PCOS have different degrees of hyperandrogenemia (HA) (2), and 44-70% of patients have insulin resistance (IR) and hyperinsulinemia (3-5). In addition, 44-70% of patients with PCOS are obese (6). Although identification of the mechanism of PCOS remains elusive, the interaction of genetic, endocrine and environmental factors likely leads to PCOS (7).

In recent years, an increasing number of studies have confirmed that androgen status, IR and obesity play notable roles in the pathogenesis of PCOS (8-10). Previous studies have suggested that hyperandrogen status (11,12), IR (13-15) and obesity (16-19) increase the rate of miscarriage and decrease the pregnancy rate among patients with PCOS. Chang *et al* (13) indicated that IR affects endometrial function and the implantation process. Patel and Carr (20) revealed that hyperandrogen status and IR are associated with adverse fertility outcomes in patients with PCOS. Cohort studies of >9,500 cycles (21-23), indicate that obesity may induce embryo implantation failure by impairing endometrial receptivity.

Endometrial receptivity is an important factor for successful embryo implantation, which is accompanied by the fluctuations of steroid hormones and endometrial receptivity-related factors, the influx of uterine natural killer cells (uNK) cells and the inhibition of inflammatory factors. Endometrial receptivity is regulated by multiple factors, such as receptors of hormones (estrogen and progesterone), pro-inflammatory cytokines [IL-6, IL-8, IL-15 and monocyte chemoattractant protein-1 (MCP-1)] and endometrial decidualization-related factors [integrin β3 (avβ3) and insulin-like growth factor binding protein-1 (IGFBP-1)] (24).

In addition, adiponectin is also a biomarker of endometrial receptivity (25,26). Adiponectin, an insulin sensitizer secreted

by adipocytes, is closely associated with the insulin signal transduction pathway (25,26). Adiponectin exerts its effects by binding to adapter protein containing PH domain, PTB domain and leucine zipper motif 1 (APPL1), together with AdipoR1 and AdipoR2, to activate different signaling pathways, such as MAPK, p38, ERK1/2, Akt and AMP-activated protein kinase (AMPK) (27). Adiponectin contains three main forms: Trimer, hexamer and high molecular weight (HMW). Some studies hypothesize that HMW adiponectin is the form most closely associated with insulin sensitivity (28). The binding of the APPL1 to adiponectin receptors is regulated by the APPL2; AdipoR1- and R2-dependent signaling is mediated through APPL 1 and APPL2. In the absence of adiponectin signal, APPL2 can bind to the adiponectin receptors or it can form an APPL1/APPL2 heterodimer which prevents the binding of APPL1/adiponectin receptors (29). The adiponectin signaling pathways play notable roles in anti-atherosclerosis, improving the insulin resistant state, reducing blood glucose and reducing anti-inflammatory effects.

Metabolic disorders (high androgen status, IR and obesity) may be the reason for embryo implantation failure, as androgen status and IR are two interacting factors. However, previous studies (13,21-23,30) did not assess different androgen status combining with different IR levels, nor consider the BMI cutoff value for Asian PCOS women. In addition, the majority of previous studies were performed in spontaneous ovulation. Therefore, based on previous findings (24,25) the present study investigated the endometrial receptivity in patients with PCOS with different metabolic abnormalities by assessing well-characterized endometrial receptivity markers, including adiponectin and its signaling protein, pro-inflammatory cytokines (MCP-1, IL-6 and IL-8) mediated through adiponectin, estrogen receptor (ERα), ERβ, progesterone receptor (PR), IL-15, avβ3 and IGFBP-1 in the endometrium during the window of implantation.

Materials and methods

Ethical approval. The present study was approved by the Clinical Scientific Research and Experimental Animal Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University [approval no. Ethics (2020) no. 422-1]. Written informed consent was obtained from all participants.

Participants. Infertile patients with PCOS attending the Department of Reproductive Endocrine of the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) from November 2020 to January 2021 were recruited. The patients' PCOS diagnoses were based on the 2003 Rotterdam criteria (31). Each participant met at least two of the following criteria: i) Oligomenorrhea and/or chronic anovulation; ii) clinical and/or biochemical hyperandrogenism; and iii) polycystic ovarian ultrasonography changes. The exclusion criteria were patients with Cushing's syndrome, congenital adrenal hyperplasia, androgen-secreting tumor, tubal effusion, intrauterine adhesions, multiple endometrial polyps, chromosomal diseases, recurrent fertilization failures, organic diseases of the uterus or ovaries (such as endometriosis or adenomyosis), history of surgery, pelvic radiotherapy and chemotherapy in ovaries, thyroid diseases, hyperprolactinemia, adrenal gland tumors, cardiovascular and cerebrovascular diseases and mental illness and associated disorders. Patients who took hormones or drugs in the past 3 months (that would affect insulin) were also excluded. The diagnosis was confirmed by a pathologist, and the pathologist was independent from the study.

Levels of testosterone (T), sex hormone-binding globulin (SHBG), oral glucose tolerance test (OGTT) and fasting insulin were measured on the 2-5 days of menstruation. High androgen status was determined when the free androgen index (FAI) (%) [T (nmol/l)/SHBG (nmol/l)] was >4.5% (32). IR was considered when the homeostasis model assessment IR (HOMA-IR) [fasting insulin (μ international unit (IU)/ml) x fasting plasma glucose (FPG) (mmol/l)/22.5] was >2.4 (33). According to the OGTT test, the present study defined FPG as ≥5.6 mmol/l and/or 2-h postprandial blood glucose (2hPBG) ≥7.7 mmol/l as impaired glucose tolerance (IGT); furthermore, FPG ≥7.0 mmol/l and/or 2hPBG ≥11.1 mmol/l was defined as diabetes mellitus (34).

The patients were classified into five different groups depending on their FAI, HOMA-IR, and OGTT results: i) Hyperandrogenemia combined with impaired glucose tolerance (HA + IGT) group (n=8), FAI ≥4.5% and HOMA-IR >2.4, 5.6 mmol/l ≤FPG ≤6.9 mmol/l, and/or 7.7 mmol/l ≤2 h PBG ≤11 mmol/l; ii) hyperandrogenemia combined with diabetes mellitus (HA + DM) group (n=8), FAI ≥4.5%, HOMA-IR >2.4, FPG ≥7.0 and/or 2 h PBG ≥11.1; iii) hyperandrogenemia combined with non-IR (HA + NIR) group (n=10), FAI ≥4.5%, HOMA-IR ≤2.4; iv) non-hyperandrogenemia androgen combined with IR (NHA + IR) group (n=8), FAI <4.5%, HOMA-IR >2.4; or v) non-hyperandrogenemia combined with non-IR (NHA + NIR) group (n=9), FAI <4.5%, HOMA ≤2.4. In addition, according to the BMI value, the subjects were divided into a lean/normal group (n=27; BMI<24 kg/m^2), an overweight group (n=8; 24≤ BMI <28 kg/m^2), and an obese group (n=8; BMI ≥28 kg/m^2) (35).

Sample collection and treatments. The number of antral follicles was measured by vaginal B-ultrasound on the 2-5 days of menstruation for all participants, and ultrasound examinations were conducted every few days to monitor the number and size of the follicles as well as the endometrial thickness. In addition, the basic information of each patient was collected on the 2-5 days of menstruation, including age, BMI, years of infertility, and blood samples. In this sample, other possible interference in the diagnosis of PCOS [such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, progesterone, testosterone, prolactin (PRL), anti-Mullerian hormone (AMH), thyroid-stimulating hormone (TSH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG) and cholesterol (CHOL)] were included on Table I.

In order to guarantee the transformation of the endometrium to the secretory phase, hormone replacement therapy (HRT) was performed: 2 mg of estradiol was given daily (Progynova®; Bayer) for endometrial proliferation support for ≥10 days. When the endometrium thickness reached 8 mm, 10 mg of dydrogesterone tablets were given twice a day (Duphaston®; Abbott Biologicals B.V.) for 5 days for endometrial transformation. On the fifth day of endometrial

Table I. Chemical and endocrine characteristics of the studied groups.

Parameters	HA + IGT	HA + DM	HA + NIR	NHA + IR	NHA + NIR	P-value
Number	8.00	8.00	10.00	8.00	9.00	
Age (years)	25.63±3.02	26.43±3.87	28.20±3.36	26.60±2.51	28.22±2.39	0.33
WHR	0.89±0.03	0.92±0.03	0.89±0.04	0.86±0.03	0.90±0.02	0.16
BMI (kg/m^2)	25.05±3.2	27.45±5.05	21.25±1.93	23.37±3.69	19.11±2.37[a]	<0.001
FSH (mIU/ml)	5.35±1.60	4.94±0.91	5.29±1.29	5.40±0.64	5.29 (4.68,5.97)	0.81
LH (mIU/ml)	7.26±4.39	7.53±2.51	8.68±4.83	40.94±11.03	7.58±3.66	0.76
E2 (pg/ml)	33.75±9.15	35.3±10.41	40.81±17.88	40.94±11.03	39.49±15.92	0.86
PRL (ng/ml)	13.01 (9.29,23.37)	17.67±8.06	15.22 (10.25,27.47)	12.41±5.29	14.39 (12.81,20.96)	0.78
AMH	11.44±5.17	7.57±4.56	11.05±5.57	10.46 (6.62,11.15)	9.04 (6.61,12.97)	0.38
TSH	1.80±1.11	1.55 (1.25,2.14)	2.16±1.09	1.52±0.63	1.97±0.58	0.62
CHOL	5.15±0.85	4.9(4.6,5.3)	5.11±0.99	4.6(4.6,5.95)	4.6±0.89	0.48
TG	1.01 (0.89,2.03)	1.3±0.65	1.05±0.39	1.11±0.43	0.85±0.13	0.42
HDL	1.28±0.36	1.24 (1.14,1.31)	1.54±0.30	1.35±0.20	1.40±0.25	0.34
LDL	3.29±0.62	3.22±0.22	1.54±0.30	1.35±0.20	1.40±0.25	0.22

[a]P<0.05 compared with HA + IGT. Data are presented as means ± SEM (normal distribution) or as medium (interquartile ranges) (non-normal distribution). HA + IGT, hyperandrogenemia combined with impaired glucose tolerance; HA + DM, hyperandrogenemia combined with diabetes mellitus; HA + NIR, hyperandrogenemia combined with non-insulin resistance; NHA + IR, non-hyperandrogenemia androgen combined with insulin resistance; NHA + NIR, non-hyperandrogenemia combined with non-insulin resistance; WHR, waist to hip ratio; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estrogen; P, progesterone; PRL, prolactin; AMH, anti-mullerian hormone; TSH, thyroid-stimulating hormone; CHOL, cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IU, international unit.

transformation, intravenous blood and endometrium samples were collected. To minimize the biopsy difference, the endometrial biopsies were collected from the superficial layer of the endometrium instead of the basal layer. The receptive phase of endometrium was determined according to Noyes histological standard (36).

High molecular weight (HMW) adiponectin ELISA detection. The sensitivity of specific ELISA kit (cat. no. ml063723, MiBio) of HMW adiponectin was 10 ng/ml. The coefficient of variation within and between plates was <10 and 15%, respectively. The serum HMW adiponectin was determined using enzyme-linked immunosorbent assay (ELISA) with an enzyme-labeled instrument (SkanIt™ software; Thermo Fisher Scientific, Inc.).

RNA extraction and PCR. The frozen endometrial samples were homogenized using TRIzol (cat. no.93289, Thermo Fisher Scientific, Inc.) reagent to obtain total ribonucleic acid (RNA). The RNA concentration was determined using spectrophotometry (A260:A280), and the integrity of RNA was determined using electrophoresis on a formaldehyde agarose gel. The tissue RNA extraction kit (EZBioscience) was used to extract 2 μg of total RNA from the endometrial sample; the RNA reverse transcription and SYBR Green qPCR Master Mix kit (EZBioscience) were used to perform the standard PCR according to the manufacturer's instructions. Human GAPDH was used as internal reference genes and as a biomarker of standardizing to the RNA load of each sample since the expression of this gene is relatively constant during the menstrual cycle (37). PCR amplification of target mRNA was assessed by using gene-specific primers in Table II. Primers were provided by Takara Biotechnology Co., Ltd. The PCR reaction was initiated with 95˚C for 5 min for amplification, melted at 95˚C for 10 sec, then annealing and extension were performed at 60˚C for 30 sec. The polymerase chain reaction products were analyzed using ethidium bromide agarose gel electrophoresis. A quantitative PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for real-time quantitative PCR and data analysis. Amplification efficiencies obtained from quantitative PCR were supported by using the method of $2^{-\Delta\Delta Cq}$ (38).

The relative expression levels of all target genes were calculated by normalizing to the reference gene (GAPDH) ($2^{-\Delta Cq}$), and the relative expression of target genes in different groups was presented as the fold-change relative to the control groups ($2^{-\Delta\Delta Cq}$). For the comparison of different metabolic disorders,

Table II. Gene-specific primer sequences.

Target genes	Primer sequences (5'-3')
GAPDH	Sense GAGTCAACGGATTTGGTCGT
	Antisense ATCCACAGTCTTCTGGGTG
Adiponectin	Sense TGCTGGGAGCTGTTCTACTG
	Antisense TACTCCGGTTTCACCGATGTC
AdipoR1	Sense AAACTGGCAACATCTGGACC
	Antisense GCTGTGGGGAGCAGTAGAAG
AdipoR2	Sense ACAGGCAACATTTGGACACA
	Antisense CCAAGGAACAAAACTTCCCA
APPL1	Sense TTAGCTGCCCGGGCCATCCATA
	Antisense ATCTTTTCCCCCTCATTGTTTG
MCP-1	Sense CAGCCAGATGCAATCAATGCC
	Antisense TGGAATCCTGAACCCACTTCT
IL-6	Sense CAGACAGCCACTCACCTCTTC
	Antisense TGCCAGTGCCTCTTTGCT
IL-8	Sense ATGACTTCCAAGCTGGCCGT
	Antisense TCCTTGGCAAAACTGCACCT
ERα	Sense GGTCAGTGCCTTGTTGGATG
	Antisense TGCCAGGTTGGTCAGTAAGC
ERβ	Sense ACTGGGATTGTGTGGTCAGC
	Antisense AGAGGATAGGCATCGGCATT
PR	Sense TTTAAGAGGGCAATGGAAGG
	Antisense CGGATTTTATCAACGATGCAG
IL-15	Sense GTCCGGAGATGCAAGTATTCA
	Antisense TCCTCACATTCTTTGCATCCA
avβ3	Sense AAGAGCCAGAGTGTCCCAAG
	Antisense AGTTTCCAGATGAGCAGGGC
IGFBP-1	Sense TTTTACCTGCCAAACTGCAACA
	Antisense CCCATTCCAAGGGTAGACGC

GAPDH, Human glyceraldehyde-3-phosphate dehydrogenase; AdipoR, Adiponectin receptor; APPL1, adapter protein containing PH domain, PTB domain and leucine zipper motif 1; AdipoR, adiponectin receptor; MCP-1, monocyte chemoattractant protein-1; IL, interleukin; ER, estrogen receptor; PR, progesterone receptor; avβ3, integrin β3; IGFBP-1, insulin-like growth factor binding protein-1.

Table III. Comparisons of HMW adiponectin levels among groups.

Groups	HMW adiponectin (ng/ml)	P-value
HA + IGT	4703.93 (4,148.54-4,768.53)	
HA + DM	5,129.82±510.88	0.11
HA + NIR	4,625.06±1,084.25	
NHA + IR	4,626.95±452.29	
NHA + NIR	4,283.51±407.41	
Lean/normal	4,520.65±779.47	
Overweight	4,736.18±505.46	0.53
Obese	4,843.26±455.09	

Data are presented as means ± SEM (normal distribution) or as medium (interquartile ranges) (non-normal distribution). HMW, High molecular weight; P, probability.

the NHA + NIR group was used as the control group; for the comparison of different BMI values, the lean/normal group was selected as a control.

uNK cell cycle analysis. On the fifth day of endometrial transformation, endometrium samples were collected using the Endometrial Sampler (TAO Brush™ IUMC). Endometrial tissue was weighed (weight, 300 μg) and immediately placed in PBS containing 0.25% collagenase from Clostridium histolyticum and 0.5% deoxyribonuclease bovine (all Sigma-Aldrich; Merck KGaA) All digestions were performed at 37˚C for 20 min with agitation. The tissue was filtered with a filter (100 um) and was centrifuged at a speed of 350 g at 4˚C for 5 min. The supernatant was removed and the endometrial cells were placed in PBS. The uNK cells are characterized by $CD3^-/CD56^+$ granular lymphocytes, while the expression of CD16 is relatively low compared with peripheral blood natural killer cells (39). Cells were stained with the following monoclonal antibodies conjugated with FITC PE and APC at 20˚C for 20 min: CD3 (FITC anti-human; cat. no. 300402 UCHT1, BioLegend), CD16 (PE anti-human cat. no. 302003 3G8, BioLegend), CD56 (APC anti-human; cat. no. 362535 NCAM, BioLegend). Cells were acquired within 24 h of staining on a BD FACSAria flow cytometer (BD Biosciences). A gate set around CD3-lymphocytes was used to measure the proportion of NK-cell subsets within each sample. In the case of the NK-cell subset, a gate set around CD56+ lymphocytes was used to exclude NK-T-cell subset (Figs. S1 and S2). CELLQuest software (version 5.1 Becton Dickinson) was used for the data analysis.

Statistical analysis. In order to obtain statistical significance results, the number of subjects were calculated: $n=2x[(t\alpha + t\beta)\sigma/\delta]^2$ (40), the test level α was set to 0.05 and the inspection power 1-β was set to 90%, checked the t boundary value table and only take one-sided, t (α=0.05)=1.645, one-sided t (β=0.1)=1.282. By consulting the literature (41), the discrimination (δ) and the standard deviation (σ) were substituted into the formula, samples required for each group was 8. SPSS software (version 20.0; IBM Corp.) was used for the statistical analysis. Normally distributed data were presented as mean ± standard deviation (SD), and the comparison between groups was performed using one-way analysis of variance. The data that was not normally distributed was presented as the median and interquartile range, and the comparison between groups was performed using the Kruskal-Wallis test. The Bonferroni test was used to analyze the post-hoc paired comparison. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic data. As presented in Table I, there was no statistical difference in other clinical and endocrine characteristics among HA + IGT, HA + DM, HA + NIR, NHA + IR and

NHA + NIR groups (P>0.05). However, only the comparison of BMI between the HA + IGT group (27.45 kg/m^2) and the NHA + NIR group (19.11 kg/m^2) demonstrated a significant difference (P<0.05).

HMW adiponectin. There was no significant difference in serum HMW adiponectin in patients across HA + IGT, HA + DM, HA + NIR, NHA + IR and NHA + NIR groups (P>0.05). In addition, no significant difference was revealed in serum HMW adiponectin in patients in lean/normal, overweight, and obese groups (P>0.05; Table III).

Endometrial receptivity-related factors. The relative mRNA expression levels of adiponectin, AdipoR1, AdipoR2, APPL1, ERα, ERβ, PR, IL-15, avβ3 and IGFBP-1 were significantly decreased, while the expression levels of IL-6 and IL-8 were significantly increased in HA + IGT, HA + DM, HA + NIR and NHA + IR groups when compared with the NHA + NIR group (all P<0.05). However, no significant difference was revealed amongst the HA + DM, HA + NIR, and NHA + IR groups (Fig. 1).

For the comparison of different bodyweight groups, the relative mRNA expression levels of adiponectin, AdipoR1, AdipoR2, APPL1, ERα, ERβ, PR, IL-15, avβ3, and IGFBP-1 were significantly lower, while the expression of MCP-1, IL-6 and IL-8 were significantly higher in the overweight and obese groups when compared with the lean/normal group (all P<0.05). Furthermore, the relative mRNA expression of PR in the obese group was significantly higher compared with that in the overweight group (P<0.01; Fig. 1).

Proportion of uNK cells. As presented in Fig. 2, the percentages of CD16$^-$/CD56$^+$ and CD16$^+$/CD56$^+$ in endometrium were comparable across HA + IGT, HA + DM, HA + NIR, NHA + IR and NHA + NIR groups (P>0.05). In addition, no significant difference was revealed among patients in the lean/normal, overweight and obese groups (P>0.05).

Discussion

Wickham *et al* (42) hypothesized that IR can reduce serum HMW adiponectin, while O'Connor *et al* (43) claimed that serum HMW adiponectin is not dependent on IR. On the other hand, studies have indicated that high androgen status may indirectly reduce serum HMW adiponectin in patients with PCOS (44,45), but the evidence was still insufficient. Garcia *et al* (41) revealed that the serum adiponectin level in obese patients with PCOS was lower compared with that in the lean or obese patient groups. Nevertheless, other studies revealed no differences in the expression of adiponectin (46) or HMW adiponectin (47) among overweight/obese or normal-weight patients between PCOS and control groups. So far, there is no consensus on whether there are differences in serum adiponectin in different types of patients with PCOS (42-47). The present study revealed no significant difference in serum HMW adiponectin levels among different high androgen statuses, insulin-resistant levels or BMI levels in patients with PCOS. The various patient group settings and the detection method used for HMW adiponectin may explain the inconsistency in results compared with previous studies. Although the present study revealed no differences in HMW adiponectin, this does not mean that there was no difference in other forms of adiponectin. The alteration in other molecular forms of adiponectin should also be considered, and this needs further exploration.

The endometrium undergoes three different phases during the normal menstrual cycle, which is induced by the steroid hormones fluctuation (48). Estrogen (E2) promotes the expression of estrogen receptor α (Erα) and estrogen receptor β (Erβ); the expression levels of these receptors are highest in the late stage of proliferation. In addition, E2, together with ER, promotes progesterone receptor (PR) expression in the endometrium. After ovulation, progesterone inhibits the expression of ER in the endometrium, thus inducing decidualization. Decidualization is a necessary transformation of endometrium for successful embryo implantation, including endometrial stromal cells proliferation, the increase of glandular epithelial secretion and natural killer cells aggregation (24). Decidualization usually happens at 5-6 days after ovulation (49). The process of decidualization is accompanied by the alteration of ER, PR and inhibition of pro-inflammatory cytokines (interleukin and MCP-1) and endometrial pathological and physiological-related factors (avβ3, IGFBP-1) (24,50). Some researchers hypothesize that the decreased expression of ERα, ERβ, PR, IL-15, avβ3 and IGFBP-1 may indicate the decline of embryo implantation rate (51-54); however, the mechanism of how these factors affect endometrial receptivity remains unclear. Furthermore, previous studies have revealed increased expression of Erα (37,55,56) and PR (56), decreased expression of IL-15 (39), avβ3 (57) and IGFBP-1 (58) and unchanged expression of Erβ (58) in secretory phase endometrium in patients with PCOS when compared with non-PCOS patients.

The present study demonstrated that the expression levels of ERα, ERβ, PR, IL-15, avβ3 and IGFBP-1 were significantly decreased in the presence of HA and/or IR and/or obesity (BMI ≥24 kg/m^2), which were consistent with previous studies by Matteo *et al* (39), Cermik *et al* (57) and Piltonen *et al* (58). Nevertheless, the results were different from the findings by Margarit *et al* (56) and Quezada *et al* (37). The difference may be due to the diversity in studied population. Apart from the distinction of the hyperandrogenemia states, the IR levels and BMI, the control group used in previous studies were different. All previous studies were carried out with in untreated patients with PCOS (with spontaneous ovulation) and non-PCOS patients. By contrast, patients in the present study were treated with HRT to guarantee the endometrial transformation.

During the menstrual cycle, adiponectin and its receptors can be detected, the mRNA expression of adiponectin increased significantly in the early stage of proliferation; however, the mRNA expression levels of AdipoR1 and AdipoR2 increased significantly in the peri-implantation period (25). These changes have been confirmed in the artificial decidua model as well (59). Adiponectin has been indicated to be reduced in the endometrium of obese and PCOS patients (60). Garcia *et al* (41) demonstrated that the obese group (compared with the lean group) have an increased expression of endometrial AdipoR1 and a decreased expression of endometrial APPL1, while the AdipoR2 expression is similar (41). In addition, they demonstrated that when treating immortalized human endometrial stromal cell lines with testosterone and

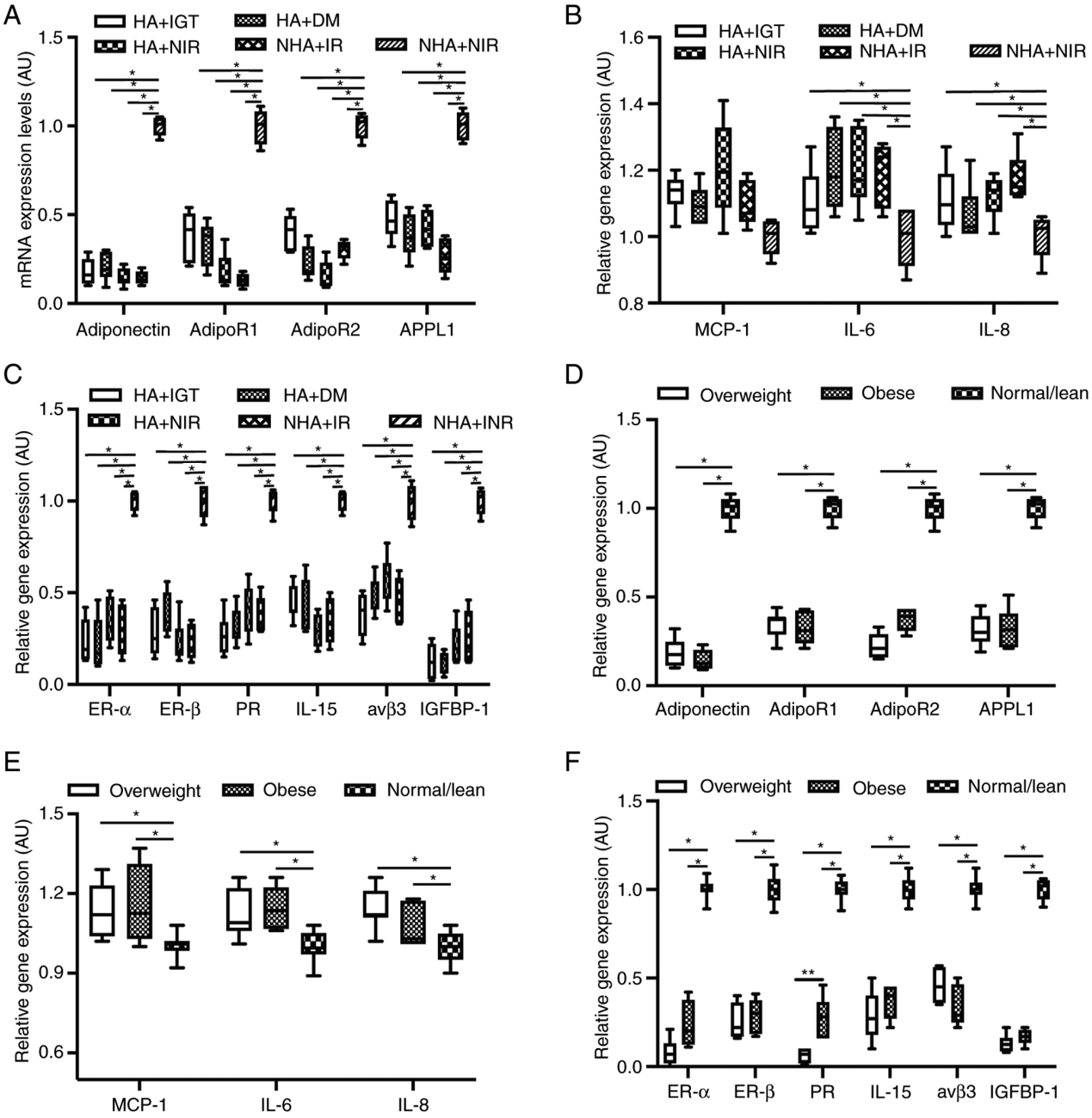

Figure 1. Relative mRNA levels of receptivity-related factors in the endometrium across studied groups. Relative mRNA levels of (A) adiponectin, AdipoR1, AdipoR2 and APPL1, (B) MCP-1, IL-6 and IL-8 and (C) ERα, ERβ, PR, IL-15, avβ3 and IGFBP-1 of receptivity-related factors in endometrium obtained from HA + IGT, HA + DM, HA + NIR and NHA + IR groups. For each gene, the mRNA expression levels were normalized to the mean value of NHA + NIR group (internal control). Relative mRNA levels of (D) adiponectin, AdipoR1, AdipoR2 and APPL1, (E) MCP-1, IL-6 and IL-8 and (F) ERα, ERβ, PR, IL-15, avβ3 and IGFBP-1 of receptivity-related factors in endometrium obtained from lean/normal, overweight and obese groups. For each gene, the mRNA expression levels were normalized to the mean value of lean/normal group (internal control). *P<0.05, **P<0.01. HA, hyperandrogenemia; IGT, impaired glucose tolerance; DM, diabetes mellitus; NIR, non-insulin resistance; NHA, non-hyperandrogenemia androgen; IR, insulin resistance; AdipoR, adiponectin receptor; APPL1, adapter protein containing PH domain, PTB domain and leucine zipper motif 1; MCP-1, monocyte chemoattractant protein-1; IL, interleukin; ER, estrogen receptor; PR, progesterone receptor; avβ3, integrin β3; IGFBP-1, insulin-like growth factor binding protein-1.

insulin, the mRNA expression levels of adiponectin, AdipoR1, AdipoR2 and APPL1 are significantly decreased (41).

Similarly, partly consistent with previous studies (37,41), the present study revealed that the mRNA expression levels of adiponectin, AdipoR1, AdipoR2 and APPL1 were significantly decreased in the presence of HA and/or IR and/or obesity (BMI ≥24 kg/m^2) when compared with the NHA + NIR group and the lean/normal group, respectively. These findings were different from the results of Garcia *et al* (41), and this may be explained by the variance in sample collection. Garcia *et al* (41) collected samples in the proliferative phase and set untreated patients with PCOS (with spontaneous ovulation) as the control group. By contrast, all the patients with PCOS in the present study were treated with HRT, and endometrium samples were collected during the window of implantation in the HRT cycle.

MCP-1, IL-6 and IL-8 are pro-inflammatory factors which are involved in the morphological and pathological changes

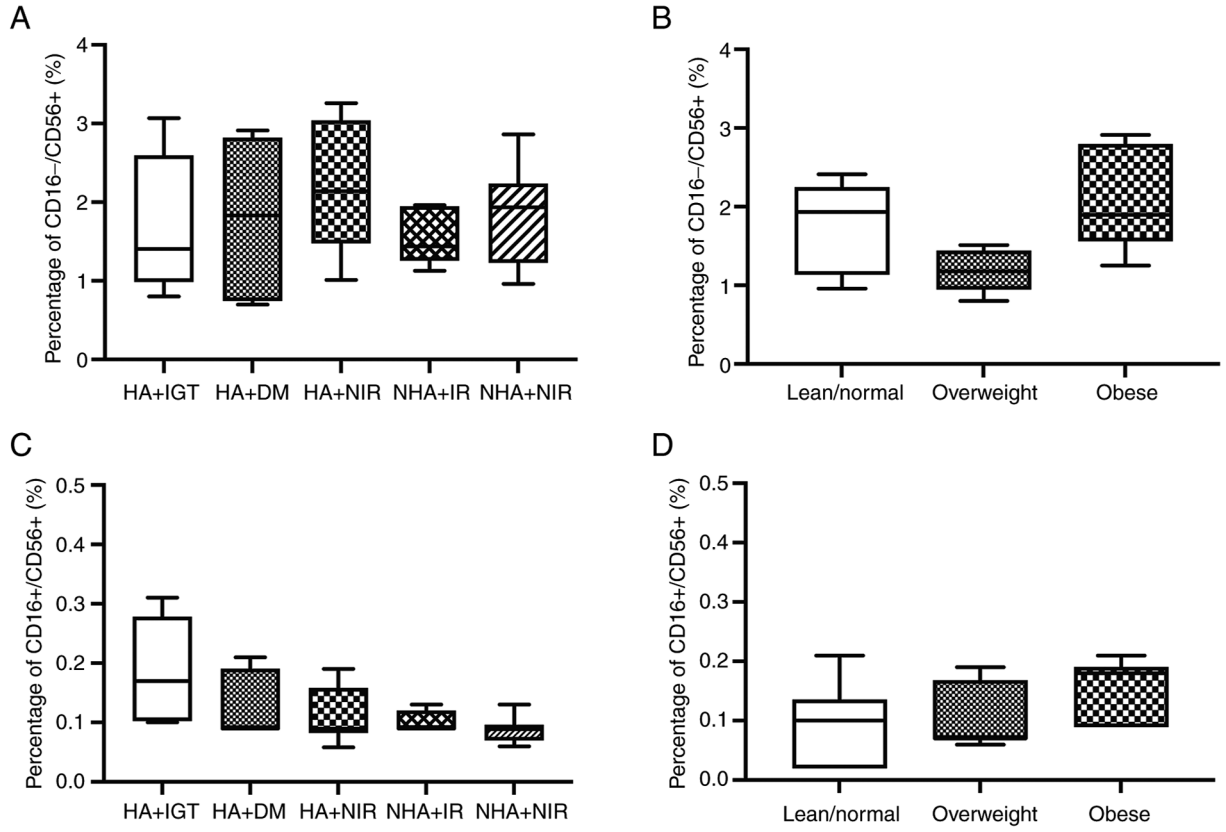

Figure 2. Percentage of subgroups of uNK cells in the endometrium across studied groups. Percentage of CD16-/CD56+ in endometrium obtained from (A) HA + IGT, HA + DM, HA + NIR and NHA + IR groups and (B) from lean/normal, overweight, and obese groups. (C) Percentage of CD16+/CD56+ in endometrium obtained from (C) HA + IGT, HA + DM, HA + NIR and NHA + IR groups and (D) from lean/normal, overweight and obese groups. HA, hyperandrogenemia; IGT, impaired glucose tolerance; DM, diabetes mellitus; NIR, non-insulin resistance; NHA, non-hyperandrogenemia androgen; IR, insulin resistance; uNK cells, uterine natural killer cells.

in the process of endometrial decidualization (61). It has been demonstrated that adiponectin exerts anti-inflammatory effects in the endometrium by inhibiting the production of pro-inflammatory cytokines (IL-6, IL-8 and MCP-1) (5). Compared with the NHA + NIR group and the lean/normal group, the expression levels of MCP-1, IL-6 and IL-8 were significantly increased in the endometrium in the presence of HA and/or IR and/or obesity (BMI ≥24 kg/m^2) of patients with PCOS. It was hypothesized that the reduction of adiponectin may explain the increased levels of IL-6, IL-8 and MCP-1 in the endometrium.

Rosenbaum *et al* (30) revealed that obesity can affect endometrial decidualization in the mouse model and human embryonic stem cells. In addition, Comstock *et al* (62) claimed that obesity can change the expression of genes involved in implantation-related chemokine signaling pathways during implantation, especially in obese patients with metabolic syndrome (63). Notably, the present study revealed that the mRNA expression level of PR in the obese group was significantly higher compared with that in the overweight group. We hypothesized that obesity increased the expression of PR as the response to progesterone resistance (53). However, the underlying mechanism remains elusive and further studies are needed.

As a factor associated with endometrial receptivity (39,64), the percentage of uNK cells fluctuates along with the hormones changing during the menstrual cycle (65,66), which increases during the secretory phase (39). However, increases in the number of peripheral blood and endometrial NK cells (CD56$^+$) have been used as an indicator to assess the risk of infertility or recurrent miscarriage (39). Piltonen *et al* (67) and Matteo *et al* (39) revealed that uNK cells decrease in the late menstrual secretion period, and the percentage of uNK cells CD16$^+$/CD56$^+$ is similar, while the percentage of CD16$^-$/CD56$^+$ is lower in the secretion phase in patients with PCOS when compared with the control group. Nevertheless, no significant difference was revealed in the present study in uNK cells among patients with PCOS with different BMI, androgen status and IR levels. Considering the different phases of the menstrual cycle, the percentage of CD16$^-$/CD56$^+$ was observed to decrease after implantation, while a previous study revealed the percentage of CD16$^-$/CD56$^+$ was lower in the late menstrual secretion period (67). But this research needs to be explored further.

To avoid the impact of actual human embryo implantation on the endometrium, the present investigation was not performed in a conception cycle but in the HRT cycles. The HRT treatment was performed in all the patients with PCOS to promote endometrial transformation and cause implantation window-related changes. Although a previous study indicated that ER and PR expression is significantly decreased in the endometrium in the early luteal phase of HRT cycles (68), all the studied patients in the present study were treated with HRT; therefore avoiding the impact of internal hormone alteration caused by ovulation, and ensuring all patients were

in the same endometrial phase. In the majority of previous studies (24,37,41) the expression of receptivity markers are measured from mRNA level. However, to clarify the differences in the expression level of theses markers, the detection from protein level is important; thus further exploration is needed to validate the present findings.

In conclusion, evidence regarding secretory endometrial receptivity factors in patients with PCOS is limited, so consistent conclusions cannot yet be made. The present study revealed that the IR status, hyperandrogenemia and obesity would impact the endometrial receptivity in patients with PCOS, which may explain the damaged embryo implantation and pregnancy outcomes. To develop the targeted therapies and improve pregnancy outcomes in patients with PCOS, further studies are needed to investigate the underlying mechanism of the impaired endometrium receptivity caused by metabolic disorders.

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Availability of data and materials

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

Authors' contributions

CW and QYM conceptualized the study and analyzed and interpreted the data. CW and YXW performed experiments and wrote the manuscript. CW and QYM confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Clinical Scientific Research and Experimental Animal Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University [approval no. Ethics (2020) no. 422-1]. Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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