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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Abnormal expressions of ADAMTS-1, ADAMTS-9 and progesterone receptors are associated with lower oocyte maturation in women with polycystic ovary syndrome

Sepide GohariTaban[1] · Iraj Amiri[2] · Sara Soleimani Asl[2] · Massoud Saidijam[3] · Mahnaz Yavangi[2] · Elham Khanlarzadeh[4] · Nooshin Mohammadpour[2] · Nooshin Shabab[3] · Tayebe Artimani[2]

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Abstract

Purpose ADAMTS-1 and 9 play a crucial role in the ovulation and their altered levels may play a role in the pathogenesis of polycystic ovary syndrome (PCOS). The aim of this study was to assess ADAMTS-1 and 9 expression and their correlation with the oocyte quality and maturity in the cumulus cells (CCs) of PCOS patients and normovulatory women during an IVF procedure.

Methods Expression of ADAMTS-1 and 9 and progesterone receptors (PRs) in the CCs containing MII and germinal vesicle (GV) oocytes of 37 PCOS patients and 37 women with normal ovulatory function who underwent IVF treatment was evaluated using qRT-PCR. Moreover, correlation between ADAMTS-1 and 9 expression and oocyte quality were also investigated.

Results mRNA expression levels of ADAMTS-1 and ADAMTS-9 were significantly reduced in the women with PCOS compared to the normovulatory women. ADAMTS-1 and ADAMTS-9 mRNA expression levels in the CCs showed a considerable correlation. Lower expression levels of ADAMTS-1 and ADAMTS-9 in PCOS patients were strongly correlated with diminished oocyte maturation. There was a remarkable association between ADAMTS-1 and ADAMTS-9 mRNA expression levels and oocyte quality. PRs (PRA and PRB) were dramatically decreased in PCOS patients when compared with the control group.

Conclusions The results of the present study indicated that ADAMTS-1 and ADAMTS-9 as well as PRs are downregulated in the human CCs in PCOS patients, which could be associated with impaired oocyte maturation and may result in a lower oocyte recovery and oocyte maturity rates, as well as lower fertilization rate.

Keywords ADAMTS-1 · ADAMTS-9 · PCOS · Progesterone receptors · Oocyte maturation

Introduction

Ovulation is a complex sequential, inflammation-like and luteinizing hormone (LH)-induced process of releasing a matured oocyte [1]. Cumulus oocyte complex (COC) provides a complex hyaluronan (HA)-rich extracellular matrix (ECM) which is indispensable for ovulation [2]. Follicles destined to ovulate must complete development and differentiation of oocyte and its nurse cells (granulosa cells (GCs) and theca cells). Simultaneously, marked changes occur in the extracellular environment, including angiogenesis and rapid growth in follicular size [3, 4].

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among women of reproductive age which is accompanied by the presence of common cysts in the ovaries, oligo-anovulation, hyperandrogenism, hyperinsulinism

✉ Tayebe Artimani
artimani@umsha.ac.ir

1 Anatomy Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

2 Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

3 Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

4 Department of Community Medicine, School of Medicine, Hamadan, Iran

and insulin resistance [5]. Impaired ovarian function, associated with aberrant folliculogenesis or steroidogenesis and also developmental disorders in the dominant follicle, as well as obesity, may be involved in the pathophysiology of PCOS [6, 7]. Abnormal intra-follicular microenvironment induced by PCOS can afflict the cytoplasmic and/or nuclear maturation of the oocyte and is capable of altering oocyte gene expression [8]. Expression patterns of GCs and cumulus cells (CCs) in PCOS patients can be used as an indirect marker for embryo selection and could clarify the complex molecular mechanisms underlying oocyte and embryo development [9].

During the maturation of pre-ovulatory follicles, the LH receptor is expressed in the GCs. The LH receptor induces the expression of several genes important for ovulation including progesterone receptor (PR), cyclooxygenase-2 (COX-2), CAAT enhancer binding protein beta (C/EBPh), and early growth regulatory factor-1 (Egr-1) [10]. It has been demonstrated that in a PR knockout mouse, ovulation may not happen, even following stimulation with exogenous hormones [10]. These findings reflect the critical role of progesterone (PG) in the ovulation process [11]. To accomplish ovulation, remodeling of some ECM components is required to allow proper formation of the expanded COC and rupture of ovarian surface epithelium. LH induces the expression of PG and its receptor in the ovaries. When PG binds to PR on the GCs, it leads to a notable elevation of A disintegrin-like and metalloproteinase with thrombospondin type-1 motif1 (ADAMTS-1) expression for breakdown of the follicular wall [5].

ADAMTS family are soluble proteinases which play very pivotal roles in the remodeling of ECM [5]. ADAMTS are inhibited by the tissue inhibitors of metalloproteinases (TIMPs) [5]. Expression of some ADAMTS family members has been reported in the corpora lutea of growing follicles, during ovulation in several mammalian species. ADAMTS-1 is mainly expressed by the GCs of rodents, cows, horses, pigs, monkeys and also humans. ADAMTS-1 may affect ovulation through the formation or stabilization of COC. ADAMTS-4, 5, 9 and 15 expressions have been identified in the CCs of rodents and/or monkeys [12]. A twofold or greater increase of the ADAMTS-1, -4, -9, and -15 mRNA expression levels has been demonstrated in the ovulatory follicles following human chorionic gonadotropin (hCG) administration. In addition, PG, estrogen and androgens increase ADAMTS-1 expression, which mediates follicular rupture through the proteolysis of syndecan and perlecan, in the ECM [13, 14].

A previous study conducted on the human ovaries during the pre-ovulatory period or early or late stages of ovulation revealed a 30- or 40-fold expression levels of ADAMTS-1 and ADAMTS-9, respectively, in the GCs, with no remarkable alteration in the theca cells [12]. Previously, it was reported that ADAMTS molecules may play a role in the pathogenesis of PCOS [15, 16].

Since ADAMTS-1 and 9 display a crucial role in ovulation and knockout of these genes results in a severe ovulatory disorder, we assumed that the dysregulation of ADAMTS-1 and 9 in PCOS patients may culminate in intrinsic oocyte abnormalities. To prove this hypothesis, we investigated and compared the differential expression of ADAMTS-1 and 9 in the CCs of PCOS patients and normovulatory women during an IVF procedure. In this study, COCs containing oocytes and CCs were analyzed at different stages, including germinal vesicle (GV, oocyte maturation arrested in the prophase of meiosis) and metaphase II (MII, second meiotic metaphase). Moreover, the correlation between ADAMTS-1 and 9 expression and oocyte quality was also assessed.

Materials and methods

Subjects and study design

This prospective study was carried out on 37 PCOS patients and 37 women with normal ovulatory function who received IVF treatment for tubal and/or male infertility during 2016–2017. PCOS was diagnosed by a gynecologist in accordance with Rotterdam criteria: hyperandrogenism, oligo/anovulation and polycystic ovaries [17]. Women with PCOS-like syndromes, such as adrenal dysfunction syndrome, congenital hyperplasia, hyperprolactinemia and systemic diseases, autoimmune diseases, diabetes and thyroid disorders were excluded from this research. The controls were infertile women with tubal obstruction, regular periods and had no clinical or biochemical profiles of hyperandrogenism or polycystic ovaries and/or their partners had male factor infertility. The age of all the participants was less than 35 years.

Basal follicle-stimulating hormone (FSH) and LH levels were measured using the electro-chemiluminescent immunoassay (ECLIA) method (Cobas, Roche, England). This study was approved by the Hamadan University of Medical Sciences Ethics Committee (IR.UMSHA.REC.1394.499) and formal written consent was obtained from all patients recruited to our study.

Ovarian stimulation and CCs isolation

All the participants were exposed to gonadotropin-releasing hormone (GnRH) agonist medication in the mid-luteal phase of the previous menstrual cycle. Ovarian stimulation was carried out during IVF cycles using recombinant FSH (Gonal-F, Merck Serono, Switzerland) and participants received injection of hCG (Choriomon, IBSA, Lugano, Switzerland). Ultrasound valuation for follicle development and

estradiol (E2) measurement in the blood samples was performed every 1–3 days. Daily dose could be adjusted after the first 3–5 treatment days based on the ovarian response. After detection of at least three dominant follicles (diameter ≥ 18 to 20 mm), 5000–10,000 IU hCG (Choriomon, IBSA, Lugano, Switzerland) was administered. Oocytes were retrieved through the transvaginal ultrasound-guided needle puncture 34–36 h after hCG treatment. COCs were isolated from all of the GV or MII oocytes taken from each patient by means of ultrasound-guided vaginal puncture. After oocyte retrieval, the cumulus was removed from the COC with hyaluronidase (SAGE, Trumbull, CT) during an intracytoplasmic sperm injection (ICSI) procedure. For the qPCR experiments, purified CCs of one patient were separately pooled to form the CCGV and CCMII groups. Pooled cells were subjected to total RNA purification. CCs were pelleted by centrifugation at 800×*g* for 8 min at room temperature, and the resulting pellets were used for extraction and purification of RNA. Denuded oocytes were injected using the ICSI method. Fertilization was confirmed by the observation of two pronuclei after 16–18 h.

RNA isolation and cDNA synthesis

Total RNA was extracted from CCs via adding 1 cc AccuZol reagent (Bioneer, South Korea), according to the instructor's protocol. Following isolation, genomic DNA contamination was removed by means of DNase 1 (Fermentas, Vilnius and Lithuania) treatment. The quality of the extracted total RNA was assessed with electrophoresis on 1% agarose gel. RNA concentration was measured at 280/260 nm with ultraviolet (UV) radiation. First-strand complementary DNA (cDNA) was synthesized with oligo (dt) primers from isolated RNA and then reverse transcribed by superscript 2 (Fermentas, Vilnius and Lithuania).

Quantitative real-time PCR

Quantitative real-time PCR was performed in triplicate for each sample on the LightCycler 96 System, Roche, in accordance with the manufacturer's protocol using SYBR Green PCR Master Mix (Takara, Japan). The primer sequences used in quantitative real-time PCR (qRT-PCR) are presented in Table 1. Quantitative real-time PCR was carried out in a total volume of 20 µl containing 10 µl SYBR Green PCR Master Mix (Takara, Japan), 7 µl water, 1 µl of each specific primer pairs and 1 µl cDNA. The thermal cycling conditions were as follows: 40 cycles at 95 °C for 5 s, 53–60 °C for 30 s and 72 °C for 30 s. Relative expression levels of the studied genes were analyzed using comparative threshold cycle method, normalized to human β-actin. The amplification specificity was confirmed using melting curve analysis and standard curves were obtained using a logarithmic dilution series of total RNA. Changes in PCOS were reported as fold differences from the values in controls at each time point as appropriate.

Assessment of parameters associated with oocyte quality

Fertilization was evaluated 16–18 h after insemination. Oocyte quality was determined by the evaluation of following variables: (1) oocyte recovery rate: number of retrieved oocytes/number of follicles during oocyte extraction; (2) oocyte maturation rate: the proportion of number of MII oocytes estimated/ the total number of oocytes recovered; (3) fertilization rate: the proportion of two pronuclei detected/ number of oocytes retrieved (2PN).

Statistical analysis

All the data were analyzed using SPSS software 16 ver. The results are presented as mean ± SE. Data with normal distribution were compared between the groups using independent sample *T* test. Mann–Whitney *U* test was used for nonparametric analyzes and Spearman coefficients were used to determine the relation between the parameters. $p < 0.05$ was considered statistically significant.

Table 1 Characteristics of the primers used in the qRT-PCR

Gene	Forward primer	Reverse primer	Accession number	Annealing temperature	Product size (bp)
ADAMTS-1	CCAGACCTTGTGCAGACCAT	TCACTTTGCCTTGCCCTCAA	NM_006988.4	60	244
ADAMTS-9	CACGAGCACACCTGGAGAAA	GAAGTCACATCACCAGCCGA	NM_001318781.1	55	88
PR-A	CTCATCCATACTTATCCTTCAC	TCCTTGTCCACTTCACTT	NM_001202474	54	206
PR-B	GGTAAGCCTTGTTGTATT	GGGTTGTAGATTTCACTC	NM_000926.4	47.7	85
β-Actin	AAGATCAAGATCATTGCT	TAACGCAACTAAGTCATA	NM_001101.4	53	177

Table 2 Clinical and endocrine parameters in the PCOS patients and normovulatory controls

Variable	PCOS	Controls	*p* value
Age (year)	28.6 ± 0.81	30.3 ± 0.93	0.17
BMI (kg/m^2)	26.51 ± 0.68	25.46 ± 0.72	0.2
Duration of infertility (year)	5.8 ± 4.2	7.2 ± 4.66	0.31
Basal FSH (IU/L)	6.04 ± 1.78	7.14 ± 2.24	0.07
LH (IU/L)	7.08 ± 5.07	5.18 ± 2.64	0.01
Total FSH dose (IU)	15,208 ± 467.03	17,234 ± 490.594	0.08

Data were presented as mean ± SEM and compared by independent-samples *T*-test

PCOS polycystic ovary syndrome, *BMI* body mass index

Table 3 Comparison of IVF-ET outcome between control and PCOS groups

Variable	PCOS	Controls	*p* value
Number of follicles punctured	13.92 ± 1.5	7.54 ± 0.71	0.001
Number of oocytes retrieved	10.65 ± 0.97	7.45 ± 0.66	0.01
Oocyte recovery rate (%)	0.85 ± 0.06	1.02 ± 0.06	0.05
MII oocytes	7.5 ± 3.4	6.2 ± 0.62	0.1
GV oocytes	3.61 ± 0.75	0.72 ± 0.13	< 0.0001
Oocyte maturity rate	0.72 ± 0.03	0.83 ± 0.02	0.03
Oocyte fertilization rate	0.54 ± 0.03	0.56 ± 0.04	0.05

PCOS polycystic ovary syndrome, *MIIs* metaphase II

Results

Clinical data and patient demographics

All PCOS patients had polycystic ovary (ovaries) confirmed by ultrasound analysis and irregular periods. In addition, among 37 PCOS patients, hyperandrogenism was observed in some of them. The main clinical parameters in normovulatory controls and women with PCOS are summarized in Table 2. There was no noticeable difference in age, body mass index (BMI), basal FSH and total rFSH dose between the PCOS and control groups. However, as with most studies, basal LH concentrations were greater in the PCOS group ($p = 0.01$).

The number of oocytes in PCOS was markedly higher than that in the control group ($p = 0.01$). Regarding the oocyte quality, as depicted in Table 3, there was a significantly higher oocyte recovery and maturity rate in the normovulatory group compared to PCOS patients. In more detail, according to the pooled results, there was no difference in the number of MII oocytes between the groups, although women with PCOS had a remarkably higher number of GV oocytes (3.61 ± 0.75) compared to the control group (0.72 ± 0.13, $p < 0.0001$). Moreover, oocyte recovery rate ($p = 0.05$), oocyte maturity rate ($p = 0.03$) and fertilization rate ($p = 0.05$) were markedly lower in the PCOS group. Nevertheless, we did not note any considerable difference in the clinical pregnancy rate between the PCOS patients and the control group.

Analysis of ADAMTS-1 and ADAMTS-9 expression between the PCOS patients and normovulatory women

Classic RT-PCR showed the expression profiles of ADAMTS-1 and ADAMTS-9 in the CCs of control and PCOS patients. All amplified products were at the expected size for the specific genes. For each of genes, control experiments confirmed no contamination of genomic DNA in the samples. Sequencing was used for identification of all amplicons. The differential expression of ADAMTS-1 and ADAMTS-9 in the CCs between the normovulatory women and PCOS patients was examined using RT-PCR. Analysis of ADAMTS-1 transcript levels exhibited a remarkable difference between the groups (Fig. 1a). ADAMTS-1 mRNA levels were noticeably greater in the controls compared to women with PCOS ($p = 0.02$). Additionally, as depicted in

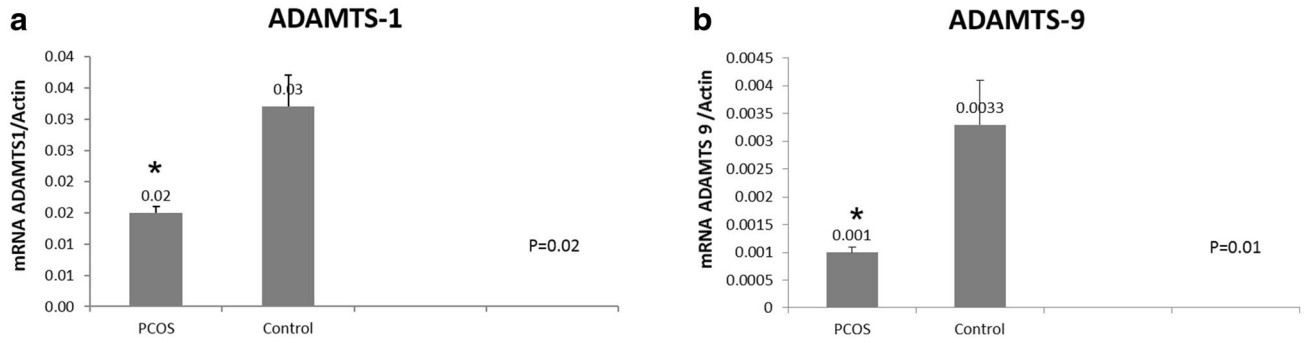

Fig. 1 Expression of ADAMTS-1 and ADAMTS-9 in cumulus cells (CCs) of polycystic ovary syndrome (PCOS) and control groups

Fig. 1b, ADAMTS-9 expression levels were notably reduced in the PCOS group compared to the control group ($p=0.01$).

Association between ADAMTS-1 and ADAMTS-9 expression and oocyte maturation

To examine a possible association between the expression levels of ADAMTS-1 and ADAMTS-9 and oocyte maturation, we assayed their expression in the CCs containing MII and GV oocytes of PCOS and control groups. ADAMTS-1 mRNA expression in CCs containing MII oocytes was dramatically elevated in comparison to those containing GV oocytes from both PCOS and control groups ($p<0.009$ and $p=0.01$, respectively, Fig. 2a, b). In addition, as depicted in Fig. 2c, d, there was a marked decline in mRNA expression levels of ADAMTS-9 in CCs containing GV oocytes compared to those containing MII oocytes taken from both PCOS and control groups ($p=0.05$).

Association between ADAMTS-1 and ADAMTS-9 expression and oocyte quality in PCOS

Correlation between ADAMTS-1 mRNA expression levels and oocyte quality in PCOS patients are presented in Fig. 3. ADAMTS-1 mRNA expression levels were positively correlated with the oocyte recovery rate ($p=0.03$, $r=0.39$, Fig. 3a). Furthermore, ADAMTS-1 mRNA expression levels were significantly associated with the oocyte maturity rate ($p=0.02$, $r=0.48$, Fig. 3b). Fertilization rate was also greatly correlated with the ADAMTS-1 mRNA levels ($p=0.04$, $r=0.48$, Fig. 3c). Figure 4 shows the association between ADAMTS-9 expression and oocyte quality in PCOS patients. There was a positive correlation between oocyte recovery rate and ADAMTS-9 mRNA levels ($p=0.006$, $r=0.78$, Fig. 4a). Moreover, the maturation rate was positively associated with the ADAMTS-9 mRNA levels ($p=0.004$, $r=0.32$, Fig. 4b). However, no significant relationship was noted between the ADAMTS-9 mRNA expression levels and fertilization rate ($p=0.07$, $r=0.38$, Fig. 4c).

Analysis of PRs expression between the PCOS patients and normovulatory women

In an effort to identify the potential nuclear receptors for PG hormone in the human CCs, PCR analysis was performed using specific primers against the PRs. Our results indicated that two PR isoforms are expressed in the CCs. All amplified products were at the expected size for these specific genes. For each of the receptors, control experiments confirmed no contamination of genomic DNA in the samples. Identification of all amplicons was confirmed by sequencing. Quantitative real-time PCR was performed on the CCs. Our result demonstrated that nuclear PRs namely, PRA and PRB, were both found to be expressed in the human CCs of the study population. The expression levels of PRA and PRB

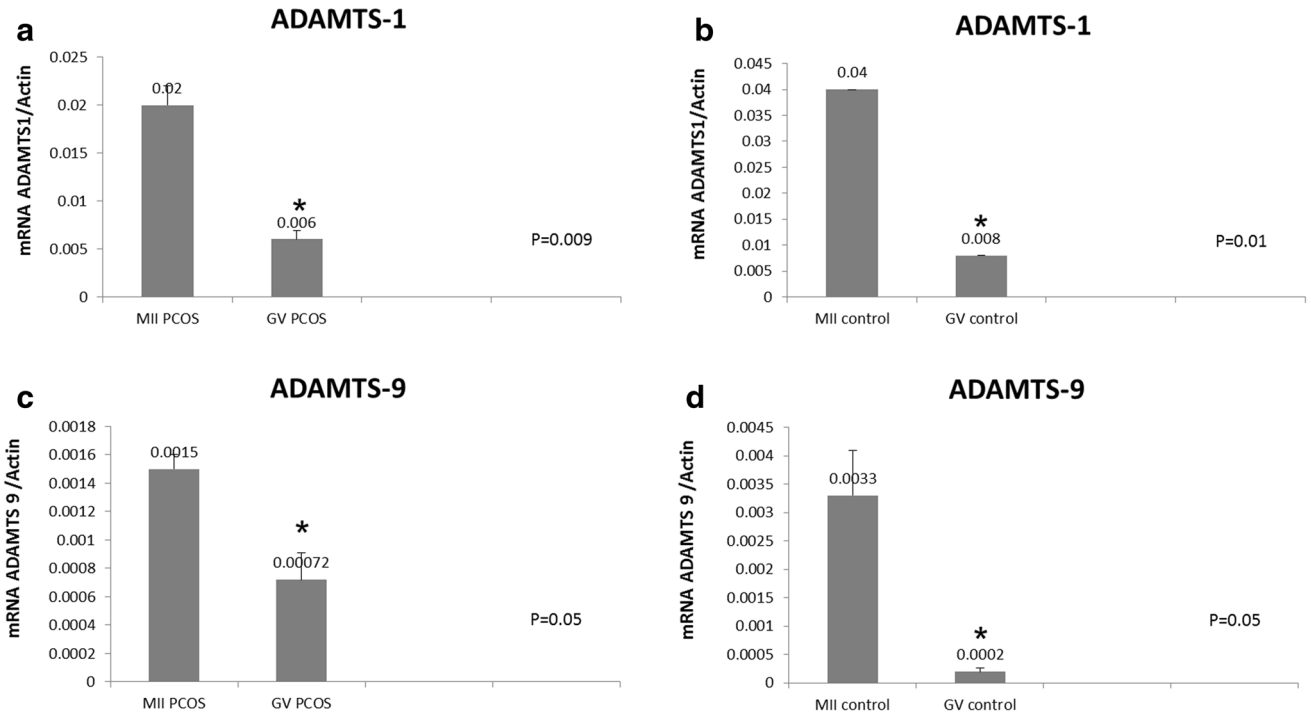

Fig. 2 Association between ADAMTS-1 and ADAMTS-9 expression and oocyte maturation

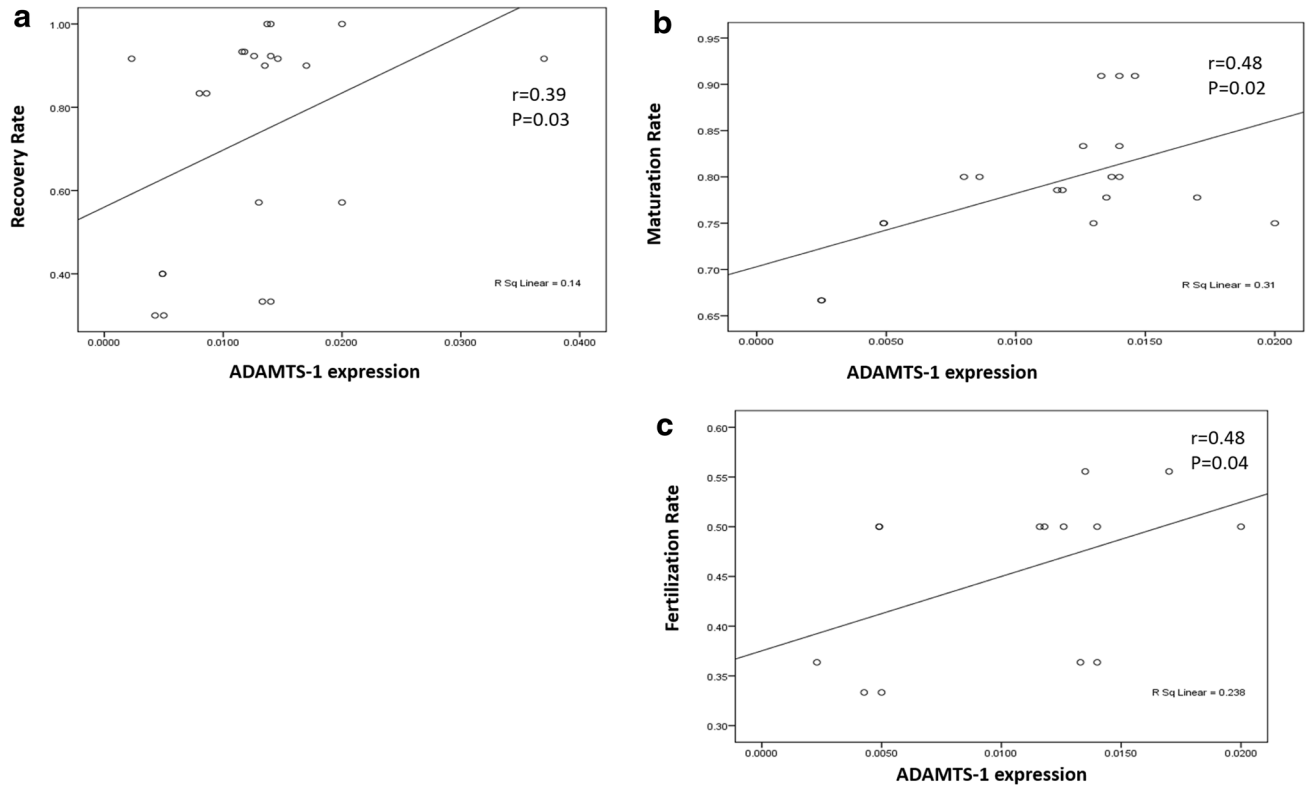

Fig. 3 Association between ADAMTS-1 expression and oocyte quality in polycystic ovary syndrome (PCOS)

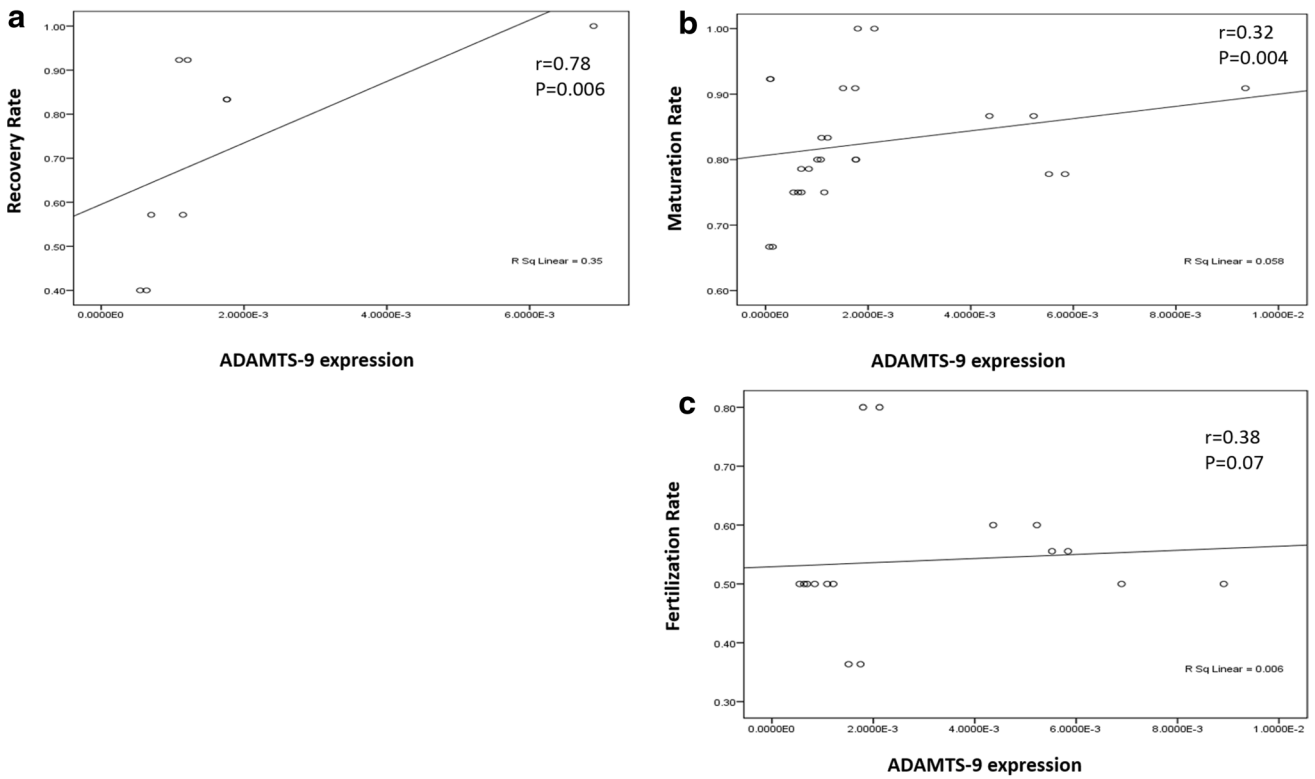

Fig. 4 Association between ADAMTS-9 expression and oocyte quality in polycystic ovary syndrome (PCOS)

were markedly lower in the PCOS patients compared to the control women ($p=0.05$ and $p=0.04$, respectively, Fig. 5).

Association between ADAMTS-1, ADAMTS-9 and PRs expression

To evaluate a possible association between the mRNA levels of ADAMTS-1 and ADAMTS -9 and PRs, bivariate correlation was used. Using Spearman test, mRNA expression levels of ADAMTS-1 and ADAMTS-9 in the CCs of PCOS patients and control group were found to be strongly correlated ($p<0.0001$, $r=0.7$, Fig. 6a). Furthermore, remarkable correlations were found between the ADAMTS-1 and PRA and PRB expression in women with and without PCOS ($p<0.0001$, $r=0.71$, and $p<0.003$, $r=0.4$, respectively, Fig. 6b, c). ADAMTS-9 mRNA expression was closely correlated with PRA expression in the study population ($p<0.008$, $r=0.4$, Fig. 6d). However, mRNA expression levels of ADAMTS-9 did not exhibit any significant correlation with PRB expression (data not shown).

Discussion

ADAMTS-1 and ADAMTS-9 play an essential role in the normal folliculogenesis and ovulatory process. Altered levels of these genes may contribute to the pathogenesis of PCOS [12, 18]. In the present study, ADAMTS-1 and ADAMTS-9 expression and their correlation were investigated in the CCs of PCOS women and women with normal ovarian function using qRT-PCR. The results of the current study demonstrated a marked reduction in the ADAMTS-1

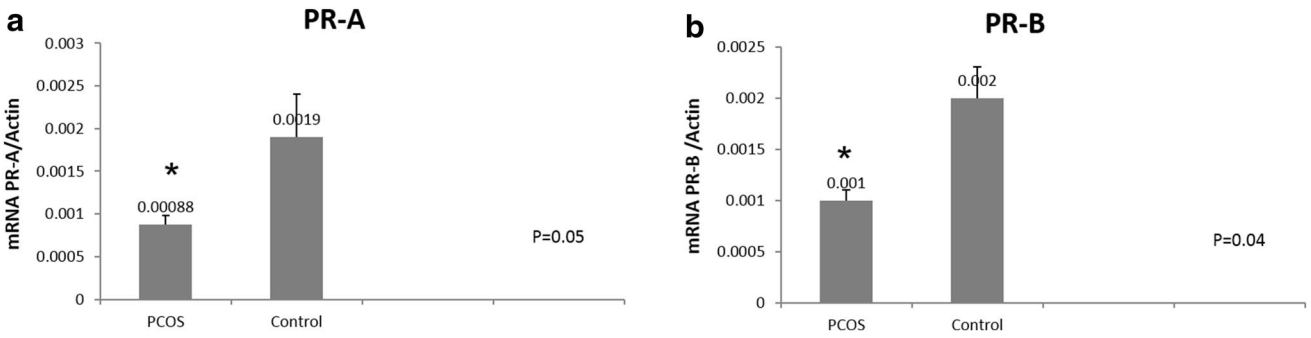

Fig. 5 Expression of PR-A and PR-B in cumulus cells (CCs) of polycystic ovary syndrome (PCOS) and control groups

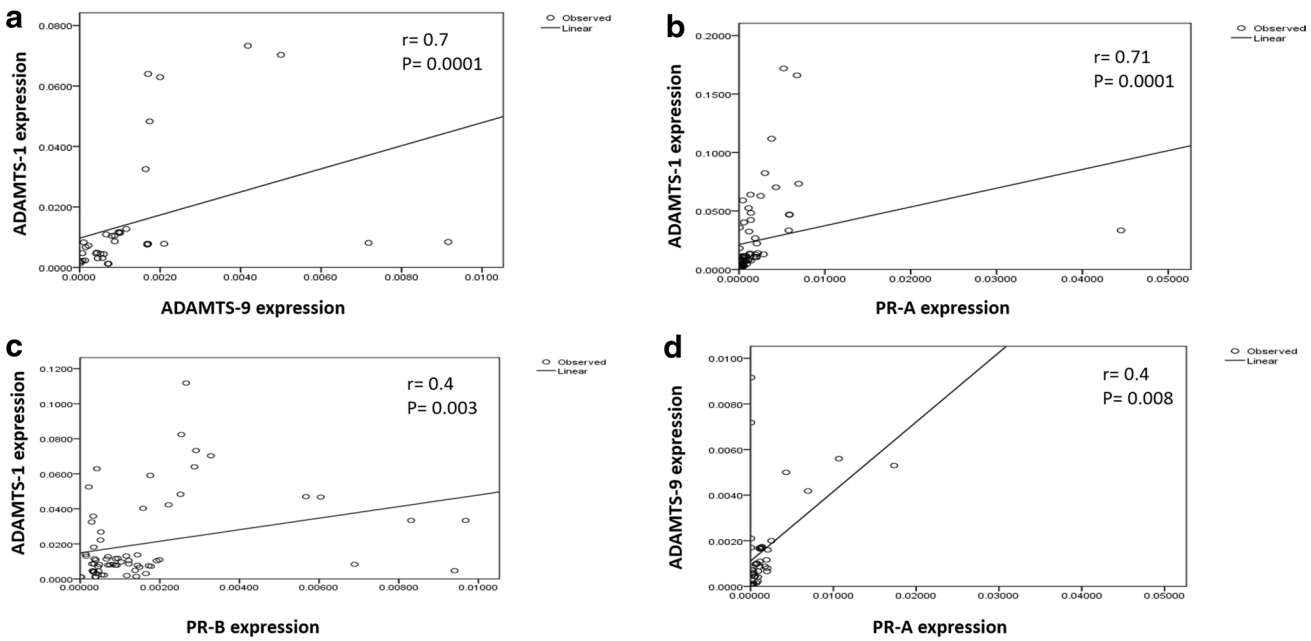

Fig. 6 Correlation between ADAMTS-1, ADAMTS-9 and progesterone receptors (PRs) expression in cumulus cells

and ADAMTS-9 expression levels of women with PCOS compared to normovulatory women. Previously, in rats treated with RU486 as an agonist for progesterone and glucocorticoid receptors, a significant downregulation of both ADAMTS-1 and ADAMTS-9 was noted [19]. It seems that downregulation of ADAMTS-1 in PCOS patients could be mediated by the high androgen levels [12]. Interestingly, the present study revealed that mRNA expression levels of ADAMTS-1 and ADAMTS-9 in the human CCs in the study population are strongly correlated. The results of this study suggest that ADAMTS-1 and ADAMTS-9 act in the same way as supported by the results of some previous studies [18, 20]. Versican is a common substrate for ADAMTS-1 and ADAMTS-9 in the ECM. Proteolytic activity of these enzymes via versican cleavage results in COC expansion, which is necessary for successful ovulation and, hence, fertility [5, 19]. Induction of ADAMTS-1 and ADAMTS-9 by hCG administration in humans imitates the periovulatory induction of these proteinases in monkey, cow, rabbit, rat and mouse and suggest a possible unity between the action of these proteinases during ovulation. The key role of ADAMTS-1 in the ovulatory process can be established by the fact that mice null for ADAMTS-1 are subfertile due to impaired ovulation [18, 20]. These animals display a 90% diminution in the ovulation rate due to a reduction in follicular development, variations in lymphangiogenesis, and changes at the end of the ovulatory process [18].

Additionally, it is believed that ADAMTS-1 expression is associated with human follicular growth and oocyte fertilization capacity rate [21, 22], so that ADAMTS-1 in the CCs of oocytes that underwent successful fertilization is expressed three times higher than in the CCs from unfertilized oocytes, highlighting the noticeable contribution of ADAMTS-1 to the human cumulus function [21].

Limited studies have been performed on the expression of ADAMTS-9 in the ovary. Rosewell et al. noted an enhancement in the ADAMTS-9 mRNA expression in the GCs during the early ovulatory period that remains high, until the time of ovulation [18]. ADAMTS-9 participates in the ECM binding and expresses extensively during the mouse embryogenesis [23]. It is of note that reduction of ADAMTS-9 expression levels in the CCs from PCOS patients could be related to the oocytes which failed to progress through meiosis [12].

Besides, in the current study, downregulation of ADAMTS-1 and ADAMTS-9 in the PCOS patients was also greatly correlated with the impaired maturation. Likewise, diminished ADAMTS-1 expression in the PCOS was associated with a lower oocyte maturity, recovery and fertilization rates. Consistent with the result of present study, Xiao et al., pointed out that ADAMTS-1 expression decreases in PCOSpatients compared to the normally ovulating women and this decline is closely associated with a lower oocyte recovery, oocyte maturity and fertilization rate [8]. Moreover, Huang et al. evaluated the transcription of ADAMTS-9 in the CCs harvested from pre-ovulatory follicles of PCOS patients before IVF. They noticed a notable difference in the CCs from oocytes at different nuclear maturation stages [9].

Several CC genes along with the ADAMTS 1 and 9 are involved in the oocyte maturation and fertilization rates of PCOS patients. Impaired oocyte maturation and downregulation of these genes might be due to the abnormalities in the microenvironment surrounding oocytes induced by PCOS which change the survival and proliferation of GCs. Therefore, the dysregulated expression of ADAMTS-1 and ADAMTS-9 accompanied by alleviated oocyte recovery, maturation and fertilization rates reported in this study may be related to poor quality of the oocytes in PCOS patient, which subsequently affect the fertilization capacity [8, 9].

Interestingly, the results of this study indicated that nuclear PRs, namely, PRA and PRB, were lower in PCOS patients compared to the control women, implying this reduction could be associated with the impaired oocyte maturation or follicular arrest [24].

To investigate a possible association between ADAMTS-1 and ADAMTS-9 and PRs expression, bivariate correlation analysis was used. Based on Spearman test, there was a positive correlation between PRs and ADAMTS-1 and ADAMTS-9 mRNA expression levels in the CCs of the PCOS and control groups. Our previous study illustrated a lower expression of PRA and PRB in the GCs of PCOS women compared to women with normal ovary function [24].

ADAMTS-1 expression in the oviduct which involves in the ovulatory events is directly or indirectly modulated by PRs [25, 26]. In mice null for PRs, the expression of ADAMTS-1 has been revealed to be downregulated in the oviducts during the periovulatory period [25].

In humans, ADAMTS-1 known as METH-1 and cathepsin L are two molecular targets of PR action [27]. Expression of ADAMTS-1 mRNA and protein was reported to be considerably decreased in the GCs of mice lacking PR. This may highlight the fundamental role of PR as an inducible co-regulator of the ADAMTS-1 gene in the GCs [4].

ADAMTS-9 is a critical downstream target gene of PRs, which contributes to ovulation. As displayed by a previous study, progesterone/PRs which is indispensable for ovulation, causes a dramatic increase in the ADAMTS-1 and -9 mRNA expression levels and accumulation of these genes prior to the ovulation in the theca interna [10]. Furthermore, it is of note that PSAT1 and ADAMTS-9 mRNA expressions in the CCMII are greater than that of CC MI/GV stages which is coordinated and modulated by LH and PR [9].

Conclusion

In summary, the present study demonstrated that the expression of ADAMTS-1 and ADAMTS-9 and PRs decrease dramatically in human CCs of PCOS patients. Downregulation of PRs is related to a decline in the ADAMTS-1 and ADAMTS-9 gene expression. Besides, diminished ADAMTS-1 and ADAMTS-9 expression in PCOS patients is closely related to impaired oocyte maturity, which may indicate the intrinsic impairment of CC. Lower ADAMTS-1 and ADAMTS-9 expressions may influence oocyte maturity and oocyte recovery rate and subsequently result in lower fertilization rates. These findings may reflect dysregulation of endocrine and intra-ovarian paracrine interactions in PCOS patients. However, further efforts are required to clarify the definite correlation between ADAMTS-1, ADAMTS-9, PRs and oocyte maturation.

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Author contributions SG project development, IA data collection, SSA data management, MS project development and data management, MY data collection, EK data analysis, NM data collection, NS data collection, TA project development, data management and manuscript writing.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of Hamadan University of Medical Sciences Ethics Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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