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# The Expression of Apolipoprotein A1 on Pregnancy Outcome in Women with Polycystic Ovary Syndrome Compared to Fertile Women: A Cross-Sectional Study

**Abstract**

**Objective:** To explore the level of apolipoprotein A1 expression in human endometrial tissues in women with polycystic ovary syndrome compared to fertile women.

**Patients and Methods:** This was a cross sectional study performed at Ain Shams University Maternity Hospital, over a 2-year period, between Jan 2014 and Jan 2016, and included 80 women divided into two groups. Group I (n=40) with polycystic ovary syndrome who were presented at the infertility clinic and group II (n=40) fertile women who were presented due to any cause other than infertility as a control group. All women were scheduled for endometrial sampling by Endosampler. Participant ages ranged from 20 to 35 years. A written informed consent was obtained from all women before participation. Endometrial apolipoprotein A1 was investigated using ELIZA. Samples were obtained from all patients in the proliferative phase (just before ovulation when the dominant follicle is 20 mm) and secretory phase (5 days after the 1<sup>st</sup> sample).

**Results:** In this study, endometrial apolipoprotein A1 expression was up regulated in patients with polycystic ovary syndrome compared to normal subjects, also the level was higher in the proliferative phase when compared to the secretory phase ( $P$  value < 0.05).

**Conclusions:** It seems that differentially expressed apolipoprotein A1 negatively affects endometrial receptivity in patients with polycystic ovary syndrome. The results showed that apolipoprotein A1 level significantly changes in the human endometrium during the menstrual cycle with minimum expression in the secretory phase, coincident with the receptive phase (window of implantation).

**Introduction**

Polycystic ovary syndrome is one of the most common endocrine disorders in premenopausal women and the most frequent cause of ovulatory infertility [1-3]. Prevalence of this condition in women of the reproductive age is approximately between 5% and 10% [1,4,5]. Polycystic ovary syndrome as a complex disorder is associated with several health complications including obesity, hyperandrogenism, metabolic syndrome, hirsutism, acne, ovarian dysfunction, and infertility [4,6,7]. Although anovulation is an obvious cause of infertility in polycystic ovary syndrome, growing evidence suggests that endometrial receptivity also contributes to infertility of these patients [1,7,8].

Endometrial receptivity means the ability of the uterine lining to accept an implanting embryo, resulting in a successful pregnancy. It seems that a shortened or absent receptive endometrium is the main cause of conception delay and lack of pregnancy [9,10]. Successful implantation depends on the regulation of protein networks that are essential for communication between the nascent embryo and endometrium. Several studies have been performed to provide

potential genes and protein candidates for endometrial receptivity. However, none of them have been verified as a clinical biomarker [1,2]. Apolipoprotein A1 is the predominant protein for high density lipoproteins [2,11]. Apolipoprotein A1 is a primary acceptor for cholesterol in extra hepatic tissues [2,11]. Apolipoprotein A1 is dysregulated in diverse tissues and body fluids in a variety of diseases [12,13]. Recent works showed that apolipoprotein A1 may also play a role in endometrial receptivity and it has been identified as a putative anti-implantation factor secreted by the differentiating endometrium [2,11,13]. A proteomic analysis of the endometrium from women with repeated implantation failure and normal fertile women showed a significantly higher apolipoprotein A1 expression in patients with repeated implantation failure [14]. Further studies confirmed that upregulation of apolipoprotein A1 is upregulated in the endometrium of women with unexplained infertility [2]. The abovementioned evidences provide a potential role for apolipoprotein A1 in female infertility associated with endometrium disturbances. Therefore, in the present study the possible *in vivo* role of apolipoprotein A1 in endometrial receptivity of patients with polycystic ovary syndrome was investigated.

## Patients and Methods

This study was carried out in Ain Shams University Maternity Hospital after the approval of the Research Ethics Committee, during the period between Jan 2014 to Jan 2016 and included 80 women divided into two groups. Group I (n=40) with polycystic ovary syndrome who were presented at the infertility clinic and group II (n=40) fertile women who were presented due to any cause other than infertility as a control group. All women were scheduled for endometrial sampling using endosampler (a product of Med Gyne Company, USA). Participant ages ranged from 20 to 35 years. A written informed consent was obtained from all women after explaining the purpose of the study. Endometrial apolipoprotein A1 was investigated using ELISA. Samples were obtained from all patients in the proliferative phase (just before ovulation when the dominant follicle is 20 mm) and secretory phase (5 days after the 1<sup>st</sup> sample).

The participants did not receive any form of hormonal therapy and none of them used an intrauterine contraceptive device during the previous 3 months. Patients with chronic anovulation, clinical and/or biochemical signs of hyperandrogenism, and those who were polycystic in the ultrasound scanning of ovaries were diagnosed as polycystic ovary syndrome. According to Rotterdam ESHRE 2004, women with PCOS should have two out of three of the following criteria: 1- oligomenorrhea or anovulation, 2-clinical and/ or biochemical signs of hyperandrogenism, 3- polycystic ovaries on ultrasound. Endometrial biopsies were taken using Endosampler under sterile conditions.

Formalin-fixed, paraffin-embedded samples were stained for apoA-I expression. Briefly, A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions. A solution of nonreacting protein, such as bovine serum albumin or casein, is added to well (usually 96-well plates) in order to cover any plastic surface in the well which remains uncoated by the antigen.

The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.

A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more antibody is bound, the faster the color will develop. A major disadvantage of the direct ELISA is the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or indirect ELISA provides a solution to this problem, by using a “capture” antibody specific for the test antigen to pull it out of the serum’s molecular mixture.

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as the sample. The use and meaning of the names “direct ELISA” and “indirect ELISA” differs in the literature and on web sites depending on the context of the experiment. When the presence of an antigen is analyzed, the name “direct ELISA” refers to an ELISA in which only a labelled primary antibody is used, and the term “indirect ELISA” refers to an ELISA in which the antigen is bound by the primary antibody which then is detected by a labeled secondary antibody. In the latter case a sandwich ELISA is clearly distinct from an indirect ELISA. When the “primary” antibody is of interest, e.g. in the case of immunization analyses, this antibody is directly detected by the secondary antibody and the term “direct ELISA” applies to a setting with two antibodies.

## Statistical methods

Data were analyzed using IBM® SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA) and XLSTAT™ version 2014.5.03 (Addinsoft™, NY, USA). Normally distributed numerical variables were presented as mean (SD) and intergroup differences were compared using the unpaired *t* test. Skewed numerical variables and discrete variables were presented as median (interquartile range) and between-group comparisons were done using the Mann-Whitney test. Categorical variables were presented as number (%) and intergroup differences were compared using the chi-squared test with Yates’ continuity correction or Fisher’s exact test, when appropriate. Ordinal data were compared using the chi-squared test for trend. A two-sided p-value <0.05 was considered statistically significant.

## Results

This current study was conducted in Ain Shams University Maternity Hospital during the period between Jan 2014 to Jan 2016 a total of 200 women with history of recurrent miscarriage were included in the study (Tables 1,2).

## Discussion

Polycystic ovary syndrome is associated with infertility not only to anovulation but also to endometrial dysfunction [9,15]. Despite many recent advances in assisted reproduction techniques even with the selection of good quality embryos, the rate of success is mainly limited because of implantation failures [7,8,16] Endometrial factors at the molecular level have been suggested to explain implantation failure and poor reproductive potential of patients with polycystic ovary syndrome [1,7].

In women with polycystic ovary syndrome, dyslipidemia and lipoprotein abnormalities are common metabolic disorders [17,18]. Apolipoprotein A1 is the main protein component of high-density

**Table 1:** The clinic-demographic criteria of women under study.

	Group I (100)	Group II (100)	P-value
Age	29.1 ± 3.6	28.7 ± 3.9	> 0.005
Menarche age	10.2 ± 3.2	10.1 ± 2.8	> 0.005
Body mass index (kg/m <sup>2</sup> )	27.1 ± 3.4	26.8 ± 2.4	> 0.005
Previous gravidity	1.3 ± 0.2	3.2 ± 2.1	< 0.005 S
Previous abortions	1.1 ± 0.1	1.3 ± 0.2	> 0.005
Education			
≤High school	22	21	> 0.005
>High school	18	19	
Occupation			
House wife	30	25	> 0.005
Employed/business Woman	10	15	

\* Analysis using independent student's t-test. NS = non-significant, S = significant.

**Table 2:** The hormonal, and clinical parameters of polycystic ovary syndrome and control women.

	Group I (40)	Group II (40)	P-value
Menstrual type			
Regular	10	27	< 0.005 S
Irregular	30	13	
LH (mu/ml)	8.2 ± 1.2	5.1 ± 1.1	< 0.005 S
FSH (mu/ml)	4.1 ± 2.4	8.2 ± 2.2	< 0.005 S
LH/FSH	2.1 ± 1.1	3.2 ± 2.1	< 0.005 S
Progesterone level	5.1 ± 2.3	12.3 ± 2.2	< 0.005 S
Testosterone (ng/ml)	3.1 ± 0.85	1.8 ± 0.41	< 0.005
ELIZA for EAPO-a	0.37 ± 0.1	Proliferative 0.21±0.05	< 0.005 S
		Secretory 0.19 ± 0.03	

\* Analysis using independent student's t-test. NS = non-significant, S = significant.

lipoprotein (HDL) [11,19]. Dysregulation of apolipoprotein A1 levels have been reported in a variety of diseases such as preeclampsia, endometriosis, and repeated implantation failure [2,12]. There are very few reports describing apolipoprotein A1 expression and function in the endometria of women with polycystic ovary syndrome. In this study, we assessed the endometrial expression of apolipoprotein A1 as a potential biomarker of nonreceptive endometria in patients with polycystic ovary syndrome without pharmacological treatment. The results showed a higher endometrial apolipoprotein in women with polycystic ovary syndrome compared to fertile women. Our work revealed that endometrial apolipoprotein A1 expression was upregulated in patients with polycystic ovary syndrome compared to normal subjects and the results were verified by western blot as a semi-quantitative technique.

Recent studies performed on human endometrial samples have revealed that elevated apolipoprotein A1 levels are associated with unexplained infertility and also repeated implantation failure [2,14]. Nyalwidhe et al. [13], demonstrated that apolipoprotein A1 is consumed by human preimplantation embryos suggesting a possible role in implantation. Indeed, embryos with the highest likelihood of implantation consume or metabolize apolipoprotein A1, which

prepares a suitable microenvironment with low apolipoprotein A1 levels for successful implantation [13]. In an *in vitro* experiment, apolipoprotein A1 was also found to be have a higher expression in nondecidualized cells compared to decidualized stromal cells [14]. Thus, important negative effects can be predicted for apolipoprotein A1 in the initiation and maintenance of the window of implantation.

Apolipoprotein A1 also has anti-inflammatory properties including inhibition of interleukin 1 (IL-1) and tumor necrosis factor alpha (TNFα) production, and suppression of neutrophil degranulation [20,21]. Inflammatory processes are implicated in the pathophysiology of endometriosis. Upregulation of endometrial apolipoprotein A1 is observed in women with endometriosis [20].

Moreover, at the time of implantation, the maternal endometrium shows characteristics of an acute aseptic inflammatory response [22,23]. Inflammatory cytokines and adhesion molecules that are expressed by the endometrium and embryo are essential for the appropriate interaction between the embryo and the endometrium for successful pregnancy [22,23]. Apolipoprotein A1 has the ability to inhibit the synthesis of inflammatory cytokines and adhesion molecules, such as selections, that have essential roles in the embryo implantation [21,24-27]. Another potential mechanism through which apolipoprotein A1 contributes to the implantation failure may be via inhibition of angiogenesis. Tissue remodeling and angiogenesis are two crucial events during implantation and decidualization [22]. Apolipoprotein A1 has inhibitory effects on angiogenesis and tissue remodeling by downregulation of matrix metalloproteinase (MMP)-9, vascular endothelial growth factor (VEGF)/basic fibroblast growth factor (bFGF) production, and dendritic cell function [28-30].

Our study demonstrates that apolipoprotein A1 expression in human endometria significantly changes during the menstrual cycle with minimum level in the secretory phase, coincident with the receptive phase (window of implantation). It appears that sex hormones regulate the expression of apolipoprotein A1 in a reverse manner. There is evidence that apolipoprotein A1 is upregulated by estradiol and is downregulated by progesterone [31]. Progesterone may protect the endometrium against the detrimental effects of apolipoprotein A1 during the critical period of the receptivity window. Endometrial apolipoprotein A1 expression is strongly inhibited by human chorionic gonadotropin (hCG), which is a key requirement for the promotion of implantation [2]. Thus, hCG treatment may improve uterine receptivity and pregnancy rate in *in vitro* fertilization (IVF) patients with polycystic ovary syndrome by decreasing apolipoprotein A1 levels.

In summary, the present study demonstrates that it appears that the mechanism of implantation failure and subfertility associated with polycystic ovary syndrome condition. Elevated apolipoprotein A1 levels can be considered as a biomarker for nonreceptive endometrium in patients with polycystic ovary syndrome. Further investigation should be performed to clarify the major clinical applications of this protein.

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