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Proteomic analysis of follicular fluids in polycystic ovarian syndrome (PCOS) patients

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의 학 과

최 범 채

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다낭성 난소 증후군 환자의 난소 여포액을 이용한 프로테오믹스 분석

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요 약 문

다낭성 난소 증후군 환자의 난소 여포액을 이용한 프로테오믹스 분석

조선대학교 대학원 의학과
박사학위과정
최 범 채

다낭성 난소증후군은 무발정에 기인한 불임의 가장 대표적인 요인이며 가임연령기 여성의 5-10%를 차지하지만 이의 원인은 아직까지 명확하게 밝혀져 있지 않다.

여포액은 인간 생식과정에서 여포의 성장과 난자 수정시 생물학적으로 중요하게 요구되는 다양한 단백질들을 함유하고 있다. 그러므로, 다낭성 난소증후군 환자의 여포액에서 단백질들의 발현양상을 조사하는 것은 다낭성 난소증후군과 관련된 원인을 규명하고 여포의 미성숙 및 난자의 미수정 등과 관련된 원인규명을 할 수 있는 간접적인 방법이 될 수 있다.

본 연구에서는 다낭성 난소증후군을 보이는 환자군과 대조군 간

의 상이한 단백질 발현양상을 조사하기 위하여 여포액 내의 단백질 발현양상을 2차 전기영동법을 이용하여 분석하였다. 다낭성 난소증후군 환자들의 여포액으로부터 6개의 단백질들(kininogen, keratin 9, antitrombin chain B fibrin beta, fibrinogen γ, a-4 type IV collagen precursor, apoliopoprotein A-IV precursor, α-1-B-glycoprotein, novel protein)이 상이하게 발현됨을 MALDI-TOF-MS와 LC-MS/MS 로 확인하였다. 상이하게 발현된 단백질들의 정량분석을 위하여 Western blot 을 실시한 결과, apoliopoprotein A-IV precursor 와 α-1-B-glycoprotein 이 다낭성 난소증후군 환자의 여포액에서 정상군에 비해 유의하게 높은 발현 양상을 나타냄을 확인하였다.

이들의 결과를 바탕으로 다낭성 난소증후군 환자의 여포액 및 혈액 등의 단백질 분석을 통하여 다낭성 난소증후군 환자발생의 분자생물학적 기전을 이해하는데 보다 도움이 될 것으로 사료되며 나아가 진단을 위한 생물학적 지표로 활용하는데 이용할 수 있을 것으로 사료된다.

I. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders, affecting 5-10% of women in reproductive age, and is characterized by hyperandrogenism, chronic anovulatory cycle, and oligomenorrhea or amenorrhea (Franks., 1995). Serum luteinizing hormone (LH) hypersecretion, insulin resistance, and compensatory hyperinsulinemia are common biochemical features of PCOS and at least 50% of the PCOS women are insulin resistant when compared with age- and weight-matched controls (Dunaif *et al.*, 1989).

Even though a previous report indicates familial clustering of PCOS (Legro *et al.*, 1998), the mode of inheritance of the disorder is still uncertain. Although a single autosomal-dominant pattern transmission was initially proposed, recent studies are indicative for a more complex mode of inheritance (Crosignami *et al.*, 2001; Legro *et al.*, 2002). Genetic studies have been difficult due to heterogeneity in the phenotype of PCOS patients.

Moreover, the availability of only small numbers of sibpairs and the lack of an unambiguous male phenotype along with the absence of an appropriate animal model made researchers difficult to elucidate the genetic basis of PCOS. Most genetic studies performed for a candidate gene approach in cultures of isolated cells, focusing on genes involved in folliculogenesis such as myeloid cell leukemia-1 (Hartley *et al.,* 2002), growth differentiation factor-9 (Teixeira *et al*., 2002) or plasma protein-A (Hourvitz *et al*., 2002), and steroidogenesis including steroid acute regulatory protein (StAR) (Jakimiuk *et al*., 2001), or cytochrome P450 (CYP 17) (Wickenheisser *et al*., 2000; Daneshmand *et al*., 2002). In addition, genes involved in insulin signaling have been investigated (Dunaif., 1997; Waterworth *et al*., 1997; Nayudu *et al*., 1989).

Follicular fluid has an essential role in the physiology of follicular growth, oocyte maturation, and ovulation. A number of studies revealed that follicular fluid inhibits zona hardening (Duran *et al*., 1997), promotes hyperactivation and acrosome

reaction of sperm (Kulin *et al*., 1994; Revelli *et al*., 1995), increases fertilization rate (Artini *et al*., 1994), and enhances preimplantation embryo development (Suchanek *et al*., 1994). Moreover, follicular fluid contains high levels of gonadotropin and growth factors that are helpful for oocyte maturation and embryo development (Fortune *et al*., 2004). Therefore, the chemical composition of fluid from dominant follicles can be used as an indicator of the secretary activities and metabolism of follicular cells, which regulate the follicular quality. This indicates that protein components in follicular fluid can provide a useful indication of the requirements for growth and maturation of cells and oocytes during development.

2-dimensional gel electrophoresis (2-DE, Figure 1) which makes it possible to simultaneously investigate hundreds of proteins in a body fluid, has been used as a powerful research technique. I have carried out 2-DE analysis using follicular fluid from normal and recurrent pregnancy loss (RPL) patients in order to screen the proteins associated with RPL disease (Kim *et al*., 2006). I

identified several proteins including coagulation factors that are differentially expressed in follicular fluid in comparison with normal controls at the polypeptide level (Kim *et al*., 2006). In this study, I have identified, for the first time two PCOS-associated proteins that are up-regulated in follicular fluid from PCOS patients using proteomic tools and confirmed by Western blot analysis.

II. MATERIALS AND METHODS

1. Chemicals

Immobiline dry strips (IPG pH 3-10 nonlinear 18 cm) and Pharmalytes were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden); Griess reagent, CHAPS, urea, dithiothreitol (DTT), Tris base, thiourea, glycine, ammonium persulfate and sodium dodecyl sulfate (SDS) were from Sigma (St. Louis, MO, USA); coomassie brilliant blue (CBB) G-250 and tetramethylethylenediamine (TEMED) were from Bio-Rad (Hercules, CA, USA). All other chemicals were of the highest grade obtained from various commercial sources.

2. Patients and samples

1) Patients

Women with PCOS (n=5) were ascertained from Fertility Center at CHA General Hospital located in Seoul, Korea. The criteria for diagnosis of PCOS rely on the combination of clinical symptoms, ultrasonographic examination, and biochemical data. The essential criteria for the diagnosis of PCOS were the presence of polycystic ovaries on ultrasound in a patient presenting with hyperandrogenism (hirsutism, acne and/or elevated serum T) and/or symptoms of anovulation (amenorrhea or oligomenorrhea). Another five healthy volunteer females were enrolled and considered as a control group. Their healthy state was determined by medical history, physical and pelvic examination, and complete blood chemistry. Their normal ovulatory states were confirmed by transvaginal ultrasonography and plasma hormone assay detected during the luteal phase of the cycle. Clinical characteristics of normal and PCOS patients are given in Table 1.

2) Follicular fluid

Depending on the human *in vitro* fertilization (IVF) programs at Fertility Center, CHA General Hospital, follicular fluids were collected from 5 women with PCOS and 5 normal women as a control. Follicular fluid from the various sizes of follicles was pooled and collected in the sterile tubes with appropriate concentration of potassium-EDTA or sodium heparin. The fluid samples were centrifuged at 3,000 rpm for 30 minutes and the supernatant was heat-inactivated at 59℃ for 35 minutes. The heated fluid was cooled and sterilized with a filter (0.22-μm-pore-size filters, Millex-GV; Millipore, Bedford, MA, USA). All samples were stored at -80℃ until use.

3. Depletion of major abundance proteins with an immunoaffinity column

Depletion of six most abundant proteins (*i.e.*, albumin, transferrin, IgG, IgA, haptoglobin and antitrypsin) in follicular fluid was carried out using a Multiple Affinity Removal Column (MARC) (Agilent, Wilmington, DE, USA). A 4.6 mm × 50 mm MARC with binding capacity for 20 μL of follicular fluid was used. Chromatographic separation of the abundance proteins by MARC was performed with a mobile phase reagent kit according to a standard liquid chromatography (LC) protocol provided by the manufacturer. Briefly, crude follicular fluid samples were diluted 5 times with Buffer A containing protease inhibitors (Complete™, Roche, Mannheim, Germany) and filtered through 0.22-μm spin filters by spinning at 16,000 x *g* at room temperature for 1-2 min. Samples were injected and flow-through fractions were collected and stored at -20℃ until use. In order to resolve depleted follicular fluid proteins on 2-DE gels,

flow-through fractions from MARC were pooled and precipitated with pre-cooled solution of 10% TCA for 1 hr at -20℃. After washing with ice-cold acetone, pellets were resolublized in the sample buffers of 2-DE.

4. 2-DE and image analysis

Isoelectric focusing (IEF) was performed using the IPGphor system (Amersham Biosciences, Uppsala, Sweden). A sample containing 1 mg of follicular fluid proteins was mixed with a sufficient volume of a rehydration buffer (7 M urea, 2 M thiourea 4.5% CHAPS, 100 mM DTE, 40 mM Tris, pH 8.8) to give a total of 350 μL. Samples were applied to 18 cm Immobiline Drystrips, pH 3-10 nonlinear by in-gel rehydration. IEF was carried out for about 80000 Vh. The second dimensional separation was performed in 9-16% linear gradient polyacrylamide gels at constant 40 mA per gel for approximately 5 hrs. After protein fixation in 40% methanol and 5% phosphoric acid for 1 hr, the gels were stained with CBB G-250 for 12 hrs. Stained gels as exampled in Figure 2 were scanned using a GS-710 imaging densitometer (Bio-Rad, Hercules, CA, USA) and analyzed with an Image Master™2D Platinum software (Amersham Bioscience, Uppsala, Sweden).

5. Protein identification

1) In-gel digestion and mass spectrometric analysis

Excised spots were destained, reduced and alkylated, and then digested with trypsin (Promega, Madison, WI, USA) as previously described (Cho *et al.*, 2005). For MALDI-TOF MS analysis (Figure 3), the tryptic peptides were concentrated by poros R2 and oligo R3 column (Applied Biosystems, Foster City, CA, USA) and eluted in α-cyano-4-hydroxycinnamic acid (Cho *et al.*, 2005). Spectra were obtained using a 4700 TOF/TOF spectrophotometer (Applied Biosystems, Foster City, CA, USA). Proteins were identified from the peptide mass maps using MASCOT (http://www.matrixscience.com/search_form_select.html), MS-Fit (http://prospector.ucsf.edu), and ProFound (http://129.85.19.192/profound_bin/WebProFound.exe) to search for the protein database, Swiss-Prot and GenBank.

2) LC-MS/MS

For nano-LC-ESI-MS/MS analysis, the peptides digested with trypsin were concentrated by poros R2 and oligo R3 column. All LC-MS/MS experiments were performed using an Agilent Nano -flow Proteomics Solution featuring an Agilent 1100 Series nano-LC for MS/MS coupled through an orthogonal nanospray ion source to an Agilent 1100 Series LC/MSC Trap XCT ion trap mass spectrometer. The nano-LC system was operated in sample enrichment/desalting mode using a ZORBAX 300SB-C18 enrichment column (0.3 x 50 mm, 5 μm). Chromatography was performed using a ZORBAX 300 SB-C18 (75 μm x 150 mm) nanocolumn. Solvent gradient started at 3% solvent B (0.1% formic acid in acetonitrile) and 97% solvent A (0.1% formic acid in water). The gradient was as follows: 3% B isocratically from 0 to 5 minute, 3 to 10% B from 5 to 10 minute, 10 to 45% B from 10 to 50 minute, 45 to 90% B from 50 to 55 minute, 90%

B isocratically from 55 to 60 minute, 90 to 3% B from 60 to 61 minute, and then washed with 3% B for 10 minute.

The LC/MSD Trap XCT was operated in the unique peptide scan auto-MS/MS mode. The ionization mode was positive nanoelectrospray with an Agilent orthogonal source. Drying gas flowed at 5 L/minute and drying gas temperature was 300°C. Vcap was typically 1800-1900 V, with skim 1 at 30 V, and capillary exit offset at 75 V. The trap drive was set at 85 V with averages of 1 or 2. ICC was on with maximum accumulation time of 150 ms, smart target was 125,000, and MS scan range was 300-2200. Automatic MS/MS was in ultra scan mode, with the number of parents 2, averages of 2, fragmentation amplitude of 1.15 V, SmartFrag on (30–200%), active exclusion on (after 2 spectra for 1 minute), prefer + 2 on, MS/MS scan range of 100-1800, and ultra scan on. Each acquired MS/MS spectrum was searched against the non-redundant protein sequence database using Spectrum Mill software tool in the protein.

3) Bioinformatics

Each MS/MS spectrum obtained was searched against the non-redundant protein sequence database using the Spectrum Mill software tool. Sequences of uninterpreted CID spectra were identified by correlation with the peptide sequences present in the protein sequence database (NCBInr 2006.10) using the Spectrum Mill MS Proteomics Workbench (Rev A.03.00.015, Agilent, Wilmington, DE, USA). Results for the SpectrumMill search were initially assessed by ‘score’ and ‘scored peak intensity (SPI)’. The software creates theoretical peptides for all or a limited group of database proteins, calculates corresponding MS/MS spectra; and compares them with an experimental spectrum (submitted for the database search) to find the match. Score means points to the matched (Bonus) or unmatched (Penalty) peaks. Bonus points are awarded for each matched peak, at one point per peak regardless of peak height. Penalty points for unmatched peaks are based on peak height/height of tallest peak. SPI is calculated as follows: from peaks remaining after peak detection, this is the percentage

of total intensity in the query set spectrum, which is matched to peaks in the library spectrum. SPI lower than 50% suggests a poor match, or presence of non-corresponding fragment ion types in the query set spectrum. Adjusting the value of minimum matched peak intensity to less than 50% (default value) will enable reporting of poorer quality matches. As a general rule, for declaring a protein hit, protein score > 13, peptide score > 10 and SPI (%) > 70 were applied throughout the data analysis procedures as suggested by the manufacturer. All the proteins identified in this paper are based on at least two peptides assignment.

6. Western blot analysis

Human follicular fluid proteins diluted to 1/10 with PBS buffer were subjected to SDS-PAGE. Albumin was removed from follicular fluid for the purpose of detection of kininogen 1, cytokeratin 9, antithrombin, fibrinogen γ chain, apolipoprotein A-IV precursor, and α-1-B-glycoprotein using Aurum serum

protein mini kit (Bio-Rad, Hercules, CA, USA). The proteins were blotted onto nitrocellulose membranes. The membranes were incubated with blocking solution, containing a 1:200 dilution of anti-kininogen 1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or anti-cytokeratin 9 (Abcam, Cambridge, UK) antibodies. In addition, antithrombin antibody (Abcam, Cambridge, UK) diluted with 1:1000 with the blocking solution was used. Also, the membranes were incubated with blocking solution, containing a 1:500 dilution of anti-fibrinogen γ (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-apolipoprotein A-IV precursor (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or anti-α-1-B-glycoprotein (Aviva Systems Biology, San Diego, CA, USA) antibodies. And then, the membranes were incubated with blocking solution containing a 1:5000 dilution of horseradish peroxidase-conjugated secondary antibodies. An ECL system (Elpis Biotech, Taejeon, Korea) was used for the detection of signals. Bands from the Western blotting were scanned and digitized using Fluor-S™ MultiImager (Bio-Rad, Hercules, CA, USA). Values are

expressed as mean ± standard error of the mean (SEM).

7. Data processing statistical analysis

All statistical analyses of the data were performed using a Student's *t*-test when two groups were compared. Numerical data are presented as mean ± SD, and a *p* value less than 0.005 was considered statistically significant.

Ⅲ. Results

1. 2-DE and image analysis of proteins in follicular fluid from PCOS patients

A typical 2-DE separation was performed in the pH range of 3-10 (Figure 4) on proteins extracted from the follicular fluid of PCOS patients and normal women as a control to identify proteins that are aberrantly expressed in PCOS patients.

2. Differential expression of proteins in follicular fluid of PCOS patients

By comparing protein expression levels in follicular fluid from 5 PCOS patients and 5 normal women as a control, the overexpressed 6 spots were selected. The expression levels were determined by examining the ratio of the relative spot volume of a protein in the gel. The identities of these different proteins

were confirmed by MALDI-TOF-MS or nano-LC MS/MS. They included kininogen 1, cytokeratin 9, antithrombin chain B, fibrinogen γ chain, apolipoprotein A-IV precursor and α-1-B -glycoprotein (Figure 5 and Table 2).

3. Western blot analysis

To confirm the expression levels of the identified spots, the follicular fluids were analyzed by Western blotting with the respective antibodies. As shown in Figure 6A, apolipoprotein A-IV precursor was detected in the follicular fluid of both PCOS and control groups. Apolipoprotein A-IV precursor (46 kDa) expression in PCOS patients was significantly higher than that in controls (1.00 *vs.* 3.21 ± 0.89, * $p < 0.005$, Figure 6B). Figure 7A shows the Western blot for α-1-B-glycoprotein (54 kDa) detected in follicular fluid of controls and PCOS patients.

α-1-B-glycoprotein expression in PCOS patients was also

increased about three-fold compared with control level (1.00 *vs.* 2.70 ± 0.31, * $p < 0.005$, Figure 7B). On the other hand, Western blotting analysis showed no significant changes of kininogen 1, cytokeratin 9, fibrinogen γ and antithrombin between PCOS and control groups as shown in Figure 8.

Ⅳ. Discussion

PCOS is a common endocrinopathy affecting 5-10% of women of reproductive age. The 2003 Rotterdam Consensus concluded that PCOS is a syndrome of ovarian dysfunction requiring two out of three of the following criteria for diagnosis: (i) oligo- or anovulation; (ii) hyperandrogenism and/or hyperandro -genemia; and (iii) polycystic ovary morphology. There is clear evidence for an underlying genetic cause for PCOS based on familiar clustering of cases. Most studies are consistent with an autosomal dominant inheritance. However, studies have been difficult due to small sample sizes, errors in statistical analysis, and differences in diagnostic criteria, an inevitable consequence of PCOS being a heterogeneous disorder. In this study, the proteins expressed in the follicular fluid of PCOS patients were investigated using 2-DE analysis and I found that two proteins apolipoprotein A-IV precursor and α-1-B-glycoprotein were aberrantly expressed in PCOS.

Human apolipoprotein A-IV is a 46 kDa glycoprotein triglyceride-rich glycoprotein (Utermann *et al*., 1979; Weisgraber *et al*., 1978). It is expressed in the small intestine, and its synthesis and secretion increase in response to a fatty meal (Kalogeris *et al*., 1994). apolipoprotein A-IV has several proposed roles, including lipid transport, lipoprotein metabolism (Goldberg *et al*., 1990), control of food intake (Fujimoto *et al*., 1993) and gastric function (Okumura *et al*., 1994). Most recently, apolipoprotein A-IV has been shown to be antiatherogenic, and there is also evidence supporting its antioxidant activity (Ostos *et al*., 2001). Two mechanisms have been suggested for apolipoprotein A-IV's antiatherogenic action: enhancement of cellular lipid efflux (i.e., "reverse cholesterol transport") (Remaley *et al*., 2001) and antioxidant activity (Ostos *et al*., 2001). I identified that the expression of apolipoprotein A-IV was significantly increased in follicular fluid from PCOS patients. This may result in aberration of lipoprotein metabolism and transport, causing the generation of polycystic ovary. However, how the

aberrant expression of apolipoprotein A-IV is involved in the generation of PCOS remains to be elucidated.

I also determined the expression level of α-1-B-glycoprotein by Western blotting analysis, and found that its expression is increased in PCOS patients compared with normal women. α-1-B-glycoprotein is a known plasma protein withunknown function and a member of the immunoglobulin superfamily. Little is known for biological functions of α-1-B-glycoprotein with regard to infertility diseases so far. Further study, therefore, is needed to explain as to why the α-1-B-glycoprotein protein is increased in PCOS patients.

Even though we identified that the expression of kininogen 1 and cytokeratin 9 was increased in follicular fluids from PCOS patients by 2-DE analysis, Western blotting analysis showed no changes between PCOS and normal patients. However, the expression level varies among normal or PCOS patients, suggesting that these proteins may be involved in etiology of PCOS in certain cases.

In addition, we identified that the expression of antithrombin and fibrinogen γ was increased in follicular fluids from PCOS patients by 2-DE analysis. Western blotting analysis, however, showed individual variation between PCOS and normal patients. In the case of antithrombin, Tsanadis *et al.* (2002) reported that the concentration difference is not statistically significant between women with PCOS and the control women, and this is in agreement with our own results. There are several results in the literature regarding the fibrinogen concentration in females with PCOS. Atiomo *et al*. (1998) have shown statistically a higher concentration of fibrinogen in women with PCOS than in normal control women. Dahlgren *et al*. (1994) have shown that the concentration of fibrinogen is less in females with PCOS. Kelly *et al*. (2002) did not find statistically significant differences between these two groups. Considering the differences in the literature with regard to the expression level of fibrinogen in PCOS patents, further studies are needed in order to investigate the detailed functions of fibrinogen in PCOS patients. not

necessary because there is no differential expression of these proteins in PCOS patients and normal women.

Ⅴ. Conclusion

Polycystic ovarian syndrome (PCOS) is a common endocrine -metabolic disorder, affecting 5-10% of women of reproductive age. The etiology still poorly understood. Follicular fluid contains a variety of biological important proteins of the requirements for oocyte fertilization and follicle maturation during mammalian reproductive process. Therefore, it can be used as a provisional source for identifying proteins involved in PCOS.

To identify the differentially expressed proteins from PCOS patients versus normal control, the protein expression in ovarian follicular fluid was analyzed using two-dimensional electrophoresis (2-DE). Over-expressed six proteins (kininogen 1, cytokeratin 9, antithrombin, fibrinogen , apoliopoprotein A-IV precursor, and α-1-B-glycoprotein) in follicular fluids from PCOS patients were identified with matrix assisted laser desorption/ionization-time or flight-mass spectrometry (MALDI-TOF-MS) and nano-LC MS/MS. Western blot analysis confirmed that the protein expression level of apoliopoprotein A-IV precursor and α-1-B-glycoprotein was increased in follicular fluid from PCOS patients compared with those from normal controls.

These results will facilitate the understanding of molecular mechanisms of PCOS and provide candidate biomarkers for the development of diagnostic tools.

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Table 1. Clinical characteristics of women with PCOS and controls

	PCOS	Normal
No.	5	5
Age (y)	33 (29-39)	31 (26-39)
BMI (kg/m^2)	23.07 (19.97-26.35)	20.94 (19.78-22.38)
FSH (IU/liter)	5.74 (2.9-8.2)	6.10 (3.7-8.2)
LH (IU/liter)	10.46 (8.0-13.0)	4.98 (2.3-6.7)
E_2 (pmol/liter)	32.60 (19.0-61.0)	27.86 (9.3-49.0)

BMI, body mass index

FSH, follicular-stimulating hormone

LH, luteinizing hormone

E_2, estradiol

Table 2. Protein identities determined by mass spectrometry

Spot ID	Accession number	Protein name	Score*	Matched peptides number(%)	Sequence coverage (%)	Theoretical MW /pI
2931	gi|4504893	Kininogen 1	89	11/44 (25)	21	47853/6.29
3074	gi|435476	Cytokeratin 9	83	17/184 (9)	41	62092/5.29
3246	gi|999514	Chain B Antithrombin III	68	17/184 (9)	53	48916/5.95
3528	gi|182489	Fibrinogen γ	68	16/165 (10)	45	49450/5.61
4093	gi|71773110	Apolipoprotein A-IV precursor	277	25/50 (50)	50	45344/5.28
6603	gi|69990	α-1-B-Glycoprotein	82	16/135 (12)	45	51908/5.65

* Score is -10 x Log (P), where P is the probability that the observed match is a random event; it is based on NCBI database using the Mascot searching program as MS/MS data.

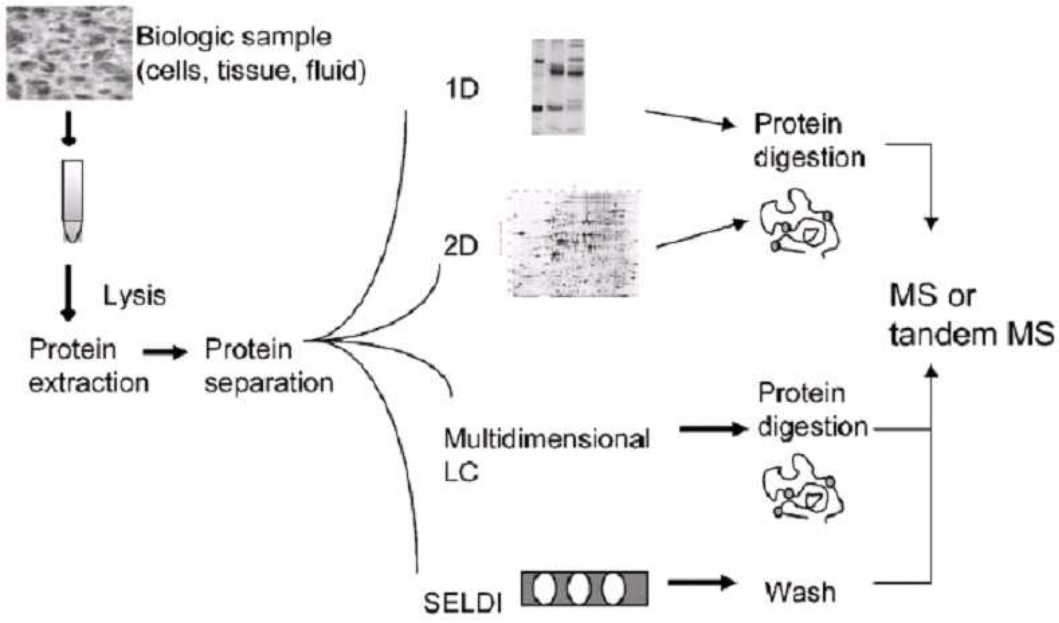

Figure 1. Overview of experimental design for mass spectrometry -based proteomic studies. Proteins are extracted from biologic samples and fractionated by a variety of separation methods. In 2-DE, proteins are separated based on isoelectric point (pI) and size.

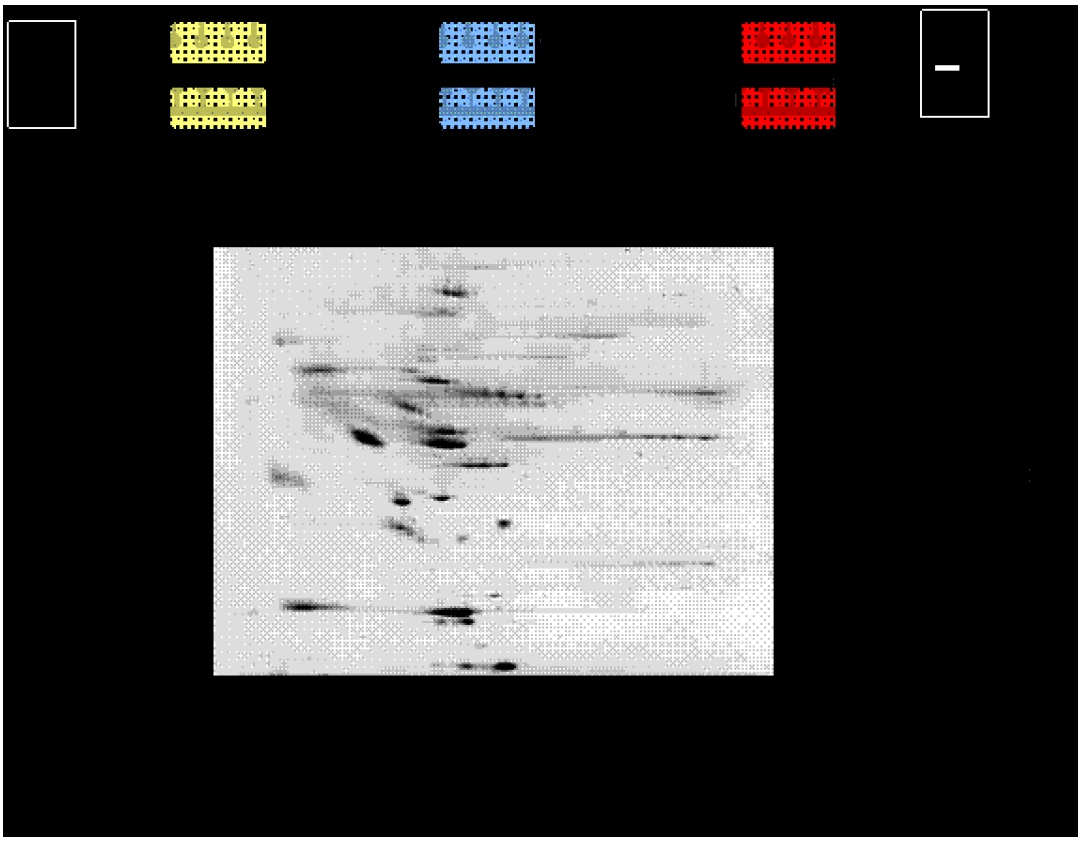

Figure 2. IPG strip and gradient SDS-PAGE for follicular fluid proteins separation.

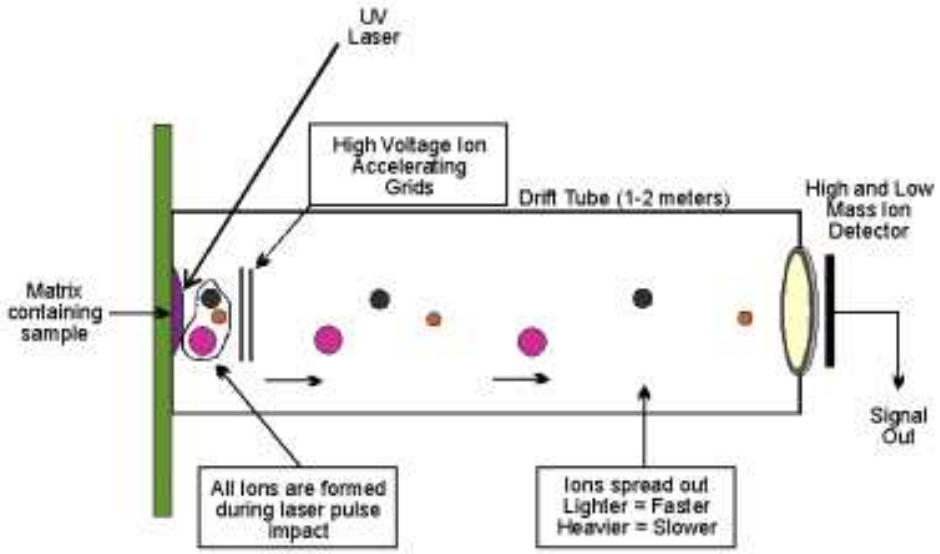

Figure 3. Protein ionization methods used for mass spectrometry -based proteomics. Matrix-assisted laser-desorption ionization (MALDI) uses analytes which are cocrystallized in a matrix composed of organic acid on a solid support. A pulse of ultraviolet laser is then used to evaporate the matrix and the analyte into gas phase resulting in generation of single charged ions.

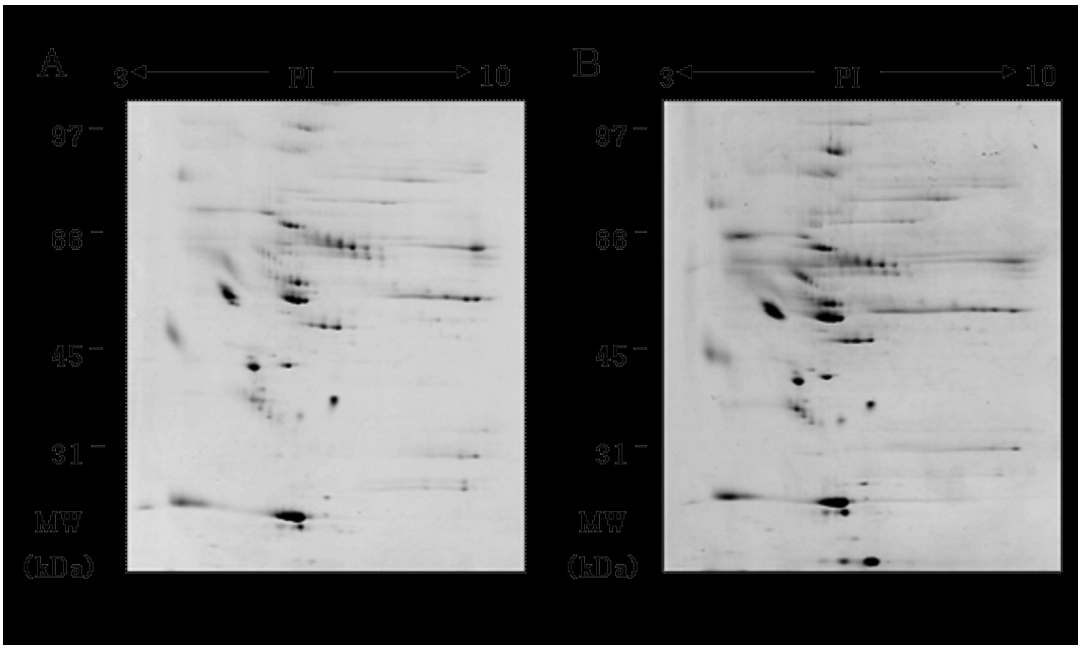

Figure 4. Master image of a 2-DE gel stained with Coomassie Blue G-250. 1 mg follicular fluid proteins were lysed and separated on 18 cm pH 3-10 IPG strip and then 9-16% gradient SDS-PAGE. (A) 2-DE gel image of follicular fluid proteins for the control. (B) 2-DE gel image of follicular fluid proteins for the PCOS patient.

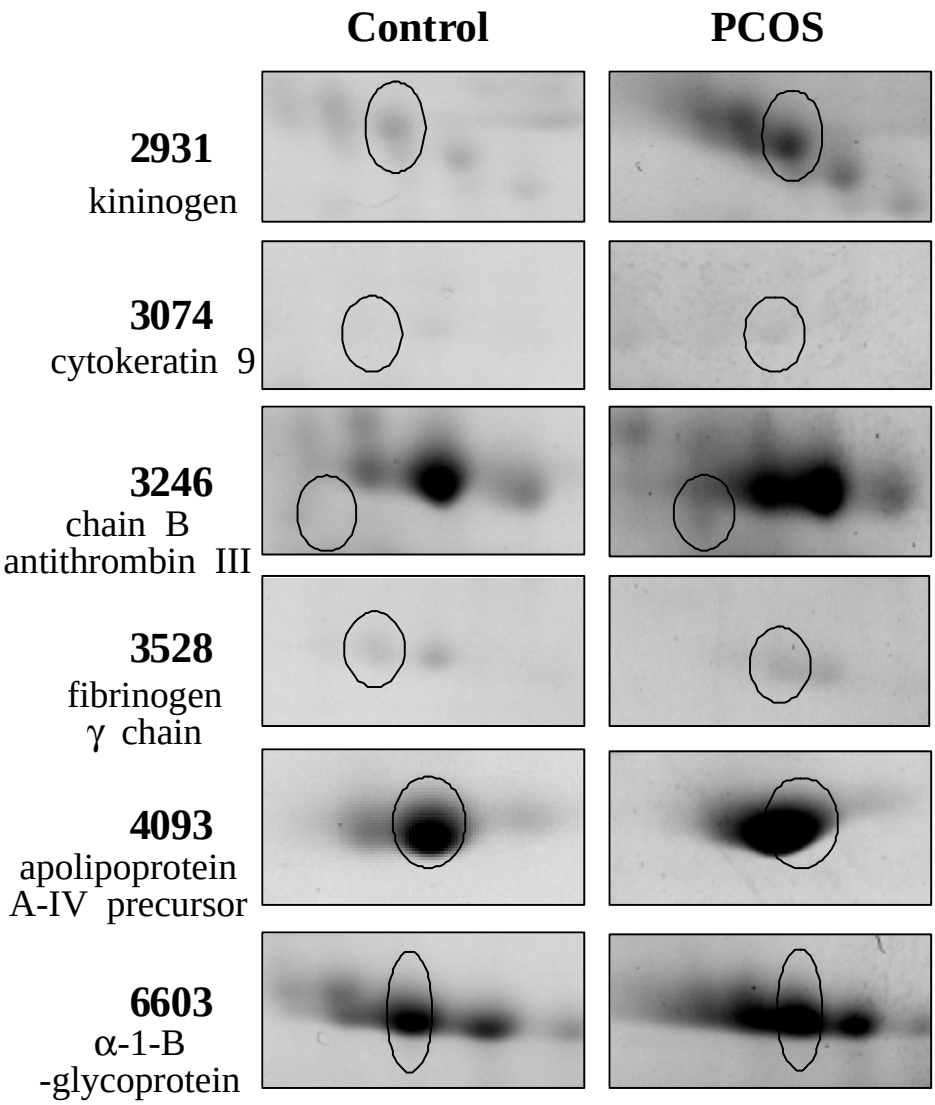

Figure 5. 2-DE image of overexpressed proteins in follicular fluid of PCOS patients. Up-regulated proteins in follicular fluids of PCOS patients. The 2-DE images of 6 up-regulated spots were analyzed using ImageMaster™ 2D Platinum Software (ver. 5.0) and magnified.

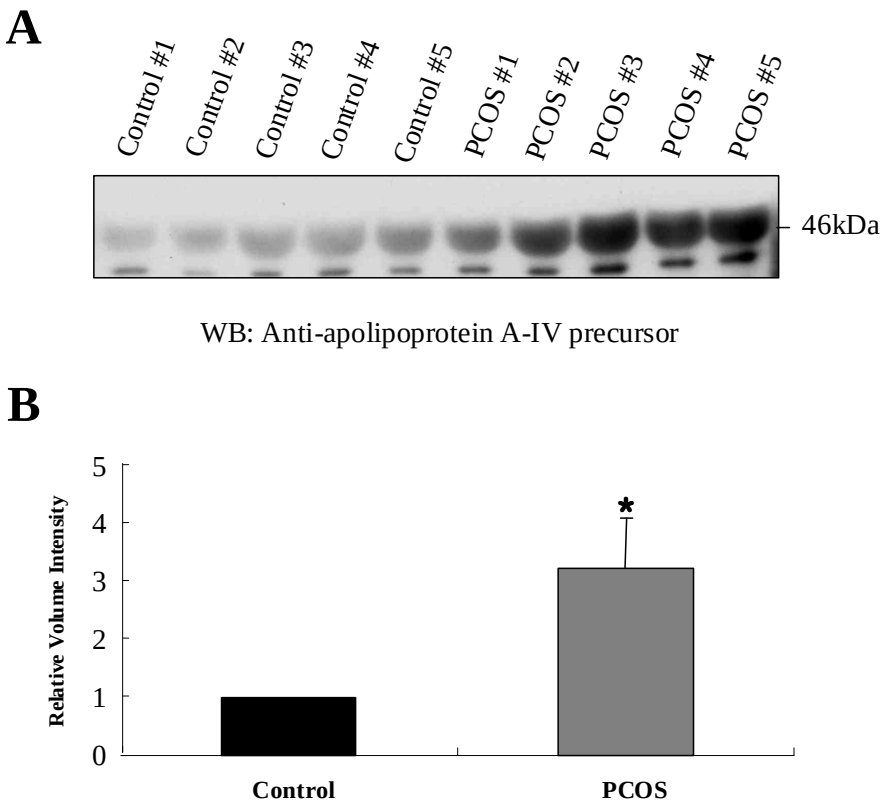

Figure 6. Western blot analysis of Apolipoprotein A-IV precursor. Western blotting for follicular fluid proteins from controls and POCS patients was carried out. (A) Western blot analysis. (B) Quantitative density of gel bands for Apolipoprotein A-IV precursor (46 kDa). The bars represent the mean ± SEM of density of gel bands determined from PCOS patients and controls. Significant difference was assessed by a Student's t-test (*$p < 0.005$).

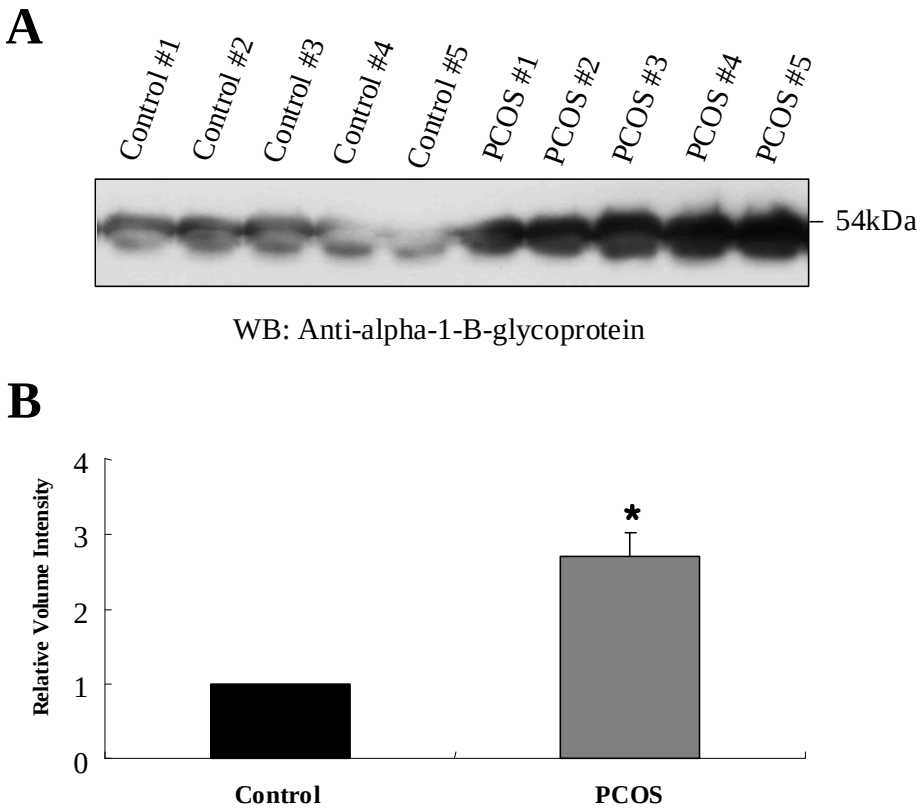

Figure 7. Expression of α-1-B-glycoprotein by Western blotting. Western blotting of follicular fluid proteins from PCOS patients and controls was carried out. A Student's t-test showed significant differences (*$p < 0.005$) between control and PCOS follicular fluid. (A) Western blot; (B) Quantitative density of gel bands in the α-1-B-glycoprotein (54 kDa). The bar represents the mean ± SEM of density of gel bands determined from controls and PCOS samples.

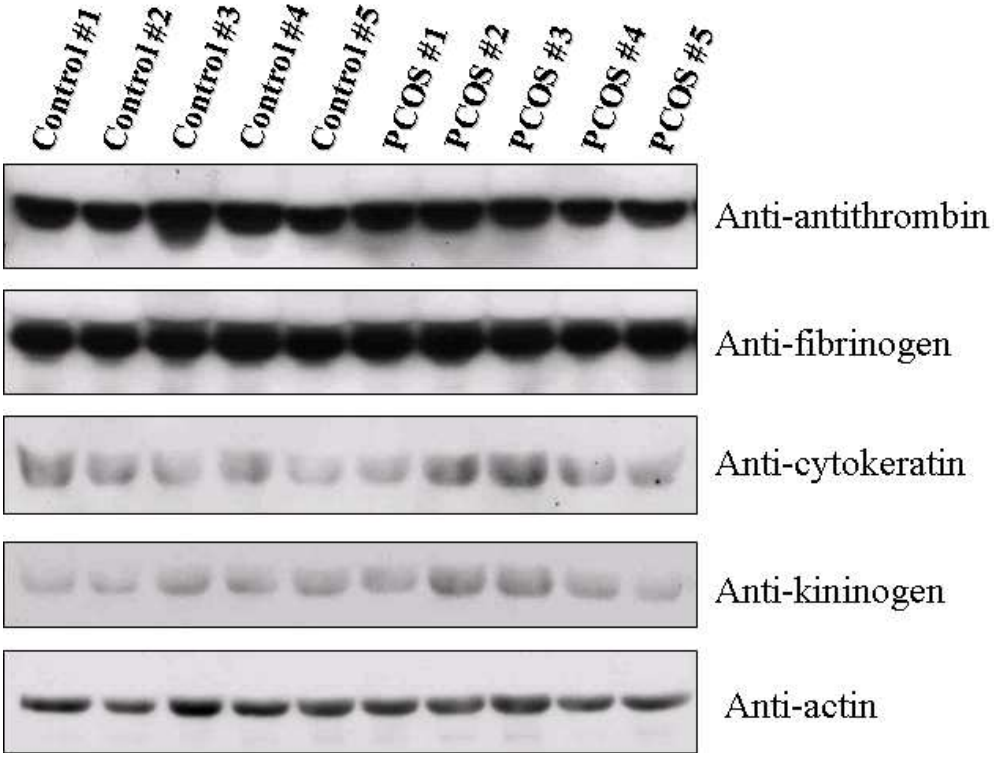

Figure 8. Western blot analysis of antithrombin, fibrinogen γ, cytokeratin and kininogen in follicular fluid of PCOS patients and normals.

저작물 이용 허락서

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논문제목	한글 : **다낭성 난소 증후군 환자의 난소 여포액을 이용한 프로테오믹스 분석** 영문 : **Proteomic analysis using follicular fluids in polycystic ovary syndrome (PCOS) patients**				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의(*0*) 조건부 동의( ) 반대( )

2006년 12 월 일

저작자: 최 범채 (서명 또는 인)

조선대학교 총장 귀하