

Original Article



Comparison of Follicular Fluid Glycosaminoglycan and Hydroxyproline Concentration in Women with Polycystic Ovary Syndrome with Healthy Women

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Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of childbearing age. As a complex structure, the extracellular matrix (ECM) and its components, including glycosaminoglycans (GAGs) and collagens, are highly affected by hormonal alterations.

Objectives: This study aimed to evaluate the level of GAG and hydroxyproline in the follicular fluid (FF) of women with PCOS.

Methods: Overall, 62 women referring to Fatemeh Hospital Infertility Center in Hamadan were evaluated in this study. FF samples were taken from 31 women with PCOS during ovarian puncture and 31 healthy women. Oocytes were evaluated in the different stages of maturation, namely, metaphase II (MII) and germinal vesicle (GV). Commercially available kits were used to measure the levels of glycosaminoglycans and hydroxyproline.

Results: The results of the present study showed that the count of oocytes in women with PCOS was significantly higher than that in healthy women. Evaluating GAG and hydroxyproline levels indicated that their levels were meaningfully higher in the FF of women with PCOS.

Conclusion: In general, the results of this study suggested that ECM changes in PCOS followed by alterations in the levels of GAGs and hydroxyproline may lead to an increase in the level of GAG and hydroxyproline in the FF. The evaluation of these markers may be the subject of further studies.

Keywords: Glycosaminoglycans, Hydroxyproline, Oocytes, Follicular fluid, Polycystic ovary syndrome



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Background

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrinopathies among women of childbearing age and the leading cause of anovulatory infertility, affecting roughly 6.5%–8% of women worldwide (1,2). It is a multifactorial disease, and its main cause is still unknown. Hyperandrogenism, infertility, menstrual dysfunction, and pregnancy complications are features of PCOS. Diagnostic criteria for the syndrome include chronic oligo-ovulation or anovulation, an increased level of androgen, and polycystic ovaries (3). Women with PCOS suffer from androgen excess, insulin resistance, and variable levels of estrogen, which affect the metabolic index, leading to an increase in lipid profiles, inflammatory factors, and oxidative stress biomarkers

(4,5). Hyperinsulinemia, obesity, and higher levels of cholesterol manifested following insulin resistance are involved in the pathogenesis of PCOS (4).

Tissue remodeling which leads to extracellular matrix (ECM) alteration is a crucial process during follicular growth in the ovaries. Additionally, changing the theca and granulosa cell composition in the developing follicle due to an alteration in the hormonal level might be the major cause of follicular growth. For example, gonadotropins, including luteinizing hormone (LH) and follicular stimulating hormone (FSH), are major endocrine factors that affect follicular growth via stimulating the enzymes responsible for androgen hormone production in the theca cells (LH), estrogen production, and growth and differentiation of granulosa



cells (FSH) (6). Consequently, any alterations in the ECM and abnormal hormonal secretion may contribute to follicular-related disorders (7). Previous studies have described that the ratio between LH and FSH usually lies between 1 and 2 in healthy women, while in women with PCOS, this ratio becomes reversed, and it might reach as high as 2 or 3 (6).

ECM is a quite stable structural material that lies under epithelia and fills the space between connective tissue cells (8). The ECM is made up of various types of proteins, including proteoglycans (PGs), glycoproteins, and collagens. These compositions facilitate cell growth and differentiation, as well as tissue morphogenesis. There are two major classes of macromolecules composing the ECM, including PGs and fibrous proteins. PGs are constructed of glycosaminoglycan (GAG) chains covalently bonded to a certain protein core (8).

ECM remodeling is a process of fractionating the existing proteins, and synthesis and accumulation of new ECM proteins. Various classes of proteolytic enzymes participate in this process, but matrix metalloproteinase (MMP) is one of the prominent ones (9). Studies have proven an increase in the circulating concentrations of MMPs in PCOS women. This increase might be associated with the pathophysiology of PCOS (10). Prolidase, a member of the MMP family and cytosolic exopeptidase, facilitates the last step of collagen degradation (by omitting hydroxyl proline), thus releasing the proline required for the synthesis of new collagen molecules. Accordingly, prolidase is assumed to take part in the remodeling process and eventually cyst formation (11). Therefore, this study sought to evaluate the level of GAGs in hydroxyproline in the follicular fluid (FF) of women with PCOS.

Materials and Methods

Subjects and Study Design

This cross-sectional study was performed on 31 women with PCOS and 31 women with normal ovulatory function who were under control for male infertility factors in their husbands and received in vitro fertilization. PCOS diagnosis was confirmed by a gynecologist according to Rotterdam criteria, namely hyperandrogenism, oligo/anovulation, and polycystic ovaries (12). All participants were within the age range of 20–40 years old. Women older than 40 years old, women with cardiovascular diseases, hypothyroidism, liver dysfunctions, cancers, diabetes, confirmed renal dysfunction, blood pressure higher than 140/90 mm Hg, use of oral contraceptive pills, and smokers were excluded from this study. The control group included women with regular menstrual cycles with no evidence of hirsutism or acne and/or whose husbands suffered from male factor infertility. The study was approved by the Ethics Committee of Hamadan University of Medical Sciences, and written formal consent was obtained from every subject recruited in our study. Afterward, they filled out a questionnaire with their demographic information.

Collection of Follicular Fluid

PCOS and controls were treated with a standard long-term protocol with a gonadotropin-releasing hormone agonist in the mid-luteal phase of the preceding menstrual cycle. Ovarian stimulation was initiated with recombinant FSH, Cinnal-F (CinnaGen Company, Iran). Transvaginal sonography scans of the ovaries were performed every 1–3 days. Human chorionic gonadotropin (hCG, Choriomon, IBSA, Lugano, Switzerland) in doses of 5000–10,000 IU was administered when more than three dominant follicles reached a diameter of 18 mm. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 hours after hCG injection. FFs were collected during follicular puncture, and after removing oocytes, the collected FFs were pooled without washing and centrifuged at 400 g for 10 minutes. The supernatant was collected and stored at -80°C for the enzyme-linked immunosorbent assay analysis.

Determination of the Level of Glycosaminoglycan in the Follicular Fluid

The GAGs level was measured using dimethyl methylene blue in comparison with different standard levels of chondroitin sulfate (13). For the determination of the GAG level, the specimens were dissolved in the papain solution in separate microtubes. Then, the mixture was centrifuged at $6000\times g$ for 15 minutes. The supernatants were isolated to be used, and the standard curve was dissolved in different concentrations of papain. Next, 30 μL of standard solution or sample solution was added to each well, and then the enzyme solution was added to a single well as a blank. In addition, 200 μL of the GAG reagent was added to every well. The plate was incubated for 60 minutes at room temperature. Finally, using a spectrophotometer, the plate's absorbance was measured at 510–560 nm.

Determination of the Level of Hydroxyproline in the Follicular Fluid

In an oven, the FF samples were hydrolyzed with HCl. Their pH was then adjusted by NaOH and acetate-citrate buffer. Subsequently, they were oxidized using chloramine-T. Eventually, the reaction was terminated by perchloric acid, and the color was generated by *para*-dimethylaminobenzaldehyde. For this purpose, 20 μL of the specimen was added to the wells, and 20 μL of the assay buffer was added to a separate well as a blank. Further, 100 μL of the oxidation solution was added to every well and incubated for 15 minutes at room temperature. Afterward, 100 μL of the chromogen solution was added to the wells and incubated for 60 minutes at 60 degrees. Ultimately, the plate's absorbance was estimated at 540–560 nm using a spectrophotometer (14).

Results

Table 1 provides the clinical characteristics and demographic information of patients. No considerable

difference was witnessed between the case and control groups in terms of age and body mass index (BMI). The number of retrieved oocytes in patients with PCOS was significantly higher than in the control group ($P < 0.001$). However, no significant difference was noticed in the number of metaphase II (MII) ($P = 0.059$) or germinal vesicle oocytes ($P = 0.187$).

The level of GAG in the FF of women with PCOS and normal women is depicted in Figure 1. Our findings revealed that women with PCOS had a significantly higher FF GAG level as compared to healthy women ($P < 0.05$).

The level of hydroxyproline in the FF of women with PCOS and normal women was also measured, and the results indicated a significantly higher level of hydroxyproline in women with PCOS ($P < 0.05$) when compared to healthy women (Figure 2).

Discussion

PCOS is a multifactorial disorder that arises from interactions between genetic, environmental, and intrauterine factors. Various studies have stated that women with PCOS have more oocytes in an immature state. It is also believed that stromal circulation in the follicular phase is richer in women with PCOS (15,16). Kazemi et al figured out that the number of oocytes formed as a result of ovulation in women with PCOS was greater than that in women with tubal infertility and normal ovaries (17). In the same manner, our study results demonstrated a significantly larger number of oocytes in women with PCOS. However, the number of immature oocytes (GV and MII) in patients with PCOS was not considerably higher than that in the patients in the control group.

Alterations in ECM components might be effective in the pathogenesis of PCOS. The ECM is an active, viable structure that determines many cellular characteristics such as proliferation, differentiation, and growth (18,19). In the present study, the levels of GAG and hydroxyproline were determined in the FF of women with PCOS and a control group of normal women. In our study, a significantly higher level of GAG was observed in patients with PCOS, which is compatible with the results of previous studies (21,23). Significantly higher levels of markers reflecting the degradation of type I (C1M), type III (C3M), and type IV (C4M, C4M12) collagens by

MMPs were detected in the serum of patients with ovarian and breast cancer in comparison with healthy individuals (20), which is an example of a destructive alteration in the ECM. The interaction between ECM and endometrium is of importance during endometrial remodeling (21). ECM is a complex structure made up of various macromolecules including collagen and elastin fibers, glycoproteins, and sulfated GAG (22). Previously, it has been suggested that GAG levels, especially chondroitin sulfate, are higher in women with PCOS (23). In this regard, recent studies have revealed that the disruption of the MMPs to tissue inhibitors of metalloproteinase balance in women with PCOS may result in the abnormal degradation of ECM components such as collagen in follicles, leading to ovulation disorders and polycystic changes in the ovary (24). In line with this study, our findings demonstrated that there was a higher hydroxyproline level as a collagen degradation product in the FFs of women with PCOS. As another component of the ECM, AG has a known role in folliculogenesis and fertility (25,26). Studies have shown that endometrium morphogenesis alterations present in PCOS might be in association with changes in GAG levels, especially heparin sulfate (18,27).

Oocyte maturation follows three developmental processes, namely, nuclear maturation, epigenetic maturation, and cytoplasmic maturation. If any of these maturations are defected, oocyte quality is affected,

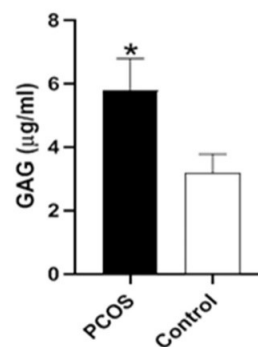


Figure 1. Glycosaminoglycan Level in the Follicular Fluid of Women With PCOS in Comparison With the Control Group. Note. PCOS: Polycystic ovary syndrome. Data are presented as means ± standard deviations. * $P < 0.05$

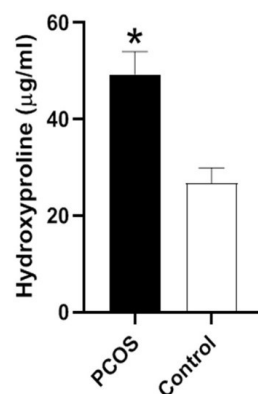


Figure 2. Hydroxyproline Level in the Follicular Fluid of Women With PCOS in Comparison With the Control Group. Note. Polycystic ovary syndrome. Data are presented as means ± standard deviations. * $P < 0.05$

Table 1. Clinical Characteristics and Demographic Information of PCOS and Control Group

Variables	Case (Mean ± SD)	Control (Mean ± SD)	P Value
Age (y)	30 ± 1.6	34 ± 1.2	0.15
BMI (kg/m ²)	35.5 ± 2.8	24.6 ± 3.1	0.026
Number of oocytes (%)	63.8%	36.2%	<0.001
Number of MII oocytes (%)	56.6%	43.4%	0.059
Number of GV oocytes (%)	37.5%	62.5%	0.187

Note. PCOS: Polycystic ovary syndrome; GV: Germinal Vesicle; MII: Metaphase II; SD: Standard deviation; BMI: Body mass index.

leading to decreased fertility potential. In spite of contradictory results, a considerable number of studies suggest impaired oocyte competence in PCOS due to the disturbance of oocyte maturation (28). The oocyte release and fertilization are defected, and infertility or sterility occurs if the zona pellucida, a transparent membrane protecting the ovum, misses the matrix molecules or their crosslinks or if they interrupt (29). Prolidase, a matrix metalloproteinase, eliminates hydroxyproline, thus catalyzing the final step in collagen degradation. Its function affects matrix remodeling and collagen metabolism, as well as cell growth and maturation (30,31). Studies have proven the prolidase level to be significantly higher in women with PCOS (10,11). Similarly, our results revealed a considerable hydroxyproline level in women with PCOS, highlighting its role in PCOS pathogenesis and defected oocyte quality. Various types of collagen take part in ECM remodeling as its basic components. By measuring the hydroxyproline level, alterations in ECM collagens at times of remodeling are possible (32,33). The abnormalities of hydroxyproline metabolism play a role in various diseases, including PCOS (34).

Overweight/obesity in women is a risk factor for inflammation and plays an effective role in the pathogenesis of disorders related to obesity, including PCOS (12,13). Our study showed that women with PCOS have a higher BMI than normal women, representing overweight in the case group. Likewise, McBreaity et al concluded that overweight and higher BMI are prevalent in women with PCOS, and they have a higher BMI than healthy women (14). Higher BMI results in metabolic changes, including peripheral aromatization of estrogens, decreased levels of sex hormone-binding globulin, which results in higher levels of estradiol and testosterone, and higher insulin levels, which may ultimately lead to increased production of androgens by the ovaries. Mentioned alterations lead to anovulation (7).

Conclusion

Overall, our findings indicated markedly elevated levels of GAG and hydroxyproline in the FF of PCOS patients. In addition, women in the PCOS group had a higher BMI. A larger number of oocytes were detected in women with PCOS. It is suggested that further studies evaluate GAG and hydroxyproline levels in women with PCOS to assess the risk of inflammatory disorders.

Authors' Contribution

Conceptualization: Iraj Amiri, Hadi Ghasemi.

Data curation: Hadi Ghasemi.

Formal analysis: Hadi Ghasemi, Kiana Kimiaei Asadi.

Funding acquisition: Iraj Amiri.

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Methodology: Iraj Amiri, Hadi Ghasemi.

Project administration: Iraj Amiri.

Resources: Iraj Amiri.

Software: Hadi Ghasemi.

Supervision: Iraj Amiri.

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Visualization: Hadi Ghasemi.

Writing—original draft: Kiana Kimiaei Asadi.

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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (Ethics code: IR.UMSHA.REC.1398.194).

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References

1. Kurdoglu Z, Ozkol H, Kurdoglu M. Serum myeloperoxidase and adenosine deaminase activities in polycystic ovary syndrome. *Gynecol Endocrinol.* 2012;28(2):115-8. doi: [10.3109/09513590.2011.583959](https://doi.org/10.3109/09513590.2011.583959).
2. Wijeyaratne CN, Balen AH, Barth JH, Belchetz PE. Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: is there a difference? *Clin Endocrinol (Oxf).* 2002;57(3):343-50. doi: [10.1046/j.1365-2265.2002.01603.x](https://doi.org/10.1046/j.1365-2265.2002.01603.x).
3. Hilali N, Vural M, Camuzcuoglu H, Camuzcuoglu A, Aksoy N. Increased prolidase activity and oxidative stress in PCOS. *Clin Endocrinol (Oxf).* 2013;79(1):105-10. doi: [10.1111/cen.12110](https://doi.org/10.1111/cen.12110).
4. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clin Biochem.* 2001;34(5):407-13. doi: [10.1016/s0009-9120\(01\)00245-4](https://doi.org/10.1016/s0009-9120(01)00245-4).
5. Aslan M, Duzenli U, Esen R, Soyoral YU. Serum prolidase enzyme activity in obese subjects and its relationship with oxidative stress markers. *Clin Chim Acta.* 2017;473:186-90. doi: [10.1016/j.cca.2017.08.039](https://doi.org/10.1016/j.cca.2017.08.039).
6. Saadia Z. Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS)-obese vs. non-obese women. *Med Arch.* 2020;74(4):289-93. doi: [10.5455/medarh.2020.74.289-293](https://doi.org/10.5455/medarh.2020.74.289-293).
7. Bhatnager R, Nanda S, Dang AS. Increased prolidase level and altered hormonal profile in women with polycystic ovarian syndrome. *Int J Biotechnol Biomed Sci.* 2016;2(2):103-6.
8. Hay ED. *Cell Biology of Extracellular Matrix.* Springer Science & Business Media; 1991.
9. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. *J Pathol.* 2003;200(4):423-8. doi: [10.1002/path.1437](https://doi.org/10.1002/path.1437).
10. Lewandowski KC, Komorowski J, O'Callaghan CJ, Tan BK, Chen J, Prelevic GM, et al. Increased circulating levels of matrix metalloproteinase-2 and-9 in women with the polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2006;91(3):1173-7. doi: [10.1210/jc.2005-0648](https://doi.org/10.1210/jc.2005-0648).
11. Syabakhash RA, Alwasiti E, Adnan E. Activity of prolidase enzyme and its association with number of antral follicle count and obesity in polycystic ovary syndrome. *Biochem Cell Arch.* 2020;20(1):1611-7. doi: [10.35124/bca.2020.20.1.1611](https://doi.org/10.35124/bca.2020.20.1.1611).
12. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci.* 2010;123(Pt 24):4195-200. doi: [10.1242/jcs.023820](https://doi.org/10.1242/jcs.023820).
13. Khan SA, Mason RW, Kobayashi H, Yamaguchi S, Tomatsu S. Advances in glycosaminoglycan detection. *Mol Genet Metab.* 2020;130(2):101-9. doi: [10.1016/j.jmgme.2020.03.004](https://doi.org/10.1016/j.jmgme.2020.03.004).
14. Robin J. Methods for measuring hydroxyproline and estimating in vivo rates of collagen synthesis and degradation. In: Varga

- J, Brenner DA, Phan SH, eds. *Fibrosis Research: Methods and Protocols*. Totowa, NJ: Humana Press; 2005. p. 189-207. doi: [10.1385/1-59259-940-0:189](https://doi.org/10.1385/1-59259-940-0:189).
15. Kodama H, Fukuda J, Karube H, Matsui T, Shimizu Y, Tanaka T. High incidence of embryo transfer cancellations in patients with polycystic ovarian syndrome. *Hum Reprod*. 1995;10(8):1962-7. doi: [10.1093/oxfordjournals.humrep.a136217](https://doi.org/10.1093/oxfordjournals.humrep.a136217).
 16. Ludwig M, Finas DF, Al-Hasani S, Diedrich K, Ortman O. Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. *Hum Reprod*. 1999;14(2):354-8. doi: [10.1093/humrep/14.2.354](https://doi.org/10.1093/humrep/14.2.354).
 17. Kazemi A, Hasan Zahraei R, Nasr-Esfahani MH, Ahmadi M, Bashardost N. Comparison of outcome of assisted reproductive methods in patient with polycystic ovaries syndrome and tubal factor. *J Shahrekord Univ Med Sci*. 2006;8(1):1-7. [Persian].
 18. Nasciutti LE, Ferrari R, Berardo PT, Souza ML, Takiya CM, Borojevic R, et al. Distribution of chondroitin sulfate in human endometrium. *Micron*. 2006;37(6):544-50. doi: [10.1016/j.micron.2005.12.005](https://doi.org/10.1016/j.micron.2005.12.005).
 19. Yue B. Biology of the extracellular matrix: an overview. *J Glaucoma*. 2014;23(8 Suppl 1):S20-3. doi: [10.1097/ijg.000000000000108](https://doi.org/10.1097/ijg.000000000000108).
 20. Brassart-Pasco S, Brézillon S, Brassart B, Ramont L, Oudart JB, Monboisse JC. Tumor microenvironment: extracellular matrix alterations influence tumor progression. *Front Oncol*. 2020;10:397. doi: [10.3389/fonc.2020.00397](https://doi.org/10.3389/fonc.2020.00397).
 21. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev*. 2000;21(2):200-14. doi: [10.1210/edrv.21.2.0394](https://doi.org/10.1210/edrv.21.2.0394).
 22. Chou CH, Chen MJ. The effect of steroid hormones on ovarian follicle development. *Vitam Horm*. 2018;107:155-75. doi: [10.1016/bs.vh.2018.01.013](https://doi.org/10.1016/bs.vh.2018.01.013).
 23. Curry TE Jr, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev*. 2003;24(4):428-65. doi: [10.1210/er.2002-0005](https://doi.org/10.1210/er.2002-0005).
 24. Han S, Wang S, Fan X, Chen M, Wang X, Huang Y, et al. Abnormal expression of prolyl oligopeptidase (POP) and its catalytic products Ac-SDKP contributes to the ovarian fibrosis change in polycystic ovary syndrome (PCOS) Mice. *Biomedicines*. 2023;11(7):1927. doi: [10.3390/biomedicines11071927](https://doi.org/10.3390/biomedicines11071927).
 25. Curry TE Jr, Smith MF. Impact of extracellular matrix remodeling on ovulation and the folliculo-luteal transition. *Semin Reprod Med*. 2006;24(4):228-41. doi: [10.1055/s-2006-948552](https://doi.org/10.1055/s-2006-948552).
 26. Engel J, Chiquet M. An overview of extracellular matrix structure and function. In: Mecham RP, ed. *The Extracellular Matrix: an Overview*. Berlin: Springer; 2011. p. 1-39. doi: [10.1007/978-3-642-16555-9_1](https://doi.org/10.1007/978-3-642-16555-9_1).
 27. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater*. 2009;5(1):1-13. doi: [10.1016/j.actbio.2008.09.013](https://doi.org/10.1016/j.actbio.2008.09.013).
 28. Chen ZJ, Cui L. Implications of polycystic ovary syndrome on oocyte quality. In: Kovacs G, Rutherford A, Gardner DK, eds. *How to Prepare the Egg and Embryo to Maximize IVF Success*. Cambridge University Press; 2019. p. 150.
 29. O'Connor BB, Pope BD, Peters MM, Ris-Stalpers C, Parker KK. The role of extracellular matrix in normal and pathological pregnancy: future applications of microphysiological systems in reproductive medicine. *Exp Biol Med (Maywood)*. 2020;245(13):1163-74. doi: [10.1177/1535370220938741](https://doi.org/10.1177/1535370220938741).
 30. Braundmeier AG, Fazleabas AT, Lessey BA, Guo H, Toole BP, Nowak RA. Extracellular matrix metalloproteinase inducer regulates metalloproteinases in human uterine endometrium. *J Clin Endocrinol Metab*. 2006;91(6):2358-65. doi: [10.1210/jc.2005-0601](https://doi.org/10.1210/jc.2005-0601).
 31. Santos Simões R, Carbonel AAF, Borges FT, Baracat MCP, da Silva Sasso GR, Simões MJ, et al. Analysis of hyaluronic acid in the endometrium of women with polycystic ovary syndrome. *Gynecol Endocrinol*. 2019;35(2):133-7. doi: [10.1080/09513590.2018.1505844](https://doi.org/10.1080/09513590.2018.1505844).
 32. Bhatnager R, Nanda S, Dang AS. Plasma prolidase levels as a biomarker for polycystic ovary syndrome. *Biomark Med*. 2018;12(6):597-606. doi: [10.2217/bmm-2017-0306](https://doi.org/10.2217/bmm-2017-0306).
 33. Goldman S, Shalev E. MMPs and TIMPs in ovarian physiology and pathophysiology. *Front Biosci*. 2004;9:2474-83. doi: [10.2741/1409](https://doi.org/10.2741/1409).
 34. Srivastava AK, Khare P, Nagar HK, Raghuwanshi N, Srivastava R. Hydroxyproline: a potential biochemical marker and its role in the pathogenesis of different diseases. *Curr Protein Pept Sci*. 2016;17(6):596-602. doi: [10.2174/1389203717666151201192247](https://doi.org/10.2174/1389203717666151201192247).