العـــدد 17S حزيران 2025
المجلة العراقية للبحوث الإنسانية والإجتماعية والعلمية
No.17S JUNE 2025 Iraqi Journal of Humanitarian, Social and Scientific Research
Print ISSN 2710-0952 Electronic ISSN 2790-1254

Quantitative Analysis of FSHR and LHR mRNA Expression in uterine fluids of Women with Functional Ovarian Cysts Using Real-Time PCR

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Abstract

Background: Functional ovarian cysts (FOC) are one of the most common benign gynecological disorders among women of reproductive age. Despite their prevalence, the molecular mechanisms underlying their development, particularly the dynamics related to gene expression within uterine fluid, remain poorly understood. **Objectives:** This study aimed to analyze the mRNA expression levels of FSHR and LHR receptors in the uterine fluid of women with FOC and compare them with those of healthy women using RT-qPCR.**Methodology:** A case-control study was conducted, including 22 women clinically diagnosed with FOC and 30 healthy women as control. Uterine fluids were collected during the follicular phase of the menstrual cycle using a sterile catheter. RNA was extracted and converted to complementary DNA (cDNA), and the expression of target genes was measured using RT-qPCR, using the GAPDH gene as a reference gene. The difference was considered statistically significant at $p < 0.05$. **Results:** The results showed a significant decrease in FSHR receptor gene expression in the FOC group (0.179 ± 0.4939) compared to the control group (1.774 ± 0.4008), $p < 0.05$. A similar decrease in LHR receptor expression (0.1986 ± 0.4476) was also observed compared to healthy women (3.828 ± 0.3704), $p < 0.05$. **Conclusion:** Reduced gene expression of FSHR and LHR receptors in uterine fluids from women with FOC suggests a possible defect in local hormonal signaling, which may contribute to the formation of these cysts.

Keywords: Functional ovarian cysts, FSHR and LHR receptors, gene expression, RT-qPCR, uterine fluids.

التحليل الكمي لتعبير mRNA عن مستقبلات FSHR و LHR في سوائل الرحم لدى النساء المصابات بأكياس المبيض الوظيفية باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي

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الخلاصة

الخلفية: تعد الأكياس الوظيفية في المبيض (Functional Ovarian Cysts, FOC)من أكثر الاضطرابات النسائية الحميدة شيوعًا لدى النساء في سن الإنجاب. وعلى الرغم من شيوعها، لا تزال الآليات الجزيئية المسؤولة عن تكوّنها، ولا سيما التغيرات في التعبير الجيني ضمن سوائل الرحم، غير

مفهومة بشكل كافٍ.الأهداف: هدفت هذه الدراسة إلى تحليل مستويات التعبير الجيني لكل من مستقبلات FSHRو LHR في سوائل الرحم لدى النساء المصابات بالأكياس الوظيفية، ومقارنتها مع النساء السليمات باستخدام تقنية RT-qPCR . المنهجية: أُجريت دراسة حالة-شاهد شملت 22 امرأة تم تشخيصهن سريريًا بوجود أكياس مبيضية وظيفية، و30 امرأة سليمة كمجموعة ضابطة. تم جمع سوائل الرحم خلال الطور الجريبي من الدورة الشهرية باستخدام قسطرة معقمة. بعد استخلاص الحمض النووي الريبي(RNA) ، تم تحويله إلى DNA متمم (cDNA) ، ثم تم قياس التعبير الجيني للأهداف المدروسة باستخدام تقنيةRT-qPCR ، مع استخدام جين GAPDH كجين مرجعي. واعتُبر الفرق ذا دلالة إحصائية عند مستوى$P < 0.05$. النتائج: أظهرت النتائج انخفاضاً معنويًا في تعبير جين مستقبل FSHRلدى مجموعة المصابات بـ FOC (0.179 ± 0.4939) مقارنةً بالمجموعة الضابطة (1.774 ± 0.4008)، مع قيمة $P < 0.05$. كما تم تسجيل انخفاض مماثل في تعبير مستقبل LHR (0.1986 ± 0.4476)مقارنةً بالنساء السليمات (3.828 ± 0.3704)، مع قيمة$P < 0.05$. الاستنتاج: يشير الانخفاض في التعبير الجيني لمستقبلات FSHR و LHRفي سوائل الرحم لدى النساء المصابات بالأكياس الوظيفية إلى احتمال وجود خلل في الإشارات الهرمونية الموضعية، مما قد يسهم في تكون هذه الأكياس.

الكلمات المفتاحية: الأكياس الوظيفية في المبيض، مستقبلات FSHR و LHRالتعبير الجيني، RT-qPCR، سوائل الرحم.

Introduction

Maintaining women's reproductive health depends greatly on hormones produced by the ovaries. The hypothalamus and pituitary gland release FSH and LH, which help ovulation by binding to receptors on the follicular and luteinizing cells in the ovary (Richards & Pangas, 2010; Barbieri, 2014; Murray & Orr, 2020). It helps develop follicles, triggers ovulation, creates the corpus luteum and releases estrogen and progesterone which are important for fertility and the menstrual cycle.

When the released of ovarian reproductive hormones were in completed or incorrect, it leads to functional ovarian cysts (FOC), although not always harmful, these follicles can remain or return formation which may lead to menstruation disruption or temporary infertility (H Ortega et al., 2016; Balen et al., 2024). Since these conditions are related to hormones, scientists have examined them on the molecular level.

Development of the ovarian follicles and corpus luteum depending on FSH and LH receptors in reproductive tissue (Kishi et al., 2018). Yet, any changes in these gene expression pathway might result ovarian hormone abnormalities that could delayed of the egg growth and release (Vannuccini et al., 2016).

Brinca et al. (2022) and Freitas et al. (2017) demonstrated that both ovarian follicular and uterine fluids contain a lot of RNA, DNA and proteins which accurately reflect the state of the ovaries, therefore the analysis of the these responsible genes in these fluids, important details about fertility and diseases connected to it can be found (Pan, Pan, & Zhang, 2024).

The uterine fluid usually reflects the hormones and immune system in the uterus, mainly when it is collected during a specific phase of the cycle. RT-qPCR is the most reliable method for studying this fluid since it offers high accuracy and sensitivity when monitoring mRNA (Kubista et al., 2006). To make sure the genes are expressed correctly, this technique checks the expression against the genes in the body at all times (Murphy & Busten, 2009).

Even using the newest tools, scientists do not know enough about the chemical changes in uterine fluid linked to FOC. This area of research is still open, as there is not much known about how the genes in this fluid contribute to the formation of healthy cysts. Therefore, it is suggested that FSHR and LHR receptor levels in the uterine fluid differ significantly between women with FOC and healthy. RT-qPCR can be used to identify these changes.

The objective of this study was to collect uterine fluid from both groups, isolate RNA, produce cDNA, measure the levels of the two target genes, and compare the findings statistically. Consequently, this study sought to determine whether FSHR and LHR can serve as simple methods for early disease detection or for selecting the best treatment for FOC patients.

Material and method

This study was conducted using a case-control study design, including a group of women with FOC (cases) and a group of healthy women (control) to compared the gene expression levels of FSHR and LHR receptors genes in uterine fluids.

Consent from participants and ethical approval.

Approval was obtained from the Innovation Committee of the College of Biotechnology, Al-Qadisiyah, Al-Qadisiyah, Iraq. Each participant received a detailed computer explanation and signed a consent form. All data were kept strictly confidential, and only names were transcribed. The following table (1) summarizes the general characterization (age, BMI, menstrual history) of the study (healthy and FOC women. This study was conducted at the Obstetrics and Gynecology Hospital in Al-Diwaniyah Governorate, in collaboration with the laboratories of the College of Biotechnology in Al-Qadisiyah, from October 2024 to February 2025.

Control group (n=30)

According to the specified inclusion and exclusion criteria, thirty samples of endometrial fluid (5 ml) were collected from healthy women with no symptoms or diagnosed with ovarian diseases.

Experiment group (n=22)

Twenty-two Endometrial fluid samples were collected from women with FOC according to case history and rottenly medical diagnosis.

Endometrial Fluid Collection

The endometrial fluid was collected using a sterile endometrial catheter during the follicular phase of the menstrual cycle (days 7–10) to ensure hormonal consistency among participants. After collection, the fluid was stored directly in RNase-free tubes and frozen at –80°C until extraction.

RNA Extraction

Total RNA was extracted from the uterine fluid using a certified commercial extraction kit (TRIzol or Qiagen kit), according to the manufacturer's protocol. Analytical reagent was added to the liquid, and a centrifuge was used to separate the phases. RNA was precipitated with ethanol and then resuspended in RNase-free water. RNA quantity and purity were determined using a NanoDrop device (NanoDrop OPTIMA®, JAPAN), ensuring an absorbance ratio of 260/280 between 1.8 and 2.0. One microgram of RNA was used to reduce it to complementary DNA (cDNA) using reverse transcriptase, according to the universal RT-PCR Kit (M-MLV, free Taq polymerase, Solarbio®, China). Oligo(dT) primers and random hexamers were supplied by Macrogen company (Macrogen®, Inc., South Korean) that used to ensure complete transcription.

Primer design

The primer sequences designed by GenBank database using the National Center for Biotechnology Information (NCBI). The http:// www. Bioinformatics. org/ sms2/ pcr_primer_stats. html) were used to check the primer pair (Table 1).

Real-Time Polymerase Chain Reaction

An real time PCR technique used to quantitatively detect the expression of FSHR and LHR genes. A reference gene, such as GAPDH, was used as a standard for comparison. The reaction mixture was added 10 µL SYBR Green Master Mix, 1 µL of each primer (forward and reverse), 2 µL of cDNA and 6 µL sterile water. The reaction program was: initial activation: 95°C for 10 min, denaturation: 95°C for 15 s, annealing: 60°C for 30 s, extension: 72°C for 30 s, run performed with 40 cycles. Used the ΔC_T method using a reference gene to determine the FSHR and LHR mRNA relative (Lorenz, 2012) by the formula:

(Ratio (reference/target) = $2^{CT(reference) - CT(target)}$).

Table 1: Primers design used for qRT-PCR.

Primer	Sequence(5' – 3')	NCBI Gene ID	Amplicon Size (pb)	Ta°C
FSHR	F: TCTGTCACTGCTCTAACAGGG R:	2492	131	55.2

	TGCACCTTTTTGGATGACTCG			
LHR	F: ACTGGGACACTGAGAAGGG R: GGAAATGAGCATGACCTTTGGTG	26012	80	54
GAPDH	F: ACAACTTTGGTATCGTGGAAGG R: GCCATCACGCCACAGTTTC	26330	116	54.5

FSHR; Follicular stimulating hormone receptor gene, LHR; Luteinizing hormone receptor gene, GAPDH; Glyceraldehyde-3-phosphate dehydrogenase (Reference gene), pb; base pairs, Ta; Annealing tem.

Statistical Analysis

The IBM SPSS Statistics 28 software was used for the analysis. The gene expression levels between experiment groups were compared using the t-test, according to distribution. Differences were considered statistically significant at $p < 0.05$.

Results

The general characteristics (Age, body mass index, and menstrual history of healthy and FOC women, we observed no significant difference in age between these groups. Furthermore, there were statistically significant differences between control and FOC groups in body mass index and cycle length (Table 2).

Table 2: Characteristics of healthy control (n=30) and FOC woman's (n=22).

Variable	Control Group (mean± SE)	FOC Group (mean± SE)	P-value
Age	26.8 ± 3.1	27.4 ± 3.6	0.45
BMI	22.5 ± 2.4	28.7 ± 3.9	0.0001
CL	28.5 ± 2.1	38.2 ± 6.5	0.00001

* Age in years, BMI; Body Mass Index, CL; Cycle Length in days, SE; Stander error.

Gene expression

The gene expression levels of both FSH-R and LH-R receptors genes were analyzed in uterine fluid from both the control and FOC group, these results showed a significant decrease in the gene expression levels of these two genes in the affected women compared to the control group. Display gene expression levels for the FSH-R receptor, the mean relative expression level in the control

group was 1.7744 ± 0.4008, while in the FOC group it was 0.1795 ± 0.4939, with a statistically significant difference ($p < 0.05$), this suggests a possible association between decreased FSH-R receptor expression and the presence of FOC (Table 3 and Figure 1) . In addition, we observed the displayed that were the gene expression regarding the LH-R receptor in the healthy group of women showed an average expression level of 3.8283 ± 0.3704, while the FOC group showed a significant decrease in expression level of 0.1986 ± 0.4476. This difference was also statistically significant ($p < 0.05$) (Table 3 and Figure 2).

Table 3: Relative FSHR and LHR expression in control and FOC samples.

Groups	Control (n=30)	FOC (n=22)	P-value
FSHR	**1.774 ± 0.4008**	**0.179 ± 0.4939**	($p < 0.05$)
LHR	**3.8283 ± 0.3704**	**0.1986 ± 0.4476**	($p < 0.05$)

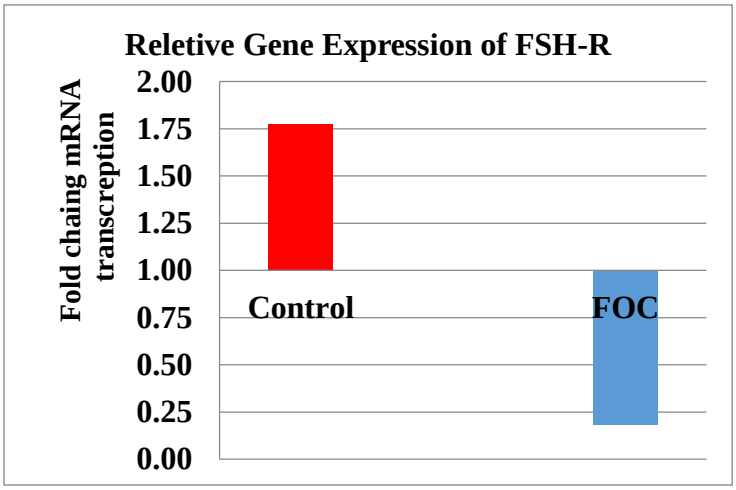

Figure (1) Relative FSHR expression in control and FOC samples.

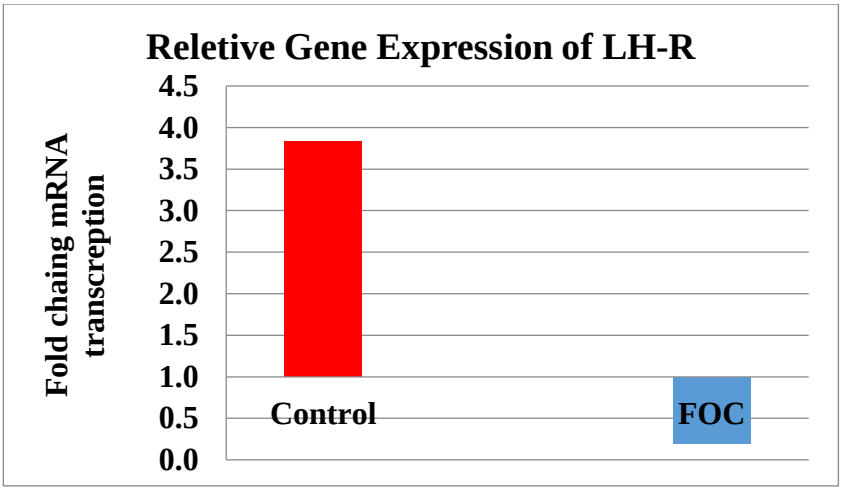

Figure 2: Relative LHR expression in control and FOC samples.

Discussion

FOC are a common condition among women of reproductive age. As shown in the table 2, the age group of adult women suffering from FOC (over 45 years) constitutes the highest percentage, based on the results of our study regarding the demographic characteristics shown in Table 1. This is consistent with the results of the survey by Henes et al. (2018), which showed that FOC is common among women in this age group. Our study results in terms of body mass index are also consistent with the study of (Holt et al., 2005). The average menstrual cycle length in our study was 30.2 ± 2.1 days, which is considered high for menstrual cycles and indicates ovulatory dysfunction. This is consistent with a study by Parazzini et al. (1996), which showed that women in the study had menstrual cycles longer than 26 days and were more likely to develop FOC. The expression of FSHR and LHR genes was significantly lower in the uterine fluid of women with FOC compared to the control group according to Table 3. When insulin levels decrease, it may indicate that hormonal activity is disturbed at the target tissue, which may contribute to the development of cysts.

FSHR and LHR are important for managing ovarian function and follicle development (Kishi et al., 2018a). FSHR promotes the growth of primary follicles and results in the production of estrogen (Achrekar et al., 2010). At the same time, LHR plays a role in ovulation and corpus luteum growth (Esteves & Alviggi, 2015). So, when these receptor genes are not working properly, it could result in no ovulation and the growth of a cyst, as the follicle does not burst (HARINI, 2019).

We found that without FSHR in granulosa cells, the growth and maturation of follicles are disturbed, which might be the reason for the occurrence of FOC. The granulosa cells can respond to FSH because they have FSHR, and this process is involved in creating follicles and producing estrogen (Desai et al., 2013). If the FSHR is present in low amounts, the process of full growth and ovulation of follicles in FOCs is unlikely to happen. Changes in the

environment and regular ovarian problems might decrease the expression of the FSHR gene. The study indicates that phthalates and bisphenol A may interfere with the function of FSHR in several ways. Some of these processes happen directly, and some happen indirectly. Such molecules may interact with the receptor at a different point from where the hormone attaches, which changes the receptor's function. In addition, these chemicals can lower receptor function and disturb vital processes of granulosa cells (Roy et al., 2021). Besides the environment, recent studies suggest that too many free fatty acids (FFAs) in the follicular fluid may decrease the activity of the FSHR gene. This mechanism gives extra details about the factors that regulate FSHR, and these details are in line with the results of our study. Palmitic acid, which is present in excess FFAs, can trigger ER stress in granulosa cells and interfere with their signaling. It is important to realize that stress on the muscle causes TRIB3 protein to rise, which shuts down the Akt signaling pathway. Therefore, Akt is less active, which allows GSK3β to become more active, and this results in a drop in the expression of the FSHR gene. As a result of this cascade, granulosa cells work less and produce less estrogen which might lead to weak follicle growth and the formation of cysts (Wang et al., 2021). This agrees with previous reports that problems with FSHR signals can result in unruptured follicles and lead to the formation of follicular or luteal cysts (Kishi et al., 2018b). Chen et al. discovered that when LHR is low, there is a higher possibility of ovarian cysts reoccurring in both animals and humans. (Papamentzelopoulou et al., 2012) also revealed that infertile women with lower LHR gene expression experienced hormonal imbalance during the follicular phase, which is in line with our hypothesis. Based on the results of our study, it seems that when LHR is lacking, the follicle responds poorly to LH, which results in issues with ovulation and continued growth of the follicle. In addition, Menon and Menon (2014) explained a different way to understand how LHR is regulated following its gene transcription. They observed that ERK1/2 released by the LH surge led LRBP to bind and get rid of LHR mRNA in normal situations. Even though the regulatory process helps control stimulation after ovulation, the sustained low level of LHR could mean the system has become too active or not working properly (Menon & Menon, 2014). If this occurs in the context of ovarian cysts, it could lead to less LH responsiveness, continued growth of follicles, and poor maturation of follicles, which is consistent with the findings of Chen et al. and Papamentzelopoulou et al., who linked low LHR to ovulation problems and cyst formation. The results of the current study indicated a significant decrease in the levels of luteinizing hormone receptor (LHR) gene expression in FOC samples, which may be attributed to multiple regulatory mechanisms, including epigenetic effects of miRNAs. A study by Liao et al. (2008) demonstrated that the LHR gene is selectively repressed by the binding of a repressor complex consisting of HDAC1 and Sin3A to its promoter region, preventing the binding of the transcription factor Sp1, which is necessary for transcriptional activation.

The study also demonstrated that activation of the PKCα/ERK signaling pathway leads to the phosphorylation of Sp1 and, consequently, the removal of the repressor complex, allowing for transcriptional reactivation. Therefore, the absence or impaired activation of this pathway may explain the observed decrease in LHR gene expression, as observed in cases of hormonal imbalance or structural changes in cellular signaling regulation, such as in some cases of functional ovarian cysts (Liao et al., 2008).

However, some studies, such as Ahn and Jeung (2023), have suggested that changes in the gene expression of these receptors may be a secondary consequence of cyst development rather than a direct cause, calling for future longitudinal studies to precisely determine the direction of the causal relationship. Based on these findings, the use of gene expression of FSHR and LHR receptors in uterine fluids could be proposed as a non-invasive biomarker for the early diagnosis or monitoring of the development of FOC. It also opens the door to studying the potential for targeted therapeutic interventions to regulate this expression through drug or gene therapies.

Conclusion

The decreased gene expression of FSHR and LHR receptors in the uterine fluids of women with FOC suggests a potential disruption of local hormonal signaling, which may contribute to the development of these cysts. These molecular changes could represent promising non-invasive biomarkers for early diagnosis and personalized treatment strategies. Future studies with larger samples and functional validation of the results are recommended.

References

Achrekar, S. K., Modi, D. N., Meherji, P. K., Patel, Z. M., & Mahale, S. D. (2010). Follicle stimulating hormone receptor gene variants in women with primary and secondary amenorrhea. *Journal of Assisted Reproduction and Genetics*, *27*(6), 317–326. https://doi.org/10.1007/s10815-010-9404-9

Ahn, C., & Jeung, E.-B. (2023). Endocrine-disrupting chemicals and disease endpoints. *International Journal of Molecular Sciences*, *24*(6), 5342.

Desai, S. S., Roy, B. S., & Mahale, S. D. (2013). Mutations and polymorphisms in FSH receptor: Functional implications in human reproduction. *Reproduction*, *146*(6), R235–R248.

Esteves, S. C., & Alviggi, C. (2015). The Role of LH in Controlled Ovarian Stimulation. In S. Ghumman (Ed.), *Principles and Practice of Controlled Ovarian Stimulation in ART* (pp. 171–196). Springer India. https://doi.org/10.1007/978-81-322-1686-5_16

H Ortega, H., U Díaz, P., R Salvetti, N., J Hein, G., E Marelli, B., M Rodríguez, F., F Stassi, A., & Rey, F. (2016). Follicular cysts: A single sign and

different diseases. A view from comparative medicine. *Current Pharmaceutical Design*, *22*(36), 5634–5645.

HARINI, H. (2019). *STUDY ON GENETIC POLYMORPHISM OF INHA, LHR, CYP19, FSHR AND ER1 GENES AND THEIR RELATIONSHIP WITH CYSTIC OVARIAN DISEASE IN HF CROSSBRED CATTLE* [PhD Thesis, KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR]. https://krishikosh.egranth.ac.in/server/api/core/bitstreams/62fcd4df-9d49-47ab-b989-20180898506a/content

Holt, V. L., Cushing-Haugen, K. L., & Daling, J. R. (2005). Risk of functional ovarian cyst: Effects of smoking and marijuana use according to body mass index. *American Journal of Epidemiology*, *161*(6), 520–525.

Kishi, H., Kitahara, Y., Imai, F., Nakao, K., & Suwa, H. (2018). Expression of the gonadotropin receptors during follicular development. *Reproductive Medicine and Biology*, *17*(1), 11–19. https://doi.org/10.1002/rmb2.12075

Liao, M., Zhang, Y., & Dufau, M. L. (2008). Protein kinase Cα-induced derepression of the human luteinizing hormone receptor gene transcription through ERK-mediated release of HDAC1/Sin3A repressor complex from Sp1 sites. *Molecular Endocrinology*, *22*(6), 1449–1463.

Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments: JoVE*, *63*, 3998.

Menon, K. M. J., & Menon, B. (2014). Regulation of luteinizing hormone receptor expression by an RNA binding protein: Role of ERK signaling. *Indian Journal of Medical Research*, *140*(Suppl 1), S112–S119.

Murray, C. M., & Orr, C. J. (2020). Hormonal regulation of the Menstrual Cycle and Ovulation. In *Maternal-Fetal and Neonatal Endocrinology* (pp. 159–167). Elsevier. https://www.sciencedirect.com/science/article/pii/B978012814823500012X

Papamentzelopoulou, M., Mavrogianni, D., Partsinevelos, G. A., Marinopoulos, S., Dinopoulou, V., Theofanakis, C., Anagnostou, E., & Loutradis, D. (2012). LH receptor gene expression in cumulus cells in women entering an ART program. *Journal of Assisted Reproduction and Genetics*, *29*(5), 409–416. https://doi.org/10.1007/s10815-012-9729-7

Parazzini, F., Moroni, S., Negri, E., La Vecchia, C., Pino, D. D., & Ricci, E. (1996). Risk factors for functional ovarian cysts. *Epidemiology*, 547–549.

Richards, J. S., & Pangas, S. A. (2010). The ovary: Basic biology and clinical implications. *The Journal of Clinical Investigation*, *120*(4), 963–972.

Roy, N., Mascolo, E., Lazzaretti, C., Paradiso, E., D'Alessandro, S., Zaręba, K., Simoni, M., & Casarini, L. (2021). Endocrine disruption of the follicle-stimulating hormone receptor signaling during the human antral follicle growth. *Frontiers in Endocrinology*, *12*, 791763.

Wang, N., Si, C., Xia, L., Wu, X., Zhao, S., Xu, H., Ding, Z., & Niu, Z. (2021). TRIB3 regulates FSHR expression in human granulosa cells under high levels of free fatty acids. *Reproductive Biology and Endocrinology*, *19*(1), 139. https://doi.org/10.1186/s12958-021-00823-z

Balen, A. H., Tamblyn, J., Skorupskaite, K., & Munro, M. G. (2024). A comprehensive review of the new FIGO classification of ovulatory disorders. *Human Reproduction Update*, *30*(3), 355-382.

Barbieri, R. L. (2014). The endocrinology of the menstrual cycle. *Human fertility: methods and protocols*, 145-169

Brinca, A. T., Ramalhinho, A. C., Sousa, Â., Oliani, A. H., Breitenfeld, L., Passarinha, L. A., & Gallardo, E. (2022). Follicular fluid: a powerful tool for the understanding and diagnosis of polycystic ovary syndrome. Biomedicines, 10(6), 1254.

Freitas, C., Neto, A. C., Matos, L., Silva, E., Ribeiro, Â., Silva-Carvalho, J. L., & Almeida, H. (2017). Follicular Fluid redox involvement for ovarian follicle growth. Journal of Ovarian Research, 10, 1-10.
H Ortega, H., U Díaz, P., R Salvetti, N., J Hein, G., E Marelli, B., M Rodríguez, F., ... & Rey, F. (2016). Follicular cysts: A single sign and different diseases. A view from comparative medicine. *Current pharmaceutical design*, *22*(36), 5634-5645.

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., ... & Zoric, N. (2006). The real-time polymerase chain reaction. Molecular aspects of medicine, 27(2-3), 95-125.

Murphy, J., & Bustin, S. A. (2009). Reliability of real-time reverse-transcription PCR in clinical diagnostics: gold standard or substandard?. Expert review of molecular diagnostics, 9(2), 187-197.

Pan, Y., Pan, C., & Zhang, C. (2024). Unraveling the complexity of follicular fluid: Insights into its composition, function, and clinical implications. Journal of Ovarian Research, 17(1), 237

Vannuccini, S., Clifton, V. L., Fraser, I. S., Taylor, H. S., Critchley, H., Giudice, L. C., & Petraglia, F. (2016). Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Human reproduction update, 22(1), 104-115

Henes, M., Engler, T., Taran, F. A., Brucker, S., Rall, K., Janz, B., & Lawrenz, B. (2018). Ovarian cyst removal influences ovarian reserve dependent on histology, size and type of operation. Women's Health, 14, 1745506518778992.