Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2023.75336

Turk J Obstet Gynecol 2023;20:53-8

The investigation of cholinergic receptor muscarinic 1 activity in the rat ovary with induced ovarian hyperstimulation

Ovaryan hiperstimülasyonu oluşturulan ratların overlerinde kolinerjik reseptör muskarinik 1 aktivitesinin araştırılması

Cengiz Şanlı[1], Remzi Atılgan[1], Tuncay Kuloğlu[2], Şehmus Pala[1], Nevin İlhan[3]

[1]*Fırat University Faculty of Medicine, Department of Obstetrics and Gynecology, Elazığ, Turkey*

[2]*Fırat University Faculty of Medicine, Department of Histology, Elazığ, Turkey*

[3]*Fırat University Faculty of Medicine, Department of Biochemistry, Elazığ, Turkey*

Abstract

Objective: We look at the immunoreactivity of cholinergic receptor muscarinic 1 (CHRM1) in the ovarian tissues of rats with ovarian hyperstimulation syndrome (OHSS) considering the possibility that the muscarinic activity may contribute to the pathophysiology of OHSS.

Materials and Methods: In this study, 14 immature female Wistar Albino rats were divided into two groups at random. The rats were 22 days old. Rats in the control group (n=7) were 22 days old, while those in the OHSS group (n=7) received 10 IU follicle-stimulating hormone subcutaneously over the course of four days and 30 IU human chorionic gonadotropin (hCG) on the fifth day to induce ovarian hyperstimulation. All the rats were sacrificed after all the groups' ovaries and blood samples were collected at the conclusion of the experiment. The left ovarian tissues were kept in aluminum foil at -80 °C, while the right ovarian tissues were kept in 10% formalin. Tissue vascular endothelial growth factor (VEGF), interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α and malondialdehyde (MDA) levels were measured by The Enzyme Linked Immunosorbent Assay technique in the ovarian tissues. CHRM1 immunoreactivity was scored immunohistochemically.

Results: Ovarian weight, tissue IL-10, TNF-α, VEGF and MDA levels, and CHRM1 immunoreactivity were significantly increased in the OHSS group.

Conclusion: Increased levels of CHRM1 activity may play a role in the pathophysiology of OHSS. With further studies, the effect of luteinizing hormone and hCG on the ovarian and hypothalamic cholinergic system can be further investigated, and useful information can be obtained in determining OHSS prevention strategies.

Keywords: Rat, OHSS, cytokine, CHRM1

Öz

Amaç: Bu çalışmada ovaryan hiperstimülasyon sendromlu (OHSS) ratların ovaryan dokularında kolinerjik reseptör muskarinik 1'in (CHRM1) immünreaktivitesine, muskarinik aktivitenin OHSS patofizyolojisine katkıda bulunma olasılığı ışığında bakmayı amaçlandı.

Gereç ve Yöntemler: Bu çalışmada 14 adet 22 günlük immatür Wistar Albino dişi rat rastgele iki gruba ayrıldı. Grup 1'deki (n=7) ratlar (n=7) 22 günlük iken, Grup 2'deki (n=7) ratlar dört gün boyunca subkütan 10 IU folikül stimülan hormone ve beşinci günde ovaryan hiperstimülasyonu indüklemek için 30 IU insan koryonik gonadotropin (hCG) aldı. Deney bitiminde tüm grupların ovaryumları ve kan örnekleri alındıktan sonra tüm ratlar sakrifiye edildi. Sol over dokuları -80 °C'de alüminyum folyo içinde, sağ over dokuları ise %10'luk formalinde saklandı. Ovaryan dokuda vasküler endotelyal büyüme faktörü (VEGF), interlökin (IL)-1β, IL-6, IL-10, tümör nekroz faktör (TNF)-α ve malondialdehid (MDA) seviyeleri Enzyme Linked Immunosorbent Assay tekniği ile ölçüldü. CHRM1 immünoreaktivitesi, immünohistokimyasal olarak skorlandı.

Bulgular: Kontrol grubu ile karşılaştırıldığında OHSS grubunda over ağırlığı, doku IL-10, TNF-α, VEGF ve MDA seviyeleri ve CHRM1 immünreaktivitesi açısından istatistiksel olarak anlamlı bir artış vardı.

PRECIS: CHRM1 activity may play a role in the pathogenesis of OHSS.

Address for Correspondence/Yazışma Adresi: Remzi Atılgan MD,
Fırat University Faculty of Medicine, Department of Obstetrics and Gynecology, Elazığ, Turkey
Phone: +90 533 629 59 81 **E-mail:** remzi_atilgan@hotmail.com **ORCID ID:** orcid.org/0000-0003-2635-7158

Received/Geliş Tarihi: 31.01.2023 **Accepted/Kabul Tarihi:** 10.02.2023

Sonuç: Artmış CHRM1 aktivitesi OHSS'nin patofizyolojisinde rol oynayabilir. Daha ileri çalışmalar ile lüteinizan hormonu ve hCG'nin over ve hipotalamik kolinerjik sistem üzerindeki etkisi daha fazla araştırılabilir ve OHSS önleme stratejilerinin belirlenmesinde faydalı bilgiler elde edilebilir.

Anahtar Kelimeler: Rat, OHSS, sitokin, CHRM1

Introduction

The ovarian hyperstimulation syndrome (OHSS), a typically fatal and iatrogenic side effect of fertility treatments, is connected to an acute, intense fluid migration from the vascular space to the third space because of elevated capillary permeability. In OHSS, there is edema in the ovarian stroma along with luteal follicle cysts, necrotic foci, and neovascularization. The risk of OHSS was dramatically decreased by employing a gonadotropin releasing hormone (GnRH) antagonist protocol instead of a GnRH agonist technique, inducing ovulation with GnRH agonists, and using vascular endothelial growth factor (VEGF) receptor blockers like cabergoline[1,2]. Even with these protective measures, severe OHSS still occurs, and doctors continue to struggle with determining the best therapeutic strategy[3]. The etiology of OHSS is significantly influenced by the shift in vascular permeability brought on by human chorionic gonadotropin (hCG). In addition to angiotensin II and other cytokines involved in angiogenesis, many vasoactive substances, including angiopoietin fibroblast growth factor, hypoxia-inducible factor, plasminogen activator, platelet-derived growth factor, protein kinase converting growth factor-, VEGF receptor, and urokinase-type plasminogen activator, play a role in the development of OHSS[4,5].

Vascular growth regulators, like VEGF, contribute significantly to inflammation through changes in vascular permeability, control over blood flow, and localized edema. The transfer of circulating immune cells to the ovulatory follicle as a classic inflammatory response is facilitated by changes in angiogenesis and vascular permeability along with increased follicular blood flow. However, it's important to terminate inflammation and vascular alterations as soon as possible, especially in the ovulatory follicle. Rapid angiogenesis and luteinization in the ovulatory follicle are made possible by tightly controlled vascular growth, which also prevents over vascularization of the corpus luteum. This might occur because angiopoietins and other vascular growth regulators have growth-restricting properties[4]. Angiogenin, some interleukins (IL) and tumor necrosis factor (TNF)-α and some cytokines increase capillary permeability and ovarian neovascularization and inflammatory response and trigger inhibition of hepatic albumin production. This results in many of the symptoms of OHSS[6,7].

The ovary maintains a balance between oxidative and anti-oxidative states. Theca cell proliferation is stimulated by mild oxidative stress (OS), but when OS increases, theca cell proliferation is inhibited[8]. Overgrown ovaries are linked to severe OHSS[9]. Pala et al.[10] suggested that hypoxia in the ovaries may increase VEGF levels in OHSS. It has also been suggested that rapid follicle growth despite moderate vascularization of granulosa cells during the ovulation stage create a hypoxic environment in the preovulatory follicle[4]. Therefore, in our study, we also evaluated tissue malondialdehyde (MDA) levels in relation to oxidative stress and some cytokines that may be associated with OHSS.

M1 receptors interact with Gq/11 type G proteins and activate phospholipases and calcium channels. It has been reported that acetylcholine (ACh), produced by granulosa cells (GCs) in human and rat oocytes, acts through muscarine (M)1, M3, and M5 receptors in their GCs[11]. Additionally, GCs form the ovarian cholinergic system by expressing muscarinic receptors for ACh. ACh, which is a part of this cholinergic system, has also been shown to stimulate ovarian growth[12,13].

In this experimental model, we investigated the role of cholinergic receptor muscarinic 1 (CHRM1) activity in the pathophysiology of OHSS.

Materials and Methods

Local Animal Ethics Committee of Fırat University approval was obtained for this experimental study (date: 16.01.2019, session no: 2019/01, decision no: 14). Many 14 immature female Wistar albino rats were obtained from Fırat University Experimental Investigations Center, Elazığ, TUrkey. All procedures performed on animals during the experiment were treated in accordance with the experimental animal care guide (NIH Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Washington, D.C.). Rats were fed ad libitum with standard diet and tap water throughout the experiment in appropriate cages, in the laboratory with a 12 h light cycle (lights from 8 am to 8 pm).

Experimental Protocol

For the OHSS model, immature rats were used as stated in previous studies. The purpose of using immature rats is that in this model the ovarian physiology can be simplified and immature rats are not affected by the corpus luteum produced in the previous cycle[14].

The Control Group (n=7): The rats in the control group were given 0.1 mL of 0.9% saline every day for 5 days (22-26 days) and were decapitated on the 27th day.

OHSS Created Group (n=7): 10 IU of pregnant mare serum gonadotropin was injected subcutaneously for four days and 30 IU of hCG on day five to initiate OHSS. Rats were decapitated on the 27th day.

Rats were monitored daily for body weight, food consumption, behavioral changes, and signs of toxicity.

The rats were anesthetized by intramuscular administration of ketamine 80 mg/kg (Ketalar, Eczacıbaşı, İstanbul, Turkey) and xylazine 10 mg/kg (Rompun Vet, Bayer AB, İstanbul, Turkey).

The rats in both groups were decapitated by the cervical dislocation method at the end of the experimental period (on the 27th day). After anesthesia was provided, the abdomen of the rats was opened and the presence of ascitic fluid in the abdomen was checked. After that, both ovarian tissues were removed, quickly cleaned from the surrounding adipose tissue and dried on filter paper. After drying, the ovaries were weighed on an analytical balance and the organ weight, expressed in mg, was recorded. Left ovarian tissue was wrapped in aluminum foil and stored at -80 °C. The right ovarian tissue was placed in 10% formol. It was then embedded in paraffin blocks.

Biochemical Examination

Biomarkers were determined with the ELISA method on a BioTek EPOCH 2 Instrument using Elisa Kits by SunRed for such factors as tissue IL-1β (SunRed Biotechnology Company Katalog No: 201 11 0120), IL-6 (SunRed Biotechnology Company, Shanghai, China, Katalog No: 201 11 0136), IL-10 (SunRed Biotechnology Company Katalog no: 201 11 0109), TNF-α (SunRed Biotechnology Company, Katolog no: 201 11 0765), MDA (SunRed Biotechnology Company, Katolog no: 201 11 0157), VEGF (VEGFR-2 ELISA kit, SunRed Biotechnology Company, Katolog no: 201 1636). The absorbance was read spectrophotometrically at 450 nm on the ELX800 ELISA reader. Bio-tek ELX50 (BioTek Instruments, USA) was used as an automatic washer for plate washing. The results obtained are shown in pg/mL unit. The dilution factor multiplied by the dilution ratio method was used to calculate the results.

Immunohistochemical Examination

Sections of 4-6 µm thick from paraffin blocks were taken on slides and deparaffinized. Then, the sections passed through the alcohol series were boiled in citrate buffer solution at pH: 6 in a microwave oven (750W) for 12 min. After boiling, the tissues kept at room temperature for cooling were washed with phosphate buffered saline (PBS), and endogenous peroxidase activity was inhibited by applying hydrogen peroxide solution for 6 min. Block solution was applied for 5 min to the tissues washed with PBS for 3x5 minutes. Then, it was incubated with primary antibodies (CHRM1 polyclonal antibody, E-AB-14000, Elabscience, China) diluted at a ratio of 1/200 for 60 min at room temperature in a humid environment. After the primary antibody application, the tissues were washed with PBS for 3x5 minutes and incubated with secondary antibody compatible with the primary antibody for 30 min at room temperature in a humid environment. Tissues were washed with PBS for 3x5 minutes after secondary antibody application, incubated with Streptavidin for 60 min at room temperature and then taken into PBS. 3-amino-9-ethylcarbazole (AEC) Substrate + AEC Chromogen solution was dripped onto the tissues. Then, after the image signal was obtained under the light microscope, all groups were washed with PBS simultaneously. Tissues that were counterstained with Mayer's hematoxylin were passed through PBS and distilled water and closed with a water-based closure solution. Preparations were evaluated and photographed using the Leica DM500 microscope (DFC295; Leica, Wetzlar, Germany). Histoscore was established based on the extent and extent of immunoreactivity in staining[15]. Histoscore = prevalence x severity [The extent of immunoreactivity (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%); the intensity of immunoreactivity (0: no, + 0.5: very little, +1: less, +2: moderate, +3: severe)].

Statistical Analysis

SPSS version 22 program was used for statistical analysis of the data. Rats' body weights in grams (g) and ovary weights in milligrams (mg), tissue cytokines, VEGF and MDA levels were given as pg/mL, and CHRM1 immunoreactivity was given as histoscore. Mean relative ovarian tissue weights were obtained from the ratio of mean ovarian tissue weights to mean body weight of mice in the same group ([Overweight/Whole body weight ($\times 10^{-3}$)]. Quantitative data were expressed as median, minimum and maximum values. Kolmogorov-Smirnov method was used to determine the normal distribution of quantitative data. Mann-Whitney U test was used for comparison between groups in non-normally distributed quantitative data. $p < 0.05$ was considered statistically significant.

Results

Ovarian Weight: The proportion of ovarian weight to whole body weight increased in the OHSS group compared with the control group (p=0.001), (Table 1).

CHRM1 Immunoreactivity: CHRM1 immunoreactivity statistically significantly increased in the OHSS group compared with the control group (p=0.001), (Table 1, Figure 1).

Biochemical Findings: According to the results of the ELISA study performed on ovarian tissues; IL-10, TNF-α, VEGF and MDA levels were significantly increased in the OHSS group compared to the control group. IL-1β and IL-6 levels were similar in both groups (Table 2).

Discussion

In this experimental study, we showed that CHRM1 immunoreactivity was significantly increased in OHSS-induced

Table 1. The ratio of ovarian tissue to body weight and CHRM1 immunoreactivity histoscore. Values are shown as median (minimum-maximum)

Groups	Ovarian weight/total body weight ($x10^{-3}$) Median (minimum- maximum)	CHRM1
Control group	0.45 (0.28-0.59)	0.20 (0.10-0.45)
OHSS group	1.15 (0.89-1.66)*	0.90 (0.60-1.20)*
P-value	0.001	0.001

*: Compared to control group (p<0.05), CHRM1: Cholinergic receptor muscarinic 1, OHSS: Ovarian hyperstimulation syndrome

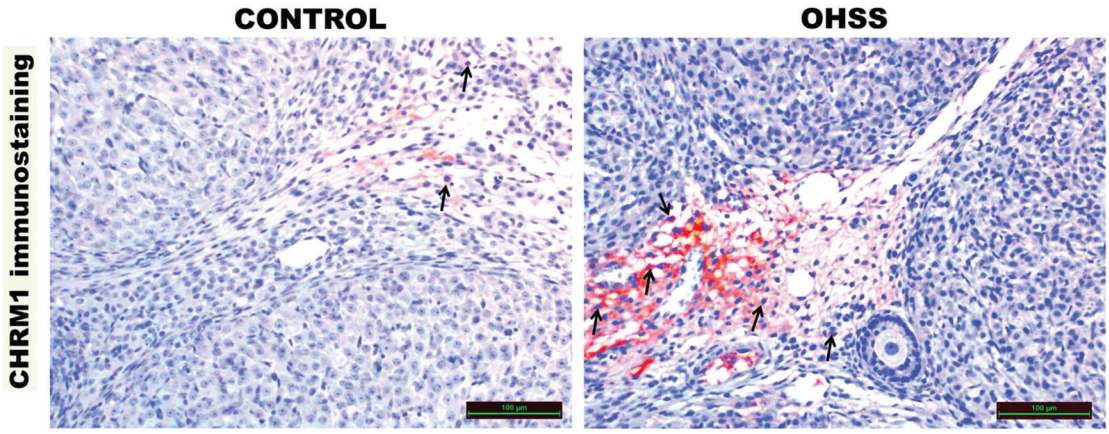

Figure 1. CHRM1 immune positive cells (black arrow) is observed

CHRM1: Cholinergic receptor muscarinic 1, OHSS: Ovarian hyperstimulation syndrome

Table 2. The tissue IL-1β, IL-6, IL-10, TNF-α, VEGF and MDA levels of all groups (pg/mL), values are shown as median (minimum-maximum)

Parameters	Control group	OHSS group	p-value
IL-1β	57.48 (43.43-140)	82.02 (56.27-351)	0.259
IL-6	22.42 (9.92-27.29)	28.88 (16.75-48.89)	0.165
IL-10	16.81 (11.29-27.47)	23.89 (19.03-41.14)	0.038*
TNF-α	39.50 (23.37-56.26)	61.84 (41.24-79.41)	0.007*
VEGF	1.12 (0.85-1.42)	1.75 (1.11-4.06)	0.017*
MDA	2.59 (1.73-4.35)	4.67 (3.42-6.85)	0.004*

*: Compared to control group, ($p<0.05$), OHSS: Ovarian hyperstimulation syndrome, IL: Interleukin, TNF: Tumor necrosis factor, MDA: Malondialdehyde, VEGF: Vascular endothelial growth factor

rats. This increase of CHRM1 immunoreactivity may indicate that CHRM1 may be a component of the mechanism that leads to excessive ovarian response in the clinical course of OHSS. Our study is the first to investigate CHRM1 immunoreactivity in the pathophysiology of OHSS.

Generally, in OHSS, the ovaries are highly enlarged and have a multi-cystic appearance[9]. We also observed significant growth and weight gain in the ovaries in our OHSS group. There is a highly active process of angiogenesis in the development of the corpus luteum. This angiogenesis process is controlled by autocrine, endocrine and paracrine factors. More than half of the total cell number of the corpus luteum is composed of endothelial cells. This makes the corpus luteum a tissue in the body where angiogenesis is most intense[16]. The high VEGF values in our study support the presence of increased angiogenesis in the OHSS group. Interestingly, in our study, we found an increase in MDA levels with the increase in VEGF levels. Based on this result, we thought that supraphysiological steroid hormone levels have harmful effects on ovarian tissue as MDA levels increase. It is unclear whether this is a consequence or a cause. TNF-α is cytotoxic for endothelial cells of the corpus luteum[17]. Therefore, we thought that high TNF-α levels in our OHSS group might cause endothelial damage in ovarian follicles. IL-10 is an anti-inflammatory cytokine. Therefore, it has been shown that IL-10 inhibits the production of many inflammatory cytokines such as IL-1, IL-2 and IL-6 by inhibiting TNF-α in monocytes[18,19]. Tissue IL-1β and IL-6 levels were similar in both groups in our study. However, we showed that IL-10 levels were increased in our OHSS group. This finding may suggest that a compensatory mechanism in ovarian tissue tries suppressing inflammation.

Endogenous acetylcholine can induce angiogenesis by acting on nicotinic and muscarinic receptors. M1 and M3-mAChR, which are found in most of the vessels, stimulate nitric oxide release because of muscarinic stimulation of the endothelium. However, overstimulation or disruption of the non-neuronal cholinergic system decreases endothelial barrier function. This reduction in the endothelial barrier creates a hyperpermeable environment for signaling molecules from the endothelium and for migrating immune system cells. This results in inflammation and an imbalance between proliferation and cell death may occur[20]. In our study, we found a significant increase in TNF-α levels, which is an inflammation marker, and MDA levels, which is an indicator of oxidative damage, with increased CHRM1 activity in our OHSS group. Additionally, we found significantly higher VEGF levels in relation to vascular permeability and neovascularization. These findings may suggest that OHSS has an inflammatory process in the ovarian tissue and that the increase in muscarinic stimulation may also contribute to this process.

Acetylcholine (ACh), produced by granulosa cells (GC) in rats and humans, has important functions in regulating ovarian

functions as part of an intraovarian system. ACh acts via M1, M3 and M5 muscarinic receptors in the GC[11]. Urra et al.[21] showed in their study that inhibition of acetylcholinesterase (AChE) with a specific AChE inhibitor, Huperzine A (Hup A), for 4 weeks, strongly changed follicular growth and reduced ovarian cyst formation due to intraovarian ACh increase. In our study, increased CHRM1 activity in the OHSS group may be associated with multifollicular development.

It has been shown that mammalian CHRMs modulate adenylate cyclase activity[22] and cAMP plays a role in the differentiation of GCs[23]. It has been shown that cAMP is central to the response to FSH by binding to the FSH receptors of newly formed primary follicles. Follicles that start to grow with neurotransmitter activity in more intensely innervated ovarian regions can enter gonadotropin control more rapidly by showing selective superiority compared to those not exposed to the effect of cAMP[24]. The stimulation we induced with FSH in our study may increase CHRM1 activity by some mechanisms.

On the day of proestrus, a signal produced by M1R-dependent acetylcholine in the follicular cells of the left ovary regulates GnRH secretion stimulates LH secretion and triggers ovulation[25]. Ovulation is a physiological process defined by the LH surge, rupture of the dominant follicle and release of the oocyte into the fallopian tube[4]. Some neurotransmission systems, similar to the cholinergic system, are involved in the increase in estradiol concentration and the regulation of the preovulation LH peak[25-28]. In an experimental study, it was shown that ovulation could be induced by injecting LHRH at 14.00 in rats treated with atropine during proestrus, while ovulation could not occur in rats with atropine implants[28]. Cruz et al.[29] showed that animals treated with atropine sulfate had a 24-hour delay in the preovulatory LH surge. In an experimental PCOS model, however, it was shown that the cholinergic system regulates steroid hormone secretion and the occurrence of ovulation depends on the presence of muscarinic receptors[30].

Our current study was not designed to describe in all detail the possible mechanisms between OHSS and cholinergic activity. In our study, we wanted to emphasize that the cholinergic system can be considered in the physiopathology of OHSS. Based on the above information, the cholinergic system has important roles in follicular growth, ovulation and ovarian steroid hormone regulation. For this reason, an antagonistic effect such as the relationship of the cholinergic system with agonist and antagonist drugs, reduction of estradiol production, suppression of ovulation can be obtained, especially in protocols in which controlled ovarian hyperstimulation is applied, and the OHSS formation can be reduced. However, this theory needs to be investigated with advanced and multidisciplinary experimental studies.

Study Limitations

The weaknesses of our study are that the population is small due to the limitations in the number of animals to prevent animal rights violations. Another weakness is that it is impossible to prove the relationship between the cholinergic system and OHSS only with our current parameters. It should not be overlooked that the results obtained from experimental studies may not match exactly with human results.

The strength of our study is that it is the first study to investigate CHRM1 activity in OHSS and the pathophysiology of OHSS with a different approach, as an example for future studies that will fill this gap in the literature. Since it is very difficult ethically to conduct studies on the ovary in humans, animal experiments are very valuable for such studies and form the basis for future clinical studies.

Conclusion

We showed that ovarian CHRM1 activity is also increased in OHSS. The cholinergic system may play a role at every stage from multifollicular development, increased angiogenesis, inflammation and vascular permeability to ovulation, which is responsible for developing OHSS. In this context, our study can investigate the use of anticholinesterase and muscarinic receptor blockers as an antagonist agent in reducing the formation of OHSS or in controlled ovarian stimulation.

*This experimental study is derived from Dr. Cengiz Şanlı's specialty thesis in medicine.

Ethics

Ethics Committee Approval: Local Animal Ethics Committee of Fırat University approval was obtained for this experimental study (date: 16.01.2019, session no: 2019/01, decision no: 14).

Informed Consent: All participants gave their informed consent before the study was conducted.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.**Ş**., R.A., N.İ., Concept: R.A., Design: R.A., T.K., N.İ., Data Collection or Processing: C.**Ş**., T.K., Ş.P., Analysis or Interpretation: R.A., T.K., Ş.P., N.İ., Literature Search: C.**Ş**., Ş.P., Writing: R.A., N.İ.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This experimental study was sponsored by Fırat University, Scientific Research Grant (Project number: 15.02.2019-TF.19.16).

References

1. Practice Committee of the American Society for Reproductive Medicine. Electronic address: ASRM@asrm.org; Practice Committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome: a guideline. Fertil Steril 2016;106:1634-47.
2. Mourad S, Brown J, Farquhar C. Interventions for the prevention of OHSS in ART cycles: an overview of Cochrane reviews. Cochrane Database Syst Rev 2017;1:CD012103.

3. Surcel M, Surcel M, Zlatescu-Marton C, Micu R, Nemeti GI, Axente DD, et al. The role of high follicular levels of angiotensin II and vascular endothelial growth factor in antipating the development of severe ovarian hyperstimılation syndrome in patients with prophylactic cabergoline therapy undergoing an in vitro fertilization procedure. Acta Endocrinol (Buchar) 2020;16:30-6.
4. Duffy DM, Ko C, Jo M, Brannstrom M, Curry TE. Ovulation: Parallels With Inflammatory Processes. Endocr Rev 2019;40:369-416.
5. Manau D, Fabregues F, Penarrubia J, Creus M, Carmona F, Casals G, et al. Vascular endothelial growth factor levels in serum and plasma from patients undergoing controlled ovarian hyperstimulation for IVF. Hum Reprod 2007;22:669-75.
6. Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohí J, Simón C. The pathogenesis of ovarian hyperstimulation syndrome: in vivo studies investigating the role of interleukin-1beta, interleukin-6, and vascular endothelial growth factor. Fertil Steril 1999;71:482-9.
7. Garcia-Velasco JA, Pellicer A. New concepts in the understanding of the ovarian hyperstimulation syndrome. Curr Opin Obstet Gynecol 2003;15:251-6.
8. Duleba AJ, Foyouzi N, Karaca M, Pehlivan T, Kwintkiewicz J, Behrman HR. Proliferation of ovarian theca-interstitial cells is modulated by antioxidants and oxidative stress. Hum Reprod 2004;19:1519-24.
9. Bartkova A, Sanak D, Dostal J, Herzig R, Otruba P, Vlachova I, et al. Acute ischaemic stroke in pregnancy: a severe complication of ovarian hyperstimulation syndrome. Neurol Sci 2008;29:463-6.
10. Pala S, Atilgan R, Ozkan ZS, Akpolat N, Ilhan N, Sapmaz E. Investigation of short- and long-term effects of ovarian hyperstimulation syndrome on ovarian reserve: an experimental study. Clin Exp Obstet Gynecol 2015;42:95-100.
11. Fritz S, Wessler I, Breitling R, Rossmanith W, Ojeda SR, Dissen GA, et al. Expression of muscarinic receptor types in the primate ovary and evidence for nonneuronal acetylcholine synthesis. J Clin Endocrinol Metab 2001;86:349-54.
12. Al-Zi'abi MO, Bowolaksono A, Okuda K. Survival role of locally produced acetylcholine in the bovine corpus luteum. Biol Reprod 2009;80:823-32.
13. Blohberger J, Kunz L, Einwang D, Berg U, Berg D, Ojeda SR, et al. Readthrough acetylcholinesterase (AChE-R) and regulated necrosis: pharmacological targets for the regulation of ovarian functions? Cell Death Dis 2015;6:e1685.
14. Ohba T, Ujioka T, Ishikawa K, Tanaka N, Okamura H. Ovarian hyperstimulation syndrome-model rats; the manifestation and clinical implication. Mol Cell Endocrinol 2003;202:47-52.
15. Carter JH, Douglass LE, Deddens JA, Colligan BM, Bhatt TR, Pemberton JO, et al. Pak-1 expression increases with progression of colorectal carcinomas to metastasis. Clin Cancer Res 2004;10:3448-56.
16. Davis JS, Rueda BR, Spanel-Borowski K. Microvascular endothelial cells of the corpus luteum. Reprod Biol Endocrinol 2003;1:89.
17. Pru JK, Lynch MP, Davis JS, Rueda BR. Signaling mechanisms in tumor necrosis factor alpha-induced death of microvascular endothelial cells of the corpus luteum. Reprod Biol Endocrinol 2003;1:17.
18. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991;174:1209-20.
19. Kawai S, Sakayori S, Kobayashi H. [The role of IL-10 in patients with SIRS (systemic inflammatory response syndrome)--in relation to TNF activity]. Kansenshogaku Zasshi 1995;69:765-71.
20. Kurzen H, Wessler I, Kirkpatrick CJ, Kawashima K, Grando SA. The non-neuronal cholinergic system of human skin. Horm Metab Res 2007;39:125-35.
21. Urra J, Blohberger J, Tiszavari M, Mayerhofer A, Lara HE. In vivo blockade of acetylcholinesterase increases intraovarian acetylcholine and enhances follicular development and fertility in the rat. Sci Rep 2016;6:30129.
22. Nathanson NM. A multiplicity of muscarinic mechanisms: Enough signaling pathways to take your breath away. Proc Natl Acad Sci U S A 2000;97:6245-47.
23. Knecht M, Ranta T, Catt KJ. Granulosa cell differentiation in vitro: induction and maintenance of follicle-stimulating hormone receptors by adenosine 3',5'-monophosphate. Endocrinology 1983;113:949-56.
24. Mayerhofer A, Dissen GA, Costa ME, Ojeda SR. A role for neurotransmitters in early follicular development: induction of functional follicle-stimulating hormone receptors in newly formed follicles of the rat ovary. Endocrinology 1997;138:3320- 9.
25. Cruz ME, Flores A, Alvarado BE, Hernández CG, Zárate A, Chavira R, et al. Ovulation requires the activation on proestrus of M1 muscarinic receptors in the left ovary. Endocrine 2015;49:809-19.
26. Mayerhofer A, Kunz L, Krieger A, Proskocil B, Spindel E, Amsterdam A, et al. FSH regulates acetycholine production by ovarian granulosa cells. Reprod Biol Endocrinol 2006;4:37.
27. Delgado SM, Escudero CG, Casais M, Gordillo M, Anzulovich AC, Sosa Z, et al. Ovaric physiology in the first oestral cycle: influence of noradrenergic and cholinergic neural stimuli from coeliac ganglion. Steroids 2010;75:685-94.
28. Vieyra E, Ramírez DA, Lagunas N, Cárdenas M, Chavira R, Damián-Matsumura P, et al. Unilaterally blocking the muscarinic receptors in the suprachiasmatic nucleus in proestrus rats prevents pre-ovulatory LH secretion and ovulation. Reprod Biol Endocrinol 2016;14:34.
29. Cruz ME, Villegas G, Domínguez-González A, Chavira R, Domínguez R. Ovulation delay induced by blockade of the cholinergic system on dioestrus-1, is related to changes in dopaminergic activity of the preoptic anterior-hypothalamic area of the rat. Brain Res Bull 2001;54:339-44.
30. Linares R, Acuña XN, Rosas G, Vieyra E, Ramírez DA, Chaparro A, et al. Participation of the Cholinergic System in the Development of Polycystic Ovary Syndrome. Molecules 2021;26:5506.