**CEOG** Clinical and Experimental Obstetrics & Gynecology

**Experimental Research**

# The effects of formoterol on the serum, peritoneal VEGF, MDA, and VEGF levels in the ovaries and endometrium of rats with OHSS

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**Summary**

*Purpose:* To investigate the effects of formoterol (a beta2-adrenoreceptor agonist) on serum and peritoneal fluid VEGF, malondialdehyde (MDA) levels, and on VEGF-stained cell counts in the ovaries and endometrium of rats with ovarian hyperstimulation syndrome (OHSS) within the framework of immunohistochemical analysis. *Materials and Methods:* A total of 28 immature female Wistar rats were randomly divided into four groups. Three groups were given ten IU pregnant mare serum gonadotropin/day on days 22–25 of life. They were administered 30 IU hCG on day 26 of life to mimic OHSS. On days 26 and 27 of life, 24 mcg/kg/day formoterol in group 3 and 48 mcg/kg formoterol in group 4 were administered intraperitoneally per animal. *Results:* Although, there were no statistically significant differences between the groups in terms of serum and peritoneal fluid VEGF or MDA levels (serum VEGF: $p = 0.281$, peritoneal VEGF: $p = 0.674$, serum MDA: $p = 0.543$, peritoneal MDA: $p = 0.506$), there was a significant difference between the control and the OHSS placebo groups ($p = 0.013$) regarding the VEGF in the ovarian cortex. There was a significant difference between the control and the other groups in terms of ovarian stroma ($p = 0.001$), and there was also a statistically significant difference between the OHSS placebo and the other groups regarding VEGF in the endometrium (OHSS placebo *vs.* control group $p = 0.002$, OHSS placebo *vs.* the formoterol 24 mcg/kg group, $p = 0.008$, and OHSS-placebo *vs.* the formoterol 48 mcg/kg group, $p = 0.001$). *Conclusions:* Formoterol represents a potential novel strategy for the management of OHSS. Further studies, including those examining the dosage of formoterol, are warranted.

*Key words:* OHSS; Prevention; VEGF; MDA; Formoterol.

## Introduction

Ovarian hyperstimulation syndrome (OHSS) is observed at a rate of 0.3% to 5% in in vitro fertilization (IVF) clinics as an iatrogenic complication of controlled ovarian hyperstimulation with gonadotropins and can lead to serious morbidity and even mortality. Within the etiopathogenesis of OHSS, fluid shifts into the extravascular space due to increased the capillary permeability (CP) that results from the vasoactive peptides [1-4]. Prevention and treatment strategies for OHSS include cycle cancellation, coasting, intravenous albumin infusion, low doses of hCG for triggering, the cryopreservation of embryos, and the use of cabergoline [5]. Although there are ample numbers of studies of the etiopathogenesis of and preventive strategies for OHSS, OHSS remains one of the most significant problems in IVF clinics.

McClure *et al.* [6] was the first to report that VEGF, also known as the permeability factor, that is secreted by the granulosa-lutein cells, causes the development of OHSS. Ensuing studies demonstrated that VEGF is an important mediator that increases angiogenesis and stimulates endothelial cell mitosis [7-10].

Malondialdehyde (MDA) is formed as the end-product of prostaglandin biosynthesis and lipid peroxidation and is used as an oxidative stress biomarker [11]. MDA levels have been shown to be elevated in the myocardial infarction, atherosclerotic coronary diseases, type 2 diabetes mellitus, and in polycystic ovary syndrome during IVF [11-13].

Formoterol fumarate (FF) is used for asthma treatment and is a highly efficient and long lasting beta2-adrenoreceptor (AR) agonist. It has been suggested that formoterol fumarate plays an immunomodulatory role in Th-1 cytokine-induced autoimmune disease by inhibiting Th-1 lymphocytes. The intraperitoneal administration of formoterol suppresses Th-1 cytokine [14].

Based on the above considerations, in the present study, the authors sought to investigate the effectiveness of the immunomodulatory beta-2 agonist FF on the serum, peri-

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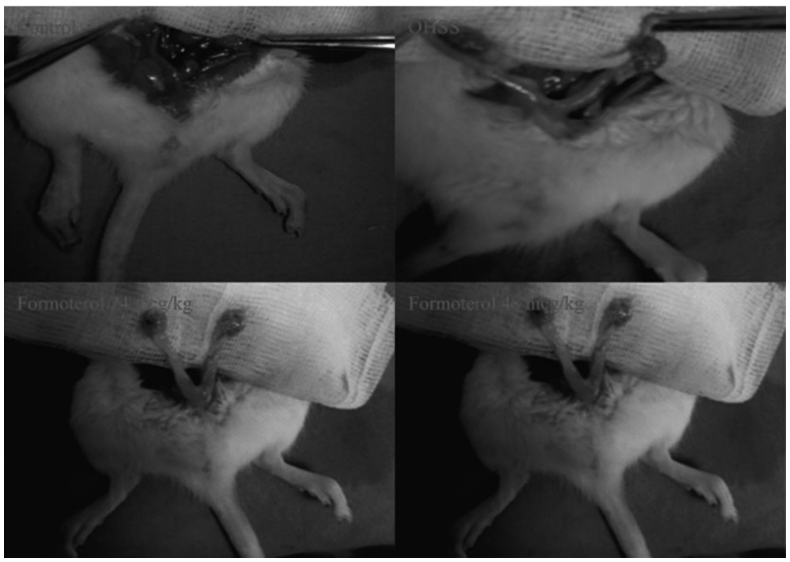

Figure 1. — Dissection of the rats.

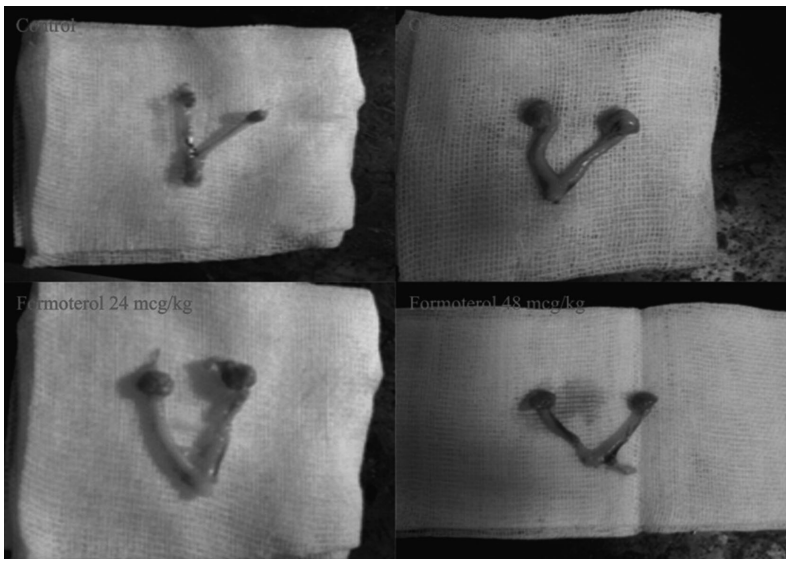

Figure 2. — Macroscopic appearance of the ovaries and uterus.

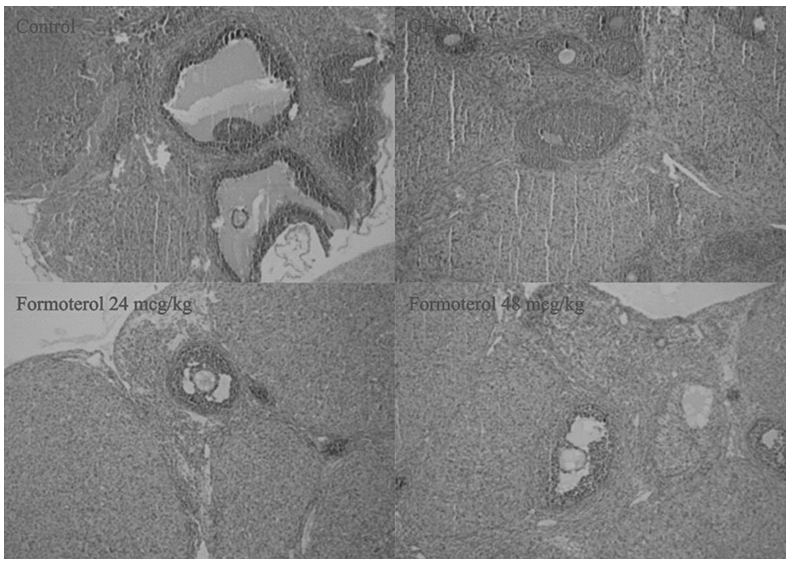


Figure 3. — Antral follicles in the ovarian tissue (H&E x100).

Table 1. — *Comparison of variables including weight gain, ovarian weights, ascite presence, primordial follicle count, antral follicle count, atretic follicle count, and corpus luteum count between the groups.*

| Variables | Group 1 (Control) | Group 2 (OHSS placebo) | Group 3 (OHSS formoterol 24 mcg/kg) | Group 4 (OHSS formoterol 48 mcg/kg) | *p* value |
|---|---|---|---|---|---|
| Weight gain (grams) | 12.07 ± f0.73 | 25.35 ± f0.74 | 19.42 ± f0.67 | 17.42 ± f0.34 | 0.001 |
| Ovarian weights (grams) | 0.34 ± f0.01 | 0.51 ± f0.02 | 0.43 ± f0.03* | 0.39 ± f0.01* | 0.001 |
| Ovarian volume ($mm^3$) | 1.72 ± f0.69 | 21.42 ± f2.30 | 8.45 ± f2.52 | 3.42 ± f1.00 | 0.001 |
| Ascite presence (%) | - | 100 | 28.6 | 14.3 | - |
| Primordial follicle count | 21.0 ± f03.65[a,b,c] | 6.43 ± f2.99[a] | 5.43 ± f1.9[b] | 10.43 ± f6.18[c] | 0.001 |
| Antral follicle count | 39.71 ± f13.42 | 29.57 ± f19.56 | 21.14 ± f8.89 | 21.86 ± f5.61 | 0.073 |
| Atretic follicle count | 8.00 ± f0.81[a,b,c] | 4.14 ± f1.06[a] | 4.43 ± f1.51[b] | 5.86 ± f1.34[c] | 0.001 |
| Corpus luteum count | 8.00 ± f1.29[a,b,c] | 52.14 ± f19.14[a] | 51.14 ± f17.89[b] | 39.57 ± f18.94[c] | 0.001 |

*There was no statistical difference between groups 3 and 4 in ovarian weight. a = The difference between groups 1 and 2 was statistically significant, b = the difference between groups 1 and 3 was statistically significant, and c = the difference between groups 1 and 4 was statistically significant.

toneal fluids, VEGF and MDA levels, and VEGF-stained cell counts in the ovarian cortex and stroma and endometrium in rats with OHSS within the framework of immunuhistochemical analysis.

## Materials and Methods

*Animals*

A total of 28 immature female Wistar rats were maintained for one week in the laboratory so that the experiments could begin with 23-d-old, 40 to 54 g animals. The animals were fed a standard diet and allowed free access to water on a 12-hour light/12-hour dark schedule. The study was conducted in conformity with the guidelines for the care and use of laboratory animals published by the United States National Institutes of Health and the Animal Care and Use Committee. This study was approved by the institutional ethics review board (reference number 2014/0019).

*The OHSS rat model*

The model was induced in rats via the administration of pregnant mare serum gonadotropin (PMSG), ten IU, subcutaneously (s.c.) for four days followed by hCG, 30 IU s.c. on the fifth day.

*Experimental design*

Twenty-eight rats were randomly divided into four groups. The study included four groups of rats as follows: group 1 – control group that was given 0.5 cc/day normal saline s.c. for four days; group 2 – OHSS placebo that was given ten IU/day PMSG s.c. for four days and 30 IU hCG s.c. on the fifth day to mimic OHSS; group 3 – formoterol 24 mg/kg group that was given ten IU/day PMSG s.c. for four days and 30 IU hCG s.c. on the fifth day to mimic OHSS. Additionally, 24 mcg/kg/day formoterol was administered intraperitoneally (ip) per animal on the fifth and sixth days; group 4 – formoterol 48 mcg/kg group that was given ten IU/day PMSG s.c. for four days, 30 IU hCG s.c. on the fifth day to mimic OHSS. Additionally, 48 mcg/kg/day formoterol was administered ip per animal on the fifth and sixth days.

*Surgical procedure*

On the 28th day of life, 48 hours after the hCG injection, all of the rats were anesthetized via the intramuscular administration of 50 mg/kg ketamine hydrochloric acid and seven mg/kg xylazine hydrochloric acid. The rats were weighed at the beginning of the study and before surgery and also, both the right and left ovaries of the rats were extracted to analyze the effects of formoterol on weight gain. Intracardiac blood was drawn from the animals before surgery to measure the serum VEGF levels. A ventral midline incision was made and peritoneal lavage with three ml saline was performed to assess the VEGF levels in the peritoneal fluid. Bilateral hysterectomy and salpingo-oophorectomy were performed to evaluate the ovarian weights and for the histopathological examination (Figures 1 and 2).

*Biochemical and histopathological evaluation*

Formalin-fixed ovarian and endometrial tissues were embedded in paraffin blocks, sectioned at five-mm thickness (four sections per sample), stained with hematoxylin and eosin and anti-VEGF receptor 2 antibody, and examined under a light microscope. The primordial, antral, atretic follicles, and corpora lutea were evaluated in the ovaries and the VEGF-positive stained cells in the endothelium of the blood vessels in the cortices and the stroma of the ovaries and the endometrium were investigated by histological examination. The VEGF and MDA levels in the peritoneal fluid and arterial blood samples were quantitatively assessed using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' instructions.

*Statistical analyses*

The statistical analyses were performed using the Statistical Package for the Social Sciences version 15.0. The variables are expressed as the means ±f the standard deviation (SDs). The normalities of the distributions of the continuous variables were assessed with Shapiro-Wilk tests. The normally distributed variables were examinated with one-way analyses of variance followed by Tukey post-hoc tests, and the non-normally distributed variables were analyzed with Kruskal–Wallis tests and Mann-Whitney U-tests with post hoc Bonferroni corrections. *P* values <0.05 were considered statistically significant.

## Results

The weight gain was greatest in the OHSS placebo group. There was a statistically significant difference between the groups ($p = 0.001$). The ovarian weights were lower in the control and formoterol treatment groups than in group 2;

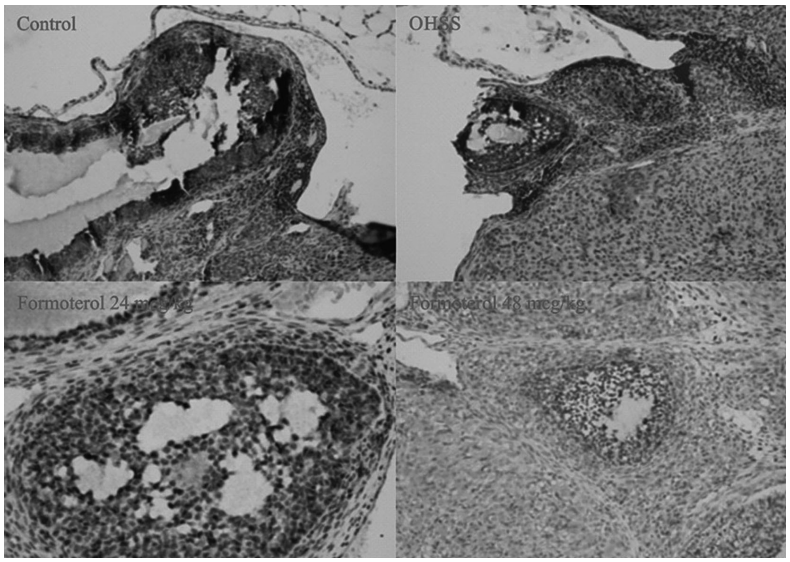


Figure 4. — VEGF-stained cells in the ovarian cortex (x200).

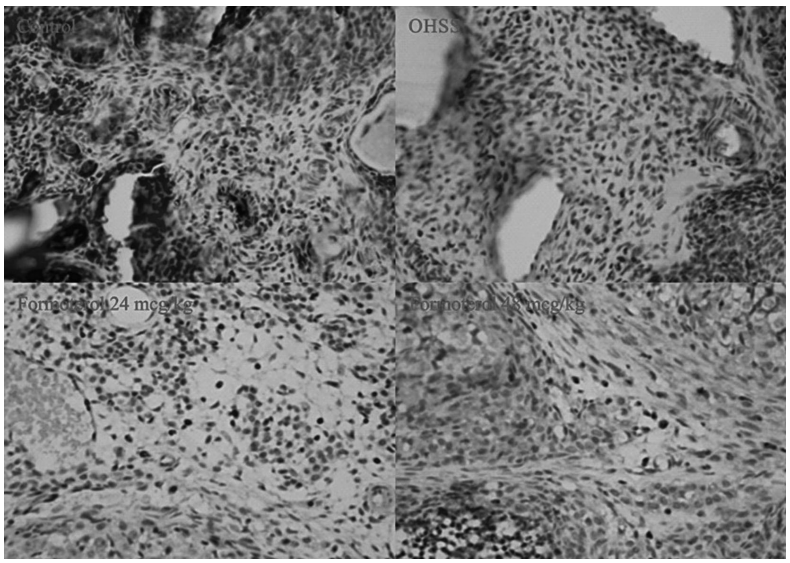


Figure 5. — VEGF-stained cells in the ovarian stroma (x400).

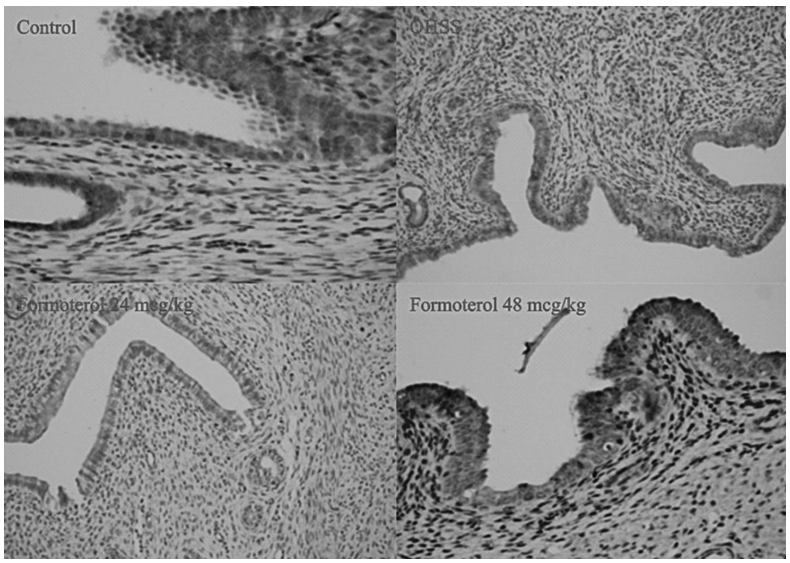


Figure 6. — VEGF-stained cells in endometrial stromal tissue (x400).

Table 2. — *Comparison of the ovarian cortical and stromal VEGF, endometrial VEGF, serum and peritoneal VEGF levels, and serum and peritoneal MDA.*

| Group | Group 1 (Control) | Group 2 (OHSS placebo) | Group 3 (OHSS formoterol 24 mcg) | Group 4 (OHSS formoterol 48 mcg) | *p* value |
|---|---|---|---|---|---|
| Ovarian cortex VEGF* | 99.14 ± f8.07 [a] | 156.57 ± f47.80 [a] | 142.86 ± f30.39 | 121.43 ± f26.24 | 0.013 |
| Ovarian stromal VEGF | 74.42 ± f15.60 [a,b,c] | 179.00 ± f17.37 [a] | 171.85 ± f17.90 [b] | 168.42 ± f10.98 [c] | 0.001 |
| Endometrial VEGF | 76.71 ± f12.47 [a] | 125.29 ± f27.47 [a,d,e] | 82.86 ± f21.10 [d] | 57.00 ± f25.41 [e] | 0.001 |
| Blood serum VEGF (mcg/ml) | 19.53 ± f6.47 | 25,83 ± f7.22 | 25.30 ± f6.00 | 24.67 ± f6.69 | 0.281 |
| Peritoneal VEGF (mcg/ml) | 22.35 ± f18.16 | 28.93 ± f7.17 | 23.52 ± f8.16 | 22.97 ± f6.98 | 0.674 |
| Blood serum MDA** (ng/ml) | 11.94 ± f2.66 | 13.37 ± f2.57 | 13.31 ± f1.88 | 12.30 ± f1.58 | 0.543 |
| Peritoneal MDA (ng/ml) | 0.72 ± f0.68 | 1.59 ± f1.38 | 1.22 ± f1.20 | 0.91 ± f0.91 | 0.546 |

*VEGF: vascular endothelial growth factor; **MDA: malondialdehyde. a = The difference between groups 1 and 2 was statistically significant, b = the difference between groups 1 and 3 was statistically significant, c = the difference between groups 1 and 4 was statistically significant, d = the difference between groups 2 and 3 was statistically significant, and e = the difference between groups 2 and 4 was statistically significant.

however, the difference between the formoterol treatment groups was not statistically different ($p = 0.066$). There were statistically significant differences between groups 1 and 2 ($p = 0.001$) and between the control and the formoterol treatment groups ($p = 0.001$, $p = 0.001$). The authors also evaluated the ovarian volumes and observed that the greatest increase in ovarian volume occurred in group 2 (21.42 ± 2.30 $mm^3$). The difference between the groups was statistically significant ($p = 0.001$). Peritoneal ascites were present in all of the rats in group 2, they were present in 28.6% (n= 2) of the group 3 and 14.3% (n= 1) of the group 4 rats (Table 1).

When the antral follicles, primordial follicles, atretic follicles, and corpora lutea counts were investigated by histopathological examination, no statistically significant difference between the groups in terms of antral follicle count was observed ($p = 0.073$) (Figure 3). However, there were statistically significant differences between the control and the other groups in the atretic follicle and corpus luteum counts ($p = 0.001$) (Table 1).

Immunohistochemical analysis of the VEGF-stained cell counts in the ovarian cortex and stroma and the endometrium revealed that there was a significant difference between the ovarian cortices of groups 1 and 2 ($p = 0.013$). Regarding the VEGF-stained cell counts in the stroma, the differences between the control and the other groups were statistically significant ($p = 0.001$) (Figures 4 and 5). The highest VEGF-stained cell count in the endometrium was observed in group 2 (125.29 ± f27.47 cells), and the differences between group 2 and the other groups were significant (group 2 *vs.* 1 $p = 0.002$, group 2 *vs.* 3 $p = 0.008$, and group 2 *vs.* 4 $p = 0.001$) (Figure 6). There were no significant differences between the control and formoterol treatment groups ($p = 0.955$ and $p = 0.372$) (Table 2).

There were no significant differences in the serum or peritoneal fluid VEGF or MDA levels (blood serum VEGF $p = 0.281$, peritoneal VEGF $p = 0.674$, blood serum MDA $p = 0.543$, and peritoneal MDA $p = 0.506$) (Table 2).

## Discussion

In the present study, the authors sought to determine the effectiveness of the beta 2 agonist FF on the serum and peritoneal fluid VEGF levels and to immunohistochemically analyze the VEGF-stained cell counts in the ovaries and the endometrium in rats with OHSS. To the best of the authors' knowledge, there have been no previous reports of the use of FF in any experimental animal OHSS model, and the present study is the first to report that FF might reduce the development of OHSS. The results of the study demonstrated that FF treatment in the hyperstimulated rat model resulted in significantly lower ovarian weights and volumes, antral follicle counts, atretic follicle counts, and endometrial VEGF-stained cell count; however, there were no significant differences in terms of serum or peritoneal fluid VEGF or MDA levels.

It has been suggested that VEGF increases capillary permeability by activating the VEGF receptor-2 (VEGFR-2) [4, 15]. Other than VEGF, prostaglandins, cytokines, the renin-angiotensin-aldosterone system, estradiol, progesterone, the kinin-kallikrein system, and nitric oxide have been implicated in the etiology of OHSS [16, 17]. There are studies in literature in which serum and peritoneal fluid VEGF levels have been shown to be reduced by the above mentioned pathways, and these effects prevented the development of OHSS [18-20]. Furthermore, Yilmaz *et al.* [21] showed that FF reduces VEGF levels in the peritoneal fluid of animals with induced endometriosis. However, the present authors were unable to identify a difference between the control and OHSS placebo groups, and the FF treatment was observed to have no effect on the serum or peritoneal VEGF levels.

Gomez *et al.* [4] and Quintana *et al.* [22] reported increased VEGF and VEGF-R expressions in the granulosa cells of rats with OHSS, and Wang *et al.* [23] and Saylan *et al.* [24] also evaluated VEGF expression in granulosa cells and reported elevated VEGF expression in OHSS situations. In accordance with the literature, the present au-

thors also observed that VEGF-stained cell counts were higher in the OHSS placebo group than in the control group. Moreover, the present authors also observed that the treatment with FF reduced VEGF levels compared with the OHSS placebo group, although this difference did not reach significance.

Ozcakır *et al.* [18] reported that VEGF immunoreactivity is stronger in the endothelium of blood vessels, the corpus luteum, and the stroma in OHSS groups compared with control groups. In line with the findings of this study, the present authors also observed VEGF-stained cell counts were higher in the OHSS placebo group than in the control group. The present results revealed that there was a significant decrease in the VEGF-stained cell counts in the endometrium due to treatment with intraperitoneal FF. Vascularized receptive endometrium needs to be present for successful implantation. It is known that VEGF regulates endometrial vascularization by increasing angiogenesis [25]. Notably, FF might negatively affect implantation because it decreased VEGF in the endometrium.

Studies of female reproductive functions have particularly shown that MDA levels are increased in unexplained infertility and have argued that the MDA levels in follicular fluid might be a potential marker for the prediction of success in ART [26]. It is known that MDA levels are elevated in patients with PCOS who are undergoing IVF [13]. It can be suggested that OHSS development will be observed more frequently in patients with PCOS, and therefore, MDA levels might be elevated in OHSS. However, in the present study, the authors were not able to observe a statistical difference between the groups in terms of serum or peritoneal fluid MDA levels.

On the one hand, a potential weakness of the present study is that the authors were not able to investigate the effects of FF on serum estradiol and progesterone levels. The strength of the present study is that they used an immunohistochemical approach to detect the effects of FF on VEGF expression and also evaluated the effects of reduced VEGF secretion in the endometrium.

Based on the findings of the present study, the authors showed that low-dose FF was as effective as high-dose FF in the prevention of OHSS. They preferred to evaluate the effects of FF on OHSS in a rat model, but they believe the results might be applicable to humans in clinical practice.

In conclusion, FF prevents OHSS by reducing ovarian weight and volume, although it does not reduce serum or peritoneal VEGF levels. The potential of FF for the treatment of OHSS requires confirmation of its efficacy, determination of the optimal effective dose, and evaluation of the effects of FF in humans. Further studies examining the dosage and routes of FF administration in terms of a potential novel strategy for the management of OHSS are warranted.

## References


[1] Chen C.D., Chen S.U., Yang Y.S.: "Prevention and management of ovarian hyperstimulation syndrome". *Best Pract. Res. Clin. Obstet. Gynaecol.*, 2012, *26*, 817.

[2] Mor Y.S., Schenker J.G.: "Ovarian hyperstimulation syndrome and thrombotic events". Am. J. Reprod. Immunol., 2014, *72*, 541.

[3] Levy T., Orvieto R., Homburg R., Peleg D., Dekel A., Ben-Rafael Z.: "Severe ovarian hyperstimulation syndrome despite low plasma oestrogen concentrations in a hypogonadotrophic, hypogonadal patient". *Hum. Reprod.*, 1996, *11*, 1177.

[4] Gómez R., Simón C., Remohí J., Pellicer A.: "Vascular endothelial growth factor receptor-2 activation induces vascular permeability in hyperstimulated rats, and this effect is prevented by receptor blockade". *Endocrinology*, 2002, *143*, 4339.

[5] Mathur R., Kailasam C., Jenkins J.: "Review of the evidence base of strategies to prevent ovarian hyperstimulation syndrome". *Hum. Fertil.*, 2007, *10*, 75.

[6] McClure N., Healy D.L., Rogers P.A., Sullivan J., Beaton L., Haning R.V.J., *et al.*: 'Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome". *Lancet*, 1994, *344*, 235.

[7] Krasnow J.S., Berga S.L., Guzick D.S., Zeleznik A.J., Yeo K.T.: "Vascular permeability factor and vascular endothelial growth factor in ovarian hyperstimulation syndrome: a preliminary report". *Fertil. Steril.*, 1996, *65*, 552.

[8] Abramov Y., Barak V., Nisman B., Schenker J.G.: "Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome". *Fertil. Steril.*, 1997, *67*, 261.

[9] 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.

[10] McLaren J., Prentice A., Charnock-Jones D.S., Millican S.A., Muller K.H., Sharkey A.M., *et al.*: "Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids". *J. Clin. Invest.*, 1996, *98*, 482.

[11] Grune T., Berger M.M.: "Markers of oxidative stres in ICU clinical settings: present and future". *Curr. Opin. Clin. Nutr. Metab. Care*, 2007, *10*, 712.

[12] Anderson D.R., Duryee M.J., Shurmur S.W., Um J.Y., Bussey W.D., Hunter C.D., *et al.:* "Unique antibody responses to malondialdehyde-acetaldehyde (MAA)-protein adducts predict coronary artery disease". *PLoS One*, 2014, *9*, e107440.

[13] Niedernhofer L.J., Daniels J.S., Rouzer C.A., Greene R.E., Marnett L.J.: "Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells". *J. Biol. Chem.*, 2003, *278*, 31426.

[14] Nishii M., Inomata T., Niwano H., Takehana H., Takeuchi I., Nakano H., *et al.*: "b2-Adrenergic agonists suppress rat autoimmune myocarditis: potential role of b2-adrenergic stimulants as new therapeutic agents for myocarditis". *Circulation*, 2006, *114*, 936.

[15] Gomez R., Gonzalez-Izquierdo M., Zimmermann R.C., Novella-Maestre E., Alonso-Muriel I., Sanchez-Criado J., *et al.*: "Low-dose dopamine agonist administration blocks vascular endothelial growth factor (VEGF)-mediated vascular hyperpermeability without altering VEGF receptor 2-dependent luteal angiogenesis in a rat ovarian hyperstimulation model". *Endocrinology*, 2006, *147*, 5400.

[16] Nastri C.O., Ferriani R.A., Rocha I.A., Martins W.: "Ovarian hyperstimulation syndrome: pathophysiology and prevention". *J. Assist. Reprod. Genet.*, 2010, *27*, 121.

[17] Brännström M., Wang L., Norman R.J.: "Effects of cytokines on prostaglandin production and steroidogenesis of incubated preovulatory follicles of the rat". *Biol. Reprod.*, 1993, *48*, 165.

[18] Ozcakir H.T., Giray S.G., Ozbilgin M.K., Uyar Y., Lacin S., Caglar H.: "Immunohistochemical detection of transforming growth factor-alpha, epidermal growth factor, and vascular endothelial growth fac-

tor expression in hyperstimulated rat ovary". *Acta Obstet. Gynecol. Scand.*, 2005, *84*, 887.

[19] Cenksoy C., Cenksoy P.O., Erdem O., Sancak B., Gursoy R.: "A potential novel strategy, inhibition of vasopressin-induced VEGF secretion by relcovaptan, for decreasing the incidence of ovarian hyperstimulation syndrome in the hyperstimulated rat model". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2014, *174*, 86.

[20] Engin-Ustun Y., Yılmaz S., Timur H., Ustun Y., Moraloglu O., Deveer R., Yilmaz N.: "Comparison of bevacizumab and cabergoline inthe treatment of ovarian hyperstimulation syndrome in a rat model". *Gynecol. Endocrinol.*, 2013, *29*, 851.

[21] Yilmaz N., Ozaksit G., Keskin R., Tapisiz O., Mollamahmutoglu L., Uysal S., *et al.*: "The effect of formoterol on peritoneal VEGF levels in rats with endometriosis". *Cytokine*, 2012, *58*, 47.

[22] Quintana R., Kopcow L., Marconi G., Young E., Yovanovich C., Paz D.A.: "Inhibition of cyclooxygenase-2 (COX-2) by meloxicam decreases the incidence of ovarian hyperstimulation syndrome in a rat model". *Fertil. Steril.*, 2008, *90*, 1511.

[23] Wang T.H., Horng S.G., Chang C.L., Wu H.M., Tsai Y.J.,Wang H.S., *et al.*: "Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with upregulation of vascular endothelial growth factor". *J. Clin. Endocrinol. Metab.*, 2002, *87*, 3300.

[24] Saylan A., Arioz D.T., Koken T., Dilek H., Saylan F., Yilmazer M.: "Prevention of ovarian hyperstimulation syndrome in a rat model: efficacy comparison between cabergoline and meloxicam". *Acta Obstet. Gynecol. Scand.*, 2010, *89*, 692.

[25] Sugino N., Kashida S., Karube-Harada A., Takiguchi S., Kato H.: "Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy". *Reproduction*, 2002, *123*, 379.

[26] Attaran M., Pasqualotto E., Falcone T., Goldberg J.M., Miller K.F., Agarwal A., Sharma R.K.: "The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization". *Int. J. Fertil. Womens Med.*, 2000, *45*, 314.

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