



Oxytocin and cabergoline alleviate ovarian hyperstimulation syndrome (OHSS) by suppressing vascular endothelial growth factor (VEGF) in an experimental model

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Abstract

Purpose Ovarian hyperstimulation syndrome (OHSS) is a life-threatening complication of ovarian stimulation in reproductive medicine. Here, we aimed to investigate the role of oxytocin (OT) and cabergoline in the prevention and alleviation of the OHSS in an animal model.

Methods Thirty-five female immature Wistar rats were randomly assigned to five groups. The control group ($n=7$) received saline only for five consecutive days. Remaining twenty-eight rats received 10 IU of pregnant mare serum gonadotropin (PMSG) followed by 30 IU of human chorionic gonadotropin (hCG) to induce OHSS. Group 2 ($n=7$) was managed with no additional intervention after the induction of OHSS. Group 3 ($n=7$) received 100 $\mu\text{g}/\text{kg}$ cabergoline 2 h before the PMSG injection for four consecutive days and 2 h before the hCG injection on the fifth day. Group 4 ($n=7$) and group 5 ($n=7$) received 80 $\mu\text{g}/\text{kg}$ and 160 $\mu\text{g}/\text{kg}$ OT after induction of OHSS, respectively. Oxytocin was administered 2 h before the PMSG injection for four consecutive days and 2 h before the hCG injection on the fifth day. Body and ovary weight, vascular permeability (VP), VEGF expression in the ovaries, and levels of VEGF in the peritoneal fluids were examined in all animals.

Results Cabergoline and OT reduced body weight, ovary weight, and VP compared to that of the OHSS group ($p < 0.05$). VEGF expressions in ovaries and peritoneal VEGF levels were decreased in cabergoline and OT groups compared to that of the OHSS groups ($p < 0.001$ for cabergoline and OT—80 $\mu\text{g}/\text{kg}$; $p < 0.00001$ for OT—160 $\mu\text{g}/\text{kg}$). However, there was no statistically significant difference in these parameters between the OT and cabergoline groups.

Conclusion Both OT and cabergoline were active in the alleviation of OHSS through suppression of VEGF and VP. Overall, we conclude that OT is effective for downregulation for VEGF and improvement in vascular permeability in OHSS.

Keywords Ovarian hyperstimulation syndrome · VEGF · Oxytocin · Cabergoline · Animal model

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most crucial and sometimes life-threatening iatrogenic complication of controlled ovarian hyperstimulation (COH) by exogenous gonadotropins for assisted reproductive techniques (ART) [1, 2]. It is characterized by cystic enlargement of the ovaries and rapid transudation of protein-rich fluid from the vascular space into the peritoneal cavity. The excess fluid produces weight gain, abdominal distension, and intravascular depletion. If the OHSS is severe, this protein-rich fluid may also be found in pleural and pericardial cavities [3, 4]. The incidence of mild, moderate, and severe forms of OHSS is 3–10% of all ART cycles. However, it can reach 20% in high-risk women [4]. The pathophysiology of OHSS is not

clearly understood. The major pathophysiological mechanism underlying OHSS is an acute vascular permeability (VP) due to exposure to exogenous and/or endogenous human chorionic gonadotropin (hCG) resulting in a fluid shift from intravascular space to third space compartments. OHSS has a wide pathophysiological spectrum ranging from mild illness to severe disease. It is usually self-limited and resolves spontaneously within several days; however, it may persist longer in conception cycles [5]. Hypovolemia in the vascular system may cause declined organ perfusion, hydrothorax, ascites, electrolyte imbalance, hemoconcentration, disseminated intravascular coagulation (DIC), and even renal and hepatic failure. In addition, venous thrombosis is a major cause of morbidity and mortality related to OHSS [6].

hCG-dependent increase in vascular endothelial growth factor (VEGF), the main regulator of vascular permeability (VP), is the cornerstone of the main pathophysiological processes in OHSS. Hence, targeting VEGF is one of the key points for the prevention of complications of OHSS and attenuating its clinical challenges. The high level of serum hCG induces mRNA expression of VEGF in the granulosa cells of the ovary. VP and capillary leakage develop after VEGF binds to its receptor (VEGFR₂) on endothelial cells [7]. The recruitment and development of a great number of antral follicles increase the production of VEGF during ART cycles. There is a notable link between the VEGF level and certain biological characteristics of capillary leakage and hemoconcentration [8]. Several other mediators, e.g., IL-2, IL-6, IL-8, histamine, elements of the ovarian renin–angiotensin system, and prostaglandins, have been suggested to be involved in the pathophysiological process of the OHSS [9, 10].

Cabergoline, an ergot derivative molecule, is potent dopamine (DA) receptor agonist on D₂ receptors. It has been used to prevent OHSS in women at high risk of OHSS undergoing ART treatments. Cabergoline reduces vascular permeability via inhibiting both VEGFR₂ phosphorylation and VEGF production [11]. In a Cochrane review, dopamine agonists were useful in the prevention of mild to severe OHSS, without reducing the live birth rates, clinical pregnancy, and abortion rates [12]. Currently, dopamine agonists are well-established agents that appear to be useful for the prevention of OHSS [13].

Oxytocin (OT) is a peptide hormone synthesized in the paraventricular nuclei of the hypothalamus and transported through the axons to the posterior pituitary. OT is released from the pituitary gland into the bloodstream to act as a neurotransmitter and a hormone on many organs. OT is the most important stimulant of the uterine contraction and is used primarily to induce or reinforce labor in obstetrics [14]. Another well-established action of OT is milk ejection during lactation. OT regulates the hypothalamic–pituitary–adrenal axis in response to stress, luteal function, pregnancy, cell

proliferation, behavioral status, emotional regulation, cardiovascular function, and neuropsychiatric disorders [15, 16]. OT is also produced by peripheral tissues, including skin, placenta, ovary, pancreas, heart, and blood vessels. OT acts through its specific receptors (OTRs) in peripheral organs. Furthermore, OT's anti-inflammatory, anti-apoptotic, and antioxidant functions have been demonstrated previously [17–19]. The potential role of VEGF, a potent angiogenic mediator, has been shown in these studies on different pathological conditions such as endometriosis, cell injury, and oxidative stress. Also, recently, Ji et al. demonstrated that OT inhibited ovarian cancer metastasis by suppressing the expression of VEGF [20]. Although several studies implicate the role of OT in anti-inflammatory, anti-apoptotic, antioxidant pathways in various pathophysiological processes, there is a lack of data with regard to the possible ameliorative effects of OT in OHSS which is a proinflammatory devastating process.

Many strategies and drugs have been described for the prevention and attenuation of OHSS complications [21, 22]. However, there is no exact method and/or drug to prevent OHSS entirely. To our knowledge, no study examined the OT for the prevention of OHSS to date. In this context, we investigated whether OT may be useful as an alternative agent for the prevention and/or alleviation of OHSS by modulating VEGF. To investigate and compare the effectiveness of OT, we used animals treated with cabergoline, which is a widely known OHSS preventive drug.

Materials and methods

Animals

In this study, 35 immature female Sprague–Dawley albino rats were used. All rats were 22 days-old and weighed 45–50 g. Animals were maintained in pairs in steel cages within a temperature-controlled room (22 ± 2 °C) and 12-h light/dark cycles. All animals were fed ad libitum. The study was approved by the Committee for Animal Research of Ege University. All animal studies strictly conform to the animal experiment guidelines of the Committee for Human Care.

Experimental design

Twenty-two-day-old female rats (weighing 45–50 g) were randomly divided into five groups: Group 1 (control, $n=7$) received 0.1 ml of saline intraperitoneally (i.p.) on five consecutive days (22–26); Group 2 (OHSS, $n=7$) was given 10 IU of pregnant mare serum gonadotropin (PMSG) (Folligon[®]-Intervet; Schering-Plough Animal Health, Pune, India) subcutaneously for four consecutive days and 30 IU hCG (Chorulon[®]-Intervet; Schering-Plough Animal Health,

Boxmeer, The Netherlands) on the fifth day to induce OHSS; Group 3 (OHSS and cabergoline; $n = 7$) was treated as the OHSS group, followed by oral gavage with cabergoline (100 $\mu\text{g}/\text{kg}$, Dostinex[®]-Pharmacia SpA, Ascoli Piceno, Italy) dissolved in 1 ml of tap water. The drug was administered 2 h before the PMSG injection for four consecutive days and 2 h before the hCG injection on the fifth day. Group 4 (OHSS and oxytocin; $n = 7$) was treated as the OHSS group, followed by i.p. injection of OT (80 $\mu\text{g}/\text{kg}$, Pituisan[®], Ege Vet, Alfasan International BV, Holland) 2 h before the PMSG injection for four consecutive days and 2 h before the hCG injection on the fifth day. Group 5 (OHSS and oxytocin; $n = 7$) was treated as the OHSS group, followed by i.p. injection of OT 160 $\mu\text{g}/\text{kg}$ 2 h before the PMSG injection for four consecutive days and 2 h before the hCG injection on the fifth day [23]. A previously published protocol was used for the OHSS model [22].

Permeability assay

All rats were anesthetized by i.p. injection of 80 mg/kg ketamine hydrochloride (Alfamine[®], Ege Vet, Alfasan International B.V., Woerden, Holland) and 4 mg/kg xylazine hydrochloride (Alfazyne[®], Ege Vet, Alfasan International B.V., Woerden, Holland) 48 h after hCG administration to evaluate vascular permeability (VP) after weighing and numbering. VP was measured as previously described [24]. Next, 0.2 ml of 5 mM Evans Blue (EB) (Sigma-Aldrich, St. Louis, MO) dye in distilled water was injected intravenously (i.v.) through an insulin injector via the femoral vein. The peritoneal cavity was filled with 5 ml saline solution (0.9% NaCl) at a 21 °C and was shaken for 30 s after a 30-min waiting period. Then, the peritoneal washing liquid was gently aspirated via a vascular catheter without damage to the tissues. Collected washing fluids were divided into two tubes with 0.05 ml of 0.1 N NaOH for VP evaluation. After centrifugation (900 \times g, 12 min), EB concentration was evaluated at 600 nm using the Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The level of extravasated dye in the collected fluid was expressed as micrograms per 100 g body weight. Finally, all the animals were killed, and ovaries harvested bilaterally and weighed on precision scales. The macroscopic images of the ovaries in rats are shown in Fig. 1.

Histopathological evaluation of VEGF expression

Each ovarian tissue was immunohistochemically stained for VEGF expression within an isolated box with formaldehyde. Ovaries were fixed in 10% buffered formaldehyde and embedded in paraffin blocks. Four-micron sections were cut from the blocks using a microtome and deparaffinized. Each section was incubated with H_2O_2 (10%) for 30 min

to remove endogenous peroxidase activity. Next, the sections were blocked with 10% normal goat serum (Invitrogen, Camarillo, CA, USA) for 1 h at room temperature and incubated with primary antibodies against VEGF (Bioss Inc., Woburn, MA, USA; dilution 1/100) for 24 h at 4 °C and stained with the Histostain-Plus Bulk Kit anti-rabbit IgG (Bioss Inc., Woburn, MA, USA) and 3,3'-diaminobenzidine (DAB).

All sections were washed in PBS and photographed with an Olympus C-5050 digital camera connected to an Olympus BX51 microscope (Olympus, Hamburg, Germany). Brown cytoplasmic staining was scored positive for VEGF. The number of VEGF-positive cells was assessed systematically by scoring at least 100 granulosa and theca cells per 10 fields of tissue sections at $\times 100$ magnification.

Biochemical evaluation

Peritoneal fluids were centrifuged (3000 rpm, 10 min at room temperature) and stored at -20 °C for VEGF measurement. The level of cytokines was determined using enzyme-linked immunosorbent assay (ELISA) kits for VEGF (Invitrogen, Camarillo, CA, USA). Samples from each animal were run in duplicates. Assays were done as recommended by the manufacturer.

Statistical analysis

Statistical analysis of the data was performed with GraphPad Prism 8.1.1 (GraphPad Software, La Jolla, CA, USA). Values were expressed as mean \pm standard deviation (SD). Kolmogorov–Smirnov test was used to determine whether variables were normally distributed, and a one-way analysis of variance (ANOVA) was used. Bonferroni's corrections were used for post hoc analysis. A value of $p < 0.05$ was considered statistically significant.

Results

Histopathological evaluation of ovaries for VEGF immunoexpression

VEGF localization in the ovaries is shown in Fig. 2 for all groups. The immunohistological examination of ovaries in the control group was found to be normal, with no staining of VEGF in granulosa cells in the antral follicles (Fig. 2a). Strong staining was observed in the granulosa cells in the OHSS group (Fig. 2b). A remarkable decrease in VEGF staining was observed in both cabergoline and OT (80 $\mu\text{g}/\text{kg}$ and 160 $\mu\text{g}/\text{kg}$) groups (Fig. 2c–f). VEGF immunoexpression percent was found to be increased in the OHSS group compared to the control group ($35.9 \pm 6.4\%$

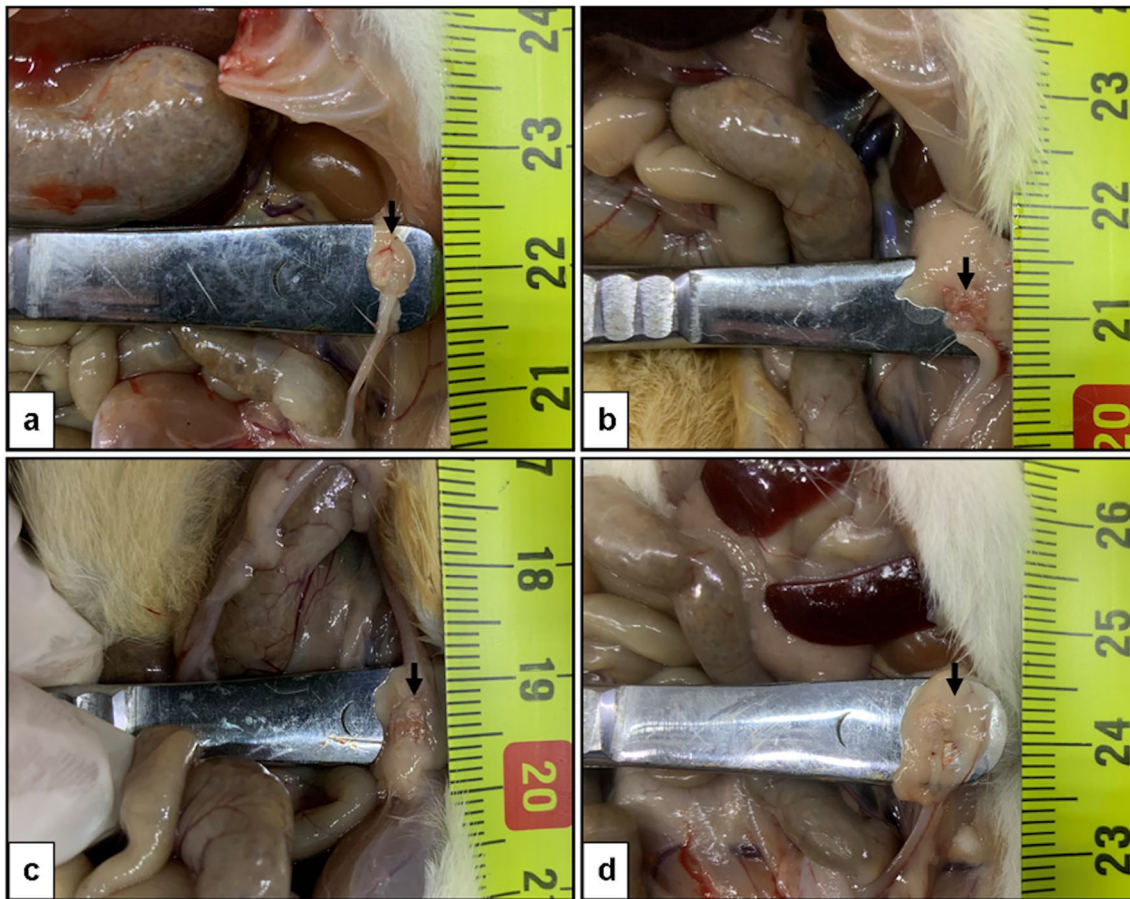


Fig. 1 Macroscopic images of the ovaries in different groups. **a** Control group, **b** OHSS group, **c** OHSS+cabergoline group, **d** OHSS+OT—160 µg/kg group; arrows indicate ovary

vs. $1.2 \pm 0.3\%$; $p < 0.0001$). VEGF immunoexpression percent was significantly lower in both OT groups (80 µg/kg and 160 µg/kg) than in the OHSS group ($12.5 \pm 2.03\%$ vs $1.2 \pm 0.3\%$, $p < 0.001$ for 80 µg/kg OT; $8.6 \pm 1.5\%$ vs $1.2 \pm 0.3\%$, $p < 0.00001$ for 160 µg/kg OT). Additionally, VEGF immunoexpression percent was significantly lower in the cabergoline group compared to the OHSS group ($10.2 \pm 1.9\%$ vs $1.2 \pm 0.3\%$, $p < 0.001$). However, there was no statistically significant difference between cabergoline and oxytocin groups ($p: 0.66$).

Measurement of ovarian weight and body weight

Ovarian weight was notably higher in OHSS groups compared to the control group (49.2 ± 8.4 mg vs 21.15 ± 2.2 mg; $p < 0.005$). Also, ovaries of rats treated with 160 µg/kg and 80 µg/kg of OT had significantly lower weight than ovaries of the OHSS groups (29.4 ± 6.07 mg and 33.6 ± 5.2 mg vs 49.2 ± 8.4 mg; $p < 0.001$ and $p < 0.01$, respectively). Similarly, ovaries of cabergoline-treated rats

had significantly lower weight than ovaries of the OHSS groups (38.5 ± 3.1 mg vs 49.2 ± 8.4 mg; $p < 0.05$).

There were significant alterations in body weight among all groups. OHSS caused significant weight gain compared to the control group (69.6 ± 7.2 g vs 46.6 ± 8.4 g; $p < 0.005$). Body weight of rats treated with cabergoline and OT (160 µg/kg and 80 µg/kg) was significantly lower than of the OHSS groups (49.05 ± 4.4 g and 55.9 ± 8.5 g for OT, 54.5 ± 4.09 g for cabergoline vs 69.6 ± 7.2 g; $p < 0.05$, respectively). However, the differences between cabergoline and OT groups were not statistically significant ($p = 0.11$).

The effects of hyperstimulation, cabergoline, and OT on the weight of ovaries and body and on VP in OHSS are presented in Table 1.

Biochemical analysis

Peritoneal VEGF levels were found to be significantly increased in the OHSS group compared to the control group (56.5 ± 8.2 pg/ml vs 4.1 ± 0.8 pg/ml; $p < 0.0001$). The level of VEGF in the peritoneal fluid was significantly

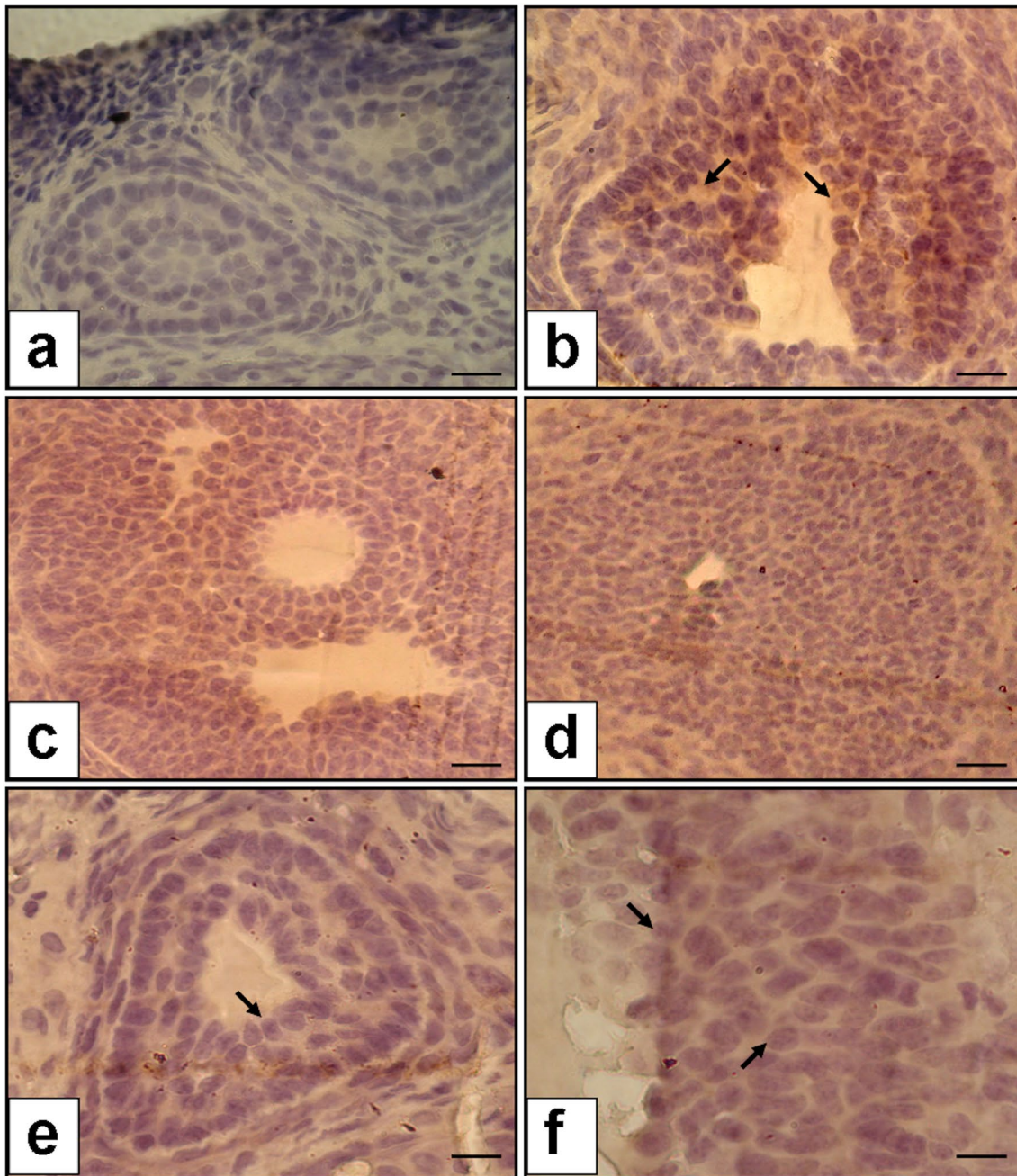


Fig. 2 VEGF immunoexpression in the ovaries (the **a–e** figure is $\times 40$ magnification, and **f** figure is $\times 100$ magnification). **a** No staining in the control group, **b** increased expression of VEGF in granulosa cells in the OHSS group; arrow indicates increased staining of VEGF, **c** decreased VEGF expression in cabergoline group, **d** decreased VEGF

expression in the group of OT—80 $\mu\text{g}/\text{kg}$; arrow indicates declined staining of VEGF, **e** and **f** decreased VEGF expression in the group of OT—160 $\mu\text{g}/\text{kg}$; arrow indicates declined staining of VEGF. Scale bars indicate 125 μm for **a**, **b**, **c**, **d**, and **e**; 250 μm for **f**

lower in both OT groups (80 $\mu\text{g}/\text{kg}$ and 160 $\mu\text{g}/\text{kg}$) compared to the OHSS group (26.5 ± 4.4 pg/ml vs. 56.5 ± 8.2 pg/ml , $p < 0.001$ for 80 $\mu\text{g}/\text{kg}$ OT; 18.5 ± 3.2 pg/ml vs. 56.5 ± 8.2 pg/ml , $p < 0.00001$ for 160 $\mu\text{g}/\text{kg}$ OT). Furthermore, VEGF levels in the peritoneal fluid were significantly decreased in the cabergoline group compared

to the OHSS group (25.06 ± 9.05 pg/ml vs 56.5 ± 8.2 pg/ml ; $p < 0.001$).

The VP was significantly increased in the OHSS group compared to the control group (34.5 ± 3.07 $\text{mM}/100$ g vs 15.06 ± 5.1 $\text{mM}/100$ g ; $p < 0.005$). When high-dose OT (160 $\mu\text{g}/\text{kg}$) group was compared with the OHSS group,

Table 1 Comparison of ovarian weight, body weight, and VP among groups

	Control (n=7)	OHSS (n=7)	OHSS + Cabergoline (n=7)	OHSS + 80 µg/kg OT (n=7)	OHSS + 160 µg/kg OT (n=7)
Ovary weight (mg)	21.15 ± 2.2	49.2 ± 8.4*	38.5 ± 3.1 [#]	33.6 ± 5.2 ^{##}	29.4 ± 6.07**
Body weight (g)	46.6 ± 8.4	69.6 ± 7.2*	54.5 ± 4.09 [#]	55.9 ± 8.5 [#]	49.05 ± 4.4 [#]
Vascular permeability (Evans Blue mM/100 g)	15.06 ± 5.1	34.5 ± 3.07*	24.3 ± 6.01 [#]	22.04 ± 6.3 ^{##}	16.9 ± 2.05**

The experimental groups (n=7) were analyzed for ovary weight, body weight, and vascular permeability. Values were expressed as mean ± standard deviation (SD). The groups were evaluated with Kolmogorov–Smirnov for normal distribution and analyzed with ANOVA test. Bonferroni correction was applied as the post hoc test

VP vascular permeability, OT oxytocin, OHSS ovarian hyperstimulation syndrome

p value < 0.05 was considered as statistically significant

*p < 0.005, OHSS group compared to control group

**p < 0.001, OT of 160 µg/kg group compared to OHSS group

[#]p < 0.05, OHSS + cabergoline or OHSS + OT group compared to OHSS group

^{##}p < 0.01, OHSS + cabergoline or OHSS + OT of 80 µg/kg group compared to OHSS group

a significant reduction was found for the high-dose OT group (16.9 ± 2.05 mM/100 g vs 34.5 ± 3.07 mM/100 g; p < 0.001). Furthermore, in rats treated with 80 µg/kg OT we observed a remarkable decline in VP compared to the OHSS group (22.04 ± 6.3 mM/100 g vs 34.5 ± 3.07 mM/100 g; p < 0.01). VP also decreased significantly in the group of cabergoline compared to the OHSS group (24.3 ± 6.01 mM/100 g vs 34.5 ± 3.07 mM/100 g; p < 0.05). However, the VP and peritoneal VEGF levels were not significantly different between the OT groups and the cabergoline group (p = 0.18). The VEGF immunopositivity percent and the levels of peritoneal VEGF are shown in Table 2 for all groups. Also, comparisons of all biochemical and immunohistochemical parameters evaluated in this study are shown in Figs. 3 and 4 as well.

Discussion

OHSS is a prominent serious complication of COH. Besides its potential detrimental complications that affect the quality of life, e.g., thromboembolic-related events, clinically many hemodynamic issues can occur. Prevention and prompt treatment of OHSS might be lifesaving. In the current study, we established a rat model of OHSS using a combination of PMSG and hCG. VEGF expressions in the ovaries and VEGF levels in the peritoneal fluid were significantly reduced in OT- and cabergoline-treated groups in comparison with OHSS groups.

Furthermore, body and ovary weight and VP decreased strikingly in the OT- and cabergoline-treated groups compared to the OHSS group. Therefore, OT is a promising agent that could potentially prevent and alleviate the potentially harmful effects of OHSS. Known main risk factors of OHSS include a history of OHSS, low body mass index,

Table 2 Comparison of ovarian expression of VEGF and peritoneal VEGF levels among groups

	Control (n=7)	OHSS (n=7)	OHSS + Cabergoline (n=7)	OHSS + 80 µg/kg OT (n=7)	OHSS + 160 µg/kg OT (n=7)
VEGF immunopositivity percent (%)	1.2 ± 0.3	35.9 ± 6.4*	10.2 ± 1.9 [#]	12.5 ± 2.03 [#]	8.6 ± 1.5**
Peritoneal VEGF (pg/ml)	4.1 ± 0.8	56.5 ± 8.2*	25.06 ± 9.05 [#]	26.5 ± 4.4 [#]	18.5 ± 3.2**

The experimental groups (n=7) were analyzed for percentage of VEGF immunopositivity and peritoneal VEGF. Values were expressed as mean ± standard deviation (SD). The groups were evaluated with Kolmogorov–Smirnov test for normal distribution. The analysis was performed with ANOVA followed by Bonferroni correction as post hoc test

VEGF vascular endothelial growth factor, OT oxytocin, OHSS ovarian hyperstimulation syndrome

p value < 0.05 was considered as statistically significant

*p < 0.0001, OHSS group compared to control group

**p < 0.00001, OT of 160 µg/kg group compared to OHSS group

[#]p < 0.001, OHSS + cabergoline or OHSS + OT of 80 µg/kg group compared to OHSS group

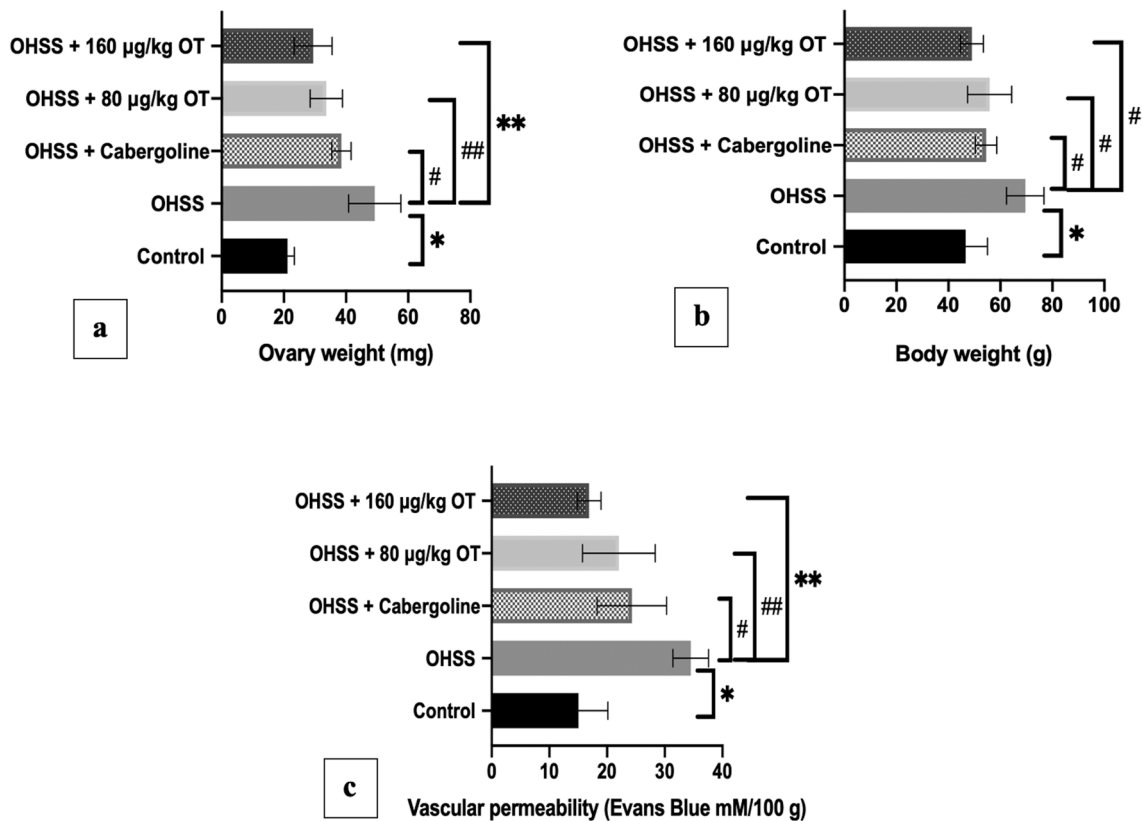


Fig. 3 a, b, c Graphical comparison of ovary weight, body weight, and VP in each group. The experimental groups for each parameter were analyzed with ANOVA, and Bonferroni correction was applied as the post hoc test. A p value <0.05 was considered as statistically significant. * $p < 0.005$, OHSS group compared to control group.

** $p < 0.001$, OT of 160 µg/kg group compared to OHSS group. # $p < 0.05$, OHSS+cabergoline or OHSS+OT group compared to OHSS group. ## $p < 0.01$, OHSS+cabergoline or OHSS+OT group compared to OHSS group. VP vascular permeability, OT oxytocin, OHSS ovarian hyperstimulation syndrome

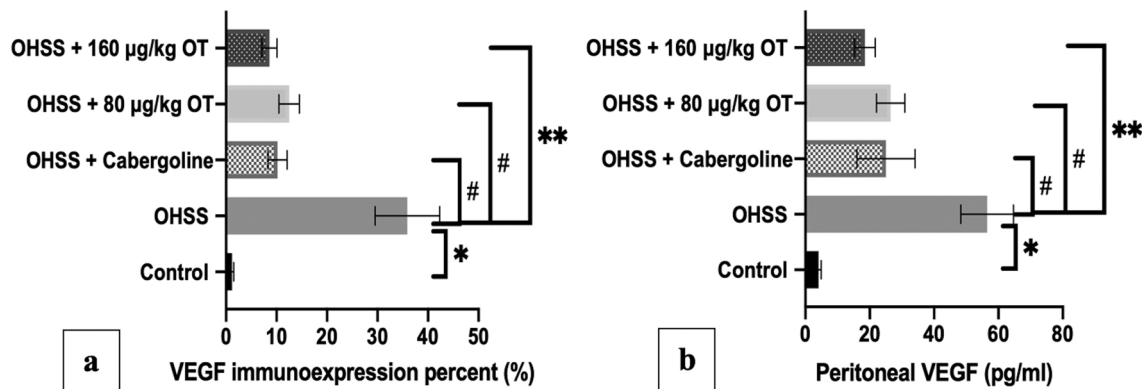


Fig. 4 a, b Graphical comparison of VEGF expression and peritoneal VEGF levels in each group. The percent of VEGF immunoeexpression (%) and peritoneal VEGF was evaluated. Each parameter was evaluated by comparing with the control group and by comparisons between experimental groups via using ANOVA test. Bonferroni correction was applied as the post hoc test. A p value <0.05 was considered as statistically significant.

* $p < 0.0001$, OHSS group compared to control group. ** $p < 0.00001$, OT of 160 µg/kg group compared to OHSS group. # $p < 0.001$, OHSS+cabergoline or OHSS+OT of 80 µg/kg group compared to OHSS group. VEGF vascular endothelial growth factor, OT oxytocin, OHSS ovarian hyperstimulation syndrome

young age, having polycystic ovaries, increased antral follicle count, or high anti-Müllerian hormone (AMH) levels, and high-dose hCG application [3]. The management of OHSS remains a subject of great importance not only for IVF providers but also for physicians who deal with affected cases in emergency departments. So far, to prevent OHSS in ART cycles, several strategies have been used in infertility practice. These include cycle cancellation, withholding exogenous gonadotropins and delaying hCG application until the serum estradiol (E_2) levels decrease to a secure level (coasting), GnRH agonist usage to launch final oocyte maturation, albumin infusion during oocyte retrieval, in vitro oocyte maturation, cryopreservation of all embryos, and use of dopamine agonists, e.g., cabergoline after oocyte pickup [25, 26]. Despite these preventive methods, no clear mechanisms have fully explained how these factors contribute to OHSS. However, it is well established that vasoactive substances released from ovaries into the circulatory system under hCG induction play the key role in this syndrome [27].

Dopamine agonists have long been used in reproductive endocrinology and infertility practice to reduce the OHSS manifestations without any maternal and/or fetal adverse effects in early periods of pregnancy [12]. In our study, we found that 100 $\mu\text{g}/\text{kg}$ cabergoline 2 h before the gonadotropin injection for four consecutive days and 2 h before the hCG injection on the fifth day significantly reduced ovary weight, body weight, VP, VEGF expressions in the ovary, and VEGF levels in peritoneal fluid. A recent randomized clinical study by Tehraninejad et al. [28] demonstrated that for women at high risk for developing OHSS cabergoline administration before oocyte pickup was superior to intravenous human albumin in the prevention of OHSS. Previously, the effectiveness of dopamine agonists, e.g., cabergoline in the treatment of OHSS, has been attributed to its ability to decrease VP related to VEGF receptor 2 (VEGFR₂) [29, 30]. Not only the treatment but also the OHSS prevention by cabergoline without compromising pregnancy has been emphasized [31]. We investigated the repression of OHSS by both cabergoline and OT in the experimental model and observed that our findings were consistent with other studies in the literature. Recently, many different agents have been tested as OHSS agents in animal models. Akman et al. [32] compared montelukast and cabergoline in the rat model of OHSS. They demonstrated a reduction in VP and VEGF expression in the cabergoline- and montelukast-treated rats in comparison with non-treated (only OHSS) controls. Also, they found that body weight, ovary weight, and peritoneal VEGF levels were significantly lower in the drug-treated groups than in the control (only OHSS) group. In another study, Sahin et al. [33] reported the effectiveness of both letrozole and cabergoline in the prevention of the OHSS through decreased VP and expression of VEGF in ovarian tissue of experimental animals. Unlike us, this team also

examined pigment epithelium-derived growth factor (PEDF) known as an antioxidant, anti-thrombogenic, and anti-angiogenic agent and an inhibitor of vascular permeability in blood vessels of animals. Levels of VP and PEDF were significantly lower in the letrozole and cabergoline group compared to the OHSS group.

In obstetrics, OT is a well-known and widely used drug. Besides, it is considered a safe drug for clinical use [34]. However, numerous potential effects of OT have been documented only in obstetrics. It is worth mentioning that besides its endocrine and paracrine activities, OT also has anti-angiogenic properties, alters immune responses, and suppresses VEGF, known as a powerful vasoactive mediator [15], which plays a crucial role in normal (e.g., embryogenesis, healing of injured tissues) or pathological angiogenesis (e.g., carcinogenesis, metastasis of tumor) and inflammation [35]. In addition, VEGF receptors are commonly found on endothelial cells where it is believed they are responsible for the transition of capillary leakage in OHSS [36]. In this regard, we have designed this experimental study on the OHSS model. To the best of our knowledge, there is no study regarding OT's possible effects on OHSS both clinical and experimental yet. We demonstrated that OT significantly reduced VEGF expression and VEGF levels in the peritoneal fluid after the induction of OHSS. In this context in our study, OT may have exerted its effects through the mechanisms as mentioned earlier. In the present study, VP and peritoneal VEGF levels were not significantly different between the OT groups and the cabergoline group. Hence, it is worth to express other properties and common side effects of both agents. The main adverse effects of oxytocin include hypersensitivity, anaphylactic reactions, flushing, cardiac arrhythmia, nausea, and vomiting. On the other hand, the main side effects of cabergoline include headache, dizziness, weakness or lack of energy, nausea, and constipation. If the consumption is higher in cabergoline, some serious adverse events (shortness of breath, chest pain, cough, and heart valve problems) might occur [37, 38]. Indeed, this was an animal model study, and therefore, before results can be generalized to human beings, clinical studies are necessary to confirm the findings. Moreover, the long-term effects of OT on pregnancy and women need to be further studied.

The study has some limitations. First, we established an animal model to investigate the effects of both OT and cabergoline on OHSS; thus, our results may not be adapted immediately to human beings without large-scale, proven clinical studies. Second, we did not measure plasma estrogen levels of the rats. Further studies are required to support and elucidate the exact mechanisms underlying the protective role of OT both before and during the COH to support our results. To the best of our knowledge, this is the first study to investigate the potential effects of the OT in an experimentally induced OHSS model. As a consequence, OT appears

to be as beneficial as cabergoline both in treating and in alleviating OHSS by modulating VEGF. The long-term effects of both OT and cabergoline, way of administration protocol, and appropriate drug dosage should be explored in further randomized clinical studies.

Author contributions IH contributed to conception and design, data extraction, and writing manuscript; EK and GO collected the data and performed interpretation and statistical analysis; GY done the histological analysis; ET and ENTG helped in supervising and critical revision; OE contributed to conception and design and performed the experiment.

Compliance with ethical standards

Conflict of interest All the authors declare that there is no conflict of interest.

Ethical approval The present study was approved by the Committee for Animal Research of Ege University, Izmir, Turkey. All procedures performed in studies involving animals were in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Informed consent The informed consent is not applicable for the current study.

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