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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

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ABSTRACT

Objective: To investigate the effects of V1A receptor antagonist through inhibition of vasopressin-induced VEGF secretion in an experimental model.
Study design: Thirty rats were randomly divided into five groups. Four groups were given 10 IU pregnant mare serum gonadotropin/day (sc) at 8:00–8:30 am on days 22–25 of life. They were administered 30 IU hCG at 8:00–8:30 am on day 26 of life. On days 26 and 27 of life at 8:00 am and 4:00 pm, (ip) per animal, 50 μg/kg/day GnRH antagonist in the GnRH antagonist group, 0.3 mg relcovaptan in the high dose relcovaptan group, and 0.15 mg relcovaptan in the low dose relcovaptan group were administered. The control group was given the same dosage of 0.9% saline solution (ip) on days 22–26 day of life. The main outcomes were weight gain, ovarian weights, peritoneal fluid VEGF values, corpus luteum count, and atretic follicle count.
Results: Weight gain was highest in the OHSS group; it was almost twice as much in the OHSS group than it was in the control group. Ovarian weights were significantly lower in all treatment groups ($p = 0.03$). There was no statistically significant difference in ovarian weights between the GnRH antagonist and relcovaptan groups ($p = 0.176$). The evaluation of peritoneal fluid VEGF-A levels revealed statistically significant differences between levels in the treatment groups and in the OHSS group ($p = 0.005$). Atretic follicle count in the OHSS group was significantly lower ($p = 0.048$). In all treatment groups, CL counts were prominently lower than they were in the OHSS group ($p = 0.002$).
Conclusion: Relcovaptan may be a novel strategy for decreasing risk of OHSS by inhibition of vasopressin-induced VEGF secretion through V1A receptor antagonist.
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Introduction

Ovarian hyperstimulation syndrome (OHSS) is a relatively common iatrogenic complication of controlled ovarian hyperstimulation, occurring in approximately 0.3–5% of cycles [1]. The severe form of OHSS is potentially lethal. Various methods have been used to prevent development of OHSS, such as cycle cancellation, coasting, intravenous albumin infusion during oocyte pickup, low doses of human chorionic gonadotrophin (hCG) for oocyte triggering, elective cryopreservation of all embryos, and cabergoline [2]. The best strategy to prevent OHSS, however, has yet to be defined. Therefore, new strategies to prevent and treat OHSS should be investigated.

The pathogenesis of OHSS is not completely understood. It seems likely that OHSS results from vasoactive peptides released from the granulosa cells in hyperstimulated ovaries. Therefore, fundamental physiologic change related to OHSS is characterized by increased vascular permeability (VP), leading to leakage of fluid from the vascular compartment, with third-space fluid accumulation and intravascular dehydration [3]. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, seems to be the principal mediator of OHSS by increasing VP [4–7]. One of the main strategies to prevent OHSS is to utilize preventive therapy modalities during stimulation. The blocking of effect of VEGF on VP could be considered a new strategy to prevent and treat OHSS by avoiding increased VP. It has been supposed that cabergoline, a dopamine agonist, prevents OHSS and reduces its severity by reducing VP with prevention of the phosphorylation of

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VEGF receptor [8]. A randomized controlled study including 200 patients showed that prophylactic treatment with cabergoline at lower doses (0.25 mg) reduces the incidence of OHSS in women at high risk undergoing IVF/ICSI treatment [9]. Relcovaptan (SR 49059) is a non-peptide, selective, and specific vasopressin V1a receptor inhibitor. Relcovaptan inhibits release of VEGF by blocking vasopressin V1a receptors. We hypothesized that relcovaptan, which acts through inhibition of vasopressin-induced VEGF secretion, may prevent and treat OHSS.

Arginine vasopressin (AVP), an antidiuretic non-peptide hormone, is responsible for regulating water and electrolyte homeostasis in the body and is considered one of several factors to increase VEGF secretion from human vascular smooth muscle cells and plays an important role in VP [10]. The biological effects of vasopressin are mediated by the vasopressin receptor subtypes V1a (vascular), V1b (pituitary, also called V3), and V2 (renal), and oxytocin receptors [11–13]. The V1a-receptor subtype is located in vascular smooth muscle cells, cardiomyocytes, hepatocytes, mesangial cells, the brain, testes, adrenal glands, spinal cord, ovary cells, and platelets [14]. It mediates vasoconstriction and vascular smooth muscle cell proliferation [15]. We hypothesized that the blocking of vasopressin V1a receptors will prevent OHSS by inhibiting VEGF (anti-VEGF effect).

The aim of our study was to evaluate the effectiveness of vasopressin V1a receptor antagonist in preventing OHSS. To our knowledge, there is no study in the literature investigating this effect.

Materials and methods

Thirty Wistar albino female rats 22 days old (33–48 g) were used for the study. All rats were provided by Gazi University Animal Reproduction Centre and housed in the Animal Laboratory of Gazi University. They were caged in a controlled environment of 22 °C with 12 h light/dark cycles. Standard rat feed and reverse-osmosis-purified water were provided ad libitum. The study protocol was approved by Gazi University Committee on the Use and Care of Animals, and all investigations complied with the National Academy of Science's Guide for Care and Use of Laboratory Animals (1996). The rats were weighed at the beginning of the study and 48 h after the HCG injection.

The 30 rats were randomly divided into five groups. Four groups were given a subcutaneous injection of 10 IU pregnant mare serum gonadotropin (PMSG-Folligon®)/day at 8:00–8:30 am on days 22–25 of life. They were administered 30 IU hCG (Chorulon®) at 8:00–8:30 am on day 26 of life. The control group was given the same dosage of 0.9% saline solution intraperitoneally (ip) on days 22–26 of life [16]. The change in body weight from pre-treatment to post-treatment was also recorded.

(i) The GnRH antagonist (GnRH anta, Cetrotide® Merck Serono, S.A., Geneva, Switzerland) group ($n = 6$) was given PMSG for 4 days (days 22–25 of life) and hCG was administered on day 26 of life. In addition, 50 μg/kg/day GnRH antagonist was administered at 8:00 am and 4:00 pm on days 26 and 27 of life.
(ii) The high dose relcovaptan (Tocris Biosciences) group ($n = 6$) was given PMSG for 4 days (days 22–25 of life) and 30 IU hCG was administered on day 26 of life. In addition, 0.3 mg relcovaptan was administered (ip) per animal at 8:00 am and 4:00 pm on days 26 and 27 of life.
(iii) The low dose relcovaptan group ($n = 6$) was given PMSG for 4 days (days 22–25 of life) and hCG was administered on day 26 of life. In addition, 0.15 mg relcovaptan was administered (ip) per animal at 8:00 am and 4:00 pm on days 26 and 27 of life.
(iv) The OHSS group ($n = 6$) was given PMSG for 4 days (days 22–25 of life) and 30 IU hCG was administered to each animal on day 26 of life.
(v) The control group ($n = 6$) was given the same dosage of 0.9% saline solution (ip) on days 22–26 of life (non-ovulating, non-OHSS group).

On day 28 of life, 48 h after the HCG injection, all the rats were anesthetized with an intramuscular administration of 50 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun; Bayer, Sisli, Istanbul, Turkey). They were immobilized on a standard rat surgery board. Using aseptic technique, a ventral midline incision was made. Peritoneal lavage with 5 ml saline was performed to assess the VEGF-A levels in the peritoneal fluid. Saline administered to the intraperitoneal cavity was collected with a cannula 30 min later. Bilateral oophorectomy was performed to evaluate the ovarian weights and for histopathologic examination. Arterial blood samples were also collected to measure serum VEGF levels. All procedures were performed by a physician blinded to the groups.

Histopathologic examination was performed by a pathologist blinded to the groups. Formalin-fixed ovarian tissues were embedded in paraffin blocks, sectioned at 5-μm thickness (four sections per sample), stained with haematoxylin and eosin, and examined under a light microscope. Atretic follicles, antral follicles, and corpora lutea were evaluated. We used the techniques described by Goto et al., Andreu et al. and Sadrkhanloo et al. [17–19].

VEGF levels in the peritoneal fluid and arterial blood samples were quantitatively assessed using commercially available enzyme-linked immunosorbent assay kits (RayBio® Rat VEGF-A ELISA Kit; Bender MedSystems Inc. Burlingame, California, USA) according to the manufacturer's instructions. The enzyme immunoassay has an intra-assay variability of 10% and inter-assay variability of 12% for VEGF. Blood serum VEGF measurements were not assessed because of technical problems.

Statistical analysis was performed using Statistical Package for the Social Sciences version 13.0 (SPSS, Chicago, IL, USA). Variables were expressed as mean ± standard deviation (SD). Normal distributions of continuous variables were assessed by Shapiro–Wilk test. Non-normally distributed metric variables were analyzed by the Kruskal–Wallis test, Mann–Whitney *U*-test with post hoc Bonferroni correction, and Wilcoxon signed rank test. *P* values of <0.05 were considered statistically significant.

Results

Weight gain was highest in the OHSS group and was similar to that in all the treatment groups; it was almost twice as much in the OHSS group as it was in the control group (Fig. 1, Table 1).

The hyperstimulation treatment caused a significant increase in ovarian weight. Ovarian weights 48 h after the HCG injection were significantly lower in all treatment groups than they were in the OHSS group (0.4 ± 0.03 g, $p = 0.01$, 0.56 ± 0.09 g, $p = 0.04$ and 0.5 ± 0.17 g, $p = 0.03$; GnRH antagonist group, high and low dose relcovaptan groups, respectively, vs. 0.72 ± 0.13 g OHSS group) (Table 1, Fig. 2). There was no statistically significant difference in ovarian weights between the GnRH antagonist and relcovaptan groups ($p = 0.176$).

The evaluation of peritoneal fluid VEGF-A levels revealed statistically significant differences between levels in the treatment groups and those in the OHSS group ($p = 0.005$), although there was no statistically significant difference between levels in the GnRH antagonist, and high and low dose relcovaptan groups ($p = 0.796$) (Fig. 3).

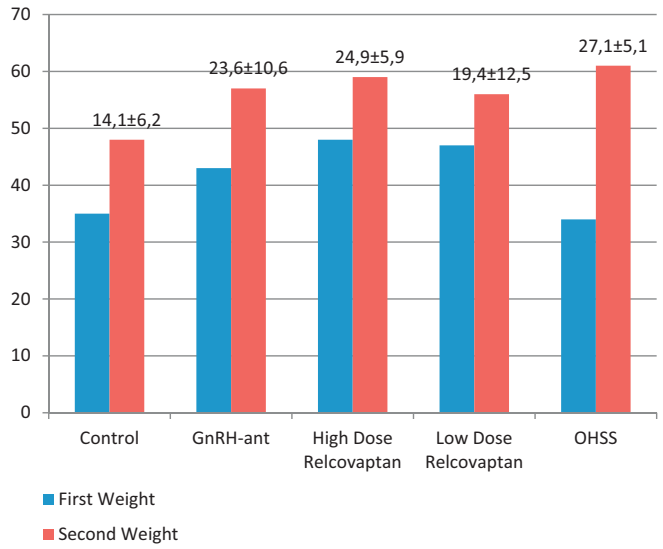

Fig. 1. Comparison of rat weight gain between groups ($p = 0.001^*$). All values are reported as mean ± standard deviation. $^*P < 0.05$, significant difference.

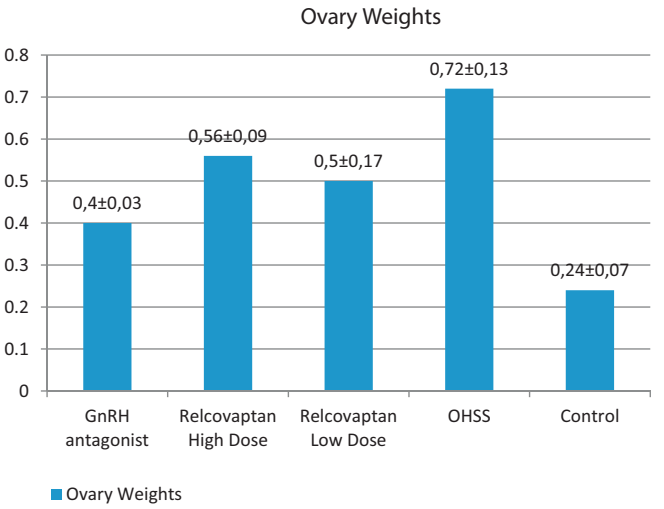

Fig. 2. Comparison of ovary weights between groups ($p = 0.001^*$ for the comparison of all groups, $p = 0.176$ for the comparison of relcovaptan groups and GnRH antagonist). All values are reported as mean ± standard deviation. $^*P < 0.05$, significant difference.

There were small numbers of antral follicles in all groups according to the histopathologic examination, and so we did not include this count in the assessment. In all treatment groups, corpus luteum (CL) counts were prominently lower than they were in the OHSS group (Table 1). The CL counts did not significantly differ between the three treatment groups ($p = 0.322$) (Figs. 4 and 5). There was no CL detected in control group rats. The number of atretic follicles was highest in the GnRH antagonist group (2.3 ± 1.2). Atretic follicle count in the OHSS group was significantly lower (0.66 ± 0.5) than it was in the treatment groups (2.3 ± 1.2 in the GnRH antagonist group, 1.8 ± 0.75 in the high dose relcovaptan group, and 1.1 ± 1.4 in the low dose relcovaptan group) (Fig. 6) ($p = 0.048$). Atretic follicle count did not significantly differ between the three treatment groups ($p = 0.193$) (Table 2).

Comments

We hypothesized that relcovaptan, which acts through inhibition of vasopressin-induced VEGF secretion, may prevent and treat OHSS. To determine whether relcovaptan is able to inhibit vasopressin-induced VEGF secretion in a hyperstimulated rat model previously used with success, we used weight gain, ovarian weight, VEGF-A levels in the peritoneal fluid, and the numbers of CL and atretic follicle in the histopathologic examination. The results of the present study showed that relcovaptan treatment in the hyperstimulated rat model resulted in significantly lower ovarian weight and significant decreases in peritoneal levels of

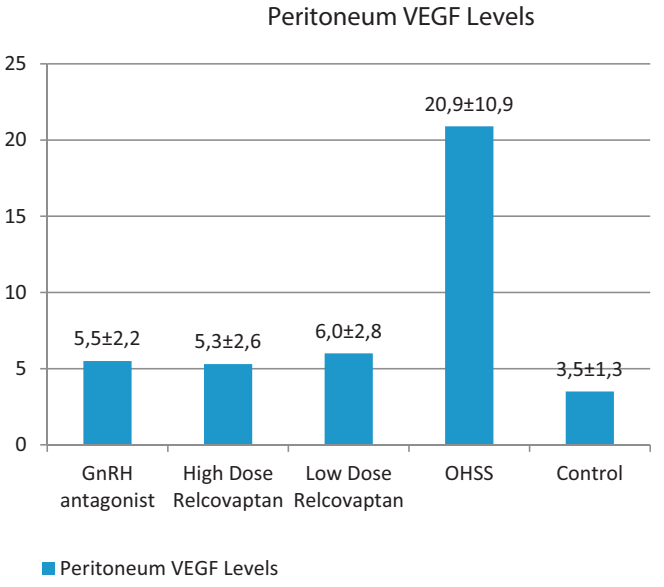

Fig. 3. Comparison of peritoneum VEGF levels between groups ($p = 0.003^*$ for the comparison of all groups, $p = 0.796$ for the comparison of relcovaptan groups and GnRH antagonist). All values are reported as mean ± standard deviation. $^*P < 0.05$, significant difference.

Table 1
Comparison of variables including weight gain, ovarian weights, peritoneal fluid VEGF values, corpus luteum count, and atretic follicle count between groups.

Variables	Group 1 (GnRHanta)	Group 2 (high dose relcovaptan)	Group 3 (low dose relcovaptan)	Group 4 (OHSS)	Group 5 (control)	*P* values
Weight gain (g)	23.6 ± 10.6	24.9 ± 5.9	19.4 ± 12.5	27.1 ± 5.1	14.1 ± 6.2	0.065
Ovarian weights (g)	0.4 ± 0.03[a]	0.56 ± 0.09[a]	0.5 ± 0.17[a]	0.72 ± 0.13	0.24 ± 0.07	0.001*
Peritoneal fluid VEGF values (pg/ml)	5.5 ± 2.2[b]	5.3 ± 2.6[b]	6.0 ± 2.8[b]	20.9 ± 10.9	3.5 ± 1.3	0.003*
Antral follicle count	1 ± 0.1	–	2 ± 05	1 ± 0.5	1 ± 0.05	0.150
Corpus luteum count	29.6 ± 15[c]	31.1 ± 11.5[c]	19.5 ± 11.9[c]	39.0 ± 14.2	–	0.002*
Atretic follicle count	2.3 ± 1.2[d]	1.8 ± 0.75[d]	1.1 ± 1.4[d]	0.66 ± 0.5	2.1 ± 1.1	0.048*

All values are reported as mean ± standard deviation.
[a] $p = 0.176$, [b] $p = 0.796$, [c] $p = 0.322$, [d] $p = 0.193$ (comparison of variables including [a]ovarian weights, [b]peritoneal fluid VEGF values, [c]corpus luteum count, and [d]atretic follicle count between relcovaptan groups and GnRH antagonist).
* $P < 0.05$, significant difference.

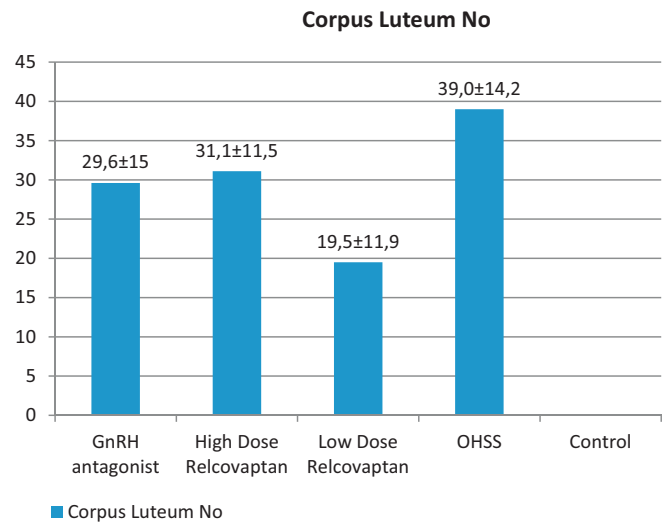

Fig. 4. Comparison of corpus luteum numbers between groups ($p = 0.002^*$ for the comparison of all groups, $p = 0.322$ for the comparison of relcovaptan groups and GnRH antagonist). All values are reported as mean ± standard deviation. $^*P < 0.05$, significant difference.

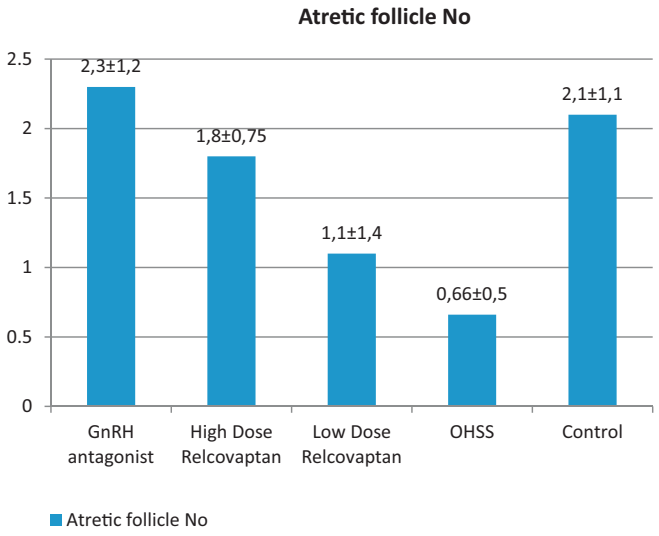

Fig. 5. Comparison of rat weight gain among groups.

VEGF, which is a marker of increasing VP. Relcovaptan treatment also caused significant decreases in the number of CL and significant increases in the number of atretic follicles as shown by the histopathologic examination compared to the OHSS group.

On the other hand, GnRH antagonist treatment has been utilized as the primary intervention for patients undergoing ovarian stimulation who have rapidly rising estradiol concentrations and are at risk for OHSS. GnRH antagonist prevents OHSS by decreasing serum estradiol concentrations, achieving excellent outcomes, while there is no adverse impact on cycles [20]. Moreover, a OHSS rat model revealed that treatment with both a GnRHanta and a GnRHa resulted in significant reductions in serum estradiol and peritoneal vascular permeability, as well as decreased ovarian expression of VEGF, and its two receptors and GnRHanta are more potent than GnRHa in preventing early OHSS through down-regulation of the expression of VEGF and its receptors in hyperstimulated ovaries [21]. The application of a GnRH agonist trigger in GnRH antagonist controlled cycles via an acute release of LH and FSH to reliably trigger final oocyte maturation also provides a unique opportunity to minimize the risk of OHSS in controlled ovarian stimulation [22].

We further compared the therapeutic effect of relcovaptan with GnRH antagonist treatment, which is usually used as a conventional prevention agent of OHSS in IVF cycles, and found that relcovaptan was as effective as GnRH antagonist treatment [23]. Our results indicate that inhibition of vasopressin V1a receptors by relcovaptan may reduce the risk of OHSS as shown by inhibition of ovarian weight increase and vasopressin-induced VEGF secretion.

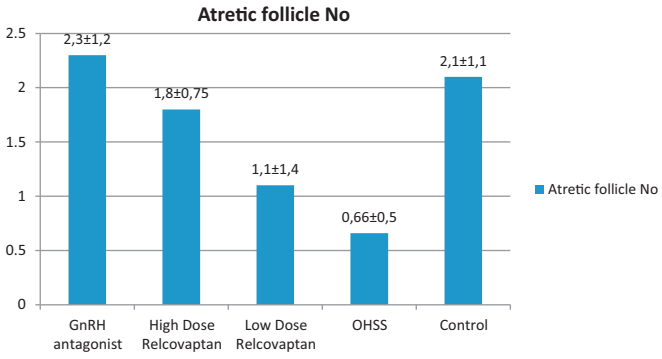

Fig. 6. Comparison of atretic follicle numbers between groups ($p = 0.048^*$ for the comparison of all groups, $p = 0.193$ for the comparison of relcovaptan groups and GnRH antagonist). All values are reported as mean ± standard deviation. $^*P < 0.05$, significant difference.

The specific molecular mechanism responsible for increased vascular permeability in OHSS has remained unclear. Vasoactive peptides which may be responsible for the pathogenesis of OHSS include VEGF, the kinin–kallikrein system, the renin–angiotensin system, and various cytokines [24–29]. Most studies have already demonstrated that VEGF plays a key role in increasing VP, leading to extravasation in the development of OHSS [30,31]. Vasopressin causes an increase in VEGF secretion from human vascular smooth muscle cells through the vasopressin V_{1A} receptor. Vasopressin-induced VEGF secretion by proliferating vascular smooth muscle cells could act as a paracrine hormone to powerfully increase VP.

Table 2
General characteristics of all groups.

Variables	Group 1 (GnRHanta)	Group 2 (high dose relcovaptan)	Group 3 (low dose relcovaptan)	Group 4 (OHSS)	Group 5 (control)	*P* values
Weight gain (g)	23.6 ± 10.6	24.9 ± 5.9	19.4 ± 12.5	27.1 ± 5.1	14.1 ± 6.2	0.065
Ovarian weights (g)	0.4 ± 0.03[a]	0.56 ± 0.09[a]	0.5 ± 0.17[a]	0.72 ± 0.13	0.24 ± 0.07	0.001
Peritoneal fluid VEGF values (pg/ml)	5.5 ± 2.2[b]	5.3 ± 2.6[b]	6.0 ± 2.8[b]	20.9 ± 10.9	3.5 ± 1.3	0.003
Antral follicle count	1 ± 0.1	–	2 ± 05	1 ± 0.5	1 ± 0.05	0.150
Corpus luteum count	29.6 ± 15[c]	31.1 ± 11.5[c]	19.5 ± 11.9[c]	39.0 ± 14.2	–	0.002
Atretic follicle count	2.3 ± 1.2[d]	1.8 ± 0.75[d]	1.1 ± 1.4[d]	0.66 ± 0.5	2.1 ± 1.1	0.048

All values are reported as mean ± standard deviation. $P < .05$, significant difference.
[a] $p = 0.176$, [b] $p = 0.796$, [c] $p = 0.322$, [d] $p = 0.193$ (outcomes were compared between relcovaptan groups and GnRH).

Some limitations of the present study need to be pointed out: (1) not investigating the effects of relcovaptan on serum estradiol and progesterone levels; (2) not using immunohistochemical methods to detect the effect of relcovaptan on expression of VEGF; and (3) not evaluating the effect of reduction in VEGF secretion on endometrium. The effect of relcovaptan on expression of VEGF and serum E2 level in a rat model should be evaluated using immunohistochemical methods in future studies to test our findings. Furthermore, in the endometrium, angiogenesis is essential to provide a vascularized receptive endometrium for implantation. VEGF is expressed in the human endometrium and regulates vascularization in the endometrium. Therefore, inhibition of vasopressin-induced VEGF secretion through relcovaptan could cause deterioration in the development of the endometrium. The effect of relcovaptan on development of the endometrium should be evaluated in future studies [32,33].

We considered it important to evaluate the dose-dependent effects of relcovaptan to determine therapeutic doses, and two different relcovaptan dosages were evaluated in our study. Since there are no reports of relcovaptan usage in any experimental OHSS model before, the different drug doses' upper and lower limits were selected as 5–10 mg/kg. Based on our finding, low dose relcovaptan was as effective as high dose relcovaptan. Furthermore, based on a human study related to relcovaptan mentioned above, we preferred to evaluate the effect of relcovaptan on OHSS in a rat model, but we suppose that it may be used clinically in humans.

In conclusion, inhibition of vasopressin-induced VEGF secretion by a V_{1A} receptor antagonist may be a potential novel strategy for decreasing the risk of OHSS and treating it. Further studies are needed, however, to confirm this mechanism and to determine the optimum effective dose and to evaluate the effects of relcovaptan in humans.

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