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Administration of a gonadotropin-releasing hormone agonist affects corpus luteum vascular stability and development and induces luteal apoptosis in a rat model of ovarian hyperstimulation syndrome

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ABSTRACT

Ovarian hyperstimulation syndrome (OHSS) is a complication of ovarian stimulation with gonadotropins followed by the administration of human chorionic gonadotropin (hCG) to trigger the final steps of oocyte maturation. Gonadotropin-releasing hormone (GnRH) analogs are thought to be effective in preventing this complication and a clinical trial has found a lower incidence of OHSS in patients treated with these molecules. Our aim was to analyze the in vivo effect of a GnRH-I agonist on corpus luteum development and regression, ANGPT-1, ANGPT-2 and Tie-2 protein expression and luteal blood vessel stabilization, the expression of the steroidogenic acute regulatory protein (StAR) and the cytochrome P450 side-chain cleavage enzyme (P450scc) and cell proliferation, in ovaries from an OHSS rat model. To this end immature female Sprague-Dawley rats were hyperstimulated and treated with a GnRH-I agonist from the start of pregnant mare serum gonadotropin (PMSG) administration until the day of hCG injection for 5 consecutive days. Blood and tissue samples were collected 48 h after hCG injection. Vascular endothelial growth factor VEGF levels were evaluated in the peritoneal fluid by ELISA. Serum progesterone and estradiol were measured by RIA. Histological features of sectioned ovaries were assessed in hematoxylin and eosin (H&E) stained slides. Luteal blood vessel stability, cell proliferation and apoptosis were assessed by immunohistochemistry for SMCA, PCNA, and TUNEL, respectively. P450scc, StAR, FLK-1, ANGPT-1, ANGPT-2, Tie-2 and PCNA protein levels were evaluated by Western blot from dissected corpora lutea (CL). The treatment with the GnRH-I agonist significantly decreased serum progesterone and estradiol levels as well as P450scc and StAR protein expression in the untreated OHSS group. In addition, the agonist significantly decreased the number of CL in the OHSS group, as compared with the untreated OHSS group. In the OHSS group, the area of periendothelial cells in the CL was larger than that of the control group. However, the treatment with the GnRH-I agonist significantly reduced the area of periendothelial cells in the CL in the OHSS group. The luteal levels of ANGPT-1 and its receptor Tie-2 significantly increased in the OHSS group when compared with the control group. Conversely, the administration of the GnRH-I agonist significantly decreased the levels of these factors in the CL from the OHSS group, as compared with the untreated OHSS group. In addition, the treatment with the GnRH-I agonist reduced the diameter of CL and decreased CL cell proliferation as compared with that observed in the untreated OHSS group. Finally, the GnRH-I agonist increased apoptosis in the CL from the OHSS group. In conclusion, these results show that GnRH-I agonist exerts diverse actions on the CL from a rat OHSS model. The decrease in P450scc, StAR, ANGPT-1 and Tie-2 expression, blood vessel stability and luteal proliferation leads to CL regression in the ovaries from OHSS rats. Moreover, our results suggest that the downregulation of ANGPT-1 and its receptor is a possible mechanism whereby GnRH-I agonists could prevent early OHSS.

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1. Introduction

The ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication associated with ovarian stimulation for the treatment of infertility (Budev et al., 2005; Rizk et al., 1997). OHSS occurs in 5–10% of patients undergoing ovulation induction therapy, and the severe form takes place in 0.5–5.0% (Aboulghar and Mansour,

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2003; Delvigne and Rozenberg, 2002). It is widely accepted that the main clinical components of this syndrome are marked enlargement of the ovaries, which contain luteal cysts, and hemorrhagic cysts along with the shifting of fluid to the third space, including the peritoneal cavity (Golan et al., 1989). Complications include renal failure, hypovolemic shock, thromboembolic episodes, and adult respiratory distress syndrome. Although the pathophysiology of OHSS has not been completely elucidated, it seems likely that increased capillary permeability, triggered by the release of vasoactive substances from the hyperstimulated ovaries under human chorionic gonadotropin (hCG) stimulation, might play a key role in the onset of OHSS.

Vascular endothelial growth factor (VEGFA), also referred to as vascular permeability factor, is a potential mediator in the development of OHSS because of its vasoactive properties (Senger et al., 1983). It has been found that the follicular fluid (FF) from women with severe OHSS presents high concentrations of VEGFA that lead to increased endothelial cell permeability and can be attenuated by VEGF antibody (Levin et al., 1998). High levels of VEGFA have also been detectable in sera (Lee et al., 1997) and ascites from OHSS patients (Chen et al., 1999). Several authors have shown that the source of VEGFA in OHSS patients seems to be the hyperstimulated ovary, since the VEGFA concentration in FF is 100 times higher than that in the serum (Geva and Jaffe, 2000; Krasnow et al., 1996; Lee et al., 1997). Accordingly, ovarian VEGFA, which originates mainly from ovarian follicular cells, is considered to play a major role in inducing OHSS. While the VEGF is the main initiator of angiogenesis, the formation and differentiation of a structurally and functionally mature vascular network probably requires the coordinated action of various factors. These include angiopoietins ANGPT-1 and ANGPT-2, which act via the tyrosine kinase receptor Tie-2 (Maisonpierre et al., 1997). Unlike VEGF, ANGPT1 is unable to stimulate endothelial cell proliferation (Davis et al., 1996) but is required for the recruitment of periendothelial cells that lead to the maturation and stabilization of newly developed capillaries (Maisonpierre et al., 1997; Suri et al., 1996). The ANGPT/Tie-2 system is expressed in ovarian follicles and corpora lutea (CL) of rodents, bovines and primates (Hayashi et al., 2003; Hazzard et al., 1999; Maisonpierre et al., 1997; Sugino et al., 2005; Wulff et al., 2001). To produce progesterone, not only high vascularization but also stabilization of blood vessels in the CL is necessary to provide luteal cells with the large amounts of cholesterol needed for progesterone synthesis. Hence, the blood vessels in the CL need to stabilize and mature to serve as functional blood vessels (Jain and Booth, 2003). It is worth mentioning that the mature CL is highly vascularized, with 50–70% of the tissue comprised of microvascular pericytes (periendothelial cells) and endothelial cells (Redmer et al., 2001; Reynolds et al., 2000). Pericytes are mesodermally derived cells that wrap around the outside of capillaries and are separated from endothelial cells by a basement membrane. Pericytes are of the vascular smooth muscle cell (SMC) lineage (Challier et al., 1995). Accordingly, ANGPTs act on vascular endothelial cells and contribute to blood vessel stabilization through interaction with perivascular cells (pericytes) (Thurston et al., 2000). However, to date, no reports have addressed the actions of GnRH-I agonists in blood vessel stability, or in ANGPT-1, ANGPT-2 and Tie-2 expression in the CL of a rat model of OHSS.

Chronic administration of GnRH-I agonists leads to pituitary desensitization and inhibition of gonadotropin and sex steroid levels. Besides their effects on the pituitary–gonadal axis, GnRH-I agonists have the potential to modulate the ovarian function through a direct effect on the ovary. Numerous studies have documented a functional ovarian GnRH-I and GnRH receptor system in rat and human ovaries (Hsueh and Schaeffer, 1985; Kang et al., 2001; Uemura et al., 1994).

We have previously demonstrated that the treatment with a GnRH-I agonist in prepubertal rats causes an increase in ovarian follicle DNA apoptotic fragmentation by interfering with the FSH, cAMP and/or growth factors pathways (Andreu et al., 1998; Parborell et al., 2001). Moreover, several studies performed in rats have demonstrated the antigonadal effect of GnRH analogs administered either in vivo or in vitro (Hazum and Nimrod, 1982; Muttukrishna et al., 1996; Sridaran et al., 1999; Yang et al., 2003). In addition, we have shown an apoptotic follicular effect of GnRH-I agonist in antral follicles, which correlates with an imbalance in the ratio of antiapoptotic:proapoptotic proteins (BCL-xL/BCL-xS) (Parborell et al., 2002, 2005). These results indicate that GnRH-I interferes with follicular development through an increase in apoptotic events mediated by an imbalance among the BCL-2 family members. Moreover, Harwood et al. (1980) have reported that GnRH-I agonists affect the luteinization process of superovulated rats.

The effect of GnRH-I agonists on ovarian angiogenesis and their regulation is still largely unknown. By using a rat model of ovarian hyperstimulation syndrome (OHSS), Kitajima et al. have shown that the treatment with a GnRH-I agonist reduces ovarian vascular permeability and that this effect seems to be mediated by an increase in the expression of the tight junction protein claudin (Kitajima et al., 2004, 2006). Moreover, we have previously reported that a decline of the levels of VEGFA and its receptor Flk-1/KDR and ANGPT-1 by effect of GnRH-I agonist administration in prepubertal pregnant mare serum gonadotropin (PMSG)-treated rats is one of the mechanisms involved in the apoptosis of ovarian cells, and that this suggests an intraovarian role of an endogenous GnRH-like peptide in follicular development induced by gonadotropins (Parborell et al., 2008).

Presently, there is no cure for OHSS, and only preventive strategies are available. GnRH antagonists and/or GnRH agonists are now used for the treatment of OHSS (Dal Prato and Borini, 2005; Humaidan, 2009; Ragni et al., 2005). However, so far, no report has studied the in vivo effect of GnRH-I agonists on luteal development, luteal blood vessel stabilization, apoptosis and proliferation in the ovary from a rat model of OHSS. Therefore, the main purpose of this study was to investigate the in vivo effects of a GnRH-I agonist on steroidogenesis, peritoneal VEGF concentrations, FLK-1, ANGPT-1, ANGPT-2 and Tie-2 protein levels, luteal vascular stability, luteal development, apoptosis and proliferation in ovaries from an immature OHSS rat model stimulated by PMSG and hCG.

2. Materials and methods

2.1. Hormones and drugs

The GnRH agonist leuprolide acetate (Lupron) was a donation from Abbot Laboratories (Buenos Aires, Argentina) in the original ampoule (2.8 mg/5 ml). PMSG (Novormon) was provided by Syntex S.A. (Buenos Aires, Argentina). hCG (Endocorion) was purchased from ELEA Laboratories (Buenos Aires, Argentina). Polyclonal primary antibodies for Flk-1 (sc-6251), PCNA (sc-7907), ANGPT-1 (sc-9360), Tie-2 (sc-9026) and actin B (sc-1616) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-rabbit and anti-mouse secondary antibodies conjugated with horseradish peroxidase were purchased from Sigma Aldrich (St. Louis, MO). All other chemicals were of reagent grade and were obtained from standard commercial sources.

2.2. Steroid hormones and VEGF assays

Serum steroid levels were measured by RIA ($n = 6$ rats/group) (Irusta et al., 2003, 2007). Progesterone (P_4) and estradiol (E_2) were measured by using specific antibodies supplied by Dr. G.D. Niswender (Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO). Under these conditions, the intra-assay and inter-assay variations were 8.0% and 14.2%, respectively. The values are expressed per ml of serum.

Levels of peritoneal VEGF were analyzed with a commercially available ELISA kit (Quantikine; R&D Systems Inc., Minneapolis, MN). This kit is designed to measure VEGF165 levels in cell culture supernates, serum, and plasma. Intra-assay and inter-assay coefficients of variation for VEGF were 5.4% and 7.3%, respectively.

2.3. OHSS induction and experimental design

Rats were cared and housed at the Instituto de Biología y Medicina Experimental (IByME), Buenos Aires, Argentina. Rats ($n = 6$/group for each treatment) were from our colony. Immature female Sprague-Dawley rats (21–23 days old) were allowed food and water ad libitum and kept at room temperature (21–23 °C) on a 12L:12D cycle. All protocols and experiments were approved by the ethics committee of the IByME and conducted according to the guide for the care and use of laboratory animals of NIH (National Institute of Health, USA).

We used an animal model that develops OHSS in immature Sprague-Dawley rats (21–23 days, 60–80 g). The model was first described by Ujioka et al. (1997) and later used by other authors (Gomez et al., 2002; Kitajima et al., 2004; Ozcakir et al., 2005; Ujioka et al., 1997).

The rats were randomly divided into three groups. The control group was injected with PMSG (10 UI, 23rd day of life at 09:00 h), and an injection of hCG 48 h later (10 UI, 25th day of life at 09:00 h). The OHSS group (hyperstimulated rats) received excessive doses of PMSG (50 UI/day) injected for 4 consecutive days (from the 21st to the 24th day of life, at 09:00 h), followed by hCG (25 UI, 25th day of life at 09:00 h). The OHSS + GnRH-I agonist group (GnRH-agonist-treated hyperstimulated rats) received the same doses of gonadotropins as the OHSS group and received injections of the GnRH agonist for 5 consecutive days (leuprolide acetate, 100 μg/kg/day, from the 21st to the 25th day of life; twice a day at 09:00 and 17:00 h), followed by hCG (25 UI, 25th day of life at 09:00 h).

Rats were then killed by CO_2 asphyxiation 48 h after the hCG injection. The ovaries were removed and cleaned of adhering tissue in culture medium, weighed, and used for subsequent assays. The blood was used for hormone assays. One of the ovaries from each of the six rats from each of the three groups ($n = 6$) was used for Western immunoblot assays and the contralateral ovary for immunohistochemical assays.

2.4. Ovarian morphology

Ovaries were extracted from the different experimental groups and immediately fixed in formalin 4% for 12 h. Histological sections were stained with hematoxylin-eosin (H&E). Five-micrometer step sections were mounted at 50 μm intervals onto microscope slides to prevent counting the same structure twice, according to the method described by Woodruff et al. (1988). Follicles were classified as either preantral follicles (PFs) or early antral follicles (EAFs) according to the presence or absence of either an antrum or preovulatory follicles (POFs). An atretic follicle (Atret. F) was defined as the follicle that presented more than 10 pycnotic nuclei per follicle; in the smallest follicles, the criterion for atresia was a degenerate oocyte, precocious antrum formation, or both (Andreu et al., 1998; Sadrkhanloo et al., 1987). Randomly selected fields from each ovarian section (six sections/ovary, $N = 6$ ovaries/group) were analyzed and expressed as percentage follicles or CL/ovary.

To assess structural luteolysis (regression of the CL), we used the technique described by Goto et al. (1999). We measured the diameters of the CL that were located mainly in the cortex of the ovary.

2.5. Immunohistochemistry

Ovaries from the different experimental groups were immediately fixed in 4% neutral buffered formalin for 12 h and then embedded in paraffin. Tissue sections were deparaffinized in xylene and rehydrated by graduated ethanol washes. Endogenous peroxidase activity was blocked with hydrogen peroxide in PBS solution and nonspecific binding was blocked with 2% bovine seroalbumin (BSA) overnight at 4 °C. Sections were incubated with rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA 1/100) or mouse monoclonal anti-smooth muscle cell α-actin (SMCA 1/100) overnight at 4 °C. SMCA is a specific marker for vascular smooth muscle and pericytes (Hirschi and D'Amore, 1996; Wulff et al., 2000). After washing, the slides were incubated with biotinylated anti-rabbit IgG or anti-mouse IgG, and then with avidin-biotinylated horseradish peroxidase complex (Vectastain ABC system from Vector Laboratories, Burlingame, CA) for 30 min. Protein expression was visualized with diaminobenzidine (DAB) staining. The reaction was stopped with distilled water, stained with hematoxylin and dehydrated before mounting (Canada Balsam Synthetic, Biopack, Argentina). Negative controls were obtained in the absence of primary antibody. No specific immunoreactivity was detected in the sections.

To perform this study, three randomly selected fields were analyzed from each ovarian section (six sections/ovary, six ovaries).

The whole area of the luteal compartment and the SMCA-positive area within the compartment were measured.

PCNA-positive cells were expressed as a percentage of the total number of cells (proliferation index).

The images were digitized using a camera (Nikon, Melville, NY, USA) mounted on a conventional light microscope (Nikon), using a magnification of 400×. Finally, the images were converted to TIFF format (bi-level scale) for their analysis. The data were processed using Image J (Image Processing and Analysis in Java, National Institute of Health, USA).

2.6. Western blots

Ovaries were removed and placed on ice. The individual CL were dissected from the ovary by ovarian microdissection under a stereoscopic microscope using a 25-gauge needle. Four or five corpora lutea/ovary were resuspended in five volumes of lysis buffer (20 mM Tris–HCl pH 8, 137 mM NaCl, 1% Nonidet P-40 and 10% glycerol) supplemented with protease inhibitors (0.5 mM PMSF, 0.025 mM N-CBZ-L-phenylalanine chloromethyl ketone, 0.025 mM N-p-tosyl-lysine chloromethyl ketone and 0.025 mM L-1-tosylamide-2-phenyl-ethylchloromethyl ketone) and homogenized with an Ultra-Turrax (IKA Werk, Breisgau) homogenizer. Samples were centrifuged at 4 °C for 10 min at 10,000 × g and the resulting pellets were discarded. Protein concentration in the supernatant was measured by the Bradford assay. After boiling for 5 min, 40 μg of protein was applied to a 12% SDS-polyacrylamide gel and electrophoresis was performed at 25 mA for 1.5 h. The resolved proteins were transferred for 2 h onto nitrocellulose membranes. The blot was preincubated in blocking buffer (5% nonfat milk, 0.05% tween-20 in 20 mM TBS pH 8.0) for 1 h at room temperature and incubated overnight with appropriate primary antibodies (P450scc 1/2000, StAR 1/1000, ANGPT-1 1/500, Tie-2 1/100, Flk-1 1/50 and PCNA 1/200 in TBS) in blocking buffer at 4 °C. The blot was then incubated with anti-mouse or anti-rabbit secondary antibodies conjugated with horseradish peroxidase (1:1000) and finally detected by chemiluminescence and autoradiography using X-ray film. The density in each band was normalized to the density of the actin B band, which was used as an internal control.

2.7. Quantification for Western blot assay

Equal amounts of protein were loaded for all samples, and different groups in one experiment were loaded on the same gel. For quantification, a screening was performed on blots with X-ray film using different times of exposure to optimize the signal. The levels of protein were compared and analyzed by densitometric studies using Scion Image for Windows (Scion Corporation, Worman's Mill, CT). Optical density data are expressed as arbitrary units ± SEM ($n = 6$).

2.8. TUNEL technique

For immunohistochemical quantification of apoptosis, formalin-fixed tissue sections were processed for in situ localization of nuclei exhibiting DNA fragmentation by the TUNEL technique (D'Herde et al., 1994) using an apoptosis detection kit (Apoptag plus peroxidase in situ Apoptosis detection kit; Chemicon International, Inc.) as previously described (Andreu et al., 1998). The 4-μm-thick tissue sections were deparaffinized and digested for 15 min at room temperature with 20 μg/ml of proteinase K (Gibco). Endogenous peroxidase was quenched with 3% hydrogen peroxide in PBS. The labeling reaction was carried out by incubating tissue sections with buffer containing digoxygenin-dUTP prior to incubation with TdT for 1 h at room temperature. Tissues were then incubated for 30 min with a peroxidase-conjugated anti-digoxygenin monoclonal antibody, and apoptotic cells were visualized as positively immunostained structures after reaction with DAB. Negative controls included TdT omission. Sections were counterstained with hematoxylin. The number of apoptotic cells was determined by counting labeled cells from CL in 400× microscopic fields (four sections/ovary; six ovaries) and expressed as the number of apoptotic cells/area.

2.9. Data analysis

Data are expressed as the mean ± SEM of at least four experiments, using 5–6 animals per group. The figures show representative gels. Differences between the groups were evaluated using one-way ANOVA, followed by Tukey's test or the multiple comparison Newman–Keuls test. Values of $p < 0.05$ were considered significant. For statistical analysis of data we used the statistical program Prism v5.0.

3. Results

3.1. Measurement of ovarian weight, steroids and VEGF and FLK-1 levels

The effects of the GnRH-I agonist on ovary weight, steroid hormone serum levels, and VEGF and FLK-1 protein levels are summarized in Table 1. The ovarian weight of the rats is expressed as the weight of individual ovaries. The ovarian weight in hyperstimulated rats (OHSS group) was significantly greater than that in the control group ($p < 0.001$). The treatment of the animals that developed OHSS with the GnRH-agonist significantly reduced their ovarian weight as compared with the OHSS group without treatment ($p < 0.001$) (Table 1).

Table 1
Comparison of ovarian weight, serum levels of steroids hormones (estradiol and progesterone), peritoneal levels of VEGF and protein expression of FLK-1 in the three experimental groups: control, OHSS and OHSS treated with a GnRH-I agonist.

	Control ($n=6$)	OHSS ($n=6$)	OHSS + GnRH-I agonist ($n=6$)
Ovarian weight (g)	0.035 ± 0.004^a	0.158 ± 0.012^b	0.075 ± 0.003^a
Serum estradiol concentration (ng/ml)	0.261 ± 0.058^a	1.429 ± 0.102^b	0.308 ± 0.074^a
Serum progesterone concentration (ng/ml)	87.890 ± 17.770^a	356.800 ± 32.680^b	77.600 ± 15.470^a
VEGF peritoneal concentration (pg/ml)	27.550 ± 1.954^a	103.400 ± 6.637^b	60.940 ± 13.660^c
FLK-1 protein expression (arbitrary units)	0.729 ± 0.129^a	2.076 ± 0.395^b	0.764 ± 0.193^a

Note: OHSS: ovarian hyperstimulation syndrome. Data are expressed as mean ± SEM; $n=6$; a vs b: $p<0.001$; b vs c: $p<0.05$; c vs a: $p<0.01$; Tukey's multiple comparison test.

Table 1 also shows the levels of steroid hormones in the rat serum of the three experimental groups. The results show that the levels of progesterone and estradiol in the OHSS group were significantly higher than those in the control group ($p<0.001$). On the other hand, we observed that both hormones were diminished in the OHSS group treated with the GnRH-I agonist as compared with the untreated OHSS group ($p<0.001$).

Knowing that VEGF is the main factor involved in the pathology of OHSS, the level of this glycoprotein was measured in the peritoneal liquid of the rats. VEGF levels in the OHSS group

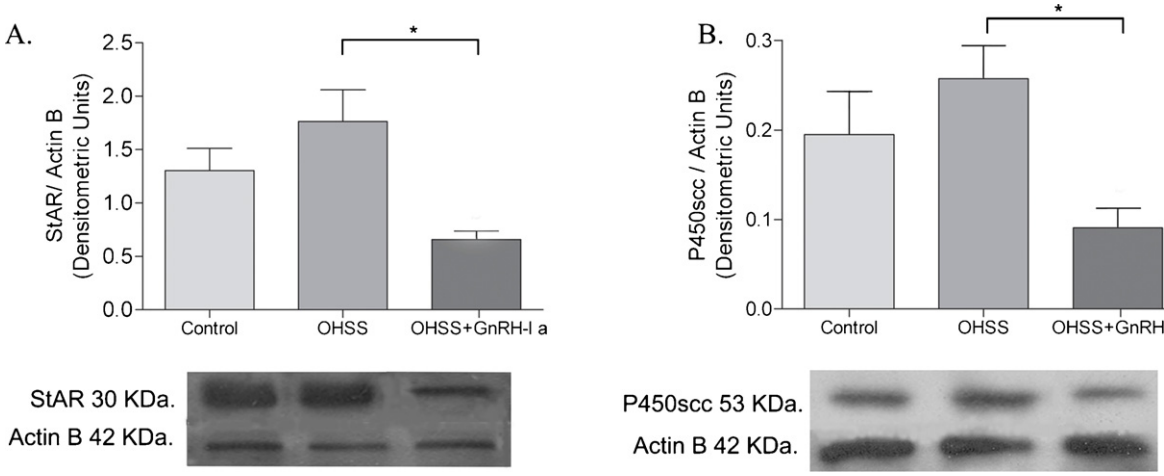

Fig. 1. Effect of treatment with a GnRH-I agonist on StAR and P450scc protein levels in corpora lutea in an OHSS rat model. (A) Densitometric quantification of StAR in corpora lutea (CL). Optical density is expressed as arbitrary units ± SEM normalized to actin B ($n=6$/group, $^*p<0.05$). Representative immunoblots of StAR content are shown in the lower panel. (B) Densitometric quantification of P450scc in CL. Optical density is expressed as arbitrary units ± SEM normalized to actin B ($n=6$/group, $^*p<0.05$). Representative immunoblots of P450scc content are shown in the lower panel.

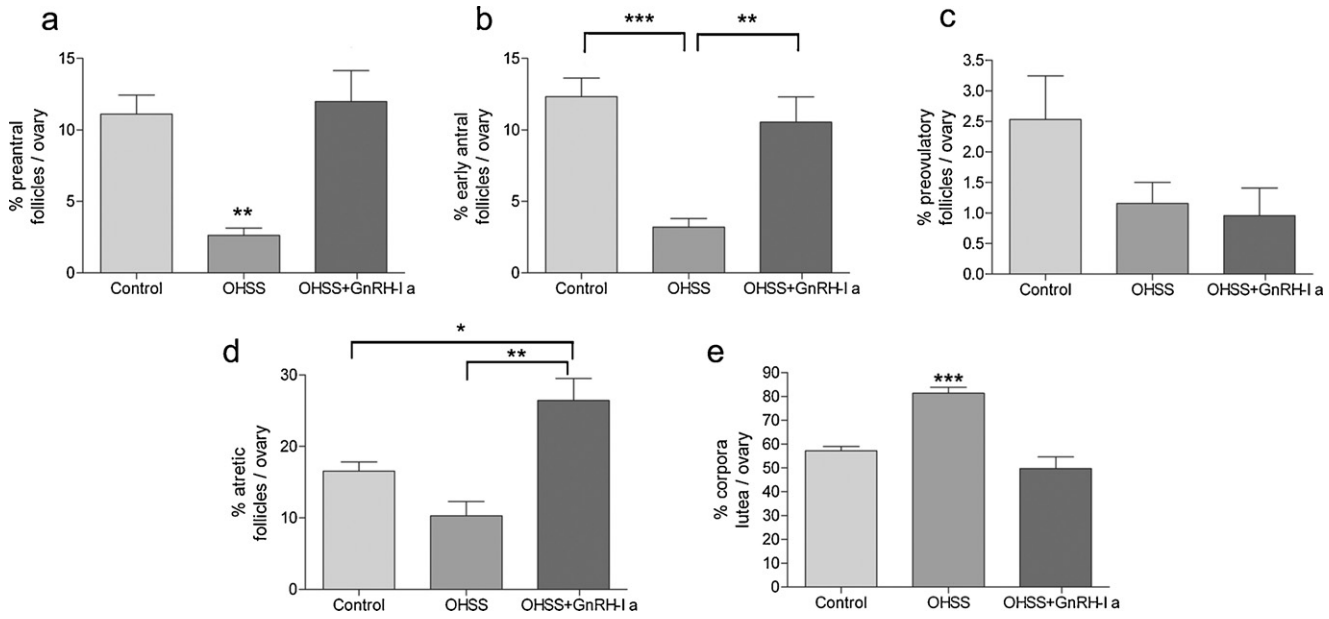

Fig. 2. Effect of treatment with a GnRH-I agonist on the ovarian morphology in an OHSS rat model. (a) Percentage of preantral follicles/ovary, PFs ($^{**}p<0.01$); (b) percentage of early antral follicles/ovary, EAFs ($^{***}p<0.001$; $^{**}p<0.01$); (c) percentage of preovulatory follicles/ovary, POFs; (d) percentage of atretic follicles/ovary, Atret. Fs ($^*p<0.05$; (b) vs $^{***}p<0.001$); (e) percentage of corpora lutea/ovary, CL ($^{***}p<0.001$). Data are expressed as the percentage of PFs, EAFs, POFs, Atret. Fs and CL per ovary ± SEM ($n=6$ ovaries/group).

increased approximately four times compared with the control group ($p < 0.001$). The treatment with the GnRH-I agonist resulted in a statistically significant decrease in the peritoneal levels of VEGF ($p < 0.05$). Also, we evaluated the expression of the main VEGF receptor, Flk-1, by Western blot. This receptor appears to be involved mainly in regulating vascular permeability, angiogenesis and vasculogenesis (Shalaby et al., 1995). There was a statistically significant increase in the levels of Flk-1 in the OHSS group as compared with the control group ($p < 0.01$). Furthermore, the treatment with the GnRH-I agonist reduced the expression of Flk-1 in hyperstimulated rats ($p < 0.01$).

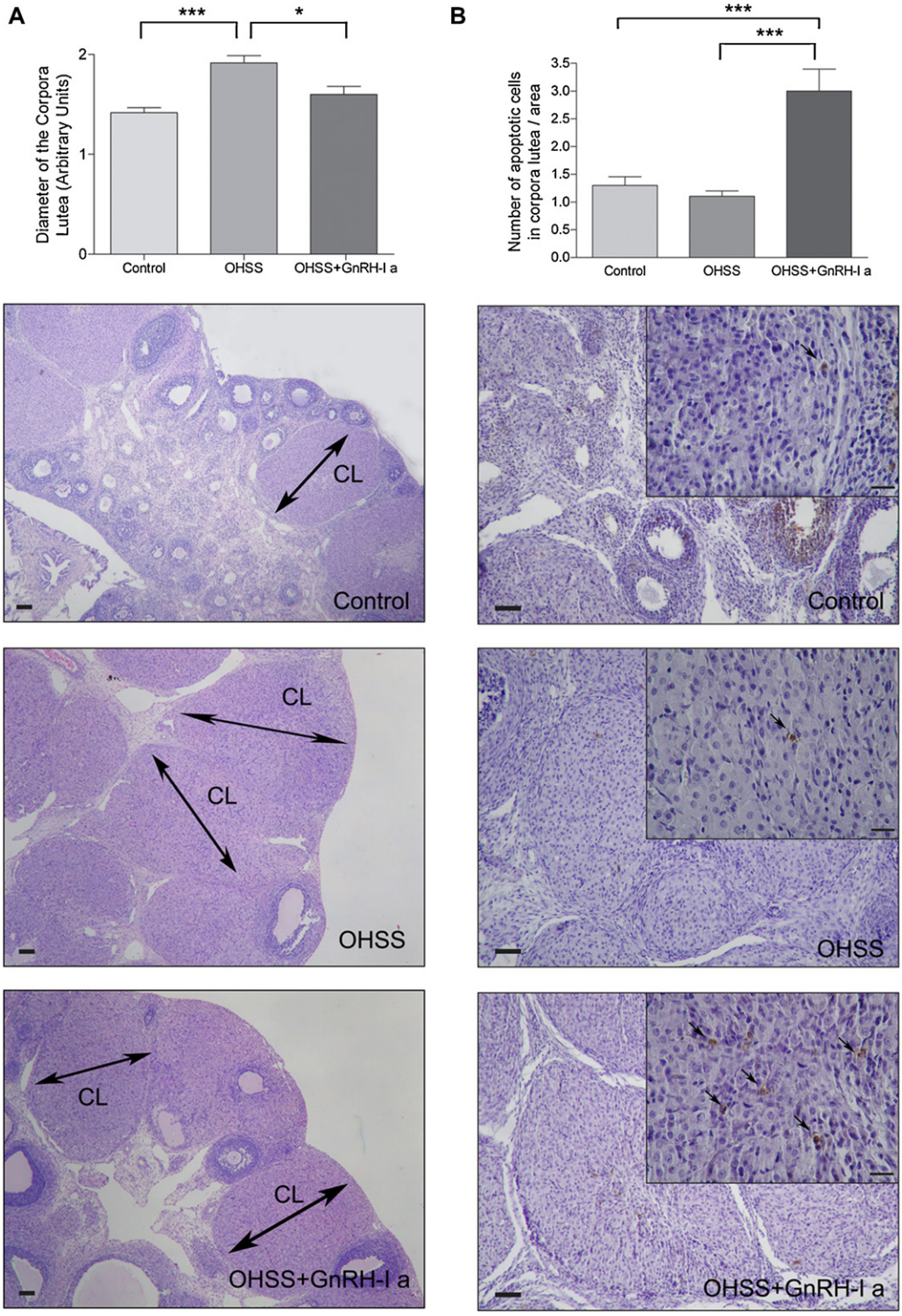

Fig. 3. Effect of treatment with a GnRH-I agonist on luteolysis in an OHSS rat model. (A) Analysis of the CL diameter ($n = 6$ ovaries/group; $^{***}p < 0.001$; $^{*}p < 0.05$). Photographs show representative fields of hematoxylin and eosin stained ovarian sections that show the diameter of the CL (solid arrows) in the three experimental groups. Original magnification 40×. CL: corpus luteum. Scale bars represent 100 μm. (B) In situ DNA fragmentation analysis (TUNEL technique). The number of apoptotic cells in the CL was determined by counting labeled cells at 400× in randomly selected fields of CL. Data are expressed as the number of apoptotic cells per field ± SEM. Three sections per ovary were analyzed ($n = 6$/group, $^{***}p < 0.001$). Photographs show representative fields of TUNEL positive ovarian sections. Scale bars represent 20 μm.

3.2. Luteal cytochrome P450scc and StAR protein expression

Since the treatment with the GnRH-I agonist significantly decreased serum progesterone and estradiol levels, we decided to study the expression of P450scc and StAR in the CL of rats from the OHSS model. In this model, the administration of the agonist significantly reduced P450scc and StAR protein expression ($p < 0.05$) (Fig. 1). These values were similar to those observed in the control group.

3.3. Ovarian morphology

In order to study the effect caused by the GnRH-I agonist on the ovarian morphology in the OHSS rat model, ovarian histological slides were stained with H&E to determine the number of different follicle stages (Fig. 2). The GnRH-I agonist significantly increased the percentages of PFs ($p < 0.01$), EAFs ($p < 0.01$) and Atret. Fs ($p < 0.05$) compared with the untreated OHSS group. The percentage of total CL in the OHSS group was significantly higher than that in control rats ($p < 0.001$). The treatment of hyperstimulated animals with the GnRH-I agonist significantly reduced the percentage of CL ($p < 0.001$).

3.4. Luteal regression

On the basis of the ovarian morphological results described above in the ovary sections, and knowing that GnRH-I agonists possess a direct apoptotic effect on follicular and luteal cells (Andreu et al., 1998; Giampietro et al., 2006; Parborell et al., 2002, 2005; Sifer et al., 2003), we decided to evaluate luteal regression in the ovaries from rats of the OHSS model.

Structural luteolysis was studied by measuring of the diameters of the CL, since this is considered as an indicator of luteal regression (Goto et al., 1999) (Fig. 3A). The diameters of the CL of the OHSS group were significantly larger than those of the control group ($p < 0.001$). The treatment with the GnRH-I agonist restored the size of the CL to control values ($p < 0.05$).

Apoptosis in the CL was evaluated by the quantification of the number of apoptotic cells by TUNEL. Sections of ovaries from the different experimental groups were evaluated for the in situ location of nuclei exhibiting fragmentation of apoptotic DNA. The results show that the treatment with the GnRH-I agonist increased the number of apoptotic cells in the CL from ovaries from OHSS rats compared with those from the untreated OHSS group ($p < 0.001$) (Fig. 3B).

3.5. Blood vessel stability in corpora lutea

To determine the effect of the GnRH-I agonist on the vascular stability of the CL from rats of the OHSS model, blood vessels of the CL were immunostained with SMCA antibody (periendothelial cell marker), and the percentage of vascular stability area was quantified (Fig. 4). The results show that the area of periendothelial cells was larger in the OHSS group than in the control group ($p < 0.001$). Furthermore, the treatment with the GnRH-I agonist significantly reduced the area of periendothelial cells in the CL ($p < 0.01$).

3.6. ANGPT-1, ANGPT-2 and Tie-2 protein levels in corpora lutea

Since ANGPTs play important roles in angiogenesis and blood vessel stabilization during luteal development, we decided to evaluate the effect of the GnRH-I agonist on protein expression of ANGPT-1, ANGPT-2 and its receptor, Tie-2, in the CL from the different groups. The luteal content of these angiogenic factors was measured by Western blotting (Fig. 5).

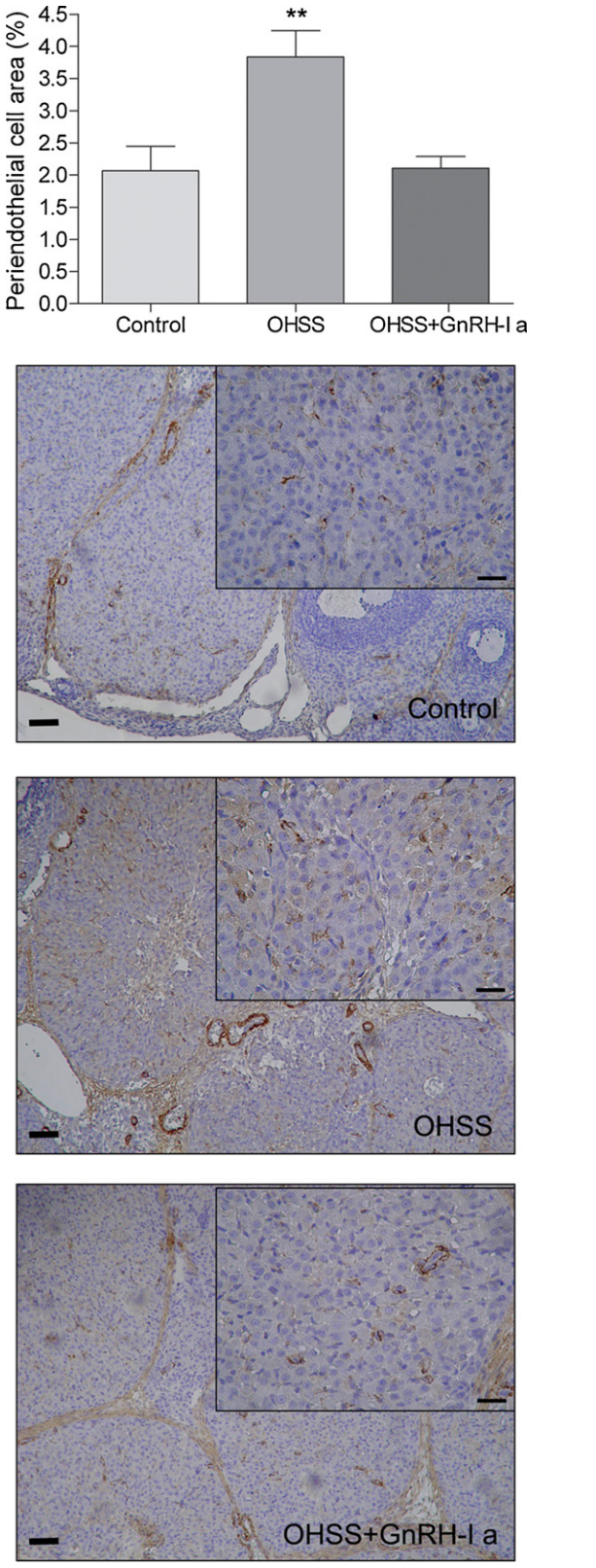

Fig. 4. Effect of treatment with a GnRH-I agonist on blood vessel stability in corpora lutea in an OHSS rat model. SMCA expression analysis in CL. The content of SMCA is expressed as the percentage of periendothelial cell area as a function of total area of randomly selected fields. Data are expressed as mean ± SEM. Three sections per CL were analyzed ($n = 6$ ovaries/group, $^{**}p < 0.01$). Photographs show representative fields of ovarian sections immunostained for SMCA in the three experimental groups. Scale bars represent 50 μm (inserts 20 μm).

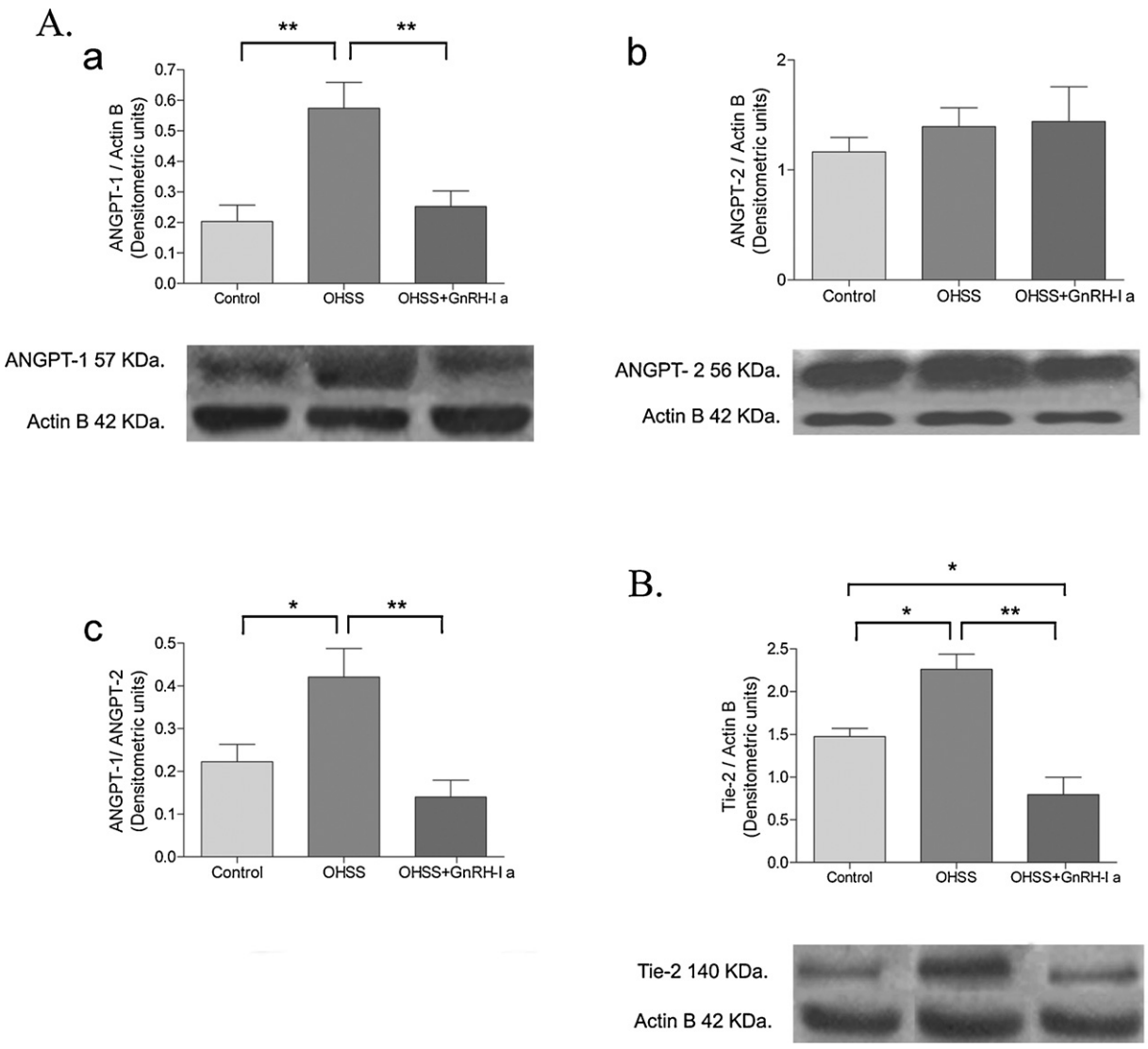

Fig. 5. Effect of treatment with a GnRH-I agonist on ANGPT-1, ANGPT-2 and Tie-2 protein levels in corpora lutea in an OHSS rat model. (A): (a) densitometric quantification of ANGPT-1 in CL (**$p < 0.01$). (b) Densitometric quantification of ANGPT-2 in CL. Optical density is expressed as arbitrary units ± SEM normalized to actin B ($n = 6$/group). Representative immunoblots of ANGPT-1 and ANGPT-2 content are shown in the lower panel. (c) ANGPT-1/ANGPT-2 ratio (*$p < 0.05$; **$p < 0.01$). (B) Densitometric quantification of Tie-2 in CL. Optical density is expressed as arbitrary units ± SEM normalized to actin B ($n = 6$/group, *$p < 0.05$, ***$p < 0.001$). Representative immunoblots of Tie-2 content are shown in the lower panel.

In the OHSS group, the ANGPT-1/ANGPT-2 ratio significantly increased compared with the control group ($p < 0.05$). However, the treatment with the GnRH-I agonist significantly decreased this ratio in the CL compared with the untreated OHSS group ($p < 0.01$). In addition, in the OHSS group, the protein levels of Tie-2 significantly increased compared with the control group ($p < 0.05$). Nevertheless, the GnRH-I agonist significantly decreased this receptor compared with the OHSS group ($p < 0.001$).

3.7. Cell proliferation in corpora lutea

To analyze whether the treatment with the GnRH-I agonist has an inhibitory effect in CL growth, cellular proliferation was studied in the OHSS model. Immunohistochemistry was used to evaluate PCNA expression in the CL. The proliferation index (PCNA-positive cells expressed as a percentage of the total number of cells) in the OHSS group was significantly greater in CL compared with the control group ($p < 0.001$). The treatment with the GnRH-I agonist diminished the proliferation index in the CL with regard to the untreated hyperstimulated group ($p < 0.05$) (Fig. 6A).

These results were corroborated by Western blot (Fig. 6B). The expression of PCNA in the OHSS group was significantly increased compared with the control group ($p < 0.05$). In addition, the treatment with the GnRH-I agonist restored the levels of PCNA to the ones observed in the control group.

4. Discussion

In the present work, we demonstrated for the first time that a GnRH-I agonist affects follicular and luteal development and steroidogenesis in the ovaries from an immature rat OHSS model and increases apoptosis and decreases cell proliferation and blood vessel stability in the CL.

We observed significant decreases in ovarian weight, serum progesterone and estradiol levels, peritoneal VEGF concentration and FLK-1 protein expression in the GnRH-I agonist-treated OHSS rats. The decrease of progesterone levels caused by this agonist could be mediated in part by the decreased expression of luteal P450scc and StAR, as demonstrated by Western blot. These results are consistent with those obtained by other

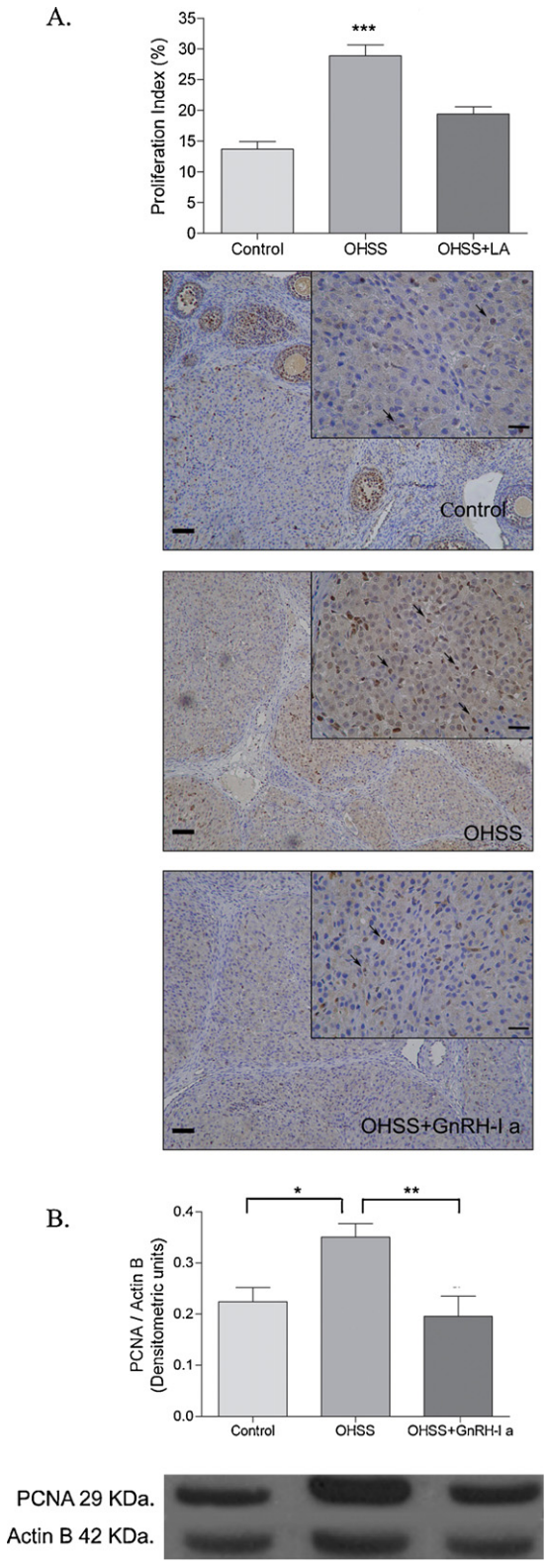

Fig. 6. Effect of treatment with a GnRH-I agonist on cell proliferation in an OHSS rat model. (A) PCNA expression analysis in CL. The proliferation index was determined by counting labeled cells at 400× in randomly selected fields of CL. Data are expressed as mean ± SEM. Four sections per CL were analyzed ($n = 6$ ovaries/group, $^{***}p < 0.001$). Photographs show representative fields of ovarian sections immunostained for PCNA in the three experimental groups. (B) Densitometric quantification of PCNA in CL. Optical density is expressed as arbitrary units ± SEM normalized to actin B ($n = 6$/group, $^{*}p < 0.05$, $^{**}p < 0.01$). Representative immunoblots of PCNA content are shown in the lower panel. Scale bars represent 50 μm (inserts 20 μm).

groups in a similar OHSS model (Kitajima et al., 2004; Tong et al., 2008).

In humans, the pathology of OHSS causes the formation of multiple CL and an increase in the levels of VEGF (Navot et al., 1992), the latter of which originate mainly from follicular and luteal cells. Our study is the first to demonstrate that a GnRH-I agonist decreases the number of CL in an OHSS rat model. Also, it is worth noting that the agonist increased the number of atretic follicles in OHSS rats compared with the untreated hyperstimulated rats. Hence, these observations suggest that GnRH-I agonists may alter luteal development, delaying folliculogenesis, leading a greater number of follicles to atresia, and therefore to a lower number of CL present in ovaries from treated OHSS rats.

It is worth mentioning that preliminary results indicated that sections of ovaries from OHSS rats presented a high number of cystic follicles, several of which were bleeding. In contrast, in the group treated with the agonist, a lower number of cysts were detected (data not shown).

Angiopoietins act on vascular endothelial cells via a tyrosine kinase receptor (Tie-2) and contributes to blood vessel stabilization not only through interaction with perivascular cells (pericytes) but also through interaction with endothelial cells. Previously, we and other authors have shown the expression of angiopoietins and its receptor Tie-2 in the follicles and CL of rats, cows, monkeys and humans, suggesting the involvement of these factors in the regulation of follicle and CL function via stabilization of blood vessels in these compartments (Abramovich et al., 2009; Hazzard et al., 2000; Tanaka et al., 2004; Wulff et al., 2000). In the present study, we found that hyperstimulation increased the stabilization of blood vessels in CL. However, administration of GnRH-I agonist to the OHSS group decreased the blood vessel stability in CL. Moreover, we evaluated the expression of the angiogenic factors ANGPT-1, ANGPT-2 and its receptor Tie-2 in CL from ovaries from OHSS rats. The treatment with the GnRH-I agonist reduced the expression of ANGPT-1 and Tie-2 in the CL from hyperstimulated rats. However, the agonist did not alter the protein level of ANGPT-2 in the OHSS group. Thus, our results suggest that the treatment with a GnRH-I agonist seems to affect the expression of ANGPT-1 and Tie-2 and, in turn, alter the stabilization of blood vessels in the CL from OHSS ovaries.

We performed different assays to gain information on the mechanisms of luteal regression induced by the GnRH agonist in ovaries from an OHSS rat model. Histology revealed that the diameters of the CL from the OHSS group were significantly larger than those from the control group. The treatment with the GnRH-I agonist restored the size of the CL to the values of the control group. In addition, the GnRH-I agonist increased the number of apoptotic cells in the CL from the OHSS group compared with the untreated OHSS group. However, the low number of luteal cells exhibiting apoptosis observed in this work may suggest that this process is not the only mechanism of cell death in CL. Fraser et al. (1999) demonstrated that, during natural or induced luteolysis, several primate luteal cells die by non-apoptotic mechanisms, including autophagocytosis and necrosis (Fraser et al., 1999). In addition, we observed that the GnRH-I agonist decreased the levels of progesterone in the OHSS group. It is worth noting that structural luteolysis is defined as involution of the CL, which is grossly characterized as a loss of tissue, and that functional luteolysis is hormonally defined as a decrease in serum progesterone and an increase in serum 20 alpha-dihydroprogesterone (McCracken et al., 1999). In agreement with our observations, several works have suggested that while GnRH agonist induces oocyte maturation and ovulation, it also completely prevents clinically significant OHSS. The mechanism of action in the context of OHSS prevention would involve luteolysis (Fauser et al., 2002; Nevo et al., 2003). Accordingly, this is the first study to show that GnRH-I agonists

induce both structural and functional luteolysis in this rat model of OHSS.

It has been reported that GnRH-I agonists affect cell proliferation in ovaries from different species. Takekida et al. (2003) demonstrated that GnRH-I agonists decrease PCNA expression in cultured porcine granulosa cells from small and medium-sized follicles. We have previously shown that GnRH-I agonists inhibit DNA synthesis in epidermal growth factor-stimulated human granulosa luteinized cell cultures (Vitale et al., 2006). In this report, we showed that the treatment with a GnRH-I agonist caused a decrease in the proliferation index of CL compared with the OHSS group. Furthermore, we observed an increase in the proliferation index in the OHSS group compared with the control group. These results are consistent with ovarian morphology, since we observed an increase in the number of CL of the OHSS group compared with the control and a decrease in these CL stages after treatment with the GnRH agonist. These observations suggest that GnRH-I agonists may play a role in the regulation of CL proliferation in this OHSS rat model.

The GnRH receptor mRNA has been identified in rat granulosa and luteal cells (Kogo et al., 1999; Whitelaw et al., 1995). Functionally, GnRH regulates steroidogenesis (Hori et al., 1998), the MAPK cascade (Kang et al., 2001), and apoptosis (Zhao et al., 2000) in human granulosa and luteal cells. In this study, we suggest that the changes observed after the treatment of OHSS rats with a GnRH-I agonist may be due mainly to an intraovarian direct effect of the GnRH-I agonist, as both we and other authors have previously shown in prepubertal eCG-treated rats (Goto et al., 1999; Kitajima et al., 2004; Parborell et al., 2001, 2002, 2005).

In conclusion, this study shows that GnRH-I agonists exert diverse actions on the CL from a rat OHSS model. The decrease in P450scc, StAR, ANGPT-1 and Tie-2 expression, blood vessel stability and luteal proliferation leads to CL regression in the ovaries from OHSS rats. Moreover, we suggest that the downregulation of ANGPT-1 and its receptor could be a possible mechanism whereby GnRH-I agonists prevent early OHSS.

Therefore, the treatment with a GnRH-I agonist decreases the severity of OHSS in an immature rat model stimulated with PMSG/hCG.

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References

Aboulghar, M.A., Mansour, R.T., 2003. Ovarian hyperstimulation syndrome: classifications and critical analysis of preventive measures. Hum. Reprod. Update 9 (3), 275–289.

Abramovich, D., Rodriguez, C.A., Hernandez, F., Tesone, M., Parborell, F., 2009. Spatiotemporal analysis of the protein expression of angiogenic factors and their related receptors during folliculogenesis in rats with and without hormonal treatment. Reproduction 137 (2), 309–320.

Andreu, C., Parborell, F., Vanzulli, S., Chemes, H., Tesone, M., 1998. Regulation of follicular luteinization by a gonadotropin-releasing hormone agonist: relationship between steroidogenesis and apoptosis. Mol. Reprod. Dev. 51 (3), 287–294.

Budev, M.M., Arroliga, A.C., Falcone, T., 2005. Ovarian hyperstimulation syndrome. Crit. Care Med. 33 (10 Suppl.), S301–S306.

Challier, J.C., Kacemi, A., Olive, G., 1995. Mixed culture of pericytes and endothelial cells from fetal microvessels of the human placenta. Cell Mol. Biol. (Noisy.-le-Grand) 41 (2), 233–241.

Chen, C.D., Wu, M.Y., Chen, H.F., Chen, S.U., Ho, H.N., Yang, Y.S., 1999. Prognostic importance of serial cytokine changes in ascites and pleural effusion in women with severe ovarian hyperstimulation syndrome. Fertil. Steril. 72 (2), 286–292.

D'Herde, K., De Pestel, G., Roels, F., 1994. In situ end labeling of fragmented DNA in induced ovarian atresia. Biochem. Cell Biol. 72 (11–12), 573–579.

Dal Prato, L., Borini, A., 2005. Use of antagonists in ovarian stimulation protocols. Reprod. Biomed. Online 10 (3), 330–338.

Davis, S., Aldrich, T.H., Jones, P.F., Acheson, A., Compton, D.L., Jain, V., Ryan, T.E., Bruno, J., Radziejewski, C., Maisonpierre, P.C., Yancopoulos, G.D., 1996. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87 (7), 1161–1169.

Delvigne, A., Rozenberg, S., 2002. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum. Reprod. Update 8 (6), 559–577.

Fauser, B.C., de Jong, D., Olivennes, F., Wramsby, H., Tay, C., Itskovitz-Eldor, J., van Hooren, H.G., 2002. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. J. Clin. Endocrinol. Metab. 87 (2), 709–715.

Fraser, H.M., Lunn, S.F., Harrison, D.J., Kerr, J.B., 1999. Luteal regression in the primate: different forms of cell death during naturaland gonadotropin-releasing hormone antagonist or prostaglandin analogue-induced luteolysis. Biol. Reprod. 61 (6), 1468–1479.

Geva, E., Jaffe, R.B., 2000. Role of vascular endothelial growth factor in ovarian physiology and pathology. Fertil. Steril. 74 (3), 429–438.

Giampietro, F., Sancilio, S., Tiboni, G.M., Rana, R.A., Di Pietro, R., 2006. Levels of apoptosis in human granulosa cells seem to be comparable after therapy with a gonadotropin-releasing hormone agonist or antagonist. Fertil. Steril. 85 (2), 412–419.

Golan, A., Ron-El, R., Herman, A., Soffer, Y., Weinraub, Z., Caspi, E., 1989. Ovarian hyperstimulation syndrome: an update review. Obstet. Gynecol. Surv. 44 (6), 430–440.

Gomez, R., Simon, C., Remohi, J., Pellicer, A., 2002. Vascular endothelial growth factor receptor-2 activation induces vascular permeability in hyperstimulated rats, and this effect is prevented by receptor blockade. Endocrinology 143 (11), 4339–4348.

Goto, T., Endo, T., Henmi, H., Kitajima, Y., Kiya, T., Nishikawa, A., Manase, K., Sato, H., Kudo, R., 1999. Gonadotropin-releasing hormone agonist has the ability to induce increased matrix metalloproteinase (MMP)-2 and membrane type 1-MMP expression in corpora lutea, and structural luteolysis in rats. J. Endocrinol. 161 (3), 393–402.

Harwood, J.P., Clayton, R.N., Chen, T.T., Knox, G., Catt, K.J., 1980. Ovarian gonadotropin-releasing hormone receptors. II. Regulation and effects on ovarian development. Endocrinology 107 (2), 414–421.

Hayashi, K.G., Acosta, T.J., Tetsuka, M., Berisha, B., Matsui, M., Schams, D., Ohtani, M., Miyamoto, A., 2003. Involvement of angiopoietin-tie system in bovine follicular development and atresia: messenger RNA expression in theca interna and effect on steroid secretion. Biol. Reprod. 69 (6), 2078–2084.

Hazum, E., Nimrod, A., 1982. Photoaffinity-labeling and fluorescence-distribution studies of gonadotropin-releasing hormone receptors in ovarian granulosa cells. Proc. Natl. Acad. Sci. U.S.A. 79 (6), 1747–1750.

Hazzard, T.M., Christenson, L.K., Stouffer, R.L., 2000. Changes in expression of vascular endothelial growth factor and angiopoietin-1 and -2 in the macaque corpus luteum during the menstrual cycle. Mol. Hum. Reprod. 6 (11), 993–998.

Hazzard, T.M., Molskness, T.A., Chaffin, C.L., Stouffer, R.L., 1999. Vascular endothelial growth factor (VEGF) and angiopoietin regulation by gonadotrophin and steroids in macaque granulosa cells during the peri-ovulatory interval. Mol. Hum. Reprod. 5 (12), 1115–1121.

Hirschi, K.K., D'Amore, P.A., 1996. Pericytes in the microvasculature. Cardiovasc. Res. 32 (4), 687–698.

Hori, H., Uemura, T., Minaguchi, H., 1998. Effects of GnRH on protein kinase C activity, Ca^{2+} mobilization and steroidogenesis of human granulosa cells. Endocr. J. 45 (2), 175–182.

Hsueh, A.J., Schaeffer, J.M., 1985. Gonadotropin-releasing hormone as a paracrine hormone and neurotransmitter in extra-pituitary sites. J. Steroid Biochem. 23 (5B), 757–764.

Humaidan, P., 2009. Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG: a pilot study. Reprod. Biomed. Online 18 (5), 630–634.

Irusta, G., Parborell, F., Peluffo, M., Manna, P.R., Gonzalez-Calvar, S.I., Calandra, R., Stocco, D.M., Tesone, M., 2003. Steroidogenic acute regulatory protein in ovarian follicles of gonadotropin-stimulated rats is regulated by a gonadotropin-releasing hormone agonist. Biol. Reprod. 68 (5), 1577–1583.

Irusta, G., Parborell, F., Tesone, M., 2007. Inhibition of cytochrome P-450 C17 enzyme by a GnRH agonist in ovarian follicles from gonadotropin-stimulated rats. Am. J. Physiol. Endocrinol. Metab. 292 (5), E1456–E1464.

Jain, R.K., Booth, M.F., 2003. What brings pericytes to tumor vessels? J. Clin. Invest. 112 (8), 1134–1136.

Kang, S.K., Tai, C.J., Nathwani, P.S., Leung, P.C., 2001. Differential regulation of two forms of gonadotropin-releasing hormone messenger ribonucleic acid in human granulosa-luteal cells. Endocrinology 142 (1), 182–192.

Kitajima, Y., Endo, T., Nagasawa, K., Manase, K., Honnma, H., Baba, T., Hayashi, T., Chiba, H., Sawada, N., Saito, H., 2006. Hyperstimulation and a gonadotropin-releasing hormone agonist modulate ovarian vascular permeability by altering expression of the tight junction protein claudin-5. Endocrinology 147 (2), 694–699.

Kitajima, Y., Endo, T., Manase, K., Nishikawa, A., Shibuya, M., Kudo, R., 2004. Gonadotropin-releasing hormone agonist administration reduced vascular endothelial growth factor (VEGF), VEGF receptors, and vascular permeability of the ovaries of hyperstimulated rats. Fertil. Steril. 81 (Suppl. 1), 842–849.

Kogo, H., Fujimoto, T., Mori, T., 1999. Evidence for gonadotropin-releasing hormone receptor mRNA expression by estrogen in rat granulosa cells. Cell Tissue Res. 297 (3), 459–465.

Krasnow, J.S., Berga, S.L., Guzick, D.S., Zeleznik, A.J., Yeo, K.T., 1996. Vascular permeability factor and vascular endothelial growth factor in ovarian hyperstimulation syndrome: a preliminary report. Fertil. Steril. 65 (3), 552–555.
Lee, A., Christenson, L.K., Stouffer, R.L., Burry, K.A., Patton, P.E., 1997. Vascular endothelial growth factor levels in serum and follicular fluid of patients undergoing in vitro fertilization. Fertil. Steril. 68 (2), 305–311.
Levin, E.R., Rosen, G.F., Cassidenti, D.L., Yee, B., Meldrum, D., Wisot, A., Pedram, A., 1998. Role of vascular endothelial cell growth factor in ovarian hyperstimulation syndrome. J. Clin. Invest. 102 (11), 1978–1985.
Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N., Daly, T.J., Davis, S., Sato, T.N., Yancopoulos, G.D., 1997. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277 (5322), 55–60.
McCracken, J.A., Custer, E.E., Lamsa, J.C., 1999. Luteolysis: a neuroendocrine-mediated event. Physiol. Rev. 79 (2), 263–323.
Muttukrishna, S., Fowler, P.A., George, L., Groome, N.P., Knight, P.G., 1996. Changes in peripheral serum levels of total activin A during the human menstrual cycle and pregnancy. J. Clin. Endocrinol. Metab. 81 (9), 3328–3334.
Navot, D., Bergh, P.A., Laufer, N., 1992. Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. Fertil. Steril. 58 (2), 249–261.
Nevo, O., Eldar-Geva, T., Kol, S., Itskovitz-Eldor, J., 2003. Lower levels of inhibin A and pro-alphaC during the luteal phase after triggering oocyte maturation with a gonadotropin-releasing hormone agonist versus human chorionic gonadotropin. Fertil. Steril. 79 (5), 1123–1128.
Ozcakir, H.T., Giray, S.G., Ozbilgin, M.K., Uyar, Y., Lacin, S., Caglar, H., 2005. Immunohistochemical detection of transforming growth factor-alpha, epidermal growth factor, and vascular endothelial growth factor expression in hyperstimulated rat ovary. Acta Obstet. Gynecol. Scand. 84 (9), 887–893.
Parborell, F., Dain, L., Tesone, M., 2001. Gonadotropin-releasing hormone agonist affects rat ovarian follicle development by interfering with FSH and growth factors on the prevention of apoptosis. Mol. Reprod. Dev. 60 (2), 241–247.
Parborell, F., Irusta, G., Rodriguez, C.A., Tesone, M., 2008. Regulation of ovarian angiogenesis and apoptosis by GnRH-I analogs. Mol. Reprod. Dev. 75 (4), 623–631.
Parborell, F., Irusta, G., Vitale, A.M., Gonzalez, O., Pecci, A., Tesone, M., 2005. GnRH antagonist antide inhibits apoptosis of preovulatory follicle cells in rat ovary. Biol. Reprod. 72, 659–666.
Parborell, F., Pecci, A., Gonzalez, O., Vitale, A., Tesone, M., 2002. Effects of a gonadotropin-releasing hormone agonist on rat ovarian follicle apoptosis: regulation by EGF and the expression of Bcl-2-related genes. Biol. Reprod. 67, 481–486.
Ragni, G., Vegetti, W., Riccaboni, A., Engl, B., Brigante, C., Crosignani, P.G., 2005. Comparison of GnRH agonists and antagonists in assisted reproduction cycles of patients at high risk of ovarian hyperstimulation syndrome. Hum. Reprod. 20 (9), 2421–2425.
Redmer, D.A., Doraiswamy, V., Bortnem, B.J., Fisher, K., Jablonka-Shariff, A., Grazul-Bilska, A.T., Reynolds, L.P., 2001. Evidence for a role of capillary pericytes in vascular growth of the developing ovine corpus luteum. Biol. Reprod. 65 (3), 879–889.
Reynolds, L.P., Grazul-Bilska, A.T., Redmer, D.A., 2000. Angiogenesis in the corpus luteum. Endocrine 12 (1), 1–9.
Rizk, B., Aboulghar, M., Smitz, J., Ron-El, R., 1997. The role of vascular endothelial growth factor and interleukins in the pathogenesis of severe ovarian hyperstimulation syndrome. Hum. Reprod. Update 3 (3), 255–266.
Sadrkhanloo, R., Hofeditz, C., Erickson, G.F., 1987. Evidence for widespread atresia in the hypophysectomized estrogen-treated rat. Endocrinology 120 (1), 146–155.
Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., Dvorak, H.F., 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219 (4587), 983–985.
Shalaby, F., Rossant, J., Yamaguchi, T.P., Gertsenstein, M., Wu, X.F., Breitman, M.L., Schuh, A.C., 1995. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376 (6535), 62–66.
Sifer, C., Blanc-Layrac, G., Bringuier, A.F., Porcher, R., Madelenat, P., Feldmann, G., Benifla, J.L., 2003. Effects of a gonadotropin-releasing hormone agonist and follicle stimulating hormone on the incidence of apoptosis in human luteinized granulosa cells. Eur. J. Obstet. Gynecol. Reprod. Biol. 110 (1), 43–48.
Sridaran, R., Philip, G.H., Li, H., Culty, M., Liu, Z., Stocco, D.M., Papadopoulos, V., 1999. GnRH agonist treatment decreases progesterone synthesis, luteal peripheral benzodiazepine receptor mRNA, ligand binding and steroidogenic acute regulatory protein expression during pregnancy. J. Mol. Endocrinol. 22 (1), 45–54.
Sugino, N., Suzuki, T., Sakata, A., Miwa, I., Asada, H., Taketani, T., Yamagata, Y., Tamura, H., 2005. Angiogenesis in the human corpus luteum: changes in expression of angiopoietins in the corpus luteum throughout the menstrual cycle and in early pregnancy. J. Clin. Endocrinol. Metab. 90 (11), 6141–6148.
Suri, C., Jones, P.F., Patan, S., Bartunkova, S., Maisonpierre, P.C., Davis, S., Sato, T.N., Yancopoulos, G.D., 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87 (7), 1171–1180.
Takekida, S., Matsuo, H., Maruo, T., 2003. GnRH agonist action on granulosa cells at varying follicular stages. Mol. Cell. Endocrinol. 202 (1–2), 155–164.
Tanaka, J., Acosta, T.J., Berisha, B., Tetsuka, M., Matsui, M., Kobayashi, S., Schams, D., Miyamoto, A., 2004. Relative changes in mRNA expression of angiopoietins and receptors tie in bovine corpus luteum during estrous cycle and prostaglandin F2alpha-induced luteolysis: a possible mechanism for the initiation of luteal regression. J. Reprod. Dev. 50 (6), 619–626.
Thurston, G., Rudge, J.S., Ioffe, E., Zhou, H., Ross, L., Croll, S.D., Glazer, N., Holash, J., McDonald, D.M., Yancopoulos, G.D., 2000. Angiopoietin-1 protects the adult vasculature against plasma leakage. Nat. Med. 6 (4), 460–463.
Tong, X.M., Zhang, S.Y., Song, T., Xu, W.H., Lin, X.N., Shu, J., Liu, L., 2008. Effects of gonadotropin-releasing hormone antagonists on the expression of vascular endothelial growth factor and its receptors in a rat model of ovarian hyperstimulation syndrome. Chin. Med. J. (Engl.) 121 (23), 2434–2439.
Uemura, T., Namiki, T., Kimura, A., Yanagisawa, T., Minaguchi, H., 1994. Direct effects of gonadotropin-releasing hormone on the ovary in rats and humans. Horm. Res. 41 (Suppl. 1), 7–13.
Ujioka, T., Matsuura, K., Kawano, T., Okamura, H., 1997. Role of progesterone in capillary permeability in hyperstimulated rats. Hum. Reprod. 12 (8), 1629–1634.
Vitale, A.M., Abramovich, D., Peluffo, M.C., Meresman, G., Tesone, M., 2006. Effect of gonadotropin-releasing hormone agonist and antagonist on proliferation and apoptosis of human luteinized granulosa cells. Fertil. Steril. 85 (4), 1064–1067.
Whitelaw, P.F., Eidne, K.A., Sellar, R., Smyth, C.D., Hillier, S.G., 1995. Gonadotropin-releasing hormone receptor messenger ribonucleic acid expression in rat ovary. Endocrinology 136 (1), 172–179.
Woodruff, T.K., D'Agostino, J., Schwartz, N.B., Mayo, K.E., 1988. Dynamic changes in inhibin messenger RNAs in rat ovarian follicles during the reproductive cycle. Science 239 (4845), 1296–1299.
Wulff, C., Wiegand, S.J., Saunders, P.T., Scobie, G.A., Fraser, H.M., 2001. Angiogenesis during follicular development in the primate and its inhibition by treatment with truncated Flt-1-Fc (vascular endothelial growth factor Trap(A40)). Endocrinology 142 (7), 3244–3254.
Wulff, C., Wilson, H., Largue, P., Duncan, W.C., Armstrong, D.G., Fraser, H.M., 2000. Angiogenesis in the human corpus luteum: localization and changes in angiopoietins, Tie-2, and vascular endothelial growth factor messenger ribonucleic acid. J. Clin. Endocrinol. Metab. 85 (11), 4302–4309.
Yang, H., Bhat, G.K., Wadley, R., Wright, K.L., Chung, B.M., Whittaker, J.A., Dharmarajan, A.M., Sridaran, R., 2003. Gonadotropin-releasing hormone-agonist inhibits synthesis of nitric oxide and steroidogenesis by luteal cells in the pregnant rat. Biol. Reprod. 68 (6), 2222–2231.
Zhao, S., Saito, H., Wang, X., Saito, T., Kaneko, T., Hiroi, M., 2000. Effects of gonadotropin-releasing hormone agonist on the incidence of apoptosis in porcine and human granulosa cells. Gynecol. Obstet. Invest. 49 (1), 52–56.