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Understanding Ovarian Hyperstimulation Syndrome

Anne Delbaere,[1,4] Guillaume Smits,[2,3] Anne De Leener,[3] Sabine Costagliola,[3] and Gilbert Vassart[2,3]

[1]Fertility Clinic, [2]Medical Genetics Department, Hôpital Erasme; [3]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM); and [4]Laboratory of Research on Human Reproduction, Faculté de Médecine, Université Libre de Bruxelles, Brussels, Belgium

The ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication of ovarian stimulation treatments. Severe forms are characterized by a massive ovarian enlargement with the formation of multiple ovarian cysts associated with extravascular fluid shifts resulting in the development of ascites, pleural and/or pericardial effusion. The pathophysiology of the syndrome has not been completely elucidated yet. The vascular fluid leakage is thought to result from an increased capillary permeability of mesothelial surfaces under the action of one or several vasoactive ovarian factor(s) produced by the multiple corpora lutea. The paper focuses on the recent identification of mutations in the FSH receptor gene that display an increased sensitivity to hCG and are responsible for the development of spontaneous OHSS occurring during pregnancy. These findings have shed light for the first time on the molecular basis of the pathophysiology of the spontaneous form of the syndrome. As spontaneous and iatrogenic OHSS share similar pathophysiological sequences including massive recruitment and growth of ovarian follicles, extensive luteinization provoked by hCG, and oversecretion of vasogenic molecules by the corpora lutea, they have also opened new research perspectives for the understanding of the much more frequent iatrogenic OHSS.

Key Words: Ovarian hyperstimulation syndrome; FSH receptor; pathophysiology.

Introduction

The ovarian hyperstimulation syndrome (OHSS) is a serious iatrogenic complication of ovarian stimulation treatments. Although traditionally classified as mild, moderate, and severe forms according to the severity of the disease, symptoms of OHSS display a continuum of clinical manifestations *(1)*. Mild forms of OHSS are characterized by abdominal discomfort and distension. Severe forms involve a massive ovarian enlargement with the formation of multiple ovarian cysts associated with extravascular fluid shifts resulting in the development of ascites, pleural and/or pericardial effusion, hypovolemia, hemoconcentration and hydroelectrolytic disorders *(2)*. Life-threatening complications of OHSS include renal failure, adult respiratory distress syndrome and thromboembolic phenomena *(3,4)*. The OHSS is typically associated with the use of exogenous gonadotropin stimulation and is rarely seen with other stimulation agents like clomiphene citrate *(5)*. It usually involves patients with an explosive response to the ovarian stimulation and is more frequent in patients suffering from polycystic ovarian syndrome *(4)*. OHSS usually resolves spontaneously within several days. However, the condition may worsen and last for longer durations in case of pregnancy.

The pathophysiology of the syndrome has not been completely elucidated yet. Human chorionic gonadotropin (hCG) is thought to play a crucial role in the development of the syndrome as severe forms are restricted to cycles with exogenous hCG (to induce ovulation or as luteal phase support) or with endogenous pregnancy-derived hCG *(6)*. Two conditions appear to be a prerequisite to the development of the syndrome: multiple follicular growth and further extensive luteinization of these follicles. The syndrome is indeed exclusively postovulatory and the vascular fluid leakage is thought to result from an increased capillary permeability of mesothelial surfaces under the action of one or several vasoactive ovarian factor(s) produced during the formation of the multiple corpora lutea *(7)*.

Mutations in the FSH Receptor Gene in Spontaneous OHSS

Spontaneous forms of OHSS are very rare and were always reported during pregnancy *(8–15)*. Several cases were observed during multiple pregnancies or hydatidiform moles known to be associated with abnormally high values of hCG *(12)*. Other cases were associated with hypothyroidism, and it was proposed that the high levels of thyroid-stimulating hormone (TSH) could stimulate the ovaries *(13)*. A series of cases were recurrent with the development of the syndrome reported in two to six consecutive pregnancies *(8–11)*.

We and another group have recently identified four different mutations in exon 10 of the follicle-stimulating hor-

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Author to whom all correspondence and reprint requests should be addressed: Anne Delbaere, Fertility Clinic, Hôpital Erasme, 808 route de Lennih, 1070 Brussels, Belgium. E-mail: adelbaer@ulb.ac.be

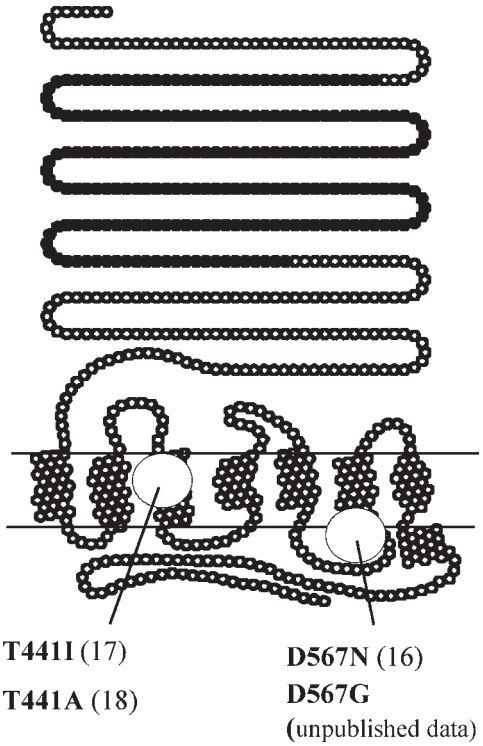

Fig. 1. Locations of the mutations identified in the FSH receptor of patients presenting spontaneous OHSS. Numbering begins at the first amino acid of the signal peptide.

mone (FSH) receptor gene of patients presenting OHSS at the end of the first trimester of each of their pregnancies *(16–18)* (Fig. 1).

The mutation described by Smits et al. *(16)* consisted of a substitution of an adenine for a guanine at the first base of codon 567 of the FSH receptor gene, resulting in the replacement of an aspartic acid with an asparagine at the cytoplasmic end of transmembrane helix VI in the serpentine domain (mutation D567N) (Fig. 1). Vasseur et al. *(17)* identified another mutation consisting of a substitution of a thymidine for a cytosine at the second base of codon 449, resulting in the substitution of isoleucine for threonine at the upper part of the third transmembrane domain of the receptor (mutation T449I) (Fig. 1). More recently, Montanelli et al. reported a third mutation also affecting codon 449 but at its first base, with a substitution of a guanine for an adenine, causing a different amino acid replacement of a threonine with an alanine (mutation T449A) *(18)* (Fig. 1). A fourth mutation was recently identified in a patient diagnosed as having a hyperreactio luteinalis at the end of the first trimester of her pregnancy *(15)*. This clinical condition appears to be similar to spontaneous OHSS when it develops in the first trimester of pregnancy. The mutation consisted of a substitution of a guanine for an adenine at the second base of codon 567 of the FSH receptor gene, resulting in the replacement of an aspartic acid with a glycine at the cytoplasmic end of transmembrane helix VI in the serpentine domain (mutation D567G) (unpublished data) (Fig. 1). This last mutation was previously reported in a hypophysectomized man, who, despite undetectable serum gonadotropin levels had normal testis volume and semen parameters *(19)*. The functional characterization of the mutant FSH receptors in transfection experiments revealed that all four mutations displayed an abnormally high sensitivity to hCG *(16–18,20)*. In addition three mutant receptors (D567N, D567G, and T449I) also showed constitutive activity together with increased sensitivity to TSH *(16,18,20)*. Initially, the T449I mutant receptor was reported to produce similar basal levels of cAMP than the wild-type receptor and was not activated by TSH *(17)*. However, contrary to the original description, site-directed mutagenesis experiments demonstrated that the T449I mutant FSH receptor also displayed constitutive activity and an abnormal responsiveness to TSH *(20)*. Unexpectedly, the four reported mutations affected the serpentine portion of the FSH receptor rather than the hormone-binding ectodomain of the protein. It was thus suggested that these mutations induce a structural change in the FSH receptor associating an increase in constitutive activity together with a decrease in the specificity barrier, rendering the low affinity interactions of hCG and TSH with the hormone–ectodomain complex functionally effective *(20)*.

How are these mutations implicated in the development of spontaneous OHSS? Normal FSH receptors are usually not or are very weakly stimulated during pregnancy, as pituitary gonadotropins fall to very low or undetectable levels in the serum. On the contrary, mutant FSH receptors expressed in the ovarian follicles would be hyperstimulated by the pregnancy-derived hCG. Accordingly, the follicles would start growing, enlarge, and finally acquire LH receptors on granulosa cells that would also be stimulated by hCG, inducing follicular luteinization together with the secretion of vasoactive molecules responsible for the development of the syndrome. From these findings, it appears that the development of spontaneous OHSS is the result of a nonphysiological interaction between placental hCG and the ovarian FSH receptor, either in the presence of normal levels of hCG with a mutated FSH receptor or in the presence of abnormally high levels of hCG as found in molar or multiple pregnancies, with a presumably normal FSH receptor *(21)*. Indeed, it has been shown recently that hCG was able to stimulate the FSH receptor in conditions that mimic high ligand concentration *(22,23)*.

These findings provided new insight into the molecular basis of the pathophysiology of spontaneous OHSS and have opened new research perspectives for the understanding of the much more frequent iatrogenic OHSS.

From Spontaneous to Iatrogenic OHSS

Although differing by the timing of their occurrence, spontaneous and iatrogenic OHSS share similar pathophysiological sequences: massive recruitment and growth of ovarian follicles, extensive luteinization provoked by hCG, and over-

secretion of vasogenic molecules by luteinized corpora lutea. In the iatrogenic form, the follicular recruitment is caused by the ovarian stimulation with exogenous FSH.

Ovarian Hyperresponse to Exogenous FSH: Role of FSH Receptor

While naturally occurring mutations appear to be rather rare, the FSH receptor and its promoter display some very common single nucleotide polymorphisms. Two non-synonymuous polymorphisms have been described in exon 10 of the FSH receptor *(24)*. The first one is A919G (Thr307 Ala) located just before the beginning of the first transmembrane helix. The second is A2039G (Asn680Ser) located intracellularly at the end of the C-terminal tail of the receptor. In the caucasian population, the two FSH polymorphisms in exon 10 almost invariably occur in two combinations, leading to two allelic variants: Thr^{307}-Asn^{680} (allele TN) and Ala^{307}-Ser^{680} (allele AS) *(24)*. Recent reports suggested that the FSH receptor genotype could play a role in vivo in the ovarian response to FSH stimulation. In particular, the analysis of the FSH receptor polymorphism at position 680 revealed that patients homozygous for Ser 680 displayed higher basal FSH levels *(25–27)*. In addition, higher requirements of exogenous FSH were necessary to achieve successful ovarian stimulation for in vitro fertilization (IVF) in patients with the Ser/Ser 680 polymorphism compared to patients with the Asn/Ser 680 or Asn/Asn 680 variants *(25)*. In another retrospective study, the presence of Ser in position 680 was associated with poor responses to gonadotropin stimulation in IVF, suggesting that the Ser/Ser 680 variant of the FSH receptor could be associated with a decreased FSH sensitivity *(28)*. However, a study conducted among normogonadotropic anovulatory patients resistant to clomiphene citrate could not establish associations between the FSH receptor genotypes and the ovarian sensitivity during ovulation induction with exogenous FSH *(27)*. It is possible that these apparently conflicting data are related to differences in the ovarian stimulation protocols: in anovulatory patients, the treatment aims at monofollicular development, while for IVF, it aims at multifollicular development. The supraphysiologic doses of FSH used during IVF stimulation protocols could therefore reveal differences in the ovarian response according to the FSH receptor genotype, which could not emerge in more physiological conditions.

These observations, together with the identification of FSH receptor mutations in spontaneous OHSS, led us to test whether coding polymorphisms of the FSH receptors could be associated with the development of iatrogenic OHSS. In particular, as the Ser 680 allele was potentially associated with poor responses to ovarian stimulation for IVF, we wanted to test whether the Asn 680 allele could be associated with hyperresponses to IVF treatments.

We conducted a study involving 37 caucasian patients who developed OHSS after an IVF cycle in our fertility clinic *(29)*. Surprisingly, the OHSS population, as the control IVF population (130 patients who did not develop OHSS after an IVF cycle) displayed higher allelic frequencies of Ser 680 in the FSH receptor gene than those observed in a caucasian control population (99 patients). However, when examining the allelic frequencies according to the severity of OHSS, a significant enrichment of Asn 680 was observed as the severity of OHSS increased. This difference persisted when the analysis was performed between mild, moderate, and severe OHSS patients who were pregnant. Although the number of OHSS patients studied was small, introducing potential sample biases, these results suggest that the genotype in position 680 of the FSH receptor cannot predict which patients will develop OHSS but could be a predictor of severity among OHSS patients. More clinical data are required to further determine the exact relationship between FSH receptor genotypes and the development of OHSS after ovarian stimulation. In addition, further experimental data are necessary to understand the in vivo association of FSH receptor alleles and the response to ovarian stimulation. So far, the in vitro functional characterization of the two FSH receptor variants (alleles TN and AS) in transfection experiments could not show significant differences in binding affinity, in neither the production of cAMP nor inositol phosphate after stimulation with FSH *(26,30)*. Several hypotheses have been proposed, as a different expression of the variants at the cell surface, differences in their turnover or in their downregulation rate, different affinity for various FSH isoforms *(25)*. On the other hand, the S^{680} N polymorphism of the FSH receptor could not play any direct functional role in the development of OHSS but could be in linkage disequilibrium with other gene polymorphisms.

Oversecretion of Vasogenic Molecules by Luteinized Corpora Lutea

Once the step of multiple follicular growth has been reached, OHSS, whether iatrogenic or spontaneous, will develop and progress only if these follicles undergo luteinization, particularly under the action of hCG. As such, the only absolute preventive measure to avoid the development of the syndrome in IVF is the cancellation of the cycle by withholding hCG in case of a hyperresponse to exogenous FSH *(5)*. Another prevention measure in IVF cycles is the cryopreservation of all embryos, avoiding exposure to endogenous hCG in case of concomittant pregnancy *(5)*. Luteinization of enlarged superstimulated ovaries induce the massive release of vasoactive mediators such as vascular endothelial growth factor (VEGF) *(31,32)*, angiotensin II *(6,33,34)*, and various interleukins *(35)*, exacerbating local inflammatory-like reactions accompanying angiogenesis during corpora lutea formation *(36,37)*. Among the studied factors, VEGF, also called vascular permeability factor, is thought to be one of the major ovarian-derived permeability agents in the development of OHSS *(31,32)*. Increased angiogenesis together with increased vascular permeability accompany the formation of corpus luteum during a normal menstrual

cycle. It is noteworthy that the kinetics of the symptoms are closely related to the lifespan of the corpus luteum: in the absence of pregnancy, symptoms will resolve spontaneously with the onset of the menses and in the presence of pregnancy, symptoms usually start to improve after the sixth week of pregnancy, before the hCG peak. Accordingly, it has been demonstrated that the activity of the corpus luteum diminishes from the fifth week of pregnancy in spite of increasing hCG levels *(38)*. OHSS can therefore be viewed as an exaggeration of the events that occur at ovulation and during the corpus luteum formation in a normal menstrual cycle. As the release of vasoactive molecules reaches a threshold, physiological control mechanisms can be overstretched, leading to the development of the symptoms of OHSS.

Perspectives

Being able to predict the individual ovarian response to exogenous FSH remains a challenge for IVF teams. The identification of patients susceptible to elicit a hyperresponse to standard stimulation treatments would allow one to adapt their treatment and to avoid, if not completely, at least to a large extent, OHSS, which remains so far the more frequent and potentially life-threatening complication of IVF. In this regard, it is very likely that the individual response to controlled ovarian hyperstimulation is a polygenic trait, as recently suggested by de Castro et al. who provided evidence of genetic interactions between FSH receptor and estrogen receptors alpha and beta genes in relation to controlled ovarian hyperstimulation outcome *(39)*. In addition, the genetic background of the patient certainly plays a role in the susceptibility to the increase of vascular permeability. Two clinical entities have been described in OHSS: early forms occurring 3–7 d after ovulation triggering by hCG and late forms developing 12–17 d after ovulation in close association with an initiated pregnancy *(40)*. The early pattern is related to an excessive response to gonadotropin stimulation, while the late pattern is more likely to be severe and is induced by endogenous hCG in conception cycles. Although the clinical sequences of the syndrome are very similar in both forms, it is possible that the susceptibility to one or the other form differs between both patterns: in the early form, the genetic background of the patient would influence the ovarian hyperresponse to exogenous FSH, while in the late form, it would more be implicated in the stimulation of the corpora lutea by hCG. The critical role of hCG in the development of iatrogenic OHSS has not been explained so far but could be related to a dual mechanism for hormone binding and signal transduction between hCG and LH on the LH/CG receptor *(41)*. In conclusion, the data reviewed here thus provide the first direct evidence for the involvement of specific genes in the pathophysiology of spontaneous and iatrogenic OHSS. Future work should aim to identify other genes involved and to understand the mechanism by which they influence ovarian function and dysfunction.

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