

# The Role of Cytokines and Hormones in the Prediction of Ovarian Hyperstimulation Syndrome

Leo Vankrieken, Eur. Eng. and  
Jennifer A. Kelly, Ph.D.

---

# The Role of Cytokines and Hormones in the Prediction of Ovarian Hyperstimulation Syndrome

Leo Vankrieken, Eur. Eng. and  
Jennifer A. Kelly, Ph.D.

Ovarian hyperstimulation syndrome (OHSS\*) arises as a serious complication of ovulation induction in women being treated with assisted reproductive technologies (ART). The syndrome severely impacts the patient's health, leading to morbidity and even mortality. The disease entails ovarian enlargement and intravascular fluid shifts to extravascular space, including the peritoneal cavity. Vascular hyperpermeability accompanies formation of ascites, hypotension, electrolyte disturbances, sodium retention, cyst formation, abdominal distention and discomfort, and liver dysfunctions.

## OHSS Classification Schemes

In 1967 Rabau first described a six-level OHSS classification scheme, which Schenker modified in 1978.<sup>1,2</sup> (See Table 1.) Three categories, mild, moderate, and severe, have two grades of severity, A and B. The mild form and stage A of the moderate form are often observed in patients following various ovarian stimulation programs in assisted reproductive technologies. The widely accepted classification scheme proposed by

---

### \*Abbreviations:

ART = assisted reproductive technologies

E2 = estradiol

FF = follicular fluid

FSH = follicle stimulating hormone

GnRH = gonadotropin releasing hormone

hCG = human chorionic gonadotropin

hMG = human menopausal gonadotropin

IL = interleukin

LH = luteinizing hormone

PCOS = polycystic ovarian syndrome

OHSS = ovarian hyperstimulation syndrome

TNF- $\alpha$  = tumor necrosis factor- $\alpha$

---

Golan in 1989 emphasizes clinical signs of the syndrome.<sup>3</sup>

## Disease Prevention and Management

Recent advances in 3-dimensional ultrasound technology combined with careful monitoring of hormone levels have helped in the prediction and management of OHSS, but complete prevention has not been achieved.<sup>4</sup>

Clinicians remain challenged to prevent OHSS and minimize its sequelae by identifying at risk patients and detecting OHSS at the early stages.

---

*"PCOS patients are characterized by an inverse LH/FSH ratio, and OHSS occurs more frequently in this population. . . [Recent studies] point to the LH/FSH ratio as a valuable predictive tool for OHSS."*

---

Patients with polycystic ovary syndrome (PCOS) are at high risk for OHSS. Other risk factors include multiple immature and intermediate follicles, young age (less than 35 years), lean (asthenic) habitus, high serum estradiol levels (>2,500 pg/mL), luteal supplementation with human chorionic gonadotropin (hCG), stimulation protocols using GnRH-agonist treatment, and early pregnancy.<sup>5,6</sup>

## Hormone Concentrations in OHSS Patients

Some patients who develop OHSS after hMG/hCG treatment have high serum levels of E2, androstenedione, and possibly other hormones.<sup>7,8</sup> The correlation between the incidence of OHSS and high estradiol (in serum and ascitic fluid) after hMG/hCG ovulation

**Table 1.** OHSS classification schemes describe three general stages of OHSS development<sup>2,3</sup>

Classification	Stage	Symptoms
<i>Golan et al., 1989</i>		
Mild	1	Abdominal distention
	2	Nausea, vomiting, diarrhea, ovarian enlargement < 12cm
Moderate	3	Mild + ascites evident by ultrasound examination
Severe	4	Moderate + clinical evidence of ascites and hypothorax
	5	Hemoconcentration, coagulation and electrolyte disorders, renal failure, ovarian enlargement > 12 cm
<i>Schenker, 1978</i>		
Mild	A	Chemical hyperstimulation, E2* < 1,500 pg/mL
	B	Chemical hyperstimulation, E2 > 1,500 pg/mL, diameter of ovaries < 6cm, abdominal heaviness, tension, swelling, pain
Moderate	A	E2 > 4,000 pg/mL, abdominal distention, ascites formation, ovaries 6 to 12 cm
	B	Stage A + gastrointestinal symptoms: vomiting, diarrhea
Severe	A	Moderate Form Stage B + E2 > 6,000 pg/mL, ovaries > 12 cm, ascites formation, hydrothorax, liver dysfunction
	B	Stage A + elevated hemoconcentration (hematocrit greater than or equal to 45%, increased blood viscosity leading to coagulation abnormalities, tension ascites, adult respiratory distress syndrome, thromboembolic phenomena, renal failure, hypovolemic shock

induction is well known. Reports also cite, however, that moderate and severe OHSS occurs in patients with normal to just slightly elevated E2 levels, and not all ART participants with high E2 suffer from OHSS.<sup>9,10</sup> Hormone measurements, examined independently, have proven insufficient to predict OHSS onset.

PCOS patients are characterized by an inverse LH/FSH ratio, and OHSS occurs more frequently in this population. Bodis *et al.* provided data supporting LH/FSH as a predictor of OHSS. They compared 12 OHSS patients to 12 healthy controls and found that most OHSS patients had LH/FSH > 1, while

most healthy women had LH/FSH < 1. (See Table 2.) The OHSS group also had higher androstenedione levels (2.3 mg/mL ± 0.7 versus 1.7 mg/mL ± 0.8, P < 0.01). Given that LH stimulates androgen production by thecal cells, high LH may be a factor in elevated androstenedione concentrations. These results point to the LH/FSH ratio as a valuable predictive tool for OHSS.

### **The Role of Cytokines in OHSS**

OHSS patients suffer from increased vascular permeability, hypotension, and shock — symptoms common to a noninfectious systemic inflammatory response. Cytokines par-

participate in the inflammatory response and perhaps OHSS as well.<sup>11</sup> IL-6, IL-8, IL-2, and TNF- $\alpha$  mediate increases in vascular permeability, which is the basic pathophysiologic event of OHSS.

**Table 2.** Most OHSS patients have LH/FSH > 1, while most healthy controls have LH/FSH < 1. The ratio may serve as an OHSS predictor.<sup>10</sup>

Ratio	OHSS Group	Control Group
LH/FSH > 1	9	1*
LH/FSH = 1	1	1
LH/FSH < 1	2	10*

\* $P < 0.001$

Cytokines have been characterized within the follicular fluid and the surrounding ovarian tissue and may originate from either leukocytes recruited into the ovary at the time of ovulation or from resident ovarian cells.<sup>12</sup> Immunohistochemical methods showed IL-6 in ovarian thecal cells, suggesting a local role for IL-6 in normal and stimulated ovarian function.<sup>13</sup> Several studies, summarized

below, indicate the potential utility of a few cytokines, especially IL-6, as OHSS markers.

*IL-6 preovulatory FF measurements distinguished OHSS from non-OHSS women even within the high risk group. IL-6 measurements may be an effective early predictor of OHSS.*

IL-6 helps mediate the acute phase response to injury including increases in vascular permeability and shifts in hepatic protein synthesis. Acute pancreatitis and acute alcoholic hepatitis, like OHSS, are marked by ascites and hypotension, and high IL-6 levels accompany both diseases.<sup>14</sup> IL-6 may contribute to the symptoms through its influence on vascular permeability. OHSS patients often have unusually low serum albumin levels, and since IL-6 suppresses hepatic albumin production in favor of acute phase reactants, low albumin might further indicate IL-6 involvement in the disease.<sup>15</sup>

**Table 3.** Cytokine levels in serum and peritoneal fluid of OHSS patients and healthy controls.<sup>16</sup>

Analyte (pg/mL)	OHSS (n=12)	Controls (n=20)	P
<i>IL-6</i>			
Serum	375 $\pm$ 244	11 $\pm$ 4.6	< 0.05
Peritoneal	3,523 $\pm$ 1,671	30 $\pm$ 5.9	< 0.001
<i>IL-8</i>			
Serum*	0 – 378	75 – 355	NS**
Peritoneal	1,695 $\pm$ 784	704 $\pm$ 538	< 0.05
<i>TNF-<math>\alpha</math></i>			
Serum*	0.2 – 26	0.5 – 6.8	NS
Peritoneal	14 $\pm$ 5.1	4.2 $\pm$ 0.99	< 0.05

\*Range of cytokine concentrations

\*\*NS = not significant ( $P \geq 0.05$ )

Table 3 lists IL-6, IL-8, and TNF- $\alpha$  concentrations in the serum and peritoneal fluid of 12 women hospitalized with OHSS. OHSS patients had higher IL-6 serum concentrations than healthy controls ( $P < 0.05$ ), but IL-8 and TNF- $\alpha$  serum levels were not significantly higher than average. (Results that had  $P < 0.05$  were considered statistically significant.) All three cytokines were elevated in the OHSS peritoneal fluid ( $P < 0.001$  for IL-6,  $P < 0.05$  for IL-8 and TNF- $\alpha$ ).<sup>16</sup>

Loret de Mola *et al.* compared IL-6 levels from 9 severely affected OHSS patients to 26 healthy women.<sup>17</sup> Both serum and peritoneal IL-6 levels were higher in OHSS patients ( $P < 0.001$ ), shown in Table 4. Although non-OHSS control patients who had undergone menotropin stimulation had slightly higher postovulatory serum IL-6 than normal controls ( $13.1 \pm 1$  pg/mL versus  $4.4 \pm 0.69$  pg/mL), the OHSS patients had higher serum IL-6 than both groups ( $18.8 \pm 1.1$ ,  $P < 0.001$ ).

Geva *et al.* examined the utility of cytokines in predicting OHSS by following 322 "high responder" ART participants — women whose serum E2 exceeded 3,000 pg/mL, placing them at high risk for OHSS.<sup>13</sup> They analyzed follicular fluid (FF) samples from

10 high responders who developed severe OHSS, 10 high responders who did not develop OHSS, and 10 low responders who did not develop OHSS. Low responders had serum E2  $< 2,000$  pg/mL and less than 10 retrieved oocytes, placing them at low risk for OHSS. Table 5 lists IL-6, IL-2, and TNF- $\alpha$  FF levels as well as serum E2 concentrations.

**Table 4.** IL-6 levels in preovulatory serum and peritoneal fluid are higher in OHSS patients than healthy controls.<sup>16</sup>

IL-6 (pg/mL)	OHSS (n=9)	Controls (n=26)	P
Serum	$18.8 \pm 1.1$	$4.5 \pm 0.8$	$< 0.001$
Peritoneal	$810 \pm 60.7$	$44.7 \pm 7.5$	$< 0.001$

IL-6 concentrations in OHSS high responders were greater than in non-OHSS high responders and normal controls ( $P < 0.05$ ). Differences in IL-2 and TNF- $\alpha$  FF levels were not significantly higher in OHSS high responders compared to non-OHSS high responders. IL-6 preovulatory FF measurements distinguished OHSS from non-OHSS

**Table 5.** Cytokine levels in follicular fluid of OHSS patients are compared to control values. E2 serum levels distinguish high responders from low responders.<sup>13</sup>

Analyte pg/mL	OHSS (n=10)	Controls	
		High E2 (n=10)	Low E2 (n=10)
E2*	$3,240 \pm 352$	$3,020 \pm 312$	$1,284 \pm 611$
IL-6	$983 \pm 305^{**}$	$343 \pm 66.2$	$73.4 \pm 1.8$
IL-2	$5.7 \pm 1.2$	$5.2 \pm 0.8$	$3.8 \pm 0.4$
TNF- $\alpha$	$4.9 \pm 2.1$	$5.4 \pm 2.4$	$3.9 \pm 0.7$

\*Serum measurements

\*\* $P < 0.05$

women even within the high risk group, demonstrating that IL-6 measurements may be an effective early predictor of OHSS.

Hormone and cytokine measurements may help physicians identify high risk ART participants and predict OHSS. Recent studies demonstrate the potential value of cytokine measurements as complements to hormone tests. Cytokines appear to participate in OHSS and may serve as valuable predictive tools. In particular, increased serum and follicular fluid IL-6 concentrations may indicate early stages of OHSS. Research also points to the utility of LH and FSH in OHSS prediction efforts. The IMMULITE test menu includes these analytes (see Table 6) and provides a valuable resource for physicians who monitor ART participants and treat OHSS patients.

## References

1. Rabau E, David A, Serr DM, Mashiach S, Lunenfeld B. Human menopausal gonadotropin for anovulation and sterility. Results of 7 years of treatment. *Amer J Obstet Gynecol* 1967;96:92-8.
2. Schenker JG and Weinstein D. Ovarian hyperstimulation syndrome: a current survey. *Fert Steril* 1978;30(3):255-68.
3. Golan A, Ron-el A, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol* 1989;44:430-40.
4. Danninger B, Brunner M, Obruca A, Feichtinger W. Prediction of ovarian hyperstimulation syndrome of baseline ovarian stimulation prior to stimulation. *Hum Reprod* 1996;11(8):1597-9.
5. Rizk B and Smitz J. Ovarian hyperstimulation syndrome after superovulation using GnRH agonists for IVF and related procedures. *Hum Reprod* 1992;7(3):320-7.

**Table 6.** *The IMMULITE test menu includes the analytes relevant to OHSS prediction and treatment.*

Analyte	Calibration Range	Volume required	Incubation time
<i>Cytokines:*</i>			
IL-1 $\beta$	up to 1,000 pg/mL	75 $\mu$ L	60 min
IL-2	3 – 2,000 pg/mL	100 $\mu$ L	60 min
IL-6	2 – 2,000 pg/mL	100 $\mu$ L	60 min
IL-8	20 – 7,500 pg/mL	50 $\mu$ L	30 min
TNF- $\alpha$	up to 1,000 pg/mL	10 $\mu$ L	60 min
<i>Hormones:</i>			
Estradiol	20 to 2,000 pg/mL	25 $\mu$ L	60 min
FSH	up to 170 mIU/mL	50 $\mu$ L	30 min
LH	up to 200 mIU/mL	50 $\mu$ L	30 min

*\* In the U.S., for research use only.*

- 
6. Elchalal U, Schenker JG. The pathophysiology of ovarian hyperstimulation syndrome — views and ideas. *Hum Reprod* 1997;12(6):1129-37.
  7. Wallach E et al. *Reproductive Medicine and Surgery. Part five: Therapy of infertility*. Mosby, 1995 (ISBN 0-8016-7504-9).
  8. Bódis J, Török A, Tinneberg H-R. LH/FSH ratio as a predictor of ovarian hyperstimulation syndrome. Letters to Editor. *Hum Reprod* 1997;12(4):869-71.
  9. Meirou D, Schenker JG, Rosler A. Ovarian hyperstimulation syndrome with low oestradiol in non-classical 17 $\alpha$ -hydroxylase, 17,20-lyase deficiency: what is the role of oestrogens ? Case Report. *Hum Reprod* 1996;11(10):2119-2121.
  10. Levy T, Orvieto R, Homburg R, Peleg D, Dekel A, Ben-Rafael Z. Severe ovarian hyperstimulation syndrome despite low plasma oestrogen concentrations in a hypogonadotrophic, hypogonadal patient. Case Report. *Hum Reprod* 1996;11(6):1177-9.
  11. Abramov Y, Schenker JG, Lewin A, Friedler S, Nisman B, Barak V. Plasma inflammatory cytokines correlate to the ovarian hyperstimulation syndrome. *Hum Reprod* 1996;11(7):1381-6.
  12. Adashi YE. The potential relevance of cytokines to ovarian physiology: the emerging role of resident ovarian cells of white blood series. *Endocrine Rev* 1990;11:454-64.
  13. Geva E, Azem F, Lessing JB, Yovel I, Lerner-Geva L, Amit A. Elevated levels of interleukin-6 in the follicular fluid at the time of oocyte retrieval for in vitro fertilization may predict the development of early-form ovarian hyperstimulation syndrome. *Fert Steril* 1997;68(1):133-7.
  14. Rizk B, Aboulghar M, Smitz J, Ron-El R. The role of vascular endothelial growth factor and interleukins in the pathogenesis of severe ovarian hyperstimulation syndrome. *Hum Reprod Update* 1997; 3(3):255-66.
  15. Friedlander MA, De Mola JR, Goldfarb JM. Elevated levels of interleukin-6 in ascites and serum from women with ovarian hyperstimulation syndrome. *Fert Steril* 1993;60:820-32.
  16. Revel A, Amit A, Barak V, Finci-Yeheskel Z, Lavy Y, Mayer M, et al. Characterization of intraperitoneal cytokines and nitrites in women with severe ovarian hyperstimulation syndrome. *Fert Steril* 1996;66(1):66-71.
  17. Loret de Mola JR, Flores JP, Baumgardner GP, Goldfarb JM, Gindlesperger V, Friedlander MA. Elevated interleukin-6 levels in ovarian hyperstimulation syndrome: ovarian immunohistochemical localization of interleukin-6 signal. *Obstet Gynecol* 1996;87(4):581-7.