

An immunohistochemical study in a fatality due to ovarian hyperstimulation syndrome

Vittorio Fineschi · Margherita Neri · Sabina Di Donato ·
Cristoforo Pomara · Irene Riezzo · Emanuela Turillazzi

Received: 18 October 2005 / Accepted: 27 April 2006 / Published online: 2 June 2006
© Springer-Verlag 2006

Abstract Ovarian hyperstimulation syndrome (OHSS) is a complication occurring in the luteal phase of a menstrual cycle in which ovulation induction or ovarian hyperstimulation for assisted reproduction techniques has been performed. Our report concerns the death of a 31-year-old woman with primary infertility on recombinant follicle stimulating hormone therapy (Gonal-F) who developed a fatal adult respiratory distress syndrome. Immunohistochemical investigation of lung and uterine samples was performed utilizing monoclonal antibodies for antisurfactant apoprotein (PE-10) and polyclonal antibodies anti-IL-6, IL-8, IL-10, TNF α for lungs, and polyclonal antibodies anti- β -HCG (human chorionic gonadotropin) for uterus. The knowledge of macroscopic and histologic findings should be of special interest for all those who perform autopsies in these victims. The exact pathogenesis of OHSS remains a mystery; so for the diagnosis of suspected OHSS, the forensic pathologist must use all the modern laboratory armament to validate the diagnosis in the extremely rare fatal cases due to this syndrome.

Keywords Adult respiratory distress syndrome · Assisted reproduction techniques · Ovarian hyperstimulation syndrome · Death · Immunohistochemistry

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic disorder arising subsequent to ovulation induction or ovarian hyperstimulation for assisted reproduction techniques (ART). It may cause severe morbidity and can even be life threatening [1].

The reported incidence of OHSS is highly variable due to the existing differences in patient populations, stimulatory agents, clinical evaluation of symptoms and clinical signs, and finally in classification used to identify the degree of this syndrome in the different reported series. According to an estimate of the World Health Organization (WHO) in developed countries, the proportion of sterile couples—the potential users of ART—varies between 15 and 20% [2]. The incidence of syndromes due to ovarian hyperstimulation, which is necessary with the so-called major assisted reproduction techniques where superovulation of varying degrees is expected, is calculated to be around 23% with 7.6% mild, 11.95% moderate, and 3.9% severe [3, 4].

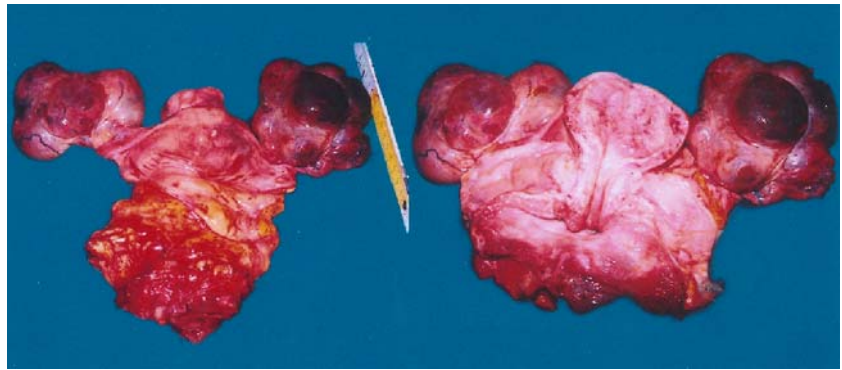
The syndrome has a broad spectrum of clinical manifestations, from mild forms requiring only careful monitoring and observation to severe disease needing hospitalization and intensive care. Although significant OHSS has a relatively low incidence ($\approx 2\%$), it may result in a potentially fatal condition as the mortality rate has been estimated to be from 1 in 45,000 to 1 in 500,000 [5].

A few deaths due to complications of OHSS have been previously reported in the literature [6–8].

Our report concerns the death of a 31-year-old woman with primary infertility on recombinant follicle stimulating hormone (FSH) therapy (Gonal-F) who developed a fatal acute respiratory distress syndrome (ARDS).

V. Fineschi (✉) · M. Neri · S. Di Donato · C. Pomara ·
I. Riezzo · E. Turillazzi
Department of Forensic Pathology, University of Foggia,
Policlinico Ospedali Riuniti, Via Luigi Pinto no. 1,
Foggia 71100, Italy
e-mail: vfinesc@tin.it

Fig. 1 Specimen of uterus and enlarged ovaries. Both ovaries were associated with the presence of multiple cysts



Case history

A 31-year-old woman with unexplained infertility underwent ovarian stimulation by recombinant FSH (rFSH). Plasma estradiol measurements were carried out during ovarian stimulation and monitoring of follicle growth was performed by vaginal ultrasound measurements. Oocyte retrieval was performed on April 3, 2004 and follicle aspiration was carried out by ultrasound-guided vaginal puncture under local anesthesia. On the same day, two administrations of albumin (20%) were applied. ICSI (intracytoplasmic sperm injection) using testicular sperm obtained by open biopsy (TESE: testicular sperm extraction) was performed and three oocytes were inseminated. On April 6, 2004, two embryos were transferred into the uterus and luteal support was given with progesterone. On April 15, 2004, the woman complained of abdominal discomfort and cystic, enlarged ovaries were visualized through vaginal ultrasound. The woman was admitted to an obstetric unit where a full clinical examination was performed, measurements of full blood count, urea, creatinine, and electrolyte concentrations.

The pelvic ultrasonography showed the presence of a uterine myoma, enlarged ovaries with multiple follicular cysts, retrohepatic gathering, and a small amount of ascitic fluid. Albumin and heparin were administered. The day after the woman complained of mild dyspnea and tachypnea, laboratory investigations showed a very low albumin level, neutrophilic leukocytosis, red cells 5,740,000/ μ l, hemoglobin 18 g/dl, and hematocrit 50.9%. No chest X-ray scans were made. On April 18, 2004, at 14.25 h the woman suddenly developed respiratory arrest and died.

Autopsy findings

A complete autopsy was performed 48 h after death. The body was that of a young woman, 161 cm in height, 63 kg in weight. Internal examination revealed 875 ml straw-colored liquid in the left pleural cavity and 1,450 ml straw-colored liquid in the right pleural cavity. The lungs were

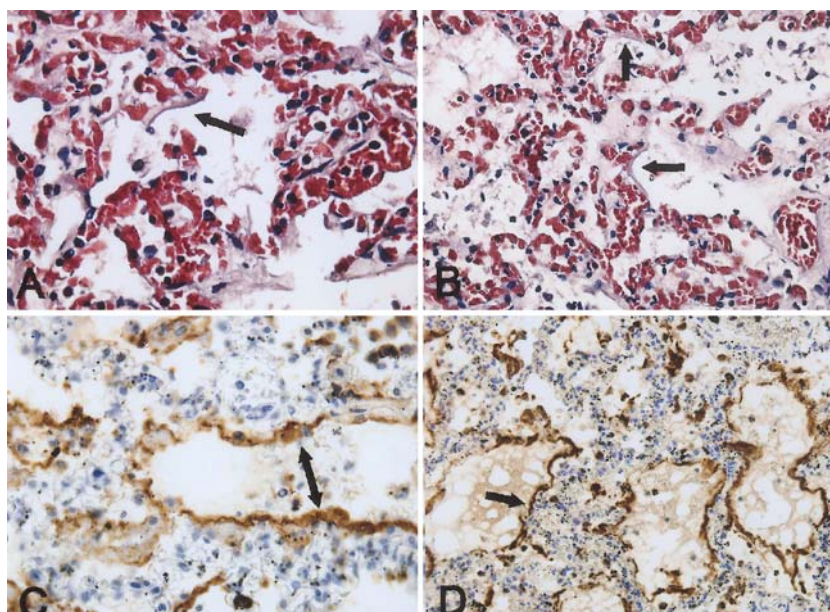
edematous, both weighing 310 g. The peritoneal cavity contained 275 ml of straw-colored liquid. The uterus was normal, both ovaries were associated with the presence of multiple cysts, the left ovary measured 12.5×9×5 cm and weighed 70 g, while the right ovary measured 14×9.5×5 cm and weighed 120 g (Fig. 1). The cardiovascular system was unremarkable and no source of emboli was localized. All the other organs did not show specific alterations except for an intense vascular congestion.

Histological findings

A routine microscopic histopathological study was performed by using formalin-fixed paraffin-embedded tissue sectioned at 4 μ m and stained with hematoxylin–eosin and Wilder (silver impregnation for reticular fibers) staining methods. Immunohistochemical investigation of lung and uterine samples was performed utilizing the monoclonal antibodies antisurfactant apoprotein (PE-10) for the lungs and polyclonal antibodies anti- β -HCG (human chorionic gonadotropin) for the uterus.

In addition, an immunohistochemical investigation of lung samples was performed utilizing polyclonal anti-IL-6, IL-8, IL-10, and TNF α . We used 4 μ m thick paraffin sections mounted on slides covered with 3-aminopropyltriethoxysilane (Fluka, Buchs, Switzerland). A pretreatment for IL-6-8-10 (5 min proteolytic enzyme Dako, Copenhagen, Denmark, 20°C), TNF α , and β -HCG (boiled in 0.1 M citric acid buffer), was necessary to facilitate antigen retrieval and to increase membrane permeability to antibodies except that the sections covered with the antibody antisurfactant apoprotein (S-AP-PE-10). The primary antibody was applied in a 1:2,000 dilution for IL-6 (Santa Cruz Biotechnology, Santa Cruz, CA), in a 1:500 dilution for IL-8 (Abcam, Cambridge, UK), in a 1:2,000 dilution for IL-10 (Peprotech EC, London, UK), in a 1:600 ratio for TNF α (Santa Cruz Biotechnology), in a 1:50 ratio for S-AP-PE-10 (Dako), and in a 1:200 dilution for anti β -HCG (Dako), and incubated for 120 min at 20°C. A secondary antibody anti-rabbit was used to link with the anti- β -HCG. The positive reaction was

Fig. 2 Diffuse alveolar damage (DAD). **a** and **b** Hyaline membranes (*arrows*) lining the denuded alveolar walls (H&E $\times 60$; H&E $\times 40$). **c** and **d** Positive reaction of the immunohistochemical dye for surfactant apoprotein (PE-10) ($\times 60$)



visualized by 3,3-diaminobenzidine (DAB) peroxidation, according to standard methods. The sections were counterstained with Mayer's hematoxylin, dehydrated, covered, and observed under a Leica optical microscope.

Histological lung examination showed alveolar septa mildly thickened by edema and capillary congestion, alveolar edema, hyaline membranes lining the denuded alveolar walls, flattened pneumocytes, fibrin thrombi in small arteries, and areas of atelectasis. Bronchial walls presented epithelial denudation. All these findings were indicative of a typical diffuse alveolar damage (DAD), confirmed by the positive results to Wilder's staining for reticular fibers and to immunohistochemical labeling for

surfactant apoprotein (PE-10) (Fig. 2). The presence of inflammatory cytokines in the lungs was highlighted with a positive reaction for antibodies anti-IL6, IL-8, IL-10, and TNF α (Fig. 3).

Routine histological examination of ovaries demonstrated follicular and stromal hyperthecosis and myxomatous change of the interstitial stroma of bilateral ovaries was detected. The uterus revealed a normal structure of the different layers of the wall, and in a sample from the uterine body, the syncytiotrophoblast composed of numerous internal multinucleated cells with a light cytoplasm was evident, and numerous external multinucleated cells with dark cytoplasm. This area showed an intensive positive

Fig. 3 Lungs: strongly positive immunohistochemical reaction for quantitative presence of inflammatory cytokines: **a** IL6, **b** IL-8, **c** IL-10, and **d** TNF α

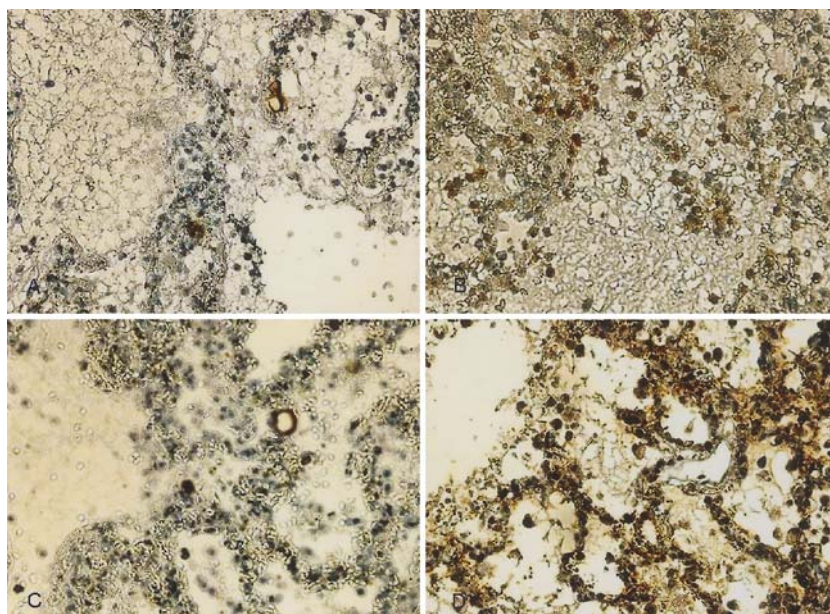
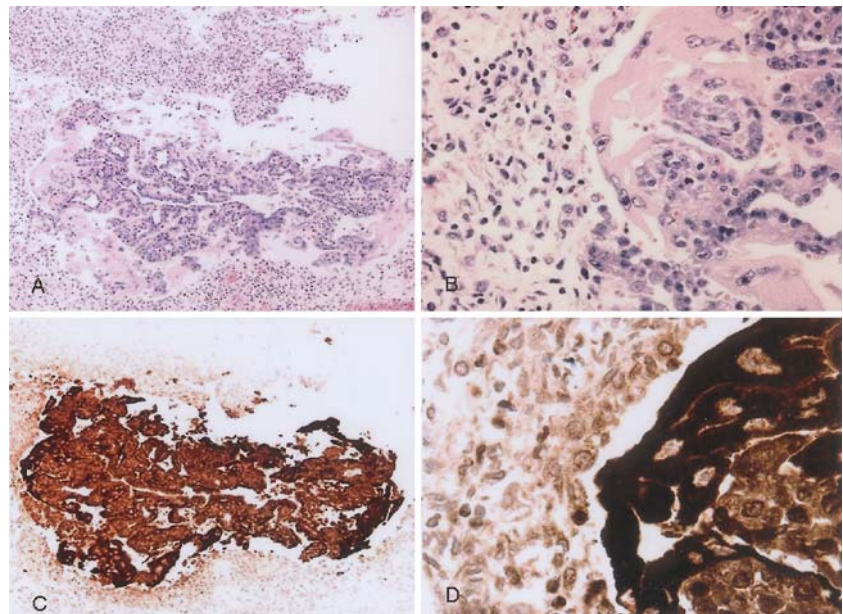


Fig. 4 The syncytiotrophoblast consisting of numerous inner multinucleated cells with a light cytoplasm, and numerous external multinucleated cells with dark cytoplasm (**a** and **b**) (H&E $\times 10$; H&E $\times 100$). Intensive positive immunohistochemical reaction to antibody anti- β -HCG (**c** and **d**) ($\times 10$; $\times 100$)



immunohistochemical reaction to the antibody anti- β -HCG, proving that pregnancy had taken place (Fig. 4).

In the other organs, there were no relevant alterations except for mild cerebral edema and polyvisceral hemostasis. The genetic analysis for thrombophilic factors was negative.

Discussion

In this case, the cause of death was attributed to ARDS due to OHSS. OHSS is a complication occurring in the luteal phase of a menstrual cycle in which ovulation induction or ovarian hyperstimulation for ART has been performed. It is characterized by cystic enlargement of the ovaries and a fluid shift from the intravascular to the third space after increased capillary permeability. These changes may cause hemoconcentration, electrolyte disturbances, liver and kidney disorders, and thromboembolic sequelae [9].

The severity of OHSS is graded according to several criteria [10–13], recently revised with further modifications by Rizk and Aboulghar [14], who first included complications (such as respiratory distress syndrome, renal shutdown, or venous thrombosis) in the classification of OHSS. The OHSS has traditionally been classified as mild, moderate, or severe (Table 1) [15]. Progression of the illness may, however, evolve to secondary complications which may be life threatening [1].

Pulmonary manifestations of severe OHSS have recently been studied in a large-scale multicenter study where ARDS incidence was reported in up to 2% of the study patients [16].

To our knowledge, only three case reports have been published reporting death due to OHSS and none reporting ARDS as cause of death [6–8]. Mozes et al. [6] described a case of ovarian hyperstimulation with cyst formation, peritoneal and pleural effusions, and acute left hemiplegia where right carotid arteriography revealed an occlusion of the left internal carotid artery extending intracranially. The patient died suddenly apparently from aspiration of gastric contents but a necropsy was not performed. Recently, clinical and pathological features of a fatal case were reported [7] where the cause of death was attributed to a cerebral infarction secondary to the blood hyperviscosity related to OHSS. Semba et al. [8] documented an autopsy case of a patient with OHSS who developed respiratory distress due to massive pleural effusions and pulmonary edema. Histological findings of the lungs were consistent with massive plasma leakage and intraalveolar hemorrhage and dilation of alveolar capillaries was described as significant.

In the case we describe, the histological features of ARDS are those of acute (exudative) DAD. The early exudative phase is characterized by alveolar and interstitial edema, capillary congestion, hyaline membranes, and infiltration of numerous inflammatory cells, such as neutrophils. The histological evaluation of lung samples stained with H&E gave evidence of a pattern of diffuse alveolar damage in the initial exudative phase, even more evident with further specific stainings. Wilder's staining for reticular fibers, demonstrated the epithelial damage showing focal areas of discontinuation and disappearance of alveolar basement membrane, while the presence of hyperplastic type II pneumocytes and hyaline membranes was confirmed by the positive reaction of the immunohis-

Table 1 Classification of ovarian hyperstimulation syndrome

Study	Mild	Moderate	Severe
Rabau et al. [10]	Grade 1: estrogen > 150 µg pregnanediol > 10 mg 24 h Grade 2: grade 1 + enlarged ovaries and possibly palpable cysts Grade 1 and 2 were not included under the title of mild OHSS	Grade 3: grade 2 + confirmed palpable cysts and distended abdomen Grade 4: grade 3 + vomiting and possibly diarrhea	Grade 5: grade 4 + ascites and possibly hydrothorax Grade 6: grade 5 + changes in blood volume, viscosity, and coagulation time
Schenker and Weinstein [11]	Grade 1: estrogen > 150 µg/24 h pregnanediol > 10 mg 24 h Grade 2: grade 1 + enlarged ovaries, sometimes small cysts	Grade 3: grade 2 + abdominal distension Grade 4: grade 3 + nausea, vomiting, and/or diarrhea	Grade 5: grade 4 + large ovarian cysts, ascites, and/or hydrothorax Grade 6: marked hemoconcentration + increased blood viscosity and possibly coagulation abnormalities
Golan et al. [12]	Grade 1: abdominal distension, discomfort Grade 2: grade 1+ nausea, vomiting, and/or diarrhea, enlarged ovaries 5–12 cm	Grade 3: grade 2 + ultrasound evidence of ascites	Grade 4: grade 3 + clinical evidence of ascites and/or hydrothorax and breathing difficulties Grade 5: grade 4 + hemoconcentration, increase blood viscosity, coagulation abnormality, and diminished renal perfusion
Navot et al. [13]			Severe OHSS: variable enlarged ovary; massive ascites ± hydrothorax; Hct > 45%; WBC > 15,000; oliguria; creatinine 1.0–1.5; creatinine clearance ≥50 ml/min; liver dysfunction; anasarca Critical OHSS: variable enlarged ovary; tense ascites ± hydrothorax; Hct > 55%; WBC ≥ 25,000; oliguria; creatinine ≥ 1.6; creatinine clearance < 50 ml/min; renal failure; thromboembolic phenomena; ARDS Grade A: dyspnea, oliguria, nausea, vomiting, diarrhea, abdominal pain, clinical evidence of ascites, marked distension of abdomen or hydrothorax, US showing large ovaries and marked ascites, and normal biochemical profile
Rizk and Aboulghar [14]		Discomfort, pain, nausea, distension, ultrasonic evidence of ascites and enlarged ovaries, and normal hematological and biological profiles	Grade B: grade A + massive tension ascites, markedly enlarged ovaries, severe dyspnea and marked oliguria, increased hematocrit, elevated serum creatinine, and liver dysfunction Grade C: complications as respiratory distress syndrome, renal shut-down, or venous thrombosis

tochemical dye for surfactant apoprotein (PE-10). The monoclonal against antisurfactant apoprotein (PE-10) antibodies positively reacted with hyperplastic type II pneumocytes, appearing as cuboidal epithelial cells (the so-called hobnail-shaped cells) lining the alveolar septal surfaces, but moreover outlined the presence of hyaline membranes, thin strands coating denuded alveolar surfaces, consisting of lamellar bodies (surfactant phospholipids) released from alveolar epithelial cells [17].

Prostaglandins and cytokines may play a role in the pathophysiology of ARDS, as they are also incriminated in the pathophysiology of OHSS [18]. The hallmark of OHSS is an increase in capillary permeability resulting in a fluid shift from the intravascular space to third space compartments [19]. Several factors have been postulated as initiators of this syndrome. Previously, the ovarian renin-angiotensin system (RAS) had been studied most extensively [1]. Most recently, several substances have been suggested to be responsible for the increased capillary permeability in OHSS, specifically endothelin-1, vascular endothelial growth factor (VEGF), angiotensin II, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), transforming growth factor (TGF) α and β , basic fibroblast growth factor (BFGF), platelet-derived growth factor (PDGF), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) [1, 19].

Interleukin-6 is markedly elevated in the peritoneal fluid and serum of patients with severe OHSS [18]. This syndrome may be precipitated by an ovarian product or cytokine secreted directly into the peritoneal cavity by the ruptured follicle and early corpus luteum. This cytokine can also gain access to the systemic circulation from the corpus luteum and serosal vessels [18].

In our case, the immunohistochemical reactions in the lungs were strongly positive for the presence of the inflammatory cytokines IL6, IL-8, IL-10, and TNF α , confirming the clinical data [1, 18]. Again, in a sample from the uterine body, the syncytiotrophoblast consisting of numerous inner multinucleated cells with a light cytoplasm, and numerous external multinucleated cells with a dark cytoplasm were evident. This area showed an intensive positive immunohistochemical reaction to antibody anti- β -HCG proving that pregnancy had taken place. Pregnancy is an increased risk factor associated with OHSS and therapeutic interruption of an early gestation may be life saving when all other therapeutic measures have failed [13].

This case deserves attention for clinical and forensic reasons. First, from a clinical viewpoint, physicians must pay attention to correctly evaluate the progression of the illness and the recurrence of respiratory difficulty (dyspnea and tachypnea) [20]. Respiratory distress in OHSS is often due to a restrictive syndrome induced by ascites, increased

ovarian size, and associated paralytic ileus. However, it may mask underlying, even more serious pulmonary complications like ARDS, pulmonary thromboembolism, and pulmonary infections which pose a diagnostic challenge in dyspnoeic patients with severe or critical OHSS [21]. Second, especially the pathologist or the forensic pathologist who is involved in such cases should be aware of these diseases. The knowledge of macroscopic and histological findings should be of special interest for all those who perform autopsies in these victims [22]. The exact pathogenesis of OHSS remains a mystery, so in the diagnostic suspicion of OHSS, the forensic pathologist must use all the modern laboratory armament to validate the diagnosis in the extremely rare fatal cases due to this syndrome [23–26].

References

1. Beerendonk CC, Van Dop PA, Braat DD, Merkus JM (1998) Ovarian hyperstimulation syndrome: facts and fallacies. *Obstet Gynecol Surv* 53:439–449
2. Fineschi V, Neri M, Turillazzi E (2005) The new Italian law on assisted reproduction technology (Law 40/2004). *J Med Ethics* 31:536–539
3. Delvigne A, Rozenberg S (2002) Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. *Hum Reprod Update* 8:559–577
4. Abramov Y, Elchalal U, Schenker JG (1999) Severe OHSS: An 'epidemic' of severe OHSS: a price we have to pay? *Hum Reprod* 14:2181–2183
5. Papanikolaou EG, Tournaye H, Verpoest W, Camus M, Vernaev V, Van Steirteghem A et al (2005) Early and late ovarian hyperstimulation syndrome: early pregnancy outcome and profile. *Hum Reprod* 20:636–641
6. Mozes M, Bogokowski H, Antebi E et al (1965) Thromboembolic phenomena after ovarian stimulation with human gonadotrophins. *Lancet* 2:1213–1215
7. Clueroe AD, Synek BJ (1995) A fatal case of ovarian hyperstimulation syndrome with cerebral infarction. *Pathology* 27:344–346
8. Semba S, Moriya T, Youssef EM, Sasano H (2000) An autopsy case of ovarian hyperstimulation syndrome with massive pulmonary oedema and pleural effusion. *Pathol Int* 50:549–552
9. Roest J, Mous HV, Zeilmaker GH, Verhoeff A (1996) The incidence of major clinical complications in a Dutch transport IVF programme. *Hum Reprod Update* 2:345–353
10. Rabau E, David A, Serr DM et al (1967) Human menopausal gonadotropins for anovulation and sterility. *Am J Obstet Gynecol* 98:92–98
11. Schenker JG, Weinstein D (1978) Ovarian hyperstimulation syndrome: a current survey. *Fertil Steril* 30:255–268
12. Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E (1989) Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv* 44:430–440
13. Navot D, Bergh PA, Laufer N (1992) Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. *Fertil Steril* 58:249–261
14. Rizk B, Aboulghar MA (1999) Classification, pathophysiology and management of ovarian hyperstimulation syndrome. In: Brinsden P (ed) *In vitro fertilization and assisted reproduction*. Parthenon, New York London, pp 131–155

15. Aboulghar MA, Mansour RT (2003) Ovarian hyperstimulation syndrome: classifications and critical analysis of preventive measures. *Hum Reprod Update* 9:275–289
16. Abramov Y, Elchalal U, Schenker JG (1999) Pulmonary manifestations of severe ovarian hyperstimulation syndrome: a multi-center study. *Fertil Steril* 71:645–651
17. Sugiyama K, Kawai T (1993) Diffuse alveolar damage and acute interstitial pneumonitis: histochemical evaluation with lectin and monoclonal antibodies against surfactant apoprotein and collagen type IV. *Mod Pathol* 6:242–248
18. Loret de Mola RJ, Arredondo-Soberon F, Randle CP, Tureck RT, Friedlander MA (1997) Markedly elevated cytokines in pleural effusion during the ovarian hyperstimulation syndrome: transudate or ascites? *Fertil Steril* 67:780–782
19. Practice Committee of the American Society for Reproductive Medicine (2003) Ovarian hyperstimulation syndrome. *Fertil Steril* 80:1309–1314
20. Venn A, Hemminki E, Watson L, Bruinsma F, Healy D (2001) Mortality in a cohort of IVF patients. *Hum Reprod* 16: 2691–2696
21. Delvigne A, Rozenberg S (2003) Review of clinical course and treatment of ovarian hyperstimulation syndrome (OHSS). *Hum Reprod Update* 9:77–96
22. Wedekind H, Schulze-Bahr E, Debus V, Breithardt G, Brinkmann B, Bajanowski T (2006) Cardiac arrhythmias and sudden death in infancy: implication for the medicolegal investigation. *Int J Legal Med* Jan 6:1–13. DOI 10.1007/s00414-005-0069-3
23. Laiho K (2004) Albumin as a marker of plasma transudation in experimental skin lesions. *Int J Legal Med* 118:282–288
24. Hayashi T, Ishida Y, Kimura A, Takayasu T, Eisenmenger W, Kondo T (2004) Forensic application of VEGF expression to skin wound age determination. *Int J Legal Med* 118:320–325
25. Reichelt U, Jung R, Nierhaus A, Tsokos M (2005) Serial monitoring of interleukin-1 β , soluble interleukin-2 receptor and lipopolysaccharide binding protein levels after death. A comparative evaluation of potential postmortem markers of sepsis. *Int J Legal Med* 119:80–87
26. Bacci S, Romagnoli P, Norelli GA, Forestieri AL, Bonelli A (2006) Early increase in TNF-alpha-containing mast cells in skin lesions. *Int J Legal Med* 120:138–142