Article

Low-dose HCG is useful in preventing OHSS in high-risk women without adversely affecting the outcome of IVF cycles

Geeta Nargund is Head of Reproductive Medicine at St George's Hospital and Medical Director of the Centre of Reproduction and Advanced Technology, London, UK. She is also Chief Executive of the Health Education Research Trust (HER Trust) UK and the President of ISMAAR (International Society for Mild Approaches in Assisted Reproduction). She has published extensively on the role of advanced ultrasound technology in reproductive medicine. She is the chief author of the first scientific paper on cumulative live birth rates of natural (unstimulated) cycle IVF and on one-stop fertility diagnosis using ultrasound technology. Her research interests include minimal approaches in assisted reproduction and advanced ultrasound technology.

Dr Geeta Nargund

Geeta Nargund[1], Lee Hutchison, Rex Scaramuzzi, Stuart Campbell
St George's Hospital and Centre for Reproduction and Advanced Technology (Create Health Clinic) London
[1]Correspondence: e-mail: geetanargund@googlemail.com

Abstract

Severe ovarian hyperstimulation syndrome (OHSS) is a rare but potentially fatal condition associated with conventional IVF treatment. It is found predominantly in women with polycystic ovaries who have an exaggerated response to exogenous FSH, leading to a large number of follicles and an overproduction of vascular endothelial growth factor with resultant excessive increases in vascular permeability. There is evidence to suggest that OHSS is also linked to the use of human chorionic gonadotrophin (HCG) to induce ovulation. Therefore, while HCG is essential for corpus luteum function, high amounts of HCG can lead to OHSS in high responders. In a pilot study, infertile patients at high risk of developing OHSS were given half the current minimum dose of HCG (i.e. 2500 IU). No woman developed moderate or severe OHSS; 13 women (61.9%) conceived and there were three twin pregnancies. In women at high risk of OHSS, a low dose of HCG appears to prevent the development of OHSS without compromising success rates.

Keywords: *HCG, IVF, OHSS, ovarian stimulation, PCO, VEGF*

Introduction

Severe ovarian hyperstimulation syndrome (OHSS) is well known to be a rare but potentially fatal condition in anovulatory women with polycystic ovaries (PCO) or polycystic ovarian syndrome (PCOS) when undergoing IVF. Low-dose stimulation is thus recommended, but it can still lead to ovarian hyperstimulation associated with high serum oestradiol concentrations by the time leading follicles reach maturity. Several methods have, therefore, been applied to prevent OHSS. First, risk assessment is made on the basis of the previous history of OHSS and the identification of women with PCO. Second, in treatment cycles a high concentration of oestradiol and three ultrasound parameters (i.e. high number of follicles, large ovarian volume, and high stromal vascularity) on the day of human chorionic gonadotrophin (HCG) are all predictive of increased risk of developing OHSS.

Under these circumstances, various methods have been adopted to prevent the development of OHSS. Abandoning cycles prior to administration of HCG or proceeding with egg collection and freezing all embryos is an inefficient method of management of treatment cycles. Another approach is to withhold FSH to reduce oestradiol concentrations (coasting), which acts through the down-regulation of vascular endothelial growth factor (VEGF) gene expression (Garcia-Velasco *et al*., 2004). Coasting up to 3 days can prevent OHSS but yields inferior pregnancy rates (Chen *et al*., 2003).

Ovulation induction with HCG in infertile patients is likely to remain the method of choice despite the availability of recombinant LH. A survey of the literature and data from various clinics indicate that there is wide variability (5000–25,000 IU) in the dose of HCG for ovulation induction, although there appears to be little evidence to support the use on any particular dose concentration. Individual clinics appear to have selected a dose of HCG on an arbitrary basis to ensure that as many follicles as

possible are ovulated and that there is a successful pregnancy outcome following embryo transfer. This study was designed to find out whether the incidence of OHSS could be reduced without compromising the outcome of IVF cycles by lowering the ovulatory dose of HCG to 2500 IU. Therefore, a pilot study in women, identified as being at high risk of OHSS, was undertaken to examine the question of the optimum dose of HCG and in particular to determine the effect of a relatively low dose (2500 IU) of HCG on ovum recovery, the incidence of OHSS and the outcome of IVF cycles.

Materials and methods

Women at high risk of severe OHSS on the day of HCG administration were offered three choices; abandonment of cycle, coasting or low-dose HCG. Patient information included the impact of HCG on OHSS and different dosages (10,000, 5000 and 2500 IU) used in accordance with ovarian response and the risk of OHSS. Patients were also given full counselling as to the different management options. All women who consented to low-dose HCG were included in this study. They had received a daily dose of 100 IU FSH (Puregon; Organon, Denmark) or 112.5 IU FSH (Gonal F; Serono, Italy) in a first attempt or 150 IU FSH in a second attempt (based on previous response) in a long pituitary down-regulation protocol.

HCG was administered when the mean diameter of the leading follicle was 18 mm. In all cases, the following characteristics were recorded at the time of HCG administration: (i) there were 4–5 follicles >16 mm and more than 20 follicles in total in each ovary; (ii) serum oestradiol concentrations were >14,000 pmol/l on the day of HCG administration; (iii) both ovaries had high volumes (combined volume >184 ml); (iv) there was high ovarian vascularity, as subjectively assessed by examining power Doppler images of the ovary (**Figure 1** (**a**, **b**).

Women exhibiting the above four criteria were given 2500 IU HCG (half of an ampoule of 5000 IU) subcutaneously (Pregnyl; Organon) and follicle aspiration was planned 34–36 h after HCG administration. All follicles were aspirated.

Embryo transfer was performed either on day 3 or 5, depending on the available embryos as per the protocol of the laboratory. A maximum of two embryos was transferred. Any good quality spare embryos were frozen.

Progesterone supplements in the form of cyclogest vaginal pessaries (400 mg twice daily) were given for luteal support. All women were followed up with telephone calls during the luteal phase regarding their well-being and serum β-HCG was carried out 2 weeks after embryo transfer to check for pregnancy.

Results

In total, 21 women, aged between 28 and 36 years and at risk of developing severe OHSS, received 2500 IU HCG. Six of them had developed severe OHSS in a previous IVF attempt, including one woman who had developed an arterial thrombotic episode with an ovulatory dose of 5000 IU HCG. The mean duration of stimulation was 9.4 days. The mean number of total follicles was 38 (22–56) and, of those, 10 were greater than 14 mm. The combined minimum ovarian volume was 184 ml. Assessment of ovarian vascularity by power Doppler ultrasound showed high stromal vascularity with colour signals from all follicles greater than 12 mm.

The total dose of FSH used per cycle ranged between 750 and 1650 IU per woman. The range for serum oestradiol was between 14,445 and 27,669 pmol/l on the day of HCG. Three women whose oestradiol concentrations were greater than 30,000 pmol/l were coasted for 3 days and their oestradiol concentrations were still above 20,000 pmol/l prior to HCG administration (**Table 1**).

The mean number of oocytes collected was 24 (range 11–32) and the fertilization rate per collected oocyte was 46.4%. Of the 21 women in the study, 11 had two blastocysts transferred and 10 had two day-3 embryos transferred. Spare embryos were frozen for future use for 10 women.

No woman developed moderate or severe OHSS and only one woman developed symptoms of mild OHSS. Pregnancies were achieved in 13 women (61.9%) and, of those, three had twin pregnancies.

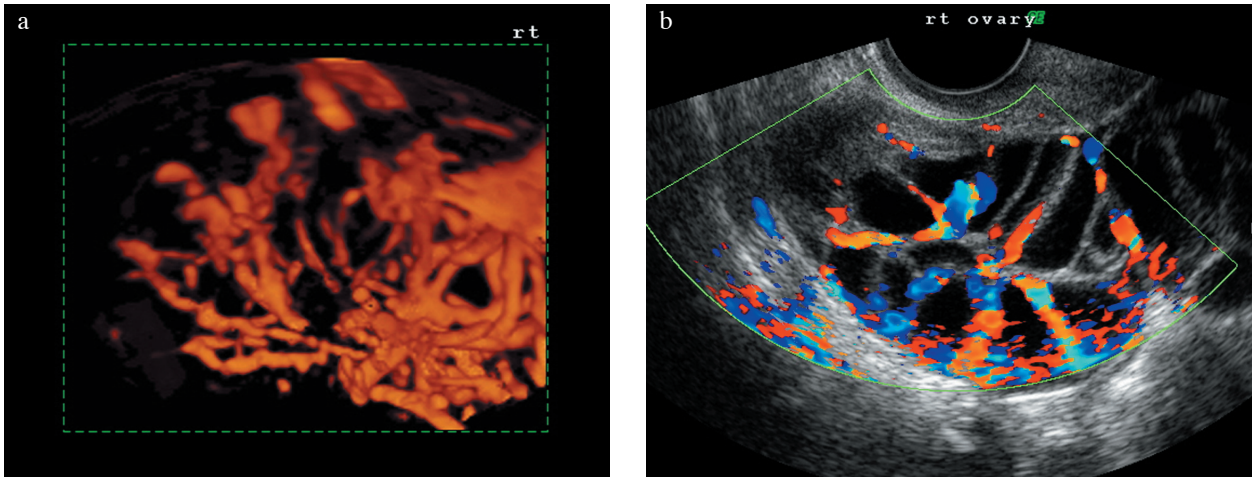

Figure 1. (**a**) High stromal vascularity as demonstrated by 3D power Doppler ultrasound. (**b**) Colour Doppler image showing high vascularity in a hyperstimulated ovary prior to human chorionic gonadotrophin administration.

Table 1. Serum oestradiol concentrations in 21 women at high risk of ovarian hyperstimulation syndrome.

Oestradiol range (pmol/l)	*No. of women*
14,000–16,000	10
16,000–21,000	3
21,000–26,000	5
26,000–31,000	2
31,000–40,000	1

Discussion

The induction of ovulation by the controlled administration of an ovulatory dose of HCG is integral to successful IVF for both conventional IVF protocols and the newer natural cycle IVF protocols. The use of HCG as a natural analogue of LH to induce ovulation takes advantage of particular pharmacokinetic properties that give HCG a longer circulating half-life than LH. The longer half-life of HCG means that it persists in the circulation well after ovulation and in this respect it is unlike LH, which is rapidly cleared from the circulation after the LH surge (Balasch and Fabregues, 2006). The persistence of HCG in the circulation is thought to be favourable to the development of a fully competent corpus luteum capable of supporting implantation and normal embryo development following embryo transfer (Neulen *et al.*, 1998). However, the persistence of HCG is also associated with OHSS, a less desirable side effect (Niederberger *et al.*, 1995; Qasim *et al.*, 1997) that, if left untreated, can be potentially life threatening.

There are strong positive associations between plasma concentrations (Abramov *et al.*, 1997; Lee *et al.*, 1997a) and follicular fluid concentrations (Lee *et al.*, 1997b) of VEGF and OHSS. The role of VEGF in the pathogenesis of OHSS appears to be due to its ability to increase vascular permeability (McClure *et al.*, 1994; Levin *et al.*, 1998). Normally, this effect of VEGF leads to paracrine-mediated 'leakiness' of the local vasculature required for late pre-ovulatory follicular enlargement. However, when a large number of mature follicles (greater than 20 in each ovary) are present as a result of exogenous FSH stimulation, there can be overproduction of VEGF and excessive increases in vascular permeability that lead to OHSS.

There is quite a compelling body of evidence to suggest that OHSS is linked to the use of HCG to induce ovulation (Albert *et al.*, 2002). The serum concentration of VEGF during FSH treatment is not a reliable indicator of OHSS (D'Ambrogio *et al.*, 1999; Artini *et al.*, 2002) whereas the serum concentration of VEGF after HCG is a good indicator of OHSS (Agrawal *et al.*, 1999; Artini *et al.*, 1998, 2002). Both LH (Gomez *et al.*, 2004) and HCG (Rizk *et al.*, 1997) stimulate production of VEGF. *In vitro*, HCG increased VEGF production by cultured granulosa-lutein cells (Lee *et al.*, 1997b) and endothelial cells (Pellicer *et al.*, 1999), while *in vivo*, HCG increases serum concentrations of circulating VEGF (Agrawal *et al.*, 1998; Pellicer *et al.*, 1999) and the concentration of VEGF in antral fluid (Agrawal *et al.*, 1998). One of the actions of VEGF is increased vascular permeability (McClure *et al.*, 1994; Levin *et al.*, 1998) and the use of excessively high doses of HCG to induce ovulation, particularly when there has been a good follicular response to FSH, will lead to high secretion of VEGF from the granulosa cells and an increased risk of OHSS.

A recent study by Gomez *et al.* (2004) showed that low-dose LH (10 IU) induces ovulation and results in significantly lower vasopressin and VEGF expression in superovulated rats. Furthermore, Schmidt *et al.* (2004) demonstrated that a reduced HCG dose of 3300 IU results in a similar proportion of mature eggs, fertilization rates and pregnancy rates compared with 5000 IU. However their study showed that 3300 IU HCG does not eliminate the risk of OHSS in high-risk groups. It, therefore, seemed to us logical that a reduced dosage of HCG at 2500 IU (half of normally used dose of 5000 IU in high responders) to induce ovulation might reduce the risks of severe OHSS.

Three of the criteria that were used to define a high risk for OHSS are well established in the literature. Higher oestradiol concentrations, a higher number of follicles (Qasim *et al.*, 1997) and a significantly higher ovarian volume (Oyesanya *et al.*, 1995) prior to the administration of HCG have been found in women who developed moderate to severe OHSS compared with women with mild or no complications. The minimal combined volume chosen in this study was 184 ml, which is 1 SD below the mean of 271 ml for the OHSS group in the study of Oyesanya *et al.* (1995). Increased stromal vascularity reflects increased angiogenic activity and VEGF concentrations (Agrawal *et al.*, 1998, 2002) and the fourth criterion of risk was the finding of high stromal vascularity on power Doppler examination.

This pilot study revealed two important findings. Firstly, at this dose of HCG, no moderate or severe OHSS was observed in these 21 women who were at high risk of severe OHSS. Secondly that a dose of HCG as low as 2500 IU will mature FSH stimulated follicles. Comparing the number of follicles seen by ultrasound examination with the numbers of oocytes recovered, fertilized and successfully implanted none of the critical post-ovulatory processes involved in IVF appeared to be impaired by this dose of HCG.

The absence of any OHSS with the low dose of HCG is noteworthy. While it is conceded that the numbers of patients in this series is perhaps too small to allow the conclusion that a lower dose of HCG is the treatment of choice in high risk patients at the present time, nevertheless it is possible to conclude that a low dose of HCG is less likely to cause OHSS because there is less VEGF produced by granulosa cells even when there has been a high level of FSH-stimulated follicle development. The lower concentration of VEGF is associated with a reduced risk of excessive increase in vascular permeability that leads to OHSS.

Conclusion

The evidence from this pilot study is that, in women at high risk of OHSS, a reduction of the current 'minimum' dose (i.e. 5000 IU) of HCG to 2500 IU appears to prevent the development of OHSS in these women without compromising success rates.

Further randomized studies are needed to establish the minimal ovulatory dose of HCG and LH required in the management of IVF cycles.

References

Abramov Y, Barak V, Nisman B, Schenker JG 1997 Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome. *Fertility and Sterility* **67**, 261–265.

Agrawal R, Jacobs H, Payne N, Conway G 2002 Concentration of vascular endothelial growth factor released by cultured human luteinized granulosa cells is higher in women with polycystic ovaries than in women with normal ovaries. *Fertility and Sterility* **78**, 1164–1169.

Agrawal R, Tan SL, Wild S *et al.* 1999 Serum vascular endothelial growth factor concentrations in in-vitro fertilization cycles predict the risk of ovarian hyperstimulation syndrome. *Fertility and Sterility* **71**, 287–293.

Agrawal R, Conway G, Sladkevicius P *et al.* 1998 Serum vascular endothelial growth factor and Doppler blood flow velocities in in-vitro fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. *Fertility and Sterility* **70**, 651–658.

Albert C, Garrido N, Mercader A *et al.* 2002 The role of endothelial cells in the pathogenesis of ovarian hyperstimulation syndrome. *Molecular Human Reproduction* **8**, 409–418.

Artini PG, Monti M, Fasciani A *et al.* 2002 Vascular endothelial growth factor, interleukin-6 and interleukin-2 in serum and follicular fluid of patients with ovarian hyperstimulation syndrome. *European Journal of Obstetric, Gynaecology and Reproductive Biology* **101**, 169–174.

Artini PG, Fasciani A, Monti M *et al.* 1998 Changes in vascular endothelial growth factor levels and the risk of ovarian hyperstimulation syndrome in women enrolled in an in-vitro fertilization programme. *Fertility and Sterility* **70**, 560–564.

Balasch J, Fabregues F 2006 LH in the follicular phase: neither too high nor too low. *Reproductive BioMedicine Online* **12**, 406–415.

Chen CD, Chao KH, Yang JH *et al.* 2003 Comparison of coasting and intravenous albumin in the prevention of ovarian hyperstimulation syndrome. *Fertility and Sterility* **80**, 86–90.

D'Ambrogio G, Fasciani A, Monti M *et al.* 1999 Serum vascular endothelial growth factor levels before starting gonadotropin treatment in women who have developed moderate forms of ovarian hyperstimulation syndrome. *Gynaecological Endocrinology* **13**, 311–315.

Garcia-Velasco JA, Zuniga A, Pacheco A *et al.* 2004 Coasting acts through downregulation of VEGF gene expression and protein secretion. *Human Reproduction* **19**, 1530–1538.

Gomez R, Lima I, Simon C, Pellicer A 2004 Administration of low-dose LH induces ovulation and prevents vascular hyperpermeability and vascular endothelial growth factor expression in superovulated rats. *Reproduction* **127**, 483–489.

Lee A, Christenson LK, Stouffer RL *et al.* 1997a Vascular endothelial growth factor levels in serum and follicular fluid of patients undergoing in-vitro fertilisation. *Fertility and Sterility* **68**, 305–311.

Lee A, Christenson LK, Patton PE *et al.* 1997b Vascular endothelial growth factor production by human luteinised granulosa cells *in vitro*. *Human Reproduction* **12**, 2756–2761.

Levin ER, Rosen GF, Cassidenti DL *et al.* 1998 Role of vascular endothelial growth factor in ovarian hyperstimulation syndrome. *Journal of Clinical Investigation* **102**, 1978–1985.

McClure N, Healy DL, Rogers PA *et al.* 1994 Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. *Lancet* **344**, 235–236.

Neulen J, Raczek S, Pogorzelski M *et al.* 1998 Secretion of vascular endothelial growth factor/vascular permeability factor from human luteinized granulosa cells is human chorionic gonadotrophin dependent. *Molecular Human Reproduction* **4**, 203–206.

Niederberger V, Rottensteiner-Grohsmann C, Turnheim K *et al.* 1995 Incidence of ovarian hyperstimulation syndrome in in-vitro fertilization treatment over a period of 11 years. *Wiener Medizinische Wochenschrift* **145**, 642–646 [in German].

Oyesanya OA, Parsons JH, Collins WP, Campbell S 1995 Total ovarian volume before human chorionic gonadotrophin administration for ovulation induction may predict the hyperstimulation syndrome. *Human Reproduction* **10**, 3211–3212.

Pellicer A, Albert C, Mercader A *et al.* 1999 The pathogenesis of ovarian hyperstimulation syndrome: in-vivo studies investigating the role of interleukin-1beta, interleukin-**6**, and vascular endothelial growth factor. *Fertility and Sterility* **71**, 482–489.

Qasim SM, Karacan M, Kemmann E 1997 An eight-year review of hospitalisation for ovarian hyperstimulation syndrome. *Clinical and Experimental Obstetrics and Gynaecology* **24**, 49–52.

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. *Human Reproduction Update* **3**, 255–266.

Schmidt DW, Maier DB, Nulsen JC, Benadiva CA 2004 Reducing the dose of human chorionic gonadotropin in high responders does not affect the outcomes of in-vitro fertilization. *Fertility and Sterility* **82**, 841–846.

Paper based on a contribution presented at the First World Congress on 'Natural Cycle/Minimal Stimulation IVF' in London, UK, December 15–16, 2006.

Received 19 March 2007; refereed 20 March 2007; accepted 11 April 2007.