Oocyte maturation employing a GnRH agonist in combination with low-dose hCG luteal rescue minimizes the severity of ovarian hyperstimulation syndrome while maintaining excellent pregnancy rates

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BACKGROUND: The traditional hCG ‘trigger’ for initiating final oocyte maturation exacerbates ovarian hyperstimulation syndrome (OHSS) in patients with an excessive follicular response because of its sustained stimulatory effect on the corpora lutea. However, a GnRH agonist trigger can produce a short duration endogenous LH surge which is adequate to initiate oocyte maturation, but allows the corpora lutea to regress, reducing the severity of OHSS. This approach produces an excellent embryology outcome, but generally results in low pregnancy rates even with the early initiation of estrogen and progesterone luteal support. The purpose of this study was to determine if a low dose of hCG (1500 IU) support on the day of oocyte retrieval can maintain good pregnancy rates, while not abolishing the protective effect of an agonist trigger on the development of severe OHSS.

METHODS: This retrospective study included 71 women who were at high risk of severe OHSS (≥14 follicles ≥12 mm) and who received an agonist trigger for final oocyte maturation.

RESULTS: The transfer of a solitary embryo produced a biochemical pregnancy rate of 60.6% and a clinical ongoing pregnancy rate of 52.1%. Only one patient was hospitalized with severe OHSS (1.4%), despite the average patient producing nearly 17 oocytes per cycle.

CONCLUSIONS: Oocyte maturation employing a GnRH agonist (protocol) in combination with low-dose hCG luteal support produces excellent clinical pregnancy rates, while not compromising the ability of GnRH agonist to prevent severe OHSS.

Key words: luteal phase / GnRH agonist/antagonist / hCG / oocyte maturation / OHSS

Introduction

Controlled ovarian hyperstimulation (COH) has become a key component of modern IVF treatment as the availability of multiple oocytes for fertilization increases a woman’s chances of successful pregnancy. However, occasionally a patient’s response to COH will be excessive, resulting in massive cystic enlargement of the ovaries and biochemical changes leading to an increase in vascular permeability and a shift of fluid from the intra-vascular compartment to the third space (Humaidan et al., 2010a). Not only is this type of ovarian hyperstimulation syndrome (OHSS) response uncomfortable for the patient, it can also lead to hospitalization and even death from venous thrombosis and embolization.

Several different approaches have been taken to minimize the development of OHSS in IVF patients (Humaidan et al., 2010b). The most common approach is to predict likely hyper-response based on medical history [polycystic ovary syndrome (PCOS) and past OHSS response] or measures of ovarian reserve [serum anti-Müllerian hormone (AMH) and antral follicle count (AFC)], and then to initiate a lower dose of gonadotropin stimulation to diminish the risk of an exaggerated response. Unfortunately this approach does not always prevent OHSS and, on many occasions, leads to an unexpected
suboptimal low oocyte response. Alternatively, if a patient develops an excessive response, gonadotrophin stimulation may be withdrawn for several days (‘coasting’) until her estrogen levels fall to a safer level before administering hCG (Humaidan et al., 2010a). The cryopreservation of all embryos is an effective treatment to prevent late-onset OHSS, but is not popular with patients and does not avoid early-onset OHSS. Finally, new pharmacological methods of minimizing OHSS severity, such as cabergoline, have been shown to be of some benefit. Unfortunately, however, none of these treatments completely removes the risk of developing OHSS, but merely reduces its incidence and severity. There is a need to develop new effective treatments to avoid severe OHSS without compromising IVF pregnancy rates.

Recently, there has been an increase in interest in the technique of oocyte maturation employing a GnRH agonist (OMEGA protocol), rather than the more traditional hCG trigger (Humaidan et al., 2011). The publication by Itskovitz et al. (1988) was the first to recognize that a GnRH agonist can stimulate a ‘flare’ gonadotrophin surge from the patient’s own pituitary which is capable of maturing oocytes, yet avoids the development of severe OHSS. Later studies suggested that the pituitary LH surge observed following an agonist trigger lasted only 24–36 h, sufficient to initiate oocyte maturation, but not to produce the prolonged stimulation of the corpus luteum as seen with the use of a traditional hCG trigger (Damewood et al., 1988; Itskovitz et al., 1991). This rapid withdrawal of LH stimulation post ovulation results in involution of the corpus luteum early in the luteal phase, an abrupt fall in steroid hormone production and a reduction in the production of vascular endothelial growth factor (VEGF), the primary cytokine felt to be primarily responsible for OHSS (Cerrillo et al., 2009).

With the recent increase in popularity of GnRH antagonist IVF therapy, it has now become possible to use a GnRH agonist trigger to stimulate final oocyte maturation (OMEGA protocol). Initial RCTs suggested that the use of an agonist trigger produced comparable or slightly superior levels of oocyte maturity, fertilization and embryo quality compared with a hCG trigger (Humaidan et al., 2005, 2009, 2010b; Kolibianakis et al., 2005; Orvieto et al., 2006; DiLuigi et al., 2010; Kol et al., 2010), while resulting in comparable pregnancy rates when these embryos were transferred into donor oocyte recipients (Acevedo et al., 2006; Erb et al., 2010). However, the majority of studies report that, during autologous oocyte IVF, the use of an agonist trigger generally results in poor clinical pregnancy rates and a high rate of pregnancy loss, despite early initiation of steroid hormone luteal support (Humaidan et al., 2005; Kolibianakis et al., 2005). Only one RCT has reported comparable clinical pregnancy rates between the traditional hCG trigger and the use of a GnRH agonist trigger (Engmann et al., 2008). Overall the majority of studies suggest an endometrial defect secondary to a lack of LH action, rather than a simple deficiency of estrogen and progesterone, and this has prompted studies using low-dose (1500 IU) hCG luteal rescue on the day of oocyte retrieval (Humaidan, 2009; Humaidan et al., 2010a). The largest RCT examining the use of an agonist trigger and low-dose hCG rescue protocol reported excellent embryology outcomes and comparable clinical pregnancy rates (Humaidan et al., 2010b), supporting the use of such an approach. However, as the participants in this study were not selected for being at high risk of OHSS, only a small proportion of them (1%) developed moderate to severe OHSS, making statistical analysis of differences in the rate of severe OHSS between this protocol and the traditional hCG trigger impossible. The purpose of this study is to describe our clinics personal experience using the OMEGA–hCG protocol in a group of patients who were all at high risk of OHSS.

Materials and Methods

Study inclusion criteria

All participants in this study were identified as having a high risk of developing severe OHSS in the late follicular phase of the IVF cycle, before administration of an oocyte maturing trigger injection. The criteria used for identification of high risk of OHSS was the presence of at least 14 follicles ≥12 mm on Day 8/9 of stimulation (or a later follow-up scan if a slow response to stimulation was encountered), as this has previously been reported to predict the development of OHSS in 87% of severe cases (Papanikolaou et al., 2005). Patients with large numbers of follicles were administered a GnRH agonist trigger, rather than the traditional hCG trigger. No upper limit of ovarian response was used to preclude the use of hCG luteal support following an agonist trigger. Our clinic commenced the use of this OMEGA–low-dose hCG luteal rescue protocol in women judged to be at high risk of OHSS in January 2010. This retrospective study summarizes our experience of 71 consecutive cases using this treatment, concluding in April 2011.

IVF treatment protocol

The GnRH antagonist treatment protocol has been the primary mode of IVF treatment used in our clinic since 2007, and now accounts for 95% of all IVF cycles. The standard gonadotrophin starting dose is 150 IU of recombinant FSH (rFSH; Puregon, MSD or Gonal F, Merck Serono) in women under 36 years of age, 200–225 IU in the 36–39 year group and a maximal dose of 300 IU in women 40 years or older. Those women identified as high risk of developing OHSS (PCOS, AFC > 20 or AMH > 30 pmol/l) are often started on a dose of 112.5–125 IU rFSH.

All gonadotrophin stimulation starts on Day 2 or 3 of the menstrual cycle and a GnRH antagonist is commenced on Day 6 of stimulation (ganiirelix 0.25 mg, MSD). A pelvic ultrasound and serum hormone assessment is performed on Day 8 or 9 of stimulation and oocyte maturation is triggered when three or more follicles ≥17 mm of diameter are present. Weekend oocyte retrievals are avoided by either a delay or advance in the day of trigger by one day from ideal, as has been previously published by our group (Tremellen and Lane, 2010).

The traditional trigger used for oocyte maturation in the vast majority of patients attending our unit is 250 µg of rhCG (Ovidrel, Merck Serono), with an agonist trigger only being used in women deemed at high risk of developing OHSS. Techniques for the minimization of OHSS such as ‘coasting’ and cabergoline therapy were not used at all in this study. The OMEGA–hCG protocol consisted of an s.c. injection of 2 mg leuprolide acetate (Lucrin, Abbott), followed by oocyte retrieval 36 h later under sedation. The patient was then given 1500 IU of hCG (Pregnyl, MSD) s.c. within an hour of the oocyte retrieval and luteal support consisting of daily vaginal progesterone (Crinone, Merck Serono) and twice daily 2 mg estradiol (E2) valerate (Progynova, Bayer), commencing on the night of the oocyte retrieval. Fertilization was undertaken using standard protocols and all embryos were cultured for 4–5 days before being transferred or cryopreserved. As all women were at high risk of OHSS, a single embryo transfer was mandated. Luteal support was continued until menstruation or 8 weeks gestation despite a previous study showing that only 2 weeks of luteal support is adequate to maintain good live birth rates (Humaidan et al., 2010a). This extended period of luteal support was used because of local practitioner’s personal concerns regarding possible
late luteal phase insufficiency from a lower dose of hCG exposure, rather than a clear evidence-based requirement.

**Outcomes and statistical analysis**

The two primary outcomes of interest for this retrospective study were clinical pregnancy rates, defined as the presence of at least one fetal heart on an 8 week ultrasound, and the incidence of severe OHSS. While many classifications of OHSS exist, we elected to adopt the classification of Navot et al. (1992) as this most closely represents our own clinical practice. Navot et al. defines severe OHSS as the presence of massively enlarged ovaries and ascites, haematocrit >0.45, white cell count >15 000, liver dysfunction and renal impairment (critically low urine output or raised serum creatinine). These indices of OHSS severity are generally the triggers used by our unit for hospitalization for fluid monitoring, thromboprophylaxis and possible surgical intervention (ascitic and/or pleural tap). In our practice, patients with lesser degrees of OHSS are normally managed adequately in an outpatient setting.

Statistical analysis was conducted using the GraphPad Prism 5 program (GraphPad, San Diego, CA, USA). A biochemical pregnancy was defined as the presence of a positive serum hCG on Day 19 post-oocyte retrieval. A clinical ongoing pregnancy was defined as the presence of at least one fetal heart on an 8 week gestation ultrasound. The miscarriage rate was defined as the percentage of cycles showing evidence of a gestational sac but no viable fetal heart on 8 week ultrasound.

**Ethical approval**

Approval for this retrospective study was obtained from the local institutional Scientific Advisory Committee, with all patients giving previous written consent for their medical notes to be accessed for retrospective quality audit studies. Full ethics committee review approval of retrospective ‘quality audits’ is not required by Australian law.

**Results**

The baseline characteristics of the study subjects are outlined in Table I. The median age of women was 32 years, with a range from 23 to 42 years. A diagnosis of PCOS was the most common aetiology of infertility, present in 45.1% of subjects. The mean serum AMH (48.3 pmol/l) and AFC (2–10 mm) of 26.9 indicate excellent ovarian reserve and a high risk of developing OHSS. The majority of women (53%) had not previously had IVF treatment before this GnRH agonist trigger treatment cycle.

Table II outlines the IVF outcomes for the study participants. All 71 participants achieved fertilization of at least one oocyte, and a single embryo transfer occurred in all cases. The overall fertilization rate of confirmed metaphase II oocytes (ICSI cases) was 73.1%, with a mean number of 9.1 embryos being produced, a mandated single embryo transfer occurring and an average of 3.4 embryos being cryopreserved.

The pregnancy outcomes for the 71 participants are summarized in Table II. In total, 35 viable singleton pregnancies and 2 twin monozygotic pregnancies were identified at the 8 week ultrasound examination. Four pregnancies failed at the biochemical stage and a further two pregnancies presented a non-viable fetus on ultrasound scan. This resulted in an overall biochemical pregnancy rate of 60.6% and a clinical viable pregnancy rate of 52.1% per embryo transfer. The clinical miscarriage rate for ultrasound confirmed pregnancies was 5.1%.

Of the 71 women at high risk of OHSS, only 1 was admitted to hospital with severe OHSS. This patient was 33 years old, undergoing her first cycle of IVF for a combination of PCOS and severe male factor infertility. Her baseline AMH of 84 pmol/l and AFC of 20 confirmed her high risk for developing OHSS. On the day of GnRH agonist trigger administration her serum E2 result was 14.7 nmol/l. A total of 17 ‘mature’ size follicles were aspirated to retrieve 13 oocytes and a solitary good quality blastocyst was transferred 5 days later. She was admitted to hospital on the 16th day post-oocyte retrieval with significant dyspnoea and abdominal swelling, ascites, a packed cell volume 0.43, hyponatremia (Na 131 mmol/l) and raised transaminases (aspartate transaminase 271 U/l). While in hospital she gained 9 kg in weight, and developed significant ascites, a mild pleural effusion and subcutaneous oedema. Following strict fluid monitoring, intravenous albumin therapy, anticoagulation (enoxaparin 40 mg daily, Sanofi-Aventis) for thromboprophylaxis and oral analgesia, she was discharged 7 days later. An ultrasound at 8 weeks gestation confirmed the presence of a viable singleton pregnancy. At 28 weeks gestation, the patient delivered a live female infant by emergency Caesarean section for placental abruption.
**Discussion**

A recent Cochrane Systematic review (Youssef et al., 2011) recommended that GnRH agonists not be used routinely as a final oocyte maturation trigger in fresh autologous IVF cycles because of a very significant reduction in live birth rates compared with the use of a traditional hCG trigger (odds ratio (OR) 0.44, 95% confidence interval (CI) 0.29–0.68). This Cochrane review analysed data from 11 RCTs and over one thousand cycles of IVF. However, only two of the studies included in this meta-analysis examined the effect of a combination of agonist trigger and low-dose luteal phase hCG rescue (Humaidan et al., 2006; 2010b). If one analyses the combined live birth rate in these two trials, no statistically significant difference between the two trigger protocols exists (24.8% in the agonist trigger hCG versus 33.3% hCG trigger, \( P = 0.11 \)). The results of this retrospective study support the conclusion that the use of an agonist trigger in combination with a low-dose hCG luteal rescue can result in excellent clinical pregnancy outcomes. While our study did not include a control arm, the observed viable clinical pregnancy rate of 52.1% from a single embryo transfer compares favourably with that observed with the use of a hCG trigger in our own general IVF population, or that reported from large national IVF registries (de Mouzon et al., 2010). Therefore, we conclude that provided a low dose of hCG luteal support is given at the time of oocyte retrieval, the use of a GnRH agonist to trigger oocyte maturation does not result in an inferior clinical pregnancy rate.

It still remains to be determined why the majority of studies suggest that estrogen and progesterone luteal support is insufficient to maintain adequate endometrial development and pregnancy rates in a GnRH agonist-triggered IVF cycle. Engmann et al. (2008) are the only investigators to report comparable clinical pregnancy success rates in a fresh embryo transfer cycle in which oocyte maturity has been triggered by a GnRH agonist, rather than the traditional hCG trigger. The reasons why this study departs from the majority view of the remaining studies outlined in the Cochrane review is not entirely clear but may reflect differences in the patient characteristics. In the Engmann study, the vast majority of participants had PCOS, where this was clearly not the case in studies reporting a negative effect of an agonist trigger on pregnancy rates. PCOS is well known to be associated with high serum LH levels and therefore it is possible that in this subgroup of patients endogenous serum LH levels are not as significantly suppressed by the IVF treatment, leading to an adequate LH drive to support normal corpora lutea function, in turn resulting in normal pregnancy rates. Alternatively, the Engmann studies more intensive and tailored luteal support regime may have an advantage over the less intensive fixed dose support regimes used by the majority of investigators. In the Engmann study, patients were administered a daily dose of 0.3 mg transdermal E2 and 50 mg intra-muscular (IM) progesterone starting on the day following the oocyte retrieval. Serum steroid levels were then closely monitored throughout the luteal phase and the dosages of both estrogen and progesterone luteal support could be increased to a maximum of 0.4 mg transdermal E2 plus 4 mg oral micronized progesterone if serum levels were found to be inadequate. This level of steroid luteal support is significantly more intense than that used by either the Humaidan [90 mg progesterone per vagina (PV), 4 mg oral E2] or Kolibianakis (600 mg progesterone PV, 4 mg oral E2) research groups and may account for the observed differences in pregnancy outcomes.

Obviously, the addition of an 'LH effect' using low-dose hCG has a positive influence on the endometrium either directly or indirectly. Previous studies have located LH receptors in the endometrium (Tesarik et al., 2003; Rao, 2006), where LH action may assist implantation by up-regulating local cytokine and growth factor production. Conversely, it is possible that hCG may boost production of corpus luteum hormones such as Relaxin, which in turn positively influence endometrial development (Goldsmith and Weiss, 2009). However, the observation that a successful pregnancy is possible in a woman with no ovaries using oocyte donation and simple estrogen/progesterone luteal support, does not support the conclusion that ovarian derived Relaxin is essential for implantation.

VEGF is thought to be the main molecular mediator of OHSS since its production is greatly enhanced during an exaggerated IVF response and it has the capacity to increase vascular permeability, thereby causing the transfer of fluid from the intra-vascular to the third space (Albert et al., 2002; Humaidan et al., 2010). VEGF production by the endothelial cells is up-regulated by hCG (Albert et al., 2002; Wang et al., 2002), with VEGF production being reduced in a GnRH agonist triggered IVF cycle compared with a hCG trigger (Cerrillo et al., 2009). These observations probably account for the significantly lower incidence of severe OHSS seen when using a GnRH agonist trigger (OR 0.10, 95% CI 0.01–0.82), as reported by the recent Cochrane review (Youssef et al., 2011). However, as the treatment protocol in this study included a small dose of hCG in the early luteal phase, there was a potential risk of exacerbating OHSS severity. Fortunately, as only one patient from 71 women at high risk of OHSS developed severe enough OHSS to warrant hospitalization, we can conclude that the addition of 1500 IU of hCG to the luteal phase does not significantly increase OHSS risk.

This study is the largest to date examining the ability of a combined GnRH agonist–low-dose hCG luteal rescue approach to prevent hospitalization for OHSS in a group of patients with an exaggerated response to gonadotrophin stimulation. All 71 subjects were at very significant risk of severe OHSS, as testified by their IVF response and baseline characteristics (high incidence PCOS, high AMH and AFC). Previous studies suggest that with these patient characteristics, approximately one-third of patients would normally develop severe OHSS with a hCG trigger. Before 2009, our clinic would typically admit to hospital 1% of patients undergoing IVF for the management of severe OHSS. However, since commencing the selective use of a GnRH agonist trigger in high responders, we have halved our OHSS admissions to only 0.45% of cycles (10 cases from 2200 cycles IVF), with nine of these OHSS admissions inadvertently receiving a hCG trigger. While the lack of an RCT precludes any absolute conclusions, the almost complete absence of severe OHSS using the combined agonist trigger-hCG approach suggests that it is an effective preventative therapy. In fact, given the extremely low rates of severe OHSS and excellent pregnancy rates seen in this study, it is doubtful whether a RCT using estrogen and progesterone luteal support without any hCG supplementation would now be ethically acceptable. However, we do acknowledge that our use of 1500 IU hCG did not completely avoid the chances of hospitalization with OHSS so a prospective study randomizing patients to dosages of hCG < 1500 IU may help determine the minimum dose of hCG required to maintain excellent pregnancy rates while completely avoiding hospitalization for severe OHSS.

In our study cohort, we did not set any upper limit on the number of oocytes collected where we would not administer hCG luteal support.
and perform a fresh embryo transfer due to the fear of an unacceptably high risk of OHSS. The maximal number of oocytes collected in our study was 41, with hCG support and a fresh embryo transfer still safely being performed without severe OHSS developing. It is our view that with the use of today’s relatively accurate markers of ovarian response to stimulation (serum AMH and AFC), excessive responses beyond 40 oocytes should be almost completely avoided by reducing the starting dose of rFSH stimulation. As such, the results of this study suggest that the vast majority of patients will be able to be protected from severe OHSS by the use of a GnRH agonist trigger/hCG support, while not compromising pregnancy outcomes. However, as the OMEGA/hCG protocol was unable to prevent hospitalization for severe OHSS with total certainty, we would suggest that a ‘freeze all’ approach with no hCG luteal support would be the preferred option in patients where any risk of severe OHSS is absolutely contraindicated due to pre-existing medical conditions (severe cardiac, respiratory or renal compromise, thrombophilia) (Griesinger et al., 2011). It must also be acknowledged that a retrospective study such as ours does not provide ‘gold standard’ level I evidence that an OMEGA/hCG protocol is capable of preventing severe OHSS while still maintaining excellent pregnancy rates. Hopefully future RCTs will be conducted to confirm our observations and help determine the minimal dose of hCG support required to maintain ideal pregnancy rates, while completely avoiding severe OHSS.

**Conclusion**

The use of OMEGA protocol in combination with a low-dose (1500 IU) hCG luteal rescue produces excellent clinical pregnancy rates, while almost totally avoiding admission to hospital with severe OHSS. Future randomized controlled trials are warranted to determine the minimal dose of luteal hCG support required to maintain excellent pregnancy rates, while completely avoiding OHSS risk. In the interim, we advocate the use of this OMEGA–hCG protocol in any woman undergoing a GnRH antagonist cycle of IVF treatment who subsequently develops an excessive ovarian response. The OMEGA/hCG protocol cannot be safely advocated in patients who have a pre-existing medical condition that makes any risk of severe OHSS absolutely contra-indicated since a complete avoidance of severe OHSS cannot be guaranteed. In this small group of patients a GnRH agonist trigger without hCG luteal support and cryopreservation of all embryos would appear to be the safest approach.

**Authors’ roles**

B.R. played a role in study design, collection of the raw data and writing of the manuscript. K.T. was involved in design of the study, statistical analysis of the data and writing of the manuscript.

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