

Consistent high clinical pregnancy rates and low ovarian hyperstimulation syndrome rates in high-risk patients after GnRH agonist triggering and modified luteal support: a retrospective multicentre study

Stamatina Iliodromiti^{1,*}, Christophe Blockeel², Kelton P. Tremellen³, Richard Fleming¹, Herman Tournaye², Peter Humaidan^{4,†}, and Scott M. Nelson^{1,†}

¹Maternal and Reproductive Medicine, School of Medicine, University of Glasgow, 3rd Floor McGregor Building, Glasgow, UK, ²University Hospital of the Free University of Brussels, Laarbeeklaan 101-1090, Brussel, ³Repromed Adelaide, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia and ⁴The Fertility Clinic, Skive Regional Hospital, Denmark and Aarhus University, Faculty of Health, Aarhus, Denmark

*Correspondence address. E-mail: stamatina.iliodromiti@glasgow.ac.uk

Submitted on May 10, 2013; resubmitted on June 11, 2013; accepted on June 28, 2013

STUDY QUESTION: Are clinical pregnancy rates satisfactory and the incidence of OHSS low after GnRH agonist trigger and modified intensive luteal support in patients with a high risk of ovarian hyperstimulation syndrome (OHSS)?

SUMMARY ANSWER: GnRH agonist trigger combined with 1500 IU hCG at the time of oocyte retrieval and subsequent estradiol and progesterone replacement in OHSS high-risk patients can facilitate fresh embryo transfer with high clinical pregnancy rates and a low risk of severe OHSS.

WHAT IS KNOWN ALREADY: Conventional luteal support packages are inadequate to facilitate a fresh transfer after a GnRH agonist trigger. A low dose of hCG (1500 IU) after oocyte aspiration can be used to replace the actions of early luteal LH to sustain implantation and the function of the early corpus luteum, although the level of risk of severe OHSS with this strategy is unclear.

STUDY DESIGN, SIZE, DURATION: This international multicentre retrospective case study, including 275 women at high risk of OHSS, was undertaken during the period January 2011–December 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women were identified as at high risk of OHSS, based on IVF response, ovarian reserve characteristics and previous history of having had treatment, in three clinical IVF centres in UK, Belgium and Australia. All three centres used a GnRH agonist trigger followed by one bolus of 1500 IU hCG 1 h after oocyte retrieval. Moreover, the luteal phase was supported with daily vaginal progesterone and twice daily estradiol valerate.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 275 autologous cycles with fresh transfer were undertaken in a cohort of high-risk women as defined by baseline characteristics [median (interquartile range)]: age 31.6 (29–35) years, antral follicle count median 25 (18–34) and anti-Müllerian hormone median 49.1 pmol/l (35.2–69.3). At the end of stimulation, the peak estradiol median of 12 000 pmol/l (9400–15 914) and the mean oocyte yield of 17.8 ± 8.4 confirmed a high response. The overall clinical pregnancy rate was 41.8% per cycle started, with only two cases of severe OHSS reported (0.72%). No significant differences in clinical pregnancy rates between centres were identified.

[†] Joint senior authors.

LIMITATIONS, REASONS FOR CAUTION: This is a retrospective study and future randomized controlled trials will be able to compare whether these outcomes can be improved upon by either segmentation of the stimulation cycle and embryo transfer or alternative aggressive luteal support strategies.

WIDER IMPLICATIONS OF THE FINDINGS: In women who are undergoing ovarian stimulation and who develop an excessive ovarian response, the use of a GnRH agonist trigger combined with modified luteal support can provide the opportunity to proceed to fresh embryo transfer with adequate clinical pregnancy rates. However, this procedure will not completely eliminate the risk of OHSS and for women with an extreme ovarian response or with significant comorbidity, where the possibility of severe OHSS is unacceptable, we recommend GnRH agonist trigger followed by a freeze-all policy to completely avoid OHSS.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: Not applicable.

Key words: GnRH agonist / GnRH antagonist / luteal support / clinical pregnancy / ovarian hyperstimulation syndrome

Introduction

Gonadotrophin-releasing hormone agonists (GnRH agonist) can be used as an alternative trigger to hCG in cycles that have been suppressed with a GnRH antagonist. Administration of a GnRH agonist displaces the GnRH antagonist in the pituitary, activating the GnRH receptor and resulting in a surge of gonadotrophins mimicking that of the natural mid-cycle surge of gonadotrophins (Itskovitz et al., 1991). However, significant differences exist between the GnRH agonist-induced surge and that of the natural cycle or the traditional hCG trigger. The LH surge of the natural cycle is characterized by three phases, with a total duration of 48 h (Hoff et al., 1983), whereas the GnRH agonist-induced surge of gonadotrophins consists of two phases only, with a duration of 24–36 h (Itskovitz et al., 1991). This leads to a significantly reduced total amount of gonadotrophins being released from the pituitary when GnRH agonist is used to trigger final oocyte maturation (Gonen et al., 1990; Itskovitz et al., 1991). Conversely, the traditional hCG trigger continues to stimulate ovarian steroid hormone production for up to 5 days, exacerbating the severity of OHSS. For the GnRH agonist trigger, these differences have no negative effect on oocyte maturation; however, do dramatically reduce the endogenous LH concentration during the early luteal phase with a marked detrimental impact on corpus luteal function and the capacity of the endometrium to sustain the function of the early implant.

The clinical implications of this are highlighted by the differences observed between oocyte donation and autologous cycles. In the oocyte donation model, avoidance of hCG exposure has been associated with complete elimination of ovarian hyperstimulation syndrome (OHSS), while recipient pregnancy rates are equivalent to those observed with hCG triggering (Galindo et al., 2009; Melo et al., 2009; Sismanoglu et al., 2009). In contrast in fresh autologous cycles, the use of a GnRH agonist trigger when combined with conventional luteal support has been associated with almost a complete reduction in OHSS, but also a marked reduction in pregnancy rates and an increased risk of miscarriage (Humaidan et al., 2005; Kolibianakis et al., 2005).

Recognition of these issues has prompted debate regarding the best approach for luteal-phase support to facilitate fresh embryo transfer in GnRH agonist triggered cycles. Two alternative strategies have been reported, which both dissociate the ovulation trigger from the luteal support. The first of these uses intensive luteal-phase support with estradiol and progesterone (Engmann et al., 2008), while the second utilizes a modified luteal-phase support with hCG (Humaidan et al., 2010a,b).

In the latter, a bolus of GnRH agonist is responsible for an endogenous surge of LH and FSH, and a low dose of hCG (1500 IU) after oocyte aspiration is used to replace the actions of early luteal LH to sustain implantation and luteal ovarian steroidogenesis. This approach was initially assessed in normo-responding women (Humaidan et al., 2006; 2010a,b), with extension to women at high risk of OHSS, with two small cohorts reporting a low incidence of OHSS and maintenance of clinical pregnancy rates [$n = 12$ (Humaidan, 2009), $n = 71$ (Radesic and Tremellen, 2011)]; however, not all cohorts have reported a similarly low incidence of OHSS [$n = 23$ (Seyhan et al., 2013)].

To date, although the use of the GnRH agonist trigger has become widespread, fresh embryo transfer in women at high risk of OHSS would appear to still be restricted to a few centres. To address this, we here report the efficiency, reproducibility and consistency of the overall outcome, i.e. clinical pregnancy rates and incidence of OHSS after GnRH agonist trigger and modified luteal-phase support in high-risk OHSS patients treated in three different infertility clinics.

Material and Methods

Study subjects

This retrospective analysis summarizes the experience from three large fertility centres using a GnRH agonist protocol to trigger final oocyte maturation and an intensive luteal support package to facilitate a fresh transfer in women deemed to be at high risk of developing OHSS. All cycles undertaken during 2011 and 2012 were eligible for inclusion. The criteria used for identifying women with a high risk of developing severe OHSS in the late follicular phase of an IVF cycle included baseline ovarian reserve measures [anti-Müllerian hormone (AMH) and antral follicle count (AFC)] and then actual ovarian response (Humaidan et al., 2010a,b). All cycles that used alternative techniques such as 'coasting' or cabergoline therapy to minimize the risk of OHSS were excluded from the study. Oocyte donation cycles were also excluded.

IVF treatment protocol

A GnRH antagonist protocol was used as the primary mode of ovarian co-treatment with gonadotrophin dose adjustment according to local protocols. The standard starting dose of recombinant FSH was 150 IU per day for women under 36 years of age. Gonadotrophin doses were, however, modified differently at each centre based on age, BMI, PCOS, AFC, AMH and previous history of OHSS, thus, starting FSH doses ranging from 112.5 to 225 IU.

Centre 1 (UK) used highly purified human menopausal gonadotrophin (Menopur; Ferring Pharmaceuticals) while Centres 2 and 3 (Belgium and Australia) used recombinant FSH (Gonal F; Merck Serono or Puregon, MSD). Stimulation started on Day 2 or 3 of the cycle, and a GnRH antagonist was added on Day 5 or 6 of stimulation. In all centres, a pelvic ultrasound was performed on stimulation day 5, with adjustment of the gonadotrophin according to ovarian response. Routine ultrasound and endocrine monitoring were initiated thereafter. A GnRH agonist trigger was administered in women with excessive follicular response at least 8 h following the last GnRH antagonist injection.

Each clinic had its own threshold for triggering with GnRH agonist rather than hCG. Specifically, Centre 1 (UK) used a GnRH agonist trigger in all women with an AMH > 40 pmol/l irrespective of ovarian response or if estradiol was $\geq 15\,000$ pmol/l on the day of trigger irrespective of the baseline AMH. Centre 2 (Belgium) used a GnRH agonist trigger when there were ≥ 14 follicles measuring ≥ 12 mm in diameter present on the day of trigger. Centre 3 (Australia) used a GnRH agonist trigger when there were ≥ 14 follicles measuring ≥ 12 mm in diameter on Day 8/9 of stimulation. For the GnRH agonist trigger Centre 1 used a subcutaneous injection of 0.5 mg busserelin (Suprefact; Sanofi-Aventis), while Centres 2 and 3 used a subcutaneous injection of 2 mg leuprolide acetate (Lucrin, Abbott). Oocyte retrieval was performed 34–36 h following GnRH agonist administration. Fertilization was undertaken using standard protocols and all embryos were cultured for 2–5 days, with a variable day of transfer and number of embryos transferred according to local protocols.

Within an hour of oocyte retrieval, Centres 1 and 3 (UK, Australia) administered luteal support consisting of 1500 IU of recombinant hCG (Merck Serono), while Centre 2 used 1500 IU of urinary hCG (Ferring). This was followed by vaginal progesterone (Crinone 90 mg daily; Merck Serono or Utrogestan 3 \times 200 mg per day, Besins) and estradiol (E2) valerate, 2 mg (Progynova, Bayer) twice daily commencing on the night of oocyte retrieval (Humaidan *et al.*, 2006, 2010a,b). Luteal support was continued until menstruation or 7–8 weeks of gestation in case of a positive pregnancy test (NICE, 2013).

Ethics approval

Full ethics committee approval was not required because of the retrospective nature of the study and the anonymized handling of the data.

Outcomes and statistical analysis

The two primary outcomes of this multicentre retrospective study were the clinical pregnancy rate, as defined by the presence of at least one fetal heart on an 8-week ultrasound, and the incidence and severity of OHSS. The Royal College of Obstetricians and Gynaecologists classification of OHSS was used across all three centres, with severe OHSS requiring hospitalization and mild or moderate OHSS managed on an outpatient basis (RCOG, 2006). A positive pregnancy test was defined as a positive serum hCG on Day 19 post-oocyte retrieval. Positive pregnancy test and clinical pregnancy rates are reported per cycle started. The miscarriage rate was defined as the percentage of cycles showing evidence of a gestational sac, but no viable fetal heart on ultrasound at 8 weeks of gestation.

Statistical analysis was done using Stata 12 and data are presented as mean \pm SD (standard distribution) when they have a Gaussian distribution or median (25th–75th range) when they are not normally distributed. Parametric and non-parametric tests were used to compare baseline characteristics and outcomes across the centres. Accumulative data from all three centres are presented on both tables. Logistic regression was performed to adjust the summary primary objectives (pregnancy rate and OHSS) for baseline characteristics and IVF centre.

Results

Demographic data

Table I shows the baseline patient characteristics, combined and for each of the three centres. Patients were in general young, slim and had high ovarian reserve indices consistent with an increased risk of an excessive ovarian response and development of OHSS if a conventional hCG trigger was used.

Reproductive outcome

Table II presents the IVF treatment outcomes, overall and broken down per centre, per cycle started. A large number of oocytes were collected across all centres with an overall mean oocyte yield of 17.8 ± 8.4 ; however, despite similar ovarian reserve indices significant differences in oocyte yield were observed between centres (Table II). Rates of fertilization and blastocyst development were similar between centres, and the significant difference in the number of embryos produced was solely due to the differing oocyte yield. In Centre 3, seven fresh embryo transfers were cancelled because of signs of early onset OHSS and one further embryo transfer was cancelled due to a premature rise in progesterone levels. No cycles were cancelled in the other two centres. There were differences in the number of embryos transferred between the centres, with elective single embryo being used in Centre 3 (Australia). Despite this, positive pregnancy test rates per cycle started did not differ across the centres with an average of 55.3% per cycle. The clinical pregnancy rate per cycle started was also similar between centres, with an overall rate of 41.8%, with 6.5% women subsequently experiencing a miscarriage.

A logistic regression model showed that the difference in baseline characteristics (BMI and age) among the patients from different centres did not affect the predictive power of the model in terms of clinical pregnancy rate.

OHSS

For OHSS, which was early onset, there were two cases of mild OHSS, four cases of moderate OHSS and one case of severe OHSS. In all seven cases of early onset OHSS, the fresh embryo transfers were cancelled in order to prevent subsequent deterioration. All cases of mild or moderate OHSS in each centre did not require hospitalization and were managed on an outpatient basis. For late onset OHSS, there were four cases of mild OHSS and one case of severe OHSS. The overall incidence of severe OHSS was 0.72% per cycle started.

Cases of severe OHSS

In Centre 1, the severe OHSS case was a 30-year old patient with a high baseline serum AMH of 91.4 pmol/l and increased AFC of 24. The stimulation protocol included a starting dose of gonadotrophin of 150 IU and resulted in a high oocyte yield of 40. Two embryos were transferred, which resulted in an ongoing twin pregnancy. In Centre 3, the patient was 26 years old, had PCOS, a BMI of 20 and a baseline AMH of 150 pmol/l. She was stimulated with a starting dose of 125 IU FSH, which resulted in 23 follicles (over 12 mm diameter) and 20 retrieved oocytes. She subsequently developed severe early OHSS requiring hospitalization, and the embryo transfer was cancelled because of the severity of the condition.

Table 1 Characteristics of women receiving GnRH agonist trigger for oocyte maturation.

	Combined	Centre 1 (UK)	Centre 2 (Belgium)	Centre 3 (Australia)	Comparison of three centres, P
Cycle number	275	68	94	113	
Age (years)	31.6 (29–35)	32.5 (31–35)	30 (27–33)	32.5 (29.2–36.1)	$P < 0.001$
BMI (kg/m ²)	25.2 ± 5.7	23.4 ± 3.9	25.5 ± 6	26.1 ± 6.2	$P = 0.007$
Aetiology					
Idiopathic	15.6%	18 (26.5%)	25 (26.6%)	–	$P < 0.001$
Male	34.2%	24 (35.3%)	32 (34%)	38 (33.6%)	
Ovulatory	2.2%	4 (5.9%)	2 (2.1%)	0	
Endometriosis	2.9%	3 (4.4%)	2 (2.1%)	3 (2.7%)	
Tubal	6.2%	3 (4.4%)	3 (3.2%)	11 (9.7%)	
PCOS	16.7%	9 (13.2%)	17 (18.1%)	20 (17.7%)	
Combined	16%	7 (10.3%)	13 (13.8%)	24 (21.2%)	
Unclassified	6.2%	–	–	17 (15.4%)	
Previous cycles	0 (0–2)	0 (0–1.75)	1 (0–3)	0 (0–1)	$P = 0.002$
AFC	25 (18–34)	25 (18.5–34)	25 (17.8–33.2)	24 (19–39.8)	$P = 0.55$
AMH (pmol/l)	49.1 (35.2–69.3)	48.6 (42.6–65.6)	49.8 (32.2–69.1)	50.7 (30.3–71.6)	$P = 0.29$

BMI was normally distributed and is presented as mean ± SD. Age and previous cycles were not normally distributed and are expressed as median (inter-quartile range). AFC, antral follicle count; AMH, anti-Müllerian hormone.

Discussion

The application of a GnRH agonist trigger in GnRH antagonist controlled cycles provides a unique opportunity to minimize the risk of OHSS in controlled ovarian stimulation. However, due to a potential detrimental impact on the pregnancy rates in autologous fresh cycles after GnRH agonist trigger (Youssef et al., 2011), overall acceptance of the possibility of a fresh transfer with intensive luteal support is still controversial and the application is restricted to relatively few centres. To examine the efficiency and consistency of clinical pregnancy rates after GnRH agonist trigger and modified luteal support, our present retrospective study was performed in a selected population of women at high risk of OHSS in various geographical regions under different legislative frameworks and treated by three different clinical and laboratory teams. Our results show consistently high clinical pregnancy rates and a low incidence of severe OHSS with only two cases of severe OHSS among 275 stimulated cycles (0.72%).

This is the largest multicentre series to present the outcomes of one of the most commonly used intensive luteal support packages for GnRH agonist trigger protocols. The results of our study support the use of a GnRH agonist trigger protocol with adequate luteal support achieved by stimulating an adequate LH surge with co-administration of one bolus of low dose of hCG for women with an excessive follicular response. Although we did not have a control arm in this study, the ongoing pregnancy rates are comparable with those reported from national IVF registries in Europe (de Mouzon et al., 2010) and previous studies using an hCG trigger in high-risk patients (Humaidan et al., 2005; Kolibianakis et al., 2005). A recent meta-analysis suggested that fresh transfer after GnRH agonist trigger protocols should not be recommended as they have a significantly lower clinical pregnancy rate [odds ratio 0.45, 95% confidence intervals 0.31–0.65; 8 randomized

controlled trials (RCTs)] (Youssef et al., 2011). The conclusions of this analysis have, however, been controversial as although eight studies with autologous cycles ($n = 713$) were included, the majority of them did not use adequate luteal support and only two used a low dose of hCG along with a GnRH agonist for oocyte maturation (Humaidan et al., 2006, 2010a,b). Future adequately powered RCTs comparing fresh transfer with intensive luteal support against a freeze-all approach and subsequent frozen embryo transfer will potentially now be feasible given potential confidence in the former strategy.

The 0.72% incidence of severe OHSS per cycle started was strikingly low compared with the 30% incidence reported for the hCG arm of the two RCTs comparing GnRH agonist and hCG triggers in high-risk women (Babayof et al., 2006; Engmann et al., 2008). Even oocyte donors who are clearly not exposed to endogenous hCG and its associated risk of late onset OHSS have a reported incidence of 4.0–8.5% if an hCG trigger is used (Galindo et al., 2009; Melo et al., 2009). The use of hCG as a luteal support mechanism was classically stopped due to the risk of developing OHSS, with potential concern that this risk would still apply even with lower doses. All three centres in our study administered 1500 IU hCG at the time of oocyte retrieval as initially suggested by Humaidan et al. (2010a,b) in his trial of GnRH agonist trigger combined with low-dose hCG luteal support versus conventional hCG. Although no cases of OHSS were reported in the GnRH agonist group, that trial was not powered to detect a difference in the incidence of OHSS. However, only one-third of patients in the GnRH agonist triggered group had at least 14 follicles ≥ 11 mm, a cut-off level previously set by Papanikolaou et al. (2005) to predict high likelihood of severe OHSS. When the same protocol was applied to high-risk women in a small cohort study, 1 case of OHSS among 12 patients was reported (Humaidan, 2009). In the current multicentre study of 275 high-risk cycles, all of whom were exposed to 1500 IU recombinant hCG, there

Table II ART treatment outcomes.

	Combined (n = 275)	Centre 1 (UK) (n = 68)	Centre 2 (Belgium) (n = 94)	Centre 3 (Australia) (n = 113)	Comparison of three centres, P
Total FSH (IU)	1425 (1200–1700)	1575 (1463–1825)	1263 (1050–1575)	1386 (1200–2000)	<i>P</i> < 0.001
Peak estradiol (pmol/l)	12 000 (9400–15 914)	14 006 (12 114–18 976)	10 477 (7544–17 514)	11 500 (10 100–15 000)	<i>P</i> < 0.001
Peak estradiol (pg/ml)	3269 (2561–4335)	3815 (3300–5169)	2854 (2055–4771)	3133 (2751–4086)	
Number of oocytes collected (<i>n</i>)	17.8 ± 8.4	12.2 ± 6.1	19.1 ± 9.7	19.9 ± 6.7	<i>P</i> < 0.001
Number of embryos produced (<i>n</i>)	10.2 ± 5.6	6.9 ± 4.5	11.1 ± 6.2	11.3 ± 4.7	<i>P</i> < 0.001
Number of cycles with embryo transfer (ET) (<i>n</i> , %)	261 (95.0)	65 (95.6)	91 (96.8)	105 (92.9)	<i>P</i> = 0.43
Number of embryos transferred (<i>n</i>)	1 (1–2)	2 (1–2)	1 (1–2)	1 (1–1)	<i>P</i> < 0.001
Elective single embryo transfer (<i>n</i> , % per ET)	169 (64.8)	31 (47.7)	45 (49.5)	93 (88.6)	<i>P</i> < 0.001
Blastocyst transferred (<i>n</i> , % per ET) ^a	186 (71.3)	32 (49.2)	49 (53.8)	105 (100)	<i>P</i> < 0.001
Number of cycles with all embryos cryopreserved (<i>n</i> , %)	8 (2.9)	0	0	8 (7.1)	<i>P</i> = 0.003
Number of embryos cryopreserved (<i>n</i>)	3 (1–6)	4 (2–5.75)	2 (0–5.25)	4 (2–7)	<i>P</i> = 0.006
At least one blastocyst formed (<i>n</i> , %)	269 (97.8)	65/68 (95.6)	91/94 (96.8)	113/113 (100)	<i>P</i> = 0.10
Positive pregnancy rate (<i>n</i> , % per started cycle)	55.3	39/68 (57.4)	52/94 (55.3)	61/113 (54)	<i>P</i> = 0.91
Clinical pregnancy rate (<i>n</i> , % per started cycle)	41.8	27/68 (39.7)	41/94 (43.6)	47/113 (41.6)	<i>P</i> = 0.88
Miscarriages (<i>n</i> , % per started cycle)	6.6	3/68 (4.4)	5/94 (5.3)	10/113 (8.8)	<i>P</i> = 0.52
OHSS severity (<i>n</i> , % per started cycle)					
Total	12 (4.4)	4 (5.9)	1 (1.1)	7 (6.2)	<i>P</i> = 0.15
Severe	2 (0.72)	1 (1.5)	0 (0)	1 (0.9)	<i>P</i> = 0.72
Moderate	4 (1.5)	0 (0)	0 (0)	4 (3.5)	<i>P</i> = 0.026
Mild	6 (2.2)	3 (4.4)	1 (1.1)	2 (1.8)	<i>P</i> = 0.10
OHSS					
Early (<i>n</i> , % per started cycle)	7 (2.6)	0	0	7 (6.2)	<i>P</i> = 0.007
Late (<i>n</i> , % per started cycle)	5 (1.8)	4 (5.9)	1 (1.1)	0	<i>P</i> = 0.015

Normally distributed variables are expressed as mean ± SD. Variables that are not normally distributed are expressed as median (25th–75th percentile). Outcome data are presented per cycle started. A clinical pregnancy was defined as the presence of at least one viable fetal heart on an 8-week ultrasound scan, while a positive pregnancy test was an embryo transfer resulting in a positive serum βhCG. Miscarriages are defined as occurring after a clinical pregnancy was established.

^aCleavage-stage embryos were transferred in the remaining cycles.

were only two cases of severe OHSS. In one of these cases, a pregnancy had occurred and therefore the woman was also exposed to increasing concentrations of endogenous hCG, an established risk factor for OHSS (Mathur et al., 2000). Interestingly, no cases of severe OHSS occurred in Centre 2 which may be attributed to an overall gentler approach followed by this centre by administering lower doses of FSH, achieving lower levels of E2 and, potentially, using a different hCG preparation during the luteal support phase (Table II). A similar approach of utilizing the lowest possible dose of recombinant FSH based on patients' baseline characteristics during conventional stimulation protocols has been demonstrated to minimize the risk of OHSS without affecting the success rate despite using a conventional recombinant hCG trigger (Yovich et al., 2012). Future trials will need to assess the effect of dose modifications of FSH on the overall OHSS risk during the GnRH agonist trigger package.

Although freezing all embryos and transferring in a frozen cycle overcomes the risk of OHSS, this procedure may not be culturally or financially acceptable and demands an optimal freezing and thawing programme which all clinics at present do not have. The impact on overall success rates is as yet unclear as pregnancy rates after frozen embryo replacement have been reported by some centres to be lower (Pinborg, 2012), and the early pregnancy loss rate is higher (Tomas et al., 2012) than after fresh transfer. However, this detrimental impact on success rates has not been observed in all centres (Shapiro et al., 2013). Furthermore, a recent meta-analysis suggested that frozen embryo transfers were associated with a reduction in adverse perinatal outcomes compared with fresh transfer (Maheshwari et al., 2012). This was despite recent concerns over potential epigenetic changes during the freeze/thaw procedure, causing a higher risk of large for gestational age babies (Henningsen et al., 2011; Pinborg, 2012). However, the malformation rate after ICSI frozen embryo transfer compared with an IVF frozen embryo transfer has been reported to be 2-fold higher (Belva et al., 2008), suggesting that a microinjected embryo could be more sensitive to the freeze/thaw procedure than an IVF embryo. Whether the optimal transfer is fresh or frozen is now being assessed in a variety of large-scale randomized controlled trials and the results are awaited with interest (Maheshwari and Bhattacharya, 2013).

The mechanism underlying our observed improvement in success rates to those equivalent of fresh cycles may be multifactorial. In addition to rescuing corpus luteal function, the one bolus of 1500 IU hCG 35 h post-GnRH agonist trigger is known to induce an endometrial gene expression similar to that of fresh cycles with a conventional hCG trigger (Humaidan et al., 2012). It may also modify the speed of embryo cleavage at the early stages of embryo development (Muñoz et al., 2013).

The finding that 1500 IU is sufficient to induce early OHSS is consistent with the recent report from Seyhan et al. (2013), where five cases of early severe OHSS occurred after a GnRH agonist trigger and 1500 IU hCG administration at the time of oocyte retrieval. However, these five cases had a mean oocyte yield of 39 oocytes (range 23–65), and in two of them, a fresh transfer still occurred. This would suggest that although the use of a GnRH agonist trigger can dramatically reduce the risk of OHSS in high-risk patients, for some patients segmentation of the cycle would still be appropriate. At present the optimal threshold for performing a freeze-all after a GnRH agonist trigger is not clear. Seyhan et al. suggested all cases of severe early OHSS had > 18 follicles measuring 10–14 mm on the day of the trigger and suggested that the number of follicles measuring 10–14 mm on the day of trigger can be predictive of the likelihood of severe early OHSS, whereas peak estradiol

and the number of follicles measuring > 12 mm were not predictive (Seyhan et al., 2013). We were unable to examine this in our multicentre study and suggest that clarification of the optimal threshold of follicular size and number for prediction of OHSS will require a prospective study with automated and accurate follicular measurements to minimize intra- and inter-observer variability (Raine-Fenning et al., 2009). The results of a large RCT, now in press, in which we have suggested > 25 follicles > 11 mm for excluding patient from fresh cycles, will provide greater clarity regarding follicular cut-off levels (Humaidan et al., 2013, in press). As reported herein, for patients without an extreme ovarian response, a fresh transfer may still be feasible; however, complete extinction of the risk of OHSS is not achieved if low-dose hCG is used as part of the intensive luteal support package.

Strengths and limitations

Although our study reflects a multicentre experience, thereby increasing its generalizability, and is the largest report to date examining pregnancy outcomes and the OHSS incidence in an OHSS high-risk population after a GnRH agonist trigger and fresh transfer, there are a number of limitations. It is retrospective in nature and each of the centres had different inclusion criteria for selecting patients for the GnRH agonist trigger package. This is evident from the baseline patient characteristics, which differed between centres, including the cause of infertility, a known determinant of IVF (Nelson and Lawlor, 2011). However, all the patients were at high risk of developing OHSS, one of the primary outcomes of our study, and were expected to respond well to exogenous gonadotrophins. Each centre utilized slightly different clinical and laboratory protocols. This is particularly striking for the markedly lower number of oocytes retrieved in Centre 1, despite similar baseline characteristics and peak estradiol. The reason for this discordance is not clear and may reflect a variety of factors. These may include: (i) the use of a different FSH preparation, as highly purified urinary gonadotrophins are associated with a slightly lower oocyte yield in women with a high AMH (Arce et al., 2013) but a higher peak estradiol despite a smaller follicular cohort (Devroey et al., 2012), (ii) that irrespective of ovarian response all women with a high baseline AMH (≥ 40 pmol/l) received a GnRH agonist trigger (Nelson, 2013) or (iii) that the clinical staff may only have punctured larger follicles and did not flush. The number of embryos transferred also varied between centres, although elective single embryo transfer dominated given the good prognosis of these patients (Lawlor and Nelson, 2012). Live birth rates were not available for all centres as there is not a statutory requirement to report these in all countries. Although one of the centres has previously reported its experience with the GnRH agonist trigger and intensive luteal support with a bolus of 1500 IU hCG, none of those patients are included in the current analysis (Radesic and Tremellen, 2011). The current outcome data are, however, very similar to that previously reported, potentially providing further confidence in the robustness of the technique.

Additional trials will now also be able to be undertaken to ascertain whether the combination of GnRH agonist trigger and the intensive luteal support package is associated with better clinical outcomes than either conventional triggering with hCG with or without freezing or use of GnRH agonist trigger and subsequent frozen embryo transfer. Another key research question for the future concerns the minimum dose of luteal hCG support required to adequately prepare the endometrium, while giving a zero incidence of severe OHSS. Dose finding

studies using hCG dosages below the 1500 IU used in this study will enable us to answer this question.

Conclusions

In women who are undergoing ovarian stimulation and who develop an excessive ovarian response, the use of a GnRH agonist trigger combined with modified luteal support can provide them with the opportunity to proceed with fresh embryo transfer with adequate clinical pregnancy rates. However, this will not completely avoid the risk of OHSS and for women with an extreme ovarian response or with significant comorbidity, where the possibility of severe OHSS is unacceptable, we recommend GnRH agonist trigger followed by a freeze-all policy to completely avoid OHSS.

Acknowledgements

We thank all patients and staff of each of the three centres.

Authors' roles

S.M.N. and P.H. conceived the study, C.B., K.P.T., H.T. and R.F. provided the original data extract from each of their centres. S.I. collated the data and performed the statistical analysis. S.I., S.M.N. and P.H. wrote the first draft of the manuscript. All co-authors contributed to the final manuscript.

Funding

No funding was received for this study.

Conflict of interest

There is no conflict of interest.

References

- Arce JC, La Marca A, Mirner Klein B, Nyboe Andersen A, Fleming R. Antimüllerian hormone in gonadotropin releasing-hormone antagonist cycles: prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. *Fertil Steril* 2013;**99**:1644–1653, e1641.
- Babayof R, Margalioth EJ, Huleihel M, Amash A, Zylber-Haran E, Gal M, Brooks B, Mimoni T, Eldar-Geva T. Serum inhibin A, VEGF and TNF alpha levels after triggering oocyte maturation with GnRH agonist compared with HCG in women with polycystic ovaries undergoing IVF treatment: a prospective randomized trial. *Hum Reprod* 2006;**21**:1260–1265.
- Belva F, Henriët S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, Liebaers I, Haentjens P, Bonduelle M. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. *Hum Reprod* 2008;**23**:2227–2238.
- deMouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, Kupka M, Nygren KG, Andersen AN, Eshre. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. *Hum Reprod* 2010;**25**:1851–1862.
- Devroey P, Pellicer A, Nyboe Andersen A, Arce JC; Menopur in GnRH Antagonist Cycles with Single Embryo Transfer Trial Group. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. *Fertil Steril* 2012;**97**:561–571.
- Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing *in vitro* fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril* 2008;**89**:84–91.
- Galindo A, Bodri D, Jose Guillen J, Colodron M, Vernaev V, Coll O. Triggering with HCG or GnRH agonist in GnRH antagonist treated oocyte donation cycles: a randomised clinical trial. *Gynecol Endocrinol* 2009;**25**:60–66.
- Gonen Y, Balakier H, Powell W, Casper RF. Use of gonadotrophin-releasing hormone agonist to trigger follicular maturation for in-vitro fertilization. *J Clin Endocrinol Metab* 1990;**71**:918–922.
- Henningsen A-KA, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. *Fertil Steril* 2011;**95**:959–963.
- Hoff JD, Quigley ME, Yen SSC. Hormonal dynamics at midcycle—a reevaluation. *J Clin Endocrinol Metab* 1983;**57**:792–796.
- Humaidan P. Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG: a pilot study. *Reprod Biomed Online* 2009;**18**:630–634.
- Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grondahl ML, Westergaard L, Andersen CY. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. *Hum Reprod* 2005;**20**:1213–1220.
- Humaidan P, Bungum L, Bungum M, Andersen CY. Rescue of corpus luteum function with peri-ovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: a pilot study. *Reprod Biomed Online* 2006;**13**:173–178.
- Humaidan P, Bredkjaer HE, Westergaard LG, Andersen CY. 1500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. *Fertil Steril* 2010a;**93**:847–854.
- Humaidan P, Quartarolo J, Papanikolaou EG. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. *Fertil Steril* 2010b;**94**:389–400.
- Humaidan P, Van Vaerenbergh I, Bourgain C, Alsbjerg B, Blockeel C, Schuit F, Van Lommel L, Devroey P, Fatemi H. Endometrial gene expression in the early luteal phase is impacted by mode of triggering final oocyte maturation in recFSH stimulated and GnRH antagonist co-treated IVF cycles. *Hum Reprod* 2012;**27**:3259–3272.
- Humaidan P, Polyzos NP, Alsbjerg B, Erb K, Mikkelsen AL, Elbaek HO, Papanikolaou EG, Andersen CY. GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: two prospective randomized controlled multi-centre studies in IVF patients. *Hum Reprod* 2013;**28**:2511–2521.
- Itskovitz J, Erlik Y, Boldes R, Kahana L, Levron J, Brandes JM. Induction of preovulatory luteinizing-hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing-hormone agonist. *Fertil Steril* 1991;**56**:213–220.
- Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K, Griesinger G. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod* 2005;**20**:2887–2892.

- Lawlor DA, Nelson SM. Effect of age on decisions about the numbers of embryos to transfer in assisted conception: a prospective study. *Lancet* 2012;**379**:521–527.
- Maheshwari A, Bhattacharya S. Elective frozen replacement cycles for all: ready for prime time? *Hum Reprod* 2013;**28**:6–9.
- Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012;**98**:368–377, e1–9.
- Mathur RS, Akande AV, Keay SD, Hung LP, Jenkins JM. Distinction between early and late ovarian hyperstimulation syndrome. *Fertil Steril* 2000;**73**:901–907.
- Melo M, Busso CE, Bellver J, Alama P, Garrido N, Meseguer M, Pellicer A, Remohi J. GnRH agonist versus recombinant HCG in an oocyte donation programme: a randomized, prospective, controlled, assessor-blind study. *Reprod Biomed Online* 2009;**19**:486–492.
- Muñoz M, Cruz M, Humaidan P, Garrido N, Pérez-Cano I, Meseguer M. The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: a time-lapse study. *Eur J Obstet Gynecol Reprod Biol* 2013;**168**:167–172.
- Nelson SM. Antimüllerian hormone: is the writing on the wall for antral follicle count? *Fertil Steril* 2013;**99**:1563–1564.
- Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from *in vitro* fertilisation: a prospective study of 144,018 treatment cycles. *PLoS Med* 2011;**8**:e1000386.
- NICE. *Fertility: Assessment and Treatment for People with Fertility Problems*. London: National Institute for Health and Care Excellence, 2013.
- Papanikolaou EG, Tournaye H, Verpoest W, Camus M, Vernaev V, Van Steirteghem A, Devroey P. Early and late ovarian hyperstimulation syndrome: early pregnancy outcome and profile. *Hum Reprod* 2005;**20**:636–641.
- Pinborg A. To transfer fresh or thawed embryos? *Semin Reprod Med* 2012;**30**:230–235.
- Radesic B, Tremellen K. 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. *Hum Reprod* 2011;**26**:3437–3442.
- Raine-Fenning N, Jayaprakasan K, Deb S, Clewes J, Joergner I, Dehghani Bonaki S, Johnson I. Automated follicle tracking improves measurement reliability in patients undergoing ovarian stimulation. *Reprod Biomed Online* 2009;**18**:658–663.
- RCOG. *The Management of Ovarian Hyperstimulation Syndrome*. London: Royal College of Obstetricians and Gynaecologists, 2006.
- Seyhan A, Ata B, Polat M, Son WY, Yarali H, Dahan MH. Severe early ovarian hyperstimulation syndrome following GnRH agonist trigger with the addition of 1500 IU hCG. *Hum Reprod* 2013;**28**:2522–2528.
- Shapiro BS, Daneshmand ST, Restrepo H, Garner FC, Aguirre M, Hudson C. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. *Fertil Steril* 2013;**99**:389–392.
- Sismanoglu A, Tekin HI, Erden HF, Ciray NH, Ulug U, Bahceci M. Ovulation triggering with GnRH agonist vs. hCG in the same egg donor population undergoing donor oocyte cycles with GnRH antagonist: a prospective randomized cross-over trial. *J Assist Reprod Genet* 2009;**26**:251–256.
- Tomas C, Alsbjerg B, Martikainen H, Humaidan P. Pregnancy loss after frozen-embryo transfer—a comparison of three protocols. *Fertil Steril* 2012;**98**:1165–1169.
- Youssef MA, Van der Veen F, Al-Inany HG, Griesinger G, Mochtar MH, Aboufoutouh I, Khattab SM, van Wely M. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist assisted reproductive technology cycles. *Cochrane Database Syst Rev (Online)* 2011;CD008046.
- Yovich J, Stanger J, Hinchliffe P. Targeted gonadotrophin stimulation using the PIVET algorithm markedly reduces the risk of OHSS. *Reprod Biomed Online* 2012;**24**:281–292.