RBMO

ELSEVIER

ARTICLE

The exogenous progesterone-free luteal phase: two pilot randomized controlled trials in IVF patients

BIOGRAPHY

Peter Humaidan is a specialist in reproductive endocrinology, and Professor at The Fertility Clinic, Skive Regional Hospital, Aarhus University, Denmark. He has focused on developing individualized treatment for the infertile patient, with an emphasis on ovulation triggering and OHSS prevention. He has authored over 217 articles and has a wide international scientific network.

Peter Humaidan[1,2,*], Birgit Alsbjerg[1,2], Helle Olesen Elbaek[1], Betina Boel Povlsen[1], Rita Jakubcionyte Laursen[1], Mette Brix Jensen[1], Alice Toft Mikkelsen[1], Lise Haaber Thomsen[1], Shahar Kol[3], Thor Haahr[1,2]

KEY MESSAGE

This RCT explored the concept of the exogenous progesterone-free HCG-based luteal phase support (LPS) in GnRHa-triggered IVF cycles. LPS was secured by two boluses of HCG. This simple and patient-friendly strategy is recommended for patients with fewer than 19 follicles ≥11 mm on the trigger day.

ABSTRACT

Research question: Is the reproductive outcome similar after gonadotrophin-releasing hormone agonist (GnRHa) trigger followed by luteal human chorionic gonadotrophin (HCG) boluses compared with HCG trigger and a standard luteal phase support (LPS)?

Design: Two open-label pilot randomized controlled trials (RCT) with 250 patients from 2014 to 2019, with a primary outcome of ongoing pregnancy per embryo transfer. Patients with ≤13 follicles on the trigger day were randomized (RCT 1) to: Group A (n = 65): GnRHa trigger followed by a bolus of 1500 IU HCG s.c. on the oocyte retrieval day (ORD) and 1000 IU HCG s.c. 4 days later, and no vaginal LPS; or Group B (n = 65): 6500 IU HCG trigger, followed by a standard vaginal progesterone LPS. Patients with 14–25 follicles on the trigger day were randomized (RCT 2) to Group C (n = 60): GnRHa trigger followed by 1000 IU HCG s.c. on ORD and 500 IU HCG s.c. 4 days later, and no vaginal LPS; or Group D (n = 60): 6500 IU HCG trigger and a standard vaginal LPS.

Results: In RCT 1, the ongoing pregnancy rate was 44% (22/50) in the GnRHa group versus 46% (25/54) in the HCG trigger group (RR 0.95, 95% CI 0.62–1.45). No ovarian hyperstimulation syndrome (OHSS) was seen in Groups A or B. In RCT 2, the ongoing pregnancy rate was 51% (25/49) in the GnRHa group versus 60% (31/52) in the HCG trigger group (RR 0.86, 95% CI 0.60–1.22). The OHSS rates were 3.3% and 6.7%, respectively.

Conclusions: Although a larger-scale study is needed before standard clinical implementation, the present study supports that the exogenous progesterone-free LPS is efficacious, simple and patient-friendly.

[1] The Fertility Clinic, Skive Regional Hospital, Resenvej 25, Skive 7800, Denmark
[2] Faculty of Health, Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 82, Aarhus 8200, Denmark
[3] IVF Unit, Elisha Hospital, Yair Kats St 12, Haifa, Israel

*Corresponding author. E-mail address: peter.humaidan@midt.rm.dk (P. Humaidan). https://doi.org/10.1016/j.rbmo.2021.03.011 1472-6483/
Outside this study, PH received unrestricted research grants from MSD, Merck, Osel Inc. and Ferring as well as honoraria for lectures from MSD, Merck, Gedeon Richter, Theramex and IBSA. TH received unrestricted research grants from Osel Inc. and Cook Medical™ as well as honoraria for lectures from Ferring, Besins, Merck and IBSA. BA received honoraria for lectures from Merck and Gedeon Richter. The study group spearheaded by PH has performed long-term research in GnRHa triggering. The remaining authors have nothing to disclose.

KEYWORDS

GnRH agonist trigger
HCG
IVF
Luteal phase support
Ovarian hyperstimulation syndrome
Progesterone

INTRODUCTION

The successful implementation of GnRH agonist (GnRHa) trigger and a freeze-all policy for ovarian hyperstimulation syndrome (OHSS) prevention has had a significant impact on safety in IVF treatment. Importantly, GnRHa trigger helped to open the 'black box' of the luteal phase, including research into the most optimal luteal phase support (LPS) during fresh as well as frozen embryo transfer (*Alsbjerg et al., 2018*; *Cédrin-Durnerin et al., 2019*; *Humaidan, 2009*; *Humaidan et al., 2005*, *2006*, *2010*, *2013*, *2014*; *Kol et al., 2011*; *Labarta et al., 2017*; *Thomsen et al., 2018*).

In GnRHa-triggered fresh transfer cycles, the so-called European LPS approach aims to boost the endogenous progesterone and oestradiol production by the corpus luteum, using one or more small boluses of human chorionic gonadotrophin (HCG) as a surrogate for luteal LH activity (*Andersen et al., 2015*; *Castillo et al., 2010*; *Fischer et al., 2019*; *Humaidan et al., 2014*; *Kol et al., 2011*). In contrast, the so-called American approach after GnRHa trigger relies on exogenous steroid support only, using intramuscular progesterone and transdermal oestradiol (*Engmann et al., 2008*; *Humaidan et al., 2014*). Both GnRHa trigger approaches have been shown to result in a lower OHSS incidence and similar reproductive outcomes as compared with HCG trigger followed by conventional LPS (*Engmann et al., 2016*; *Haahr et al., 2017*; *Humaidan et al., 2015*). Importantly, several trials and a recent meta-analysis concluded that GnRHa trigger yields more metaphase II (MII) oocytes and a higher number of good quality embryos compared with HCG trigger (*Haahr et al., 2017*; *Humaidan et al., 2011*, *2010*; *Pereira et al., 2017*; *Reddy et al., 2014*).

Vaginal progesterone and oral oestradiol were part of the LPS protocols in the vast majority of the above-mentioned trials. However, in recent years, it has been shown that the resorption of progesterone from the vagina seems to be highly individual (*Alsbjerg et al., 2018*; *Cédrin-Durnerin et al., 2019*; *Labarta et al., 2017*), making luteal progesterone monitoring and subsequent LPS intervention a potential tool to optimize live birth rates (LBR) in fresh (*Thomsen et al., 2018*) as well as frozen embryo transfer cycles (*Alsbjerg et al., 2018*; *Labarta et al., 2017*). As regards vaginal application of progesterone, this may cause bothersome side-effects such as vaginal leakage and itching in 10–51% of patients, depending on the specific drug reported (*Kleinstein and the Luteal Phase Study Group, 2005*; *Simunic et al., 2007*). Although there is solid physiological and clinical evidence to stop the LPS 14 days after embryo transfer (*Nyboe Andersen et al., 2002*; *Schmidt et al., 2001*), the widespread global practice remains to continue long-term LPS after HCG trigger (*Di Guardo et al., 2020*). This policy may expose patients to unnecessary side-effects for an extended period during early pregnancy. Moreover, a negative aspect of the gold standard HCG trigger is the immediate steep, non-physiological rise in early luteal serum progesterone, which may cause endometrial advancement, hampering implantation and early pregnancy (*Fatemi et al., 2013*; *Humaidan et al., 2012*; *Vuong et al., 2020*).

In an attempt to design a more physiological IVF protocol, a small uncontrolled proof-of-concept study (n = 15) had previously been performed, exploring the so-called HCG-based/exogenous progesterone-free (EPF) LPS (*Kol et al., 2011*). In that study 15 normal responder IVF patients underwent GnRHa trigger, followed by a total of two boluses of 1500 IU HCG, administered on the oocyte retrieval day (ORD) and 4 days later. Neither progesterone nor oestradiol were administered for LPS, and a high ongoing clinical pregnancy rate (47%) was reported. This concept was later explored further in a small three-arm proof-of-concept RCT in 93 normal responder IVF patients, using daily boluses of 125 IU HCG s.c. from ORD until the day of the pregnancy test (*Andersen et al., 2015*). Once again, neither progesterone nor oestradiol were administered for LPS, and a mean ongoing clinical pregnancy rate of 38% was seen in the two GnRHa-triggered EPF–LPS groups, as compared with 41% in the HCG-triggered, standard LPS group.

From the most recent study, it was concluded that apart from a non-significant difference in reproductive outcomes, the EPF–LPS concept also resulted in more physiological progesterone, oestradiol and HCG concentrations during the early and mid-luteal phases, as well as higher patient satisfaction. Nevertheless, it was found that daily administration of HCG was inconvenient and laborious for patients.

The objective of the present pilot RCT was to inform the design for future large-scale trials, exploring whether GnRHa trigger and HCG-based LPS, consisting of two luteal HCG boluses only, and modified according to the number of follicles ≥11 mm on the ovulation trigger day results in reproductive outcomes similar to those of HCG trigger, followed by a standard LPS.

MATERIALS AND METHODS

This study consisted of two RCT that were prospectively registered on 31 March 2014 (EudraCT trial registration numbers 2014-000448-13 and 2014-000447-32). The project was monitored by the Good Clinical Practice (GCP) Unit at Aarhus University, Denmark, ensuring compliance with the International Conference on Harmonisation GCP guidelines. The first patient was enrolled in November 2014 and the last patient in August 2019.

Eligibility

The inclusion criteria were: (i) females aged between 18 and 40 years; (ii) body mass index (BMI) >18 and <30 kg/m^2, (iii) sperm quality suitable for intracytoplasmic sperm injection (ICSI) or IVF, according to the study centre's standard clinical criteria. Patients were excluded from the trial if they had: experienced OHSS previously, a previous poor ovarian response to stimulation (<4 oocytes retrieved in a previous cycle), uterine abnormalities or chronic medical diseases, e.g. diabetes mellitus or Crohn's disease.

Patients were eligible for inclusion in RCT 1 if they developed ≤13 follicles ≥11 mm after ovarian stimulation on the day of ovulation trigger. These patients were considered 'normal responders' at low risk of OHSS development. Patients who developed 14–25 follicles ≥11 mm on the day of ovulation trigger were eligible for inclusion in RCT 2. Finally, patients who developed more than 25 follicles ≥11 mm on the day of ovulation trigger were excluded from participation in the trial and underwent GnRHa trigger and cycle segmentation outside the study.

Stimulation

Ovarian stimulation was initiated, using a fixed dose of recombinant FSH: either 150 or 200 IU per day for the first 4 days, according to the antral follicle count on cycle day 2. After 4 days of stimulation, the FSH dose was adjusted according to the ovarian response. A fixed gonadotrophin-releasing hormone (GnRH) antagonist protocol was used, commencing from stimulation day 5. From this day onwards 0.25 mg/day of the GnRH antagonist ganirelix (Orgalutran; MSD, Skovlunde, Denmark) was administered up to and including the day of ovulation trigger. Ovulation was triggered as soon as two follicles reached a diameter of 17 mm.

Randomization

Two different randomization lists were used, depending on the number of follicles on the final day of ovarian stimulation: one for patients with ≤13 follicles ≥11 mm diameter and one for patients with 14–25 follicles ≥11 mm. Patients were randomized 1:1 in a parallel-group design. Random sequence generation was performed by a computer-generated code in blocks of 10, to ensure equal distribution between groups until 100 patients had undergone embryo transfer in each RCT. A started block of 10 needed to be completed before the end of the study. Allocation was performed by a study nurse on the day of ovulation trigger after obtaining written informed consent, using sealed, opaque, unlabelled envelopes containing a unique study number. Each patient could only participate once. After group allocation, the study was open label for patients, nurses and doctors.

Randomization of patients at low OHSS risk (RCT 1)

In RCT 1 patients were randomized into two groups (A and B) as follows. Group A: ovulation trigger with a bolus of 0.5 mg buserelin (Suprefact®; Sanofi A/S, Copenhagen, Denmark), followed by a bolus of 1500 IU HCG (Pregnyl®; MSD, Skovlunde, Denmark) after oocyte retrieval and an additional bolus of 1000 IU HCG (Pregnyl) on ORD+4. Group B: ovulation trigger with 6500 IU HCG (Ovitrelle®; Merck A/S, Søborg, Denmark) followed by 100 mg vaginal progesterone (Lutinus®; Ferring, Copenhagen, Denmark) three times daily until the pregnancy test (14 days after ORD), after which LPS was stopped.

Randomization of patients at risk of OHSS (RCT 2)

In RCT 2, patients were randomized into two groups (C and D) as follows. Group C: ovulation trigger with a bolus of 0.5 mg buserelin (Suprefact), followed by a bolus of 1000 IU HCG (Pregnyl) after oocyte retrieval and an additional bolus of 500 IU HCG (Pregnyl) on ORD+4. Group D: ovulation trigger with 6500 IU HCG (Ovitrelle), followed by 100 mg vaginal progesterone (Lutinus) three times daily until the pregnancy test (14 days after oocyte retrieval), after which LPS was stopped.

Due to discontinuation of Pregnyl production during the study period, until 26 March 2018 Group A (RCT 1) received Pregnyl, 1500 and 1000 IU during the early luteal phase, and Group C (RCT 2) received 1000 IU HCG (Pregnyl) after oocyte retrieval and 500 IU HCG (Pregnyl) on ORD+4. After this date the study medication was changed to Ovitrelle. Two dosing clicks of Ovitrelle equal 520 IU HCG, four clicks equal 1040 IU HCG, and six clicks equal 1560 IU HCG. Ten patients were treated with Ovitrelle in Group A and 12 patients in Group C. The research protocol can be seen in FIGURE 1.

Oocyte retrieval and embryo transfer

All patients underwent oocyte retrieval 36 h after ovulation trigger and a maximum of two embryos was transferred on day 3 or day 5 after retrieval, following national criteria of single embryo transfer. A freeze-all policy was used in patients who developed ≥25 follicles from trigger day to ORD.

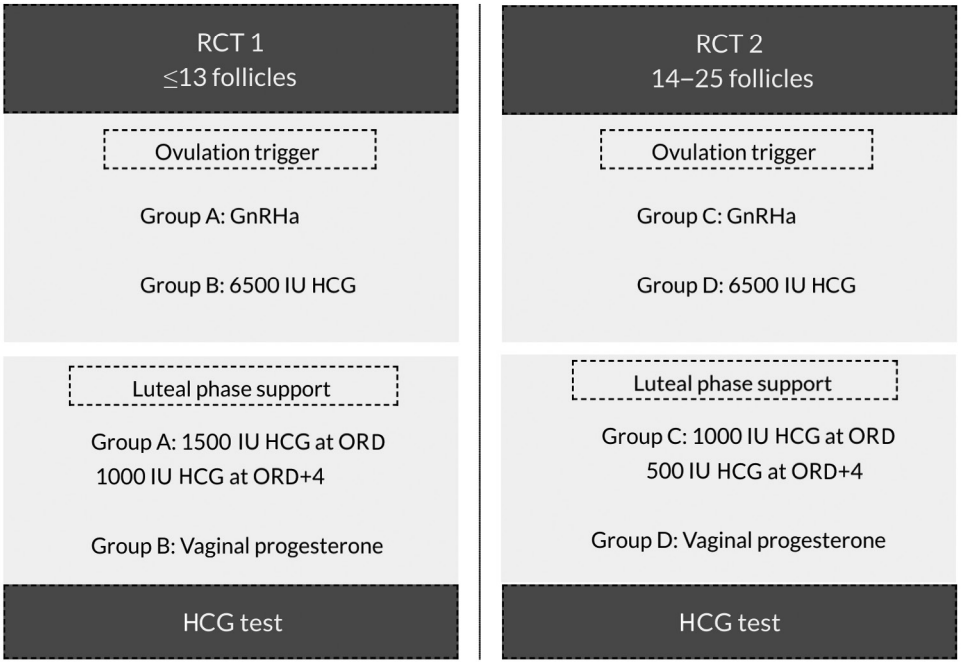

FIGURE 1 Study protocol. GnRHa = gonadotrophin-releasing hormone agonist; HCG = human chorionic gonadotrophin; ORD = oocyte retrieval day; RCT = randomized controlled trial.

Outcome measures

The primary outcome of the study was ongoing pregnancy rate at gestational week 10. Secondary outcome measures were biochemical pregnancy rate, clinical pregnancy rate at week 7, early pregnancy loss rate, LBR, OHSS rate and luteal steroids (LH, FSH, progesterone, HCG and oestradiol concentrations). Moreover, for this study a new efficacy measure of the trigger bolus was post hoc defined in terms of the trigger day follicle:oocyte index (T-FOI). T-FOI was considered low if the number of oocytes retrieved was less than 50% of the pre-ovulatory follicle number. A pre-ovulatory follicle was defined as a follicle >11 mm on ORD. Unless otherwise described, the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) definitions were adhered to (*Zegers-Hochschild et al., 2017*).

Thus, a positive HCG test was defined by a plasma beta-HCG >10 IU/l on day 14 after oocyte retrieval. A clinical pregnancy was defined as an intrauterine gestational sac with a heartbeat 3 weeks after a positive HCG test. An ongoing pregnancy was defined as a viable pregnancy at week 10 of pregnancy. A live birth was defined as a live birth after 22 gestational weeks. Early pregnancy loss was defined by a non-viable HCG positive pregnancy at 10 weeks.

Classification of OHSS

Moderate OHSS was defined as abdominal distension and discomfort, nausea with or without vomiting, ultrasound evidence of ascites, ovarian diameters of 8–12 cm, haematocrit <45% and weight gain <2 kg. Severe OHSS was defined as ovarian enlargement, ascites with or without hydrothorax, haematocrit >45%, weight gain >2 kg, white blood cell count >15,000, oliguria, creatinine of 1.0–1.5, creatinine clearance of >50 ml/min, liver dysfunction and oedema anasarca (*Navot et al., 1992*).

Blood sampling and hormone assays

Blood sampling was performed: on the day of trigger, 7 days after oocyte retrieval and on day 14 after oocyte retrieval. Blood samples were divided in two after centrifugation at 1107*g* for 10 min, and serum samples were frozen immediately at –80°C for subsequent analysis of oestradiol, FSH, LH, progesterone and HCG. LH and FSH were measured by electrochemiluminescence immunoassay on the Cobas® e411 system (Roche Diagnostics) according to the manufacturer's instructions and following local validation. Oestradiol and HCG were measured using the Cobas e801 system (Roche Diagnostics) according to the manufacturer's instructions and following local validation. Serum progesterone was measured according to the manufacturer's instructions and following local validation on the Immulite® 2000 XPi system (Siemens Healthcare).

Sample size

This project was planned in 2013. At that time, only a small uncontrolled study had explored the exogenous progesterone-free luteal phase (*Kol et al., 2011*). Thus, the sample sizes of the present pilot RCT were based on the aim of informing potential future large-scale trials. Statistical evidence for sample size in pilot trials based on anticipated main trials suggested that at least 50 participants per group was advisable for a pilot RCT (*Sim and Lewis, 2012*). Subsequently, it was decided to randomize patients in blocks of 10 until a total of 100 patients in the GnRHa trigger and HCG trigger groups, respectively, had undergone embryo transfer.

Statistics

As this study investigated the HCG-based LPS protocol, the primary analysis was the modified intention to treat (ITT) analysis (*White et al., 2011*) of all randomized patients having an embryo transfer, under the assumption that missing outcome data (patients not having an embryo transfer) were missing conditionally at random. Additionally, both per protocol and strict ITT analyses were carried out for comparison. This approach is consistent with a frequently cited methods framework in the British Medical Journal (*White et al., 2011*). A binary regression model was used to calculate the crude relative risks and relative differences (cRR, cRD) for the primary outcome. As an exploratory post hoc analysis the adjusted relative risks and relative differences (aRR, aRD) were calculated according to adjustment for female age and number of oocytes retrieved as continuous parameters. Hormonal outcome analysis was performed by use of Student's *t*-test or Mann–Whitney *U*-test, depending on the normality and variance of the data. Normal distribution was assessed by quantile–quantile plots and equal variance was tested using the *F*-test. $P < 0.05$ was considered statistically significant. Analyses were performed using Stata Statistical Software, Release 16.0 (StataCorp LP, College Station, TX, USA).

RESULTS

A total of 275 IVF patients were assessed for eligibility and 250 patients were subsequently recruited for the study (FIGURE 2). In RCT 1, a total of 130 patients were randomized, whereas 120 patients were randomized in RCT 2. No patient was lost to follow-up. Demographic data and baseline endocrinology for RCT 1 and RCT 2 are presented in TABLE 1.

RCT 1: oocytes and embryos, Groups A and B (≤13 follicles)

A significantly higher number of follicles at oocyte retrieval ($P = 0.007$), more MII oocytes retrieved ($P = 0.03$) and a higher number of transferable embryos ($P = 0.02$) were obtained in Group B as compared with Group A (TABLE 2). The median number of good transferable embryos was 1 (IQR 1–2) and 2 (IQR 1–3) for the GnRHa group and the HCG trigger group, respectively ($P = 0.02$). Consequently, the number of patients having fresh embryo transfer was lower in Group A ($n = 50$) compared with Group B ($n = 54$), but this difference was not statistically significant ($P = 0.38$). The single embryo transfer (SET) rate was 90% and 89%, of which 36% and 37% were day 5 transfers in the two groups, respectively (TABLE 2, Supplementary Table 1).

RCT 1: reproductive outcomes and OHSS, Groups A and B (≤13 follicles)

The reproductive outcomes per embryo transfer and per randomized patient are shown in TABLE 3 and Supplementary Table 1, respectively. No statistical difference in reproductive outcomes between Groups A and B was seen. Thus, the ongoing pregnancy rate per embryo transfer was 44% (22/50) versus 46% (25/54), comparing Group A to Group B, cRR 0.95 (95% CI 0.62 to 1.45, $P = 0.81$). The corresponding cRD was –2% (95% CI –21% to –17%). When adjusting for female age and number of oocytes retrieved, the ongoing pregnancy rate per embryo transfer was similar, aRR 0.86 (95% CI 0.56 to 1.33, $P = 0.51$) and the aRD was –5% (95% CI –25% to –14%). The ITT analysis yielded similar effect estimates between groups, cRR 0.88 (95% CI 0.55 to 1.39, $P = 0.59$) and aRR 0.86 (95% CI 0.54 to 1.37, $P = 0.54$). LBR per embryo transfer was 40% (20/50)

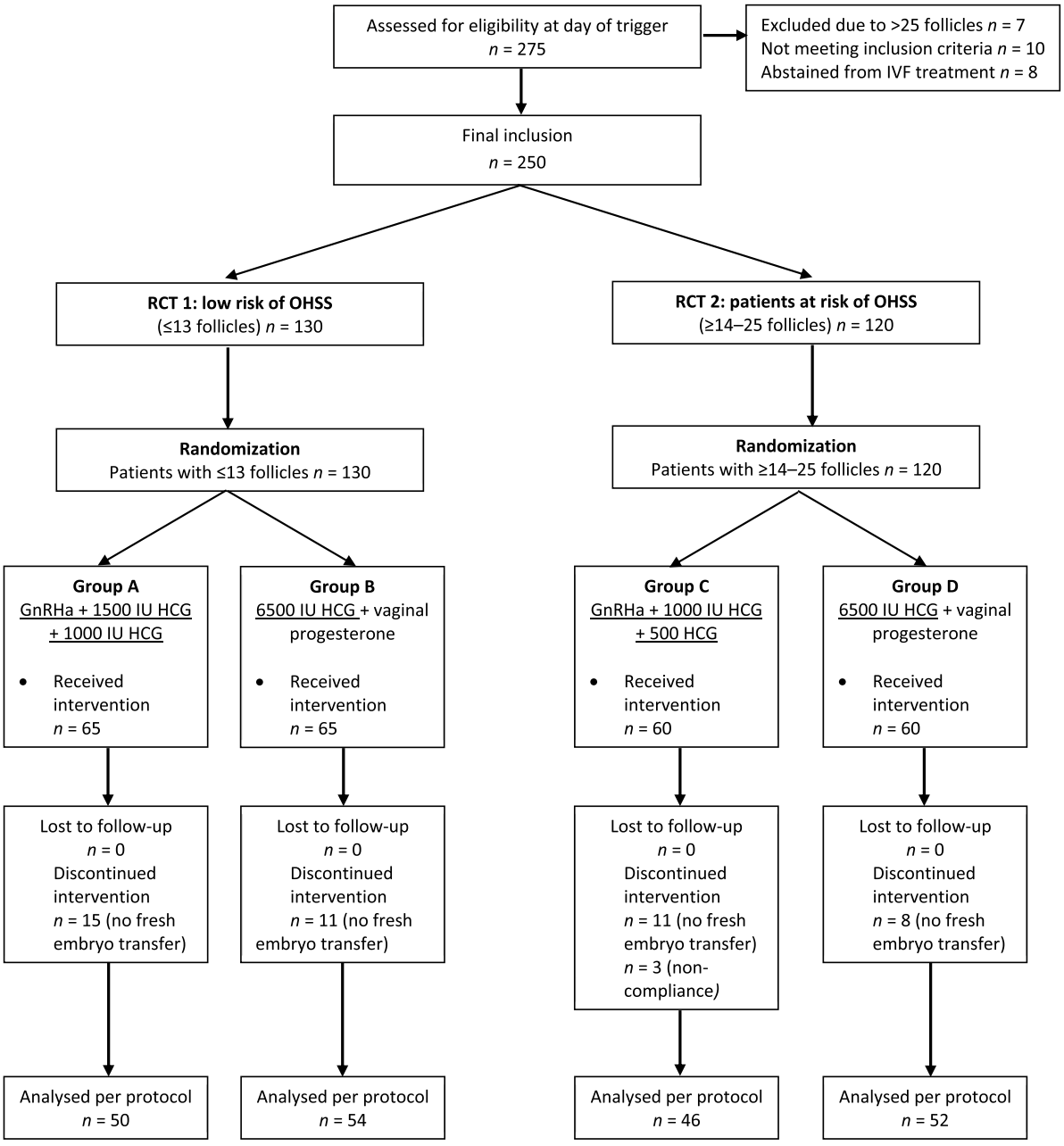

FIGURE 2 In RCT 2, three patients did not undergo trial per protocol (all in Group C) due to non-compliance (3). Finally, a total of 26 and 19 patients did not undergo fresh embryo transfer in RCT 1 and in RCT 2, respectively, due to no embryos available for transfer or cycle segmentation for safety reasons (see also *Table 2*).

versus 46% (25/54), $P = 0.52$. No OHSS was seen in Groups A or B.

RCT 2: oocytes and embryos, Groups C and D (14–25 follicles)

No statistical difference between Groups C and D was seen as regards number of follicles at oocyte retrieval, MII oocytes retrieved and number of transferable embryos. The number of good transferable embryos did not differ significantly between groups ($P = 0.69$) (TABLE 2). Slightly fewer patients received fresh embryo transfer in Group C ($n = 49$) versus Group D ($n = 52$), however, this difference was not statistically significant ($P = 0.45$). The SET rate was 98% and 94%, of which 53% and 52% were day 5 transfers in the groups, respectively (TABLE 3 and Supplementary Table 1).

RCT 2: reproductive outcomes and OHSS, Groups C and D (14–25 follicles)

In RCT 2, the ongoing pregnancy rate per embryo transfer was 51% (24/49) in Group C as compared with 60% (31/52) in Group D, cRR 0.86 (95% CI 0.60 to 1.22, $P = 0.39$). The cRD was –9% (95% CI –28% to –11%) in the modified ITT analysis per embryo transfer. When adjusting for female age and number of oocytes retrieved, the estimate for ongoing pregnancy per embryo transfer was aRR 0.85 (95% CI 0.60 to 1.21, $P = 0.38$) and aRD was –8% (95% CI –27% to –11%). In the ITT analysis the results were similar, cRR 0.81 (95% CI 0.55 to 1.19,

TABLE 1 BASELINE CHARACTERISTICS

	RCT 1 (≤13 follicles)		RCT 2 (14–25 follicles)	
	Group A (*n* = 65)	Group B (*n* = 65)	Group C (*n* = 60)	Group D (*n* = 60)
Age	29.5 (4.0)	30.6 (3.7)	28.9 (3.9)	28.9 (3.9)
BMI[a]	23.8 (22.3–28.1)	25.8 (21.7–29.0)	24.7 (22.9–27.6)	24.7 (22.0–27.5)
Basal FSH	6.7 (2.2)	6.1 (1.9)	5.8 (1.7)	5.7 (2.1)
Basal LH[a]	5.1 (3.9–6.5)	5.1 (3.6–6.6)	5.6 (4.3–7.7)	6.2 (4.4–8.6)
One previous failed IVF/ICSI cycle	3 (5)	9 (14)	3 (5)	7 (12)
PCOS	0	1 (2)	5 (8)	9 (15)
Cause of infertility				
Tubal factor	5 (8)	4 (6)	4 (7)	5 (8)
Male factor	26 (40)	23 (35)	21 (35)	20 (33)
Single/lesbian	22 (34)	23 (35)	18 (30)	12 (20)
Idiopathic	4 (6)	4 (6)	1 (2)	4 (7)
Other	8 (12)	11 (17)	16 (27)	19 (32)
Antral follicles, stimulation day 1[a]	13 (10–16)	14 (12–17)	17 (14–26)	18 (15–25)

Data are presented as *n* (%) or mean (SD).

Cause of infertility may have multiple categories; only primary cause listed.

BMI = body mass index; ICSI = intracytoplasmic sperm injection; PCOS = polycystic ovary syndrome; RCT = randomized controlled trial.

[a] Skewed values presented as medians (IQR).

P = 0.28) and aRR 0.81 (95% CI 0.55 to 1.19, *P* = 0.28) for Group C versus Group D. Moreover, LBR did not differ significantly between groups, 51% (25/49) versus 58% (30/52), *P* = 0.50 (TABLE 3). Per protocol numbers are shown in Supplementary Table 2, and the per protocol analysis resulted in similar results to the ITT analyses (data not shown).

OHSS

Two OHSS cases (3.3%) were reported in Group C (GnRHa trigger): one late moderate and one late severe. In Group D (HCG trigger), four OHSS cases (6.7%)

TABLE 2 FOLLICLES, OOCYTES, EMBRYOS, OHSS AND STIMULATION

	RCT 1 (≤13 follicles)		*P*-value	RCT 2 (14–25 follicles)		*P*-value
	Group A (*n* = 65)	Group B (*n* = 65)		Group C (*n* = 60)	Group D (*n* = 60)	
No of follicles ≥11 mm on the day of trigger	8.5 (2.4)	9.0 (3.0)	0.28	17 (15–20)	16 (15–18)	0.26
Follicles on ORD[a]	9 (6–12)	11 (9–13)	0.007	19.4 (5.5)	19.9 (5.5)	0.68
Low T-FOI[b]	5 (8)	1 (2)	0.21	2 (3)	2 (3)	1.00
Oocytes[a]	7 (5–10)	9 (7–11)	0.08	15.5 (4.5)	15.0 (4.3)	0.52
MII oocytes[a]	6 (4–8)	7 (5–10)	0.03	12.3 (4.4)	12.2 (4.6)	0.84
2PN	4.3 (2.7)	4.9 (2.8)	0.24	8.6 (3.5)	8.4 (4.0)	0.70
Transferable embryos[a]	1 (1–2)	2 (1–3)	0.02	3 (1–5)	3 (2–6)	0.69
No of embryos available for transfer	14 (22)	7 (11)	0.09	4 (7)	5 (8)	1.00
Segmentation/freeze-all[b]	1 (2)	4 (6)	0.37	7 (12)	3 (5)	0.20
OHSS[d]	0	0	N/A	2 (3)	4 (7)	0.68
Total dose of FSH[a]	2000 (1700–2325)	1800 (1500–2400)	0.11	1500 (1349–1819)	1588 (1294–1975)	0.53
Duration of FSH (days)	12.3 (1.4)	12.1 (1.5)	0.40	12.3 (1.5)	12.7 (1.4)	0.21

Data are presented as *n* (%) or mean (SD).

2PN = two-pronuclear; MII = metaphase II; OHSS = ovarian hyperstimulation syndrome; ORD = oocyte retrieval day; RCT = randomized controlled trial.

[a] Skewed values presented as medians (IQR).

[b] T-FOI = trigger day follicle:oocyte index (low <50%).

[c] In RCT 1: segmentation due to hydrosalpinx (*n* = 1) (Group B), >25 follicles at oocyte retrieval (*n* = 1) (Group B), polyp (*n* = 1) (Group B), patient request (*n* = 1) (Group A), and intraperitoneal bleeding after oocyte retrieval (*n* = 1) (Group B).

In RCT 2: segmentation due to >25 follicles at oocyte retrieval (*n* = 5 in Group C and *n* = 3 in Group D), unexpected azoospermia (*n* = 1) (oocyte freeze) (Group C), and bleeding during oocyte retrieval (*n* = 1) (Group C).

[d] A total of two OHSS cases was reported in Group C: one late severe and one late moderate. In Group D, four OHSS cases: one early severe, and three late moderate OHSS cases were reported.

TABLE 3 REPRODUCTIVE OUTCOMES PER EMBRYO TRANSFER

	RCT 1 (≤13 follicles)		*P*-value	RCT 2 (14–25 follicles)		*P*-value
	Group A (*n* = 50)	Group B (*n* = 54)		Group C (*n* = 49)	Group D (*n* = 52)	
Single embryo transfer	45 (90)	48 (89)	1.00	48 (98)	49 (94)	0.62
Cleavage (day 2–3)	32 (64)	34 (63)	0.91	23 (47)	25 (48)	0.91
Blastocysts (day 5)	18 (36)	20 (37)	0.91	26 (53)	27 (52)	0.91
Positive HCG	28 (56)	30 (56)	0.97	29 (59)	33 (63)	0.66
Clinical pregnancy rate	23[b] (46)	26[b] (48)	0.83	27 (55)	31[b] (60)	0.65
Ongoing pregnancy week 12	22[b] (44)	25[b] (46)	0.81	25 (51)	31[b] (60)	0.39
Early pregnancy loss rate[a]	6 (21)	5 (17)	0.74	4 (14)	2 (6)	0.41
Live birth rate	20[b] (40)	25[b] (46)	0.52	25 (51)	30[b] (58)	0.50

Unless otherwise stated, numbers are *n* (%).

[a] Calculated by subtracting ongoing from HCG positive pregnancies.

[b] One twin pregnancy.

HCG = human chorionic gonadotrophin; LBR = live birth rate; RCT = randomized controlled trial.

were reported: three late moderate and one early severe OHSS case. The early severe OHSS case in the HCG trigger group underwent cycle segmentation for safety reasons due to the presence of >25 follicles at oocyte retrieval. The four moderate OHSS cases were treated on an outpatient basis, whereas the late severe OHSS case (Group C) was hospitalized for 8 days (TABLE 4).

RCT 1: luteal phase endocrinology, Groups A and B (≤13 follicles)

LH, FSH, progesterone, HCG and oestradiol were compared between Groups A and B (TABLE 5), showing significantly higher serum progesterone, HCG and oestradiol concentrations (all $P < 0.00001$) on ORD+7 in Group A versus Group B. All other hormone concentrations were not statistically different between groups on the respective days.

RCT 2: luteal phase endocrinology, Groups C and D (14–25 follicles)

Significantly higher FSH ($P = 0.016$), progesterone ($P < 0.00001$), HCG ($P = 0.0003$) and oestradiol ($P = 0.003$) concentrations on ORD+7 were seen in Group C as compared with Group D (TABLE 5). All other hormone concentrations were not statistically different between groups on the respective days.

DISCUSSION

This pilot RCT explored an HCG-based only LPS in IVF patients. Two small luteal HCG boluses were used in the GnRHa-triggered groups for LPS, and so no vaginal supplementation with progesterone and oral oestradiol was used. The comparator was HCG trigger and a standard vaginal progesterone support. Using this LPS concept, a non-significant difference was seen in ongoing clinical pregnancy rate as well as LBR when comparing the GnRHa-triggered groups to the HCG-triggered groups. No OHSS was seen in the OHSS low-risk groups, whereas in the group of patients at risk of OHSS, OHSS occurred in two and four cases after GnRHa and HCG trigger, respectively. In both RCT the per transfer analysis (modified ITT analysis) was closer to unity between GnRHa- and HCG-triggered groups, showing that patients actually completing the HCG-based LPS protocol had similar ongoing pregnancy rates compared with the HCG-triggered standard LPS groups.

The reintroduction of GnRHa for ovulation trigger in IVF, apart from introducing the era of segmentation (elective freeze-all) and the concept of the 'OHSS-free clinic' (*Devroey et al., 2011*), also boosted interest and research into the luteal phases of GnRHa-triggered as well as HCG-triggered IVF cycles (*Humaidan, 2009*; *Humaidan et al., 2005*, *2006*, *2010*, *2013*, *2014*; *Kol et al., 2011*; *Thomsen et al., 2018*; *Vuong et al., 2020*). As for GnRHa trigger, it became evident that modifications of the standard LPS were necessary if acceptable ongoing pregnancy rates and LBR were to be obtained (*Humaidan et al., 2005*), and several modifications were introduced, one of which was low-dose luteal HCG, usually accompanied by a standard vaginal progesterone support and oral oestradiol.

The present LPS protocol was based on the findings of a small uncontrolled proof-of-concept study (15 patients), using two small luteal boluses of HCG

TABLE 4 OHSS

Group	Grade	Follicles, trigger day	Follicles, ORD	Oocytes	Other information
C	Late, severe	22	25	18	Hospitalized 8 days
C	Late, moderate	19	12	11	Ascites puncture × 1
D	Early severe	20	30	30	Segmentation, ascites puncture × 2
D	Late, moderate	25	18	10	Ascites puncture × 2
D	Late, moderate	19	24	11	Ascites puncture × 1
D	Late, moderate	22	18	19	Ascites puncture × 1

OHSS = ovarian hyperstimulation syndrome; ORD = oocyte retrieval day.

TABLE 5 TRIGGER DAY AND LUTEAL PHASE ENDOCRINE PARAMETERS

	RCT 1 (≤13 follicles)		*P*-value	RCT 2 (14–25 follicles)		*P*-value
	Group A (*n* = 65)	Group B (*n* = 65)		Group C (*n* = 60)	Group D (*n* = 60)	
Progesterone (nmol/l), trigger day	1.97 (1.44–2.91)	2.01 (1.40–2.86)	0.60	2.13 (1.48–3.09)	2.08 (1.59–2.99)	0.69
Progesterone (nmol/l), ORD+7	231 (120–317)	74 (52–115)	<0.00001	249 (120–313)	87 (53–212)	<0.00001
Progesterone (nmol/l), HCG day	37 (2–267)	28 (20–68)	0.60	96 (2–410)	61 (25–195)	0.59
LH (IU/l), ORD+7	0.1 (0.1–0.1)	0.1 (0.1–0.2)	0.17	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.79
FSH (IU/l), ORD+7	0.54 (0.44–0.89)	0.52 (0.37–0.67)	0.17	0.4 (0.3–0.5)	0.3 (0.2–0.5)	0.016
Oestradiol (nmol/l), trigger day	3.9 (2.6–6.0)	3.7 (2.4–6.3)	0.95	6.4 (4.1–9.0)	5.6 (3.5–7.9)	0.30
Oestradiol (nmol/l), ORD+7	4.7 (2.9–6.1)	3.2 (1.7–3.8)	<0.00001	5.1 (4.2–7.0)	3.9 (2.1–6.0)	0.003
Oestradiol (nmol/l), HCG day	1.4 (0.2–4.5)	0.22 (0.12–2.09)	0.03	2.7 (0.2–9.4)	1.8 (0.1–4.5)	0.12
HCG (IU/l), ORD+7	8.9 (5.4–11.2)	2.1 (1.1–2.9)	<0.00001	3.1 (2.0–4.3)	1.9 (1.2–2.9)	0.0003

Data reported as median (interquartile range).

HCG = human chorionic gonadotrophin; ORD = oocyte retrieval day; RCT = randomized controlled trial.

and no progesterone or oestradiol supplementation for LPS after GnRHa trigger (*Kol et al., 2011*). With this concept in the present RCT, it was possible to corroborate the findings of the proof-of-concept study in terms of a non-significant difference in ongoing clinical pregnancy rates between the two GnRHa trigger groups and the two HCG trigger groups; however, although non-significant, a difference of 2% in ongoing clinical pregnancy rate in the OHSS low-risk groups and 9% in the group at risk of OHSS was in favour of HCG trigger, suggesting that further minor luteal modifications in terms of, for example, adjustments of the dose and the day of HCG administration could be justified in the GnRHa-triggered groups. However, the fact that ongoing pregnancy rates were closer to unity in the modified ITT analysis points towards an efficient LPS in the low-risk OHSS patient (≤13 follicles), whereas LPS modifications are warranted in the group with 14–25 follicles. Importantly, based on the OHSS cases – predominantly late moderate, treated on an outpatient basis – in Groups C and D of the present study, and as discussed later, it would be advisable to perform a freeze-all in all patients with ≥19 follicles ≥11 mm on the day of trigger (*Griesinger et al., 2016*). Furthermore, an obligatory SET policy for the OHSS risk patient would reduce the OHSS risk even further.

The difference in fresh embryo transfer rate and number of transferable embryos in RCT 1 may have been caused by a higher number of patients with a low (<50%) trigger day follicle:oocyte index (T-FOI), comparing Group A to Group B (5 patients versus 1 patient). However, a more plausible explanation is that significantly more follicles (11 versus 9) were present at oocyte retrieval in Group B compared with Group A, despite equal numbers on the day of trigger (the day of randomization). In contrast, the difference in fresh embryo transfer rate in RCT 2 is most likely a random difference, unrelated to trigger method. As regards early pregnancy losses, no significant difference was seen between GnRHa-triggered and HCG-triggered groups.

Mid-luteal serum progesterone concentrations and correlations between progesterone concentrations and the reproductive outcome during fresh embryo transfer, following HCG trigger and a 'standard' LPS, recently attracted attention (*Thomsen et al., 2018*; *Tu et al., 2020*). From the *Thomsen et al. (2018)* paper, which explored a total of 602 IVF/ICSI cycles, the optimal serum progesterone cut-off for live birth was suggested to be 150–250 nmol/l; interestingly, not only lower, but also higher progesterone concentrations resulted in poorer LBR. More recently, *Tu et al. (2020)*, in their retrospective analysis of 1402 IVF/ICSI cycles, reported a lower cut-off of 127 nmol/l (40 ng/ml) on the day of embryo transfer +6 (ORD+9) for live birth. In that analysis, patients with very low progesterone concentrations (<32 nmol/l, ≤10 ng/ml) were supplemented with daily oral synthetic progesterone from embryo transfer +6, resulting in LBR similar to those of the most optimal mid-luteal progesterone subgroup.

Over the years, by modifications of the LPS after GnRHa trigger, it has been possible to continuously increase the mean mid-luteal progesterone concentration (ORD+5) from 39 nmol/l (*Humaidan et al., 2005*), using vaginal micronized progesterone, 90 mg daily, only to 74 nmol/l (*Humaidan et al., 2010*) with the addition of 1500 IU HCG on ORD – and finally to 440 nmol/l using two boluses of 1500 IU HCG – one on ORD and another bolus on ORD+5. These LPS modifications significantly increased ongoing pregnancy rates from 6% (*Humaidan et al., 2005*) to 39% (*Humaidan et al., 2013*) and, equally importantly, significantly reduced early pregnancy losses from 79% (*Humaidan et al., 2005*) to 9% (*Humaidan et al., 2013*), showing the significant importance of progesterone for implantation and early pregnancy.

The present study achieved median progesterone concentrations on ORD+7 of 231 and 249 nmol/l, in the GnRHa-triggered groups of RCT 1 and RCT 2, respectively, significantly higher than those of the HCG-triggered, standard LPS groups on ORD+7 (74 and 87, respectively). Interestingly, although progesterone concentrations on ORD+7 were higher in the GnRHa-triggered groups, there was a non-significant difference in ongoing pregnancy rate of 2% and 9% in favour of HCG trigger and the standard LPS. As regards oestradiol concentrations on ORD+7, these were significantly higher in the GnRHa-triggered groups of RCT 1 and RCT 2 as compared with the HCG-triggered groups, showing a clear stimulatory effect on the corpora lutea of a mid-luteal HCG

bolus (1000 or 500 IU) administered on ORD+4.

As expected, circulating HCG concentrations on ORD+7 were also higher in the GnRHa-triggered groups as compared with the HCG-triggered groups (*Vuong et al., 2020*). Taken together, the current exogenous progesterone-free luteal phase protocol secured high circulating mid-luteal progesterone and oestradiol concentrations.

An important issue with luteal HCG supplementation is obviously to use the minimal dose needed to secure the reproductive outcome without increasing the risk of OHSS. In the present study a dose of 1500 IU on ORD and an additional dose of 1000 IU on ORD+4 was chosen for patients considered at low risk (≤13 follicles ≥11 mm) of OHSS development, compared with a 6500 IU HCG trigger. This regimen was designed based on a previous trial (*Humaidan et al., 2013*), in which patients considered at low risk of OHSS development received a total of two boluses of 1500 IU HCG during the luteal phase after GnRHa trigger in addition to vaginal progesterone support. In that study, although the reproductive outcome was similar to the HCG comparator, two late-onset OHSS cases developed in the GnRHa-triggered group. Taking the lessons learned from the previous study into account (*Humaidan et al., 2013*), the second bolus of HCG on ORD+4 was reduced to 1000 IU in the present study to avoid OHSS.

Patients at risk of developing OHSS (14–25 follicles ≥11 mm) were randomized to either GnRHa trigger followed by a bolus of 1000 IU HCG on ORD and an additional bolus of 500 IU on ORD+4 – without any further luteal support – or HCG trigger (6500 IU) and a standard vaginal LPS. This resulted in two late OHSS cases in the GnRHa trigger group (3.3%) and four OHSS cases in the HCG trigger group (6.7%), mainly moderate OHSS. Importantly, only one of these patients required hospitalization. This was a late OHSS in a 24-year-old woman triggered with GnRHa (Group C) with 22 follicles at ovulation induction and 18 oocytes retrieved.

In hindsight and considering more recent scientific evidence on follicle cut-off levels for OHSS prevention (*Griesinger et al., 2016*; *Steward et al., 2014*), published after the present study was designed, this patient as well as the four other patients experiencing late moderate OHSS might have been avoided, using a GnRHa trigger and freeze-all policy.

A previous RCT (*Humaidan et al., 2013*) used the same definition and upper cut-off for inclusion as in the present trial (>25 follicles ≥11 mm on trigger day). The HCG trigger dose was 5000 IU as compared with 6500 IU in the present trial, and in the previous trial two moderate late-onset cases occurred in the HCG trigger group (3.4%) considered at risk of OHSS development (14–25 follicles), whereas no OHSS occurred in the GnRHa trigger group. Thus, the increase in OHSS between the two studies, from 3.4% to 6.7% after HCG trigger, might be explained by the slight increase in the HCG trigger dose. Furthermore, whereas OHSS did not occur in the GnRHa trigger group at risk of OHSS in the *Humaidan et al. (2013)* study, in the present trial and with the present LPS, two OHSS cases occurred.

With current knowledge of OHSS prevention, based on evidence from studies published after the design of the present trial, the cut-off for fresh embryo transfer previously suggested for HCG trigger, 19 follicles ≥11 mm on the trigger day (*Griesinger et al., 2016*), also seems to apply for GnRHa trigger followed by modified LPS and fresh embryo transfer.

A limitation of this study is that, although a non-significant difference (9%) in ongoing pregnancy rate was observed between Groups C and D, the sample size required to confirm this difference with a power of 80% would be 478 patients in each group. Thus, a larger study is needed before the current concept can be considered for standard clinical practice. Another limitation could be the time used to finalize the present trial; however, no significant changes were performed in the embryology laboratory during the study period, and the time spent for this large GCP-monitored single-centre trial is not uncommon for a medium-sized European IVF unit. Finally, the lack of blinding could be considered a limitation, albeit this was not possible with the present set-up.

The strength of the study, however, is that it is a single-centre study and also that it is the largest RCT performed to explore the HCG-based LPS concept at a time when fresh embryo transfer seems more relevant than ever due to the findings of large RCT and meta-analyses (*Roque et al., 2019*; *Stormlund et al., 2020*) reporting no difference in reproductive outcomes when comparing fresh embryo transfer to freeze-all.

LPS vaginal support during IVF treatment is usually considered cumbersome and inconvenient by patients, mainly due to vaginal leakage and itching. In this pilot RCT, LPS was secured by two small boluses of HCG, making vaginal progesterone for LPS redundant. Although ongoing pregnancy rates and LBR were non-significantly different between GnRHa and HCG trigger, and the luteal phase steroid profiles were in favour of GnRHa trigger and HCG-based LPS, the results of the present study need to be corroborated by future large-scale trials. However, the present study supports the notion that the exogenous progesterone-free LPS is efficacious, simple and patient-friendly.

ACKNOWLEDGEMENTS

The authors acknowledge lab technician Anette Tyrsted and the OPEN initiative at Odense University Hospital for assistance in biobanking and analysing blood samples. Moreover, the authors acknowledge the patients who entered this trial.

This study was funded by an unrestricted research grant from MSD (Organon). The funders had no role in the design, conduct or interpretation of the study.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2021.03.011.

REFERENCES

Alsbjerg, B., Thomsen, L., Elbaek, H.O., Laursen, R., Povlsen, B.B., Haahr, T., Humaidan, P. **Progesterone levels on pregnancy test day after hormone replacement therapy-cryopreserved embryo transfer cycles and related reproductive outcomes.** Reprod. BioMed. Online 2018; 37: 641–647. doi:10.1016/j.rbmo.2018.08.022

Andersen, C.Y., Elbaek, H.O., Alsbjerg, B., Laursen, R.J., Povlsen, B.B., Thomsen, L., Humaidan, P. **Daily low-dose hCG stimulation during the luteal phase combined with GnRHa triggered IVF cycles without exogenous progesterone: a proof of concept trial.** Human Reproduction (Oxford, England) 2015; 30: 2387–2395. doi:10.1093/humrep/dev184

Castillo, J.C., Dolz, M., Bienvenido, E., Abad, L., Casan, E.M., Bonilla-Musoles, F. **Cycles triggered with GnRH agonist: Exploring low-dose HCG for luteal support.** Reprod. BioMed. Online 2010; 20: 175–181. doi:10.1016/j.rbmo.2009.11.018

Cédrin-Durnerin, I., Isnard, T., Mahdjoub, S., Sonigo, C., Seroka, A., Comtet, M., Herbemont, C., Sifer, C., Grynberg, M. **Serum progesterone concentration and live birth rate in frozen-thawed embryo transfers with hormonally prepared endometrium.** Reprod. BioMed. Online 2019; 38: 472–480. doi:10.1016/j.rbmo.2018.11.026

Devroey, P., Polyzos, N.P., Blockeel, C. **An OHSS-Free Clinic by segmentation of IVF treatment.** Hum. Reprod. 2011; 26: 2593–2597. doi:10.1093/humrep/der251

Di Guardo, F., Midassi, H., Racca, A., Tournaye, H., De Vos, M., Blockeel, C. **Luteal phase support in IVF: comparison between evidence-based medicine and real-life practices.** Front. Endocrinol. 2020; 11. doi:10.3389/fendo.2020.00500

Engmann, L., Benadiva, C., Humaidan, P. **GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis.** Reprod. BioMed. Online. 2016; 32: 274–285. doi:10.1016/j.rbmo.2015.12.007

Engmann, L., DiLuigi, A., Schmidt, D., Benadiva, C., Maier, D., Nulsen, J. **The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: a prospective randomized study.** Fertil. Steril. 2008; 89: 554–561. doi:10.1016/j.fertnstert.2007.04.006

Fatemi, H.M., Polyzos, N.P., van Vaerenbergh, I., Bourgain, C., Blockeel, C., Alsbjerg, B., Papanikolaou, E.G., Humaidan, P. **Early luteal phase endocrine profile is affected by the mode of triggering final oocyte maturation and the luteal phase support used in recombinant follicle-stimulating hormone-gonadotropin-releasing hormone antagonist in vitro fertilization cycles.** Fertil. Steril. 2013; 100: 742–747. doi:10.1016/j.fertnstert.2013.05.028

Fischer, R., Nakano, F.Y., Roque, M., Bento, F.C., Baukloh, V., Esteves, S.C. **A quality management approach to controlled ovarian stimulation in assisted reproductive technology: the 'Fischer protocol.** Panminerva Med. 2019; 61: 11–23. doi:10.23736/S0031-0808.18.03549-8

Griesinger, G., Verweij, P.J.M., Gates, D., Devroey, P., Gordon, K., Stegmann, B.J., Tarlatzis, B.C. **Prediction of ovarian hyperstimulation syndrome in patients treated with corifollitropin alfa or rFSH in a GnRH antagonist protocol.** PLoS ONE 2016; 11e0149615. doi:10.1371/journal.pone.0149615

Haahr, T., Roque, M., Esteves, S.C., Humaidan, P. **GnRH agonist trigger and LH activity luteal phase support versus hCG trigger and conventional luteal phase support in fresh embryo transfer IVF/ICSI cycles – a systematic PRISMA review and meta-analysis.** Front. Endocrinol. 2017; 8: 116. doi:10.3389/fendo.2017.00116

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., Ejdrup Bredkjaer, H., Bungum, L., Bungum, M., Grøndahl, M.L., Westergaard, L., Andersen, C.Y. **GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study.** Hum. Reprod. 2005; 20: 1213–1220. doi:10.1093/humrep/deh765

Humaidan, P., Bungum, L., Bungum, M., Yding Andersen, C. **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., Ejdrup Bredkjaer, H., Westergaard, L.G., Yding Andersen, C. **1,500 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. 2010; 93: 847–854. doi:10.1016/j.fertnstert.2008.12.042

Humaidan, P., Engmann, L., Benadiva, C. **Luteal phase supplementation after gonadotropin-releasing hormone agonist trigger in fresh embryo transfer: the American versus European approaches.** Fertil. Steril. 2015; 103: 879–885. doi:10.1016/j.fertnstert.2015.01.034

Humaidan, P., Engmann, L., Benadiva, C. **Luteal phase supplementation after gonadotropin-releasing hormone agonist trigger in fresh embryo transfer: The American versus European approaches.** Fertil. Steril. 2014; 103: 879–885. doi:10.1016/j.fertnstert.2015.01.034

Humaidan, P., Polyzos, N.P., Alsbjerg, B., Erb, K., Mikkelsen, A.L., Elbaek, H.O., Papanikolaou, E.G., Andersen, C.Y. **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. doi:10.1093/humrep/det249

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. doi:10.1093/humrep/des279

Humaidan, P., Westergaard, L.G., Mikkelsen, A.L., Fukuda, M., Yding Andersen, C. **Levels of the epidermal growth factor-like peptide amphiregulin in follicular fluid reflect the mode of triggering ovulation: a comparison between gonadotrophin-releasing hormone agonist and urinary human chorionic gonadotrophin.** Fertil. Steril. 2011; 95: 2034–2038. doi:10.1016/j.fertnstert.2011.02.013

Kleinstein, J. **for the Luteal Phase Study Group. Efficacy and tolerability of vaginal progesterone capsules (Utrogest 200) compared with progesterone gel (Crinone 8%) for luteal phase support during assisted reproduction.** Fertil. Steril. 2005; 83: 1641–1649. doi:10.1016/j.fertnstert.2004.11.073

Kol, S., Humaidan, P., Itskovitz-Eldor, J. **GnRH agonist ovulation trigger and hCG-based, progesterone-free luteal support: a proof of concept study.** Hum. Reprod. 2011; 26: 2874–2877. doi:10.1093/humrep/der220

Labarta, E., Mariani, G., Holtmann, N., Celada, P., Remohí, J., Bosch, E. **Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: a prospective study.** Hum. Reprod. 2017; 32: 2437–2442. doi:10.1093/humrep/dex316

Navot, D., Bergh, P.A., Laufer, N. **Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment.** Fertil. Steril. 1992; 58: 249–261. doi:10.1016/s0015–0282(16)55188–7

Nyboe Andersen, A., Popovic-Todorovic, B., Schmidt, K.T., Loft, A., Lindhard, A., Højgaard, A., Ziebe, S., Hald, F., Hauge, B., Toft, B. **Progesterone supplementation during early gestations after IVF or ICSI has no effect on the delivery rates: a randomized controlled trial.** Hum. Reprod. 2002; 17: 357–361. doi:10.1093/humrep/17.2.357

Pereira, N., Kelly, A.G., Stone, L.D., Witzke, J.D., Lekovich, J.P., Elias, R.T., Schattman, G.L., Rosenwaks, Z. **Gonadotropin-releasing hormone agonist trigger increases the number of oocytes and embryos available for cryopreservation in cancer patients undergoing ovarian stimulation for fertility preservation.** Fertil. Steril. 2017; 108: 532–538. doi:10.1016/j.fertnstert.2017.06.027

Reddy, J., Turan, V., Bedoschi, G., Moy, F., Oktay, K. **Triggering final oocyte maturation with gonadotropin-releasing hormone agonist (GnRHa) versus human chorionic gonadotropin (hCG) in breast cancer patients undergoing fertility preservation: an extended experience.** J. Assist. Reprod. Genet. 2014; 31: 927–932. doi:10.1007/s10815–014–0248–6

Roque, M., Haahr, T., Geber, S., Esteves, S.C., Humaidan, P. **Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes.** Hum. Reprod. Update 2019; 25: 2–14. doi:10.1093/humupd/dmy033

Schmidt, K.L., Ziebe, S., Popovic, B., Lindhard, A., Loft, A., Andersen, A.N. **Progesterone supplementation during early gestation after in vitro fertilization has no effect on the delivery rate.** Fertil. Steril. 2001; 75: 337–341. doi:10.1016/s0015–0282(00)01709-x

Sim, J., Lewis, M. **The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency.** J. Clin. Epidemiol. 2012; 65: 301–308. doi:10.1016/j.jclinepi.2011.07.011

Simunic, V., Tomic, V., Tomic, J., Nizic, D. **Comparative study of the efficacy and tolerability of two vaginal progesterone formulations, Crinone 8% gel and Utrogestan**

capsules, used for luteal support. Fertil. Steril. 2007; 87: 83–87. doi:10.1016/j.fertnstert.2006.05.067

Steward, R.G., Lan, L., Shah, A.A., Yeh, J.S., Price, T.M., Goldfarb, J.M., Muasher, S.J. **Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles.** Fertil. Steril. 2014; 101: 967–973. doi:10.1016/j.fertnstert.2013.12.026

Stormlund, S., Sopa, N., Zedeler, A., Bogstad, J., Prætorius, L., Nielsen, H.S., Kitlinski, M.L., Skouby, S.O., Mikkelsen, A.L., Spangmose, A.L., Jeppesen, J.V., Khatibi, A., la Cour Freiesleben, N., Ziebe, S., Polyzos, N.P., Bergh, C., Humaidan, P., Andersen, A.N., Løssl, K., Pinborg, A. **Freeze-all versus fresh blastocyst transfer strategy during in vitro fertilisation in women with regular menstrual cycles: multicentre randomised controlled trial.** BMJ 2020; 370: m2519. doi:10.1136/bmj.m2519

Thomsen, L.H., Kesmodel, U.S., Erb, K., Bungum, L., Pedersen, D., Hauge, B., Elbæk, H.O., Povlsen, B.B., Andersen, C.Y., Humaidan, P. **The impact of luteal serum progesterone levels on live birth rates – a prospective study of 602 IVF/ICSI cycles.** Hum. Reprod. 2018; 33: 1506–1516. doi:10.1093/humrep/dey226

Tu, J., Lin, G., Gong, F. **Additional luteal support might improve IVF outcomes in patients with low progesterone level in middle luteal phase following a GnRH agonist protocol.** Gynecol. Endocrinol. 2020; 37: 132–136. doi:10.1080/09513590.2020.1756252

Vuong, L.N., Ho, T.M., Pham, T.D., Ho, V.N.A., Andersen, C.Y., Humaidan, P. **The early luteal hormonal profile in IVF patients triggered with hCG.** Hum. Reprod. 2020; 35: 157–166. doi:10.1093/humrep/dez235

White, I.R., Horton, N.J., Carpenter, J., Pocock, S.J. **Strategy for intention to treat analysis in randomised trials with missing outcome data.** BMJ 2011; 342: d40. doi:10.1136/bmj.d40

Zegers-Hochschild, F., Adamson, G.D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., Rienzi, L., Sunde, A., Schmidt, L., Cooke, I.D., Simpson, J.L., van der Poel, S. **The International Glossary on Infertility and Fertility Care, 2017.** Hum. Reprod. 2017; 32: 1786–1801. doi:10.1093/humrep/dex234

Received 13 December 2020; received in revised form 22 February 2021; accepted 11 March 2021.