

GnRH agonist for triggering of final oocyte maturation: time for a change of practice?

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Submitted on August 25, 2010; resubmitted on December 18, 2010; accepted on February 22, 2011

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BACKGROUND: GnRH agonist (GnRHa) triggering has been shown to significantly reduce the occurrence of ovarian hyperstimulation syndrome (OHSS) compared with hCG triggering; however, initially a poor reproductive outcome was reported after GnRHa triggering, due to an apparently uncorrectable luteal phase deficiency. Therefore, the challenge has been to rescue the luteal phase. Studies now report a luteal phase rescue, with a reproductive outcome comparable to that seen after hCG triggering.

METHODS: This narrative review is based on expert presentations and subsequent group discussions supplemented with publications from literature searches and the authors' knowledge. Moreover, randomized controlled trials (RCTs) were identified and analysed either in fresh IVF cycles with embryo transfer (ET), oocyte donation cycles or cycles without ET; risk differences were calculated regarding pregnancy rate and OHSS rate.

RESULTS: In fresh IVF cycles with ET (9 RCTs) no OHSS was reported after GnRHa triggering [0% incidence in the GnRHa group: risk difference 5% (with 95% CI: -0.07 to 0.02)]. Importantly, the delivery rate improved significantly after modified luteal support [6% risk difference in favour of the HCG group (95% CI: -0.14 to 0.2)] when compared with initial studies with conventional luteal support [18% risk difference (95% CI: -0.36 to 0.01)]. In oocyte donation cycles (4 RCTs) the OHSS incidence is 0% [10% risk difference (95% CI: 0.02 – 0.40)].

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CONCLUSIONS: GnRHa triggering is a valid alternative to hCG triggering, resulting in an elimination of OHSS. After modified luteal support there is now a non-significant difference of 6% in delivery rate in favour of hCG triggering.

Key words: GnRH agonist / GnRH antagonist / hCG / *in vitro* fertilization / OHSS

Introduction

Human chorionic gonadotrophin (hCG) has been the gold standard for ovulation induction as a surrogate for the mid-cycle LH surge for several decades. Due to structural and biological similarities, hCG and LH bind to and activate the same receptor, the LH/hCG receptor (Kessler et al., 1979). An important difference, however, exists between the half-life of LH and hCG, as the half-life of LH is ~ 60 min (Yen et al., 1968) whereas that of hCG is >24 h (Damewood et al., 1989). Due to its prolonged circulatory half-life, hCG exerts a sustained luteotropic activity, and may induce the occurrence of ovarian hyperstimulation syndrome (OHSS) (Delvigne and Rozenberg, 2002). Moreover, studies have reported adverse effects of hCG in terms of a reduced endometrial receptivity and a negative impact on oocyte quality (Forman et al., 1988; Valbuena et al., 2001).

When GnRH antagonist protocols were introduced for the prevention of a premature LH surge (Albano et al., 1997; Itskovitz-Eldor et al., 1998; Borm and Mannaerts, 2000) it became possible to trigger final oocyte maturation and ovulation with a single bolus of a GnRH agonist (GnRHa) as an alternative to hCG (Nakano et al., 1973). The GnRH antagonist occupies the GnRH receptor without causing down-regulation and once the GnRHa displaces the GnRH antagonist from the receptor, the receptor becomes activated which induces a release of gonadotrophins (flare up). Although the GnRHa induced surge effectively stimulates ovulation and oocyte maturation, differences exist regarding the duration and profile of the GnRHa induced surge of gonadotrophins when compared with that of the natural cycle (Gonen et al., 1990; Itskovitz et al., 1991). Thus, the GnRHa induced surge (Fig. 1) consists of two phases: a short ascending limb (>4 h) and a long descending limb (>20 h), in total ~24–36 h (Itskovitz et al., 1991). In contrast, the mid-cycle surge of the natural cycle (Fig. 1) is characterized by three phases: a rapidly ascending phase lasting for 14 h, a plateau of 14 h and a descending phase of 20 h, in total ~48 h (Hoff et al., 1983). Thus, the total amount of gonadotrophins released during the surge is

significantly reduced when GnRHa is used to trigger ovulation when compared with the natural cycle. However, a possible advantage of GnRHa for triggering of final oocyte maturation in comparison with hCG is the simultaneous induction of a FSH surge comparable to the surge of the natural cycle. The role of the mid-cycle FSH surge in the natural cycle is not fully understood, but FSH has been shown to induce LH receptor formation in the luteinizing granulosa cells, thus optimizing the function of the corpus luteum. Moreover, FSH specifically seems to promote oocyte nuclear maturation, i.e. resumption of meiosis (Zelinski-Wooten et al., 1995; Yding Andersen et al., 1999) and cumulus expansion (Stickland and Beers, 1976; Eppig, 1979). Interestingly, several studies reported the retrieval of more mature oocytes after GnRHa trigger, which could be an effect of a more physiological surge including a FSH surge as well as an LH surge (Imoedemhe et al., 1991a; Humaidan et al., 2005, 2009a, 2010; Oktay et al., 2010). Importantly, although large-scale studies do not exist, small studies have suggested that triggering of ovulation with GnRHa prevents OHSS (Itskovitz et al., 1988; Kol et al., 1996; Kol and Itskovitz-Eldor, 2000; Kol, 2003, 2004; Orvieto, 2005; Griesinger et al., 2007a; Hernandez et al., 2009). The study by Itskovitz et al. (1988) was the first to suggest GnRHa triggering after ovarian stimulation as a means to avoid OHSS, as two of the five included patients had previously developed severe OHSS after conventional triggering with hCG, but did not show any hyperstimulation signs after GnRHa triggering.

Despite potential advantages by the use of GnRHa to trigger final oocyte maturation, previous randomized controlled trials (RCTs) reported a poor clinical outcome with an extremely high early pregnancy loss rate when GnRHa was used to trigger ovulation (Fauser et al., 2002; Humaidan et al., 2005; Kolibianakis et al., 2005; Table I; Fig. 2a). The poor results were attributed to a luteal phase insufficiency despite standard luteal phase support (LPS) with progesterone and estradiol. However, following these first disappointing reports several studies now report a luteal phase rescue after modified LPS, resulting in a reproductive outcome comparable to that seen after hCG triggering (Humaidan et al., 2006, 2010; Pirard et al., 2006; Engmann et al., 2008a; Papanikolaou et al., 2011; Table I; Fig. 2b). In terms of delivery rate there is thus now a non-significant difference of 6% in comparison with hCG triggering (Table I; Fig. 2b).

However, until now the greatest advantage of GnRHa triggering in ovarian hyperstimulation, is the total elimination of OHSS (Table II; Figs 3 and 4). This has led to the fact that GnRHa triggering is now the method of choice in many oocyte donation programmes, resulting in a high good quality oocyte yield, an elimination of OHSS, a higher degree of patient convenience and an excellent pregnancy rate in recipients (Hernandez et al., 2009; Bodri et al., 2009).

Finally, an additional indication for GnRHa triggering has recently been reported in women with breast cancer. GnRHa triggering in this subgroup of patients decreased the post-trigger estradiol exposure and significantly increased the number of mature oocytes and embryos (Oktay et al., 2010).

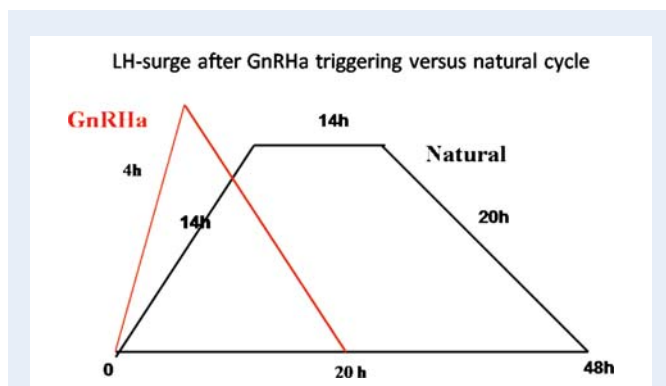


Figure 1 Differences in LH-surge after GnRH-agonist triggering when compared with a natural cycle.

Table 1 Delivery rate in GnRH antagonist IVF cycles according to final oocyte maturation regimen (GnRHa versus HCG).

Studies	Study design	Patients type	Luteal support	Agonist used	AGONIST triggering arm	HCG triggering arm
Conventional luteal support						
Fauser <i>et al.</i> (2002)	RCT	Normovulatory	IM progesterone	Leuprorelin 0.5 Triptorelin 0.2	18.7% (06/32) ^a	13.3% (02/15) ^a
Humaidan <i>et al.</i> (2005)	RCT	Normovulatory	Vag progesterone Estrogen pos	Buserelin 0.5	3.9% (03/55)	36.0% (24/67)
Kolibianakis <i>et al.</i> (2005)	RCT	Normovulatory	Vag progesterone Estrogen pos	Triptorelin 0.2	3.9% (02/52) ^a	27.7% (15/54) ^a
			Rate difference: -0.18, 95% (CI: -0.36 to 0.01)		7.9% (11/139)	30.1% (41/136)
Modified luteal support						
Humaidan <i>et al.</i> (2006)	RCT	Normovulatory	Vag progesterone Estrogen pos 1500 bolus HCG	Buserelin 0.5	38.0% (05/13)	53.0% (08/15)
Babayof <i>et al.</i> (2006)	RCT	PCOS	IM progesterone Estrogen pos adjustable	Triptorelin 0.2	6.6% (01/15)	15.0% (02/13)
Pirard <i>et al.</i> (2006)	RCT	Normovulatory	Buserelin only Different doses	Buserelin 0.2	16.6% (02/12)	16.6% (01/06)
Engmann <i>et al.</i> (2008a,b)	RCT	PCOS	IM progesterone Estrogen patch + Estrogen pos	Leuprorelin 1.0	48.5% (16/33)	43.7% (14/32)
Humaidan <i>et al.</i> (2010)	RCT	Normovulatory	Vag progesterone Estrogen pos 1500 bolus HCG	Buserelin 0.5	23.7% (36/152)	31.3% (47/150)
Papanikolaou <i>et al.</i> (2011)	RCT	Normovulatory	Vag progesterone Rec-LH 6 doses	Triptorelin 0.2	22.2% (04/18)	23.5% (04/17)
			Rate difference: -0.06, 95% (CI: -0.14 to 0.02)		26.3% (64/243)	32.6% (76/233)

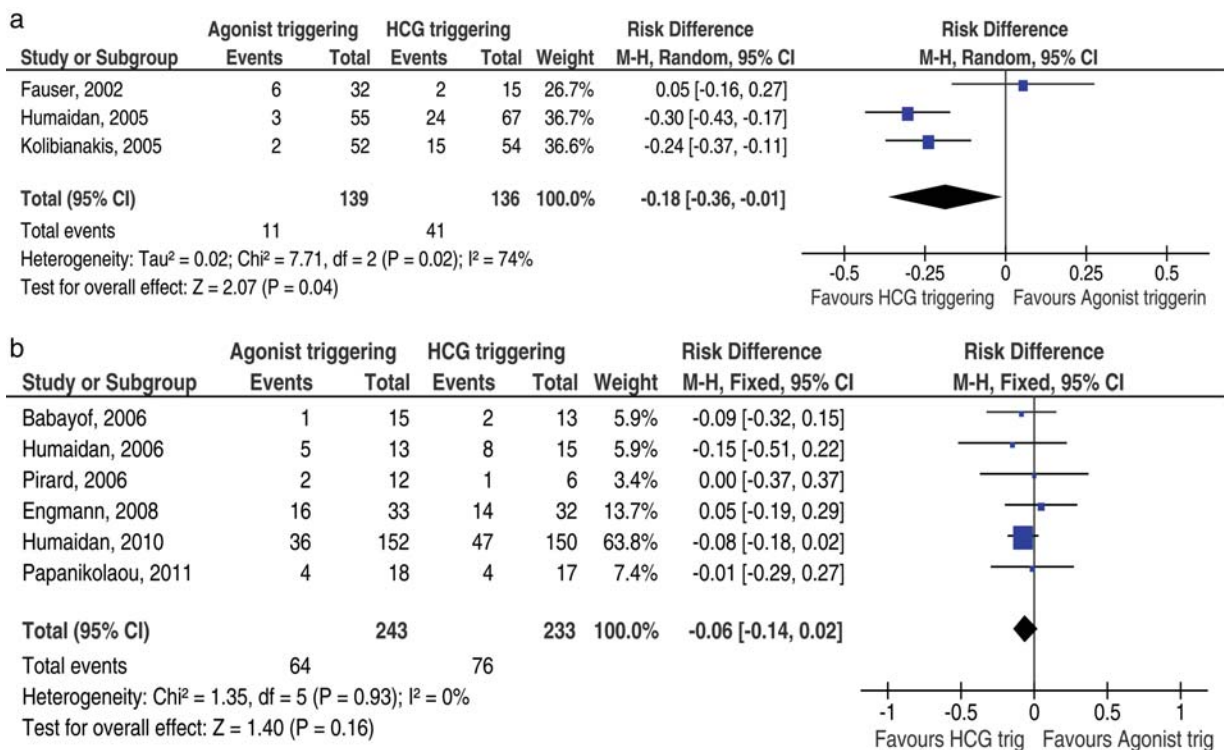
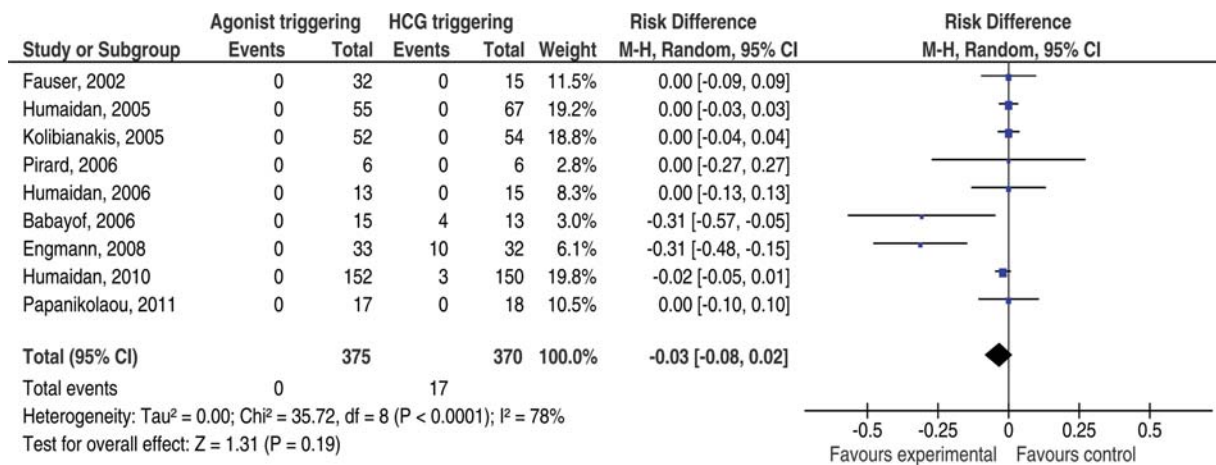
^aOngoing pregnancy rates (12–18 weeks) provided.**Figure 2** (a) Ongoing pregnancy rate after conventional luteal support. (b) Delivery rate after modified luteal support.

Table II OHSS incidence after GnRHa triggering of final oocyte maturation versus HCG triggering in published trials.

Studies	Study design	OHSS risk	AGONIST triggering arm	HCG triggering arm
<i>Fresh IVF cycles with Embryotransfer (ET)</i>				
Fauser et al. (2002)	RCT	Normal	0% (0/32)	0% (0/15)
Humaidan et al. (2005)	RCT	Normal	0% (0/55)	0% (0/67)
Kolibianakis et al. (2005)	RCT	Normal	0% (0/52)	0% (0/54)
Pirard et al. (2006)	RCT	Normal	0% (0/06)	0% (0/06)
Humaidan et al. (2006)	RCT	Normal	0% (0/13)	0% (0/15)
Babayof et al. (2006)	RCT	High	0% (0/15)	31.0% (4/13)
Engmann et al. (2008a, b)	RCT	High	0% (0/33)	31.0% (10/32)
Humaidan et al. (2010)	RCT	Normal/high	0% (0/152)	2.0% (3/150)
Papanikolaou et al. (2011)	RCT	Normal	0% (0/17)	0% (0/18)
<i>Donor IVF cycles (no ET)</i>				
Acevedo et al. (2006)	RCT	Normal	0% (0/30)	17.0% (5/30)
Galindo et al. (2009)	RCT	Normal	0% (0/106)	8.5% (9/106)
Melo et al. (2009)	RCT	Very high	0% (0/50)	4.0% (2/50)
Sismanoglu et al. (2009)	RCT	Very high	0% (0/44)	6.8% (3/44)
<i>Total embryo freezing (no ET)</i>				
Griesinger et al. (2007a, b)	Observ	Very high	0% (0/20)	–
Manzanares et al. (2010)	Observ	Very high	0% (0/42)	–

**Figure 3** OHSS rate in fresh IVF cycles with embryo transfer.

The First Copenhagen Interest Group meeting was held on 30 November–1 December 2009 to evaluate the existing evidence on the use of GnRHa to trigger final oocyte maturation. We here report the discussions and expert opinions based on presentations summarizing current literature and areas for future research.

Methods

Prior to The Copenhagen GnRHa triggering group meeting, specialists in reproductive medicine and scientists prepared presentations based on their own published research, ongoing research and published literature on the subject of GnRHa triggering. At the meeting the presentations

were followed by group discussion to reach conclusions on the topics presented. The contents of this report are based on the presentations and the following discussions during the meeting. The discussions related to each topic were supplemented with an electronic literature search via PubMed for articles in the English language. However, a more detailed description of the search strategy follows as a meta-analysis was performed regarding pregnancy rate and OHSS rate, including the RCTs published so far.

Search strategy and eligibility criteria

Two independent investigators (E.P. and J.G.V.) searched PubMed and the Cochrane Library without language restriction through November 2010,

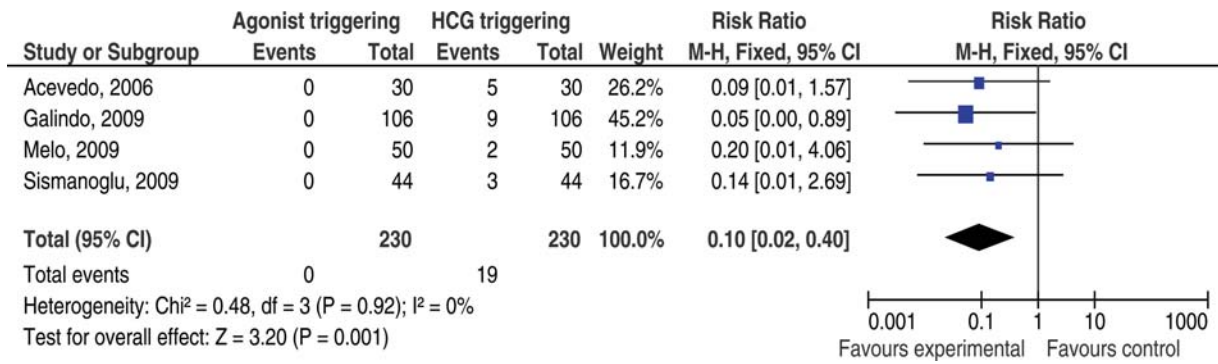


Figure 4 OHSS rate in oocyte donation IVF cycles.

using the search algorithm 'GnRH agonist OR Leuprorelin OR Triptorelin OR Buserelin OR nafarelin AND ovulation triggering OR ovulation induction OR oocyte maturation'. In addition the references of all eligible trials were reviewed and cross-searches in PubMed were performed, using the names of the investigators who were lead authors in at least one eligible trial.

All randomized, prospective or retrospective controlled trials that allocated patients undergoing IVF/ICSI cycles to receive either a GnRH_a or hCG for final oocyte maturation were considered eligible for the narrative review. Only RCTs were eligible regarding the meta-analysis.

Data extraction and analysis

Two independent investigators (D.B. and J.C.C.F.) were involved in the data extraction. The following data were extracted from each arm of the eligible trials: authors' names, journal and year of publication, country of origin, enrolment year, protocol used, type of gonadotrophin used for ovarian stimulation, ovulation triggering medication, incidence of OHSS, ongoing pregnancy rate and delivery rate.

The primary outcome was delivery rate or ongoing pregnancy rate above 12 weeks of gestation. Secondary outcome was OHSS rate.

Meta-analyses included only RCTs. Two-by-two tables were constructed, calculating the risk difference for each primary study to estimate relative risks among GnRH_a triggering and HCG triggering groups. In order to test the homogeneity of the estimates of risk differences among eligible studies, we used the χ^2 test with a level of significance of 0.1, and we further quantified the degree of heterogeneity by using the I^2 test. Data across studies were synthesized, using the fixed effects (Mantel–Haenszel) model whenever no statistical heterogeneity was observed, or using the random effects model (DerSimonian and Laird).

A prespecified subgroup analysis was performed according to the type of LPS adopted within eligible trials (Table 1; Fig. 2a and b) as a modified luteal support was involved in the studies published after 2006 in order to reverse the previously reported low reproductive outcome. Data were analysed using RevMan 5 software. All *P*-values were two-tailed with a level of significance <0.05.

Results

Eligible trials characteristics

A total of 1725 reports were retrieved through searches in PubMed (1393 hits) and Cochrane Central trials Registry (332 hits). Finally

through careful scrutiny of the results obtained and the references of all eligible trials, 30 trials were considered eligible.

Among 30 eligible trials, 9 RCTs involving a GnRH-Antagonist protocol were available including 745 patients, 375 allocated to GnRH_a triggering and 370 allocated to hCG triggering. The GnRH_a regimen used to induce final oocyte maturation differed among them; four trials using buserelin (0.2–0.5 mg), three triptorelin 0.2 mg, one trial with Leuprorelin 1.0 mg and one trial using either Leuprorelin 0.5 or triptorelin 0.2 mg. LPS also differed among eligible randomized trials; three of them used conventional LPS (Fauser *et al.*, 2002; Kolibianakis *et al.*, 2005; Humaidan *et al.*, 2005) whereas six used modified LPS (Babayof *et al.*, 2006; Humaidan *et al.*, 2006; Pirard *et al.*, 2006; Engmann *et al.*, 2008a, b; Humaidan *et al.*, 2010; Papanikolaou *et al.*, 2011).

Follicular fluid

Follicular fluid composition after GnRH_a triggering

Following the reports from the first RCTs on GnRH_a versus hCG triggering (Humaidan *et al.*, 2005; Kolibianakis *et al.*, 2005), the question was asked whether the poor reproductive outcome seen in these trials could be related to the surge of gonadotrophins elicited by a bolus of GnRH_a, leading to an insufficient follicular maturation and thus oocytes with a reduced developmental competence. Speaking against this theory, however, was the fact that significantly more MII oocytes (16%) were retrieved after GnRH_a triggering (Humaidan *et al.*, 2005).

In a study comparing hormonal characteristics of follicular fluid (FF) after GnRH_a and hCG triggering, respectively, a total of 138 FF from 69 patients were examined (Andersen *et al.*, 2006). Significantly higher levels of FSH and LH, a 25% lower level of P4 and a total absence of hCG was seen in FF after GnRH_a triggering, confirming previous findings (Yding Andersen *et al.*, 1993). In the hCG triggering group the total lower level of gonadotrophins (LH and FSH) was compensated for by hCG levels more than one order of magnitude higher than LH and FSH in the GnRH_a group. No differences were seen regarding inhibin-A and inhibin-B between groups (Andersen *et al.*, 2006). The authors concluded that GnRH_a triggering secures a proper preovulatory maturation leading to the release of non-compromised mature

oocytes. Thus, these findings focused the attention of a possible luteal phase insufficiency after GnRHa triggering.

Following the above-mentioned study, a study was performed to examine possible differences in FF Amphiregulin (AR), a member of the Epidermal Growth Factor-like (EGF) family, when either GnRHa or hCG was used for triggering (Humaidan et al., 2009a).

AR is synthesized in granulosa cells in response to the mid-cycle surge of gonadotrophins and is considered to be the mediator (transmitter) of LH effects inside the follicle during the periovulatory period. The expression of this growth factor is rapidly and transiently increased in FF in response to LH/hCG and it is considered to be involved in oocyte maturation (Park et al., 2004; Ashkenazi et al., 2005; Hsieh et al., 2007; Lindbloom et al., 2008). The study included 73 FF samples obtained after GnRHa triggering and 73 FF obtained after hCG triggering. Significantly lower levels of AR were found in FF after GnRHa triggering, approaching AR levels of the natural cycle. Moreover, 14% more MII oocytes and 11% more transferable embryos were seen in the GnRHa group, suggesting that oocyte competence is linked to AR levels (Humaidan et al., 2009a).

In conclusion, although significant differences exist in FF depending on mode of triggering, recent research seems to indicate a beneficial effect of GnRHa triggering over hCG triggering in terms of oocyte maturity and a higher embryo quality.

Vascular mediators in follicular fluid after GnRHa triggering

More than a decade ago, it was shown that once hCG is administered, the ovaries will release vasoactive substances that increase vascular permeability which may result in ascites formation (Balasch et al., 1998). Vascular endothelial growth factor (VEGF) is thought to be the main mediator behind this increase in permeability (McClure et al., 1994; Abramov et al., 1997; Pellicer et al., 1999; Gómez et al., 2002). VEGF production by endothelial cells is up-regulated by hCG (Albert et al., 2002). The increased permeability elicited by VEGF may be reversed by pharmacological inhibition of the VEGF receptor type 2. Apart from VEGF, other key players in the physiopathology of OHSS include soluble vascular endothelial (sVE)-cadherin, which is expressed in endothelial cells (Breviario et al., 1995), and angiopoietin 1 (Ang-1) and 2 (Ang-2), which affects vascular integrity (Fraser, 2006; Molskness et al., 2006).

The shorter duration of the endogenous LH surge induced by GnRHa triggering compared with the continuous LH/hCG receptor stimulation for an estimate of 7–9 days with a bolus of 10 000 IU hCG in combination with a negative impact of the supraphysiological steroid level on LH secretion by the pituitary is the most plausible explanation behind the reduced risk of OHSS when GnRHa is used to trigger final oocyte maturation. In a further effort to understand the possible benefits of GnRHa triggering to avoid OHSS, research was conducted to explore the differential modulation of vascular mediators like VEGF, Ang-2 and sVE-cadherin in volunteers undergoing ovarian stimulation, followed by either hCG or GnRHa to induce final oocyte maturation.

VEGF proves to be significantly lower in patients receiving GnRHa instead of hCG, both at the mRNA and protein level (Cerrillo et al., 2009; Cerrillo et al., 2010). These findings suggest that the endogenous LH surge induced by GnRHa is not only of shorter duration, but

also a weaker activator of the LH/hCG receptor. Although differences in plasma or serum VEGF levels (Manau et al., 2007) may not be so clear, and may even lead to differing conclusions (Babayof et al., 2006), VEGF levels are better evaluated by directly studying FF and/or granulosa cells.

Another signal transduction pathway of interest, controlling endothelial cell survival and vascular maturation is the angiopoietin/Tie (tyrosine kinases containing Ig and EGF domains) system (Augustin et al., 2009; Thomas and Augustin, 2009). While Ang-1 induces Tie2 phosphorylation and vessel stabilization, Ang-2 acts as an antagonist of Ang-1, inducing vascular destabilization, Ang-2 may act synergistically with VEGF. Thomas and Augustin (2009) recently showed that in the presence of VEGF, Ang-2 mediated vascular leakage to the third space. Interestingly, Cerrillo et al. (2010) were not able to find differences in Ang-2 mRNA or protein expression when either hCG or GnRHa was used for final oocyte maturation, although a non-significant decrease in Ang-2 gene expression in the GnRHa group was observed.

Another family of molecules involved in ovarian physiology are cell adhesion molecules (Campbell et al., 1995; Abramov et al., 2001). The regulating role of the cell-specific cadherin VE-cadherin (also known as CD144/cadherin-5) on endothelial cell permeability and migration (Nagafuchi et al., 1993; Breviario et al., 1995) as well as capillary hyperpermeability was recently shown—a process modulated by hCG and VEGF (Villasante et al., 2007). Monitoring serum levels of VE-cadherin may serve as an indicator of corpus luteum function (Villasante et al., 2008). When evaluating whether hCG or GnRHa had a differential effect on VE-cadherin expression, a non-significant trend towards higher serum levels in the group receiving hCG was observed, confirming previous assertions (Cerrillo et al., 2010).

Finally, the luteal phase of oocyte donors having GnRHa triggering for final oocyte maturation was explored in a pilot study (García-Velasco et al., 2010). The luteal phase was supplemented with either low-dose hCG, 500 IU i.m. on days hCG +4, +7 and +10 or estradiol patches, 100 mg, twice per week and progesterone 200 mg twice daily, vaginally.

In comparison with the group of patients having estradiol and progesterone for LPS, patients receiving low dose hCG for LPS had significantly higher serum levels of steroids, lower endogenous LH levels, significantly higher amount of free fluid in the pouch of Douglas and a larger ovarian size measured by transvaginal 2D-ultrasonography throughout the luteal phase. These differences suggest that even low-dose hCG luteal has an effect on vascular permeability, ovarian steroid secretion, luteal phase function and patient convenience.

In conclusion, differences in the regulation of vascular mediators are found between GnRHa and hCG triggering. These differences in the expression levels of vascular mediators could be responsible for the reduced/eliminated OHSS rate seen after GnRHa triggering.

The luteal phase

The luteal phase of the stimulated versus the natural cycle

The luteal phase of all stimulated IVF/ICSI cycles is abnormal (Edwards et al., 1980; Fatemi et al., 2007a, b). In comparison, only 8.1% of natural cycle luteal phases are abnormal in normo-ovulatory

patients with primary or secondary infertility (Rosenberg *et al.*, 1980). According to recent research, the most plausible reason for the luteal phase insufficiency seen after ovarian stimulation with gonadotrophins is the combination of a multifollicular development and triggering of ovulation with hCG with its prolonged half-life, resulting in supra-physiological levels of progesterone and estradiol. The supra-physiological steroid levels (mainly progesterone) directly inhibit the LH secretion from the pituitary via negative feedback actions at the level of the hypothalamic–pituitary axis (Tavaniotou *et al.*, 2001, 2003; Fauser and Devroey, 2003; Tavaniotou and Devroey, 2006; Fatemi, 2009), resulting in luteal phase LH levels below the detection limit (Fatemi *et al.*, 2008).

In the luteal phase LH plays a crucial role, being totally responsible for the steroidogenic activity of the corpus luteum (Casper and Yen, 1979), up-regulation of growth factors like vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2) (Sugino *et al.*, 2000; Wang *et al.*, 2002) and cytokines involved in implantation (Licht *et al.*, 2001). Moreover, LH stimulates extragonadal LH receptors present in the endometrium (Rao, 2001; Tesarik *et al.*, 2003). As a result a significant reduction in LH levels will lead to luteolysis, implantation failure and shortening of the luteal phase (Duffy *et al.*, 1999). Thus, LPS with progesterone for at least 15 days enhances the reproductive outcome in all stimulated IVF/ICSI cycles (Schmidt *et al.*, 2001; Nyboe Andersen *et al.*, 2002).

The luteal phase after GnRHa triggering - conventional LPS

Within the first 12 h after the administration of GnRHa, an LH/FSH surge is induced; a process associated with an acute rise in progesterone and estradiol levels. Following the initial rise, levels of progesterone and estradiol then decrease during the next 24 h preceding follicle aspiration. Following oocyte retrieval, a second rise in levels of progesterone and a continuous fall in estradiol takes place, as ovarian steroidogenesis shifts from follicular to luteal phase.

Importantly, serum levels of estradiol and progesterone during the luteal phase are significantly lower after GnRHa triggering than those obtained following the administration of hCG (Itskovitz *et al.*, 1991), which is most certainly caused by the longer half-life of hCG when compared with that of LH.

The corpus luteum is recognized as the major source of inhibin A and pro- α C production during the luteal phase (Lockwood *et al.*, 1998; Yamoto *et al.*, 1991) and during early pregnancy (Treetampinich *et al.*, 2000). Moreover, the number of follicles and corpora lutea is correlated with levels of inhibin A and pro- α C levels in circulation (Eldar-Geva *et al.*, 2000). Finally, it has been shown that inhibin A, but not pro- α C, is produced by the fetoplacental unit during early pregnancy (Illingworth *et al.*, 1996; Muttukrishna *et al.*, 1997).

To study the luteolytic process induced by a mid-cycle injection of GnRHa, levels of inhibin A and pro- α C, were measured in IVF patients randomized to final oocyte maturation with either GnRHa or hCG (Nevo *et al.*, 2003). GnRHa trigger dramatically decreased levels of these markers in comparison with hCG, and pregnancy did not correlate with a rise in the levels of the markers in the late luteal phase. It was, therefore, concluded that GnRHa trigger results in a complete luteolysis. Moreover, if pregnancy occurs, the corpus luteum cannot

be rescued by the time the endogenous hCG produced by the implanting embryo appears in circulation.

Normal function of the corpus luteum is dependent on the pulsatile release of LH from the anterior pituitary (Mais and Yen, 1986). It is, therefore, reasonable to suggest that the significantly shorter duration of the LH surge after a mid-cycle injection of a GnRHa in combination with supra-physiological steroid levels during the luteal phase and possibly also the down-regulation of pituitary GnRH receptors collectively result in a reduced LH support for the developing corpora lutea which will lead to early luteolysis.

A previous study on GnRHa triggering stopped the LPS when a positive hCG was measured in serum on Day 12 after transfer, as this was the usual procedure used after hCG triggering (Humaidan *et al.*, 2005) while others continued the LPS until the seventh week of gestation (Kolibianakis *et al.*, 2005). Interestingly, there was no difference in the early pregnancy loss rate between these two studies.

Previous studies performed in a GnRH antagonist protocol, showed that after hCG triggering there is no effect of supplementing the luteal phase with estradiol (Fatemi *et al.*, 2007a, b; Kolibianakis *et al.*, 2008). However, as the estradiol level after GnRHa triggering is reduced by more than 50% compared with hCG triggering (Humaidan *et al.*, 2005), a beneficial effect of estradiol supplementation after GnRHa triggering cannot at present be excluded. Thus, for the time being LPS in the form of progesterone and estradiol until the luteo-placental shift around the seventh gestational week (Scott *et al.*, 1991) should be recommended in all IVF/ICSI patients having final oocyte maturation with GnRHa.

Reproductive outcome of GnRHa triggering versus hCG triggering with conventional luteal support (Meta-analysis 2006)

A meta-analysis in 2006 summarized the results of three RCTs comprising a total of 275 patients (Griesinger *et al.*, 2006). Although the occurrence of OHSS was significantly reduced when GnRHa was used to trigger final oocyte maturation, the best estimate from the pooled data from all the randomized studies also showed a significant reduction in the clinical pregnancy rate (7.9% in the GnRHa group versus 30.14% in the HCG group, $P = 0.02$; Griesinger *et al.*, 2006). The negative impact was ascribed to a luteal phase deficiency (reduced endogenous progesterone production and low endogenous LH secretion around the time of implantation) and not to a poor oocyte quality, which was supported by good birth rates in frozen-thawed embryo replacement cycles in which the embryos derived from GnRHa triggered cycles (Eldar-Geva *et al.*, 2007; Griesinger *et al.*, 2007b).

In the initial studies included in the above meta-analysis, the luteal support was a standard support with vaginal progesterone and oral estradiol. Interestingly, the initial serum hCG levels, indicative of implantation, were comparable between GnRHa and hCG triggering (Humaidan *et al.*, 2005; Kolibianakis *et al.*, 2005). However, during the following weeks a high early pregnancy loss was seen in the GnRHa group. Therefore, it was speculated that not only the luteal endocrine environment, but possibly also the luteal endometrial

milieu after GnRHa triggering might differ from what had previously been observed after hCG triggering. For that reason the conventional luteal progesterone support, although supplemented with estradiol seemed to be insufficient, indicating a need for a more intense luteal steroid treatment or supplementation with LH activity.

Luteal phase rescue after GnRHa triggering: modified LPS

One bolus of hCG

After the disappointing reports regarding GnRHa triggering in IVF/ICSI cycles despite conventional LPS (Humaidan et al., 2005; Kolibianakis et al., 2005), focus was directed towards the luteal phase insufficiency following GnRHa triggering. As LH is totally responsible for the steroidogenic activity of the corpus luteum (Casper and Yen, 1979), the up-regulation of growth factors like VEGF-A, FGF2 (Sugino et al., 2000; Wang et al., 2002), the up-regulation of cytokines involved in implantation (Licht et al., 2001) and the activation of LH receptors in the endometrium (Rao, 2001; Tesarik et al., 2003), it was hypothesized that low endogenous luteal phase LH levels would negatively affect all these parameters, resulting in an increased early pregnancy loss rate and a low live birth rate—as noted in previous studies. As a result, the question was asked: when and in which formulation should LH activity be introduced after GnRHa triggering to most optimally rescue the luteal phase?

On the basis of two small pilot studies in IUI patients (Penarrubia et al., 1998; Empeiraire et al., 2004) a number of trials were conducted in IVF/ICSI patients, using GnRHa to trigger final oocyte maturation in GnRH antagonist co-treated cycles, supplementing patients with one bolus of 1500 IU hCG on the day of ovum pick-up (OPU) in addition to a standard LPS with vaginal progesterone and oral estradiol (Humaidan et al., 2006, 2010; Humaidan, 2009b). The small supplementary bolus of hCG clearly rescued the luteal phase after GnRHa triggering, resulting in a normal reproductive outcome. Thus, in the latest study—the largest randomized trial until now including a total of 302 IVF/ICSI cycles—a non-significant difference in delivery rate was seen between GnRHa triggering supplemented with a bolus of 1500 IU hCG after OPU and 10 000 IU hCG (Humaidan et al., 2010). Interestingly, no OHSS case was seen in the GnRHa group versus 2% in the 10 000 IU hCG group, despite the fact that more than one-third of patients in each group had ≥ 14 follicles ≥ 11 mm on the day of triggering; a level previously set to predict 87% of severe OHSS cases (Papanikolaou et al., 2005).

In conclusion, GnRHa triggering supplemented with one bolus of hCG rescues the luteal phase, and dramatically reduces the high early pregnancy loss rate previously seen after GnRHa triggering, without increasing the OHSS rate.

Repeated boluses of hCG

In a further effort to find the optimal dose of hCG necessary during the luteal phase to secure the reproductive outcome and at the same time avoid OHSS, a non-randomized trial was performed in patients at risk of developing OHSS (Castillo et al., 2010). The trial included 192 patients with high response to ovarian stimulation on the day of triggering final oocyte maturation. All patients had triggering

with GnRHa (leuprolide acetate, 1.5 mg). The LPS started on the day after OPU with a fixed dose of hCG every third day (OPU + 1, OPU + 4 and OPU + 7), a total of three doses; in addition progesterone, 600 mg vaginally, was administered to all patients. Three different doses of hCG for LPS were evaluated: Group A ($n = 44$): 1000 IU; Group B ($n = 115$): 500 IU and Group C ($n = 33$): 250 IU.

A non-significant difference in pregnancy rates between groups A, B and C was seen (47.7, 42.6 and 39.4%, respectively). In the total population, moderate OHSS was seen in 4.2% of patients (8/192) and severe OHSS in 3.6% (7/192) of patients; no significant difference was seen between groups. The vast majority—six out of seven—of severe cases were late onset OHSS, and four out of six occurred in twin pregnancies.

The study indicates that repeated low doses of hCG during the luteal phase after GnRHa triggering effectively normalizes the reproductive outcome in a high-risk group of IVF/ICSI patients. Regarding OHSS, there was a clear trend for fewer cases, the lower the hCG dose used. Importantly, there was a distinct relationship between severe OHSS (late onset) and multiple pregnancies due to the increased levels of circulating endogenous hCG—a further argument for single embryo transfer (SET), especially in the high-responder patient.

Regarding the dose of exogenous hCG, fewer OHSS cases were seen in patients supplemented with either 500 or 250 IU when compared with 1000 IU. This finding suggests that three doses of 1000 IU hCG during the luteal phase in high-responder patients is unadvisable.

In conclusion, it seems that three doses of 500 IU of hCG as luteal support post GnRHa triggering in GnRH antagonist protocols is a safe and efficient alternative for treatment of the high-responder patient. This strategy allows embryo transfer in fresh cycles without apparently increasing the OHSS rate and at the same time securing a good reproductive outcome; combining this strategy with SET at the blastocyst stage further increases the observational period of the patient, allowing cancellation of transfer if necessary. However, sufficiently powered RCTs are needed to confirm the results.

Recombinant LH

Another way of adding LH activity to the LH insufficient luteal phase after GnRHa triggering could theoretically be repeated doses of recombinant LH (rLH). The advantage of rLH over hCG is the significantly shorter half-life, which could further reduce the risk of OHSS.

To explore this concept, a pilot study was performed by Papanikolaou et al., 2011. Ovarian stimulation was performed using a fixed dose of 187.5 IU FSH starting on cycle Day 2 in a fixed stimulation Day 6 GnRH antagonist protocol in a total of 35 IVF/ICSI patients. Ovulation triggering was performed with 0.2 mg triptorelin administered as soon as three follicles of 17 mm were present. Six alternate doses of 300 IU rLH were administered starting on the day of OPU and repeated on days OPU + 2, OPU + 4, OPU + 6, OPU + 8 and OPU + 10—in addition to vaginally administered micronized progesterone (600 mg daily). The control group ($n = 17$), consisted of patients undergoing the same stimulation protocol, having hCG to trigger ovulation. A comparable number of oocytes (13.8 versus 11.7) and embryos (8.3 versus 7.9) were seen in the GnRHa and hCG group, respectively. All patients underwent elective single blastocyst transfer, resulting in a non-significant difference in

delivery rate in the rLH group versus the hCG group [22.20% ($n = 4/18$) versus 23.5% ($n = 4/17$) $P = 0.91$, respectively]. No OHSS case was recorded in either group, but a larger sample size is necessary to draw conclusions regarding the efficacy and dose of rLH for luteal support after GnRHa triggering.

Intensive progesterone and estradiol

GnRHa triggering results in low progesterone and estradiol levels during the luteal phase and it could be claimed that most previous studies utilized a suboptimal luteal phase steroidal supplementation (Fauser *et al.*, 2002; Humaidan *et al.*, 2005; Kolibianakis *et al.*, 2005). Moreover, as the ideal type of LPS after GnRHa has not yet been clearly defined, another point of action could be an intensive LPS along with close monitoring of serum steroid levels after GnRHa triggering. This concept was evaluated in a RCT including a total of 59 OHSS high-risk patients, randomized to either dual pituitary suppression (OCP overlapping with GnRHa) followed by triggering of final oocyte maturation with hCG in a dose ranging from 3.300 to 10 000 IU depending on follicular response and serum estradiol levels (hCG group; 32 patients), or a GnRH antagonist protocol followed by final oocyte maturation with a bolus of GnRHa (leuprolide acetate, 1 mg) (GnRHa group; 33 patients). The luteal phase supplementation consisted of 50 mg of i.m. progesterone daily starting the day after oocyte retrieval until ~10 weeks of gestation. Additionally, the patients received three 0.1 mg estradiol transdermal patches every other day, commencing the day after oocyte retrieval. Serum estradiol and progesterone levels were measured on the day of embryo transfer, 1 week after oocyte retrieval and weekly thereafter. The dose of transdermal estradiol patches was increased, if necessary, to a maximum of four 0.1 mg patches every other day, and/or addition of oral micronized estradiol to maintain the serum estradiol level above 200 pg/ml. The IM progesterone dose was also increased, if necessary, to a maximum of 75 mg daily and/or addition of micronized vaginal progesterone to maintain serum progesterone levels above 20 ng/ml. This protocol resulted in an ongoing pregnancy rate after GnRHa triggering of 53% and no OHSS in the GnRHa group versus 34% OHSS in the control group (10/29): five mild, four moderate and one severe case (Engmann *et al.*, 2008a).

Although results are promising in terms of the reproductive outcome and the total elimination of OHSS in a group of PCO/PCOS patients, the findings need to be confirmed in a significantly larger group of high-risk patients. Furthermore, it also needs to be explored whether this protocol applies to the normogonadotropic patient as serum LH is significantly increased (30–90%) during not only the follicular, but importantly also during the luteal phase of the PCOS patient when compared with the normogonadotropic woman; the reason being an increase in the LH pulse frequency and amplitude elicited by the GnRH pulse generator (reviewed in McCartney *et al.*, 2002). This in combination with a decreased sensitivity of the GnRH pulse generator to inhibition by ovarian steroids—in particular progesterone—leaves the PCOS patient with a significantly higher LH level during the luteal phase when compared with the normogonadotropic patient (reviewed in McCartney *et al.*, 2002). A fact which might be a plausible explanation for the results of the Engmann *et al.* study (2008a).

However, the results (Engmann *et al.*, 2008a) are contrasted in a similar study by Babayof *et al.* (2006) in which OHSS high-risk patients were randomized to either hCG (13 patients) or GnRHa triggering (15 patients), followed by intensive luteal support with i.m. progesterone. Moreover, oral estradiol (4 mg) was given to patients with serum E2 concentration below 200 pmol/l during the luteal phase. This protocol resulted in a disappointingly low ongoing pregnancy rate of 6% and a high early pregnancy loss rate (80%) in the group of patients who received GnRHa to trigger final oocyte.

The optimal LPS, mode of administration and length after controlled ovarian hyperstimulation (COH) still needs to be determined (Penzias, 2002; Pritts and Atwood, 2002; Daya and Gunby, 2004), however, several recent publications confirm that there is no superiority of i.m. progesterone over vaginally administered progesterone in COH cycles triggered with hCG (Zarutskie and Phillips, 2009; Fatemi, 2009; Mitwally *et al.*, 2010). Moreover, as previously mentioned evidence is conflicting estradiol supplementation after COH the (Kolibianakis *et al.*, 2008; Engmann *et al.*, 2008b; Jee *et al.*, 2010), but it might be essential after GnRHa triggering due to significantly lower circulating estradiol levels in the luteal phase after GnRHa triggering when compared with hCG triggering.

Meta analysis 2010 with modified luteal support

Analysing the six RCTs published after 2005 we noticed that all studies implemented a luteal support scheme consisting of either intensive luteal supplementation with estradiol and progesterone or luteal LH activity supplementation. With this modified luteal support, the delivery rates increased remarkably to rates comparable to those seen after hCG triggering. However, a 6% difference in delivery rate still exists in favour of hCG triggering (Table I; Fig. 2b). Importantly, OHSS was completely eliminated after GnRHa triggering contrasted by a 7% OHSS incidence after hCG triggering (Table II; Fig. 3).

These findings indicate that the modified luteal support has had a significant positive impact on the reproductive outcome after GnRHa triggering without an increase in the OHSS rate. Nevertheless, the most optimal LPS still has to be explored.

GnRHa triggering in oocyte donors

Oocyte donation has been practiced for more than two decades and currently has wide indications (Sauer and Kavic, 2006). This has led to an increased demand, and growth of oocyte donation cycles worldwide. Although serious short-term complications are reported to occur at a relatively low rate <1% (Bodri *et al.*, 2008; Maxwell *et al.*, 2008), it is universally recognized that ovarian stimulation and oocyte retrieval *per se* might involve significant inconvenience and discomfort as well as risk for the donor. Oocyte donors are only exposed to early-onset OHSS due to the absence of a subsequent pregnancy. This risk of OHSS, however, should not be underestimated as donors are typically young women selected to have a good ovarian reserve and with a yield of large numbers of oocytes.

Several retrospective cohort studies (Shapiro *et al.*, 2007; Bodri *et al.*, 2008; Hernandez *et al.*, 2009) evaluated the outcome of oocyte donation cycles after GnRHa triggering. Although studies vary largely in size (ranging from 32 to 2077 cycles) all report no

significant differences in the key outcome variables such as the proportion of mature oocytes, fertilization rates and subsequent implantation and pregnancy rates in the recipient. More importantly, no moderate/severe OHSS cases were reported after GnRHa trigger whereas in the hCG group the incidence was as high as 8%. These retrospective series have an inherent bias between the examined treatment groups because GnRHa triggering was preferentially applied to cycles with a significantly higher ovarian response.

A number of methodologically more appropriate randomized clinical trials (Acedo *et al.*, 2006; Galindo *et al.*, 2009; Melo *et al.*, 2009; Sismanoglu *et al.*, 2009) have evaluated the same variables (Table II). They included a total number of oocyte donors ranging from 60 to 212 per study. No significant differences were observed in the number of retrieved oocytes (total and mature), fertilization rates, and embryo quality and pregnancy rates in corresponding recipients. Importantly, OHSS was not reported after GnRHa triggering, whereas the OHSS incidence after hCG triggering was between 4 and 17% (Table II; Fig. 4). Hence data from both retrospective cohort studies and controlled clinical trials suggest that GnRHa triggering completely eliminates early-onset OHSS in oocyte donors without adversely affecting the quality of retrieved oocytes or the implantation potential of the resulting embryos.

Apart from elimination of OHSS, additional benefits include a shorter duration of the unsupported luteal phase (4–6 days), a reduced ovarian volume (Engmann *et al.*, 2008a) and diminished abdominal distension; factors that altogether substantially decrease the treatment burden of the oocyte donor (Cerrillo *et al.*, 2009; Hernandez *et al.*, 2009). Furthermore, the use of GnRHa triggering in oocyte donation cycles has also important practical consequences in terms of diminished cycle monitoring and no need for cycle cancellation or interventions reducing the risk of OHSS (Hernandez *et al.*, 2009). These factors simplify the everyday management of oocyte donation cycles for the clinician as well as for the donor.

In summary, extensive recent evidence supports the use of GnRHa triggering for final oocyte maturation in oocyte donors as this mode of triggering apart from eliminating the risk of any clinically significant OHSS secures a good reproductive outcome in the recipient.

GnRHa triggering and OHSS

Does GnRHa triggering completely eliminate OHSS?

The notion that GnRHa trigger eliminates OHSS in high-risk patients was first introduced by Itskovitz *et al.* (1988)—10 years before the GnRH antagonist era. The culprit of this robust approach was that it was too good to ethically justify randomized controlled studies. Instead, Lewit *et al.* (1996) published a study in which 16 women who previously developed severe OHSS following a routine long GnRHa protocol and hCG triggering were triggered in a subsequent cycle with GnRHa: none developed OHSS. Imoedemhe *et al.* (1991b) described 38 women considered at risk of OHSS, having serum estradiol higher than 4000 pg/ml following ovarian stimulation: none developed OHSS. The same group extended their series to 708 polycystic ovarian syndrome patients (PCOS), high-responder IVF patients with a mean estradiol on the day of GnRHa trigger of 7817 pg/ml. Ovulation was effectively triggered in 682 cases (96%).

One patient (0.1%) developed severe OHSS. However, probably by mistake, this patient had received hCG for luteal support, as the protocol dictated progesterone-only luteal support. Interestingly, in 26 patients the GnRHa induced LH surge was judged as 'inadequate'. In 18 of these patients, hCG trigger was used, resulting in 11 (61%) cases of severe OHSS. This last figure reflects the large number of severe OHSS cases prevented in this series by GnRHa triggering.

Although, initially there were some doubts whether GnRHa trigger always prevents OHSS, this issue was fully addressed in a 'Debate' (Kol *et al.*, 1996).

Not surprisingly, GnRHa trigger did not gain much interest until GnRH antagonists became clinically available. Preliminary reports confirmed the ability of GnRHa trigger to prevent OHSS in GnRH antagonist-based stimulation protocols with high-risk patients (Itskovitz-Eldor *et al.*, 2000). There were some hopes that the introduction of the GnRH antagonist protocol in itself would dramatically decrease the OHSS incidence, however, these hopes did not materialize in the general setting (Papanikolaou *et al.*, 2006), or in a clinical trial setting using a long-acting FSH preparation (Devroey *et al.*, 2009), despite specific measures taken to lower the incidence of OHSS.

As for GnRHa triggering in GnRH antagonist cycles, evidence from observational uncontrolled trials and randomized studies during the last decade—published in English in peer-reviewed journals—shows an absence of OHSS (Table II). This is truly remarkable. No other OHSS prevention strategy comes even close to this result. Of special note is the attempt to rescue the luteal phase with hCG post GnRHa trigger in high-risk patients, resulting in one late onset OHSS out of 12 patients (Humaidan, 2009b).

Two randomized prospective studies merit recognition: Babayof *et al.* (2006) and Engmann *et al.* (2008a), randomizing OHSS high-risk patients to either GnRHa or hCG triggering. Both reported 30% OHSS incidence in the hCG group versus 0% in the GnRHa triggering group. However, pregnancy rates were low in the study by Babayof *et al.* (2006), in contrast to the study by Engmann *et al.* (2008a), although both studies employed a very similar LPS.

Profound luteolysis after GnRHa triggering necessitates modified LPS to secure the reproductive outcome. However, another option in OHSS high-risk patients is GnRHa triggering followed by cryopreservation and transfer in subsequent frozen thaw cycles. This approach has resulted in a good reproductive outcome (Manzanares *et al.*, 2010) and may have several advantages:

- (i) Avoiding intensive LPS.
- (ii) Avoiding 'out of phase' endometrium, which is common in high-responder patients.
- (iii) Allowing embryo transfer in a natural cycle where applicable.

In summary: GnRHa dramatically reduces OHSS in high-risk patients. Therefore, there is a clear benefit of choosing a GnRH antagonist-based protocol, particularly in young, ovarian stimulation-naïve patients and even more so if a long-acting FSH preparation is used.

GnRHa triggering: practical issues

Regarding the dose and type of GnRHa used to trigger final oocyte maturation, a previous study by Parneix *et al.* (1996) explored different types, doses and modes of administering GnRHa in 123 women. The

Table III GnRHa to trigger ovulation: target groups and current recommendations.

Type of patient	Advantages by using GnRHa	Luteal phase support	Disadvantages
Oocyte donor	OHSS is avoided Good oocyte quality Menses after 4–6 days Reduction in luteal ovarian size Reduction in luteal free fluid accumulation	Not required	No disadvantages-considered to be the protocol of choice
High-responder patient	OHSS is avoided Good oocyte quality Reduction in luteal ovarian size Reduction in luteal free fluid accumulation	IM prog + E2patches adjusted according to serum levels 1500 IU hCG, 35 h after GnRHa trigger Repeated bolus of 500 IU hCG Repeated bolus of rec-LH Repeated bolus of GnRHa Freeze all embryos	The most optimal LPS still to be determined
Normo-responder patient	OHSS is avoided More MII oocytes Good oocyte quality	1500 IU HCG, 35 h after GnRHa trigger Repeated bolus of rec-LH IM prog + E2patches adjusted according to serum levels Freeze all embryos	Minor adjustment in LPS still needed
Fertility preservation (oocyte freezing prior to chemotherapy)	OHSS is avoided More MII oocytes Good oocyte quality Low luteal phase estradiol	Not required	No disadvantages-considered to be the protocol of choice

authors reported that no regimen was superior to the other in inducing ovulation successfully.

Most recent studies have used single doses of the following types of GnRHa: either triptorelin 0.2 mg (Bodri *et al.*, 2009; Hernandez *et al.*, 2009; Papanikolaou *et al.*, 2011), buserelin 0.5 mg (Humaidan *et al.*, 2005, 2006, 2009b, c, 2010), leuprolide acetate 1 mg (Engmann *et al.*, 2008a) or leuprolide acetate 1.5 mg (Castillo *et al.*, 2010).

The timing of the OPU after GnRHa administration should be the same as after hCG triggering (34–36 h).

Regarding the LPS after GnRHa triggering in fresh transfers, the majority of studies support supplementation with LH activity in addition to standard LPS with estradiol and progesterone. LH activity could be supplemented in the form of either one bolus of 1500 IU hCG administered on the day of OPU, a total of three boluses of hCG (250–500 IU) during the luteal phase or rLH 300 IU administered every second day during the luteal phase until a positive pregnancy test.

Future research

There is a need to perform more studies exploring the possibility to further optimize the LPS after GnRHa triggering in the normo-responder patient; thus the ideal dose of LH activity either LH or hCG sufficient to provide similar live birth rates without increasing the OHSS rate still needs to be determined. Another interesting question is whether an 'optimal' progesterone threshold exists during the

luteal phase which should be reached in order to secure the early implanting embryo. Furthermore, the promising results after intensive LPS in OHSS high-risk patients (Engmann *et al.*, 2008a) as well as the duration of the luteal support should be confirmed in larger studies.

Although smaller dose finding studies exist in GnRHa triggering (Parneix *et al.*, 1996; Shalev *et al.*, 1996), there is a need for future dose–response studies, related to different types and doses of GnRHa. Finally, as the luteal phase is still not fully understood, the endometrium of the oocyte donor is an important source to study endometrial histology, gene expression and receptivity factors in our striving to increase implantation in ART.

Conclusions

The members of The Copenhagen GnRH Agonist Triggering Workshop Group agreed that the time has come for a paradigm shift in the ovulation triggering concept in ART. In fresh IVF cycles with ET (9 RCTs) OHSS was eliminated when GnRHa was used to trigger ovulation [0% incidence in the GnRHa group, risk difference 3 (95% CI: –8%, 2%)], moreover the delivery rate has improved significantly after modified luteal support [6% risk difference in favour of HCG arm (95% CI: –14%, 2%)] when compared with the initial studies using conventional luteal support [18% risk difference (95% CI: –36%, –1)].

GnRHa triggering is more physiological, resembling the natural mid-cycle surge of gonadotrophins, without the prolonged action of hCG, resulting in luteal phase steroid levels closer to those of the natural cycle and the reported retrieval of more mature oocytes when compared with hCG triggering. GnRHa triggering significantly reduces or eliminates the OHSS risk after ovarian stimulation for IVF/ICSI in comparison with hCG triggering. Moreover, GnRHa triggering provides a higher patient convenience in the luteal phase, reducing the treatment burden.

GnRHa triggering should be used in all oocyte donation cycles, due to a total elimination of OHSS. Moreover, GnRHa triggering is preferable over hCG triggering in cancer patients undergoing ovarian stimulation and oocyte retrieval for fertility preservation prior to chemotherapy due to a decrease in the post-trigger estradiol exposure, and an increase in the yield of mature oocytes. Table III summarizes current recommendations and target groups of patients.

In the normo-responder patient, the future paramount aim should be to further improve the reproductive outcome by optimizing the LPS after GnRHa triggering without increasing the risk of OHSS. Until the optimal protocol of luteal supplementation is defined an alternative option is to freeze all oocytes/embryos and transfer in subsequent natural or stimulated cycles.

Finally, the members of the group agreed that success in ART should be redefined: the achievement of a pregnancy with no OHSS, leading to a singleton live term birth of a healthy child.

Authors' roles

P.H.: design, draft of article, statistical analysis, revision, final approval. S.K.: design, draft of article, revision, final approval. E.G.P.: design, draft of article, revision, statistical analysis, final approval. J.I., B.T.: draft of article, revision, final approval. C.Y.A., P.D., C.B., E.J.M., E.R.: draft of article, revision, final approval. L.E., J.C.C.F.: data interpretation, draft of article, revision, final approval. J.A.G.V.: data collection, draft of article, revision, final approval. D.B.: data collection, draft of article, revision, statistical analysis, final approval. P.H. is the corresponding author. P.H., E.G.P. and S.K. accept direct responsibility for the manuscript. All members of the consensus group actively participated in the design, acquisition and interpretation of data, drafting and final approval of the manuscript.

Acknowledgements

The First Copenhagen Workshop Group Meeting on GnRHa triggering of final oocyte maturation (30 November–1 December 2009) was organized to advance the concept of ovulation induction with GnRHa in women undergoing controlled ovarian stimulation, co-treated with a GnRH antagonist and was supported by an unrestricted grant from MSD—Denmark. This paper is based on the presentations and the vivid discussions of the Copenhagen meeting. The meeting was chaired by P Humaidan (Skive Regional Hospital, Skive, Denmark), E.G. Papanikolaou (Assisted Reproduction Unit, Aristotle University of Thessaloniki) and S. Kol (Department of Obstetrics and Gynecology, IVF Unit, Rambam Medical Center, Haifa, Israel). The speakers included P. Devroey (Free University Brussels, Brussels, Belgium), J. Itskovitz-Eldor (Department of Obstetrics and Gynecology,

Rambam Medical Center, Haifa), B.C. Tarlatzis (Assisted Reproduction Unit, Aristotle University of Thessaloniki), P. Humaidan (Skive Regional Hospital, Skive, Denmark), J. Garcia Velasco (Instituto Valenciano de Infertilidad Madrid and Rey Juan Carlos University, Madrid, Spain), J. Castillo Farfan (Assisted Reproduction Unit, Department of Obstetrics and Gynecology, Hospital Clinico Universitario de Valencia), E.G. Papanikolaou (Assisted Reproduction Unit, Aristotle University of Thessaloniki), L. Engmann (Center for Advanced Reproductive Services, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of Connecticut Health Center, Farmington, USA), S. Kol (Department of Obstetrics and Gynecology, IVF Unit, Rambam Medical Center, Haifa, Israel) and D. Bodri (Clínico EUGIN, calle Entença 293–295, 08029 Barcelona, Spain). Moreover C. Yding Andersen (University Hospital of Copenhagen, Copenhagen, Denmark), C. Benadiva (Center for Advanced Reproductive Services, University of Connecticut School of Medicine, Department of Obstetrics and Gynecology, Farmington, Connecticut, USA), E. Margalioth (IVF Unit, Department of Obstetrics and Gynecology, Shaare Zedek Medical Centre, Ben-Gurion University of the Negev, Jerusalem, Israel) and E. Ricciarelli (Clinica 'FivMadrid', Juan Alvarez de Mendizabal, Madrid, Spain) attended the meeting.

Funding

The meeting was supported by an unrestricted educational grant from MSD, Denmark.

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