ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

Issue 5

Myths and facts in controlled ovarian stimulation II

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CONTENTS

abbreviations

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AMH	Anti-Müllerian Hormone
ART	Assisted Reproductive Technology
ASRM	American Society for Reproductive Medicine
CLBR	Cumulative Live Birth Rate
COS	Controlled Ovarian Stimulation
GnRH	Gonadotropin-Releasing Hormone
hCG	Human Chorionic Gonadotropin
hMG	Urinary Human Menopausal Gonadotropin
ICSI	Intracytoplasmic Sperm Injection
IVF	*In Vitro* Fertilization
LH	Luteinizing Hormone
OHSS	Ovarian Hyperstimulation Syndrome
rFSH	Recombinant Follicle-Stimulating Hormone
SET	Single Embryo Transfer
IM	Intramuscularly
SC	Subcutaneously

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expert insights

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The first successful IVF-embryo transfer (IVF-ET) was carried out in 1978, then after the treatment of infertility has advanced significantly.

A significant milestone in the development of controlled ovarian stimulation (COS) was the implementation of gonadotropin-releasing hormone (GnRH) agonists or antagonist protocols. GnRH agonists are mainly used for pituitary suppression, from the mid-luteal phase of the prior cycle until the completion of the COS process (long protocol).

In the current practice, the GnRH antagonists has several potential advantages over GnRH agonists. Among these advantages are: 1) shorter duration of injectable drug treatment, 2) decreased gonadotropin requirement per cycle, 3) improved patient convenience, 4) lower treatment cost, 5) prevent premature rise of luteinizing hormone (LH).

GnRH antagonists have been shown to be an effective first-line of treatment in women undergoing COS for IVF in multiple meta-analyses and clinical studies. GnRH antagonists have also been used effectively in patients who have a poor prognosis or who have shown a diminished ovarian response to COS.

As clinicians gain experience with these drugs, optimal treatment paradigms will likely emerge.

Myth 1: GnRH agonists* and antagonists are similar in terms of efficacy and safety

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Role of pituitary down-regulation in COS cycles

Ying Y, Yang T, Zhang H, et al. Prolonged pituitary down-regulation with full-dose of gonadotropin-releasing hormone agonist in different menstrual cycles: a retrospective cohort study. Peer J. 2019;7:e6837.

Badawy A, Wageah A, Gharib MEL, et al. Strategies for pituitary down-regulation to optimize IVF/ICSI outcome in poor ovarian responders. J Reprod Infertil. 2012;13(3):124–130.

- Over the last three decades, gonadotropin-releasing hormone agonists (GnRH-a) were the most commonly used drugs for controlled ovarian stimulation (COS) in assisted reproductive procedures.
- Utilizing GnRH-a has been considered the gold standard for COS. However, the ovarian stimulation of poor responders remains a challenging task for clinicians.
- There are numerous strategies that have been suggested to improve the outcome in poor responders but there is still no single pituitary down-regulation protocol that best suits all women with such condition.
- Approaches like reduction of GnRH agonist doses, "stop" protocols, and microdose GnRH agonist flare regimes all appear to improve the clinical outcomes. Recently, conducted study by *Ying* et al reported that prolonged pituitary down-regulation achieved by utilizing a full-dose of GnRH-a administrated in either phase of the menstrual cycle can have a positive effect on ongoing pregnancy rate and live-birth rate (LBR) per fresh embryo transfer cycle.

Mechanism of action of GnRH agonists and GnRH antagonists

Ortmann O, Weiss JM, Diedrich K. Gonadotrophin-releasing hormone (GnRH) and GnRH agonists: Mechanisms of action.Reprod Biomed Online. 2002;5 (Suppl 1):1–7.

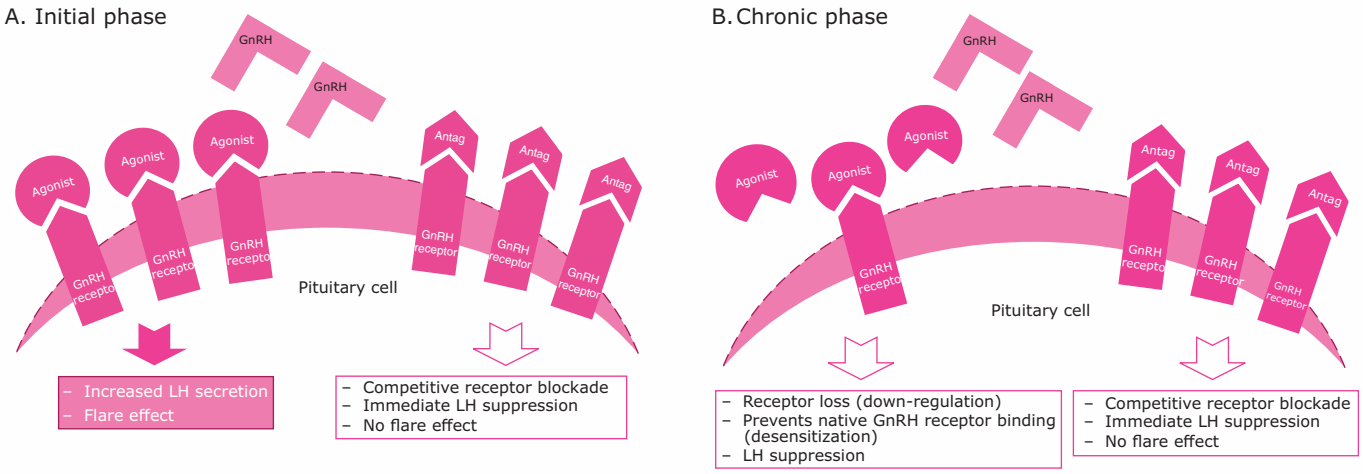
Tur-Kaspa I and Ezcurra D. GnRH antagonist, cetrorelix, for pituitary suppression in modern, patient-friendly assisted reproductive technology. Expert Opin Drug Metab Toxicol. 2009;5(10):1323–36.

Van Loenen AC, Huirne JA, Schats R, et al. GnRH agonists, antagonists, and assisted conception. Semin Reprod Med. 2002;20(4):349–364.

- The hypothalamic decapeptide GnRH binds to specific receptors on pituitary gonadotrophs.
- These receptors belong to the family of G protein-coupled receptors.
- Their activation leads to phosphoinositide breakdown with generation of inositol 1,4,5-trisphosphate and diacylglycerol.
- These second messengers initiate Ca2+ release from intracellular stores and activation of protein kinase C, both of which are important for gonadotrophin secretion and synthesis.
- Prolonged activation of GnRH receptors by GnRH leads to desensitization and consequently to suppressed gonadotrophin secretion. This is the primary mechanism of action of agonistic GnRH analogues (Figure 1).
- Unlike GnRH agonists, the antagonists do not induce an initial hypersecretion of gonadotropins but instead cause an immediate and rapid, reversible suppression of gonadotropin secretion (Figure 2).
- GNRH agonists are approved for use only in some cases of prostate cancer, uterine leiomyomas, central precocious puberty, breast cancer and endometriosis.

* GnRH agonists are approved for use only in some cases of prostate cancer, uterine leiomyomas, central precocious, puberty, breast cancer and endometriosis.

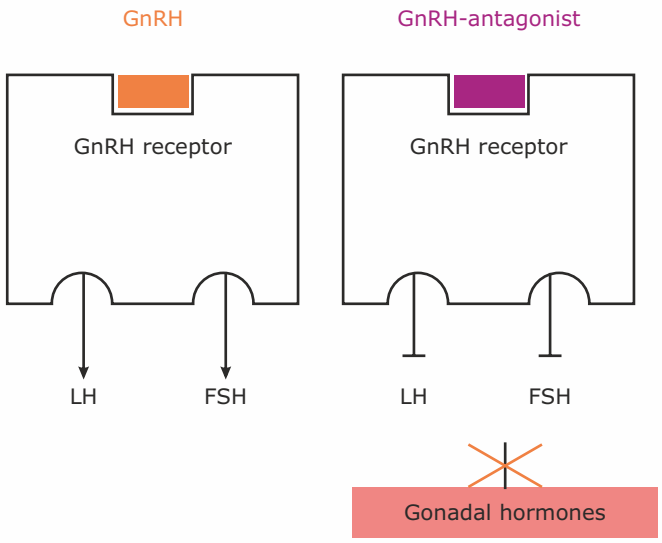
Figure 1. Mechanism of action of GnRH analogs



Antag: Antagonist; GnRH: Gonadotropin-releasing hormone; LH: Luteinizing hormone

- **GnRH agonists exert an initial stimulatory effect on gonadotropin secretion, which leads to the so-called 'flare effect'.**
- **The use of GnRH antagonists offers several advantages over agonists.**
- **GnRH antagonists produce a rapid and reversible suppression of LH and FSH, with no initial flare effect.**

Figure 2. The principal mechanism of action of GnRH antagonists



Activation of GnRH receptors by GnRH leads to gonadotrophin secretion. This is the primary mechanism of action of agonistic GnRH analogues. By contrast, GnRH antagonists compete with GnRH for receptors on gonadotroph cell membranes, inhibit GnRH-induced signal transduction and consequently gonadotrophin secretion.

Efficacy of agonists *vs.* antagonists

Comparison of GnRH antagonist and long GnRH agonist protocols in elective single embryo transfer (eSET) practice

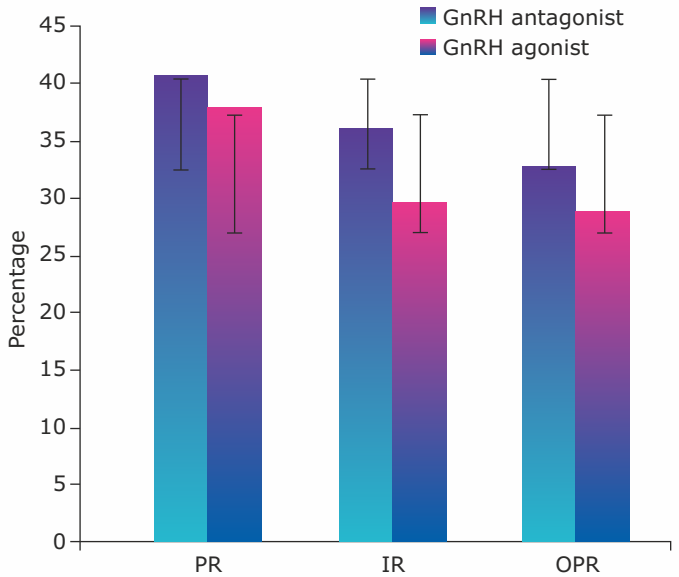
Dahdouh EM, Gomes FL, Granger L, et al. Is the flexible GnRH antagonist protocol better suited for fresh eSET cycles? J Obstet Gynaecol Can. 2014;36(10):885–891.

Dahdouh et al conducted a prospective cohort analysis to evaluate the efficacy of the flexible GnRH antagonist protocol in comparison with the long GnRH agonist protocol in elective single embryo transfer (eSET) practice. Primary outcomes studied were rates of biochemical pregnancy, implantation, and ongoing pregnancy.

Ovarian stimulation (OS) protocol

- Daily injections of gonadotropin were initiated on the second or third day of the menstrual cycle, after baseline transvaginal ultrasound had been performed.
- Daily subcutaneous administration of 0.25 mg GnRH antagonist was initiated when at least one follicle reached ≥14 mm in mean diameter on transvaginal ultrasound.
- Gonadotropin injections included recombinant follicle-stimulating hormone (rFSH), human menotropin (hMG) and others as needed.
- Ovulation was triggered with a subcutaneous injection of human chorionic gonadotropin (hCG) when at least three follicles reached a mean diameter ≥18 mm.
- Mean duration of stimulation for GnRH antagonist is 9.8 days and for GnRH agonist is 10.7 days .

Figure 3. Pregnancy rates (PR), implantation rates (IR), and ongoing pregnancy rates (OPR) in fresh eSET cycles using a GnRH antagonist or a GnRH agonist protocol



- Compared with the long GnRH agonist protocol, treatment using a GnRH antagonist is shorter, rapidly reversible, requires fewer injections, and appears to require a lower dose of gonadotropins, which is likely to lead to improved compliance and lower costs.
- GnRH antagonists have been used safely and effectively in a wide range of women undergoing *in-vitro* fertilization (IVF), such as those with a poor prognosis (due to baseline FSH>15 mIU/mL or a smaller number of mature oocytes retrieved <4), those with a high-risk of ovarian hyperstimulation syndrome (OHSS).
- The GnRH antagonist protocol is easy to use and well-tolerated. Therefore, the GnRH antagonist protocol appears to offer a promising alternative and ideal choice of therapy to the long mid-luteal GnRH agonist regimen during COS in fresh IVF treatment cycles.

Key objectives and conclusions of four meta-analyses of data on the use of cetrorelix in COS

Study	Objective	Findings
Ludwig et al (cetrorelix *vs*. ganirelix)	To evaluate if there is a reduction in the incidence of OHSS and/or a reduction in pregnancy rates with cetrorelix or ganirelix compared with long agonist protocols.	Compared with a long agonist protocol, cetrorelix but not ganirelix, is associated with a: • Significantly lower incidence of OHSS. • Similar pregnancy rate.
Kolibianakis et al	To determine whether the choice of GnRH analog for pituitary suppression during COS affects LBR	GnRH agonists and antagonist protocols result in similar LBR
Al-Inany et al	To update the comparative evidence on the efficacy of GnRH antagonists *vs.* standard long agonist protocols for COS.	GnRH antagonist protocols are short and simple, and associated with good clinical outcomes Compared with GnRH agonists, antagonists are associated with a: • Significant reduction in the incidence of OHSS. • Significantly lower consumption of gonadotropins.

efficacy and safety in pcos patients

Flexible GnRH antagonist protocol vs. GnRH agonist long protocol in patients with polycystic ovary syndrome (PCOS) treated for IVF

Lainas TG, Sfontouris IA, Zorzovilis IZ, et al. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: A prospective randomized controlled trial (RCT). Hum Reprod. 2010;25(3):683–689.

- The present study evaluated the comparative efficacy of the flexible GnRH antagonist and the long GnRH agonist down-regulation protocol in PCOS patients treated for IVF. The primary endpoint of the study was ongoing pregnancy rate.
- Ongoing pregnancy rates were similar in the two protocols, although the GnRH antagonist protocol was associated with significantly lower incidence of Grade II OHSS.
- Significantly lower FSH dose and shorter stimulation period with GnRH antagonist compared to GnRH agonist.

OHSS (%)	Agonist group (%)	Antagonist group (%)
Grade I	34.5	55.5
Grade II	60	40
Grade III	5.5	4.5

- **GnRH antagonist protocol is associated with a:**
  - Similar ongoing pregnancy rate.
  - Lower incidence of OHSS.
  - Lower gonadotrophin requirement.
  - Shorter duration of stimulation compared with GnRH agonist.
- The antagonist protocol is more patient friendly as compared with the agonist, GnRH antagonists might be the protocol of choice for patients with PCOS.

Differences in safety

Tur-Kaspa I, Ezcurra D. GnRH antagonist, cetrorelix, for pituitary suppression in modern, patient-friendly assisted reproductive technology. Expert Opin Drug Metab Toxicol. 2009;5(10):1323–1336.

Onofriescu A, Bors A, Luca A, et al. GnRH Antagonist IVF Protocol in PCOS. Curr Health Sci J. 2013;39(1):20–25.

- Given the pharmacologic and physiologic effects of GnRH antagonists, their use has been postulated to reduce the risk of adverse effects associated with long GnRH agonist protocols, such as hormone withdrawal symptoms and OHSS. Clinical evidence shows that cetrorelix (in multiple or single-dose protocols) is generally well-tolerated in women undergoing COS.
- Clinical trials and meta-analyses have shown that GnRH antagonists are associated with similar live birth rates but a reduced treatment burden (in terms of cycle duration and side effects) and a lower risk of OHSS compared with long agonist protocols.
- OHSS rate was significantly more in agonist group.

	Agonist group (%)	Antagonist group (%)
OHSS rate (p=0.003)	28%	4%

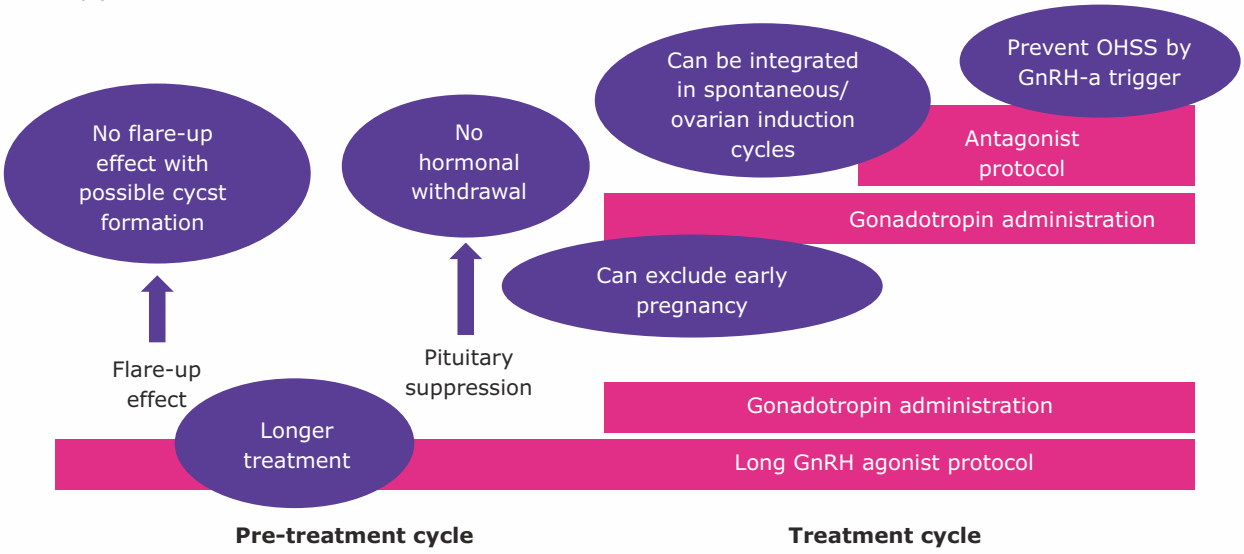
Health of children born after COS for IVF using the luteinizing hormone–releasing hormone antagonist cetrorelix

Ludwig M, Riethmüller-Winzen H, Felberbaum RE, et al. Health of 227 children born after controlled ovarian stimulation for in vitro fertilization using the luteinizing hormone-releasing hormone antagonist cetrorelix. Fertil Steril. 2001;75(1):18–22.

- *Ludwig* et al demonstrated that conventional IVF and intracytoplasmic sperm injection (ICSI) have no adverse effect in terms of the rate of malformations among children born after these procedures.
- Cetrorelix has no detrimental effect on the pregnancy course of women or on the birth characteristics and developmental competence of children.

GnRh agonist vs. antagonist protocol

Van Loenen AC, Huirne JA, Schats R, et al. GnRH agonists, antagonists, and assisted conception. Semin Reprod Med. 2002 ;20(4):349–364.



Secondary benefits of GnRH antagonists over GnRH agonists

Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: A review of the literature. Reprod Biol Endocrinol. 2013;11:20.

No cyst formation as seen with GnRH agonists administration

GnRH antagonist treatment does not produce an initial flare of gonadotrophins

Cyst formation lowers oocyte quality, fertilization rate, number of oocytes retrieved and embryo quality; increases the probability of cycle cancellation and is associated with decrease in implantation and pregnancy rate

No hot flushes are observed with GnRH-antagonists because their use does not result in the profound hypoestrogenemia observed with GnRH-agonists

Shorter duration of OS protocols

GnRH antagonist dose requirements are decreased as compared with GnRH agonists

GnRH antagonist mild protocol of COS could be the best method of choice in good prognosis patients

GnRH antagonist mild protocol of OS could be the method of choice to stimulate the ovaries of good prognosis patients without a risk of compromising the outcome of IVF cycle

Patient populations benefiting from GnRH antagonist protocols

- Patients undergoing first-line COS
- Patients with a poor prognosis
- Patients at risk for OHSS
- Patients with PCOS
- Patients taking oral contraceptive to regulate menstrual cycles

Busted myth 1: GnRH agonists and antagonists are similar in terms of efficacy, however, GnRH antagonists demonstrated superior safety profile with less incidence of OHSS and many secondary benefits over GnRH agonists.

Myth 2: Progesterone rise has negative impact on COS outcomes

Progesterone elevation (PE): Definition

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

- Progesterone elevation (PE) is defined as a threshold of more than 0.9 ng/mL. Meanwhile, evidence mounts that progesterone levels above 1.5 ng/mL on the day of final oocyte maturation may lead to reduced pregnancy rates when the embryo transfer is carried out in the same cycle.

Difference between PE and premature luteinization (PL)

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in-vitro fertilization/ICSI cycles. Front Endocrinol (Lausanne). 2018;9:201.

Nagaraja N, Talwar P, Mukherjee B, et al. Correlation between serum progesterone level on the day of ovulation trigger during In vitro fertilization and its effect on treatment outcome. J Hum Reprod Sci 2019;12:136–140.

PE phenomena

- PE is not a universal phenomenon with evidence indicating that its detrimental consequences on pregnancy outcomes do not affect all patient populations equally.
- Researchers showed that the incidence of PE (>1.5 ng/mL) was 13.3%, but ongoing pregnancy rate (OPRs) were not significantly different between patients with normal progesterone levels and PE (27.0 *vs.* 19.0%).
- Progesterone concentration was strongly associated with the number of follicles and retrieved oocytes. There was no significant association between the late-follicular phase progesterone concentration and clinical pregnancy rate.
- Progesterone levels neither had a negative impact on the oocyte quality and endometrial receptivity nor did it affect pregnancy success.
- Only in the group of progesterone level >1.80 ng/mL there was a marginally significant negative impact on pregnancy rates (OR: 0.73, 95% CI: 0.61–0.99).

Premature luteinization (PL) is defined as a premature rise in serum progesterone concentration on or before the day of ovulation trigger with hCG. Studies by *Bosch* et al., and *Papanikolaou* et al., have shown the negative effect of PL for pregnancy outcome when the progesterone level was >1.5 ng/mL on the day of ovulation trigger.

- PL may have an adverse effect on endometrial receptivity, poor endometrial receptivity may be explained by premature endometrial maturation which may lead to asynchrony between the embryo and endometrium.
- PL affects the endometrial gene expressions and it is known to occur with increased number and size of follicles, a higher dose of gonadotropin and poor ovarian response with increased luteinizing hormone (LH) sensitivity.
- PL is also associated with increased activity of FSH stimulated, granulosa cells, and LH stimulated theca cells.

cut-off values of progesterone for cycle cancellation

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, et al. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: An analysis of more than 10,000 cycles. Fertil Steril.2012;97(6):1321–1327.e1–4.

Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: A cohort analysis of 3296 IVF cycles. Hum Reprod. 2015;30(3):684–691.

Griesinger G, Mannaerts B, Andersen CY, et al. Progesterone elevation does not compromise pregnancy rates in high responders: a pooled analysis of in vitro fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. Fertil Steril. 2013;100(6):1622–1628.e1–3.

- In the literature, the relationship between PE and pregnancy rate has been analyzed by using different thresholds of serum progesterone on the day of hCG.
- The thresholds have been arbitrarily chosen and lie between 0.4 ng/mL and 3 ng/mL.
- Nevertheless, following the analysis of a large series the optimal progesterone threshold over which a detrimental effect on IVF outcome might be observed has been estimated at 1.5 ng/mL.

[A vaginal gel (8%) in the frozen embryo transfer cycles was administered at a dose of 90 mg/day or sometimes 90 mg twice a day to patients].

progesterone thresholds and impact on IVF outcome

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

- Some researchers have reported that raised progesterone levels (>1.5 ng/mL, estimated at automated immunoassay platforms BioScale) would be detrimental and thus a "freeze-all" embryos policy should be adopted.

- The progesterone cut-off points associated with decreased pregnancy outcomes in fresh embryo transfer cycles were:

1.5 ng/mL Poor responders	1.75 ng/mL Intermediate responders	2.75 ng/mL High-responders

In this article it was observed that the progesterone mean values differed according to ovarian response.

1.18 ng/mL Poor responders	1.47 ng/mL Intermediate responders	1.89 ng/mL High-responders

- PE is more common in the high ovarian response group than intermediate and poor ovarian response groups.
- A PE does not uniformly mean a failed implantation, because there are still clinical pregnancies recorded in cycles with high progesterone levels. Hence, there is a need to identify the subgroup of patients who have a good chance of conception despite elevated progesterone levels.
- *Griesinger G* et al has suggested that there is a subgroup of high-responders in whom elevated progesterone does not negatively affect the outcome.
- Although PE has been associated with decreased pregnancy rates in several studies, PE does not seem to affect all patient populations equally with high responders with PE achieving similar pregnancy success than counterparts without PE.

Role of gonadotropins in progesterone rise

1.Bergh C, Howles CM, Borg K, et al. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F) versus highly purified urinary FSH (Metrodin HP): Results of a randomized comparative study in women undergoing assisted reproductive techniques. Hum Reprod. 1997; 12(10):2133–2139.

2.Frydman R, Howles CM, Truong F, et al. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. Hum Reprod. 2000;15(3):520–525.

3.Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: A randomized assessor-blind controlled trial. Hum. Reprod. 2006; 21(12): 3217–27.

4.Trew GH, Brown AP, Gillard S, et al. In vitro fertilisation with recombinant follicle stimulating hormone requires less IU usage compared with highly purified human menopausal gonadotrophin: Results from a European retrospective observational chart review. Reprod Biol Endocrinol. 2010;8:137.

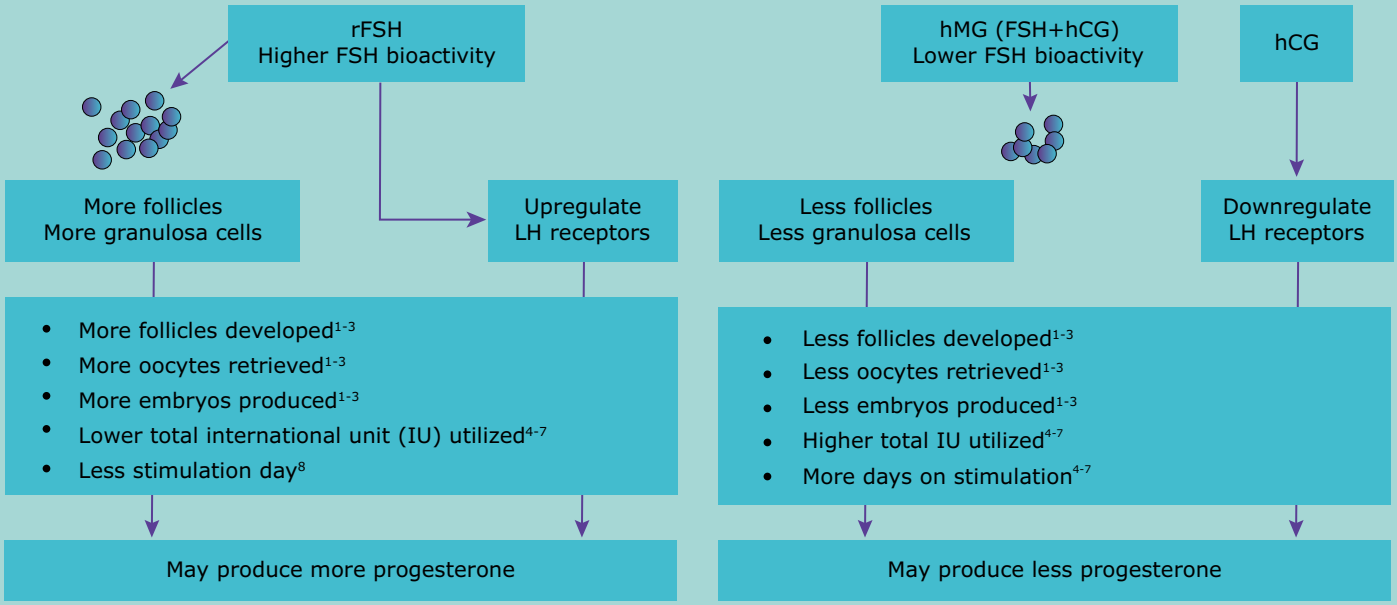
5.Nyboe Andersen A, Devroey P, Arce JC, et al. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum Reprod. 2006;21(12):3217–3227

6.Devroey P, Pellicer A, Nyboe Andersen A, et al. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. Fertil Steril. 2012;97(3):561–571.

7.Frydman R, Howles CM, Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. The French Multicentre Trialists. Hum. Reprod. 2000; 15(3):520–525.

8.EMEA Final decision July 30 2009 - SmPC approved amendments of marketing authorization for Gonal-f. Available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_ -_Scientific_Discussion/human/000071/WC500023744.pdf. Accessed on July 24, 2018.

Recombinant follicle stimulating hormone (rFSH) *vs.* human menopausal gonadotropin (hMG)



Comparison between rFSH and hMG in progesterone rise in ART cycles

Bosch E, Vidal C, Labarta E, et al. Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists—A randomized study. Human Reproduction. 2008;23(10):346–2351.

Highly purified human menopausal gonadotropin (hp-hMG) and rFSH have been widely and successfully used for OS in infertile women undergoing treatment for IVF or ICSI and embryo transfer.

Table 1. Ovarian stimulation outcome

	hp-hMG (n=122)	rFSH(n=126)	p value
Total gonadotropin dose (IU)	2481±994	2624±801	0.22
Serum progesterone (P) on day of hCG (ng/mL)	**0.73±0.42**	**0.99±0.48**	**<0.001**
Number of COCs collected	11.3±6.0	14.4±8.1	0.001
Number of metaphase II (ICSI)	7.8±4.0	9.7±6.0	0.004
Fertilization rate	69.8±26.4	68.9±22.3	0.765

Values are expressed by mean±SD. Gn: Gonadotrophin; COC: Cumulus-oocyte complexes

A similar outcome was observed for hp-hMG and rFSH when used for stimulation in GnRH antagonist cycles. Increased progesterone levels have been already related to FSH administration, in either GnRH antagonist cycles or GnRH agonist long protocol cycles.

MEGASET study (rFSH vs. hp-hMG) antagonist cycles

Devroey P, Pellicer A, Nyboe AA, et al. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with the compulsory single-blastocyst transfer. Fertil Steril. 2012;97(3):561–571.

Devroey P et al compared the efficacy and safety of hp-hMG and rFSH for COS in a GnRH antagonist cycle with the compulsory single-blastocyst transfer. In this trial, COS with hp-hMG or rFSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer on day 5 in one fresh or subsequently frozen blastocyst replacement in natural cycles initiated within 1-year of each patient's start of treatment.

Table 2. Clinical parameters from the stimulation phase to embryo transfer

	hp-hMG (n=374)	rFSH (n=375)	p value
Follicles ≥12 mm	3.6±2.8	4.2±3.1	0.011[a]
E2 (pmol/L)	2,626±1,405	2,973±1,702	0.003[b]
Progesterone (nmol/L)	2.2±1.9	2.8±10.8	0.025[b]
Progesterone (ng/mL) Day of oocyte retrieval	**0.69**	**0.88**	**0.025[b]**
Number of oocytes retrieved	9.1±5.2	10.7±5.8	**<0.001**
Metaphase II oocytes/ oocytes retrieved	77±23%	78±19%	0.798

Note: Numbers are mean±SD unless otherwise indicated. a-Wilcoxon test. b-Test for treatment difference based on log transformed values.

Thus, it is important to note that progesterone values were <1.5 ng/mL in hp-hMG and rFSH groups, implying statistically significant values could be clinically irrelevant.

The average serum progesterone level and the proportion of patients with serum progesterone concentrations above 1.25 ng/mL at the end of stimulation (16% in the hp-hMG group and 14% in the rFSH group) were similar between the treatment groups. In this study, the threshold value for defining serum PE was 1.0 ng/mL.

summary of impact of progesterone rise on cycle outcomes

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

PE is not a universal phenomenon with evidence indicating that its detrimental consequences on pregnancy outcomes do not affect all patient populations equally.

Late-follicular-phase PE, commonly defined as progesterone levels of 1.5 ng/mL or greater at the day of hCG trigger, has been reported in 6–30% of COS cycles.

Researchers showed that the incidence of progesterone level (>1.5 ng/mL) was 13.3%, but on-going pregnancy rate (OPRs) were not significantly different between patients with normal progesterone levels and PE levels. (27.0 *vs*. 19.0%).

Progesterone concentration was strongly associated with the number of follicles and retrieved oocytes. There was no significant association between the late-follicular-phase progesterone concentration and clinical pregnancy rate.

Progesterone levels neither had a negative impact on the oocyte quality and endometrial receptivity nor did it affect pregnancy success.

It is important to note that progesterone elevation values were <1.5 ng/mL in both the groups (rFSH treated group *vs.* hMG), implying statistically significant values could be clinically irrelevant.

Busted myth 2: Progesterone elevation is not a universal phenomena, its deleterious effects on pregnancy outcomes seems to be not similar to all patient population equally. PE failed to demonstrate the consistent negative impact on oocyte quality and endometrial receptivity. An individualized approach is recommended in cases of PE.

Myth 3: GnRH agonist triggering can be effectively used for all patients in COS

Alyasin A, Mehdinejadiani S, Ghasemi M. GnRH agonist trigger versus hCG trigger in GnRH antagonist in IVF/ICSI cycles: A review article. Int J Reprod Biomed (Yazd). 2016;14(9):557–566.

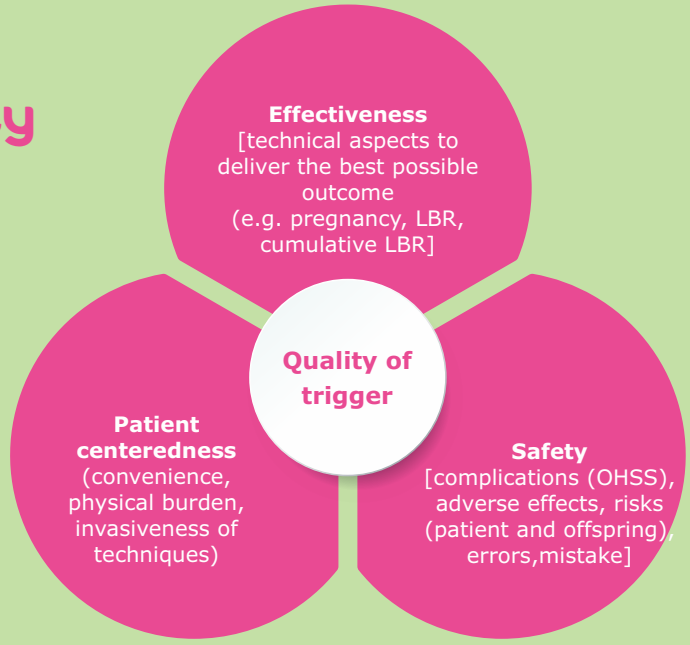
Su HW, Yi YC, Wei TY, Chang TC, Cheng CM. Detection of ovulation, a review of currently available methods. Bioeng Transl Med. 2017;2(3):238–246.

- Assisted reproductive technology (ART) consisting IVF, ICSI and intrauterine insemination (IUI) are based on the exact timing of ovulation, oocyte pick-up before ovulation and then insemination of oocyte.
- Due to biological activity of hCG like LH, since the mid-1970s exogenous hCG has been used to trigger the final oocyte maturation. The release of oocyte occurs usually 36–40 hours after induction of ovulation like natural ovulation.
- The persistent high level of estrogen induces an abrupt release of LH from the pituitary gland, and this hormonal surge then triggers ovulation. After ovulation, the dominant follicle transforms into a corpus luteum, which secretes estrogen and progesterone and collapses, initiating menstruation.
- Detection and monitoring of ovulation have long been practiced by women pursuing or avoiding pregnancy. The fertility window begins approximately 3–5 days (sperm lifespan) before ovulation and continues to a point approximately 1–2 days (oocyte lifespan) after ovulation.
- Identifying this window, rather than simply identifying or detecting ovulation, is vital for encouraging or discouraging contraception. For physicians or women who wish to know if a menstrual cycle is normal or to evaluate ovarian function, a test that retrospectively confirms ovulation should suffice, but for artificial reproductive techniques, the time of ovulation and the fertility window must be defined clearly.

Conventional methods used to monitor trigger timings are

- Ultrasonography.
- Urinary LH.
- Serum progesterone and urinary pregnanediol 3-glucuronide.
- Urinary FSH.
- Basal body temperature.
- Cervical mucus.

Evaluation of the quality of trigger methods depend on



Features of currently available Methods to detect ovulation

Su HW, Yi YC, Wei TY, et al. Detection of ovulation, a review of currently available methods. Bioeng Transl Med. 2017;2(3):238–246.

	Cost	Accuracy	Accessibility	Invasion	Detect before ovulation	Features/disadvantage
POC methods available						
Urinary LH	Low-cost of kits	High (97%)	High (OTC)	No	Yes	Repeated purchases of kits
Computerized monitor (urinary LH + E1-3-G)	Moderate cost of device	High (95.8–97%)	High (OTC)	No	Yes	Evidence to improve pregnancy rate Repeated purchases of sticks
Basal body temperature	Low-cost of thermometer	Low (22.1%)	High	No	No	Not easily interpreted Affected by environmental factors
Cervical mucus	No-cost	Moderate (48–76%)	High	No	Yes	Unable to perform while vaginal infection
Salivary ferning	Low-cost of kits	Moderate (42–53%)	High (OTC)	No	Yes	High percentage of unpredictable result
POC methods unavailable						
Transvaginal ultrasound	High	High (standard reference examination)	Low (performed by physician)	Yes (introduce vaginal probe)	Yes	May be uncomfortable during exam
Serum progesterone	N/A	High (89.6%)	Low (need laboratory)	Yes (venipuncture)	No	Confirms ovulation
Urinary PDG	N/A	High (92.2%)	Low (need laboratory)	No	No	Confirms ovulation

[a]These two exams are not commonly performed. The cost may vary in different country. E1-3-G: Estrone-3-glucoronide; LH: Leutinizing hormone; N/A: Not applicable; OTC: Over the counter; PDG: Pregnanediol 3-glucuronide; POC: Products of conception.

An ideal method to detect ovulation should be (a) noninvasive, (b) inexpensive, (c) easily available and easy to use (as a point-of-care method), (d) precise in determining ovulation, and (e) precise in determining the fertility window.

Types of ovulation triggering agents

Role of exogenous gonadotropins in ovulation induction. Available at https://www.ijogr.com/blog/2017/role-exogenous-gonadotropins-ovulation-induction/. Accessed on November, 2019.

Raju GA, Chavan R, Deenadayal M, et al. Luteinizing hormone and follicle stimulating hormone synergy: A review of role in controlled ovarian hyper-stimulation. J Hum Reprod Sci. 2013;6(4):227–234.

Endogenous	Exogenous
LH	• hCG (most commonly used in clinical practice, recommended dose a single injection of 5,000–10,000 IU IM or SC, is administered at a dose of 250 µg SC, which corresponds to approximately 6,000–7,000 IU hCG). • GnRH agonist.

kisspeptin*: future of trigger in IVF

Castillo JC, Humaidan P, Bernabéu R. Pharmaceutical options for triggering of final oocyte maturation in ART. Biomed Res Int. 2014;2014:580171.

- Kisspeptins (KP) involve a group of recently discovered peptide hormones, which play a key role in the neuroendocrine regulation of human reproduction.
- KP are potent stimulators of the hypothalamic pituitary-gonadal axis.
- KP signals directly to the GnRH neurons, which in turn stimulates the secretion of both LH and FSH from the anterior pituitary that can induce a physiological final follicular maturation.
- Recently, in IVF cycles, *Abbara* et al described that KP were able to effectively elicit an LH surge to induce final oocyte maturation with subsequent successful achievement of live births.
- This new trigger agent may, therefore, offer a completely new, "natural" pharmacological option for ovulation induction in ART. Importantly, the risk of OHSS might be eliminated.

strengths, and opportunities of GnRHa trigger

Castillo JC, Humaidan P, Bernabéu R. Pharmaceutical options for triggering of final oocyte maturation in ART. Biomed Res Int. 2014;2014:580171.

- The administration of hCG to induce final oocyte maturation has been used for decades and has been considered the gold standard during OS for IVF cycles.
- Recently, however, it has been suggested that the time has come for a paradigm shift in triggering policies.
- Although hCG effectively induces oocyte maturation and maintains excellent pregnancy rates during the IVF process, the prolonged half-life of hCG compared with natural LH promotes supraphysiological luteal steroid levels and the development of multiple corpora lutea, resulting in a potential increased risk of OHSS.
- Therefore, the use of alternate modalities to induce oocyte maturation to prevent OHSS, such as GnRH agonist has been the focus of research for years.

Strengths

- Physiological endogenous gonadotrophin surge.
- Similar pregnancy rates using 'modified luteal support'.
- May prevent OHSS risk.
- Less luteal phase patient discomfort.
- Improved oocyte yield in immature oocyte syndrome and empty follicle syndrome.

Opportunities

- Development of individualized luteal phase regimens.
- Improves safety of patients having risk of OHSS.
- Ideal protocol for specific clinical scenarios.
- Improved performance of embryo cryopreservation programmes.

*Still in research

ovulation triggering with hcg: benefits and problems

Hershko Klement A, Shulman A. hCG triggering in ART: An Evolutionary Concept. Int J Mol Sci. 2017;18(5):1075.

Humaidan P, Kol S, Papanikolaou EG, et al. GnRH agonist for triggering of final oocyte maturation: Time for a change of practice? Hum Reprod Update. 2011;17(4):510–524.

Shapiro BS and Andersen CY. Major drawbacks and additional benefits of agonist trigger--not ovarian hyperstimulation syndrome related. Fertil Steril. 2015;103(4):874–878.

Benefits

- 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.
- An important difference, however, exists between the half-life of LH and hCG, as the half-life of LH is equal to 60 minutes whereas that of hCG is >24 h.
- hCG have a role in triggering ovulation of small follicles (10–14 mm in diameter).
- hCG levels are elevated even after 6 days of administration.

Gonadotrophin-releasing hormone agonist (GnRHa) has been suggested as an alternative to hCG for triggering ovulation, while reducing risk of OHSS.

Problems

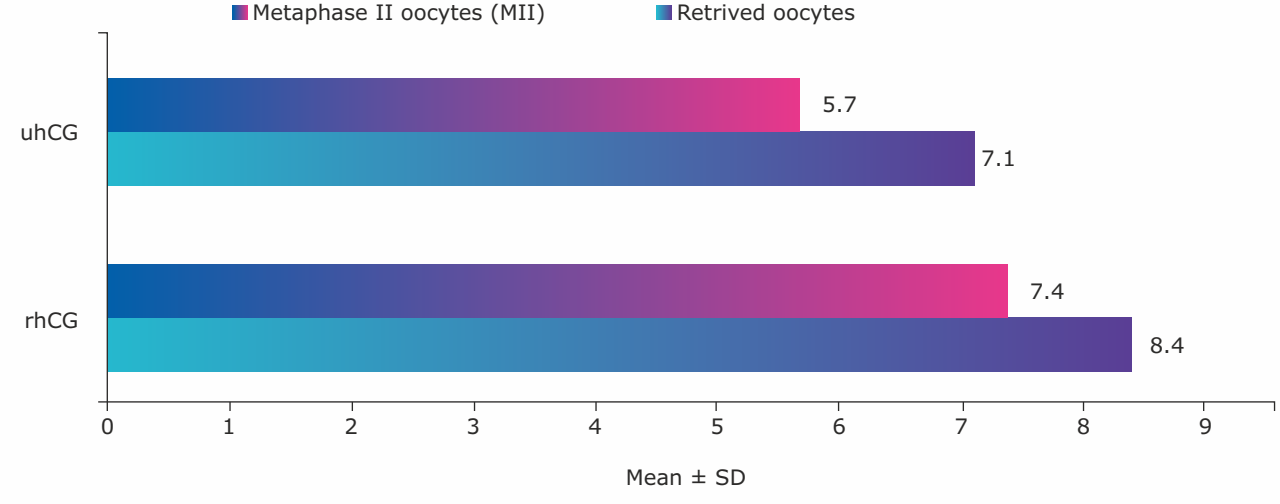
- Due to its prolonged circulatory half-life, hCG exerts a sustained luteotropic activity, and may induce the occurrence of OHSS.
- In high responders, an alternative trigger agent was needed to safely induce oocyte maturation in such patients.

comparing rhcg vs. uhcg

Farrag A, Costantini A, Manna C, Grimaldi G. Recombinant HCG for triggering ovulation increases the rate of mature oocytes in women treated for ICSI. J Assist Reprod Genet. 2008;25(9-10):461–466.

A prospective randomized study was conducted in order to investigate the effect of recombinant hCG (rhCG) on oocyte nuclear and cytoplasm maturity compared to urinary hCG (uhCG), for inducing ovulation in women treated with ICSI for male factor infertility.

Figure 3. Data of patients treated with rhCG or uhCG



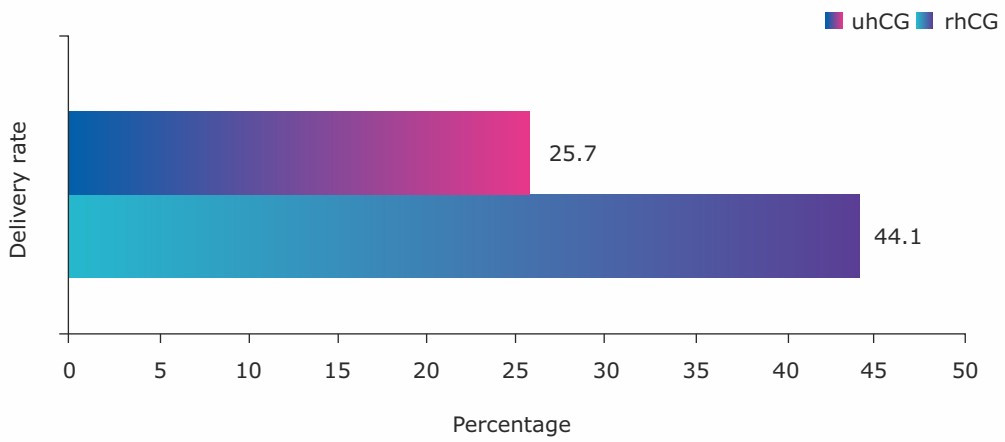
rhCG increases the rate of metaphase II oocytes, the number and the rate of MII oocytes with mature cytoplasm compared to uhCG.

Higher birth rate after rhCG triggering compared with uhCG in single-blastocyst IVF antagonist cycles: A randomized controlled trial

Papanikolaou EG, Fatemi H, Camus M, et al. Higher birth rate after recombinant hCG triggering compared with urinary-derived hCG in single-blastocyst IVF antagonist cycles: A randomized controlled trial. Fertil Steril. 2010;94(7):2902–2904.

- In a prospective randomized controlled trial, 119 patients were randomized to receive either rhCG (250 µg) or uhCG (10,000 IU) for final oocyte maturation in an antagonist protocol with a fixed-dose of rFSH (187.5 IU) and predefined single blastocyst transfer.

Figure 4. Data of patients treated with rhCG or uhCG



The delivery rate was improved in the rhCG group compared with the uhCG group (44.1 *vs.* 25.7, respectively)

Effectiveness of hCG *vs.* agonist triggering ovulation triggering with GnRH agonists

Lewit N, Kol S, Manor D, Itskovitz-Eldor J. Comparison of gonadotrophin-releasing hormone analogues and human chorionic gonadotrophin for the induction of ovulation and prevention of ovarian hyperstimulation syndrome: a case-control study. Hum Reprod. 1996;11(7):1399–1402.

- A retrospective, case-self-control study was conducted to compare GnRHa with hCG. A group of 16 IVF patients who had severe OHSS in previous cycles, in which hCG was given to trigger ovulation, were studied in subsequent cycles in which GnRHa was used.
- Study findings: OS and retrieval of mature oocytes were successfully accomplished with GnRHa in 22 cycles in patients who developed OHSS in previous cycles, during which hCG was used to induce ovulation, yet not a single case of severe OHSS was observed.

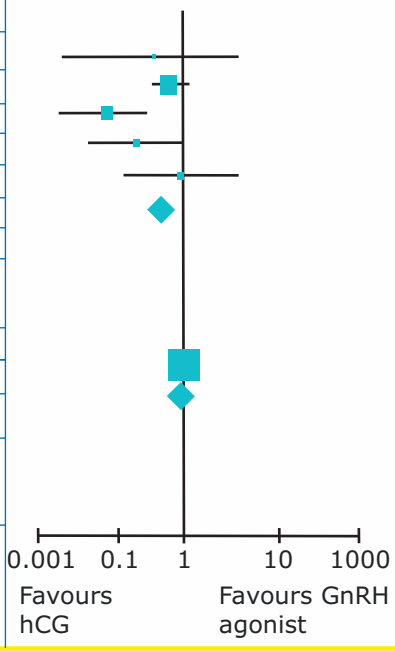
The comparison of the two strategies for ovulation triggering (hCG and GnRHa) in the same patients clearly shows the advantage offered by the latter in reducing the risk of OHSS.

Comparison of GnRH agonist vs. hCG: Cochrane review

Youssef MA, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev. 2014;(10):CD008046.

GnRH agonist vs. hCG for oocyte maturation triggering: Live birth rate (LBR)

Study or subgroup	GnRH agonist group		hCG group			Odds Ratio M-H,Fixed, 95% CI
	Events	Total	Events	Total	Weight	
1.1.1 Fresh autologous cycles						
Babayof 2006	1	15	2	13	2.9%	0.39 [0.03, 4.92]
Humaidan 2010	36	152	47	150	51.6%	0.68 [0.41, 1.13]
Humaidan 2005	3	55	24	67	29.3%	0.10 [0.03, 0.37]
Humaidan 2006	7	30	8	15	11.7%	0.27 [0.07, 1.00]
Papanikolaou 2010	4	18	7	17	4.6%	0.93 [0.19, 4.50]
Subtotal (95% CI)		270		262	100.0%	0.47 [0.31, 0.70]
Total events	51		85			
Heterogeneity: Chi^2 =8.99, df=4 (p=0.06); I^2=56% Test for overall effect: Z=3.69 (p=0.0002)						
1.1.2 Donor cycles						
Galindo 2009	40	106	42	106	100.0%	0.92 [0.53, 1.61]
Subtotal (95% CI)	40	106	42	106	100.0%	0.92 [0.53, 1.61]
Heterogeneity not applicable Test for overall effect: Z=0.28 (p=0.78)						
Test for subgroup differences: Chi^2 =3.82, df=1 (p=0.05); I^2=73.8%						

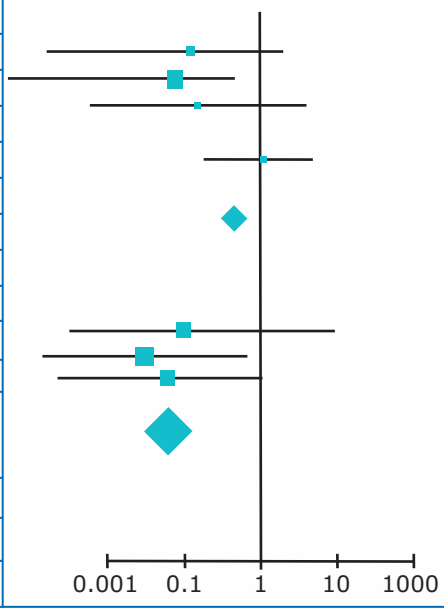


- In fresh autologous cycles GnRH agonist trigger was associated with a lower LBR than was seen with hCG (OR 0.47, 95% CI 0.31 to 0.70; five RCTs, 532 women, I^2 = 56%).
- The LBR varied from 15% to 53% in the hCG group and from 5% to 24% in the agonist group.

This means that for a woman with a 31% chance of achieving LBR with the use of hCG, the chance of a LBR with the use of a GnRh agonist will be between 12% and 24%.

GnRH agonist vs. hCG for oocyte maturation triggering: OHSS incidence

Study or subgroup	GnRH agonist group		hCG group			Odds Ratio M-H,Fixed, 95% CI
	Events	Total	Events	Total	Weight	
Fresh autologous cycles						
Babayof 2006	0	15	4	13	22.4%	0.07 [0.00, 1.41]
Engmann 2008	0	34	10	32	51.4%	0.03 [0.00, 0.56]
Humaidan 2010	0	152	3	150	16.9%	0.14 [0.01, 2.70]
Humaidan 2006	0	30	0	15		Not estimable
Humaidan 2013	0	185	2	199	9.2%	1.08 [0.15, 7.72]
Kolibianakis 2005	2	52	0	54		Not estimable
Papanikolaou 2010	0	18	0	17		Not estimable
Pirard 2006	0	17	0	6		Not estimable
Subtotal (95% CI)		**503**		**486**	**100.0%**	**0.15 [0.05, 0.47]**
Total events	2		19			
Heterogeneity: Chi^2 =5.21, df=3 (p=0.16); I^2=42% Test for overall effect: Z=3.29 (p=0.0010)						
Donor cycles: mild, moderate or serere OHSS						
Acevedo 2006	0	30	5	30	22.3.0%	0.08 [0.00, 1.44]
Galindo 2009	0	106	10	106	43.0%	0.04 [0.00, 0.75]
Melo 2007	0	50	8	50	34.7.0%	0.05 [0.00, 0.88]
Subtotal (95% CI)		**186**		**186**	**100.0%**	**0.05 [0.01, 0.28]**
Total events	0		23			
Heterogeneity Chi^2 =0.08, df=2 (p=0.96); I^2=0% Test for overall effect: Z=3.46 (p=0.0005)						
Test for subgroup differences: Chi^2 =1.09, df=1 (p=0.30); I^2=8.6%						



In women undergoing fresh autologous cycles GnRH agonist was associated with lower risk of OHSS than was seen with hCG (OR 0.15, 95% CI 0.05 to 0.47; eight RCTs, 989 women, I² = 42%). This suggests that for a woman with a 5% risk of OHSS using hCG, the rate would be between nil and 2% with the use of a GnRH agonist.

Delivery rate in GnRH agonist vs. hCG triggering in OHSS low-risk patients

Humaidan P, Kol S, Papanikolaou EG, et al. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update. 2011;17(4):510–24.

Studies	Study design	Patients type	Luteal support	Agonist used	Agonist triggering arm	hCG triggering arm
Fauser et al., 2002	RCT	Normovulatory	IM Progesterone	Leuprorclin 0.5 Triptoreline 0.2	18.7% (06/32)[a]	13.3% (02/15)[a]
Humaidan et al., 2005	RCT	Normovulatory[a]	Vaginal progesterone Estrogen pos	Buserelin 0.5	3.9% (03/55)	36.0% (24/67)
Kolibianakis et al., 2005	RCT	Normovulatory	Vaginal progesterone Estrogen pos	Triptoreline 0.2	3.9% (02/52)[a]	27.7% (15/54)[a]
[a]Rate difference: -0.18, 95% (CI: -0.36 to 0.01)					7.9% (11/139)	30.1% (41/136)

RCT: Randomized controlled trial; IM:Intramuscular

GnRH agonists dramatically reduces OHSS in high-risk patients. Therefore, there is a clear benefit of choosing a GnRH antagonist based protocol, particularly in young, OS naive patients

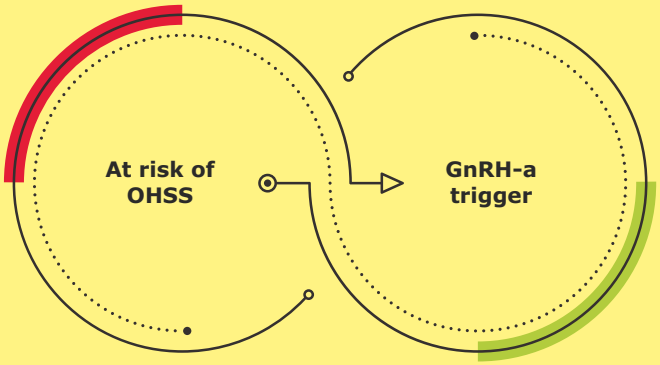
Use of GnRH agonist for trigger is not free of OHSS risk

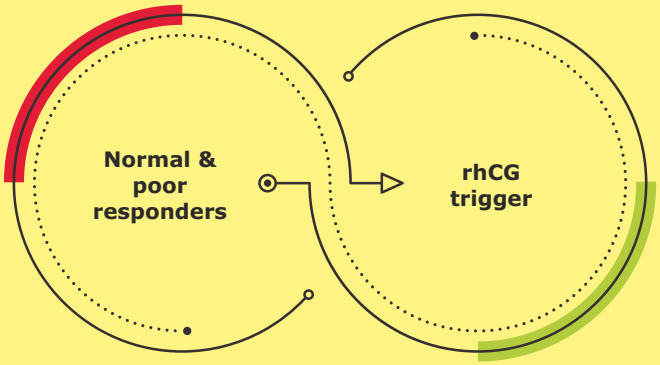
Friedler S and Grin L. Luteal phase support with GnRH agonist does not eliminate the risk for ovarian hyperstimulation syndrome. Gynecol Endocrinol. 2019;35(5):368–369.

Friedler et al report a case of early, severe OHSS following GnRH agonist trigger for final oocyte maturation despite luteal support with a GnRH agonist. Minimizing the risk of OHSS by GnRH agonist triggering in GnRH antagonist cycles has been incorporated in the armamentarium of IVF practitioners during the last decade. As pregnancy rates may be impaired after GnRH triggering, due to ensuing luteal phase defect, in patients that opt for fresh embryo transfer, it has been recognized that the optimal luteal support is debatable.

Presently, one must admit that none of the luteal phase supports can promise abolition of OHSS. The precise cause for this phenomenon is yet to be elucidated, but surely, it is related to the various individual expression of the vascular endothelial growth factor (VEGF) receptor genes in the vasculature of the follicles in each patient, which play a cardinal role in the trigger and clinical manifestation of OHSS. In these circumstances, only segmentation, a concept introduced by *Devroey* et al may offer an efficient mean to achieve an absolute risk-free clinic.

Individualizing of trigger options and patient stratification





Busted Myth 3

rhCG trigger

- ☐ More effective and patient-centered
- ☐ More mature oocytes
- ☐ Lesser empty follicles
- ☐ More LBR
- ☐ Higher ongoing pregnancy rate
- ☐ Indicated in majority of patients: Normo responders and poor responders

GnRH agonist trigger

- ☐ Lesser risk of OHSS
- ☐ Higher early miscarriage rate
- ☐ Beneficial for patients at risk of OHSS

FEEDBACK FORM

ALIVE
ART LEARNING INITIATIVES FOR EXPERTS

Issue 5 | January 2020

Thank you for going through the contents of **Alive Newsletter Issue 5.** To ensure that future issues will be of interest to you, we would greatly appreciate your feedback on the format and content of this issue.

Name: .....

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Contact No: .....

Satisfaction Score for ALIVE Newsletter Issue 5

Myths and Facts in Controlled Ovarian Stimulation II : Issue 5; January 2020

Rating Scale	Poor ----------Excellent (Please circle the appropriate rating)									
Scientific content	1	2	3	4	5	6	7	8	9	10
Relevance of the topic	1	2	3	4	5	6	7	8	9	10
Impact on my daily practice	1	2	3	4	5	6	7	8	9	10
Innovation	1	2	3	4	5	6	7	8	9	10
Overall level of satisfaction	1	2	3	4	5	6	7	8	9	10

What aspects of the Newsletter issue 5 did you find particularly interesting and/or informative?

.....

Please suggest topics/areas that you would like to be covered in future issues of the Alive Newsletter?

.....

How can the subsequent Newsletter issues be improved?

.....

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THREE CENTURIES OF TRANSFORMATION

MERCK
350th ANNIVERSARY

2018

2015 Acquisition of Sigma-Aldrich

2014 Acquisition of AZ Electronic Materials

2011 We take up the fight against schistosomiasis, aiming to eliminate this tropical disease in Africa

2010 Acquisition of Millipore

2007 Acquisition of Serono

1995 Initial public offering

1969 The Institute for Experimental Drug Research is established

1955 We enter the field of bio-technology, assuming a pioneering role in Germany

1950/60 Our subsidiaries are founded or reestablished around the world

1945 All foreign subsidiaries are lost and the company starts up again from the ruins in Darmstadt

1917 Our U. S. subsidiary Merck & Co, is expropriated as a consequence of World War 1

1900 Merck from Darmstadt, Germany is represented on all continents

1827 Emanuel Merck begins production on an industrial scale

1668 Friedrich Jacob Merck purchases the "Angel pharmacy" (Engel-Apotheke) in Darmstadt

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