ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

Issue 4

Myths and facts in controlled ovarian stimulation

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abbreviations

AFC	Antral Follicle Count
AMH	Anti-Müllerian Hormone
ART	Assisted Reproductive Treatment
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
WOI	Window of Implantation

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expert insights

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Infertility is a common problem in India. Gonadotropin therapy and selection of gonadotropin is an essential component of infertility management. The use of gonadotropins for multiple follicular developments has significantly improved the outcomes *in vitro* fertilization (IVF) through controlled ovarian stimulation (COS).

Currently, there is the availability of purified, highly purified urinary, and recombinant gonadotropin preparations and clinicians are having several myths and doubts about the clinical efficacy, safety for ovarian stimulation in women undergoing assisted reproductive therapy.

The development of recombinant follicle-stimulating hormone (rFSH) was thought to be a breakthrough that would revolutionize the management of infertility. The comprehensive review of the literature suggests that the recombinant gonadotropins might be superior to the urinary gonadotropins in COS.

This issue was drafted to understand the popular myths and facts in COS and to highlight the different roles of urinary and recombinant gonadotropins with their clinical recommendations so to prevent the confusion among the clinical experts.

An overall idea was to "debunk the myth, create a gap, and to fill the gap with facts."

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Myths and facts in controlled ovarian stimulation

Myth

- A widely held but false belief or idea
- A misrepresentation of the truth
- An exaggerated or idealized conception of a thing

Fact

- Verifiable truth; reality

Introduction

Alviggi C, Conforti A, Esteves SC, et al. Understanding Ovarian Hypo-Response to Exogenous Gonadotropin in Ovarian Stimulation and Its New Proposed Marker—The Follicle-To-Oocyte (FOI) Index. Front. Endocrinol. 2018; 9:589.

Sunkara SK, Rittenberg V, Raine-Fenning N, et al. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.

Ovarian stimulation (OS) is an essential step in assisted reproductive technology (ART). The conventional OS approaches lead to adequate follicular growth and proper estrogen levels in most women.

An appropriate ovarian response to controlled ovarian stimulation (COS) is crucial for the success of ART. The number of oocytes retrieved at the end of stimulation is the parameter most often used to assess ovarian response to exogenous gonadotropin and can be directly linked to the predicted live birth rate.

To date, most clinical decisions on ovarian stimulation in *in vitro* fertilization (IVF) have been based on ovarian reserve tests which are good at predicting numbers of eggs retrieved which also allow the clinicians to link the (predicted) number of eggs to live birth, and to optimize IVF outcomes by preventing the complications.

Do all women respond same to COS?

Conforti A, Esteves SC, Di Rella F. The role of recombinant LH in women with hypo-response to controlled ovarian stimulation: a systematic review and meta-analysis. Reproductive Biology and Endocrinology. 2019; 17:18.

Based on ovarian biomarkers and oocyte number, women are classically defined as poor, normal or hyper-responders. However, there is a specific subgroup of women termed "hypo-responders" who have an unexpectedly poor or suboptimal response to gonadotropin therapy despite adequate ovarian pre-stimulation parameters.

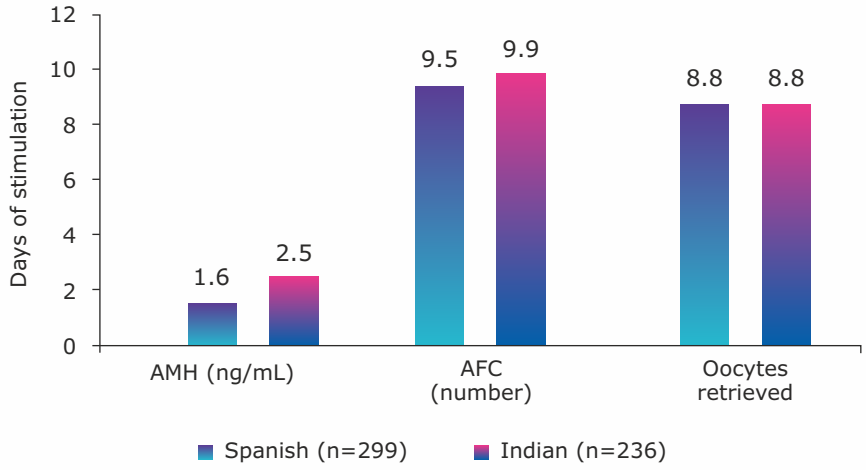
COS outcomes in Indian women: Role of ethnicity

Iglesias C, Banker M, Mahajan N, et al. Ethnicity as a determinant of ovarian reserve: Differences in ovarian aging between Spanish and Indian women. Fertil Steril. 2014;102(1):244–249.

Ethnicity has been consistently shown to affect ART outcomes

- Iglesias *et al.,* investigated the differences in ovarian reserve markers, anti-Mullerian hormone (AMH) and antral follicle count (AFC) in Indian and Spanish women (Figure 1).
- The mean age of women undergoing their first or second IVF cycle was significantly higher in Spanish than in Indian women (37.5 *vs.* 31.5).
- **Ovarian aging:** Indian women demonstrate an accelerated ovarian aging and their ovaries age 6 years earlier than white Spanish women.

Figure 1. Correlation between ovarian markers and stimulation cycle in Indian and Spanish patients



Ethnicity should be a risk factor for diminished ovarian reserve, because it was observed that ART outcomes were less optimal in a population of infertile Indian women compared with white Spanish women.

The influence of ethnicity on ART outcomes

Jayaprakasan K, Pandian D, Hopkisson J, et al. Effect of ethnicity on live birth rates after in vitro fertilisation or intracytoplasmic sperm injection treatment. BJOG. 2014;121:3000–7.

- In ART, ethnicity is important considering age as a relevant prognostic factor. In a retrospective study, Jayaprakasan *et al.,* evaluated the effect of ethnicity of women on the outcome of IVF or intracytoplasmic sperm injection (ICSI) treatment and confirmed that ethnicity is an independent and a determining factor of live birth following IVF treatment.
- In some ethnic groups, lower live birth rates were observed in southeast Asian, African, and Middle-Eastern women than in white European women. Variations in ovarian aging patterns have recently been associated with specific ovarian genotypes and sub-genotypes of the gene encoding.

Goals of IVF therapy

Edward E and Wallach MD. Controlled ovarian hyperstimulation protocols for in vitro fertilization: two decades of experience after the birth of Elizabeth Carr. Fertility and Sterility. 2005; 84 (3):555–569.

The major goals of IVF therapy are:

1. To obtain multiple fertilizable oocytes of good quality than can lead to fertilization and early embryo development
2. To establish a single, healthy (euploid) pregnancy following embryo transfer to the uterine cavity; and to cryopreserve excess embryos of good quality to optimize the total reproductive potential.

COS is therefore, a principal step of IVF therapy.

standard practices for COS in IVF

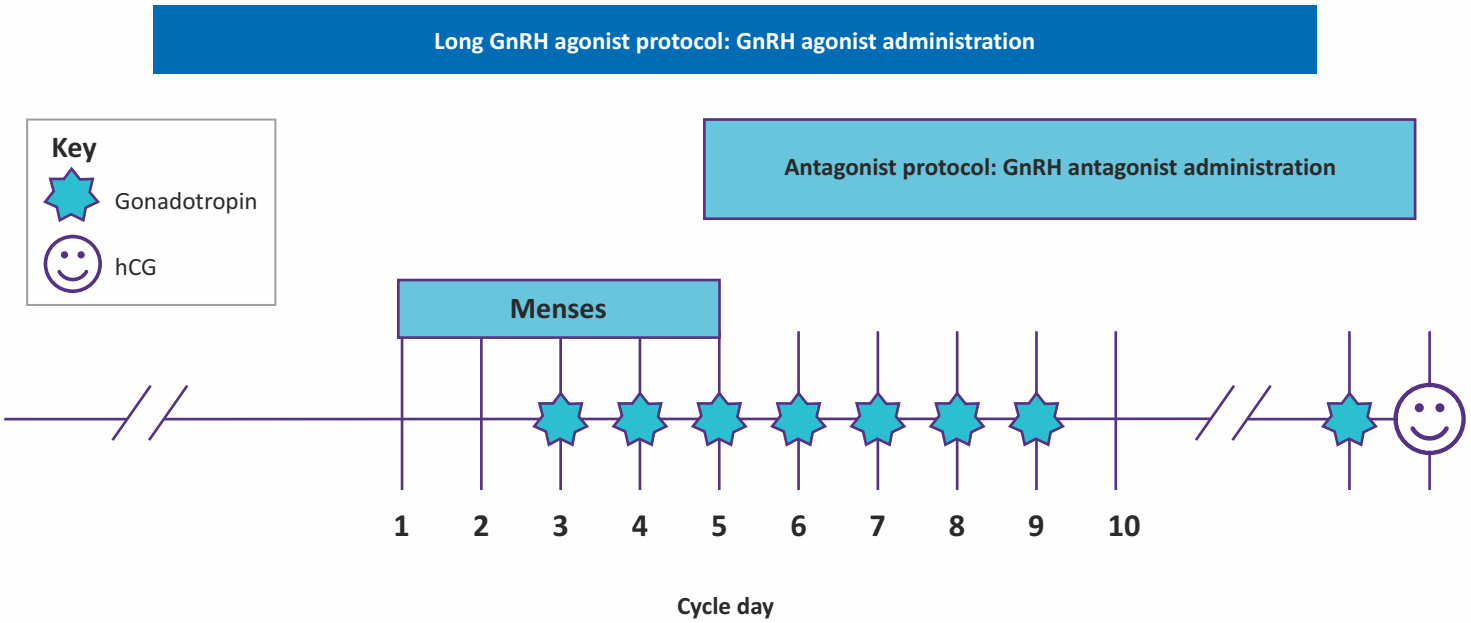
Jungheim ES, Meyer MF, Broughton DE. Best practices for controlled ovarian stimulation in in vitro fertilization. Semin Reprod Med. 2015;33(2):77–82.

The most commonly used protocols for COS are using long agonist protocol and antagonist protocol.

These protocols are essential to achieve adequate cycle control and to prevent premature luteinizing hormone (LH) surge.

Components of these protocols are illustrated in Figure 2.

Figure 2. COS protocols using long agonist and GnRH antagonist



GnRH: Gonadotropin-releasing hormone

Although this field continues to evolve at a very rapid pace, ART practitioners still face major dilemmas about some myths related to COS protocols. Thus, in this Newsletter issue, we will try to address some widely held and misinterpreted myths regarding to COS protocol and present the evidence-based reality of these myths.

Myth statement 1: urinary and recombinants gonadotropins are equally recommended in cos

Gonadotropin preparations used for cos

Currently used gonadotropins are derived either from the urinary source or using recombinant technology, but these are different with respect to: **1) Efficacy, 2) Purity and 3) Consistency**

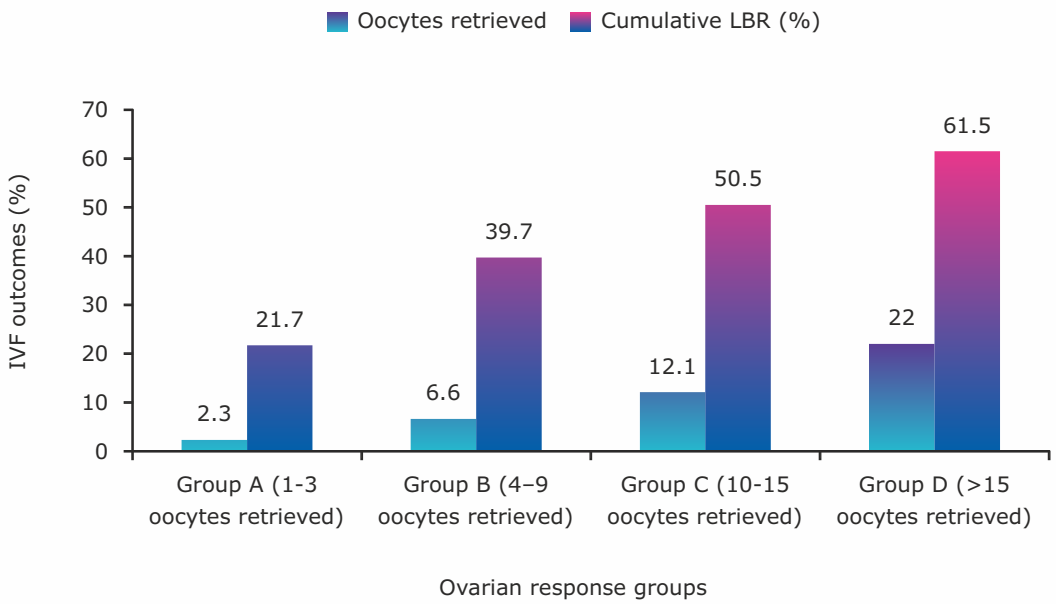
Efficacy

Ovarian response on cumulative live birth rates in women undergoing their first ovarian stimulation cycle planned to undergo single embryo transfer (SET)

Drakopoulos P, Blockeel C, Stoop D, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos. Hum Reprod. 2016;31(2):370–6.

- The increased number of follicles, and consequently the number of oocytes retrieved, improved pregnancy rates in women undergoing IVF/ICSI, not only by increasing the number of available embryos but also by allowing extended embryo culture and enabling the selection of the best quality embryo for transfer.
- This study included 1099 eligible consecutive women 18–40 years old undergoing their first IVF cycle and planned to undergo SET in their fresh cycle. To evaluate the impact of oocyte yield on fresh live birth rate (LBR) and on cumulative LBR after utilization of all cryopreserved embryos, patients were categorized into four groups according to the number of oocytes retrieved: 1–3 (Group A), 4–9 (Group B), 10–15 (Group C) or >15 oocytes (Group D).
- The cumulative LBR significantly increased the number of oocytes retrieved (x2 test for trend p<0.001). High responders (>15 oocytes) demonstrated a significantly higher LBR not only versus poor (0–3 oocytes) (p<0.001) and suboptimal (4–9) responders (p<0.001), but also versus women with normal (10–15) ovarian response (p=0.014).

Figure 3. The relationship between oocytes retrieved and cumulative LBR



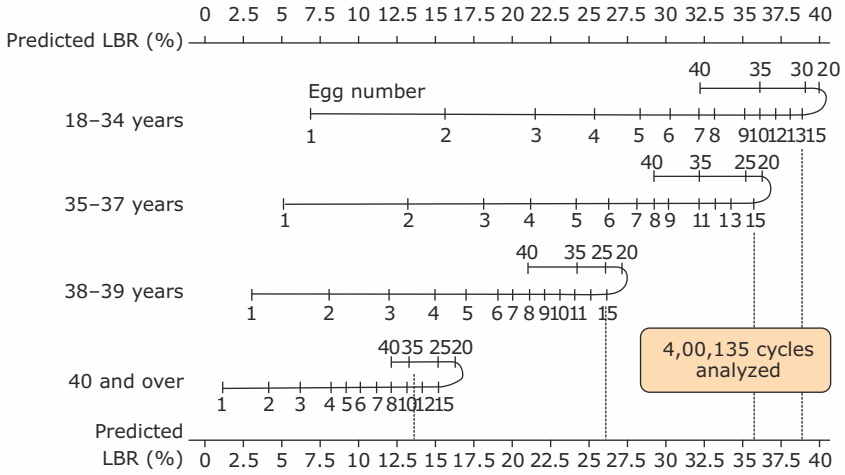
Women undergoing COS for their first IVF/ICSI cycle and planned SET should be informed that, although the number of oocytes retrieved does not affect LBR in the fresh cycle, the higher the oocyte yield, the higher is the probability of achieving LBR after utilization of all cryopreserved embryos

Association between the number of eggs and LBR in IVF treatment: An analysis of 4,00,135 treatment cycles

Sunkara SK, Rittenberg V, Raine-Fenning N, et al. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.

- Sunkara *et al.*, reported that the LBR is the principal clinical outcome following IVF treatment, the number of eggs retrieved following ovarian stimulation is used as a surrogate outcome in clinical practice and research.
- The overall LBR was 21.3% per fresh IVF cycle. There was a strong association between the number of eggs and LBR. The predicted LBR for women with 15 eggs retrieved in age groups 18–34, 35–37, 38–39 and 40 years and over was 40, 36, 27 and 16%, respectively (Figure 4).
- More oocytes allow for more frozen embryo transfers, thereby increasing cumulative pregnancy & LBR. One more oocyte makes a difference in LBR for all age categories.

Figure 4. Predicted LBR probability with given egg number and age

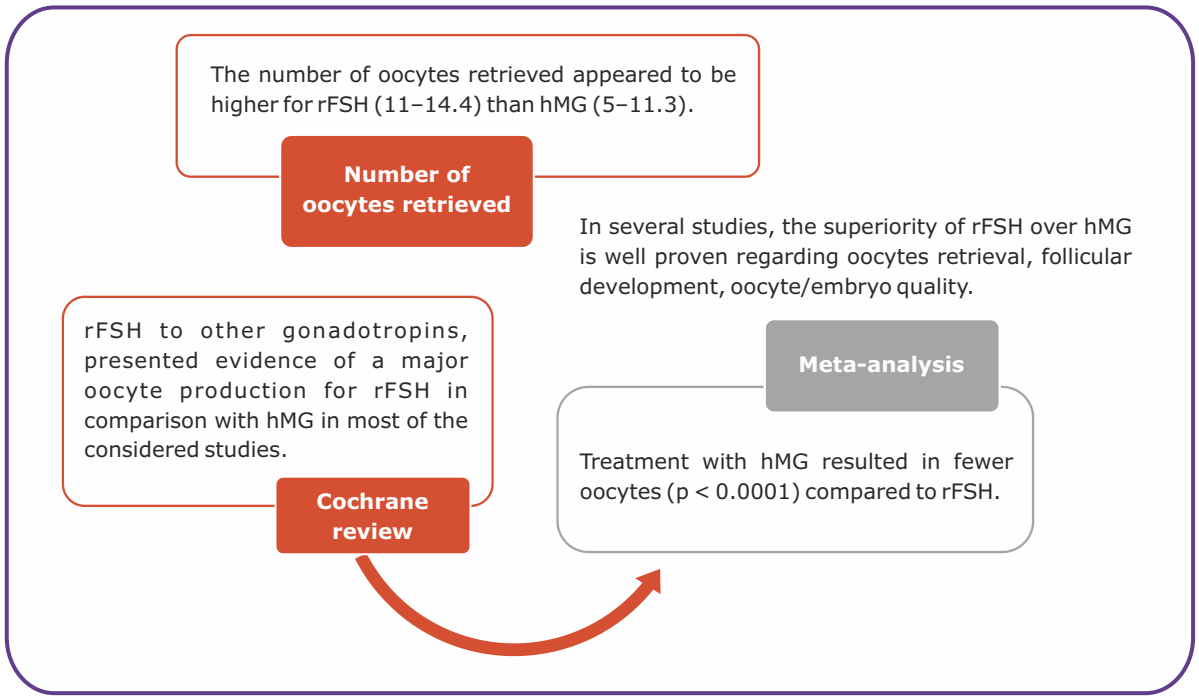


- **Fertility treatment should be managed on time to avoid over-or under-treatment.**
- **The relationship between the number of eggs and LBR, across all female age groups, suggests that the number of eggs in IVF is a robust surrogate outcome for clinical success.**

Human recombinant follicle-stimulating hormone (rFSH) compared to urinary human menopausal gonadotropin (hMG) for ovarian stimulation in ART

Levi Setti PE, Alviggi C, Colombo GL, et al. Human recombinant follicle stimulating hormone (rFSH) compared to urinary human menopausal gonadotropin (HMG) for ovarian stimulation in assisted reproduction: a literature review and cost evaluation. J Endocrinol Invest. 2015;38(5):497–503.

The review was conducted selecting prospective, randomized, controlled trials comparing the two gonadotropin medications from a literature search of several databases. The outcome measure used to evaluate efficacy was the number of oocytes retrieved per cycle.

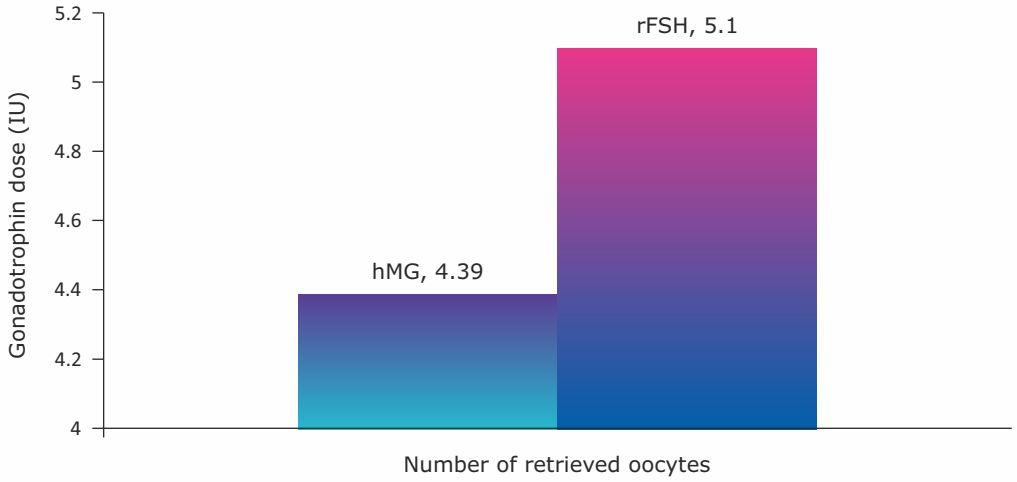


rFSH produces more oocytes with a lower total dose per cycle in ART compared with uMG: A meta-analysis

Lehert P, Schertz JC, Ezcurra D. Recombinant human follicle-stimulating hormone produces more oocytes with a lower total dose per cycle in assisted reproductive technologies compared with highly purified human menopausal gonadotrophin: a meta-analysis. Reprod Biol Endocrinol. 2010;8:112.

- The findings of the present meta-analysis agree with previous meta-analyses regarding the number of oocytes, consistently found to be higher for rFSH in almost all the studies and all the meta-analyses.
- The authors estimated ratio of the number of oocytes/1000 IU of gonadotropin dose to be 4.39 and 5.10 for hMG and rFSH, respectively, with a mean difference favoring rFSH of 0.70 oocytes/1000 IU (95% CI: 0.10 to 1.30; p=0.021) (Figure 5).

Figure 5. The number of oocytes retrieved per 1000 IU total gonadotrophin dose



In terms of a number of oocytes retrieved and total gonadotrophin dose, the differences between the two treatments are significant (p=0.021).

Impact on embryo and oocyte and quality: Embryo developmental kinetics

P-504. The type of gonadotropins used for controlled ovarian stimulation affects embryo developmental kinetics. Munoz, M. Cruz, P. Humaidan, N. Garrido, I. Perez-Cano, M. Meseguer. IVI Alicante, Alicante, Spain; The Fertility Clinic, Odense, Denmark.

Munoz *et al.,* examined 751 embryos developed using either hMG or rFSH in the stimulation protocols for calculating embryo developmental kinetics.

It was observed that embryos obtained after stimulation with rFSH divided significantly earlier (T2 (2-cell embryos), T3 (3-cell embryos) as compared to those after hMG stimulation.

	rFSH (n=43)	CI 95%	hMG (n=58)	CI 95%	p value
T2 (h)	27.0	26.5–27.5	28.3	27.9–28.7	<0.05
T3 (h)	38.8	38.1–39.5	40.1	39.6–40.6	<0.05

Munoz *et al.,* also noticed a significant increase in embryo quality in cycles stimulated with rFSH.

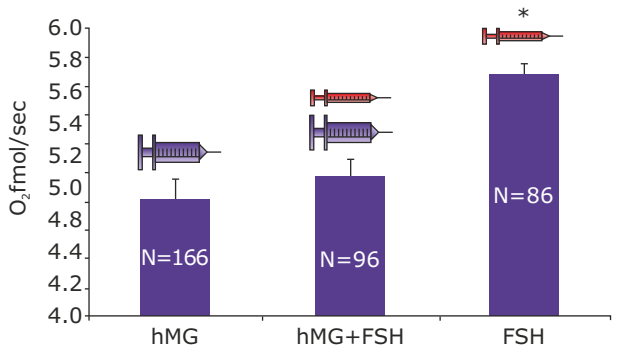
Considering the number of oocytes retrieved as the best direct measure of efficient ovarian stimulation and considering the strong correlation between egg number and live birth, rFSH resulted to be more effective in comparison with hMG.

Oxygen consumption as a marker of oocyte quality

Tejera A, Herrero J, Santos MJ, et al. Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimens. Fertil Steril. 2011;96:618–23.

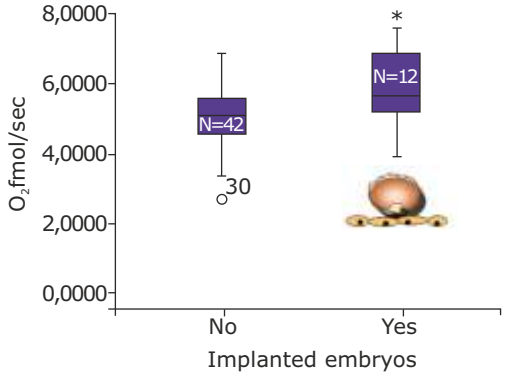
- Tejera *et al.,* evaluated 349 oocytes from 56 IVF treatment cycles in our oocyte donation program. These oocytes were retrieved using rFSH, hMG or the combination of hMG and FSH.
- It was observed that the oxygen consumption for oocytes retrieved using hFSH was higher.
- Also, higher oxygen consumption was observed for those oocytes which generated embryos that implanted compared with those that did not implant.

Figure 6. Oocyte oxygen consumption rate and ovarian stimulation protocols



A) Oxygen consumption and gonadotropin use, *p<0.05

Figure 7. Oocyte consumption levels in oocytes before ICSI that produced embryos that were transferred and implanted *vs.* those transferred and not implanted



B) Higher implantation rates and oxygen consumption, *p<0.05

Measurement of oxygen consumption rates for individual oocytes before fertilization provides a non-invasive marker of oocyte quality and hence a quantitative assessment of the reproductive potential for the oocyte.

Thus, use of rFSH in COS leads to retrieval of not only higher number of oocytes but also oocytes and embryos with better quality.

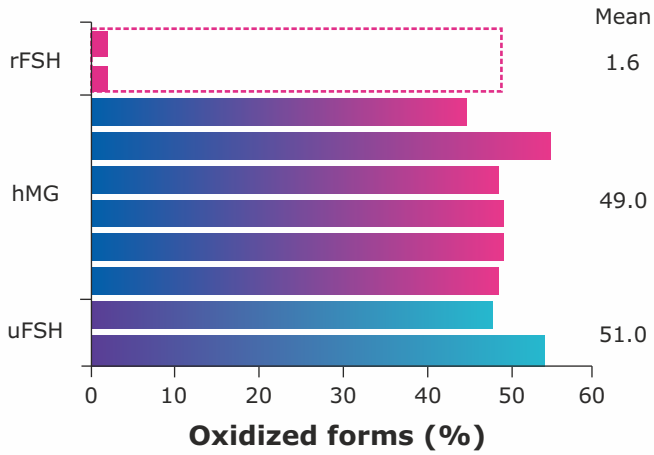
2. purity

Comparison of urinary gonadotropins vs. recombinant formulations

Bassett R, Lispi M, Ceccarelli D, et al. Analytical identification of additional impurities in urinary-derived gonadotrophins. Reprod Biomed Online. 2009;19(3):300–13

- The purity of two purified hMG and hFSH, was compared with a preparation of rFSH.
- It has been shown that rFSH has high product purity with no other detectable proteins, together with a uniform isoform profile and low oxidation level (1.6%).
- rFSH preparations consistently deliver the expected dose from batch to batch, free of undetectable proteins.

Figure 8. Proportion of oxidized forms of gonadotropins in rFSH, hMG and uFSH



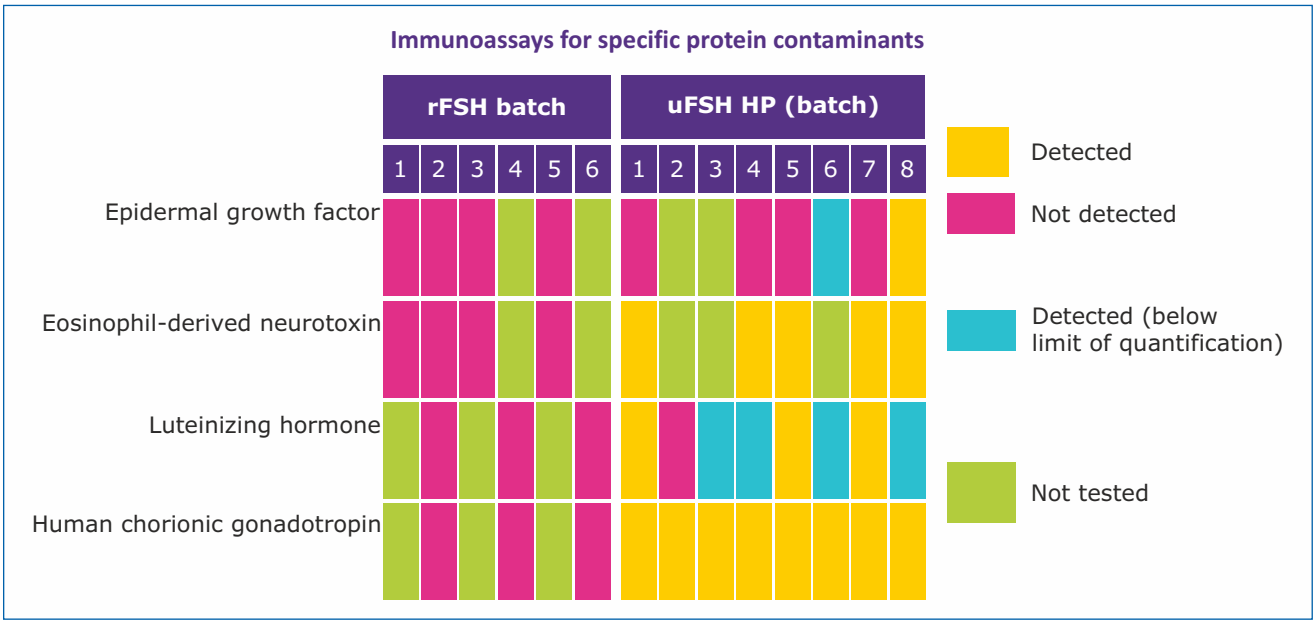
**Oxidation affects the quality of the product – the less oxidation, the more bioavailability

By using a comprehensive proteomic approach, it has been shown that the rFSH preparation has greater purity than either of the urine-derived preparations

Comparative assessment of the consistency and quality of a highly purified FSH extracted from human urine (urofollitropin) and a recombinant human FSH (follitropin alpha)

Lispi M, Bassett R, Crisci C, et al. Comparative assessment of the consistency and quality of a highly purified FSH extracted from human urine (urofollitropin) and a recombinant human FSH (follitropin alpha). Reprod Biomed Online. 2006;13(2):179-93.

- It has been demonstrated that the highly purified urofollitropin contains variable levels of urine-derived contaminant proteins and demonstrates a variable level of FSH purity, FSH isoforms, and delivered dose. These variable factors may contribute to the control of OS.
- The relative purity, variable consistency and the presence of contaminants indicates that the urofollitropin is, at best, a partially purified uFSH that is not able to meet the quality attributes of follitropin alpha (hFSH).



3) consistency

Improvement in consistency of response to ovarian stimulation with rFSH

Recombinant follicle stimulating hormone: development of the first biotechnology product for the treatment of infertility. Recombinant Human FSH product development group. Hum Reprod Update. 1998;4(6):862–81.

Hugues JN, Barlow DH, Rosenwaks Z, et al. Improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method for calibrating the therapeutic preparation. Reprod Biomed Online. 2003;6(2):185–90.

rFSH is manufactured and purified by a unique 'immunoaffinity chromatography purification' process that results in high purity (99%) and batch-to-batch consistency.

- rFSH is also manufactured by using a Filled by Mass (FbM) technology which provides superior batch to batch consistency with variability of 1.6%, while the batch to batch variability with urinary gonadotropins varies from 10–20%. Other advantages of FbM technology are that rFSH manufactured by this process has low levels of degradation and consistent isoform distribution.

High batch-to-batch variability in COS negatively impacts COS outcomes as there is a very strong correlation between the batch FSH bioactivity and the number of mature follicles.

Production and characterization methods of gonadotropin FSH reveals the superiority of FbM (newer) over FbIU (conventional) methods with following advantages:

- Low intrinsic variabilities.
- Consistent isoforms.
- Opportunities to eliminate one variable.
- Reduce risk of adverse events.
- End results of more effective treatment with respect to higher number of oocytes retrieved and higher pregnancy rates.
- Clinical impact: higher consistency of ovarian response.

Busted Myth statement 1

- Overall, as per the review of literature on largest meta-analysis and randomized controlled trials of r-rFSH and hMG, it was found that use of rFSH does show a significant difference in terms of clinical effectiveness, quality of oocytes retrieved, and embryos transferred, as compared with urinary gonadotropins. With improved extraction and purification procedures, the recombinant preparations show significant safety and efficacy. Recombinants are more potent and result in an increased oocyte yield.
- Increasing oocyte yield is an opportunity to have more euploid embryos, which positively affect implantation and pregnancy.
- **Thus, use of urinary and recombinants gonadotropins may not be equally recommended in COS.**

Myth statement 2: LH activity driven by rLH and hCG produces no major differences in endocrine environment

Casarini L, Riccetti L, De Pascali F, et al. Estrogen modulates specific life and death signals induced by LH and hCG in human primary granulosa cells in vitro. Int J Mol Sci. 2017;18(5):926.

LH and human chorionic gonadotropin (hCG) are glycoprotein hormones used for assisted reproduction acting on the same receptor (LHCGR) and mediating different intracellular signaling.

Role of LH during folliculogenesis

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.

Early follicular phase steroidogenesis

- FSH receptor expression in glucocorticoids (responsiveness ↑)
- Act synergistically with insulin-like growth factor 1 (IGF-1) (growth ↑)
- Increase in pre-antral and antral follicles (recruitability ↑)

Mid-follicular phase onwards

- Expression of LH receptors in granulosa
- Sustain of FSH-dependent granulosa activities, including aromatase induction and growth factors release (IGF-1, epidermal growth factor [EGF])
- Regulation of final follicle/oocyte maturation
- Steroidogenesis

The ovary comprises of two cellular components, which are stimulated independently by LH and FSH, leading to the production of ovarian steroids. Androgen production from cholesterol and release during folliculogenesis is dependent on the stimulation of the theca cells by LH and FSH.

Figure 9. Two-cell, two-gonadotropin theory

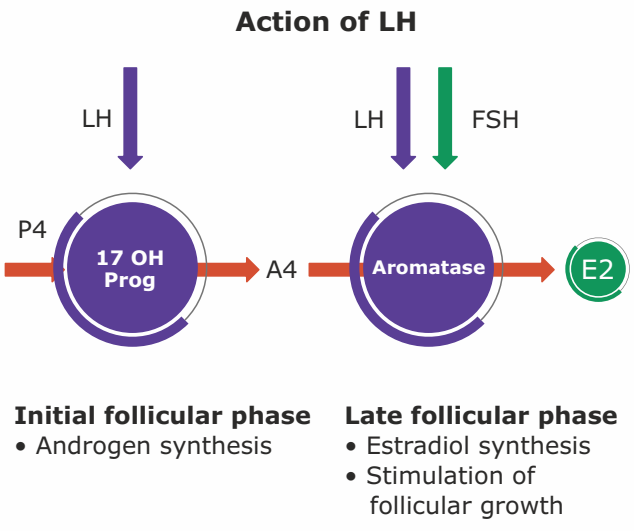
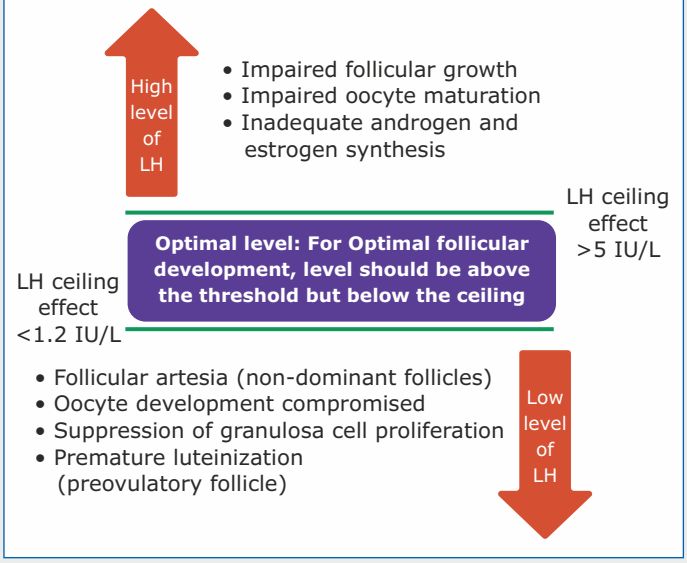


Figure 10. LH therapeutic window



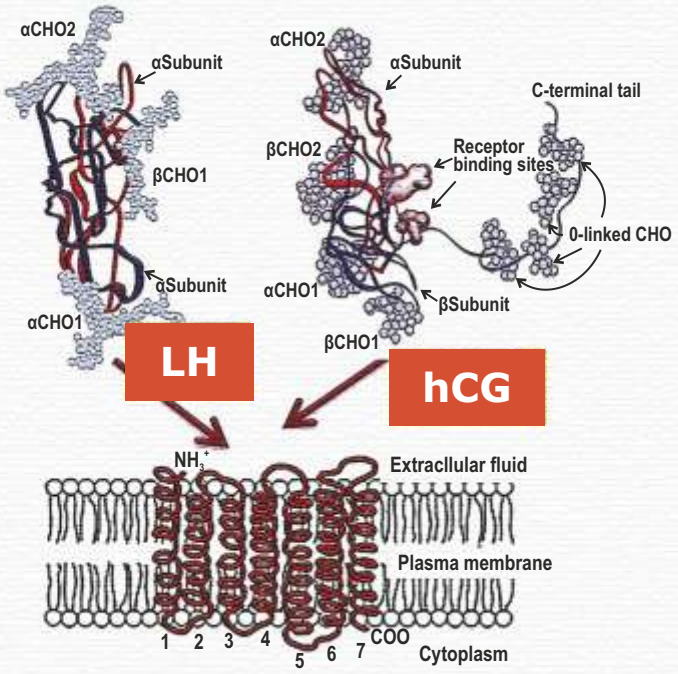
Although FSH can induce follicular growth even without LH, there is an evidence that the follicles may have developmental deficiencies like abnormally reduced estradiol production and lack of ability to luteinize and rupture, following hCG stimulus. Hence, a certain amount of LH exposure is necessary for an optimal follicular development.

Comparison of LH and hCG at the molecular level

Esteves SC. Efficacy, efficiency and effectiveness of gonadotropin therapy for infertility treatment. Medical Express (São Paulo, online). 2015 ;2(3):M150302.
Casarini L, Riccetti L, De Pascali F, et al. Estrogen modulates specific life and death signals Induced by LH and hCG in human primary granulosa cells in vitro. Int J Mol Sci. 2017;18(5):926.
Kahyaoğlu S, Yılmaz B, Işık AZ. Pharmacokinetic, pharmacodynamic, and clinical aspects of ovulation induction agents: A review of the literature. J Turk Ger Gynecol Assoc. 2017;18(1):48–55.
Ezcurra and Humaidan. A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. Reproductive Biology and Endocrinology. 2014,12(95): 1-12.
Lea˜o RB and Esteves SC. Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. Clinics. 2014;69(4):279–293.
Riccetti L, Yvinec R, Klett D, et al. Human luteinizing hormone and chorionic gonadotropin display biased agonism at the LH and LH/CG receptors. Sci Rep. 2017;7(1):940.

In assisted conception, hCG from urine has traditionally been used to trigger final oocyte maturation. hCG has been used as a surrogate for midcycle LH peak to induce final oocte maturation before oocyte retrieval in ART. Despite obtaining a stimulus for final oocyte maturation, ovulation triggering with hCG has no beneficial effect on endometrial receptivity and oocyte quality when compared with spontaneous ovulation. There are considerable structural similarities between hCG and human LH (hLH), and both hormones stimulate the same receptor. However, recombinant LH (rLH) preparations are derived from genetically engineered Chinese hamster ovary (CHO) cells through recombinant DNA technology. Though, hCG can mimic the bioactivity of LH, there are differences between LH and hCG at the molecular level.

Figure 11. LH and hCG molecules



LH: Luteinizing hormone; hCG: human chorionic gonadotropin; CHO: Chinese hamster ovary

Thus, in COS, use of rLH treatment promotes growth and proliferation and prevents apoptosis of granulosa cells, whereas use of hCG leads to higher levels of cyclic adenosine monophosphate (cAMP) and decreased proliferation of granulosa cells.

hCG	LH
Display higher potency than LH on cAMP/phospho-kinase A (PKA) pathway as well on steroidogenesis.	Display more potency than hCG on extracellular signal-regulated kinase (ERK1/2) and serine/threonine-specific protein kinase (AkT) phosphorylation as well as on related gene expression.
Downstream action: Reduced proliferation of granulosa cells.	**Downstream action:** Reduced granulosa cell apoptosis and increased survival.

Difference in pharmacokinetic parameters: LH vs. hCG

Esteves SC. Efficacy, efficiency and effectiveness of gonadotropin therapy for infertility treatment. Medical Express (São Paulo, online). 2015 ;2(3):M150302.
Kahyaoğlu S, Yılmaz B, Işık AZ. Pharmacokinetic, pharmacodynamic, and clinical aspects of ovulation induction agents: A review of the literature. J Turk Ger Gynecol Assoc. 2017;18(1):48–55.
Youssef MA, Abou-Setta AM, and Lam WS. Recombinant versus urinary human chorionic gonadotrophin for final oocyte maturation triggering in IVF and ICSI cycles. Cochrane Database of Systematic Reviews. 2016; 4. Art. No.: CD003719.

- LH and hCG differ in the composition of their carbohydrate moieties; this, in turn, affects bioactivity and half-life.
- LH activity in serum is 30 times higher when hCG is used due to its higher binding affinity to LH receptors, in comparison to rLH.

Table 1A. Pharmacokinetic parameters between sources of LH activity

Molecule	rLH	hCG
Binding affinity	Lower (less punch)	Higher (strong punch x 2/3)
Half-life (hours)	14 ± 8	29 ± 6
Equivalency	6–8 IU	1 IU
T-max (hours)	3–6	12–24
Accumulation	No	Yes

Table 1B. Differences between sources of LH activity

	rLH	hMG
LH content (%)	99.9	<3
LH content (IU)	75	2.3–40
hCG content (IU)	0	9.7–1.6
Urinary impurities (µg)	0	300–1250
LH activity half-life (hours)	13	31 (hCG)

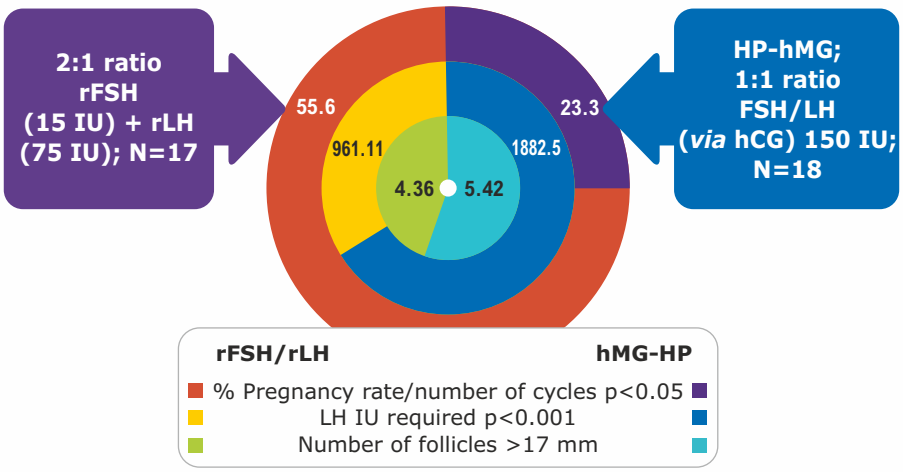
clinical significance of rFSH *vs.* hMG

The superiority of LH in supporting FSH-induced follicular development in WHO group I anovulatory women

Carone D, Caropreso C, Vitti A and Chiappetta R. Efficacy of different gonadotropin combinations to support ovulation induction in WHO type I anovulation infertility: Clinical evidences of human recombinant FSH/human recombinant LH in a 2:1 ratio and highly purified human menopausal gonadotropin stimulation protocols. J Endocrinol Invest. 2012;36:996–1002.

This study was conducted to compare the efficacy of rFSH plus (rLH) in a 2:1 ratio with highly purified human menopausal gonadotropin (hMG-HP) urinary extract, containing LH-like activity, in women with hypogonadotropic hypogonadism (HH).

Figure 12. Comparative effect of rFSH *vs.* rLH



From this study, the group receiving rFSH plus rLH achieved:

- A significantly higher pregnancy rate/number of cycles (55.6% *vs.* 23.3%, p<0.05)
- A smaller number of follicles ≥17 mm (4.4 *vs*. 5.4, p=0.6)
- Significantly less LH required (p<0.001).
- The results from this study suggest that better quality oocytes are produced, although in smaller number, by patients treated with rFSH/rLH.

It was suggested that patients may be more satisfied with the recombinant treatment due to the higher pregnancy rate requiring fewer stimulation cycles

Busted Myth statement 2

1. LH and hCG act on same receptors, activate different pathways. hCG is a pro-apoptotic and LH is anti-apoptotic towards granulosa cells and helps in steroidogenesis.
2. Pharmacokinetic parameters: hCG has a longer half-life, stays in blood and strongly bind with receptors.

3. The superiority of LH compared to hCG is well-evident *via* supporting FSH-induced follicular development in WHO type I anovulation infertility HH women.
4. Thus, use of LH and hCG produce major differences in endocrine environment during COS.

Myth statement 3: GnRH agonists and GnRH antagonists have similar outcomes on reducing time to live birth

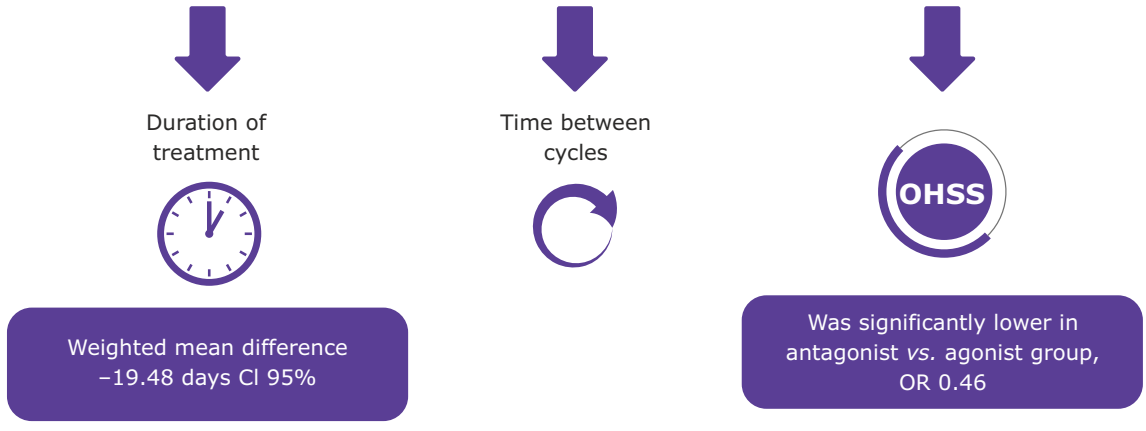
Devroey P, Aboulghar M, Garcia-Velasco J. Improving the patient's experience of IVF/ICSI: a proposal for an ovarian stimulation protocol with GnRH antagonist co-treatment. Hum Reprod. 2009 ;24(4):764–74.

Meta-analyses comparing GnRH agonists and antagonists have calculated almost identical odds ratios (0.82–0.86) for the probability of live birth, although the difference was statistically significant in one analysis and not in another. The difference is unlikely to be of clinical significance.

The contribution of GnRH antagonists in reducing time to pregnancy (TTP): Systematic review and meta-analysis

Kolibianakis EM, Collins J, Tarlatzis BC, et al. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. Hum Reprod Update 2006;12(6):651–671.

- As per the Cochrane review, there is no difference in (LBRs) between GnRH antagonists and long-course agonist protocols (OR 1.02, 95% CI 0.85–1.23; n=2303).
- The subgroup analysis carried out in from the current meta-analysis suggested no association between the type of analogue used and the probability of live birth.



OHSS: Ovarian hyperstimulation syndrome

Busted Myth statement 3

Compared with GnRH agonists, GnRH antagonist protocols may drive a reduction in time to birth by reducing the duration of treatment, the time between cycles and ovarian hyperstimulation syndrome (OHSS) stimulation.

Myth statement 4: Freeze-all policy can be adopted universally for all patient subgroups

The rationale of freeze-all strategy

Roque M, Valle M, Kostolias A, Sampaio M, Geber S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod. 2017;21(1):49–53.
Labarta E, Martínez-Conejero JA, Alamá P, et al. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. Hum Reprod. 2011;26(7):1813–25.
Roque M, Nuto Nóbrega B, Valle M, et al. Freeze-all strategy in IVF/ICSI cycles: an update on clinical utility. Panminerva Med. 2019;61(1):52-57.
Papanikolaou EG, Bourgain C, Kolibianakis E, et al. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. Hum Reprod. 2005;20(6):1541–7.

- It is well evident that, IVF success depends not only on embryo quality but also on endometrial receptivity and on the embryo-endometrium interaction.
- Labarta *et al.*, (2011) found that hyperstimulation might be detrimental to implantation, by altering genes that are crucial for the endometrium-embryo interaction. COS associated endometrial modifications may have consequences not only on implantation rates during IVF treatments, but also be associated with obstetric and perinatal complications.
- Furthermore, in COS it may lead to differences in the timing of endometrial maturation compared with natural cycles. This genomic delay may be of interest to define gene targets for the understanding of endometrial development under COS and search for the optimal stimulation treatments that better mimic the gene expression profile of the natural cycle.
- Several studies, continued to show receptivity impairment in stimulated cycles, due to histologic advancement, glandular-stromal desynchrony, genomic dysregulation, and an increased occurrence of uterine contractions.
- The idea of the "window of implantation" (WOI) is driven by programmed progesterone (P4) exposure after enough estrogen exposure. In a natural 28-day cycle, the WOI is supposed to occur during days 22 to 24. However, it seems that compared to natural cycles WOI is different in stimulated cycles.

Thus, it has been suggested that transfer of frozen-thawed embryos in a non-stimulated cycle is more conducive to early placentation and embryogenesis.

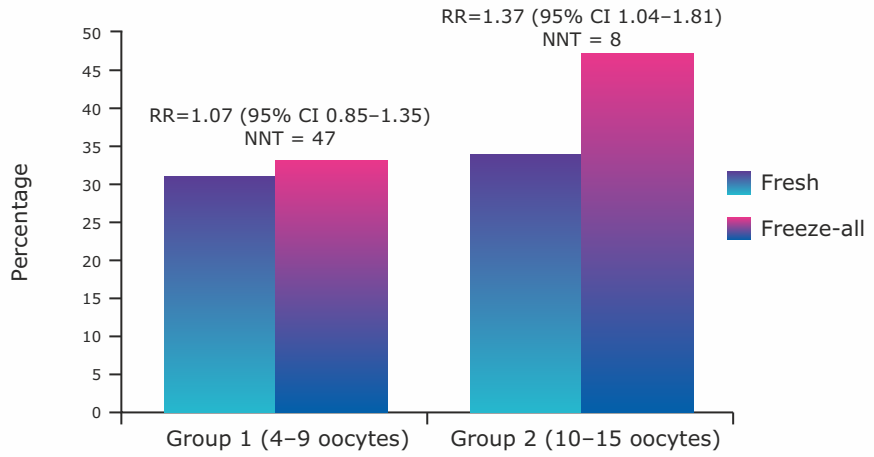
Impact of fresh embryo transfer on pregnancy outcomes

Roque M, Valle M, Guimarães F, et al. Freeze-all cycle for all normal responders? J Assist Reprod Genet. 2017;34(2):179–185.

Fresh embryo transfer is still a routine practice in IVF cycles. However, patients with poorer ovarian response do not benefit from the freeze-all strategy. Since last decade, cryopreservation techniques have improved, which means that the quality and potential for an implantation of frozen embryos are like those for fresh embryos.

Study purpose	Study design	Study findings
• To evaluate the freeze-all strategy in subgroups of normal responders.	• It was an observational, cohort study performed in a private IVF center. • A total of 938 IVF cycles were included in this study. • The comparison between the fresh embryo transfer (n=523) and the freeze-all cycles (n=415) was performed in the present study. • The analysis was performed in two subgroups of patients based on the number of retrieved oocytes: Group 1 (4–9 oocytes) and Group 2 (10–15 oocytes).	• In Group 1 (4–9 retrieved oocytes), the implantation rates (IR) were 17.9 and 20.5% (p=0.259) in the fresh and freeze-all group, respectively; the ongoing pregnancy rates (OPR) were 31 and 33% (p=0.577) in the fresh and freeze-all group, respectively. • In Group 2 (10–15 oocytes), the IR were 22.1 and 30.1% (p=0.028) and the OPR were 34 and 47% (p=0.021) in the fresh and freeze-all groups, respectively.

Figure 14. Ongoing pregnancy rates with its relative risk (RR) and the number needed to treat (NNT) in fresh *vs.* freeze-all cycles



As per the above study, the freeze-all policy in normal responders is strongly associated with better IVF outcomes than fresh embryo transfer, although there is no benefit in performing the strategy in suboptimal responders.

overall benefits of freeze-all strategy

Vuong L, Dang V, Huynh BG, et al. IVF transfer of fresh or frozen embryos in women without polycystic ovaries. New England Journal of Medicine. 2018; 378 (2): 137–47.

Roque M, Valle M, Kostolias A, Sampaio M, Geber S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod. 2017;21(1):49–53.

Coates A, Kung A, Mounts E, et al. Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial. Fertil Steril. 2017;107(3):723–730.

RATIONALE

- Hyperstimulation
- Evolving endometrium-embryo interaction
- Altered gene expression
- Adverse effects of COS
- Progesterone elevation cut-off levels (at present, 1.5 ng/mL)

CLINICAL BENEFITS

- Obstetric outcome is better than the fresh transfers
- Increases ongoing pregnancy rates and live birth rates
- Improves implantation rates

SAFETY

- Prevent the development of ovarian hyperstimulation syndrome
- Reduces the risk of low-birth weight and pre-term birth

busted myth statement 4

- Freeze-all policy may not benefit all patient subgroups.
- Overall, the above benefits reflect a trend toward favoring the freeze-all option as a preferred transfer strategy when using known euploid embryos.
- Although the freeze-all policy may be related to better IVF outcomes in normal responders, these potential advantages decrease with worsening ovarian response
- Patients with poorer ovarian response do not benefit from the freeze-all strategy.

summary of facts

Fact 1	• rFSH do show a significant difference as compared to urinary gonadotropins in terms of clinical effectiveness, quality of oocytes retrieved, and embryos transferred. • The recombinant preparations show significant purity, less contamination, safety and efficacy. • The use of urinary and recombinants gonadotropins may not be equally recommended in COS.
Fact 2	• Various studies demonstrated that the two molecules LH and hCG interact with the same receptor, activating different mechanisms. • Recombinant LH is superior in clinical action with better pharmacokinetic parameters. • Use of recombinant LH and hCG produce major differences in an endocrine environment during COS.
Fact 3	• Use of GnRH antagonist compared to agonists may drive a reduction in time to birth by reducing the duration of treatment, the time between cycles and ovarian hyperstimulation syndrome (OHSS) stimulation.
Fact 4	• Freeze-all policy may not benefit all patient subgroups. • Freeze-all strategy is recommended in hyper-responders with OHSS, progesterone rise and preimplantation genetic testing for aneuploidies (PGT-A). • It is recommended as a preferred transfer strategy when using known euploid embryos.

FEEDBACK FORM

ALIVE
ART LEARNING INITIATIVES FOR EXPERTS

Issue 4 | August 2019

Thank you for going through the contents of **Alive Newsletter Issue 4.** 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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Satisfaction Score for ALIVE Newsletter Issue 4

Myths and Facts in Controlled Ovarian Stimulation: Issue 4; August 2019

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 4 did you find particularly interesting and/or informative?

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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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