ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

Issue 8

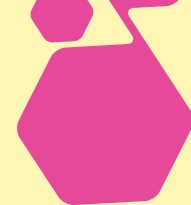## FSH and LH deficiency in ART and management options

January 2022

MERCK

A crisp audio summary of ALIVE newsletter issue-8.

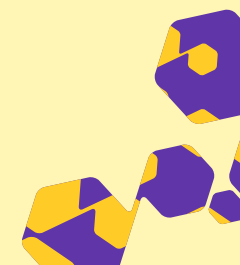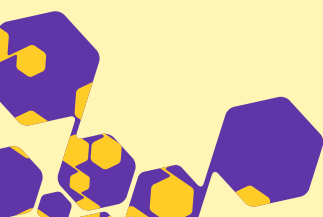Please click on the link below or scan the QR code to listen to the audio.

Link:
https://hcp.merckgroup.com/content/dam/web/health care/biopharma/fertility/india/hcp/resources/ALIVE% 20NL%20Issue%208%20Voice%20Over.mp3

CONTENTS

Dr. G. A. Rama Raju

Founder & Director,
Krishna IVF Clinic,
Visakhapatnam, India.

Dr. Manish Banker

Medical Director,
Nova IVF Fertility,
Ahmedabad.

Dr. Rohit Gutgutia

Medical Director,
NOVA IVF Fertility (East),
Kolkata.

Although the IVF technology at its helm in the last decade, infertility rates in India are still at an alarming level. Despite the advent of technology, many couples are failing to achieve the success in the IVF in the first cycle itself. Many couples painfully resort to the second cycle with much distress and low hopes. The two main reasons for this problem are women of advanced maternal age undergoing IVF cycles and their hypo-response to the ovarian stimulation in the ART.

Currently, despite the availability of a wide choice in the gonadotropin market including urinary and recombinant preparations of FSH, LH, hCG, and hMG - alone or in various combinations, there is a need to further evaluate the failures in the IVF at the genetic level.

Although the researchers are planning for the personalized reproductive medicine, there exits an issue at the primary level itself–LH and FSH deficiency in ART, which is much less talked about.

The polymorphisms with LH and FSH and their receptors are further making the IVF success process complex as they lead to reduced fertility in terms of gonadotropin resistance and Ovarian stimulation.

So, this Newsletter aims to discuss about the FSH and LH deficiency in the ART, the different glycosylation and genetic variants of FSH and LH and the reason for their deficiency, reduced action in women of advanced maternal age and also their hypo-response to ovarian stimulation.

An effort is also made to discuss about the impact of polymorphisms and management strategies with the pharmacogenomic approach in the IVF cycles to improve the reproductive outcomes.
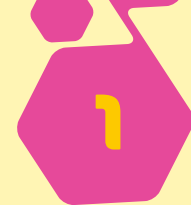
FSH and LH deficiency in ART and Management options

Introduction

1. 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.
2. Nasser AM, Al-Jumaili EF and Alhusni ZK. The effect of pituitary hormones levels and relationship to female infertility in Baghdad province. J. Phys.Conf. Ser. 2021; 1853 012015.
3. Yilmaz S, Ozgu-Erdinc AS, Yumusak O et al. The reproductive outcome of women with hypogonadotropic hypogonadism undergoing in vitro fertilization. Syst Biol Reprod Med. 2015;61(4):228-232.
4. Zegers-Hochschild F, Adamson GD, Dyer S et al. The International Glossary on Infertility and Fertility Care. Hum Reprod. 2017;32(9):1786-1801.
5. Santi D, Casarini L, Alviggi C et al. Efficacy of Follicle-Stimulating Hormone (FSH) Alone, FSH+Luteinizing Hormone, Human Menopausal Gonadotropin or FSH+Human Chorionic Gonadotropin on Assisted Reproductive Technology Outcomes in the "Personalized" Medicine Era: A Meta-analysis. Front Endocrinol (Lausanne). 2017 Jun 1;8:114.
6. Bosch E, Alviggi C, Lispi M et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.

Luteinizing hormone (LH) in synergy with follicle stimulating hormone (FSH) stimulates follicular growth and ovulation. Normal follicular growth is the result of separate but complementary actions of FSH and LH.

A reduction in female fertility ensues with the deficiency in LH and FSH production.

Hypogonadotropic hypogonadism (HH) is a rare disease that is characterized by low levels of FSH and LH.

The International Glossary on Infertility and Fertility Care, 2017, now defines Hypogonadotropic hypogonadism as gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotropin production or action.

Although it is very well implicated that the role of these primary gonadotropins is of prime importance in the Assisted reproductive technology (ART), their deficiency has received less attention in ART.

This article discusses how the association of the FSH and LH deficiency in ART is associated with a reduced quantitative and qualitative response to ovarian stimulation (OS). It also discusses about the management options in women for improvement of FSH and LH deficiency undergoing ART.

LH and FSH action in physiological and altered conditions

The hypothalamic-pituitary-gonadal axis

1. Clavijo RI, Hsiao W. Update on male reproductive endocrinology. Transl Androl Urol. 2018;7(Suppl 3):S367-S372.
2. Marques P, Skorupskaite K, George JT et al. Physiology of GNRH and Gonadotropin Secretion. [Updated 2018 Jun 19]. In: Feingold KR, Anawalt B, Boyce A et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279070/ as on 04.08.2021.
3. Moore AM, Coolen LM, Lehman MN. Kisspeptin/Neurokinin B/Dynorphin (KNDy) cells as integrators of diverse internal and external cues: evidence from viral-based monosynaptic tract-tracing in mice. Sci Rep. 2019;9(1):14768.
4. Berga S, Naftolin F. Neuroendocrine control of ovulation. Gynecol Endocrinol. 2012 Mar;28 Suppl 1:9-13.
5. Marshall JC, Dalkin AC, Haisenleder DJ et al. Gonadotropin-releasing hormone pulses: regulators of gonadotropin synthesis and ovulatory cycles. Recent Prog Horm Res. 1991; 47:155-187.
6. Barbotin AL, Peigné M, Malone SA et al. Emerging Roles of Anti-Müllerian Hormone in Hypothalamic-Pituitary Function. Neuroendocrinology. 2019;109(3):218-229.

The hypothalamic-pituitary-gonadal (HPG) axis is comprised of the hypothalamus, the pituitary gland and the ovaries.

*rFSH/rLH in 2:1 ratio is indicated for the stimulation of follicular development in adult women with severe LH and FSH deficiency.
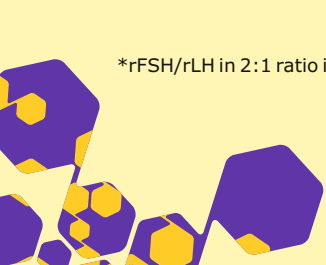
The hypothalamus secretes gonadotrophin releasing hormone (GnRH) in a pulsatile fashion, which stimulates the anterior pituitary gland to secrete two hormones vital for reproduction, FSH and LH. Research in the last decade suggests that kisspeptin-neurokinin B-dynorphin (KNDy) neuronal network plays a pivotal role in the regulation of pulsatile GnRH secretion.

Neurons in the hypothalamic arcuate nucleus (ARC) that co-express kisspeptin, neurokinin B and dynorphin (KNDy cells) are essential for mammalian reproduction as key regulators of gonadotropin releasing hormone secretion. They provide an ideal nodal point through which peripheral signals can alter gonadotropin secretion and reproductive capacity.

The main hormone regulating this HPG axis is Estradiol along with Progesterone and inhibin. Failure of the orderly progression of hypothalamic GnRH → pituitary gonadotrophins → ovarian steroids and inhibin → hypothalamus/pituitary results in anovulation/amenorrhea.

Figure 1 outlines the HPG axis interaction, feedback system and disrupting factors.
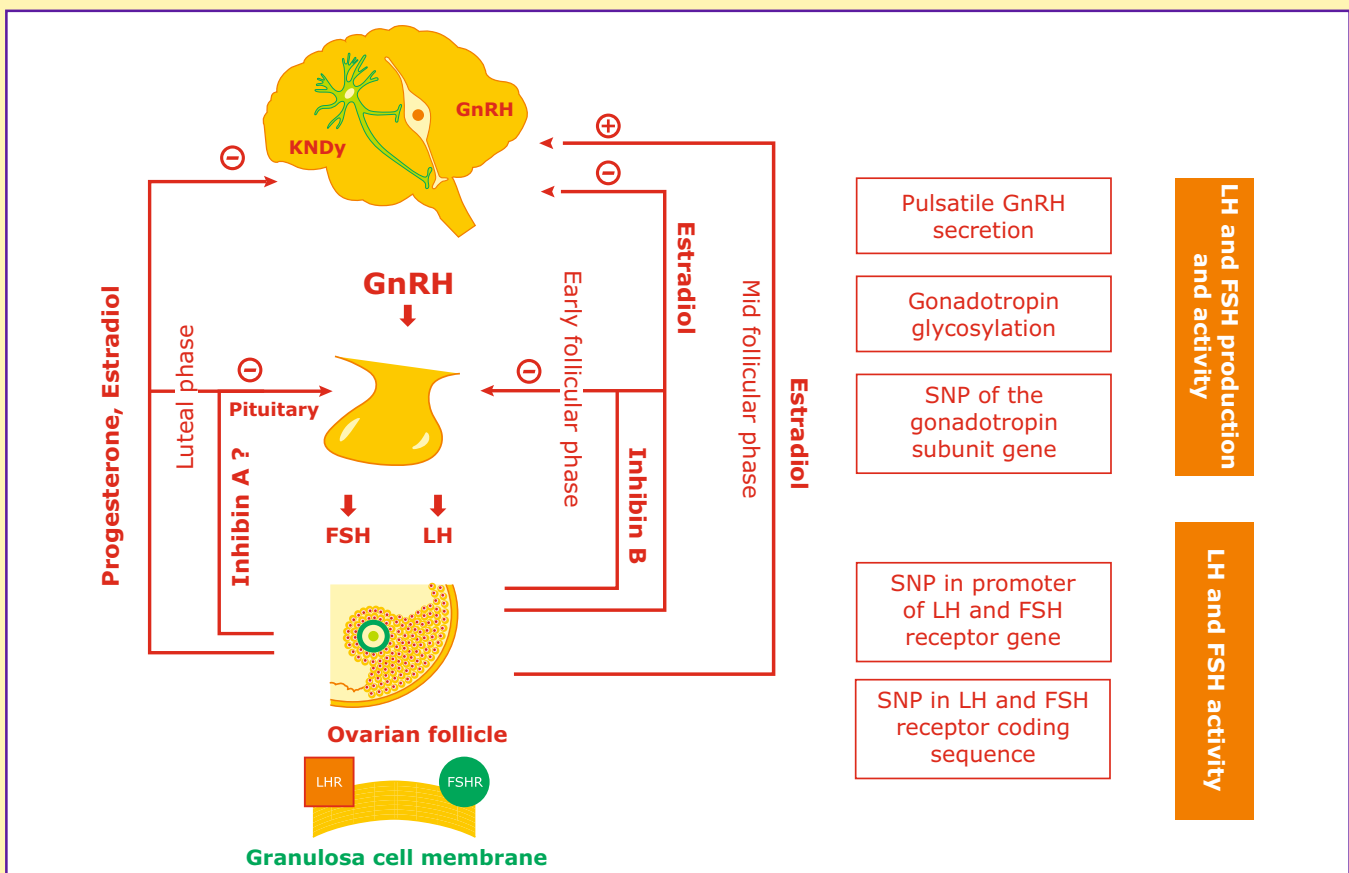
Figure 1. The hypothalamus–pituitary–gonadal axis interaction, feedback system and disrupting factors
KNDy, kisspeptin, neurokinin B, and dynorphin; SNP, single nucleotide polymorphism
Figure adapted from Hum Reprod. 2021;36(6):1469-1480.

In the early follicular phase of the menstrual cycle, the initial increase in FSH stimulates follicular recruitment and maturation. Estradiol (E_2), consequently secreted, selectively inhibits FSH release and maintains rapid GnRH pulsatility during the late follicular phase.

The persistent rapid GnRH pulses increases LH. LH further stimulates E_2 secretion, culminating in positive E_2 feedback to produce the mid-cycle LH surge. The GnRH levels, during the LH surge, appear to be consistently elevated and remain elevated as LH declines. Once the ovulation sets in,

there is a reduction in the GnRH pulses frequency due to progesterone secretion. Finally, there is a fall in the E_2, progesterone and inhibin levels due to the demise of corpus luteum. The GnRH pulse frequency increases, leading to follicular maturation in the next cycle.

Of late, the novel neuroendocrine actions of Anti-Müllerian hormone (AMH) have started to be elucidated.

Emerging role of AMH

1. Cimino I, Casoni F, Liu X et al. Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. Nat Commun. 2016 Jan 12;7:10055.
2. Durlinger AL, Gruijters MJ, Kramer P et al. Anti-Müllerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142(11):4891-4899.

AMH plays crucial roles in sexual differentiation and gonadal functions. AMH signals by binding to a specific type-II receptor (AMHR2) that heterodimerizes with one of several type-I receptors (ALK2, ALK3 and ALK6), and recruiting Smad proteins that are translocated to the nucleus to regulate target gene expression.

AMH is the only known ligand of AMHR2, suggesting that tissues that express this receptor are likely to be targets of AMH.

Cimino et al., in their study demonstrated that a significant subset of GnRH neurons both in animals and humans express the AMH receptor, and that AMH potently activates the GnRH neuron firing in animals. Combining *in vivo* and *in vitro* experiments, they showed that AMH increases GnRH-dependent LH pulsatility and secretion, supporting a central action of AMH on GnRH neurons.

Another experimental study by Durlinger et al., showed that AMH is one of the factors determining the sensitivity of ovarian follicles for FSH and that AMH is a dominant regulator of early follicle growth.

Action of LH and FSH in follicles
LH-The Master regulator - ERK pathway

1. Kahnamouyi S, Nouri M, Farzadi L et al. The role of mitogen-activated protein kinase-extracellular receptor kinase pathway in female fertility outcomes: a focus on pituitary gonadotropins regulation. Ther Adv Endocrinol Metab. 2018;9(7):209-215.
2. Rama 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.
3. Filicori M, Cognigni GE, Pocognoli P. Current concepts and novel applications of LH activity in ovarian stimulation. Trends Endocrinol Metab. 2003;14(6):267-273.
4. Casarini L, Riccetti L, De Pascali F et al. Follicle-stimulating hormone potentiates the steroidogenic activity of chorionic gonadotropin and the anti-apoptotic activity of luteinizing hormone in human granulosa-lutein cells in vitro. Mol Cell Endocrinol. 2016;422:103-114.
5. Feng X, Zhang M, Guan R et al. Heterodimerization Between the Lutropin and Follitropin Receptors is Associated With an Attenuation of Hormone-Dependent Signaling. Endocrinology. 2013; (154)10:3925-3930.
6. Casarini L, Simoni M. Recent advances in understanding gonadotropin signaling. Fac Rev. 2021;10:41.

FSH and LH are heterodimeric hormones consisting of α and β subunits. FSH and LH signaling implement several signaling pathways in ovarian granulosa cells such as mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK). Precise functions of MAPKs in the regulation of gonadotropins are not yet fully understood. However, Luteinizing hormone (LH) plays a crucial role in ovulation and has the capability of ERK1/2 activation.

"Two-cell, two-gonadotropin" theory: Two cellular components are seen in the ovary, 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 1).
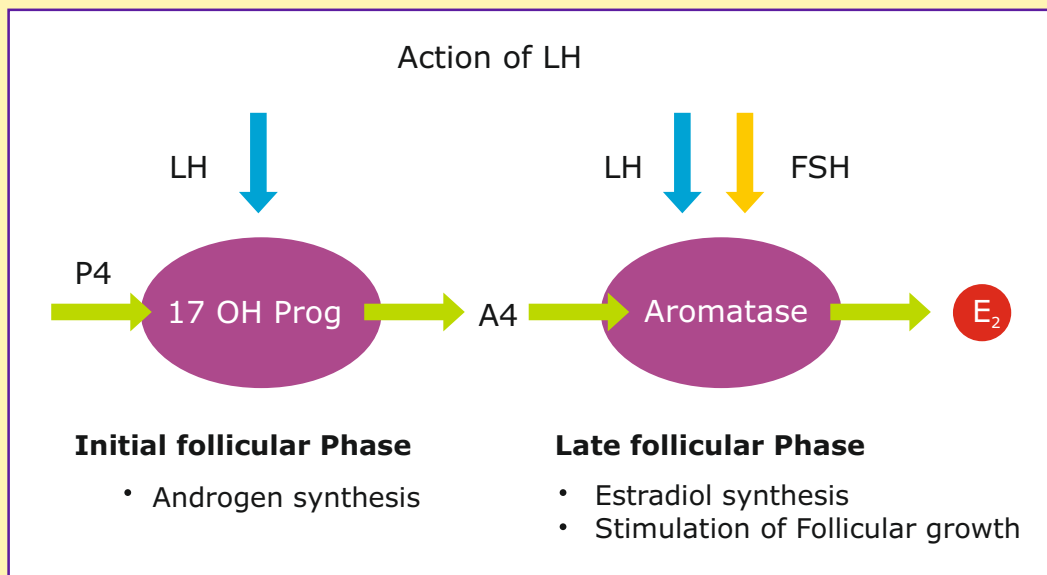
Figure 1. Two-cell, two-gonadotropin theory
Figure adapted from J Hum Reprod Sci. 2013;6(4):227-234.

Luteinizing hormone is thus a crucial physiological regulator of the human ovulatory cycle. LH is therapeutically advantageous, particularly in the support and modulation of ovarian folliculogenesis.

In a study, Casarini et al., demonstrated that the different modulatory activity of FSH on LH and human chorionic gonadotropin (hCG) action corresponds to their different physiological functions. In this *in vitro* study, FSH potentiated the steroidogenic activity of chorionic gonadotropin and the anti-apoptotic activity of LH in human granulosa-lutein cells.

Studies have also shown that there is also a synergy between the LH and FSH at the receptors level. The LH receptor (LHR) and FSH receptor (FSHR) are each G protein-coupled receptors that play critical roles in reproductive endocrinology.

Feng et al., in their study demonstrated that the co-expression of the LHR and FSHR enables heterodimerization between the 2 gonadotropin receptors and results in an attenuation of signaling through each receptor.

(Heterodimerization: Dimerization of G protein-coupled receptors (GPCRs) is a well-documented and accepted phenomenon. In addition to homodimerization, many GPCRs have been shown to form heterodimers. In some cases, this has been shown to lead to alterations in the functional properties of the heterodimer as compared with the individual GPCRs).

The two gonadotropin receptors have common signaling pathways. The overlapping of FSHR- and LHCGR-dependent (luteinizing hormone/chorionic gonadotropin receptor) intracellular signaling pathways would be due to structural similarities between the two receptors. The two receptors mediate different and irreplaceable physiological effects although they induce partially overlapping signal transduction pathways.

Effect of different LH and FSH glycosylation variants and female age on LH and FSH action

1. Wide L, Eriksson K. Dynamic changes in glycosylation and glycan composition of serum FSH and LH during natural ovarian stimulation. Ups J Med Sci. 2013;118(3):153-164.
2. Wide L, Eriksson K. Low-glycosylated forms of both FSH and LH play major roles in the natural ovarian stimulation. Ups J Med Sci. 2018;123(2):100-108.

3. Anobile CJ, Talbot JA, McCann SJ et al. Glycoform composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state. Mol Hum Reprod. 1998;4(7):631-639.
4. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol 2014;383:203-213.
5. Bousfield GR, May JV, Davis JS et al. In Vivo and In Vitro Impact of Carbohydrate Variation on Human Follicle-Stimulating Hormone Function. Front Endocrinol (Lausanne). 2018;9:216.
6. Wide L, Naessén T, Sundström-Poromaa I et al. Sulfonation and sialylation of gonadotropins in women during the menstrual cycle, after menopause, and with polycystic ovarian syndrome and in men. J Clin Endocrinol Metab. 2007;92(11):4410-4417.

N-glycosylation is a post-translational process in which an oligosaccharide is transferred and attached to asparagine on a nascently translated polypeptide. Glycosylation and glycan composition are of fundamental importance for the biological properties of FSH and LH.

FSH and LH are glycoprotein hormones. Fully glycosylated FSH has four glycans (FSHtetra), two on the alpha-subunit and two on the beta-subunit, and fully glycosylated LH has three glycans (LHtri), two on the alpha subunit, but only one on the beta-subunit. The circulatory half-life of all glycoforms is expected to be short at the beginning and the end of the ovarian and menstrual cycles. Studies show that low-glycosylated forms of both FSH and LH, have a higher biopotency than those which are fully glycosylated and play major roles in the natural ovarian stimulation.

Serum FSH and LH governing the natural ovarian stimulation process exhibited dynamic changes of glycosylation and glycan composition.

Anobile et al., in their study showed that during the normal menstrual cycle, the glycoforms of FSH and LH become less complex (simpler) at midcycle compared with the follicular and luteal phases. The charge of FSH glycoforms also changed at midcycle such that fewer acidic forms were present.

During the reproductive life cycle, variations in LH isoform composition are also observed. In general, LH isoforms with shorter half-lives but increased biopotency are present in younger post-pubertal women, whereas LH species with longer half-lives are the predominant form detected in post-menopausal women.

Hypo-glycosylated (or partially glycosylated) FSH variants exhibit higher association rates, greater apparent affinity, and greater occupancy than fully glycosylated FSH. Partially glycosylated pituitary FSH shows an age-related decline in abundance that may be associated with decreased fertility.

The LH isoforms are more basic in women. With ageing, they change to less bioactive isoforms, i.e. more sialylated and less sulfonated LH isoform.

Androgen production

Mushayandebvu T, Castracane VD, Gimpel T et al. Evidence for diminished midcycle ovarian androgen production in older reproductive aged women. Fertil Steril. 1996;65(4):721-723.

Studies show diminished midcycle ovarian androgen production in older reproductive aged women as the hormones are produced in lesser quantity at midcycle.

The effect of genetic variants of LH, FSH and their receptors on LH and FSH action

1. Beate K, Joseph N, Nicolas de R et al. Genetics of isolated hypogonadotropic hypogonadism: role of GnRH receptor and other genes. Int J Endocrinol. 2012;2012:147893.
2. Achermann JC, Ozisik G, Meeks JJ et al. Genetic causes of human reproductive disease. J Clin Endocrinol Metab. 2002;87:2447-2454.
3. Latronico AC, Chai Y, Arnhold IJ et al. A homozygous microdeletion in helix 7 of the luteinizing hormone receptor associated with familial testicular and ovarian resistance is due to both decreased cell surface expression and impaired effector activation by the cell surface receptor. Mol Endocrinol. 1998;12(3):442-450.

Mutations of the β-subunits of LH or FSH are rare causes of hypogonadotropic hypogonadism. Heterozygous LHβ gene changes have been described in women with infertility. Women with homozygous inactivating mutations of LHR (LH receptor) have severe menstrual irregularities (secondary amenorrhea, oligomenorrhea) and infertility.

Delayed puberty due to Mutations

Layman LC, Lee EJ, Peak DB et al. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. N Engl J Med. 1997;337(9):607-611.

Delayed puberty and hypogonadism have been reported due to mutations in the FHS-β-subunit gene.

FSH receptor and FSH receptor gene

1. George JW, Dille EA, Heckert LL. Current concepts of follicle-stimulating hormone receptor gene regulation. Biol Reprod. 2011;84(1):7-17.
2. Perez Mayorga M, Gromoll J, Behre HM et al. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000;85(9):3365-3369.
3. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. Hum Reprod Update. 2002;8(5):413-421.
4. Desai SS, Roy BS, Mahale SD. Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. Reproduction. 2013;146(6):R235-R248.
5. Ruth KS, Beaumont RN, Tyrrell J et al. Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and impact female reproductive health. Hum Reprod. 2016;31(2):473-481.
6. Rull K, Grigorova M, Ehrenberg A et al. FSHB -211 G>T is a major genetic modulator of reproductive physiology and health in childbearing age women. Hum Reprod. 2018;33(5):954-966.

To transmit its signal, FSH must bind to its receptor (FSHR) located on Sertoli cells of the testis and granulosa cells of the ovary. Thus, both the magnitude and the target of hormone response are controlled by mechanisms that determine FSHR levels and cell-specific expression, which are supported by transcription of its gene (FSHR gene).

Mayorga et al., demonstrated that the ovarian response to FSH stimulation depends on the FSHR genotype.

FSH receptor genotype can influence the ovarian response to FSH stimulation. The polymorphisms in FSH and LH and their receptors may lead to reduced fertility in terms of resistance to gonadotropins and ovarian stimulation.

Studies on polymorphisms in the FSHR gene have shown variability in clinical outcome among women treated with FSH.

Evidenced data shows that the T allele of the FSHB promoter polymorphism decreases FSH levels, results in longer menstrual cycles and age at menopause.

In women, the FSHB-211 G>T represents a key genetic modulator of circulating gonadotropin, leading to various possible downstream effects on reproductive physiology.

Clinical presentation of LH and FSH deficiency

1. Bosch E, Alviggi C, Lispi M et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.
2. Warren MP. Endocrine manifestations of eating disorders. J Clin Endocrinol Metab. 2011;96(2):333-343.
3. Gordon CM, Ackerman KE, Berga SL et al. Functional Hypothalamic Amenorrhea: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2017;102(5):1413-1439.
4. Meczekalski B, Katulski K, Czyzyk A et al. Functional hypothalamic amenorrhea and its influence on women's health. J Endocrinol Invest. 2014;37(11):1049-1056.
5. Boehm U, Bouloux PM, Dattani MT et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11(9):547-564.
6. Dodé C, Hardelin JP. Kallmann syndrome. Eur J Hum Genet. 2009;17(2):139-146.

7. Cano Sokoloff N, Misra M, Ackerman KE. Exercise, Training, and the Hypothalamic-Pituitary-Gonadal Axis in Men and Women. Front Horm Res. 2016;47:27-43.
8. Hajder M, Hajder E, Dervisefendic M et al. Prolactinomas in infertile women: clinical and endocrine characteristics before and after 24 months of treatment with bromocriptine. Med Arch. 2013;67(3):181-184.
9. Kazmi SRH, Can AS. Luteinizing Hormone Deficiency. [Updated 2021 Jul 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562219/ as on 06.08.2021.
10. Conforti A, Esteves SC, Cimadomo D, et al. Management of Women With an Unexpected Low Ovarian Response to Gonadotropin. Front Endocrinol (Lausanne). 2019;10:387.

Different factors cause reduction in gonadotropin production or action which leads to LH and FSH deficiency (Figure 1).
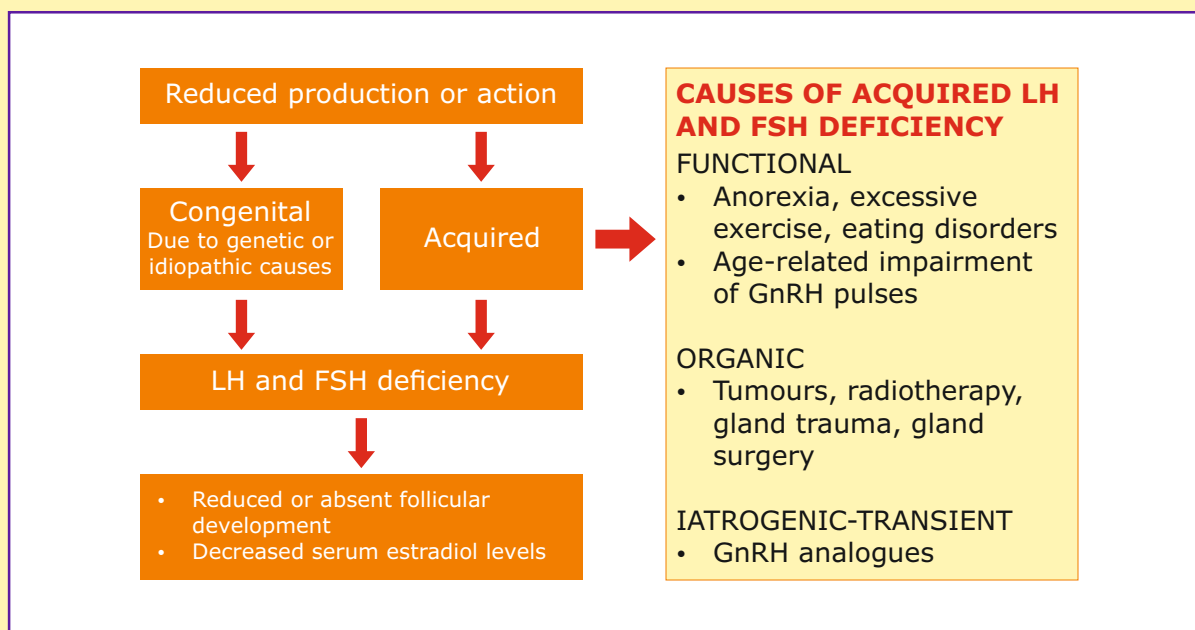

Figure 1. LH and FSH deficiency is a spectrum of conditions with various aetiologies.
Women with LH and FSH deficiency have low/normal gonadotropin levels and low estradiol levels
Figure adapted from Hum Reprod. 2021;36(6):1469-1480.

Suppressed gonadotropin secretion is also seen in anorexia/eating disorders.

In addition, poorly controlled diabetes and thyroid levels may present as oligomenorrhea or amenorrhea from reduced GnRH drive.

For the restoration of the reproductive function, patients should be carefully diagnosed and properly managed to prevent both short- and long-term medical consequences.

Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder caused by the deficient production, secretion or action of gonadotropin-releasing hormone. Clinically, the disorder is characterized by an absence of puberty and infertility.

The Kallmann syndrome (KS), a clinically and genetically heterogeneous disease, combines hypogonadotropic hypogonadism with anosmia.

KAL1 gene, encoding the extracellular glycoprotein anosmin-1, is responsible for the X chromosome-linked recessive form of the disease.

Among the acquired conditions, there is evidence that suppression of the HPG axis could be due to a decrease in GnRH production and a reduced response of LH and FSH during habitual exercise.

Among other acquired conditions, prolactinomas are the most common tumours of the pituitary gland causing gonadal dysfunction and infertility.

High level of prolactin inhibits the secretion of FSH and LH from the anterior pituitary and result in hypogonadism, infertility and galactorrhea.

Sheehan syndrome also leads to panhypopituitarism and the absence of pituitary reproductive hormones (FSH and LH) causing hypogonadotropic hypogonadism.

Overall, in ART, a combination of factors like advance maternal age, genetic polymorphisms affecting the gonadotropins and their receptors might affect the LH and FSH production thus resulting in adverse ovarian stimulation outcomes.

LH and FSH deficiency in Medically Assisted Reproduction

Severe FSH and LH deficiency due to WHO type I anovulation (Hypogonadotropic Hypogonadism, HH)

1.Carone D, Caropreso C, Vitti A et al. 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;35(11):996-1002.

In a study by Carone D et al, the efficacy of r-hFSH plus r-hLH in a 2:1 ratio was compared with hMG-HP in women with Hypogonadotropic Hypogonadism. Participants received 150 IU of r-hFSH and 75 IU r-hLH daily (2:1) or 150 IU hMG-HP daily. Patients were initially treated for one cycle (series A), and who did not become pregnant during the first cycle were treated for a further optional one (series B), or two series (series C) of cycles, with the same criteria of randomization. In the r-hFSH/r-hLH group, 15 out of 17 patients became pregnant (88%) and only 27 stimulation cycles were needed on three repeated series. While in the hMG-HP group, a total of 43 cycles were necessary on three repeated series to obtain 10 pregnant patients out of 18 (Figure 1). There was

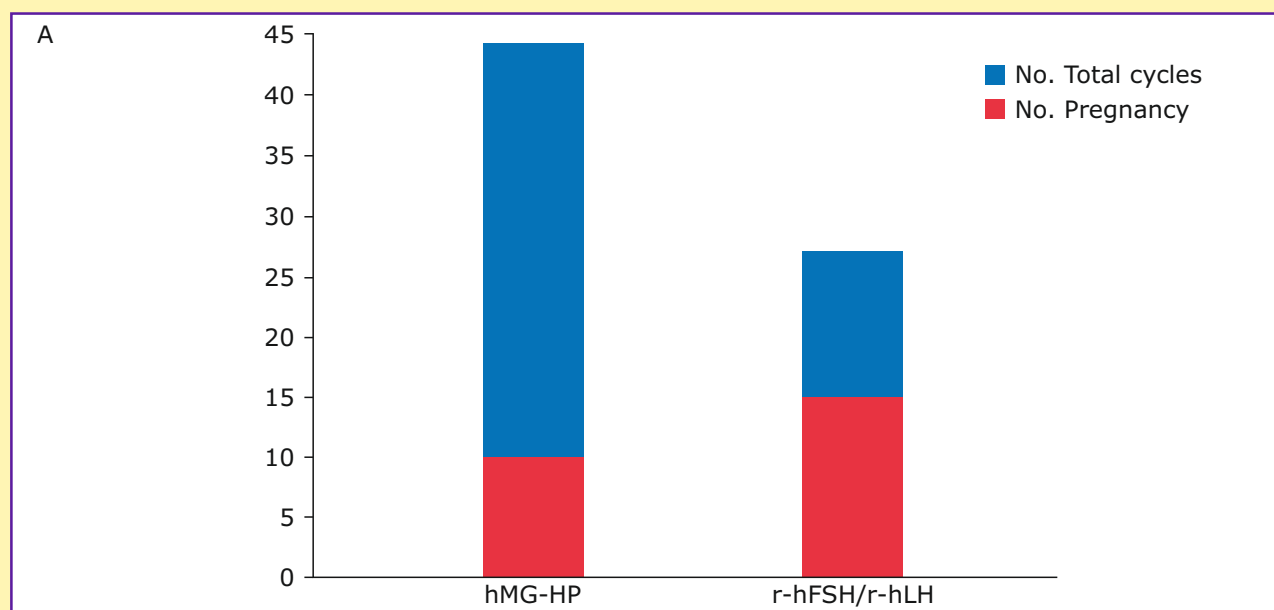Figure 1. Panel A - Improved pregnancy rate and lesser stimulation cycles in the r-hFSH/r-hLH group vs hMG-HP group

a non-significant difference in the number of follicles (no. follicles: 4.4 vs 5.4; p=0.06) between both the arms, with requirement of significantly less IU of LH (961.1 IU vs 1882.5 IU; p<0.001) with rFSH/rhLH in 2:1 ratio vs hMG-HP. Use of rFSH/rhLH in 2:1 ratio produced a significantly

higher pregnancy rate (55.6% vs 23.3%; p<0.05) compared to the hMG-HP arm (Figure 2).
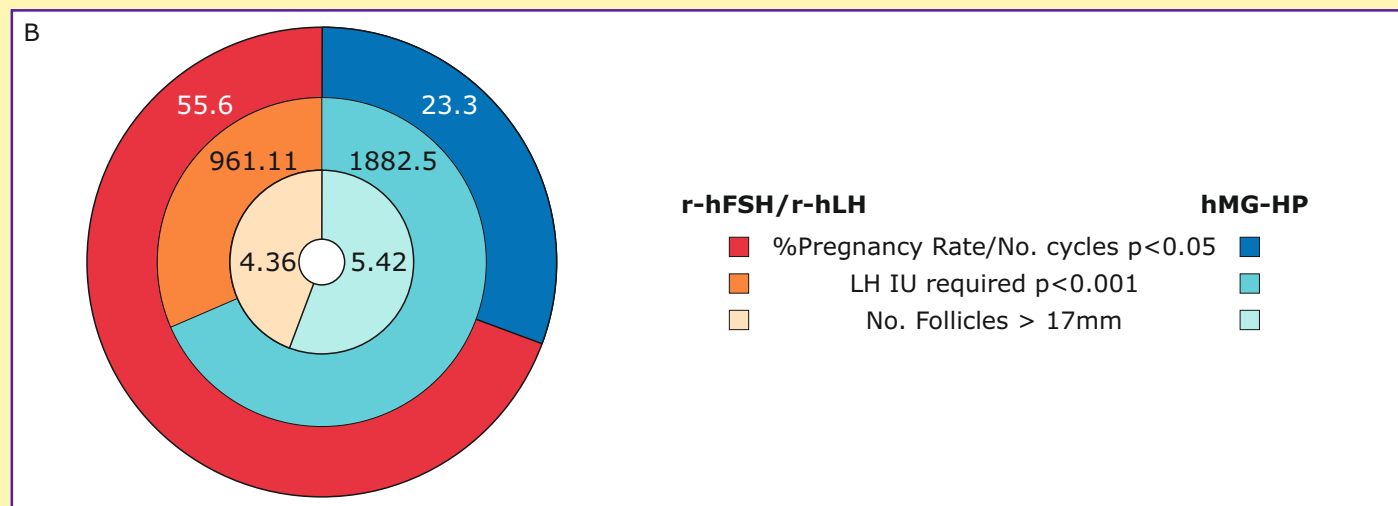

Figure 2. Panel B - Significantly higher pregnancy rate and lesser IU of LH in the r-hFSH/r-hLH group vs hMG-HP group

This study showed the superiority of LH compared to hCG (hMG-HP)in supporting FSH-induced follicular development in HH women.

LH and FSH deficiency induced by GnRH analogue protocols

1. Huirne JA, van Loenen AC, Schats R et al. Dose-finding study of daily GnRH antagonist for the prevention of premature LH surges in IVF/ICSI patients: optimal changes in LH and progesterone for clinical pregnancy. Hum Reprod. 2005;20(2):359-367.
2. Speroff L et al. (1999) Clinical Gynecologic Endocrinology and Infertility. 6th edn. Lippincott Williams & Wilkins.
3. Sonntag B, Kiesel L, Nieschlag E, Behre HM. Differences in serum LH and FSH levels using depot or daily GnRH agonists in controlled ovarian stimulation: influence on ovarian response and outcome of ART. J Assist Reprod Genet. 2005;22(7-8):277-283.
4. Hugues JN, Massé-Laroche E, Reboul-Marty J et al. Impact of endogenous luteinizing hormone serum levels on progesterone elevation on the day of human chorionic gonadotropin administration. Fertil Steril. 2011;96(3):600-604.
5. Depalo R, Jayakrishan K, Garruti G et al. GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET). Reprod Biol Endocrinol. 2012 Apr 13;10:26.
6. Kumar P, Sharma A. Gonadotropin-releasing hormone analogs: Understanding advantages and limitations. J Hum Reprod Sci. 2014;7(3):170-174.
7. Kol S. Individualized treatment from theory to practice: the private case of adding LH during GnRH antagonist-based stimulation protocol. Clin Med Insights Reprod Health. 2014;8:59-64.
8. Kol S, Homburg R. Change, change, change: hormonal actions depend on changes in blood levels. Hum Reprod. 2008;23(5):1004-1006.
9. Garcia-Velasco JA, Coelingh Bennink HJ, Epifanio R et al. High-dose recombinant LH add-back strategy using high-dose GnRH antagonist is an innovative protocol compared with standard GnRH antagonist. Reprod Biomed Online. 2007;15(3):280-287.
10. Pezzuto A, Ferrari B, Coppola F et al. LH supplementation in down-regulated women undergoing assisted reproduction with baseline low serum LH levels. Gynecol Endocrinol. 2010;26(2):118-124.
11. Alviggi C, Conforti A, Esteves SC et al. for the International Collaborative Group for the Study of r-hLH (iCOS-LH). Recombinant luteinizing hormone supplementation in assisted reproductive technology: a systematic review. Fertil Steril. 2018;109(4):644-664.
12. 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 (Lausanne). 2018;9:589.
13. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod. 2000;15(5):1003-1008.
14. Lahoud R, Al-Jefout M, Tyler J et al. A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates. Hum Reprod. 2006;21(10):2645-2649.
15. Balasch J, Vidal E, Peñarrubia J et al. Suppression of LH during ovarian stimulation: analysing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2001;16(8):1636-1643.
16. Humaidan P, Bungum L, Bungum M et al. Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod. 2002;17(8):2016-2021.

A certain LH threshold needs to be achieved for adequate folliculogenesis and steroidogenesis, which is required for successful fertilization and implantation. However, published evidence says that severe suppression of LH is associated with impaired IVF outcome. The figure outlines the GnRH analogue protocols impact circulating LH (Figure 1).
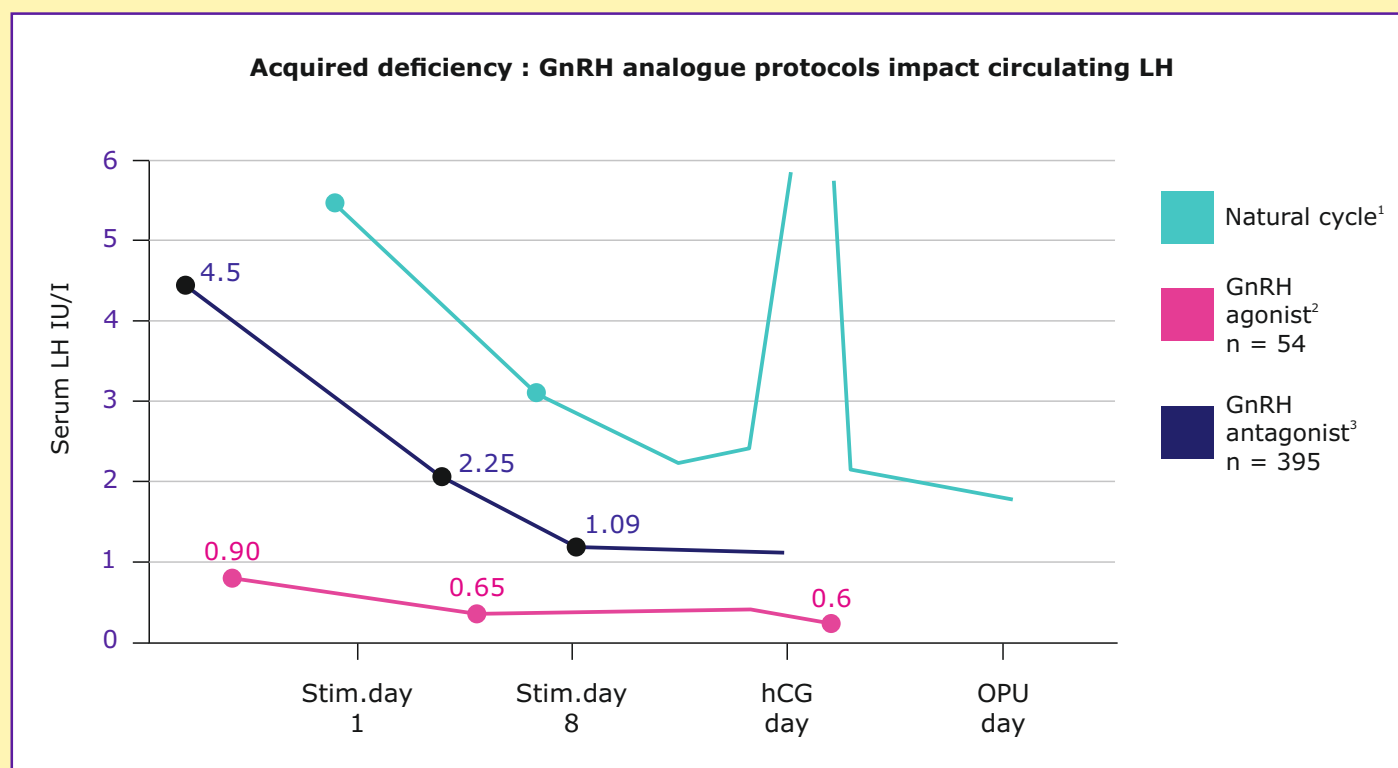

Acquired deficiency : GnRH analogue protocols impact circulating LH

Figure 1. Serum LH in GnRH analogue protocols vs natural cycle
Figure adapted from 1. Speroff L, et al. (1999) Clinical Gynecologic Endocrinology and Infertility. 6th edn. Lippincott Williams & Wilkins. 2. Sonntag B et al. J Assist Reprod Genet. 2005;22:277-83. 3. Hugues J et al. Fertil Steril. 2011;96:600-604.

Several protocols are actually available for IVF and ET. GnRH agonists and GnRH antagonists are used for suppression of FSH and LH secretion resulting in enhancement of follicular recruitment, allowing the recovery of a larger number of oocytes.

The actions of GnRH agonists and GnRH antagonists differ. GnRH agonists produce an initial stimulation of pituitary gonadotrophs that results in secretion of FSH and LH and the expected gonadal response. This response is followed by down-regulation and inhibition of the pituitary-gonadal axis.

On the contrary, GnRH antagonists promptly suppress pituitary gonadotropin by GnRH-receptor competition, thereby avoiding the initial stimulatory phase of the agonists. Discontinuation of GnRH antagonist treatment leads to a rapid and predictable recovery of the pituitary-gonadal axis. Studies have reported about the profound suppression of LH with use of GnRH antagonists.

Kol et al., in a prospective, single center, non-randomized, proof-of-concept study showed that there was an "over suppression" (if LH was <50% of the pre-injection level) of LH in 26% patients after GnRH antagonist administration compared to the "normal" responders. In these patients, there was a significant decrease in estradiol rise during the first 24 hours after initial antagonist administration (Table 1). This effect was reversed for the rest of the stimulation period during which recombinant LH was added to the "over-suppressed" population.

Table 1. Comparison of LH and E_2 in the study

	Over Suppressed n = 12 Mean ± SD	Normal Responders n = 34 Mean ± SD	p^c
E_2 before cetrorelix (pmol/L)	2138 ± 1500	1749 ± 736	0.24
*P before cetrorelix (nmol/l)	1.57 (1.19–2.4)	2.36 (1.9–3.0)	0.053
*LH before cetrorelix (IU/l)	3.05 (1.5–5.8)	2.08 (1.40–2.80)	0.051
E_2 24 hours after cetrorelix (pmol/L)	2452 ± 1755	2384 ± 1021	0.88
*P 24 hours after cetrorelix (nmol/l)	1.60 (1.19–2.09)	2.40 (1.81–3.48)	0.006
*LH 24 hours after cetrorelix (IU/l)	1.1 (0.8–1.55)	1.5 (1.07–2.01)	0.11
*Decrease in LH levels 24 hours after cetrorelix (%)	63 (55.3–79.0)	29.5 (14.5–33.0)	<0.0001
*E_2 increment per oocyte first 24 h[a] (pmol/L)	25.4 (4.04–51.05)	57.7 (27.8–102.1)	0.009
E_2 trigger day (pmol/L)	6571 ± 3678	5454 ± 2436	0.25
E_2 increment per oocyte total[b] (pmol/L)	428.4 (155.5–704.5)	276.5 (185.4–394.2)	0.29

Notes: [a]E_2 level 24 hours after the first cetrorelix dose, minus E_2 before the first cetrorelix dose divided by oocytes retrieved.
[b]E_2 level on trigger day minus E_2 level 24 hours after the first cetrorelix dose divided by oocytes retrieved. [c]p value by Student's t-test. *Statistical significant by Mann–Whitney U test (median, 25%–75% Interquartile).
Abbreviations:P, progesterone; LH, luteinizing hormone; FSH, follicle stimulating hormone.

Table adapted from Clin Med Insights Reprod Health. 2014;8:59-64.

There is no clarity why the strong suppression of LH levels in response to GnRH antagonists is observed only in some subgroups of patients. It might also be perceived that it is due to the individual response of the patient.

Researchers hypothesize that the drop in LH level is clinically significant, not the absolute level itself. The severity of the LH deficiency caused by GnRH antagonist treatment is linked to the magnitude of suppression over time versus the baseline.

Studies of Garcia-Valesco etal., and Pezzuto et al., have shown that with GnRH antagonist or agonist protocol, a combination of r-hFSH and r-hLH can improve ovarian stimulation in women with temporary LH and FSH deficiency.

Researchers opine that woman of advanced maternal age or the hypo responders to ovarian stimulation who are more prone to LH and FSH deficiency should be considered to evaluate the benefits of recombinant LH and FSH protocols.

In a retrospective study, Westergaard et al., evaluated the impact of suppressed concentrations of circulating LH during OS on the outcome of IVF or ICSI treatment. The study included 200 consecutive, normogonadotrophic women. A standard stimulation protocol with mid-luteal GnRH agonist down-regulation and ovarian stimulation with recombinant FSH was used in all cases.

A threshold value of serum LH of 0.5 IU/l on stimulation day 8 (S8) was chosen to discriminate between women with low or 'normal' LH concentrations.

The results showed a lower serum estradiol concentration, which on S8 was significantly lower than in the normal LH group (1349 ± 101 (<0.5 IU/l) vs. 2908 ± 225 (>0.5 IU/l) pmol/l; p<0.001). There was a significant 5 times increased pregnancy loss in the low LH group and a significantly poorer chance of delivery than in the normal LH group (Table 2). The researchers concluded that in women with profoundly suppressed LH levels, there was a compromise in the treatment outcome.
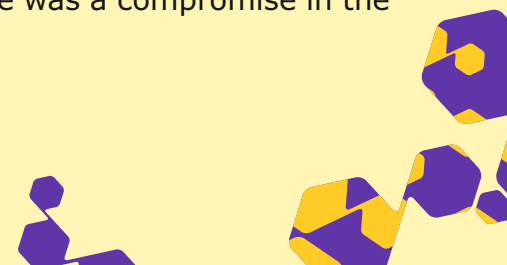
Table 2. Pregnancy loss and deliveries in the study

	Concentration of LH (IU/l) on stimulation day 8		
	<0.5	>0.5	Total
Early pregnancy loss[a] (n)	13	3	16
% of positive tests	45[c]	9[c]	25
% of clinical pregnancies[b]	36[c]	3[c]	17
Deliveries (n)	15	27	42
% of positive tests	52[d]	77[d]	66
% of clinical pregnancies	60	82	72

[a]Including biochemical pregnancies and clinical spontaneous abortions, but excluding ectopic pregnancies; [b]Including clinical spontaneous abortions but excluding biochemical and ectopic pregnancies; [c]$p < 0.005$; [d]$p < 0.05$.

Table adapted from Hum Reprod. 2000;15(5):1003-1008.

In a prospective study, Lahoud et al., evaluated the effect of early and mid-follicular LH concentrations on the ovarian response and pregnancy outcomes in 701 women receiving pituitary down-regulation with a GnRH agonist and OS with rFSH during IVF/ICSI treatment. There were 2 groups in the study on the basis of LH concentrations on stimulation day 7/8: LH < 1.2 IU/l (n = 179) and LH ≥ 1.2 IU/l (n = 522). Cycle outcomes were also compared on the basis of a ratio of mid- to early-follicular LH concentrations (≤0.5, n= 210; >0.5, n=491).

According to the results, a reduction of ≥50% early-to-mid follicular LH concentrations resulted in significant reduction in live birth rates (Table 3).

Table 3. Reduction in live birth rate with reduction of ≥50% early-to-mid follicular LH concentrations

	LH ratio > 0.5 (n = 491)	LH ratio ≤ 0.5 (n = 210)	Statistical comparison (p-value)
Live birth rate per transfer	27.3% (109/399)	19.0% (33/174)	<0.05
Live birth rate per cycle started	22.2% (109/491)	15.8% (33/209)	<0.05

Table adapted from Hum Reprod. 2006;21(10):2645-2649.

A drop in LH level is clinically significant during the ovarian stimulation and can result in sudden drop in precursor availability resulting in insufficient E_2 production by the growing follicles, manifested as a drop in circulating E_2 levels.

However, 2 studies by Balasch et al., and Humaidan et al., showed no differences in ovarian response and pregnancy outcome with suppression of LH during OS.

These studies are suggestive that there is no consensus regarding the extent of the serum LH levels that can predict positive outcome with GnRH agonist cycles. It should also be noted that the threshold values of the LH varied in the three studies discussed (from <0.5 to <1.5 IU/l LH).

Reduced LH and FSH action in advanced maternal age women

1. Vihko KK, Kujansuu E, Mörsky P et al. Gonadotropins and gonadotropinreceptors during the perimenopause. Eur J Endocrinol. 1996;134:357-361.
2. Mitchell R, Hollis S, Rothwell C et al. Age related changes in the pituitary-testicular axis in normal men; lower serum testosterone results from decreased bioactive LH drive. Clin Endocrinol (Oxf). 1995;42(5):501-507.
3. Marrs R, Meldrum D, Muasher S et al. Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. Reprod Biomed Online. 2004;8(2):175-182.

4. Humaidan P, Bungum M, Bungum L et al. Effects of recombinant LH supplementation in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with recombinant FSH: an opening study. Reprod Biomed Online. 2004;8(6):635-643.
5. Bosch E, Labarta E, Crespo J et al. Impact of luteinizing hormone administration on gonadotropin-releasing hormone antagonist cycles: an age-adjusted analysis. Fertil Steril. 2011;95(3):1031-1036.
6. Marrama P, Montanini V, Celani MF et al. Decrease in luteinizing hormone biological activity/immunoreactivity ratio in elderly men. Maturitas. 1984;5(4):223-231.
7. Piltonen T, Koivunen R, Ruokonen A et al. Ovarian age-related responsiveness to human chorionic gonadotropin. J Clin Endocrinol Metab. 2003;88(7):3327-3332.
8. Hurwitz JM, Santoro N. Inhibins, activins, and follistatin in the aging female and male. Semin Reprod Med. 2004;22(3):209-217.
9. Younis JS, Izhaki I, Ben-Ami M. The effect of rLH supplementation to the GnRH-antagonist protocol on endocrine dynamics in the advanced reproductive age. J Endocrinol Invest. 2017;40(8):831-839.
10. Matorras R, Prieto B, Exposito A et al. Mid-follicular LH supplementation in women aged 35-39 years undergoing ICSI cycles: a randomized controlled study. Reprod Biomed Online. 2009;19(6):879-887.
11. Ata B, Kaplan B, Danzer H et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. Reprod Biomed Online. 2012;24(6):614-620.
12. Ruvolo G, Bosco L, Pane A et al. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. Fertil Steril. 2007;87(3):542-546.
13. Huang Y, Zhao Y, Yu Y et al. Altered amphiregulin expression induced by diverse luteinizing hormone receptor reactivity in granulosa cells affects IVF outcomes. Reprod Biomed Online. 2015;30(6):593-601.

LH and age-related changes: Figure 1 outlines the need of LH supplementation in advanced age.
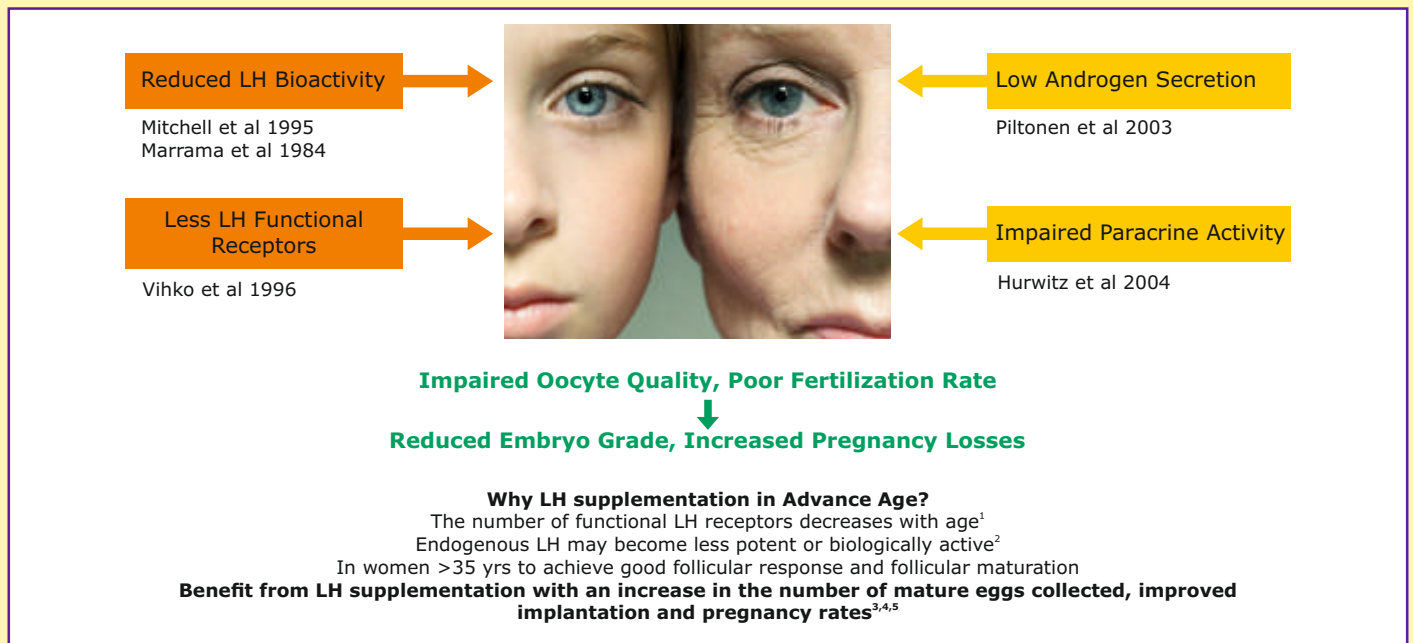
Figure 1. LH Activity addition in poor responders & advanced age group
Figure developed from 1.Vihko KK et al. Eur J Endocrinol. 1996;134:357-361; 2.Mitchell R et al. Clin Endocrinol (Oxf).1995;42(5):501-507; 3.Marrs R et al. Reprod Biomed Online. 2004;8(2):175-182; 4.Humaidan P et al. Reprod Biomed Online. 2004;8(6):635-643; 5.Bosch E et al. Fertil Steril. 2011;95(3):1031-1036.

A plethora of studies have shown the reduced endogenous LH concentrations in women of advanced maternal age and the supplementation of LH/rLH to FSH/rFSH (co-treatment) would lead to improved ART outcomes. Endogenous concentrations of LH are reduced in women undergoing down-regulation with gonadotrophin-releasing hormone agonists (GnRHa) and ovarian stimulation with recombinant human FSH (r-hFSH).

A study by Humaidan et al., showed that combination of r-hFSH and r-hLH seemed to benefit treatment outcome for women ≥35 years of age with significantly reduced total FSH consumption and significantly increased implantation rates (Table 1).

Table 1. Reduced total FSH consumption and increased implantation rates with combination of r-hFSH and r-hLH

	LH supplementation		No supplementation	
	<35 years	≥35 years	<35 years	≥35 years
Duration of FSH stimulation (days)	11.2	10.6	11.2	10.9
Implantation rate (%)	44/143 (30.8)	12/33 (36.4/)[a]	49/157 (31.2)[b]	4/30 (13.3)[a,b]

[a]p < 0.05 (Fisher's exact test), [b]p < 0.03 (Fisher's exact test)

Table adapted from Reprod Biomed Online. 2004;8(6):635-643.

In a study by Younis et al, addition of rLH to rFSH following GnRH-antagonist treatment in women with advanced maternal age compensated for the LH deficiency observed in women above 35 years.

Among women receiving rFSH only, serum LH levels dropped ≤2, ≤1 and ≤0.5 mIU/mL in 71.4, 46.4, and 28.6% of cases, while this occurred only in 38.7% (p=0.01), 6.5% (p=0.0004) and 3.2% (p=0.007) of women receiving combined rFSH and rLH treatment, respectively (Figure 2).
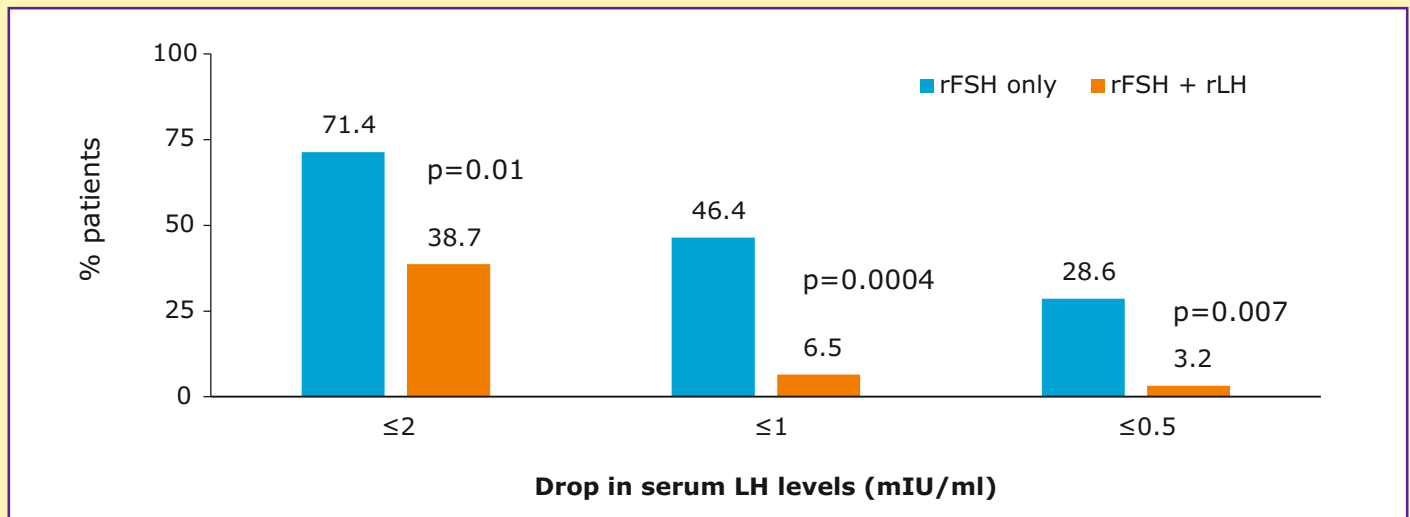

Figure 2. Compensation of LH deficiency in patients receiving combined rFSH and rLH treatment

Motarras et al., in a single-centre, randomized, parallel group, comparative study aimed to identify potential benefits of mid-follicular recombinant human LH (r-hLH) supplementation in women aged 35-39 years undergoing ovarian stimulation for ICSI.

The results showed higher rates of implantation and live birth per started cycle with r-hLH supplementation than with r-hFSH alone (Table 2).

Table 2. Higher Implantation rate and Live birth rate with r-hFSH+r-hLH vs. r-hFSH alone

	r-hFSH (n = 68)	r-hFSH + r-hLH (n = 63)	p-value
Implantation rate	11.3 (16/141)	18.1 (26/144)	0.049
Live birth rate per started cycle	7.4 (5/68)	19.0 (12/63)	0.047
Live birth rate per transfer	9.3 (5/54)	21.4 (12/56)	NS

Table adapted from Reprod Biomed Online. 2009;19(6):879-887.

In a randomized, open-label, controlled trial performed in two age subgroups [(up to 35 years old (n=380) and aged 36 to 39 years (n=340)], rFSH versus rFSH + rLH administration was compared.

Recombinant LH administration significantly increased the implantation rate in patients aged 36 to 39 years (rFSH + rLH group: 26.7% versus 18.6% rFSH alone, OR 1.56 (95% CI 1.04–2.33; Figure 3).
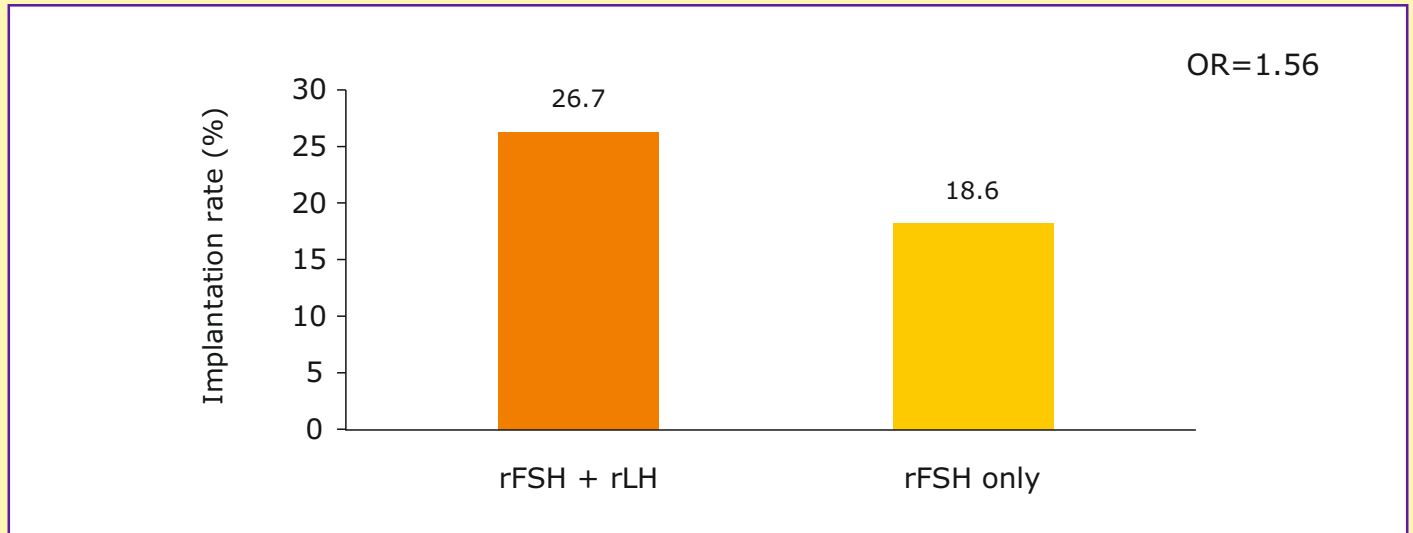

Figure 3. Improved implantation rate in patients receiving rFSH + rLH vs rFSH alone

Ata et al., in a retrospective study showed that increasing female age was associated with a significant decrease in euploidy rate of day-3 and day-5 embryos.

LH supplementation benefits in women on advance maternal age may be attributed to its several beneficial effects on oocytes and embryo.

Studies have shown that supplementation with r-LH improves the chromatin quality of cumulus cells involved in the control of oocyte maturation.

Huang et al., demonstrated that altered AREG (Amphiregulin, specific gene involved in peri-ovulatory signalling pathways) expression induced by diverse LH receptor reactivity in granulosa cells may provide a useful marker for oocyte developmental competency.

Hypo-response to ovarian stimulation due to reduced LH and FSH action

1. Conforti A, Esteves SC, Cimadomo D et al. Management of Women With an Unexpected Low Ovarian Response to Gonadotropin. Front Endocrinol (Lausanne). 2019;10:387.
2. 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 (Lausanne). 2018;9:589.
3. Bosch E, Alviggi C, Lispi M et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.
4. Conforti A, Esteves SC, Di Rella F et al. The role of recombinant LH in women with hypo-response to controlled ovarian stimulation: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2019;17(1):18. [published correction appears in Reprod Biol Endocrinol. 2019 Mar 14;17(1):31].
5. Genro VK, Grynberg M, Scheffer JB et al. Serum anti-Müllerian hormone levels are negatively related to Follicular Output RaTe (FORT) in normo-cycling women undergoing controlled ovarian hyperstimulation. Hum Reprod. 2011;26(3):671-677.

Ovarian hypo-responsiveness to gonadotropin stimulation remains an undervalued issue in ART. Approximately 10% of women defined as normal responders require a higher than expected total dosage of gonadotropin to promote adequate follicular development. The pathophysiology

mechanisms explaining the hypo-response remains poorly understood.

Ovarian hypo-response can be defined as an unexpected slow response or stagnated follicle growth during OS with adequate FSH monotherapy or higher than expected dose of gonadotropins (FSH) depending on age, BMI and ovarian reserve.

A systematic review and meta-analysis study by Conforti et al., showed that significantly higher clinical pregnancy rates (Figure 1), implantation rates and number of oocytes retrieved were observed in hypo-responders supplemented with recombinant LH versus hypo-responders who underwent FSH monotherapy.
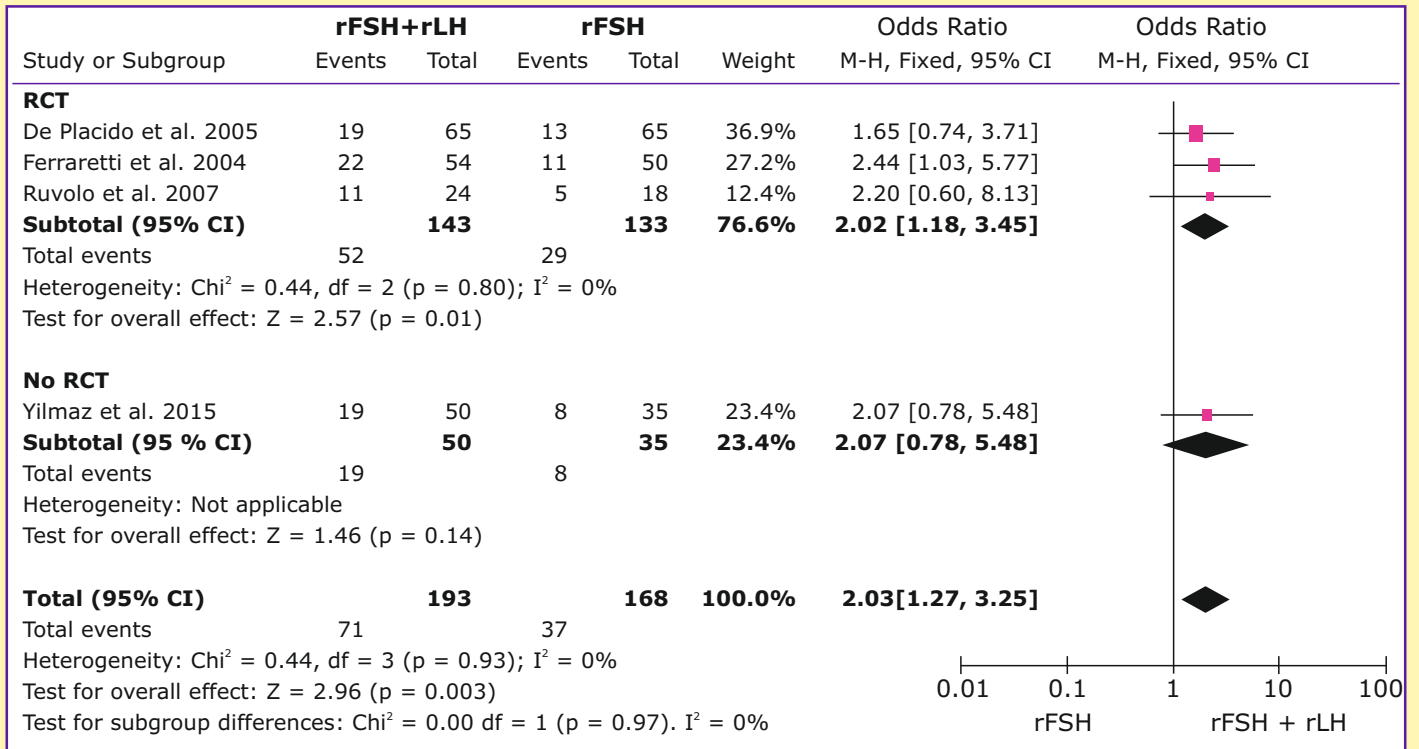
Study or Subgroup	rFSH+rLH		rFSH		Weight	Odds Ratio M-H, Fixed, 95% CI	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total			
RCT							
De Placido et al. 2005	19	65	13	65	36.9%	1.65 [0.74, 3.71]	
Ferraretti et al. 2004	22	54	11	50	27.2%	2.44 [1.03, 5.77]	
Ruvolo et al. 2007	11	24	5	18	12.4%	2.20 [0.60, 8.13]	
Subtotal (95% CI)		**143**		**133**	**76.6%**	**2.02 [1.18, 3.45]**	
Total events	52		29				
Heterogeneity: Chi^2 = 0.44, df = 2 (p = 0.80); I^2 = 0%							
Test for overall effect: Z = 2.57 (p = 0.01)							
No RCT							
Yilmaz et al. 2015	19	50	8	35	23.4%	2.07 [0.78, 5.48]	
Subtotal (95 % CI)		**50**		**35**	**23.4%**	**2.07 [0.78, 5.48]**	
Total events	19		8				
Heterogeneity: Not applicable							
Test for overall effect: Z = 1.46 (p = 0.14)							
Total (95% CI)		**193**		**168**	**100.0%**	**2.03[1.27, 3.25]**	
Total events	71		37				
Heterogeneity: Chi^2 = 0.44, df = 3 (p = 0.93); I^2 = 0%							
Test for overall effect: Z = 2.96 (p = 0.003)							
Test for subgroup differences: Chi^2 = 0.00 df = 1 (p = 0.97). I^2 = 0%							

0.01 0.1 1 10 100
rFSH   rFSH + rLH

Figure 1. Forest plot of Odds Ratio for the clinical pregnancy rate in rFSH+ rLH vs rFSH alone treatment
Figure adapted from Reprod Biol Endocrinol. 2019;17(1):18.

Recently, two indices based on individual ovarian reserve have been proposed to measure the ovarian response to OS. **One is the FORT and the other is FOI.**

FORT – (Follicular Output RaTe) = ratio of pre-ovulatory follicle count on day of hCG × 100/small antral follicle count at baseline.

FOI ([Follicle to Oocyte Index) = Oocyte number/Antra Follicle Count x 100, which is the ratio between the total number of oocytes collected at the end of OS, and the number of antral follicles available at the start of stimulation.

These two indices help to identify the direct response of the individual to OS and can help to identify a hypo-response to OS better than oocyte number alone.

Reduced LH and FSH action in ovarian stimulation due to genetic variants

Perez Mayorga M, Gromoll J, Behre HM et al. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000;85(9):3365-3369.

Although the pathophysiology of the hypo-response to gonadotropin stimulation is not fully understood, it is believed that in some cases a hypo-response may be associated with LH and FSH single nucleotide polymorphisms (SNPs) and their receptors.

LH gene polymorphisms/variants

Alviggi C, Pettersson K, Longobardi S et al. A common polymorphic allele of the LH beta-subunit gene is associated with higher exogenous FSH consumption during controlled ovarian stimulation for assisted reproductive technology. Reprod Biol Endocrinol. 2013 Jun 1;11:51.

Alviggi et al., demonstrated that a common polymorphic allele of the LH beta-subunit gene (V betaLH) was associated with higher exogenous FSH consumption during COS.
The v-betaLH polymorphism represents a biologically less active form of LH, unable to adequately support FSH activity during follicular stimulation.

FSH gene polymorphisms

1. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. Hum Reprod Update. 2002;8(5):413-421.
2. Schuring AN, Busch AS, Bogdanova N et al. Effects of the FSH-b-subunit promoter polymorphism -211G->T on the hypothalamic-pituitary-ovarian axis in normally cycling women indicate a gender-specific regulation of gonadotropin secretion. J Clin Endocrinol Metab 2013;98:E82–E86.
3. Rull K, Grigorova M, Ehrenberg A et al. FSHB -211 G>T is a major genetic modulator of reproductive physiology and health in childbearing age women. Hum Reprod. 2018;33(5):954-966.
4. Ruth KS, Beaumont RN, Tyrrell J et al. Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and impact female reproductive health. Hum Reprod. 2016;31(2):473-481.
5. Trevisan CM, de Oliveira R, Christofolini DM et al. Effects of a Polymorphism in the Promoter Region of the Follicle-Stimulating Hormone Subunit Beta (FSHB) Gene on Female Reproductive Outcomes. Genet Test Mol Biomarkers. 2019;23(1):39-44.
6. La Marca A, Sighinolfi G, Argento C et al. Polymorphisms in gonadotropin and gonadotropin receptor genes as markers of ovarian reserve and response in in vitro fertilization. Fertil Steril. 2013;99(4):970-8.e1.

Some SNPs in LH and FSH can lead to reduced fertility. Simoni et al, suggested that the FSH receptor genotype can influence the ovarian response to FSH stimulation.
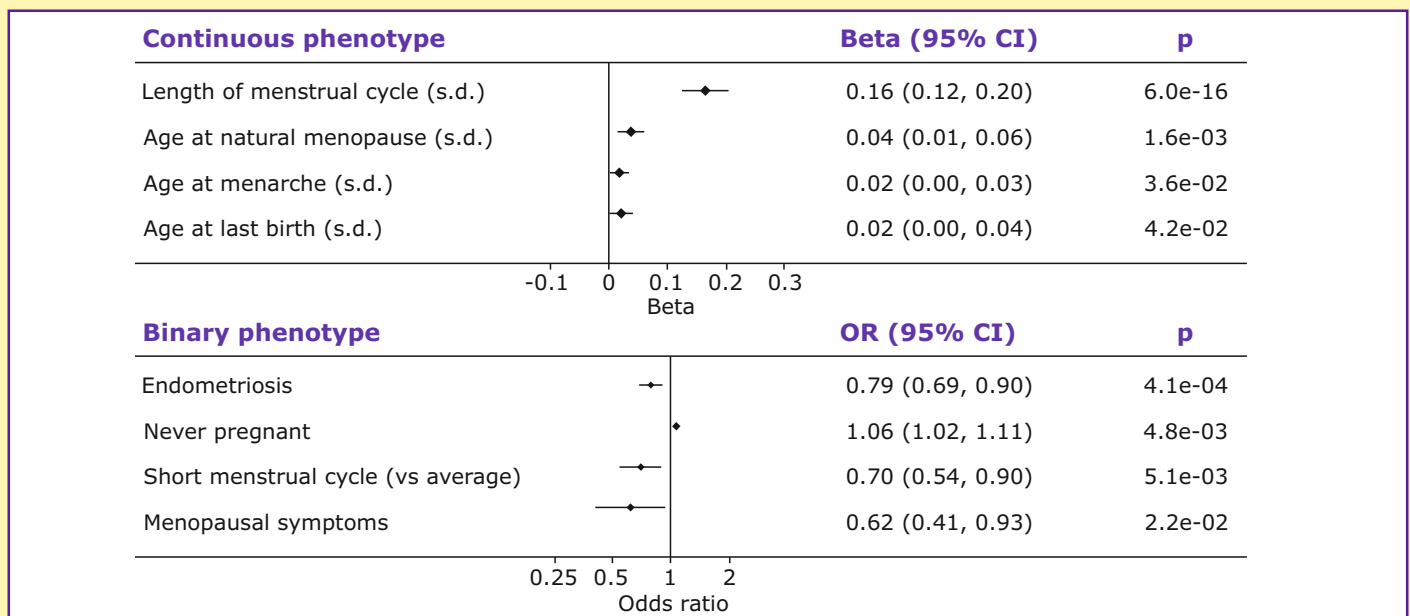
Continuous phenotype	Beta (95% CI)	p
Length of menstrual cycle (s.d.)	0.16 (0.12, 0.20)	6.0e-16
Age at natural menopause (s.d.)	0.04 (0.01, 0.06)	1.6e-03
Age at menarche (s.d.)	0.02 (0.00, 0.03)	3.6e-02
Age at last birth (s.d.)	0.02 (0.00, 0.04)	4.2e-02

-0.1   0   0.1   0.2   0.3
Beta

Binary phenotype	OR (95% CI)	p
Endometriosis	0.79 (0.69, 0.90)	4.1e-04
Never pregnant	1.06 (1.02, 1.11)	4.8e-03
Short menstrual cycle (vs average)	0.70 (0.54, 0.90)	5.1e-03
Menopausal symptoms	0.62 (0.41, 0.93)	2.2e-02

0.25   0.5   1   2
Odds ratio

Figure 1. Forest plot of phenotypes associated ($p < 0.05$) with the FSH-lowering T allele of rs10835638 (c.-211G>T). For continuous variables, effects (β) are in standard deviations of the inverse-normally transformed variable to enable effect size comparisons. CI, confidence interval; OR, odds ratio.
Figure adapted from Hum Reprod. 2016;31(2):473-481.

Schuring et al., in their study reported on polymorphism of FSHB-211GT on the hypothalamic pituitary-ovarian axis in women pointing to a gender-specific compensatory mechanism of gonadotropin secretion.

In a study by Rull et al., accumulating data suggested that the FSHB-211 G>T not only represented a key genetic modulator of female serum gonadotropin concentrations and reproductive physiology but also was a possible contributing factor to other gynaecological diseases.

A cross-sectional study involving 63,350 women showed that a T allele of the FSHB promoter polymorphism was associated with longer menstrual cycles, later age at menopause and lower risk of endometriosis (Figure 1).

Trevisan et al., showed that women carrying the GT genotype (FSH gene) had a poorer response more frequently to COS compared to individuals with the GG genotype.

Different combinations of genetic variants in FSH beta-subunit (FSHB) gene and of FSHR affect menstrual Day 3 serum FSH levels in women of reproductive age.

FSH Receptor Polymorphisms

1. La Marca A, Sighinolfi G, Argento C et al. Polymorphisms in gonadotropin and gonadotropin receptor genes as markers of ovarian reserve and response in in vitro fertilization. Fertil Steril. 2013;99(4):970-8.e1.
2. Desai SS, Roy BS, Mahale SD. Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. Reproduction. 2013;146(6):R235-R248.
3. Alviggi C, Conforti A, Santi D et al. Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: a systematic review and meta-analysis. Hum Reprod Update. 2018;24(5):599-614.
4. Bosch E, Alviggi C, Lispi M et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.
5. Achrekar SK, Modi DN, Desai SK et al. Follicle-stimulating hormone receptor polymorphism (Thr307Ala) is associated with variable ovarian response and ovarian hyperstimulation syndrome in Indian women. Fertil Steril. 2009;91(2):432-439.
6. Trevisan CM, Peluso C, Cordts EB, de Oliveira R, Christofolini DM, Barbosa CP, Bianco B. Ala307Thr and Asn680Ser polymorphisms of FSHR gene in human reproduction outcomes. Cell Physiol Biochem. 2014;34(5):1527-1535.

La Marca et al., showed that the common FSHR polymorphism is a well-established determinant of response to FSH in IVF programs.

SNPS of the FSHR gene have been reported and some of them affect fertility, mainly in females.

It has been observed that specifically, a common SNP of the FSH receptor (FSHR, rs6166) has been associated with increased basal levels of FSH and increased consumption of FSH during COS.

According to the published data, FSHR -29G>A (rs1394205) allele A carriers were found to have fewer FSHRs than allele G carriers. This leads to higher FSH consumption during OS. Researchers have observed that FSHR (rs1394205) AA carriers have a higher FSH consumption in COS than carriers of the GG and AG haplotypes, and therefore, AA carriers may have an impaired response to ovarian stimulation.

Studies also have shown that FSHR c.G919A(rs6165) variant may affect ovarian response but not ovarian reserve.

LH Receptor Polymorphisms

1. Alviggi C, Clarizia R, Pettersson K et al. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. Reprod Biomed Online. 2009;18(1):9-14.
2. Alviggi C, Pettersson K, Longobardi S et al. A common polymorphic allele of the LH beta-subunit gene is associated with higher exogenous FSH consumption during controlled ovarian stimulation for assisted reproductive technology. Reprod Biol Endocrinol. 2013 Jun 1;11:51.
3. Alviggi C, Conforti A, Cariati F et al. Impact of polymorphisms of gonadotropins and their receptors on controlled ovarian stimulation: a prospective observational study. Abstract P-729. Abstracts of the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology. Pi443. Accessed from website https://iris.unimore.it/retrieve/handle/11380/1164896/201141/Alviggi%20et%20al._ESHRE_2016.pdf as on 08.08.2021.

4. Lindgren I, Bååth M, Uvebrant K et al. *Combined assessment of polymorphisms in the LHCGR and FSHR genes predict chance of pregnancy after in vitro fertilization. Hum Reprod. 2016;31(3):672-683.*
5. Bosch E, Alviggi C, Lispi M et al. *Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.*
6. Ramaraju GA, Cheemakurthi R, Prathigudupu K et al. *Role of Lh polymorphisms and r-hLh supplementation in GnRh agonist treated ART cycles: A cross sectional study. Eur J Obstet Gynecol Reprod Biol. 2018;222:119-125.*

Variants of the LH beta chain and of LHCGRs that affect OS have also been identified. The most common polymorphism of LH (LH-B variant: v-BLH) is known to lead to suboptimal response to GnRHa long protocol and also lead to higher exogenous FSH consumption during COS.

A prospective observational study in 100 normogonadotropic IVF/ICSI patients evaluated the impact of polymorphisms of gonadotropins and their receptors on COS.

The results showed that the presence of allele C on both FSHR-min29 and LHCGR-291 caused an increased ratio between the cumulative r-FSH consumption and the total number of oocytes as well as mature oocytes. Specifically, the presence of allele C on these three genes [FSHR-min29 (rs1394205), FSHR (rs6166), and LHCGR-291 (rs12470652)] was related to an increased ratio between the cumulative FSH consumption and the total number of oocytes or mature oocytes.

Lindgren et al., showed that LHCGR S312N (rs2293275) carriers required a higher FSH dosage during OS versus asparagine carriers.

It is thus imperative that function of both gonadotropins is crucial during OS as the polymorphisms of the LH beta chain and of LHCGRs could reduce ovarian sensitivity to FSH.

A cross sectional study by Ramaraju et al., evaluated the role of LH polymorphisms and r-hLH supplementation in GnRH agonist treated ART cycles. The study determined the association between the r-hLH supplementation and LHCGR N312S polymorphism and clinical pregnancy. The study results showed a consistent association between LHCGR N312S polymorphism and a higher requirement for r-hLH in women homozygous and heterozygous for serine.

There was also a significant increase in clinical pregnancy rate in women homozygous or heterozygous for G allele compared to women homozygous for A allele, (GG-56%, AG-57.1% vs. AA-40.8%) after excluding the women with PCOS and endometriosis (p < 0.04).

Impact of polymorphisms: Role of genetic variants and their impact on management strategies

1. Ramaraju GA, Cheemakurthi R, Prathigudupu K et al. *Role of Lh polymorphisms and r-hLh supplementation in GnRh agonist treated ART cycles: A cross sectional study. Eur J Obstet Gynecol Reprod Biol. 2018;222:119-125.*
2. Ramaraju GA, Cheemakurthi R, Kalagara M et al. *Effect of LHCGR Gene Polymorphism (rs2293275) on LH Supplementation Protocol Outcomes in Second IVF Cycles: A Retrospective Study. Front Endocrinol (Lausanne). 2021;12:628169.*

The role of r-hLH supplementation in relation to LHCGR polymorphisms is not studied extensively when compared to FSHR polymorphisms.

In a study on LHCGR N312S polymorphism, the researchers found a strong association between LHCGR polymorphism and the requirement of r-hLH in COS protocols that resulted in higher clinical pregnancy outcome.

Published data indicates that r-hLH and r-hFSH co-administration improves follicle to oocyte yield and pregnancy rates in hypo-responders rather than in poor responders, establishing an individualized pharmacogenomics tool based on LHCGR polymorphism.

Ramaraju et al., examined the impact of a personalized pharmacogenomic approach on LH supplementation on the pregnancy and live birth rate outcomes in comparison with the traditional approaches. About 193 women who underwent a second cycle followed by first unsuccessful IVF cycle were included in the analysis. Patients were divided into two groups: Group-I consisted of 78 patients receiving LH supplementation (LH dosage of 75 IU/day supplemented from day-6

onwards) while Group-II patients were provided with LH supplementation based on their SNP profile in LHCGR (N312S) polymorphism (A/A, A/G, and G/G alleles).

In the Group I, the average biochemical pregnancy rates observed were 43.75%, 34.15%, and 23.81% in patients with genotype A/A, A/G, and G/G, respectively (Figure 1).
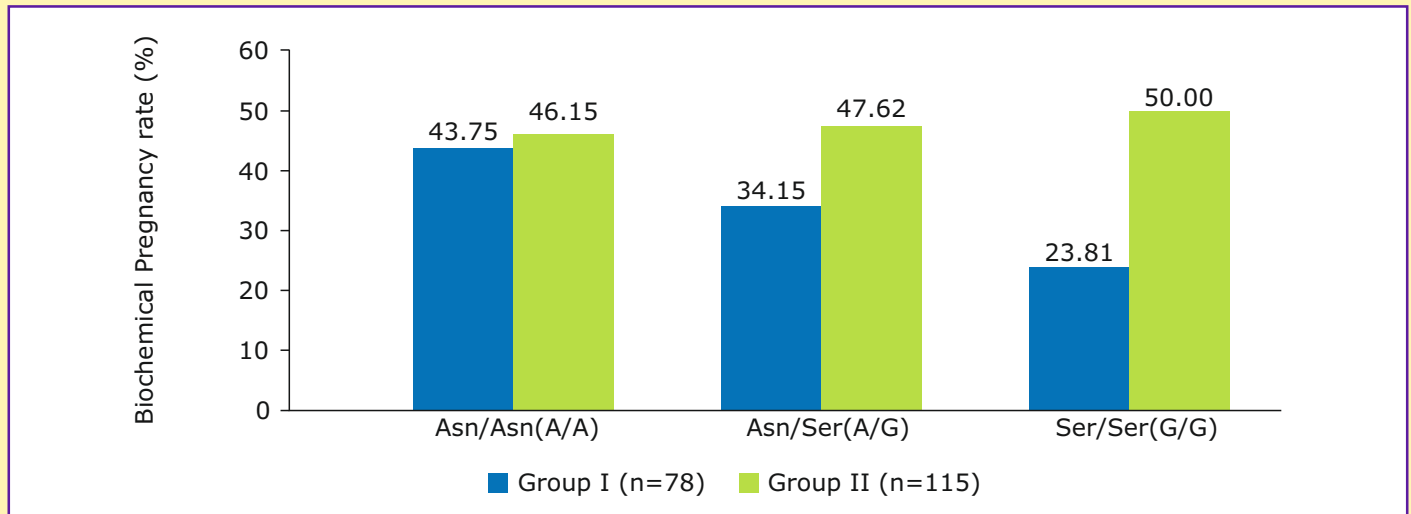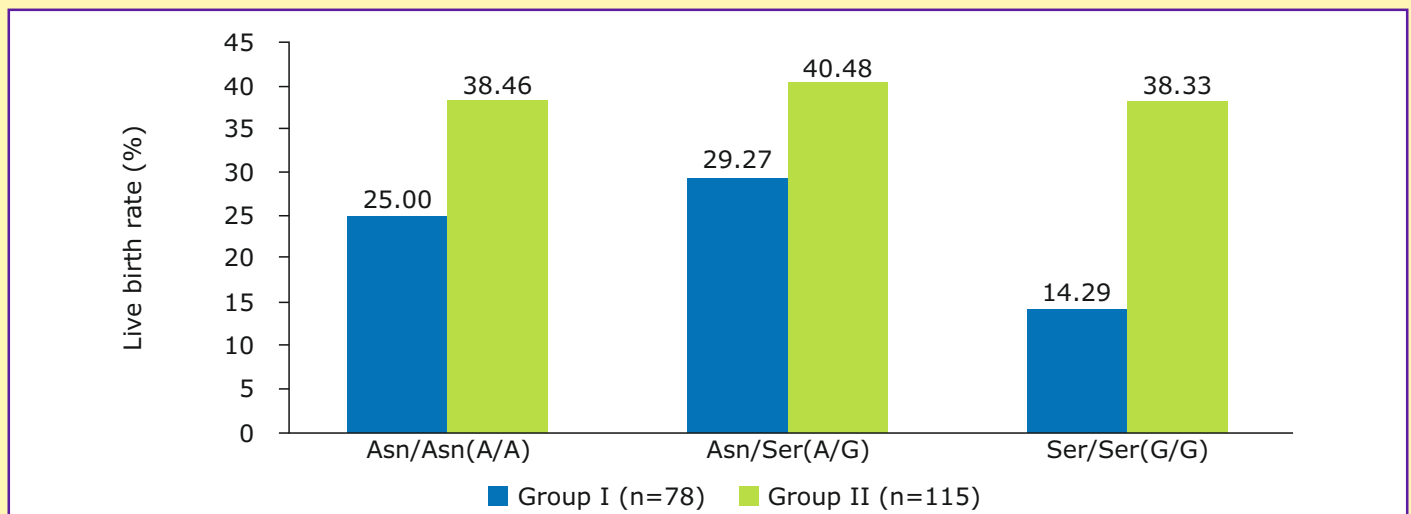

Figure 1. Biochemical pregnancy rate for patients in Groups-I and II in the second cycle based on LHCGR (Asn312Ser) variations
Figure adapted from Ramaraju GA et al. Front Endocrinol (Lausanne). 2021;12:628169.

The biochemical pregnancy rate in Group II did not show significant difference for A/A genotype but showed improved clinical pregnancy rates of 47.62% and 50%, for genotypes A/G and G/G respectively.

The live birth rates in Group-I had 25%, 29.27%, and 14.29% success rate in the second IVF cycle for genotypes A/A, A/G, and G/G, respectively (Figure 2).

An improved live birth rate was observed in Group-II with 38.46%, 40.48%, and 38.33% rates were observed for the three genotypes A/A, A/G, and G/G, respectively.

Figure 2. Live birth rate for patients in Groups-I and II in the second cycle based on LHCGR (Asn312Ser) variations
Figure adapted from Ramaraju GA et al. Front Endocrinol (Lausanne). 2021;12:628169.

This study delivered a strong evidence in support of supplementation of LH to women undergoing IVF, based on their SNP profile (rs2293275) of LHCGR. Th study confirms that personalized interventions in IVF may improve the success rate of fertilization and live births.

Implications for future research

1. Alviggi C, Conforti A, Esteves SC for International Collaborative Group for the Study of r-hLH (iCOS-LH). Recombinant luteinizing hormone supplementation in assisted reproductive technology: a systematic review. Fertil Steril. 2018;109(4):644-664.
2. Bosch E, Alviggi C, Lispi M et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021;36(6):1469-1480.
3. Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), Alviggi C, Andersen CY, Buehler K et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril. 2016;105(6):1452-1453.
4. Genro VK, Grynberg M, Scheffer JB et al. Serum anti-Müllerian hormone levels are negatively related to Follicular Output RaTe (FORT) in normo-cycling women undergoing controlled ovarian hyperstimulation. Hum Reprod. 2011;26(3):671-677.
5. 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 (Lausanne). 2018;9:589.
6. Vermey BG, Chua SJ, Zafarmand MH et al. Is there an association between oocyte number and embryo quality? A systematic review and meta-analysis. Reprod Biomed Online. 2019;39(5):751-763.
7. Esteves SC, Alviggi C, Humaidan P, et al. The POSEIDON Criteria and Its Measure of Success Through the Eyes of Clinicians and Embryologists. Front Endocrinol (Lausanne). 2019;10:814.
8. Alviggi C, Conforti A, Santi D et al. Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: a systematic review and meta-analysis. Hum Reprod Update. 2018;24(5):599-614.
9. Mol BW, Bossuyt PM, Sunkara SK et al. Personalized ovarian stimulation for assisted reproductive technology: study design considerations to move from hype to added value for patients. Fertil Steril. 2018;109(6):968-979.

LH and FSH deficiency could be caused due to various factors as discussed above.

In women of advanced maternal age and with a hypo-response to OS unfavorable outcomes may ensue due to various underlying causes of LH and FSH deficiency. In this class of patients, a combination of r-hFSH:r-hLH used for OS is known to improve the ART outcomes.

An application of these aspects in larger population studies hallmarks the beginning of a scientific based approach for the identification of LH and FSH deficiency at the genetic level.

The concept of POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) classification, clinical endpoints like FOI and FORT are driving the ART programs towards more individualized approach and stratifying the patients based on their response to gonadotropin stimulation and retrieving the highest number of oocytes for each cycle and each patient. Such an approach may lead to increased probability of live birth as a recent meta-analysis on 291,752 ART cycles confirms a strong positive association between oocytes retrieved and top/good-quality day 2/3 embryos.

However, in reality, low gonadotropin dosing or suboptimal gonadotropin regimen might result in hypo-response and the retrieval of fewer than expected oocytes. This phenomenon can be better appreciated in POSEIDON groups 1 and 2, who despite adequate pre-stimulation ovarian parameters end up having a poor or suboptimal oocyte yield, possibly due to inappropriate gonadotropin dosing/regimen and/or the presence of **genetic polymorphisms affecting the gonadotropins and their receptors.**

A recently published meta-analysis by Alviggi et al., showed that specific SNPs of the gonadotrophins and their receptors influence COS outcomes. This evidence is supported by a large number of trials mainly devoted to FSHR (rs6165) and FSHR (rs6166) polymorphisms.

While a personalized reproductive medicine is still in its infancy, efforts are already made to develop an approach for the personalized Ovarian Stimulation in ART taking into cognizance which aspects could be personalized.

Those days are not far when a tailor-made individualized management strategy is in the offing for those patients with LH and FSH deficiency due to various reasons.

feedback form

ALIVE
ART LEARNING INITIATIVES FOR EXPERTS

Issue 8 | January 2022

Thank you for going through the contents of **Alive Newsletter Issue 8.** 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:_____

Email ID:_____

Contact No:_____

Satisfaction Score for ALIVE Newsletter - FSH and LH deficiency in ART and

Management options : Issue 8; January 2022

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 8 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?
_____

Steps to scan QR code

1) Open the Camera application either from the lock screen or tapping on the icon from your home screen.
2) Hold your device steady for 2-3 seconds towards the QR Code.
3) Click on the notification to open the content of the QR Code.

MERCK

Merck Specialities Pvt. Ltd.
Godrej One, 8th Floor, Pirojshanagar Nagar, Eastern Express Highway,
Vikroli East, Mumbai, Maharashtra 400079, India.