



OPEN ACCESS

EDITED BY

Human Mousavi Fatemi,
ART Fertility Clinics, United Arab Emirates

REVIEWED BY

Tsung-Hsien Lee,
Chung Shan Medical University, Taiwan

*CORRESPONDENCE

Livio Casarini
✉ livio.casarini@unimore.it

RECEIVED 15 September 2025

REVISED 09 December 2025

ACCEPTED 16 December 2025

PUBLISHED 12 January 2026

CITATION

Casarini L and Simoni M (2026) Commentary:
FSH receptor N680S genotype-guided
gonadotropin choice increases
cumulative pregnancy and live
birth rates after *in vitro* fertilization.
Front. Endocrinol. 16:1706120.
doi: 10.3389/fendo.2025.1706120

COPYRIGHT

© 2026 Casarini and Simoni. This is an open-
access article distributed under the terms of
the [Creative Commons Attribution License
\(CC BY\)](#). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication
in this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Commentary: FSH receptor N680S genotype-guided gonadotropin choice increases cumulative pregnancy and live birth rates after *in vitro* fertilization

Livio Casarini^{1,2*} and Manuela Simoni^{1,2,3}

¹Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy, ²Center for Genomic Research-University of Modena and Reggio Emilia, Modena, Italy, ³Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria di Modena-Baggiovara Hospital, Modena, Italy

KEYWORDS

FSH, FSHR polymorphism, hMG, recombinant, urinary

A Commentary on

FSH receptor N680S genotype-guided gonadotropin choice increases cumulative pregnancy and live birth rates after *in vitro* fertilization

By Hjelmér I, Nilsson M, Henic E, Jędrzejczak P, Nenonen H, Ozegowska K, Giwercman A, Kitlinski ML and Giwercman YL (2025) *Front. Endocrinol.* 16:1576090. doi: 10.3389/fendo.2025.1576090

Introduction

This is a comment on the findings described by Hjelmér et al., 2025 (1) and their proposal to use urinary FSH (uFSH) preparations to improve assisted reproduction technique (ART) outcomes in women homozygous for the FSH receptor (FSHR) “680Ser” polymorphic variant (rs6166). We aimed to appraise methodological flaws and interpretative issues in the study by Hjelmér et al. Relevant concerns arise from the interpretation of the results and their use to support the conclusion that uFSH should be recommended to treat genotyped FSHR 680Ser carriers.

Inaccurate terminology leads to misinterpretation of results

The commercial preparation Menopur (Ferring Pharmaceuticals SA, Saint-Prex, Switzerland), improperly defined by the authors as a uFSH, is a mixture of FSH and LH obtained from menopausal women, with the addition of hCG (2), and should be indicated as “menopausal gonadotropins” (hMG). uFSH drugs contain exclusively FSH molecules purified from urine, which is not the case for Menopur (3). Although this may appear to be a semantic

issue, inaccurate terminology may have led to the misinterpretation that FSH of urinary origin elicits an *in vivo* response that could be, at least in part, attributable to LH and hCG. This may underlie some of the result misinterpretations discussed below.

In vitro data do not support authors' conclusion

In their study, Hjelmér and colleagues performed *in vitro* experiments intended to support their clinical observations. They measured cyclic adenosine monophosphate (cAMP) production in transfected COS-1 cells treated with recombinant FSH (rFSH) and hMG. However, these cells express only FSHR, preventing the action of LH/hCG molecules, which exert their effects through the LH/hCG receptor (LHCGR). Therefore, this model is not representative of clinical ovarian stimulation, in which granulosa cells express both FSHR and LHCGR, and theca cells express LHCGR (4). LH/hCG are present in Menopur but absent in the rFSH reference drug (Gonal-F, Merck KGaA, Darmstadt, Germany) (2). The efficacy of FSH and hCG varies considerably depending on whether LHCGR is co-expressed with FSHR [see Table 2 in ref (2)]. However, the *in vitro* system used by Hjelmér et al. does not allow detection of the contribution of LH/hCG to cAMP activation because of the lack of LHCGR expression.

Once it is established that the cellular response shown in Figure 2 (1) is mediated by FSH molecules only, it is not surprising that cAMP levels differ between the hMG and rFSH groups in FSHR-expressing COS-1 cells. These considerations raise concerns about the actual FSH concentration administered *in vitro* and how these doses were determined. Specifically, the authors used international units (IU) rather than molarity, introducing a bias: IU is not a measure of hormone quantity, as it reflects *in vivo* activity in rodents (5), whereas molarity is more appropriate for *in vitro* experiments because it reflects the number of molecules. There is no direct proportionality between Menopur and Gonal-F IU values, as they are based on the activity of different molecules [Table 2 in ref. (2)]. By using IU *in vitro*, the authors applied different concentrations of FSH, with a clear overdosing in the Menopur-treated group. In addition, no information is provided on the stimulation volume, making it impossible to determine the actual hormone concentration used, while the reported quantity (up to 90 IU) appears extremely high for *in vitro* experiments.

The *in vitro* data also present methodological and statistical issues, as a t-test was inappropriately used to analyze multiple groups. The authors compared three groups (treatments: “uFSH,” “rFSH,” and “no FSH”) across two variables (FSH dose: 0, 1, 10, and 90 IU). A two-way ANOVA, or a *non*-parametric equivalent such as the Kruskal–Wallis test, should have been used instead of a t-test, which is limited to two-group comparisons and may lead to type I errors when applied to multiple testing (6). Finally, the number of independent replicates and the buffer volume used for cell treatment are not reported, and no data are shown for COS-1 cells expressing FSHR 680Asn or FSHR 680Asn/680Ser.

In summary, insufficient information is provided to allow the *in vitro* experiments to be reproduced or correctly interpreted. Therefore, these results do not support the authors' conclusions.

Major flaws of the clinical study

The main problem is the lack of a clinical study registration number, which prevents evaluation of whether the experimental design was modified *a posteriori* and biased by data selection, potentially performed after the initial analyses did not reveal any difference. This observation is particularly relevant given the retrospective nature of the study (1).

Other key information is missing. For instance, the drug used to treat *non*-genotyped women is not indicated, preventing its use as a control group. Results may be biased by gonadotropin dosing, which is higher in the hMG group [see Table 1 in the paper (1)]. Details on how adjustments for BMI, age, and fertilization method were performed are also missing. Other relevant data impacting ART prognosis are not reported, such as antral follicle count (AFC) (7). In summary, the study design is not appropriate for testing the authors' hypothesis.

Opposite data

The authors did not reconcile their results with the existing literature.

There is no clear evidence demonstrating that uFSH elicits different responses than rFSH, weakening the rationale of the study by Hjelmér et al. Commercial FSH preparations are mixtures of different glycoforms of the same molecule, but they rarely trigger clear preparation-specific responses due to the lack of substantial diversity in overall composition and half-life among these mixtures (3, 8, 9). Many clinical studies support or refute the superiority of rFSH vs uFSH, and vice versa (10–15). However, they have not produced converging evidence for the superiority of one drug in improving clinical outcomes (14). Instead, previous findings consistently support that differences between rFSH and hMG are due to LH/hCG (16), as these ligands modulate the response to FSH through LHCGR activation (17), regardless of FSHR genotype. This concept was demonstrated in a large *in vitro* dataset (18), in which human primary granulosa lutein cells were treated with rFSH in the presence and absence of recombinant LH (Luveris, Merck KGaA). LH improved cellular responses regardless of FSHR genotype, suggesting that LH/hCG addition may have an indication in poor/sub-responder patients, rather than in a subgroup of FSHR 680Ser carriers (18) (see Supplementary Tables S3, S4). Rigorous meta-analysis (19) and a registry-based study (20) consolidated this view, showing that LHCGR ligands may be used to personalize FSH treatments when the ovarian response to FSH needs to be optimized.

In conclusion, the study by Hjelmér et al. fails to provide indications for the use of hMG—and even less so uFSH—based on FSHR polymorphism. Improper study design, missing key information, lack of support from *in vitro* experiments, and data misinterpretations undermine the authors' conclusions. Converging data suggest that LH/hCG addition to FSH may benefit ART patients with poor/sub-response to FSH, regardless of FSHR genotype.

Author contributions

LC: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. MS: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Hjelmér I, Nilsson M, Henic E, Jędrzejczak P, Nenonen H, Ozegowska K, et al. FSH receptor N680S genotype-guided gonadotropin choice increases cumulative pregnancy and live birth rates after *in vitro* fertilization. *Front Endocrinol (Lausanne)*. (2025) 16:1576090. doi: 10.3389/FENDO.2025.1576090
- Casarini L, Riccetti L, Paradiso E, Benevelli R, Lazzaretti C, Sperduti S, et al. Two human menopausal gonadotrophin (hMG) preparations display different early signaling *in vitro*. *Mol Hum Reprod*. (2020) 26:894–905. doi: 10.1093/MOLEHR/GAAA070
- Riccetti L, Sperduti S, Lazzaretti C, Klett D, De Pascali F, Paradiso E, et al. Glycosylation pattern and *in vitro* bioactivity of reference follitropin alfa and biosimilars. *Front Endocrinol (Lausanne)*. (2019) 10:503. doi: 10.3389/fendo.2019.00503
- Liu YX, Hsueh AJW. Synergism between granulosa and theca-interstitial cells in estrogen biosynthesis by gonadotropin-treated rat ovaries: Studies on the two-cell, two-gonadotropin hypothesis using steroid antisera. *Biol Reprod*. (1986) 35:27–36. doi: 10.1095/biolreprod35.1.27
- Steelman SL, Pohley FM. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology*. (1953) 53:604–16. doi: 10.1210/endo-53-6-604
- Mitchell PJ. *Experimental Design and Statistical Analysis for Pharmacology and the Biomedical Sciences*. John Wiley & Sons, Inc (2022). doi: 10.1002/9781119437642
- Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, Esteves SC, 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) 105:1452–3. doi: 10.1016/J.FERTNSTERT.2016.02.005
- Le Cotonneq JY, Porchet HC, Beltrami V, Khan A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone (FSH). I. Comparative pharmacokinetics with urinary human FSH. *Fertil Steril*. (1994) 61:669–78. doi: 10.1016/S0015-0282(16)56644-8
- Karlsson MO, Wade JR, Loumaye E, Munafa A. The population pharmacokinetics of recombinant- and urinary-human follicle stimulating hormone in women. *Br J Clin Pharmacol*. (1998) 45:13–20. doi: 10.1046/J.1365-2125.1998.00644.X
- Matorras R, Osuna C, Exposito A, Crisol L, Pijoan JI. Recombinant FSH versus highly purified FSH in intrauterine insemination: A systematic review and meta-analysis. *Fertil Steril*. (2011) 95:1937–42. doi: 10.1016/j.fertnstert.2011.02.030
- Abd-Elaziz K, Duijkers I, Stöckl L, Dietrich B, Klipping C, Eckert K, et al. A new fully human recombinant FSH (follitropin epsilon): Two phase I randomized placebo

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

and comparator-controlled pharmacokinetic and pharmacodynamic trials. *Hum Reprod*. (2017) 32:1639–47. doi: 10.1093/humrep/dex220

12. Lledó B, Dapena P, Ortiz JA, Morales R, Llacer J, Bernabeu R. Clinical efficacy of recombinant versus highly purified follicle-stimulating hormone according to follicle-stimulating hormone receptor genotype. *Pharmacogenet Genomics*. (2016) 26:288–93. doi: 10.1097/FPC.0000000000000215

13. Bergandi L, Canosa S, Carosso AR, Paschero C, Gennarelli G, Silvagno F, et al. Human recombinant FSH and its biosimilars: Clinical efficacy, safety, and cost-effectiveness in controlled ovarian stimulation for *in vitro* fertilization. *Pharmaceuticals*. (2020) 13:1–23. doi: 10.3390/ph13070136

14. Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T, Sunkara SK. The development of gonadotropins for clinical use in the treatment of infertility. *Front Endocrinol (Lausanne)*. (2019) 10:429. doi: 10.3389/fendo.2019.00429

15. Liu X, Hao C, Wang J. Efficacy of highly purified urinary FSH versus recombinant FSH in Chinese women over 37 years undergoing assisted reproductive techniques. *Int J Fertil Steril*. (2015) 8:385–92. doi: 10.22074/ijfs.2015.4178

16. Bosch E, Alamá P, Romero JL, Mari M, Labarta E, Pellicer A. Serum progesterone is lower in ovarian stimulation with highly purified HMG compared to recombinant FSH owing to a different regulation of follicular steroidogenesis: A randomized controlled trial. *Hum Reprod*. (2024) 39:393–402. doi: 10.1093/humrep/dead251

17. Casarini L, Riccetti L, De Pascali F, Nicoli A, Tagliavini S, Trenti T, 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) 422:103–14. doi: 10.1016/J.MCE.2015.12.008

18. Sperduti S, Paradiso E, Anzivino C, Lazzaretti C, Limoncella S, D'Alessandro S, et al. LH increases the response to FSH in granulosa-lutein cells from sub/poor-responder patients. *in vitro*. *Hum Reprod*. (2023) 38:103–12. doi: 10.1093/HUMREP/DEAC246

19. Santi D, Casarini L, Alviggi C, Simoni M. 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) 8:114. doi: 10.3389/FENDO.2017.00114

20. Arvis P, Massin N, Lebert P. Effect of recombinant LH supplementation on cumulative live birth rate compared with FSH alone in poor ovarian responders: a large, real-world study. *Reprod BioMed Online*. (2021) 42:546–54. doi: 10.1016/J.RBMO.2020.08.035