Dose-dependent modulation of luteinizing hormone receptor expression in human granulosa cells and progesterone production during ovarian stimulation

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Objective: To assess the impact of different recombinant follicle-stimulating hormone (recFSH) dosages in ovarian stimulation (OS) on luteinizing hormone (LH)–receptor and FSH-receptor expression in granulosa cells (GCs) and progesterone level.
Design: Prospective interventional study (07/2023–02/2024).
Subjects: Six volunteers with regular cycles and normal ovarian reserve undergoing two OS cycles for oocyte vitrification as a means of elective fertility preservation.
Exposure: Different recFSH dosages (150 IU vs. 300 IU) in two OS cycles, performed in a gonadotropin-releasing hormone–antagonist protocol.
Main Outcome Measures: The LH receptor expression on GCs.
Results: Volunteers had a median age of 34 years (range: 28–36), a median antimüllerian hormone–level of 2.28 ng/mL (interquartile range [IQR]: 2.41–3.35) and a median antral follicle count of 13.5 (IQR:12–14) in the 150 IU and 13 (12–15) in the 300 IU OS cycle. Median total recFSH dosages were 1650 IU (IQR: 1350–1800IU)/3150 IU (IQR: 3000–3300) in the 150 IU/300 IU OS cycle, respectively. The number of retrieved cumulus-oocyte-complexes was (median and IQR) 11.5 (10–14)/12.5 (7–15), the number of mature oocytes (median and IQR) of 10.5 (7–12)/10 (6–12), respectively, without a statistically significant difference.

Besides the stimulation start, serum FSH levels of the 300 IU-cycle were significantly higher compared with the 150 IU-cycle. Estradiol levels were significantly higher in the 300 IU-group on day 5 and at follicle size of 14–16 mm. Progesterone (P4) levels were not statistically significantly different between the stimulation cycles.

The LH receptor expression in the GCs at oocyte-pick up was significantly higher and FSH-receptor expression was significantly lower in the 300 IU-group compared with the 150 IU-group. The P4 levels on oocyte-pick up day were significantly associated with LH receptor levels after adjusting for the recFSH dose. Furthermore, a significant and independent association of the recFSH dose between the P4 levels and the LH receptor levels was seen on the day of oocyte retrieval.
Conclusion: Higher recFSH dosages during OS are associated with increased LH receptor expression, and LH receptor count had a significant association with serum P4 levels. (F S Sci® 2026;7:27–34. ©2025 by American Society for Reproductive Medicine.)

Key Words: Ovarian stimulation, FSH dosage, LH receptor expression, granulosa cells, progesterone elevation

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F&S SCIENCE CLINICAL QUICK TAKE

What clinical problem is addressed by this study?

- The mechanism underlying late follicular progesterone (P4) elevation during ovarian stimulation with gonadotropins for multifollicular development remains inadequately elucidated. Intense gonadotropin dosing is hypothesized to increase luteinizing hormone (LH) receptor expression in the granulosa cells of the growing follicle.

What are the key findings?

- This study, conducted with a limited cohort of healthy volunteers with normal ovarian reserve, revealed that higher recombinant follicle-stimulating hormone (recFSH) dosage resulted in an increase in LH receptors on granulosa cells. Furthermore, an association between serum P4 levels and LH receptors after controlling for recFSH dosage was seen.

How do these findings apply to human fertility or the reproductive process?

- Premature P4 elevation may result in embryo-endometrial asynchrony, potentially affecting outcomes in fresh embryo transfer cycles. Therefore, reducing recFSH dosage during the late follicular phase of ovarian stimulation may decrease the incidence of premature P4 elevation.

Ovarian stimulation (OS) with gonadotropins for multifollicular development is a crucial component of assisted reproductive technologies treatment. Multifollicular development is linked to supraphysiologic levels of progesterone (P4) and estradiol (E2) and progesterone elevation in the late follicular phase may occur in up to 38% of stimulation cycles (1). In fresh embryo transfer cycles, the detrimental effects of late follicular P4 elevation on endometrial receptivity and the pregnancy rate have been well-established (2,3); therefore, in settings in which a fresh embryo transfer is planned, P4 elevation should be avoided.

The physiological basis for the development of a single dominant follicle in a natural cycle is the intricate interplay between the trajectory of the serum follicle-stimulating hormone (FSH) levels and the changes in the receptor composition of the granulosa cells (GCs): during follicle growth and the follicle's preparation for ovulation a transition occurs from a predominance of FSH-receptors in the early follicular phase to a predominance of luteinizing hormone (LH)–receptors in the late follicular phase (4). The effect of the systemic FSH levels is mediated by FSH- and LH receptors of the GCs of the follicles (5). Although in natural cycles FSH levels decline during the follicular phase, in OS systemic FSH levels are kept constantly above the required threshold to achieve multifollicular growth (6). According to the findings of a retrospective clinical and laboratory-based study, late follicular P4 elevation appears to be caused by high FSH dosage used during OS (7, 8).

To improve our understanding of the effects of the recombinant FSH (recFSH) dosages on the LH receptor expression on the GC, we conducted a prospective cross-over study in healthy women without a history of infertility and with the desire for elective fertility preservation and who underwent two OS cycles with different recFSH dosages. Furthermore, we investigated the effect of the recFSH stimulation dosage on Inhibin A, Inhibin B, Activin A, Activin B, Activin AB, and Follistatin as they contribute to the regulation of the menstrual cycle and the follicle growth.

MATERIALS AND METHODS

This prospective interventional study included six volunteers with regular cycles and a normal ovarian reserve according to antimüllerian hormone (AMH) (inclusion criteria range 1.3–6.25 ng/mL) who underwent two OS cycles for oocyte vitrification as a means of elective fertility preservation between July 2023 and February 2024. The volunteers did not have a history of infertility.

Both OS cycles were performed in a fixed gonadotropin-releasing hormone (GnRH)–antagonist cycle with recFSH (Puregon) as gonadotropin and Ganirelix (Orgalutran) for pituitary suppression, starting on day 5 of stimulation. A daily dose of 150 IU of recFSH was given consistently during the first OS cycle, and in the second OS cycle, a daily dose of 300 IU was given. Between the two OS cycles, there was a washout period of at least 1 month.

Monitoring of the OS cycles was performed with ultrasound and hormonal evaluation as follows: at the time of commencing OS (day 2/3), on day 5 of OS before administration of the GnRH-antagonist (only hormonal evaluation) and then throughout the stimulation according to the follicle development.

Hormonal evaluation of LH, FSH, E2, P4, Inhibin A, Inhibin B, Activin A, Activin B, Activin AB, and Follistatin was performed at five timepoints during the stimulation: start of stimulation, day 5 of stimulation (before administration of the GnRH-antagonist), at a follicle size (FS) of 14–16 mm of the leading follicle, the day of final oocyte maturation (before administration of the trigger medication) and on the OPU day. One follicle was punctured during each OS cycle at leading FS of 14–16 mm and 17 mm (before administration of the final oocyte maturation medication), and follicular fluid (FF) and GC were collected. For final oocyte maturation, 0.3 mg GnRH-agonist was used in all OS cycles. On the OPU day, FF and GCs were aspirated. Mature oocytes (oocytes at the metaphase II stage) were vitrified.

Total stimulation dosages, stimulation duration, hormonal levels of LH, FSH, E2, P4, Inhibin A, Inhibin B, Activin A, Activin B, Activin AB, and Follistatin, as well as the number of oocytes retrieved and mature, were recorded.

Hormonal measurements

Progesterone analysis. The Elecsys Progesterone III assay uses sheep monoclonal antibodies because of their higher

TABLE 1

Characteristics of the six volunteers and outcomes of ovarian stimulation cycles.

Variables	reFSH 150 IU	recFSH 300 IU	*P*[a]
AFC (n)	13.5 (12–14)	13 (12–15)	.643
Gonadotropin dosage (IU)	1,650 (1,350–1,800)	3,150 (3,000–3,300)	< .001
Stimulation days (n)	10.5 (9–12)	10.5 (10–11)	.724
COC (n)	11.5 (10–14)	12.5 (7–15)	.866
MII (n)	10.5 (7–12)	10 (6–12)	.710

Note: [a]Mixed-effects Poisson regression for count variable (Stimulation days, AFC, COC and MII) and mixed-effects linear regression for gonadotropin dose. Variables are presented as median and interquartile range. AFC = antral follicle count, COC = cumulus-oocyte-complex, MII = mature oocytes, recFSH = recombinant FSH.

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specificity toward progesterone. The measuring range is 0.159–191 nmol/L or 0.05–60 ng/mL. For the detection of analytical specificity, cross-reactivities toward other hormones were used, with a maximum cross-reactivity of 3.93% toward 11-deoxycorticosterone and a minimum cross-reactivity of 0.001% toward Danazol.

Oestradiol analysis. The Elecsys Estradiol III assay employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-oestradiol. Endogenous oestradiol released from the sample by mesterolone competes with the added oestradiol derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody. Results are determined via a calibration curve instrument, specifically generated by two-point calibration and a master curve provided via the reagent barcode or e-barcode.

FSH analysis. The Elecsys FSH assay employs two different monoclonal antibodies specifically directed against human FSH. Cross-reactivity with LH, thyroid-stimulating hormone (TSH), human chorionic gonadotropin (hCG), human growth hormone (hGH), and human placental lactogen (hPL) was negligible. Results were determined via a calibration curve instrument, specifically generated by two-point calibration and a master curve provided via the reagent barcode or e-barcode.

LH analysis. The Elecsys LH assay employs two monoclonal antibodies specifically directed against human LH. The two specific antibodies used recognize particular conformations, with the biotinylated antibodies detecting an epitope constructed from both subunits, whereas the antibody with the ruthenium complex, label detects an epitope from the β-subunit. As a result, the Elecsys LH assay shows negligible cross-reactivity with FSH, TSH, hCG, hGH, and hPL.

Inhibin A, Inhibin B, Activin A, Activin B and Activin AB, Follistatin analysis. Commercially available immunoassays from AnshLabs, Texas were used to detect levels of Inhibin A (AL-123), Inhibin B (AL-107), Activin A (AL-110), Activin B (AL-150), Activin AB (AL-153), and Follistatin (AL-117) in frozen/thawed serum samples. The enzyme-linked immunosorbent assays (ELISAs) were analyzed at AnshLabs LLC (Webster, Texas) as per the instructions for use provided in their respective ELISAs.

The description of the test specifics is presented in Supplemental Table 1, available online.

Evaluation of LH receptor (LHR) gene expression

RNA purification. Total ribonucleic acid (RNA) was purified under RNase-free conditions at room temperature for each sample using a combination of Tri Reagent (Sigma–Aldrich) and the RNAeasy Mini Kit (Qiagen, Hilden, Germany).

Each cell sample was lysed by the addition of 1 mL Tri Reagent followed by incubation at room temperature for 5 min. Approximately 200 μL 1-bromo-3-chloro-propane (Sigma–Aldrich) were added to the lysed cells, shaken vigorously for 15 s and incubated for 5 min at room temperature. Subsequent centrifugation at 15,000 $\times$ g for 15 min at 4 °C separated the solution into an upper aqueous phase containing RNA and a lower phenol-containing organic phase. The upper phase was transferred to a clean, RNase-free Eppendorf tube, and RNA purification was continued by following the RNeasy Mini Kit protocol. The final elution step was repeated, and the purified total RNA was stored at −80 °C. The quality and degradation level of the total RNA samples were analyzed using a Qsep400 Bio-Fragment Analyzer and NR1 Cartridge, according to the manufacturer's instructions (NR1 Cartridge, Bioptic, Taiwan). The quantity of total RNA in each sample was measured using a Qubit 4 Fluorometer (Thermo Fisher Scientific).

Complementary deoxyribonucleic acid synthesis and quantitative polymerase chain reaction analysis. First-strand complementary deoxyribonucleic acid (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Briefly, a master mix containing 2.0 μL 10 $\times$ RT Buffer, 0.8 μL 25 $\times$ dNTP Mix (100 mM), 2.0 μL 10 $\times$ RT Random Primers, 1.0 μL MultiScribe Reverse Transcriptase (50 U/μL) and 3.2 μL nuclease-free (DEPC) water was prepared, and for each 20 μL reaction 10 μL master mix was added to an equal volume of total RNA. All steps were performed on ice. Samples were centrifuged briefly at 12,000 $\times$ g and then incubated at room temperature for 10 min, followed by 37 °C for 2 h and finally, 85 °C for 5 s. First-strand cDNA was stored at −80°C until quantitative polymerase chain reaction (qPCR) analysis.

Evaluation of gene expression levels was achieved by relative quantification (RQ)–PCR analysis using TaqMan technology (Applied Biosystems). Follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor

(LHR) TaqMan Gene Expression Assays (predesigned) as well as the Endogenous Control Assay for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems (Assay id-No.: GAPDH:#4333764F; FSHR:#Hs00174865_m1; LHR:#Hs00174885_m1). Sample duplicates were prepared according to the manufacturer's instructions. A total reaction volume of 10 μL was prepared on ice containing 5.0 μL TaqManFast Advanced PCR Master Mix, 0.5 μL 20 $\times$ TaqMan Gene Expression Assay Mix, 2 μL undiluted cDNA, and RNase-free water up to 10 μL. The samples were then centrifuged at 2,800 $\times$ g at 4 °C for 5 min. The cDNA was amplified using the QuantStudio 5 Real-Time PCR System (Applied Biosystems) under the following thermal cycling conditions: 95 °C for 20 min, 45 cycles of 95 °C for 15 s, and 60 °C for 1 min.

RNA quality and integrity assessment

The RNA integrity was evaluated using Qsep 400 Bio-Fragment Analyzer, which reports the RNA Quality Number (RQN) analogous to the Agilent Bioanalyzer RIN scale. Although an integrity threshold of RIN/RQN $\geq$ 5 is generally recommended for reliable reverse transcription quantitative polymerase chain reaction (RT-qPCR) performance (9,10), we adopted a slightly lower cut-off of RQN $\geq$ 4 to increase sample inclusion, given the limited number of available samples. Previous reports and manufacturer guidelines indicate that RT-qPCR is largely tolerant of moderate RNA degradation, particularly when amplicon sizes are short (70–120 bp) and appropriate reference normalization is applied. To further mitigate the effects of partial degradation, we also considered the DV200 (%) metric, which reflects the percentage of RNA fragments longer than 200 nucleotides and provides a more robust indicator of RNA suitability for downstream analyses. Samples meeting these combined criteria (RQN $\geq$ 4 and DV200 $\geq$ 50%) were therefore included in RT-qPCR experiments.

Data processing. The data were normalized against the stably expressed housekeeping gene GAPDH and RQ according to the Comparative CT Method (QuantStudio Design & Analysis Software v1.5.2) was used to quantify gene expression.

Statistical analysis

Variables are presented as median and interquartile range (IQR) for continuous variables and count and percentage of the total for categorical variables. Statistical significance was tested with mixed-effects regression models using random intercepts for repeat measurements of the same patient and appropriate distribution links (Logit, Poisson, Gaussian). The P values below .05 were considered statistically significant. All analyses were conducted in R for Statistical Computing Software (v.4.2.0).

Ethical approval

The study was approved by the Department of Health in Abu Dhabi, United Arab Emirates, with reference number DOH/CVDC/2023/467, the local Ethical Committee with number REFA078 and REFA078a-2107-ABU-010-BL, and clinicaltrials.gov with identifier NCT05330130.

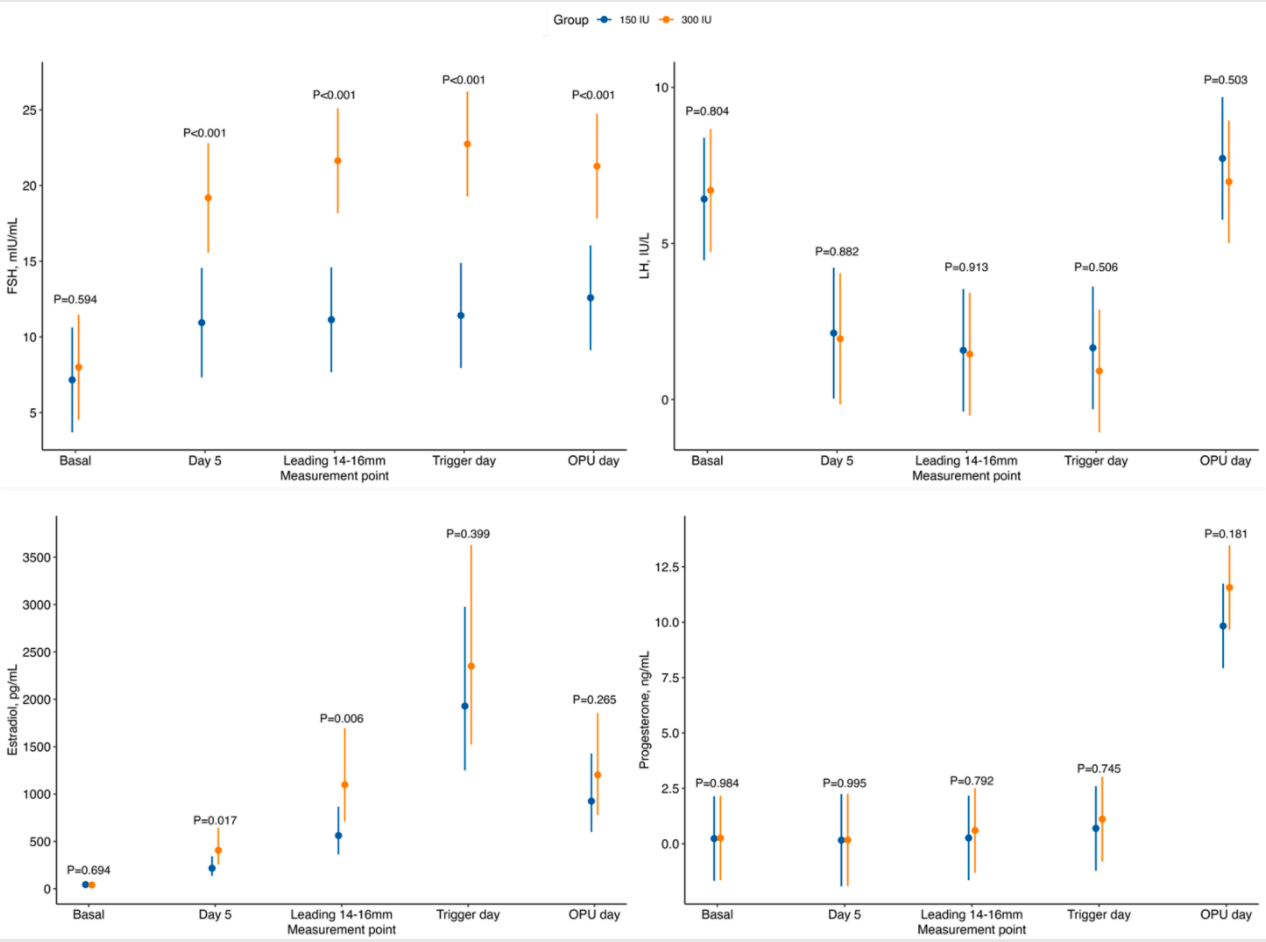
RESULTS

The volunteers' median age was 34 years (28–36 years) with a median AMH of 2.28 ng/mL (IQR: 2.41–3.35 ng/mL). At the onset of the OS cycles, median AFC values were 13.5 (IQR:12–14) in the 150 IU and 13 (IQR: 12–15) in the 300 IU-cycle. Median total stimulation dosages were 1,650 IU (IQR: 1,350–1,800 IU)/3150 IU (IQR: 3,000–3,300) in the 150 IU and 300 IU cycles, respectively. The number of cumulus-oocyte-complexes (COCs) were (median and IQR): 11.5 (IQR: 10–14) in the 150 IU and 12.5 (IQR: 7–15) in the 300 IU OS cycle, with a number of mature oocytes (MII) (median and IQR) of 10.5 (IQR: 7–12) and 10 (IQR: 6–12) respectively with no significant differences for the outcomes (nonsignificant [n.s.] for all) between the 150 IU and 300 IU stimulation cycles (Table 1).

The comparison of the different dosages on the individual time points showed the following results: serum FSH levels within the 150 IU-cycle were significantly higher at all time points compared with the stimulation start on day 2/3 and significantly higher at all time points in the 300 IU-cycle compared with the same time points of the 150 IU-cycle ($P<.001$ for all). The LH levels on D5, FS 14–16 mm and trigger day were lower compared with the stimulation start (day2/3) but not on the OPU day, in both 150 IU and 300 IU cycles. The LH levels were similar between the 150 IU and the 300 IU cycles at all time points (P=n.s.). In both cycles, E2 levels during the stimulation were higher at all time points compared with the stimulation start, and they were significantly higher in the 300 IU-group on day 5 and at FS of 14–16 mm ($P=.017$/$P=.006$, respectively), but not on the trigger and the OPU day. The P4 levels were not statistically significantly different within the 150 IU stimulation cycle, and only on OPU day, a trend but without statistical significance toward higher P4 levels in the 300 IU OS group was seen. The P4:E2 ratio on the day of final oocyte maturation was 0.35 in the 150 IU and 0.47 in the 300 IU stimulation cycle. A summary of the results is presented in Figure 1. Results of serum FSH, LH, E2, P4, Inhibin A, Inhibin B, Activin A, Activin B, Activin AB and Follistatin levels throughout both stimulation cycles are summarized in Supplemental Table 2.

The LH receptor expression in the GCs, which were retrieved together with the COCs at oocyte collection, was significantly higher in the 300 IU OS group compared with the 150 IU OS group (Mean difference: 0.56, 95% confidence interval [CI]: 0.16–0.95; P=.007) (Fig. 2). The FSHR expression on the GCs were significantly lower in the 300 IU stimulation cycle compared with the 150 IU-cycle (Mean difference: −0.54, 95 CI: −1.0 to −0.09; $P=.021$) (Fig. 2). The P4 levels on the OPU day were significantly associated with LHR levels after adjusting for the recFSH dose ($P=.034$). Furthermore, a significant and independent association of the recFSH dose between the P4 levels and the LHR levels was seen on the day of oocyte retrieval ($P=.003$) (Table 2).

FIGURE 1



FSH, LH, estradiol and progesterone levels throughout the ovarian stimulation cycles, performed with 150 IU and 300 IU of gonadotropins. P values are derived from mixed-effects regression and show the significance of the difference between 150 IU and 300 IU stimulation at the measurement point. FSH = follicle-stimulating hormone, LH = luteinizing hormone.

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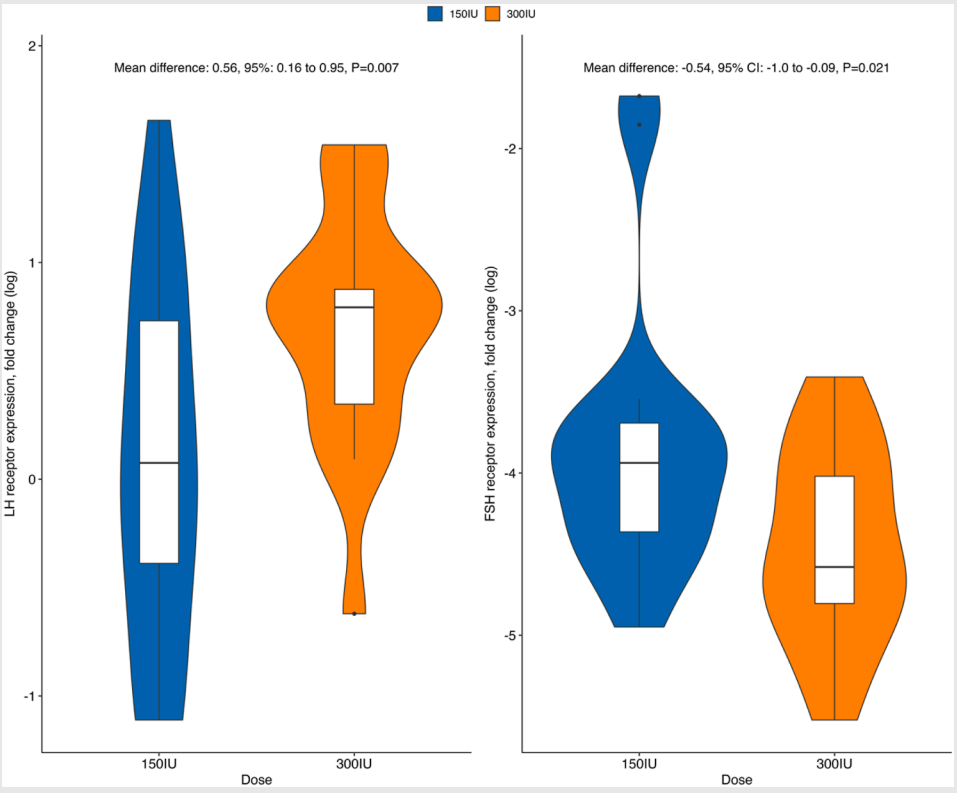
For Inhibin A, Inhibin B, Activin B and partially for Activin A, AB and Follistatin, significant differences throughout the 150 IU OS course have been seen compared with the starting day; however, for most parameters, no significant differences were seen between the two recFSH stimulation courses. Results are presented in Supplemental Table 3.

DISCUSSION

Our study demonstrated in a small sample size of six volunteers that the use of higher recFSH stimulation dosages is associated with an increase in LHR expression of the GCs of the growing follicle. Furthermore, serum P4 levels were significantly associated with LHR levels after adjusting for the recFSH dosage (Table 2). These results are consistent with earlier research showing that higher dosages of recFSH stimulation appear to affect the expression of LH receptors and may therefore be the reason for the increase in progesterone in the late follicular phase (7,8). In addition to inducing and activating LH receptors, FSH also interferes in the regulation and enzymatic activity of enzymes involved in the biosynthesis of progesterone, increasing P4 synthesis through other routes (8).

Contrary to the effect of the stimulation dosage on the LH receptor expression, no effect of the different stimulation dosages was seen for the Inhibins, the Activins, and Follistatin. These factors are key regulators of follicular development, and they act primarily through modulation of FSH activity and intraovarian signaling (11). Inhibin B is predominantly synthesized by smaller follicles, whereas Inhibin A concentrations rise with increasing FS. Changes were observed during the stimulation course as FS increased; however, no differences were noted between the stimulation cycles performed with 150 and 300 IU recFSH. This may be attributed to the comparable number of growing follicles in

FIGURE 2



LH and FSH-receptor expression (fold change, log) in 150 IU and 300 IU groups on oocyte-pick-up day. FSH = follicle-stimulating hormone, LH = luteinizing hormone.

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both stimulation cycles; however, the small sample size could also play a role. Although some papers assessed the potential use of Inhibins in OS planning and monitoring, they are not commonly utilized and are primarily employed in research contexts, and additional studies are required to validate their role in OS monitoring (12,13). Given that Activins and Follistatin function primarily as paracrine factors in the ovary (14), no alterations in hormonal levels were anticipated; their measurement was conducted purely for scientific inquiry.

In OS, the number of retrieved oocytes is key to the success of an in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment (15). To achieve multifollicular growth, serum FSH levels need to be kept constantly above the required threshold according to the "FSH threshold and window concept" (6,16). The FSH start dosages are usually

TABLE 2

Association between progesterone levels on oocyte-pick-up day and gonadotropin dose with LH receptor levels (log).

Variables	Mean difference (95% CI[a])	Adjusted mean difference (95% CI[b])
recFSH dose		
150 IU	Reference	Reference
300 IU	0.62 (0.13–1.11, $P = .014$)	0.38 (−0.03–0.78, $P = .034$)
Progesterone levels, ng/mL		
150 IU	Reference	Reference
300 IU	0.10 (0.05–0.16, $P < .001$)	0.09 (0.02–0.15, $P = .003$)

Note: [a]Univariable mixed-effects regression analysis. [b]Multivariable mixed-effects regression analysis including gonadotropin dose and progesterone levels on OPU day. CI = confidence interval, LH = luteinizing hormone, OPU = oocyte-pick up, recFSH = recombinant FSH.

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chosen according to the ovarian reserve parameters (17, 18). However, not only the "optimal" starting dose, but also the required dosage throughout the stimulation course might be difficult to determine (19). Although starting FSH dose needs to be sufficiently high to ensure multifollicular response, high FSH levels in the later follicular phase appear unnecessary because of the above-mentioned shift in the receptors of the GCs and continuously high FSH levels might be even harmful as they seem to cause late follicular phase P4 elevation.

Clinically, the shift toward LH receptors and the decreased need of FSH for further follicle growth in OS was demonstrated by Filicori et al. (20): in 40 women, OS was initiated using 150 IU FSH and after 7 days of stimulation, patients were randomly assigned to four different treatment groups with varying administration of decreasing FSH and increasing hCG administration. The hCG was used because of its similarity with LH and to foster LH activity. Their study indicates that hCG/LH activity can sustain follicle growth in the case of adequate follicle development after FSH-priming. Progesterone levels at the end of OS, however, were unaffected by the full replacement of FSH by LH activity starting on day 8, with no discernible difference in progesterone levels between the FSH-only and the hCG/LH-only groups. The lack of any significant change in the progesterone levels within the 150 IU stimulation cycle, and only a trend (without statistical significance) toward higher P4 levels in the 300 IU OS group on OPU day, could be the result of the small sample size (six patients only in each group).

A higher incidence of late follicular phase P4 elevation was seen in a retrospective study comparing OS cycles with corifollitropin alfa (CFA) vs. daily recFSH administration (7). As the FSH trajectory of CFA resembles that one of a natural cycle with an initial increase, followed by a slow decline of FSH activity, it was concluded that overexposure and constant FSH stimulus might be the primary cause of premature progesterone rise in stimulated IVF cycles. Besides the possible impact of higher FSH levels during stimulation on the incidence of P4 elevation, it also seems that higher FSH levels on the day of final oocyte maturation do not increase the number of retrieved oocytes in women with an adequate ovarian reserve (21).

Although it is not surprising that in our small study group of young women with a normal ovarian reserve, doubling the recFSH dosage from 150 IU to 300 IU resulted in significantly higher serum FSH levels throughout the entire stimulation duration; higher serum FSH levels did not translate into an increased number of retrieved and mature oocytes. This result supports the European Society of Human Reproduction and Embryology guidelines on OS, which recommend moderate stimulation dosages for women with a sufficient ovarian reserve (18).

Despite the transition to a "freeze-all" approach potentially rendering the discussion of P4 elevation in the late follicular phase and its effects on endometrial receptivity obsolete, the influence of progesterone elevation on embryo quality remains a subject of ongoing debate (22–24). Moreover, for several reasons, it is highly unlikely that the practice of fresh embryo transfers will be entirely abandoned. Consequently, minimizing the incidence of late follicular progesterone elevation through the avoidance of forced recFSH stimulation may offer a straightforward solution to this issue.

The main limitation of the study is the small sample size of six volunteers, and the study might be underpowered to detect some differences because of sample size. Moreover, the small sample size also limited the possibility of performing a further sensitivity analysis on other sampling parameters. Although mixed-effects models mitigate intraindividual variability, they do not overcome the fundamental constraint of a small sample size on the generalizability of the results. Consequently, the results should be interpreted as exploratory and hypothesis-generating. The generalizability of the results to women with infertility may be constrained because of the study population comprising young, healthy, noninfertile women, and the absence of reproductive outcomes since oocyte vitrification was performed. All participants underwent the identical order of stimulation cycles, commencing with 150 IU, followed by 300 IU of recFSH as the stimulation dosage, after a minimum 1-month washout interval. Notwithstanding the washout interval, this approach may still be susceptible to residual effects that could have been mitigated by reversing the sequence of stimulation dosages for half of the subjects.

This study is the first to demonstrate the impact of increased recFSH dosage during OS on LH receptor expression in GCs. The results presented here offer a physiological and mechanistic explanation for the elevation of progesterone during the late follicular phase in OS using recFSH as the gonadotropin. However, the implications for clinical outcomes, such as oocyte competency, require further investigation. The strength of the study lies in its evaluation of the effects of varying stimulation dosages across two OS cycles conducted within the same individual. This approach eliminates the possibility that other individual characteristics affect the impact of increased recFSH dosage on LH receptor expression.

CONCLUSION

In summary, this study demonstrated the impact of increased recFSH dosage on LH receptor expression in the GCs. Future research should assess whether decreasing recFSH dosage during the late follicular phase can not only reduce the incidence of elevated progesterone levels but also potentially lessen the treatment burden for patients by minimizing the required recFSH dosage.

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CRediT Authorship Contribution Statement

Barbara Lawrenz: Writing – original draft, Methodology, Conceptualization. **Sahar Ghafari:** Writing – review & editing, Investigation. **Erkan Kalafat:** Writing – review & editing, Formal analysis. **Araz Raberi:** Writing – review & editing, Methodology. **Jonalyn Edades:** Writing – review & editing, Project administration. **Bhanu Kalra:** Writing – review & editing, Investigation. **Noreen Hourani:** Writing – review & editing, Investigation. **Virginia Ferracuti:** Writing – review & editing, Investigation. **Asina Bayram:** Writing – review & editing, Investigation. **Human Fatemi:** Writing – review & editing, Conceptualization.

Declaration of Interests

B.L. has nothing to disclose. S.G. has nothing to disclose. E.K. has nothing to disclose. A.R. has nothing to disclose. J.E. has nothing to disclose. B.K. has nothing to disclose. N.H. has nothing to disclose. V.F. has nothing to disclose. A.B. has nothing to disclose. H.F. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.xfss.2025.11.002.

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