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How FSH and LH analogs affect fertility hormone results?

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Abstract

Infertility is defined as the inability of a couple to achieve pregnancy within a certain period despite regular sexual intercourse. With technological advances, infertility treatments have also advanced, and assisted reproductive technologies have become increasingly widespread. In this study, the potential interference effects of different gonadotropin preparations used during controlled ovarian stimulation in in vitro fertilization treatment on hormone levels were experimentally investigated. The effects of urine-derived (menotropin and urofollitropin) and recombinant (follitropin and follitropin + lutropin) gonadotropin preparations on the hormone levels were evaluated. Interference effects on follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, prolactin, testosterone, thyroid-stimulating hormone, free T3, and free T4 hormone levels were analyzed using immunoassay techniques. The results showed significant deviations, especially in the FSH and LH tests, whereas the interference levels varied among the different preparations. The highest interference was observed in the FSH assays, with deviations reaching up to 292,138%. Significant differences were also observed in estradiol and thyroid hormones (free T3 and free T4). These findings suggest that gonadotropin drugs containing high concentrations of FSH and LH may interfere with routine immunoassay-based hormone tests, leading to errors in clinical interpretation. This study emphasizes the need for careful interpretation of hormone assays in patients undergoing in vitro fertilization treatment, and the importance of considering possible interference effects.

Abbreviations: E2 = estradiol, FSH = follicle-stimulating hormone, FT3 = free T3, FT4 = free T4, HMG = human menopausal gonadotropin, HP = highly purified, LH = luteinizing hormone, PRL = prolactin, PROG = progesterone, rec = recombinant, TESTO = testosterone, TSH = thyroid-stimulating hormone.

Keywords: controlled ovarian stimulation, false results, gonadotropin, hormone, infertility, IVF

1. Introduction

Infertility is defined as a couple's inability to achieve pregnancy despite regular sexual intercourse for a certain period. In line with these technological advances, infertility treatments are evolving simultaneously. Assisted reproductive technology includes various medical procedures in which oocytes, sperms, or embryos are used to achieve fertility or pregnancy. In in vitro fertilization treatment, controlled ovarian hyperstimulation is used to stimulate the development of multiple follicles. Urinary and recombinant gonadotropins are widely used to stimulate oocytes. Urinary-derived preparations include menotropins (highly purified-human menopausal gonadotropin [HP-HMG]) and urofollitropins (highly purified follicle-stimulating hormone [HP-FSH]), whereas preparations obtained using recombinant techniques include follitropin (recFSH) and follitropin + lutropin (recFSH + recLH) combinations. In the clinical evaluation of the woman, the optimal controlled ovarian stimulation protocol and dosage were determined by considering factors such as age, ovarian reserve status, previous infertility treatment history, body mass index, and likely suggestive hormone concentrations.

The alpha subunits of luteinizing hormone (LH), FSH, thyroid-stimulating hormone (TSH), and human chorionic gonadotropin glycoproteins are identical and consist of 96 amino acids, whereas the beta subunits differ.[1,2] The complex structure of hormone molecules increases their interaction with other molecules. Interference from other molecules in immunoassays, although rare, can cause inaccurate laboratory results, which can significantly affect patient management. Interacting molecules include proteins, glycoproteins, immunoglobulins, food extracts, and xenobiotics. In immunoassay techniques used for measurements, the results of such interactions may appear as measurement errors. Although many studies have been conducted on this subject, the interference effect of highly concentrated FSH and LH analogs has not yet been investigated.

Errors in hormone tests caused by such interactions between gonadotropic preparations and hormone levels may lead clinicians to different clinical interpretations of infertility treatment stages. The aim of this study was to experimentally investigate the effects of 4 different drugs: menotropin (HP-HMG), urofollitropin (HP-FSH), recombinant follitropin (follitropin),

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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and recombinant follitropin combined with lutropin (follitropin + lutropin), on FSH, LH, estradiol (E2), progesterone (PROG), prolactin (PRL), testosterone (TESTO), TSH, free T3 (FT3), and free T4 (FT4) levels.

2. Materials and methods

2.1. Materials

Control solutions are commercial solutions with a structure similar to the blood matrix and are free of other interfering substances that may be present in human blood. When using patient blood, we used a control solution similar to the blood matrix, which would not pose a risk, to exclude potential interference from food, drugs, or herbal extracts.

"Multichem IA plus (Abbott, Wiesbaden, Germany, lot: 37109220)" control solution (contains 86 analytes including fertility and thyroid hormones, steroid hormones, cardiac markers, anemia markers, therapeutic drugs, adrenal markers, bone metabolism markers and tumor markers) was used in the study. Hormone control material (Bio-Rad Lyphochek Immunoassay Plus Control, Irvine) was used. The trade names of the drugs were kept confidential and coded as Drug 1, Drug 2, Drug 3, and Drug 4. Contents:

Drug 1, urofollitropin (HP-FSH) –150 IU FSH analog (1 mL lyolifized flk, 1 mL solvent solution, SC).

Drug 2, recombinant follitropin alpha –900 IU FSH analog (1.5 mL of injectable solution (SC).

Drug 3, recombinant follitropin alfa combined with lutropin alfa –150 IU FSH analog, 75 IU LH analog for injection (1 mL lyophilized flk, 1 mL solvent solution, SC).

Drug 4, menotropin (HP-HMG) –75 IU FSH analog, 75 IU LH analog (1 mL lyophilized flk, 1 mL solvent solution, SC).

2.2. Measurement devices

Hormone tests were performed by chemiluminescent microparticle immunoassay using an Abbott Alinity (USA) immunoassay autoanalyzer. All tests were calibrated and the control values were within acceptable limits.

2.3. Sample preparation

A prespecified amount of aforementioned control solution (1800 µL) was mixed with 4 different FSH and LH analogs by adding 200 µL of the drugs to 4 different godes. The solutions were vortexed for 30 seconds before the study. The obtained samples were analyzed using an Abbott Alinity biochemistry analyzer (Siemens, Marburg, Germany). The same procedure was repeated 3 times, with the addition of distilled water (200 µL) to the control solution to determine the target value. All measurements were repeated 3 times, and the mean values were recorded. A bias formula was used to calculate the deviation of the results from target values. This study does not require ethics committee approval as no blood or tissue samples were used and it is an experimental study. The names of the drugs used in the routine were not disclosed and were coded as Drug 1, Drug 2, Drug 3, and Drug 4. Statistical calculations were performed using Microsoft Excel software (Microsoft® Excel® MSO in Microsoft 365, Version 2502; Microsoft Corporation, Redmond). In the Bias (%) formula used to calculate deviation rates from the target value, C1 refers to the measurement result obtained from the distilled water mixture, and C2 refers to the measurement result prepared with the antibody. Bias (%) = ((C2 - C1)/C1) × 100.

3. Results

Of the 4 different drugs used in the study, Drug 1 and Drug 2 were FSH analogs and Drug 3 and Drug 4 were FSH and LH analogs. Bias deviation values were calculated according to the target values listed in Table 1. The highest bias after Drug 1 administration was observed in the FSH test (133383%). Drug 1 caused a bias of 7.31% in E2. Drug 1 caused deviations in other tests (LH, PROG, PRL, TESTO, TSH, FT3, and FT4) between –2.03% and 3.88%. The highest deviation from Drug 2 occurred for FSH (292,138%). Drug 2-induced deviation of –13.39% in the FT3 test and 7.61% in the FT4 test, respectively. Other tests (LH, PROG, PRL, TESTO, E2, and TSH) deviated by –1.74% to 1.01%. Drug 3 caused a deviation of 167,449% in the FSH test and 7180% in the LH test. Drug 3 caused a deviation of –2.08% to 4.79% in other tests (PROG, PRL, TESTO, E2, TSH, FT3, and FT4). Drug 4 caused a deviation of 56,851% in the FSH test and 62.90% in the LH test. Drug 4 caused a deviation of –2.63% to 5.58% in other tests (PROG, PRL, TESTO, E2, TSH, FT3, and FT4).

4. Discussion

In clinical laboratories, hormone tests are routinely performed using immunoassay techniques because technologies such as liquid chromatography-tandem mass spectrometry are expensive.[3,4] The fact that hormones are large molecules with protein and steroid structures makes their analysis difficult, and interference problems arise by interacting with other molecules.[5,6] The most important negative aspect of this measurement method is that the antibodies in the kit may interact with endogenous or exogenous molecules in the blood and cause false high or false low results. Some studies and case reports have shown that factors such as heterophile antibodies, rheumatoid factor, herbal remedies, paraproteins, carryover, reactive contamination, contrast agents, drugs, drug metabolites, supplements, hemolysis, lipemia, and microclots can affect the biochemical test results.[7–10] The large size of hormone molecules complicates the accuracy of their analysis. Although kit manufacturers have conducted research and development studies to produce monoclonal antibodies with specific labeling for each molecule, some interferants may have affected the results.[11]

The FSH content of the drugs used in this study was 56,851% and 292,138% higher than that in normal individuals. The amounts of LH were 62.9% and 7180% higher than the target values. As such high hormone concentrations are not observed in normal individuals, these hormones are not expected to interact with other tests. The fact that menotropin and follitropin contain very high levels of FSH and LH increases the possibility that other hormones may also be affected. Follitropins were extracted from human urine samples or were produced using recombinant DNA technology. They are composed of 2 glycoproteins, alpha and beta subunits. Structural analysis showed that although the amino acid sequence of follitropins is identical to that of the natural human FSH, the oligosaccharide side chains may differ. However, this small difference did not affect bioactivity.

Although Drug 3 and Drug 4 are menotropins and contain the same amount of FSH and LH, the LH content of Drug 4 was detected much lower than that of Drug 3. This difference may be due to the difference in the molecules obtained due to the production technology and their different affinities for the detection antibodies. A clinical comparative study showed that patients treated with Drug 3 had significantly higher numbers of mature and fertilized oocytes, with a non-significantly lower pregnancy rate per transfer, than those treated with Drug 4. According to that study by Kirshenbaum et al and our clinical experience, although there was no significant difference between Drug 3 and Drug 4 in terms of treatment efficacy, the LH difference can be evaluated as the inability of the detection kit to detect it.[12] This facilitates the detection of LH in this assay, resulting in better results.

The follitropin drug Drug 1 had a positive effect on E2 levels (7.31%). E2 deviated from Drug 2 by –0.68%, Drug 3 by

Table 1

Hormon tests and relationships with drugs, and bias deviation values.

	Unit	Target cons	Drug 1			Drug 2			Drug 3			Drug 4		
			Cons	Diff	BİAS %	Cons	Diff	BİAS %	Cons	Diff	BİAS %	Cons	Diff	BİAS %
FSH	IU/L	32.08	42180	42147.92	**131,383**	93,750	93717.92	**292,138**	53,750	53717.92	**167,449**	18,270	18237.92	**56,851**
LH	IU/L	38.63	40.13	1.5	3.88	38.72	0.09	0.23	2812.5	2773.87	**7180.61**	62.93	24.3	**62.90**
PROG	ng/mL	17.82	18.5	0.68	3.82	18	0.18	1.01	18.49	0.67	3.76	17.49	-0.33	-1.85
PRL	ng/mL	36.52	36.25	-0.27	-0.74	36.66	0.14	0.38	35.9	-0.62	-1.70	35.56	-0.96	-2.63
TESTO	ng/mL	831.03	844.33	13.3	1.60	836.9	5.87	0.71	835.5	4.47	0.54	837.66	6.63	0.80
E2	pg/mL	438	470	32	**7.31**	435	-3	-0.68	459	21	4.79	430	-8	-1.83
TSH	mIU/mL	17.77	18.39	0.62	3.49	17.46	-0.31	-1.74	18.3	0.53	2.98	18.5	0.73	4.11
FT3	pg/mL	8.66	8.63	-0.03	-0.35	7.5	-1.16	**-13.39**	8.48	-0.18	-2.08	8.98	0.32	3.70
FT4	ng/mL	1.97	1.93	-0.04	-2.03	2.12	0.15	**7.61**	1.98	0.01	0.51	2.08	0.11	5.58

E2 = estradiol, FSH = follicle-stimulating hormone, FT3 = free T3, FT4 = free T4, LH = luteinizing hormone, PRL = prolactin, PROG = progesterone, TESTO = testosterone, TSH = thyroid-stimulating hormone.

4.79%, and Drug 4 by –1.83%. The fact that Drug 1 caused more deviation than the others may require a better interpretation of the E2 results when using this drug. Falsely high E2 levels may partially mask low E2 levels, which negatively affect fertility. Drug 2 caused a deviation of –13.39% in FT3 and 7.61% in FT4 from thyroid hormones. Drugs 1, 3, and 4 caused much lower deviation. Drug 2 caused the highest deviation in FSH levels. This may be due to the fact that FSH has a much higher binding effect than the others. The amount of FSH in Drug 2 may be attributed to the fact that it exceeded the binding threshold in the FT3 and FT4 kits. This may also be due to differences in production technology.

Despite the high concentrations of FSH and LH, the rates of interference in PROG, PRL, TESTO, and TSH tests were <4.7%. This may be due to the low binding affinity of the detection antibodies used in FSH and LH tests.

Webster et al reported erroneously high FSH and LH test results because of heterophilic and autoimmune autoantibodies in the blood. High results may also be caused by antibodies formed after contact with animals.[13] Although the interference effect of heterophilic antibodies has decreased with improved kit technology, anti-animal antibodies still interfere with FSH and LH levels.[14] In addition, the results may be erroneously high in patients with high Macro FSH levels. A guideline published in 2021 reported what should be done to achieve the correct result for macro FSH detection.[5]

A limitation of this study is that it was conducted in vitro. The inclusion of patients undergoing in vitro fertilization treatment may be inconvenient in terms of ethical approval and because of the possibility that the uneasiness it may cause in the patient may affect the success of the treatment. Molecular interactions in the in vitro environment may also produce different results owing to the effect of the thermodynamic properties of the molecule in the in vivo environment and the binding energy level variations caused by blood matrix differences.

5. Conclusion

The possibility that menotropins and follitropins contain very high levels of FSH and LH, which may interfere with the routine kits, should always be considered. The amounts of drug ingredients above a certain threshold may be associated with doses that could interfere with other kits. We believe that FT3 and FT4 test results should be interpreted with caution when using drugs containing FSH analogs.

Author contributions

Conceptualization: Ebru Ersoy.
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Methodology: Ataman Gonel.
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