

Efficacy, efficiency and effectiveness of gonadotropin therapy for infertility treatment

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Gonadotropin therapy is an essential element in infertility treatments involving assisted reproductive technology. In recent years there have been outstanding advances in the development of new gonadotropins, particularly with the production of gonadotropins using biotechnological resources. Recombinant gonadotropins have higher specific activity compared with urinary counterparts, thus allowing subcutaneous administration of minimal amounts of glycoprotein. As a result, recombinant formulations have a better safety profile despite an overall similarity in terms of efficacy for pregnancy, as reported in many randomized controlled trials and meta-analyses. Gonadotropins stimulate the ovaries to develop follicles and oocytes, which are the raw material for fertilization and embryo production. The resulting embryos are transferred (fresh or frozen-thawed) to achieve pregnancy. The efficiency of a gonadotropin should therefore be measured by the amount of drug used, the number of oocytes/embryos produced, and the number of pregnancies achieved by transferring fresh and/or frozen-thawed embryos to the uterus (cumulative pregnancy). Comparisons between different gonadotropin preparations should also take into account other important quality indicators in reproductive medicine, such as safety and patient-centeredness. Altogether, the aforementioned quality indicators favor biotech gonadotropins over biologic products in infertility therapy.

KEYWORDS: Gonadotropins; Controlled ovarian stimulation; Assisted reproductive technology biotechnology; Infertility.

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INTRODUCTION

Gonadotropin therapy has a central role in ovarian stimulation for infertility treatment. Its introduction in medical practice dates from almost one century ago, and represents a major upgrade in the treatment of infertility. Follicle stimulating hormone (FSH) was originally derived from animal (pregnant mare serum) or human (post-mortem pituitary glands) sources, but these preparations were abandoned because of safety concerns.¹⁻³ Gonadotropins were first extracted from urine in the 1940s, more exactly, human chorionic gonadotropin (hCG) in 1940 and human menopausal gonadotropin (hMG) in 1949. Over a decade later, the first urinary forms of hCG and hMG became commercially available.^{2,3}

Further improvements in the purification methods led to the production of FSH-only products in the 1980s, and subsequent development of highly-purified urinary FSH (HP-hFSH), which became available 10 years later, in 1993.^{2,3} In the 1970s and 1980s, advances in DNA technology enabled the development of recombinant human FSH (rec-hFSH), which became commercially available in 1995.²⁻⁴ In 2000, recombinant human luteinizing hormone (rec-hLH) became available and, with the launching of recombinant human hCG (rec-hCG) in 2001, the complete recombinant gonadotropin portfolio became available.^{2,3}

In this article, I critically analyze the effectiveness and efficiency of commercially available gonadotropins, specifically recombinant FSH and LH, and human menopausal gonadotropin, taking into account the three most important quality indicators in infertility care, namely effectiveness, safety and patient centeredness.

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Understanding the structure of gonadotropins

FSH, LH and hCG are proteins covalently linked to a carbohydrate (glycoproteins). They are composed of two non-covalently linked protein subunits, the alpha and beta. The three-dimensional structure and the active conformation of the subunits are maintained by internal disulfide bonds.⁵ The alpha subunit contains 92 amino acids and is identical in FSH, LH and hCG. In contrast, beta subunits are distinct and confer unique receptor specificity as well as differential biological and immunological properties.⁶ Protein subunits alone have no biologic activity; the latter is provided by the attachment of carbohydrate moieties forming heterodimers.³ In general, protein glycosylation plays a very important regulatory role in determining protein activity and function. The extent and pattern of glycosylation convey the differential spectrum of charges, bioactivities and half-lives of each glycoprotein.⁷ Glycoproteins have two basic types of glycosylation patterns, the O-linked and the N-linked. O-linked glycosylation is characterized by attachment of carbohydrate N-acetylgalactosamine (GalNAc) to the hydroxyl group of an amino acid, serine or threonine. N-linked glycosylation involves attachment of N-acetyl glucosamine (GlcNAc) to the amide group of asparagine (Asn), as shown in Figure 1.⁸

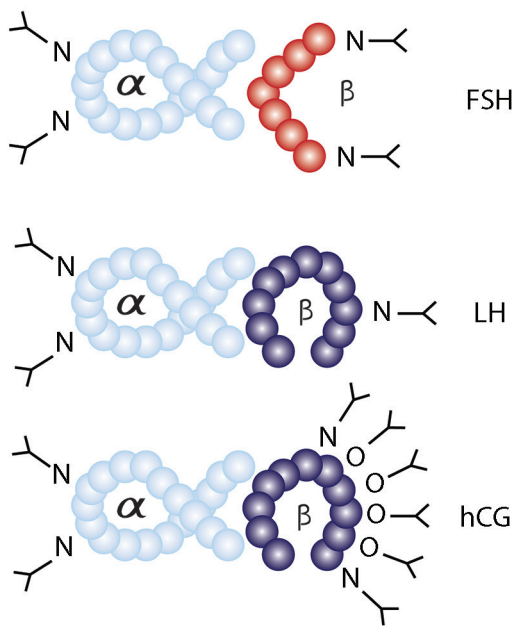


Figure 1 -Glycosylation patterns of FSH, LH and hCG. The alpha subunits of each hormone are identical in amino acid sequence and contain two sites of N-linked glycosylation. The beta subunit confers hormone specificity and contains variable amounts of N-linked glycosylation. LH beta subunit contains a single site of N-linked glycosylation, while FSH and hCG beta subunits contain two sites of N-linked glycosylation. In addition, hCG has an extended C-terminal that contains four sites of O-linked glycosylation.

Gonadotropins are further modified *in vivo* by the addition of a sialic acid (sialylation) or a sulfonic group (sulfonation) to the carbohydrate moieties. Both sialylation and sulfonation are physiological processes with major roles in gonadotropin biological activity modulation.^{9,10} The oligosaccharides often terminate with sialic acid and/or sulfonated β 1-4-linked GalNAc ($\text{SO}_3\text{-4GalNAc}$).^{9,11} Molecules with an increased number of sulfonated Gal-NAc disappear faster from the circulation than less sulfonated isoforms, due to their affinity to specific $\text{SO}_3\text{-4GalNAc}$ receptors in the liver.^{9,12} On the other hand, an increased number of sialic acids enhances half-life.^{9,13}

Removal of the carbohydrate moieties of either subunit diminishes gonadotropic activity; however, experimental data indicate that carbohydrate chains have no role in gonadotropin binding to their receptors.¹⁴ Nevertheless, carbohydrate components affect the biologic activity of the hormone-receptor complex after binding, thus playing a critical role in activation (coupling) of the adenylate cyclase system.¹⁵

Follicle-Stimulating Hormone (FSH)

The alpha subunit of FSH contains 92 amino acids, as is the case for LH and hCG. The beta subunit, however, is unique: it is composed of 111 amino acids with four N-linked glycosylation sites, two on the alpha subunit, added to Asn52 and Asn78, and two on the beta subunit (Asn7 and Asn24).^{8,16} Thus, each subunit is attached to two carbohydrate moieties with variable compositions that, in turn, create different isoforms, as shown in Figure 1.^{3,8} These multiple isoforms of FSH differ in their plasma half-lives (ranging from 3 to 4 hours) and their bioactivity.³

Although both sialic acid and sulfonated GalNAc residues may modulate the half-lives of human gonadotropins, sialic acid residues are much more common in FSH than sulfonated residues.¹³ Increased sialylation enhances FSH metabolic stability by decreasing both glomerular filtration and clearance by sialoglycoprotein receptors in the liver, which is the major site for gonadotropin clearance.^{17,18} It means that the greater the sialic acid content, the longer the hormone remains in circulation.^{9-11,13,17,18}

Production of different isoforms is controlled by a combination of steroidal feedback and gonadotropin-releasing hormone (GnRH).^{10,19} The higher the estradiol levels, the lower the FSH sialylation, as shown in Table 1.^{3,20} Therefore, the pattern of circulating FSH during the menstrual cycle is dynamic with respect not only to its quantity (concentration) but also to isoform distribution (quality).²¹ The isoform profile is more acidic during early follicular to midfollicular phase, and become more basic shortly before ovulation.^{3,21,22} These dynamic changes in sialylation are not mimicked by exogenous gonadotropin formulations, and it is unknown whether the absence of such fluctuations during controlled ovarian stimulation would affect oocyte quality.³

Table 1 - Characteristics of native FSH isoforms

Isoform	Sialic acid content	Biologic Activity	<i>In vivo</i> half-life	Predominance
Acid	High	Low	Long	Early/mid-follicular and luteal phase
Basic	Low	High	Short	Late follicular/pre-ovulatory phase

Luteinizing Hormone

Although the LH alpha subunit is identical to that of FSH, the beta subunit contains more amino acids (121 amino acids) than FSH, a difference that confers its specific biologic activity and is responsible for its interaction with the LH receptor.³ Elimination of LH from circulation is modulated by the number of both SO3-GalNAc and sialic acid residues attached to the carbohydrate moieties.¹⁷ LH β -subunits contain a single site of N-linked glycosylation (Asn 30) and less sialic acid residues (only 1 or 2); as such, native LH has a short half-life of only 20 to 30 minutes.¹⁷ Furthermore, LH molecules with increased number of SO3-4GalNAc disappear faster from the circulation due to binding of sulfonic groups to specific SO3-4GalNAc receptors at the hepatic endothelial cells.^{9,12}

Similar to FSH, LH shows fluctuations in isoform profile during the menstrual cycle. More basic LH isoforms are seen at midcycle due to considerably decreased sulfonation, concomitant with slightly increased sialylation. Both changes increase LH half-life in the circulation, thus explaining the increased levels of serum LH at this period. This change in isoform profile seems to be physiologically important for the triggering of ovulation.¹³

Human Chorionic Gonadotropin

As aforementioned, the alpha subunit of hCG is identical to those of LH and FSH. Although hCG amino acid sequence is similar to that of LH, a notable difference is the presence of a long carboxyterminal segment with 24 amino acids containing four sites of O-linked oligosaccharides, as shown in Figure 1.^{3,8} In addition, hCG beta subunits contain two sites of N-linked glycosylation, compared with a single site in LH. Due to the higher number of both glycosylation sites and sialic acid residues (approximately 20) than LH, native hCG exhibit a markedly longer terminal half-life of 24 hours after intravenous injection in comparison with approximately 30 minutes for LH, as shown in Table 2.¹⁷

Gonadotropin preparations used for controlled ovarian stimulation

Human menopausal gonadotropin

Human menopausal gonadotropin (hMG), or menotropin, was first extracted from the urine of postmenopausal women in 1949.² Urine was originally obtained from an Italian nunnery, and early preparations contained varying amounts of FSH, LH, and hCG with only 5% of pure forms.³

Improvements in the purification techniques standardized FSH and LH activities to 75 IU for each type of gonadotropin in 1963, as measured by standard *in vivo* bioassays (Steelman-Pohley assay). The first hMG preparation was registered in Italy in 1950, but clinical trials only started ten years later.³ Human menopausal gonadotropin preparations have both FSH and LH activity, but the latter is primarily derived from the hCG component present in postmenopausal urine and concentrated during purification.^{2,18,19} Sometimes, hCG is added to achieve the desired amount of LH-like biological activity.² In 1999, purified hMG gonadotropins were introduced, allowing its subcutaneous (SC) administration.^{3,20,21} Both conventional and highly-purified hMG (HP-hMG) are commercially available in an FSH:LH ratio of 1:1.²¹

Urinary FSH

In the 1980s, pure urinary FSH preparations were produced by removing LH with polyclonal antibodies. The production process was essentially passive, because LH was separated from the bulk material, and FSH, together with some other urinary proteins, was collected and lyophilized. Despite being a biologically purer urinary gonadotropin, urofollitropin or purified urinary follicle-stimulating hormone (hFSH-P) still contained high amounts of urinary proteins.^{21,22} Further technological advances made it possible to use highly specific monoclonal antibodies to extract FSH and produce HP-hFSH. The latter became commercially available in 1993 and is available to date. Such preparations contain < 0.1 IU of LH and < 5% of unidentified urinary proteins. FSH specific activity is approximately 10,000 IU/mg protein compared to 100-150 IU/mg protein in the earlier urinary hMG preparations (Table 3). Similarly to HP-hMG, the enhanced purity of HP-hFSH enabled subcutaneous administration.³ Subcutaneous gonadotropin administration represented an important gain for patients. Consistently better tolerability (less pain at injection site) was reported with subcutaneous injections compared with the intramuscular route. More importantly, it allowed self-administration which is more convenient and less time-consuming, as patients require fewer visits to the clinic or hospital for the injections.²³

Recombinant FSH preparations

Recombinant technology has fulfilled the need for a more reliable source of FSH. The procedures use the genes coding for the human FSH alpha subunit and beta-subunit which are incorporated into the nuclear DNA of a host cell via a plasmid vector, using spliced DNA strings containing the FSH gene and segments of bacterial DNA.^{2,3,25} The Chinese hamster ovary cell line has been chosen to produce gonadotropins because it is genetically stable, fully characterized and easily transfected with foreign DNA. Furthermore, it can be grown in cell cultures on a large scale and produce adequate levels of biologically active rec-hFSH.^{2,25}

Table 2 - Structural characteristics of native FSH, LH and hCG molecules

	Alpha subunit	Beta subunit	N-linked glycosylation sites (alpha)	N-linked glycosylation sites (beta)	O-linked glycosylation sites (beta)	Carboxyl terminal segment	Terminal half-life (t _{1/2})*
FSH	92 AA	111 AA	2	2	-	Absent	3-4h
LH	92 AA	121 AA	2	1	-	Absent	20-30'
hCG	92 AA	145 AA	2	2	4	Present	24h

AA: Amino acids; t_{1/2} = time that it takes for the concentration in blood plasma of a substance to reach one-half of its steady-state value; * Following intravenous infusion.

Table 3 - Differences between hMG and FSH formulations

	Purity (FSH content)	Mean specific FSH activity U/mg protein)	LH activity (IU/vial)	Injected protein per 75 IU (mcg)
hMG	< 5%	~100	75	~750
HP-hMG	< 70%	2,000-2,500	75	~33
rec-hFSH				
Follitropin beta	> 99%	7,000-10,000	0	8.1
Follitropin alfa	> 99%	13,645	0	6.1

rec-hFSH: Recombinant human follicle-stimulating hormone; hMG: Human menopausal gonadotropin; HP-hMG: Highly purified human menopausal gonadotropin.

In 1995, the first rec-hFSH (follitropin alfa) was licensed for clinical use in the European Union. One year later, a similar rec-hFSH (follitropin beta) was made available.² In the manufacturing process of follitropin alfa, two separate vectors, one for each subunit, are used to build the master cell bank of FSH-producing cell line, unlike follitropin beta in which a single vector contains the coding sequences of both subunit genes.^{21,25} The subsequent production steps are similar for both preparations. First, a working cell bank is established by growing cells from a single vial that contains identical cell preparations. An aliquot from the selected clone of Chinese hamster ovary cells is grown in T-flasks, then subcultured into roller bottles and allowed to expand for up to 36 days. The cells are then mixed with a suspension of microcarrier beads and transferred to a bioreactor vessel with continuous culture media infusion for an average of 34 days. The cell culture supernatant medium, containing the proteins secreted by the cells, is collected from the bioreactor. The harvested 'crude FSH' is stored at 48 °C until purification.² Lastly, the protein is purified by chromatography, followed by ultrafiltration. The downstream purification process differs for the two commercially available recombinant FSH preparations. The follitropin beta process uses a series of anion and cation exchange chromatography steps, hydrophobic chromatography and size exclusion chromatography. A similar series of chromatography steps are used in the production of follitropin alfa, in addition to an immunoaffinity step with a specific monoclonal antibody that is similar to the one used in the production of HP-hFSH.^{2,25} Each purification step is rigorously controlled in order to ensure batch-to-batch consistency of the final purified product.² While the production of urine-derived gonadotropins is often performed in open, non-sterile

environments, the production of rec-hFSH takes place in closed, sterile environments, such as a bioreactor. Both the production and the purification of rec-hFSH are subject to continuous quality control assessments, ensuring a pure, consistent and high-quality product.²⁵ These same concepts, highlighted above, are now used in the manufacturing process of other recombinant gonadotropins including LH and hCG.^{2,3,21}

Recombinant LH preparations

Currently, there are three groups of commercially available gonadotropin preparations containing LH activity, namely, (i) urinary hMG, in which LH activity is dependent on hCG rather than on pure LH glycoprotein, (ii) pure LH glycoprotein produced by recombinant technology (lutropin alfa), and (iii) a combination of pure FSH and LH glycoproteins in a fixed ratio of 2:1 also manufactured by recombinant technology, as shown in Table 4.³

Recombinant LH (rec-hLH; lutropin alfa) was introduced in the market in the year 2000 for use in women with gonadotropin insufficiency. The manufacturing process of rec-hLH is similar to rec-hFSH. Lutropin alfa is highly pure and has high biological activity.³ It is intended to be used subcutaneously in daily injections. Up to date, lutropin alfa is the only recombinant form of human LH developed for use in ovarian stimulation. It is presented in vials of 82.5 IU of lyophilized pure glycoprotein powder to be reconstituted with diluent before administration, using a conventional syringe and needle (75 IU of lutropin alfa is delivered per vial).

Rec-hLH is used not only to support follicular development during controlled ovarian stimulation (COS) in hypogonadotropic hypogonadic woman but also in other categories of female infertility, including poor responders,

Table 4 - Differences in LH activity of gonadotropins commercially available

	Purity (LH content)	FSH activity (IU/vial)	LH activity (IU/vial)	hCG content (IU/vial)	Specific activity (LH/ mg protein)
Lutropin alfa	> 99%	0	75 ^a	--	9,000
Follitropin alfa + lutropin alfa 2:1 ratio	> 99%	150	75	--	9,000
HP-hMG	Unknown*	75	75*	~8	--

^a 1 µg of Lutropin alfa = 22 IU; * Derives primarily from the hCG component, which is preferentially concentrated during the purification process but sometimes added to achieve the desired amount of LH-like biological activity; HP-hMG: highly purified human menopausal gonadotropin

women over > 35 years of age and hypo-responders.^{2,21,26-28} Recombinant LH has three major differences compared to urinary products containing LH activity: (i) it has higher purity and specific activity because it is manufactured using recombinant technology; (ii) it is associated with better dose precision due to filled-by-mass (FbM) technology that virtually eliminates batch-to-batch variation;^{2,29,30} (iii) LH activity is derived directly from pure LH glycoprotein, unlike hMG, in which hCG is concentrated during purification or added to achieve the desired amount of LH-like biological activity.² LH and hCG differ in the composition of their carbohydrate moieties; this, in turn, affects bioactivity and half-life. As mentioned earlier, LH activity in serum is 30 times higher when hCG is used due to its higher binding affinity to LH receptors. After subcutaneous administration, recombinant human LH is eliminated with a terminal half-life of approximately 24 hours in contrast to 5-7 days of hCG.^{17,31} It has been shown that the expression of the LH/hCG receptor gene, as well as genes involved in the biosynthesis of cholesterol and steroids in granulosa cells, are lower in patients treated with hMG preparations.³² Such effects are caused by a constant ligand exposure during the follicular phase due to longer half-life and higher binding affinity of hCG compared with rec-hLH. In animal models, down-regulation of LH receptors is maintained for up to 48h after hMG administration.³³ These findings indicate that the granulosa cells have lower LH-induced cholesterol uptake, a decrease in the novo cholesterol synthesis and a decrease in steroid synthesis, thus explaining the observed lower serum progesterone levels achieved in patients treated with hMG.^{32,33} The clinical implications of these findings, however, have not been fully elucidated.

In 2007, a new fixed combination of rec-hFSH and rec-hLH at 2:1 ratio was launched (follitropin alfa + lutropin alfa) as an alternative for those women who need LH supplementation.³⁴ The 2:1 ratio of FSH and LH in a fixed dose combination was obtained by recombinant technology and vial filling using FbM. The use of FbM as opposed of filled-by-bioassay was possible because the specific activity, isoform distribution and sialylation profile of both gonadotropins are highly consistent among manufactured batches.²⁹ The bioequivalence of rec-hFSH and rec-hLH administrated alone or in combination has been similar.^{34,35}

Comparison of gonadotropin formulations

Clinical efficacy, efficiency and effectiveness

Efficacy, efficiency and effectiveness are frequently used as synonymous, but technically they represent different concepts. Efficacy in relation to medication can be defined as the extent to which a drug has the ability to bring about its intended effect under controlled situations, such as in randomized controlled trials.³⁶ To establish efficacy, a drug should be at least as good as other available ones to which it has been compared. Efficiency, on the other hand, is the capability of a drug to effectively produce a specific outcome with a minimum amount of quantity while effectiveness estimates the extent to which a drug achieves its intended effect in the usual clinical setting.³⁶ Effectiveness measures the degree of beneficial effect under "real world" clinical settings, i.e., based on conditions of routine clinical practice and on outcomes essential for clinical decisions.³⁷

A number of meta-analyses of randomized controlled trials have compared the efficacy of different gonadotropin products, as can be seen in Table 5.³⁸⁻⁴⁵ Despite the heterogeneity of several of these meta-analyses pertaining the different stimulation protocols and choice of fertilization with standard *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), the overall conclusion is that both urinary gonadotropins, mainly hMG preparations, and recombinant gonadotropins have similar efficacy in terms of achieving a pregnancy or live birth per treatment cycle. While some of these studies were in favour of hMG preparations,^{38,39,41} in spite of the fact that the confidence limits were just 1% lower, others reported no differences in pregnancy outcomes between the two treatments.^{40,43-45} Furthermore, no significant differences were noted for spontaneous abortion, multiple pregnancy, cycle cancellation and ovarian hyperstimulation syndrome (OHSS) rates.³⁸⁻⁴⁵

Although live birth per started cycle is an important endpoint in assisted reproductive technologies (ART), it is confounded by many variables, apart from the stimulation protocol itself, which are difficult to ascertain. The intended effect of gonadotropin therapy is to stimulate the ovaries to develop follicles and oocytes, which are the raw material for fertilization and embryo production. The resulting embryos are transferred (fresh or frozen-thawed) to achieve a

Table 5 - Meta-analyses comparing pregnancy/live birth rates in ART cycles using urinary and recombinant gonadotropins

Authors and Year	Gonadotropins	Number of RCTs included	Number of participants	Conclusions
Coomarasamy et al., 2008	rec-hFSH vs. hMG	7	2,159	hMG preparations yielded a significantly higher clinical pregnancy (relative risk [RR] = 1.17, 95% CI: 1.03 to 1.34) and live birth rate (RR = 1.18, 95% CI: 1.02 to 1.38; p = 0.03). The observed difference represented a 4% increase in live birth rate with hMG when compared with rec-hFSH. No significant differences in spontaneous abortion, multiple pregnancy, cycle cancellation and OHSS rates.
Al Inany et al., 2009	rec-hFSH vs. hMG/HP-hMG	6	2,371	No significant differences in clinical, ongoing pregnancy or live birth rates. After grouping the treatment cycles by method, the ongoing pregnancy/live-birth rate favored the HP-hMG group (OR = 1.31, 95% CI: 1.02 to 1.68; p = 0.03).
Jee et al., 2010	rec-hFSH vs. HP-hMG	5	2,299	No difference in ongoing pregnancy rate per started cycle (RR = 1.10, 95% CI: 0.96 to 1.26) or per embryo transfer (RR = 1.13, 95% CI: 0.99 to 1.29), as well as in the live birth rates per embryo transfer (RR = 1.14, 95% CI: 0.98 to 1.33).
Lehert et al., 2010	rec-hFSH vs. hMG/HP-hMG	16	4,040	The absolute risk difference (RD) for hMG minus r-hFSH was 0.03 (95% CI: -0.01 to 0.07; p = 0.051), and the relative risk (RR) was 1.10 (95% CI: 0.97 to 1.25; p = 0.06). When adjusting for baseline conditions, the RR for hMG versus r-hFSH was 1.04 (95% CI: 0.89 to 1.15; p = 0.49) and the absolute RD was 0.01 (95% CI: -0.02 to 0.04; p = 0.34).
Van Wely et al., 2011	rec-hFSH vs. hFSH-P/HP-hFSH/hMG/HP-hMG	28	7,339	Overall, no significant differences in live birth or OHSS rates. Comparing only hMG/HP-hMG to rec-hFSH, significantly fewer clinical pregnancies (OR = 0.85, 95% CI: 0.74 to 0.99; I ² = 0%; 12 trials, n = 3,775; p = 0.03) and live births/ongoing pregnancies (OR = 0.84, 95% CI: 0.72 to 0.99; I ² = 0%; 11 trials, n = 3,197; p = 0.04) were obtained with rec-hFSH compared with hMG.
Gerli et al., 2013	rec-hFSH vs. hFSH-P/HP-hFSH	8	955	No significant differences in clinical pregnancy (OR = 0.85, 95% CI: 0.68 to 1.07) and live-birth rate (OR = 0.84; 95% CI: 0.63 to 1.11)

RCT: Randomized controlled trial; rec-hFSH: Recombinant human follicle-stimulating hormone; hMG: Human menopausal gonadotropin; HP-hMG: Highly purified human menopausal gonadotropin; hFSH-P: Purified urinary follicle-stimulating hormone; HP-hFSH: Highly purified urinary follicle-stimulating hormone; COS: Controlled ovarian stimulation; OHSS: Ovarian hyperstimulation syndrome.

pregnancy. The number of oocytes retrieved is therefore a highly relevant endpoint directly resulting from ovarian stimulation, and where the drug effect may be estimated with the best sensitivity. The higher the number of retrieved oocytes, the higher is the likelihood of having more embryos available both for transfer and cryopreservation, which ultimately impact on the cumulative pregnancy rates. The use of cumulative pregnancy rates per treatment instead of rates per fresh transfers is also becoming an important endpoint to assess differences in efficacy and effectiveness.

Regarding the efficacy of gonadotropins to yield oocytes following ovarian stimulation with hMG and recombinant FSH, Lehert et al., in a meta-analysis that included sixteen randomized controlled trials and 4,040 patients, showed that treatment with hMG resulted in fewer oocytes (mean difference [MD]: -1.54; 95% CI: -2.53 to -0.56; p < 0.0001) compared to rec-hFSH. When adjusting for baseline conditions, the MD estimate was

-2.10 (95% CI: -2.83 to -1.36; p < 0.001). Moreover, a higher total dose of hMG is necessary to achieve the intended effects (MD: 235.46 IU; 95% CI: 16.62 to 454.30; p = 0.03).⁴⁵ Devroey et al. in a RCT involving more than 700 patients compared HP-hMG (n = 374) with rec-hFSH (n = 375) in antagonist cycles with single blastocyst transfer. The authors retrieved more oocytes in the group treated with rec-hFSH (10.6 ± 5.8 vs. 9.1 ± 5.2; p < 0.001).⁴⁶ Although pregnancy outcomes did not differ between the treatment methods in both studies, none of them reported on the cumulative pregnancy rates associated with the transfer of fresh and frozen embryos. In a recent study, Wex and Abou-Setta added to the literature by pooling randomized controlled trials and meta-analyses that compared highly-purified hMG with rec-hFSH in fresh and frozen IVF/ICSI cycles.⁴⁷ Not surprisingly, the authors showed no significant difference in live births per started cycle (odds ratio 0.82, 95% confidence interval

[CI] 0.66-1.01), but a greater number of oocytes with rec-hFSH (mean difference [MD] 1.96, 95% CI 1.02-2.90). Using a model for economic evaluation that accounted for embryo availability, survival following thawing, and patient dropout, the investigators showed that rec-hFSH was cost-saving compared with HP-hMG in a combination of fresh and frozen cycles. Differences were equivalent to €315 per patient starting treatment or 6.4% of the total treatment cost.⁴⁷

We have compared the effectiveness of different gonadotropin products for controlled ovarian stimulation in one of the largest observational studies to date.²⁰ Our study was intended to assess treatment effectiveness, which cannot be measured in controlled trials because the act of inclusion in a study is a distortion of usual practice. Effectiveness can be evaluated through observational studies of real practice, thus allowing practice to be assessed in qualitative as well as quantitative terms. In our aforementioned study, we compared normogonadotropic down-regulated women undergoing ICSI by type of gonadotropin, follitropin alfa FbM (n = 236), hMG (n = 299) and HP-hMG (n = 330). Overall, pregnancy rates did not differ among the three treatment groups. The clinical pregnancy rate per initiated cycle was 34.7%, 35.5% and 40% for rec-hFSH, hMG and HP-hMG, respectively while the live birth rate per initiated cycle was 30.1%, 24.4% and 32.4%, respectively. However, the total dose of gonadotropin used for ovarian stimulation was significantly lower in women who received rec-hFSH ($2,268 \pm 747$ IU) compared with those who received hMG ($2,685 \pm 720$ IU) or HP-hMG ($2,903 \pm 867$ IU; $p < 0.001$). The difference in favor of rec-hFSH was also reflected in the amount of gonadotropin needed per live birth, as significantly less rec-hFSH was required compared with hMG (52% reduction) and HP-hMG (21% reduction).²⁰

In summary, when comparing gonadotropins it is important to take into account the amount of drug used, the number of oocytes/embryos produced, and the number of pregnancies obtained by transferring fresh and frozen-thawed embryos. Assuming a similar quality, the quantity of oocytes and embryos produced, including those available for cryopreservation, will impact the chances of a patient to have a baby.

Quality indicators of infertility care

Quality indicators are measurable elements for which there is evidence that they assess the quality of health care.^{48,49} While the majority of quality indicators includes safety and effectiveness of care,^{49,50} it is argued that indicator sets should fully represent healthcare quality and, therefore, cover the six dimensions of quality of care, including effectiveness, safety, efficiency, timeliness, equity and patient centeredness.^{49,51} In a recent study involving doctors, nurses and infertility patients, the

relative importance of the six aforesaid quality dimensions of care was analyzed by the Delphi method. The workgroup agreed that safety, effectiveness and patient-centeredness were the most important quality dimensions for infertility care.⁵¹

Safety

Urine-derived gonadotropins require large amounts of human urine as a primary source for manufacturing. In the 1960s and 1970s, when the demand for gonadotropins was still low, most urine was collected in Italy and the Netherlands. However, the development of new clinical indications for gonadotropins combined with the expansion of infertility treatment on a worldwide basis led to a rapid increase in demand. Urine collection was expanded to countries such as Spain, China, Brazil and Argentina. As the demand for gonadotropins began to rise exponentially in the 1980s, the ability to control the source material became more difficult. Unlike blood collection, human urine collection is not subject to specific collection regulations. Moreover, urine is collected at home from tens of thousands of individual donors and pooled. A medical questionnaire is usually the only source of health information. Because urine is pooled, the donor source cannot be traced. As the pool is constantly changing, standardization is difficult to ascertain. Transportation (from urine collection sites to processing facility) is poorly monitored and therefore quality cannot be checked throughout all manufacturing steps.^{22,52,53} Although sophisticated purification techniques are currently available, which allow the safe use of urinary formulations, extraneous urinary proteins may account for more than 30% of the protein content in high-purified hMG products even, as demonstrated by high-performance liquid chromatography analysis.³⁰ Following protein identification by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry, a total of 23 non-gonadotropin-related proteins have been identified at variable levels in different batches of the urine-derived preparations.⁵⁴ In other studies, two-dimensional gel analysis demonstrated that protein impurities were composed of leukocyte elastase inhibitor, protein C inhibitor and zinc- α_2 -glycoprotein. These proteins are involved in receptor activity, immune response, protein metabolism, and cell growth. Tumor necrosis factor-binding protein I, transferrin and immunoglobulin-related proteins were also present in both hMG and urinary FSH preparations. Lastly, recent data has shown that some of these impurities are prion proteins, which are a matter of great concern for health regulatory agencies because of their association with transmissible spongiform encephalopathy diseases.⁵⁵ A prion is a mis-folded isoform of a normal cellular protein found in the brain. When a prion comes into contact with a normal version of this protein, it induces the normal protein to adopt the mis-folded shape. The body is unable

to recognize and break down the abnormally folded protein. As a result, prions accumulate in the central nervous system, interfering with normal brain function. Conversion of normal cellular protein into the abnormal form can occur spontaneously or following infection. Abnormal prions include PrP^{sc}, the protein associated with scrapie, and PrP^{Res}, the protein resistant to enzyme degradation found in patients with Creutzfeldt-Jakob disease (CJD).⁵⁵ Inactivation of prions in urine-derived material may denature proteins, including FSH. For instance, urea, a commonly used denaturant, destroys the dimeric structure of proteins. This is one reason why urine-derived gonadotropins cannot be as pure as recombinant ones. Cross-contamination is another concern in urine-derived products. A rogue element in one individual donation of urine may spread through a complete batch and potentially cause problems in the final product. Quality control is only possible through stringent donor collection, transportation and production. In fact, several regulatory agencies have set limitations to urine-derived products.^{22,52} Although the clinical significance of most protein contamination from urinary gonadotropins is unknown, it is certain that these contaminants are not needed to induce optimal follicle development. More importantly, these findings underscore the poor quality of urinary sources and stress the need for more reliable proteins.^{22,52} Many of the risks associated with biologically extracted proteins are avoided when the protein is produced synthetically.

In the manufacturing process of recombinant gonadotropins, vectors are used to build the master cell bank of a specific gonadotropin-producing cell line.^{21,25} A working cell bank is established by growing cells from a single vial that contains identical cell preparations. The cell culture supernatant medium, containing the proteins secreted by the cells, is collected from the bioreactor, and the protein is purified.^{21,25} Both the production and the purification of recombinant gonadotropins are subject to continuous quality control assessments, ensuring a pure, consistent and high-quality glycoprotein as demonstrated by SDS-PAGE and western blot analysis.⁵⁴ Given its high purity, each product batch of recombinant gonadotropin is routinely characterized and controlled using physicochemical techniques, including size exclusion high performance liquid chromatography (SE-HPLC), which allows assessment of both the integrity and the amount of glycoproteins; and isoelectric focusing and glycan mapping, which characterize protein glycoforms present in each preparation.^{56,57}

Patient-centeredness

Recombinant gonadotropins are presented in ready-to-use solutions; they include pen devices, so that the gonadotropin can be self-administered subcutaneously. In contrast, urinary gonadotropins consist of a

freeze-dried lysosphere containing either 75 IU of FSH/hMG or 5,000/10,000 IU of hCG that need to be dissolved in sterile water before injection.¹² Intramuscular administration is the route of choice for most urinary gonadotropins, although it is possible to apply highly-purified urine-derived gonadotropins subcutaneously.³⁰

Earlier studies have shown that drug delivery by pen devices was bioequivalent to that of conventional syringe injections. However, due to unavoidable losses during syringe filling and/or removing excess air, about 18% of the FSH amount is lost in the conventional syringe application when compared to a ready-for-use solution with a pen device.³⁵

Studies addressing satisfaction indicate that patients clearly prefer pen injections. Outcomes including self-administration and patient satisfaction, the overall incidence of local reactions, overall pain score, and burning sensation at the injection site clearly favor the pen device group.^{35,58} In a study focussing on patient-centeredness, patients and their partners received nurse-led training on three gonadotropin presentations: (i) powdered urofollitropin with conventional needles and syringes for administration, (ii) follitropin beta in a premixed and prefilled cartridge with a reusable injection device, and (iii) follitropin alfa in a disposable, premixed and prefilled injection device. A total of 123 participants attended the training and were asked to complete a post-training questionnaire. More participants expressed a preference for using pen injectors compared with conventional syringes (84.6% versus 5.7%; $p < 0.0001$). Of the 94 participants who preferred a particular device, more preferred the follitropin alfa prefilled pen (68.1%) than the follitropin beta cartridge and pen (24.5%; $p < 0.0001$) or urofollitropin with needle-free reconstitution device and conventional syringe (7.4%; $p < 0.0001$).⁵⁹

Recently in 2011, a new family of pen (FoP) devices has been approved in the European Union, Canada and Australia, intended to deliver recombinant preparations.^{60,61} The new device is like the pen used to administer follitropin alfa, but explaining its use to patients is simpler and quicker, which reduces the risk of errors. In addition, these novel devices offer dosing increments in very small amounts, thus allowing the dosage to be fine-tuned at any point after treatment has started.^{60,61} In summary, the introduction of recombinant technology, filling method by mass, and pen devices for gonadotropin administration represent an important step forward in terms of patient-centeredness in infertility treatments.

CONCLUSIONS

Gonadotropin therapy has a central role for ovarian stimulation in infertility treatments. Efforts have been made to improve gonadotropin preparations over the last century, which led to the development of

recombinant products using biotechnology. Recombinant gonadotropins are purer than urinary-derived gonadotropins, and the introduction of FbM technology virtually eliminated batch-to-batch variations and enabled accurate dosing in small increments; all of which allowed the introduction of individualized controlled ovarian stimulation. Biotech gonadotropins have better clinical efficiency and effectiveness compared with urinary products, owed to the production of more oocytes using lower amounts of the drug. An increased number of oocytes/embryos produced will positively impact the chances of patients to have a baby, by offering them the highest cumulative pregnancy rate. Biotech gonadotropins are also in line with important quality indicators of infertility care, including safety and patient-centeredness. Recombinant gonadotropin formulations have a better safety profile owing to purity, and the introduction of prefilled pen devices simplified infertility treatment for patients, doctors and nurses alike.

EFICÁCIA, EFICIÊNCIA E EFETIVIDADE DA TERAPIA GONADOTRÓFICA PARA O TRATAMENTO DA INFERTILIDADE

RESUMO: A terapia gonadotrófica é elemento essencial nos tratamentos de infertilidade que envolvem tecnologia de reprodução assistida. Nos últimos anos houve avanços notáveis no desenvolvimento de novas gonadotrofinas, principalmente com a produção de gonadotrofinas via recursos biotecnológicos. As gonadotrofinas recombinantes têm maior atividade específica em comparação com os suas homólogas urinárias, permitindo, assim, a administração subcutânea de quantidades mínimas de glicoproteína. Como resultado, as formulações recombinantes tem um melhor perfil de segurança, apesar de semelhança em termos de eficácia para a gravidez, como relatado em diversos ensaios clínicos randomizados e meta-análises. As gonadotrofinas estimulam os ovários a desenvolver folículos e ovócitos, que são a matéria-prima para a fertilização e produção de embriões. Os embriões resultantes são transferidos (frescos ou congelados/descongelados) para produzir gravidez. Comparações entre as gonadotrofinas devem, portanto, ser medidas não somente pela eficácia clínica de produzir gravidezes pela transferência de embriões a fresco, mas sobretudo pela eficiência na produção de ovócitos e embriões em relação à quantidade de droga administrada, e efetividade na obtenção de gravidezes pela transferência de embriões frescos e congelados/descongelados (taxa de gravidez cumulativa). As comparações entre diferentes preparações de gonadotrofinas também devem levar em conta outros indicadores importantes de qualidade em medicina reprodutiva, como a segurança e o interesse do paciente. Estes indicadores de qualidade favorecem as gonadotrofinas biotecnológicas em relação aos produtos biológicos na terapia da infertilidade.

UNITERMOS: gonadotrofinas; estimulação ovariana controlada; tecnologia reprodutiva assistida

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