Gonadotropin preparations: past, present, and future perspectives

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This Educational Bulletin offers past, present and future prespectives on gonadotropin preparations. (Fertil Steril® 2008;90:S13–20. ©2008 by American Society for Reproductive Medicine.)

Use of gonadotropin therapy is so central to infertility treatment that it is easy to overlook the considerable discovery and research that preceded production of the effective and safe products available today. The history underpinning this development spans close to 100 years and provides a splendid example of how basic animal experimentation and technological advances have progressed to clinical application.

The consistent driving force beyond the early demonstration that pituitary extracts could stimulate follicular development, and therefore have potential utility in infertility treatment, has been that gonadotropin products must be safe and effective. Gonadotropin treatment for induction of ovulation in anovulatory women began in the 1960s, and for stimulating multifollicular development in ovulatory women, began in the 1980s.

The objectives of this educational bulletin are [1] to outline the landmark discoveries leading to routine clinical application of gonadotropin therapy for follicular stimulation; [2] to describe the structure of glycoprotein hormones; [3] to review the development of gonadotropin preparations from crude animal extracts to currently available products; and [4] to consider future pharmaceutical preparations for follicular stimulation. The overarching purpose of this bulletin is to provide a concise reference document for practitioners using gonadotropin therapy in the treatment of infertility.

LANDMARK STUDIES IN THE DEVELOPMENT OF GONADOTROPIN THERAPY

Pituitary Regulation of Ovarian Function

Historically, the development of gonadotropin preparations began with discovery that the pituitary regulates gonadal function (1, for review). As early as 1910, studies with dogs showed that partial ablation of the pituitary resulted in gonadal atrophy (2). By 1930, numerous studies with several other species had shown that implantation of anterior pituitary tissue from sexually mature females into sexually immature animals conferred reproductive function (3) and that reproductive function was lost after complete pituitary ablation (4). Shortly thereafter, Zondek described the cyclical secretory dynamics of two gonadotropins (prolan A and B) in the blood and urine of women, now known as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (1). Taken together, these observations laid the foundation for using gonadotropins for ovarian stimulation, both in animals for experiments on oogenesis and embryogenesis and in women for the treatment of infertility.

The Discovery of Pregnant Mare's Serum Gonadotropin (PMSG) and Human Chorionic Gonadotropin (hCG)

Pregnant mare's serum gonadotropin (PMSG) is found at the maternal–fetal interface in pregnant mares (5) and can be extracted from their blood to obtain large quantities of purified, stable powder that can be sterilized for use in laboratory and clinical studies. In equids, PMSG has only LH-like activity and is known as equine chorionic gonadotropin (6). In nonequine species, PMSG has both FSH and LH activity and stimulates follicular development, ovulation, and corpus luteum formation (7, for review).

The discovery of hCG arose from the observation made 80 years ago by Ascheim and Zondek that the urine of pregnant women contained a gonad-stimulating substance that induced both follicular maturation and ovarian stromal luteinization when injected into immature mice (1). Subsequently, hCG was shown to be secreted by placental tissue in vitro (8), localized to the syncytiotrophoblast (9), and derived from giant syncytiotrophoblast cells (10). The hormone first was made commercially available in 1931 under the label Pregnon®, later changed to Pregnyl® in 1932. Remarkably, Pregnyl® still is available today.

Superovulation in Rodents

Regimens involving consecutive injections of PMSG and hCG were used first by Runner (11) to induce estrus, ovulation, and coitus in immature mice. The landmark studies by Fowler and Edwards (12) were published in 1957. Using adult mice injected with PMSG, followed 48 hours later by hCG, they observed and defined the time required for entering estrus, for progression through the successive meiotic stages from prophase I through to emission of the first polar body at metaphase II, and for ovulation and implantation. The work culminated in the first live birth in any adult mammalian species primed with gonadotropins.

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Follicular Stimulation in Women

Follicular stimulation in women was attempted as early as 1931 using PMSG (13). Impure preparations induced an ovarian response, but ovulation was not achieved (14), even when purified PMSG was used (15).

Animal Pituitary Extracts

In parallel to the studies with PMSG, there were attempts to stimulate follicular development with pituitary extracts from several species, including swine, sheep, and cows. Such extracts, either alone or in conjunction with PMSG, were used in Europe and in the United States through the early 1960s, despite the earlier discovery that treatment resulted in the production of neutralizing antibodies ("anti-hormones") that ultimately rendered the ovaries unresponsive (16, for review). Several pregnancies were reported, some resulting from use of hCG to stimulate ovulation in an early two-step protocol (17, for review). Nevertheless, concerns regarding adverse effects of the animal extracts led to formation of a group (the "G Club") that was founded to coordinate development and purification of human gonadotropins for therapeutic use (1).

Human Pituitary Gonadotropin

Human pituitary gonadotropin (hPG), first isolated in 1958 (18), was used with some success to stimulate ovulation. However, the supply of human pituitaries could not meet the demand for production. By the mid-1980s, cases of dementia and death due to iatrogenic Creutzfeldt-Jacob disease were linked to human pituitary growth hormone, thereby forcing hPG off the market (19).

STRUCTURE OF GLYCOPROTEIN HORMONES

The glycoprotein hormones FSH, LH, hCG, and thyroid-stimulating hormone (TSH) are composed of two non–covalently linked protein subunits, the alpha and beta subunits, to which carbohydrate moieties are attached (Figs. 1 and 2). The alpha subunit is identical among the four hormones and is composed of 92 amino acids. In contrast, the beta subunits are distinct and confer the unique biological and immunological properties and the receptor specificity of each of these glycoproteins (20). The subunits alone have no known biologic activity. It is the formation of the heterodimer that provides the hormonal activity through attachment of the carbohydrate moieties, and the extent of glycosylation, especially sialylation, that conveys the spectrum of differences in charge, bioactivities, and elimination half-lives (21).

FSH

The beta subunit of FSH is composed of 111 amino acids. There are four asparagine-linked glycosylation sites (two on the alpha subunit and two on the beta subunit). Each subunit is attached to two carbohydrate moieties with variable compositions that, in turn, create different isoforms. These multiple forms of FSH differ in their plasma half-lives (range: 3 to 4 hours) due to variations in their binding potentials. When FSH isoforms have more sialic residues, they have decreased receptor affinity and therefore remain in circulation longer. It is unknown whether these lower clearance rates translate into increased bioactivity. The distribution of isoform types is under endocrine control, and the amount of sialic acid present is influenced mainly by estradiol (E_2) levels (22)—the higher the E_2 levels, the less glycosylated the FSH, the shorter the half-life, but the greater the receptor affinity (Table 1). Therefore, the isoform profile is more acidic during the early follicular to midfollicular phase of the menstrual cycle, but shifts to become more basic shortly before ovulation (23). These dynamic changes in sialylation are not mimicked by current controlled ovarian gonadotropin stimulation regimens. It is unknown whether such modifications in follicular stimulation protocols would affect oocyte quality.

LH and hCG

Although the alpha subunits of LH and hCG are identical to that of FSH, the beta subunits are different. Luteinizing hormone has a beta subunit containing 121 amino acids that confers its specific biologic action and is responsible for its interaction with the LH receptor. This beta subunit of LH contains the same amino acids in sequence as the beta subunit of hCG, but the hCG beta subunit contains an additional 23 amino acids. The two hormones differ in the composition of their carbohydrate moieties which, in turn, affects bioactivity and half-life. The half-life of LH is 20 minutes, and that for hCG is 24 hours.

DEVELOPMENT OF GONADOTROPIN PREPARATIONS FOR FOLLICULAR STIMULATION

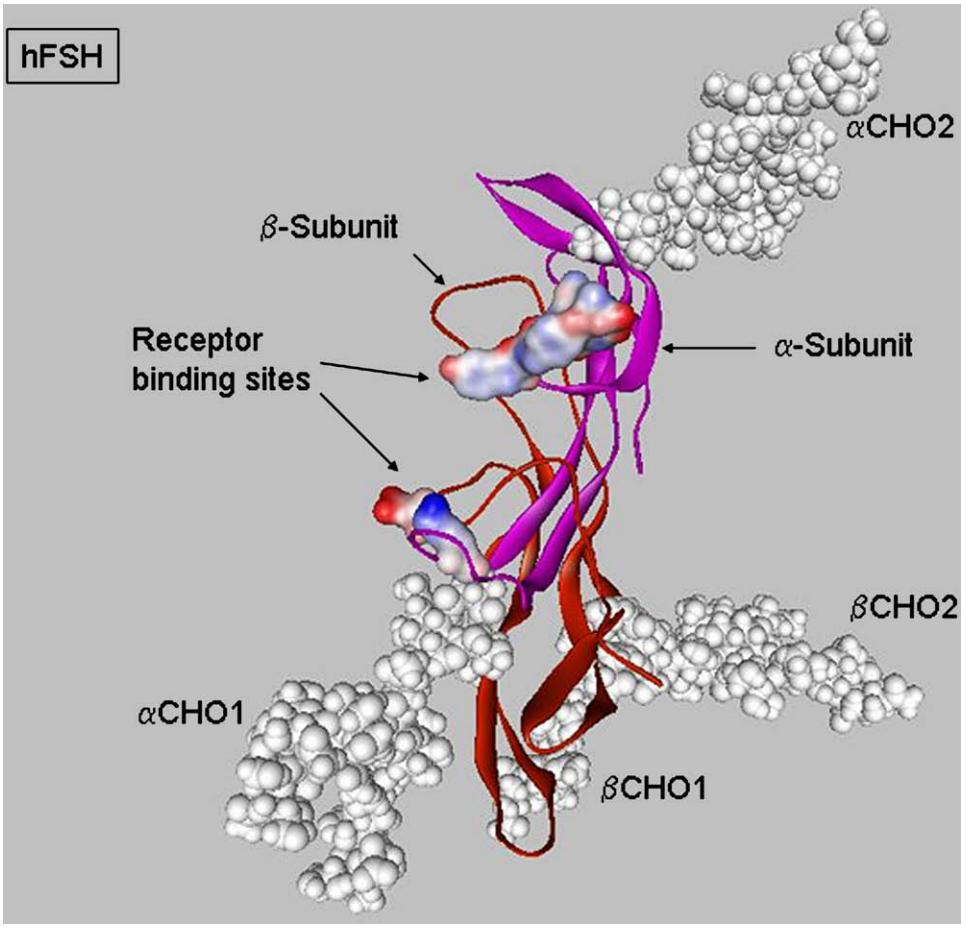
Research underlying production of the current gonadotropin preparations has spanned at least five decades. Gonadotropin manufacture has evolved from extraction of urine to application of recombinant techniques to yield a variety of FSH preparations for ovarian stimulation (Table 2). The result is a broad choice of effective drugs with similar clinical efficacy (25). Because both urinary and recombinant purification processes involve exposure to impurities, preparations devoid of extraneous biological materials cannot be guaranteed. However, all products appear to be safe.

Human Menopausal Gonadotropin

Human menopausal gonadotropin (hMG), or menotropin, is derived from postmenopausal urine. The urine originally was obtained from a single nunnery in Italy but later collection was expanded to numerous centers in a wide variety of other countries. The early preparations were originally only about 5% pure and contained varying amounts of FSH, LH, and hCG. Improvements in purification techniques resulted in standardization of the FSH and LH activity to 75 IU for each type of gonadotropin, although extraneous urinary proteins still existed and are present even in the hMG products

FIGURE 1

Human follitropin and human choriogonadotropin consist of two subunits, the alpha subunit (*purple*) and the beta subunit (*red*). There are four carbohydrate attachment sites in follicle-stimulating hormone (FSH). As carbohydrate beyond GlcNac2 was not visible in the FSH crystal structure (1FL7), the structures represented were attached to each site by grafting those structures on to Asn and GlcNAc1 molecules at each site. The carbohydrate chain that if removed does not prevent binding but abrogates signal transduction is seen at the 7 o'clock position (αCHO1). At the 4 o'clock and 5 o'clock positions are the two chains (βCHO1, βCHO2) that are resident on the beta subunit and differ in glycosylation as women age (older women have mostly two chains at this site, and younger women have a mixture of two or no chains at this site). Residues critical for receptor binding are rendered with surfaces (receptor-binding sites) as verified in the hFSH/hFSHR crystal structure (PDB files 1XWD). *Source:* Courtesy James A. Dias, Ph.D.



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available today. The LH-activity in hMG derives primarily from the hCG component, which preferentially is concentrated during the purification process and sometimes was added to achieve the desired amount of LH-like biological activity. Clinical use of hMG began in 1950, but clinical trials were not started until the early 1960s (26, 27).

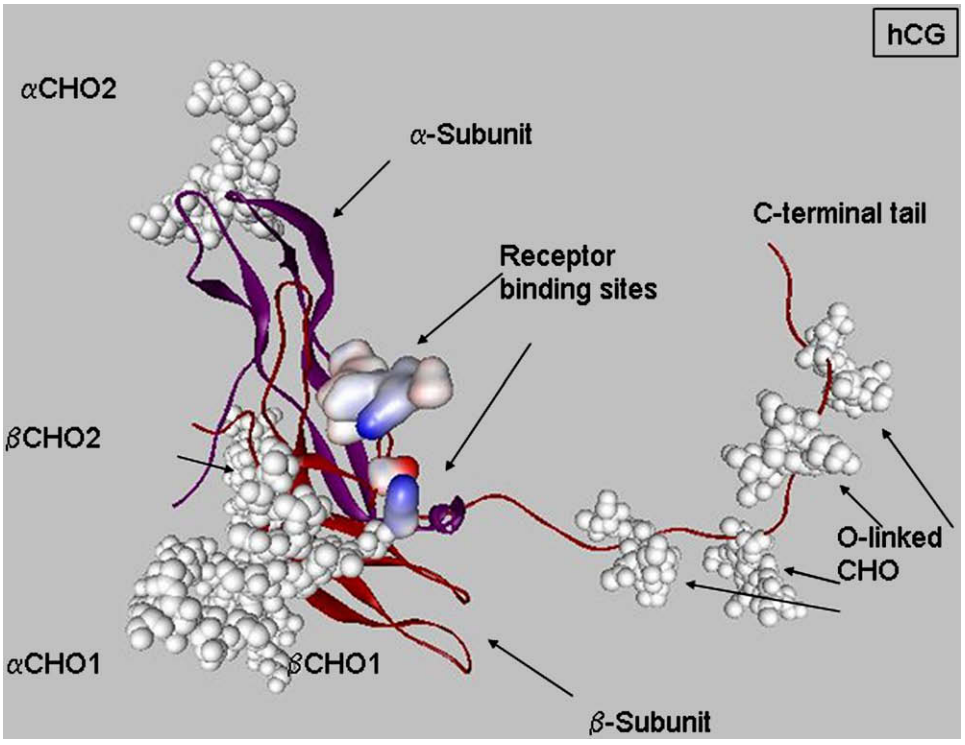
Urinary FSH

Removal of the LH with polyclonal antibodies resulted in a biologically pure urinary FSH preparation (urofollitropin), but one that still contained urinary proteins (28).

Use of monoclonal antibodies specific to FSH resulted in further refinements in manufacture, and the production of highly purified (HP) urinary FSH. Such preparations contain $<$0.1 IU LH and $<$5% of unidentified urinary proteins. Furthermore, the specific activity of the FSH is increased from 100–150 IU/mg protein in purified urinary FSH preparations to approximately 10,000 IU/mg protein in the HP product. The enhanced purity and increasing specific activity of HP-FSH enable subcutaneous delivery in very small volumes, in addition to virtual elimination of batch-to-batch variation.

FIGURE 2

Human chorionic gonadotropin (hCG) is similar in structural attributes compared with follitropin. A notable exception is the presence of a long carboxy terminal segment that is O-glycosylated (O-linked CHO). This segment is not visible in the hCG crystal structure (PDB files 1XUN and 1HCN), but it is shown here for illustrative purposes. Of importance is that this extension confers a long half-life to hCG; when grafted onto the hFSH beta subunit, this extension likewise confers a longer circulatory half-life to hFSH. The C-terminal extended hFSH is currently in clinical trials. *Source:* Courtesy James A. Dias, Ph.D.



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Recombinant FSH Preparations

The advent of recombinant DNA technology opened the door for production of recombinant FSH preparations. Recombinant preparations are manufactured by inserting the genes encoding for the alpha and beta subunits of FSH into expression vectors that are transfected into Chinese hamster ovary cell lines (24, for review).

The two recombinant FSH preparations currently available are marketed as follitropin alpha and follitropin beta. Both are structurally identical to native FSH and, despite being named follitropin alpha and follitropin beta, each comprises one alpha and one beta glycoprotein chain. These dissimilar glycoprotein chains are noncovalently linked, being conjoined by electrostatic and hydrophobic forces, attached to two complex carbohydrate structures. The posttranslational glycosylation process and purification procedures of the two recombinant FSH preparations are not identical (29), resulting in different sialic acid residue compositions and thus different isoelectric coefficients. The subtle differences in structure have not resulted in any proven differences in clinical performance.

In contrast to the FSH content of urinary-derived FSH, that of recombinant FSH preparations can be quantified by protein content (mass in μg) rather than by biological activity. Nevertheless, the biological activity of all FSH-containing preparations is still confirmed at some point in the manufacturing process by the classic Steelman-Pohley ovarian bioassay (30). Formulations of both follitropin alpha and follitropin beta are now available based on the fill-by-mass

TABLE 1

Follicle-stimulating hormone (FSH) isoforms.

Type of FSH isoform	E_2 level	Sialic acid content	Receptor affinity	Half-life in vivo
Acidic	Low	High	Low	Long
Basic	High	Low	High	Short

Source: Adapted from Howles 1996 (24).

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TABLE 2

Characteristics of exogenous follicle-stimulating hormone (FSH) preparations.

Gonadotropin preparation	U.S. brand name[a] (Year of FDA approval)	Marketing status	FSH activity (IU/ampule)	LH activity (IU/ampule or vial)	% Protein contamination	Source	Technology used	Route of Administration
hMG (menotropins)	Pergonal® (1975)	Discontinued	75 or 150	75 or 150	>95	Urine	Chemical extraction	IM
	Humegon® (1994)	Discontinued	75 or 150	75 or 150	>95	Urine	Chemical extraction	IM
	Repronex® (1999)				>95	Urine	Chemical extraction	IM or SC
		Available	75	75				
		Discontinued	150	150				
	Menopur® (2004)	Available	75	75	<5	Urine	Chemical extraction	SC
Urinary FSH (urofollitropins)	Metrodin® (1986)	Discontinued	75 or 150	Negligible	>95	Urine	Chemical extraction + PAB	IM
Highly purified urinary FSH	Fertinex® (1986)	Discontinued	75 or 150	Negligible	<5	Urine	Chemical extraction + MAB	IM or SC
	Bravelle® (2002)	Available	82.5 (75[a])	Negligible	<5	Urine	Chemical extraction + MAB	IM or SC
Recombinant FSH follitropin-alpha	Gonal-F® (1997)					Transfected CHO cells	Recombinant DNA	
	RFF vial	Available	75	0	Unknown			SC
	Multidose	Available	450	0	Unknown			SC
	RFF-pen	Available	300, 450, or 900	0	Unknown			SC
Follitropin-beta	Follistim® (1997)					Transfected CHO cells	Recombinant DNA	
	AQ vial	Available	75 or 150	0	Unknown			IM or SC
	AQ cartridge + pen	Discontinued	175 (150[b])	0	Unknown			SC
		Available	350 (300[b])	0	Unknown			
		Available	650 (600[b])	0	Unknown			
		Available	975 (900[b])	0	Unknown			

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TABLE 2

Continued.

Gonadotropin preparation	U.S. brand name[a] (Year of FDA approval)	Marketing status	FSH activity (IU/ampule)	LH activity (IU/ampule or vial)	% Protein contamination	Source	Technology used	Route of Administration
Urinary hCG	Profasi® (early 1960s) Chorionic gonadotropin® (1973)	Discontinued	Negligible	10,000	<5	Urine	Chemical extraction	IM
		Available	Negligible	10,000	<5	Urine	Chemical extraction	IM
		Discontinued	Negligible	2000; 5000, 15,000, 20,000	<5	Urine	Chemical extraction	IM
Highly purified urinary hCG	Pregnyl® (1976)	Available	Negligible	10,000	<5	Urine	Chemical extraction	IM
	Choragon® (1996)	Available	Negligible	5000	<5	Urine	Chemical extraction	IM
Recombinant hCG	Ovidrel® (2003), syringe	Available	0	250 μg delivered	Unknown	Transfected CHO cells	Recombinant DNA	SC
Recombinant LH	Luveris® (2004)	Available	0	75 delivered	Unknown	Transfected CHO cells	Recombinant DNA	SC

Abbreviations: CHO = Chinese hamster ovary; IM = intramuscular; MAB = monoclonal antibodies; PAB = polyclonal antibodies; SC = subcutaneous.

[a] Brand names and formulations may vary in different countries.

[b] Indicates actual amount of FSH delivered.

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method (31). The conversion factor suggested for these products is that 75 IU of FSH assessed by the Steelman-Pohley assay corresponds to between 5.0 and 5.5 μg of a fill-by-mass product. Delivery systems for recombinant FSH products include pen-shaped administration devices that either are pre-filled or can be adjusted to deliver variable amounts of gonadotropin contained in vials (32). All preparations are packaged as lyophilized powder with the exception of the liquid formulations found in cartridges or pens. Neither the packaging nor the method of delivery has translated into superior pregnancy rates for either urinary or recombinant FSH preparations.

Recombinant LH Preparation

Recombinant LH has been available for clinical use since 1993 in vials with syringes that together are designed to deliver 75 IU. The product may enhance follicular development when used in conjunction with FSH in patients suffering from hypogonadotropic hypogonadotropism who have profound LH deficiency (33). Among otherwise normal women undergoing stimulation for assisted reproductive technologies (ART), a recent meta-analysis revealed that the pregnancy rate is not increased when LH is used in "add-back" therapy (odds ratio, 0.92; confidence interval, 0.65–1.31; P=.35) (34).

Chorionic Gonadotropin Preparations

Chorionic gonadotropin is used to promote the final stages of follicular maturation and progression of the immature oocyte at prophase I (the germinal vesicle stage) through meiotic maturation to reach metaphase II. Approximately 36 hours is required for completion of the meiotic process (35), and, in the absence of follicular aspiration at oocyte retrieval, ovulation will ensue approximately 4 hours later. Chorionic gonadotropin can be human derived from urine of pregnant women (hCG) or manufactured using recombinant technology. Preparations of hCG are marketed in vials of 5000 or 10,000 IU. Recombinant chorionic gonadotropin syringes contain 250 μg of product, which is equivalent to 5000–6000 IU of hCG.

FUTURE DEVELOPMENTS IN GONADOTROPIN PREPARATIONS

Molecular engineering provides the technology to modify FSH preparations to prolong their half-lives and therapeutic actions, thereby reducing the number of injections required to achieve optimal follicular growth. To this end, novel FSH preparations with modifications in FSH glycosylation (36) or replacements of the carboxy-terminal peptide (CTP) of FSH (37) are undergoing clinical trials. Additional future developments may employ high-throughput screening of large chemical libraries to identify orally active small molecule agonists of human FSH or LH receptors that might obviate the need to inject gonadotropins.

SUMMARY

- Landmark studies underpinning the clinical application of gonadotropins include the discovery of pituitary control of ovarian function, the identification and purification of PMSG and hCG, and the development of treatment regimens to stimulate superovulation in rodents.
- Production of gonadotropin preparations has focused on improving purity, safety, efficacy, and consistency while ensuring adequate availability to meet market needs.
- Compared with earlier crude animal extracts, modern highly purified urinary and recombinant gonadotropin products have clearly superior quality, specific activity, and performance.
- There are no confirmed differences in safety, purity, or clinical efficacy among the various available urinary or recombinant gonadotropin products.
- New, longer acting gonadotropin preparations are under development and hold promise for improving patient satisfaction while maintaining efficacy.

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