Reproductive Biology Insights

REVIEW

What is the Clinical Relevance of Follicle-Stimulating Hormone Isoforms in Fertility Treatment?

Claus Yding Andersen[1] and Diego Ezcurra[2]

[1]Laboratory of Reproductive Biology, University Hospital of Copenhagen, University of Copenhagen, Blegdamsvej 9, Copenhagen, DK-2100, Denmark. [2]Fertility and Endocrinology Business Unit, Merck Serono S.A., Geneva, 9 Chemin de Mines, Geneva CH-1202, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany).
Corresponding author email: diego.ezcurra@emdserono.com

Abstract: Follicle-stimulating hormone, both naturally synthesized and as commercial preparations, exists as different isoforms. Variation in the process of glycosylation, particularly in the number of terminal sialic-acid residues, gives rise to isoforms of varying acidic profiles with differences in half-life and bioactivity. Based on the known follicle-stimulating hormone isoform variation across the reproductive cycle, it is possible that the follicle-stimulating hormone isoform profile used in controlled ovarian stimulation may impact follicular recruitment and clinical treatment outcomes. In light of the uncertainty regarding the clinical relevance of follicle-stimulating hormone isoforms in fertility treatment, published studies exploring this topic are reviewed.

Keywords: follicle-stimulating hormone, isoform, gonadotrophin, heterogeneity

Reproductive Biology Insights 2011:4 1–10

doi: 10.4137/RBI.S7362

This article is available from http://www.la-press.com.

Introduction

In mammals, the anterior pituitary gland is responsible for the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), two critical hormones (gonadotrophins) involved in the regulation of gonadal function. Under the influence of FSH and LH, ovarian follicles develop from antral to preovulatory stages; at ovulation, mature oocytes that are capable of being fertilized are released. Structurally, gonadotrophins are heterodimers that include two non-covalent protein subunits; an alpha subunit (common to all gonadotrophins) and a beta subunit (different for each gonadotrophin and responsible for the specific biological activity of each molecule).[1] Each alpha and beta chain is associated with two heterogeneous oligosaccharide chains that play a crucial role in the in vivo and in vitro bioactivity of the hormone. FSH binds to G protein-linked cell surface receptors belonging to the G protein-coupled receptor super-family, which can also display a high degree of molecular heterogeneity (through single nucleotide polymorphisms) that could potentially impact on receptor functionality.[2,3]

Despite the structural complexity of gonadotrophins and their receptors, these molecules may exhibit a high degree of elasticity, and this could allow the formation of distinct ligand–receptor complexes; these complexes are potentially capable of selectively activating or deactivating a variety of signalling pathways that could trigger different clinical outcomes. Knowledge of the structural differences of these molecules and the implications at both the functional and clinical levels are paramount to understand and individualize controlled ovarian stimulation and maximize outcomes for the benefit of patients.

FSH Heterogeneity

FSH plays a key role in the regulation of ovarian follicular development in women. During the synthesis of FSH in the anterior pituitary gland, post-translational glycosylation takes place. The composition and structure of the added carbohydrate/oligosaccharide chains are highly variable and give rise to different isoforms; at least 15 FSH isoforms have been identified.[4]

The variable proportion of negatively charged sialic acid residues on the carbohydrate/oligosaccharide chains creates heterogeneity in electric charge: more acidic isoforms are isolated from urinary products, contain higher numbers of sialic acid residues and have a longer half life than the less acidic isoforms (referred to from here as more basic isoforms). The more basic isoforms are recombinant molecules that demonstrate higher receptor binding affinity and higher biopotency in vivo than the more acidic isoforms.[5–7] This difference in charge enables separation of isoforms using techniques such as isoelectric focusing. The isoelectric point (pI) is the pH at which the protein has no net charge; it is high for basic proteins and low for acidic proteins. The pI of individual FSH isoforms ranges between 3.5 and 7; for the majority of isoforms, the pI is between 4.5 and 5.0.[1]

The added oligosaccharides determine a number of properties of glycoprotein hormones, including alpha/beta subunit assembly and intracellular trafficking.[8] Molecular and structural differences also influence the interactions of different FSH isoforms with their target cell receptors, in terms of inducing biological responses in vitro and in vivo and survival in the circulation in vivo.[9] More basic isoforms, which predominate in recombinant human FSH (r-hFSH), exhibit a higher receptor-binding affinity in heterologous cell assay systems, such as those employing rat granulosa cells or testicular membranes.[10,11] Consistent with their receptor-binding affinity, more basic isoforms exhibit higher in vitro bioactivity than the more acidic isoforms in heterologous assay systems.[10,11] In cultured rat granulosa cells, more basic isoforms induced higher cAMP release, estrogen production and tissue-type plasminogen activator enzyme activity than their more acidic counterparts.[6] Experiments employing a human embryonic kidney-derived cell line transfected with human FSH receptor cDNA suggest that in homologous cell systems, FSH bioactivity may also be influenced by factors other than receptor-binding affinity, such as media half life; more acidic isoforms show a longer half-life than more basic isoforms.[11]

The relative proportions of more acidic and more basic isoforms present in human FSH products, either urinary-derived (u-hFSH) or r-hFSH, depends on the manufacturing process and the origin of the raw material. Furthermore, due to their specific isoform profile, different commercial preparations of FSH may deliver different qualitative and/or quantitative signals to the follicle and contribute to different clinical efficacy outcomes.[12]

The clinical relevance of the structural and functional isoform differences of FSH products used for ovarian stimulation has yet to be fully elucidated since the data are few and disparate. The objective of this article is to summarize the published literature on this topic and highlight unresolved issues that are yet to be fully addressed in clinical trials.

Natural FSH Isoforms and their Physiological Relevance

Under neuro-endocrine control, changes in the distribution of circulating FSH isoforms occur during different physiological states, such as at the onset of puberty,[13–15] after menopause[16,17] and during the peri-ovulatory period of the menstrual cycle.[1,16,17] These findings suggest that FSH heterogeneity is functionally relevant and of physiological importance.[18] It has been suggested that subtle changes in distribution of FSH isoforms may alter the net potency of the signal delivered to the target cell, and may even elicit divergent responses at the receptor level;[19] this may provide a fine-tuning mechanism by which gonadal function can be closely controlled.[1]

The pH-distribution profiles of serum FSH during different phases of the menstrual cycle indicate that heavily sialylated, more acidic isoforms are predominant in the circulation during the follicular phase. This is followed by a progressive shift towards the less sialylated isoforms that are found during the late follicular and peri-ovulatory phase.[17,20,21] This shift may represent an important mechanism to regulate the intensity and/or duration of the FSH stimulus during the final stages of follicular maturation.[22] It may also reflect the changing requirements of the follicle through different stages of development, with the secreted FSH isoform profile matched to support specific follicular developmental stages.[23–26] More basic FSH isoforms may provide short-lived potent stimuli for the initiation of dynamic events, such as ovulation.[27]

The follicle that is destined to ovulate usually becomes selected in the mid-follicular phase of the menstrual cycle. The selected follicle vastly increases its capacity to produce estradiol; shortly after selection, >90% of the estradiol present in the body is derived from this follicle.[28] Recent evidence suggests that the estradiol secreted by this follicle plays an important role in directing the pituitary output of FSH isoforms.[28] Pituitary expression of different glycosyltransferases, including 2,3 α-sialyltransferase (an enzyme that catalyses the incorporation of sialic acid residues into sugar residues attached to the FSH molecule), is down-regulated by estradiol.[29] Recent studies have shown that estradiol administered to post-menopausal women shifts pituitary FSH release to more basic FSH isoforms compared with non-treated women.[28]

Thus, in addition to the fine-tuned interplay between the selected developing follicle and pituitary release of gonadotrophins, it appears that the follicle is capable also of directing the isoform profile released.

Isoforms in FSH Preparations

FSH is used therapeutically to stimulate follicular development during ovulation induction and controlled ovarian stimulation. Commercial preparations of FSH, including human menopausal gonadotrophin (hMG), u-hFSH and r-hFSH, vary in their degree of purity and specific activity.[30] During the commercial preparation of FSH, the steps involved in purification (such as those based on ion exchange) may affect the isoform composition of the final product. For urine-derived products, the degree of sialylation is dependent on the physiological status of the donor when the sample is collected, whereas for recombinant products, the glycosylation pattern depends on the cell culture system utilized, which is controlled and consistently the same for every batch produced. As the pI values of commercial preparations of FSH are generally much more acidic than pituitary-derived FSH, commercial preparations have a longer half-life and, hence, remain in the blood for several days after a single injection.[11,31]

Differences in the isoform profile of different FSH preparations have been observed with respect to charge heterogeneity. Bassett et al have shown that the isoform profile of urinary gonadotrophin preparations is more acidic (pI 3.0–5.2) than that of recombinant products (pI 3.5–6.0).[30] It is thought that the types of isoforms that constitute u-hFSH (highly purified; Fertinorm P®, Serono International SA, Geneva, Switzerland [now known as Merck Serono S.A.—Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany]) and r-hFSH (Follistim®, Organon, Roseland, NJ, USA) could be the same and that the predominance of more

basic isoforms in r-hFSH can be mainly attributed to differences in the levels of the basic isoforms of the alpha subunits (Fig. 1).[32]

Using a pI cut-off value of four to distinguish more acidic (<4) from more basic (≥4) FSH isoforms, Robertson et al have characterized the FSH isoform distribution for a range of commercial FSH preparations using chromatofocusing.[33,34] u-hFSH (Metrodin® HP [Serono International SA]) was reported to contain a higher proportion of more acidic isoforms than r-hFSH, while r-hFSH (follitropinalfa, GONAL-f®, Merck Serono S.A.—Geneva, Switzerland [an affiliate of Merck KGaA, Darmstadt, Germany] and follitropin beta (Puregon®, Organon, Roseland, NJ, USA) were reported to have a higher proportion of more basic isoforms (Table 1).

Bagatti et al have compared the isoform distribution of the two different r-hFSH products by Western-blot analysis following isoelectric focusing.[35] As above, follitropinalfa was found to comprise a less heterogeneous and more acidic range of isoforms than follitropin beta; in particular, the isoforms in the pI range 5.5–6 were not present in follitropinalfa. In a comparison of the electrospray mass spectroscopy patterns obtained with the alpha subunit of both recombinant products, follitropin beta appeared to have a higher proportion of isoforms with a few sialic acid residues, whilst follitropinalfa had a higher proportion of more heavily sialylated isoforms.[35] The slight differences in the isoform profile of these two recombinant products may be related to the differences in the culture system and/or purification process employed during production.

Although each commercial FSH preparation is characterized by a specific isoform profile, the profiles may vary from batch to batch[36] and this is particularly true of urinary-derived products.[36] Manufacturing of a filled-by-mass formulation of follitropinalfa has resulted in the availability of a product that exhibits high batch-to-batch consistency in terms of isoform profile and glycan-species distribution.[36,37]

The isoform profile of FSH preparations relates not only to charge heterogeneity, but also to the complexity of the oligosaccharides attached to the peptide backbone and to in vitro bioactivity.[34] The proportion of simple FSH isoforms is higher in recombinant products than in urinary-derived ones[33] (Table 1). However, the physiological impact of variations in complexity of oligosaccharides is not yet clear. Independent of whether the FSH has been derived from urine or produced by recombinant technology, the more acidic FSH isoforms appear to exhibit less in vitro bioactivity than the more basic isoforms[38] (Table 1).

In summary r-hFSH products are more basic than urinary ones due to their oligosaccharide type and number of sialic acid residues; even though they have a shorter half-life, they showed higher binding affinity for the receptor and higher bioactivity compared with u-hFSH.

Clinical Efficacy of Different FSH Isoforms

Potential functional differences between various naturally occurring FSH isoforms in the circulation are discussed above. Given the differences in the range of FSH isoforms used for ovarian stimulation, it would

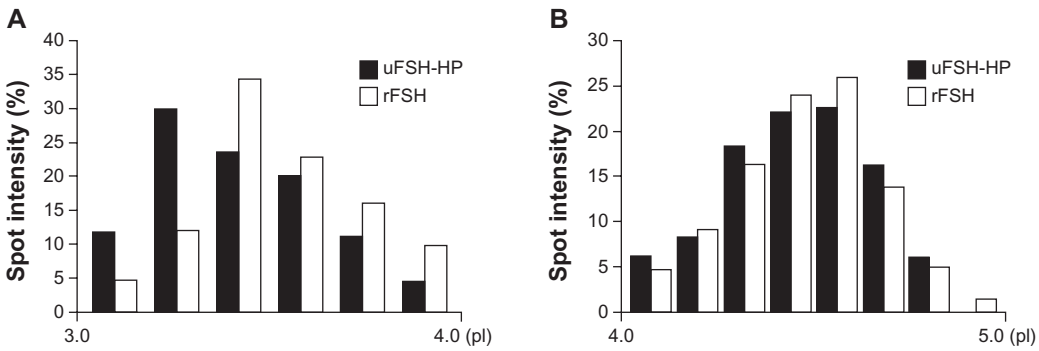

Figure 1. Comparison of quantitative ratios of isoforms in urinary and recombinant preparations of FSH using two-dimensional gel electrophoresis mapping. Based on fluorescence-labeled protein spot intensities, r-hFSH contained higher amounts of the basic isohormones of the FSH alpha subunit than uFSH-HP, with a less significant difference seen for the FSH beta subunit. (**A**) FSH alpha subunit; (**B**) FSH beta subunit. Reproduced with permission.[32]
Abbreviations: FSH, follicle-stimulating hormone; pI, isoelectric point; uFSH-HP, highly purified urinary-derived FSH (Fertinorm P®); r-FSH, recombinant FSH (Folistim®).

Table 1. FSH isoform distribution, biopotency and complexity of commercial FSH preparations.[33,38]

Name of preparation	FSH isoform distribution*		Proportion of simple FSH isoforms (%)†	Biopotency‡ Mean (SEM) ED_{50}
	pI < 4, (%)	pI ≥ 4, (%)		IU/L
Metrodin®	40	60	6	4.7 (1.1)
Metrodin®HP	74	26	5	13.2 (0.7)
Puregon®	~24	~76	19	2.2 (0.5)
GONAL-f®	9	91	24	ND

Notes: *Total percentage of FSH with a pI < 4 (more acidic isoforms) or ≥4 (more basic isoforms) is given. †Defined by their ability to bind to immobilized concanavalin A, FSH preparations divided into simple, intermediate or complex carbohydrate moieties. ‡FSH concentration required to induce a half-maximal response in the rat Sertoli cell assay.
Abbreviations: FSH, follicle-stimulating hormone; SEM, standard error of mean; ED_{50}, effective dose, 50%; pI, isoelectric point; ND, not determined.

be of interest to reproductive medicine specialists to know whether these differences may influence oocyte development, and be reflected in clinical outcome. Indeed, there is evidence from in vitro studies that different FSH isoforms exhibit differences with respect to their influence on the embryonic development of cultured follicles.[39] It has also been reported for other therapeutic recombinant proteins that the precise structure of the attached oligosaccharides can influence biological efficacy.[40]

The published literature on the clinical efficacy of different FSH isoforms can be divided into two types—those in which isoforms were the main focus of the study (see *Clinical evidence* below) and those in which FSH efficacy was studied without reference to the isoform profile (see *Indirect evidence* below).

Clinical evidence

Few published reports directly explore the clinical effects of different isoforms of therapeutic FSH in female fertility treatment. Andersen et al have performed a meta-analysis of data from five randomized, controlled, clinical trials in 1,297 women undergoing assisted reproductive technologies (ART).[34] These trials compared two products that differ profoundly in FSH-isoform profile (and, therefore, half-life): u-hFSH (Metrodin®HP; more acidic isoform profile) and r-hFSH (follitropinalfa; more basic isoform profile). Overall, there was a lower total dose requirement (weighted mean difference [WMD] –3.04 ampoules, 95% confidence interval [CI]: –3.78 to –2.31) and a shorter duration of treatment (WMD –1.18 days, 95% CI: –1.43 to –0.92) with r-hFSH. Ovarian stimulation outcomes revealed a greater number of follicles (WMD 2.28 > 10 mm follicles, 95% CI: 1.61–2.95) and a greater number of oocytes retrieved (WMD 2.25, 95% CI: 1.55–2.94) for r-hFSH. However, no significant difference was found in clinical pregnancy rate per patient (odds ratio [OR]: 1.18, 95% CI: 0.93 to 1.51), clinical pregnancy rate per embryo transfer (ET) (OR: 1.05, 95% CI: 0.82 to 1.36), ongoing pregnancy rate per patient (OR: 1.27, 95% CI: 0.98 to 1.65) and ongoing pregnancy rate per ET (OR: 1.17, 95% CI: 0.90 to 1.53). These data suggest that FSH preparations with a predominance of acidic isoforms were, overall, less efficient than those with more basic isoforms, when considering ovarian stimulation outcomes, but are no different when considering pregnancy rates. The authors suggested that a lack of significance for clinical pregnancy outcomes may be related to the fact that only a fraction of the FSH effect is taken into account when comparing pregnancy rates: as 2–3 embryos are routinely transferred fresh in ART procedures, the quality of only the best 2–3 embryos from each patient is assessed, and the remainder of the oocytes/embryos produced are not considered. In fact, to have a clear reflection of the difference between treatments, the results must be expressed combining the pregnancies achieved with both fresh and frozen/thawed transfers (cumulative pregnancy rates).

Selman et al have explored the use of an ovarian stimulation protocol based on the natural menstrual cycle.[22] This utilized r-hFSH (follitropinalfa), which contains a high proportion of more basic isoforms (pI 3.5–4.5) and u-hFSH (Fostimon®, IBSA, Geneva, Switzerland) enriched with more acidic isoforms (pI 3.0–4.0), for ovarian stimulation in patients undergoing in vitro fertilization (IVF) in a prospective open-label study. The two FSH formulations were used in a sequential protocol to mimic the physiological cycle. A total of 188 women

were randomized to one of three treatment groups: group A received u-hFSH until day 6 and then received r-hFSH; group B received r-hFSH alone throughout; and group C received u-hFSH alone throughout. No significant differences were observed between groups in the mean number of oocytes retrieved (10.6, 10.7, 10.6; $P = 0.879$). However, in group A vs. group B, a significantly greater proportion of mature (metaphase II) oocytes were retrieved (64.1 vs. 45.5%; $P < 0.004$) and grade I quality embryos found (55.2 vs. 39.3%; $P < 0.003$). No significant differences were observed between groups A and C for proportion of mature oocytes or grade I embryos. In terms of clinical outcomes, significantly higher implantation rates were observed in groups A and C vs. group B (24.5%, 20.4% and 17.3%; $P < 0.008$) and significantly higher pregnancy rates were observed in groups A and C vs. group B (43.5%, 39% and 33.3%; $P < 0.009$). No significant differences were observed between groups A and C in terms of pregnancy or implantation rates. The study concluded that ovarian stimulation using a sequential protocol was highly efficacious in terms of oocyte quality, embryo quality, and pregnancy and implantation rates compared with stimulation using more basic r-hFSH alone. The authors suggested that the higher proportion of mature oocytes and good quality embryos resulting from stimulation with the sequential protocol may reflect the positive effect of more acidic FSH on oocyte follicular growth during recruitment and the follicular growth phase.

Limitations of the findings of Selman et al are that they were based on a subjective assessment of embryo quality and the fact that the study was not adequately powered to show differences in implantation and pregnancy rates, even though the authors make this claim. At present, oocyte and embryo quality are evaluated using subjective morphological parameters, such as those relating to the cumulus corona cells, the zonapellucida, the perivitelline space, the presence, appearance and genetics of the first polar body, the granulated appearance of the cytoplasm and the meiotic spindle.[41] Why and how oocyte morphology should be used as a prognostic factor for embryo development and implantation, however, is unfortunately not clear in the literature and whether oocyte morphology assessment should be taken into account for embryo assessment is debatable.[41] Objective markers are needed to clearly define a good quality oocyte. The new -omics methodologies currently being explored (eg, genomics, transcriptomics, proteomics and metabolomics) may allow the quality of oocytes produced under different ovarian stimulation protocols to be more objectively defined in the future.[42,43]

Indirect evidence

The only two objective parameters that can be used to measure the efficacy of controlled ovarian stimulation are the amount of drug used and the total number of oocytes and mature oocytes that are retrieved. A number of large controlled studies suggest a higher clinical effectiveness of r-hFSH vs. urinary-derived FSH formulations (u-hFSH or hMG) in terms of dose required and number of oocytes retrieved, both in ovulation induction and ART.[12,44–59] Assessing endpoints which are more closely associated with the stimulation period (eg, the number of oocytes retrieved) can be expected to give a more accurate assessment of gonadotrophin efficacy than more distant endpoints such as live birth rate.

In a study to compare r-hFSH (follitropinalfa) and u-hFSH (Metrodin® HP) in 278 women undergoing ART,[12] a lower mean (standard deviation [SD]) total dose (27.6 [10.2] vs. 40.7 [13.6] ampoules; $P = 0.0001$) and a shorter mean (SD) treatment period (11.7 [1.9] vs. 14.5 [3.3] days; $P = 0.0001$) were needed with r-hFSH to trigger follicular maturation; however, a significantly higher mean (SD) number of oocytes was retrieved (11.0 [5.9] vs. 8.8 [4.8]; $P = 0.002$).

In a randomized multi-national trial of r-hFSH (follitropinalfa) compared with purified hMG (Menopur®, Ferring Pharmaceuticals A/S, Copenhagen, Denmark), which included more than 700 women undergoing IVF,[44] a significantly higher mean (SD) number of oocytes was retrieved from women treated with r-hFSH compared with those receiving hMG (11.8 [5.7] vs. 10.0 [5.4]; $P < 0.001$). In addition, significantly more follicles and embryos on day 3 were produced, and a significantly lower mean (SD) total dose (IU) of gonadotrophin was required in the r-hFSH group compared with the hMG group: (2385 [622] vs. 2508 [729]; $P = 0.006$).

In an observational study of almost 25 000 ART cycles in women who received either r-hFSH (follitropinalfa) or hMG (menotropin) following a long

down-regulation protocol, considerably less gonadotrophin was used than with hMG for each birth achieved with r-hFSH (175.8 vs. 245.3 ampoules). Overall, 39.5% more gonadotrophin was required, per birth, in the hMG group than in the r-hFSH group.[50]

A retrospective chart review of databases from four European countries investigated the effects of r-hFSH and hMG on outcomes in IVF cycles. Results demonstrated that r-hFSH yielded statistically more oocytes, and more mature oocytes, while using significantly less IU per cycle than hMG.[59]

A series of meta-analyses have examined studies comparing hMG and r-hFSH in IVF/ICSI cycles,[60–64] but these focused on live birth rates or pregnancy rates and gave heterogeneous results. In a more recent, and potentially more sensitive meta-analysis (16 studies, n = 4040) where the number of oocytes was the main endpoint, significantly fewer oocytes were retrieved with hMG compared with r-hFSH (−1.54, 95% CI: −2.53 to −0.56; $P < 0.0001$) and a higher total dose of hMG was necessary compared with r-hFSH (standardized mean difference 0.33, 95% CI: 0.08 to 0.58; $P = 0.01$).[65]

Together these studies suggest that the more acidic FSH isoform profile of u-hFSH may be less effective in recruiting antral follicles than r-hFSH (which is more basic), as shown by the production of fewer oocytes and the requirement for higher doses. Furthermore, although hMG differs from u-hFSH in that it retains LH activity in the form of hCG, this does not appear to compensate for the lower potency of its more acidic FSH isoform profile. Whether administered as u-hFSH alone or in combination with LH activity (as in hMG), higher doses are needed and may result in fewer oocytes than r-hFSH.[44–60,65] This again highlights that the more basic isoform profile of r-hFSH may be more effective in recruiting follicles than u-hFSH or hMG (which contain less potent, more acidic FSH isoforms) and thereby results in the retrieval of higher numbers of oocytes.

Although the studies described here have compared formulations of FSH for which the source of raw material is different (urinary or recombinant), there is no evidence to suggest there are any differences at the protein level between FSH molecules derived from either of the two sources.[34] It is likely, therefore, that the difference in FSH isoform profile between the two product types plays a significant role in the observed differences. Further studies are needed that control for other factors which may potentially differ between FSH preparations.

Conclusions and Future Perspectives

The variation seen in the isoform profiles of different gonadotrophins was first reported in the scientific literature decades ago. There is no question that the more acid and more basic isoforms of FSH have different pharmacodynamin properties, however, there is some controversy as to which isoforms are more efficient. Limited published data are available on the direct clinical effects of different isoforms of therapeutic FSH in fertility treatment. Studies that directly address this topic suggest that the isoform profile of the FSH used does have an influence on some of the outcomes obtained; however, the data are not clear-cut. The claims of better outcomes are generally associated with imprecise subjective parameters, such as oocyte and embryo quality, and are based on studies that are underpowered to make reliable conclusions relating to pregnancy.

However, there are many studies that show that r-hFSH (follitropinalfa) with a more basic isoform profile is more potent in the clinical setting than formulations with more acidic isoforms, particularly in terms of the number of oocytes retrieved. The differences between urinary and recombinant preparations may relate not only to the source of the FSH molecule, but also to the specific FSH isoform profile of each individual preparation.

It is clear that additional well-designed, randomized, controlled trials are required to evaluate the effect of different FSH isoforms on clinical outcomes in fertility treatment. These studies should directly address oocyte quality immediately after retrieval, using objective assessments (such as evaluation of a neuploidy rate of polar bodies, or whole-genome micro array analysis of metaphase stage-II oocytes) and defining objective markers of embryo quality, which is critical when making conclusions regarding clinical efficacy. Ideally, such studies should control for factors related to IVF/intracytoplasmic sperm injection techniques, taking into consideration cumulative pregnancy rates, in order to allow a greater understanding of the extent to which study outcomes can be accurately attributed to different FSH formulations and their isoform profiles.

Conflicts of Interest

C. Yding Andersen has no conflicts of interest. D. Ezcurra is an employee of Merck Serono S.A.—Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany).

Funding

This work was supported by Merck Serono S.A.—Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany).

Authors' Contributions

CYA has made a substantial contribution to the conception and design, and acquisition and interpretation of data and has been involved in drafting the article and revising it critically for important intellectual content. DE has made a substantial contribution to the conception and design, acquisition of data, analysis and interpretation of data and has been involved in drafting the article and revising it critically for important intellectual content. Both authors approved the final manuscript.

Acknowledgements

The authors take full responsibility for the content of this article, but thank Jane Davies, Carol Cooper and MaiLee Wong of Caudex Medical (supported by Merck Serono S.A.—Geneva, Switzerland [an affiliate of Merck KGaA, Darmstadt, Germany]) for their assistance in the preparation of this review article.

Disclosure

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

References

1. Ulloa-Aguirre A, Midgley AR, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: characterization and physiological relevance. *Endocr Rev*. 1995;16:765–87.
2. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. *Hum Reprod Update*. 2002;8:413–21.
3. Wunsch A, Sonntag B, Simoni M. Polymorphism of the FSH receptor and ovarian response to FSH. *Ann Endocrinol (Paris)*. 2007;68:160–6.
4. Stanton PG, Burgon PG, Hearn MT, Robertson DM. Structural and functional characterisation of hFSH and hLH isoforms. *Mol Cell Endocrinol*. 1996;125:133–141.
5. Wide L, Eriksson K, Sluss PM, Hall JE. Serum half-life of pituitary gonadotropins is decreased by sulfonation and increased by sialylation in women. *J Clin Endocrinol Metab*. 2009;94:958–64.
6. Barrios-de-Tomasi J, Timossi C, et al. Assessment of the in vitro and in vivo biological activities of the human follicle-stimulating isohormones. *Mol Cell Endocrinol*. 2002;186:189–98.
7. Andersen CY, Westergaard LG, van Wely M. FSH isoform composition of commercial gonadotrophin preparations: a neglected aspect? *Reprod Biomed Online*. 2004;9:231–6.
8. Ulloa-Aguirre A, Timossi C, Damián-Matsumura P, Dias JA. Role of glycosylation in function of follicle-stimulating hormone. *Endocrine*. 1999;11:205–15.
9. Ulloa-Aguirre A, Timossi C, Barrios-de-Tomasi J, Maldonado A, Nayudu P. Impact of carbohydrate heterogeneity in function of follicle-stimulating hormone: studies derived from in vitro and in vivo models. *Biol Reprod*. 2003;69:379–89.
10. Cerpa-Poljak A, Bishop LA, Hort YJ, et al. Isoelectric charge of recombinant human follicle-stimulating hormone isoforms determines receptor affinity and in vitro bioactivity. *Endocrinology*. 1993;132:351–56.
11. Zambrano E, Zarinan T, Olivares A, Barrios-de-Tomasi J, Ulloa-Aguirre A. Receptor binding activity and in vitro biological activity of the human FSH charge isoforms as disclosed by heterologous and homologous assay systems: implications for the structure-function relationship of the FSH variants. *Endocrine*. 1999;10:113–21.
12. Frydman R, Howles CM, Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. The French Multicentre Trialists. *Hum Reprod*. 2000;15:520–5.
13. Padmanabhan V, Mieher CD, Borondy M, et al. Circulating bioactive follicle-stimulating hormone and less acidic follicle-stimulating hormone isoforms increase during experimental induction of puberty in the female lamb. *Endocrinology*. 1992;131:213–20.
14. Phillips DJ, Albertsson-Wikland K, Eriksson K, Wide L. Changes in the isoforms of luteinizing hormone and follicle-stimulating hormone during puberty in normal children. *J Clin Endocrinol Metab*. 1997;82:3103–6.
15. Olivares A, Soderlund D, Castro-Fernandez C, et al. Basal and gonadotropin-releasing hormone-releasable serum follicle-stimulating hormone charge isoform distribution and in vitro biological-to-immunological ratio in male puberty. *Endocrine*. 2004;23:189–98.
16. Wide L, Naessen T. 17 beta-oestradiol counteracts the formation of the more acidic isoforms of follicle-stimulating hormone and luteinizing hormone after menopause. *Clin Endocrinol (Oxf)*. 1994;40:783–9.
17. Anobile CJ, Talbot JA, McCann SJ, Padmanabhan V, Robertson WR. Glycoform composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state. *Mol Hum Reprod*. 1998;4:631–9.
18. Ulloa-Aguirre A, Timossi C, Mendez JP. Is there any physiological role for gonadotrophin oligosaccharide heterogeneity in humans? I. Gondatrophins are synthesized and released in multiple molecular forms. A matter of fact. *Hum Reprod*. 2001;16:599–604.
19. Timossi CM, Barrios-de-Tomasi J, Gonzalez-Suarez R, et al. Differential effects of the charge variants of human follicle-stimulating hormone. *J Endocrinol*. 2000;165:193–205.

20. Padmanabhan V, Lang LL, Sonstein J, Kelch RP, Beitins IZ. Modulation of serum follicle-stimulating hormone bioactivity and isoform distribution by estrogenic steroids in normal women and in gonadal dysgenesis. *J Clin Endocrinol Metab*. 1988;67:465–73.
21. Wide L, Bakos O. More basic forms of both human follicle-stimulating hormone and luteinizing hormone in serum at midcycle compared with the follicular or luteal phase. *J Clin Endocrinol Metab*. 1993;76:885–9.
22. Selman H, Pacchiarotti A, El-Danasouri I. Ovarian stimulation protocols based on follicle-stimulating hormone glycosylation pattern: impact on oocyte quality and clinical outcome. *Fertil Steril*. 23 Nov 2009. [Epub ahead of print].
23. Beitens I, Padmanabhan V. Bioactive follicle stimulating hormone. *Trends in Endocrin and Metab*. 1991;2:145–51.
24. Chappel SC, Ulloa-Aguirre A, Coutifaris C. Biosynthesis and secretion of follicle-stimulating hormone. *Endocr Rev*. 1983;4:179–211.
25. Nayudu PL, Vitt UA, Barrios De Tomasi J, Pancharatna K, Ulloa-Aguirre A. Intact follicle culture: what it can tell us about the roles of FSH glycoforms during follicle development. *Reprod Biomed Online*. 2002;5:240–53.
26. West CR, Carlson NE, Lee JS, et al. Acidic mix of FSH isoforms are better facilitators of ovarian follicular maturation and E2 production than the less acidic. *Endocrinology*. 2002;143:107–16.
27. Flack MR, Bennet AP, Froehlich J, Anasti JN, Nisula BC. Increased biological activity due to basic isoforms in recombinant human follicle-stimulating hormone produced in a human cell line. *J Clin Endocrinol Metab*. 1994;79:756–60.
28. Wide L, Naessen T, Eriksson K. Effects of 17beta-oestradiol and norethisterone acetate on sulfonation and sialylation of gonadotrophins in postmenopausal women. *Ups J Med Sci*. 2010;115:97–106.
29. Damian-Matsumura P, Zaga V, Maldonado A, Sanchez-Hernandez C, Timossi C, Ulloa-Aguirre A. Oestrogens regulate pituitary alpha2,3-sialyltransferase messenger ribonucleic acid levels in the female rat. *J Mol Endocrinol*. 1999;23:153–65.
30. Bassett R, Lispi M, Ceccarelli, et al. Analytical identification of additional impurities in urinary-derived gonadotrophins. *Reprod Biomed Online*. 2009; 19:300–13.
31. Baird DT. Is there a place for different isoforms of FSH in clinical medicine? IV. The clinician's point of view. *Hum Reprod*. 2001;16:1316–8.
32. Kuwabara Y, Mine K, Katayama A, Inagawa T, Akira S, Takeshita T. Proteomic analyses of recombinant human follicle-stimulating hormone and urinary-derived gonadotropin preparations. *J Reprod Med*. 2009;54: 459–66.
33. Robertson WR. Gonadotrophin isoform pattern in different pharmaceutical preparations. In *Gonadotrophin Isoforms: Facts and Future*. *Volume 2*. Edited by Kahn J. 1997:53–60.
34. Andersen CY, Westergaard LG, van Wely M. FSH isoform composition of commercial gonadotrophin preparations: a neglected aspect? *Reprod Biomed Online*. 2004;9:231–6.
35. Bagatti G, Crisci C, Datola A. Characterisation and comparison of recombinant human follicle-stimulating hormones. *J Clin Res*. 2001;4:91–104.
36. Bassett RM, Driebergen R. Continued improvements in the quality and consistency of follitropin alfa, recombinant human FSH. *Reprod Biomed Online*. 2005;10:169–77.
37. Driebergen R, Baer G. Quantification of follicle stimulating hormone (follitropin alfa): is in vivo bioassay still relevant in the recombinant age? *Curr Med Res Opin*. 2003;19:41–46.
38. Lambert A, Rodgers M, Mitchell R, et al. In-vitro biopotency and glycoform distribution of recombinant human follicle stimulating hormone (Org 32489), Metrodin and Metrodin-HP. *Hum Reprod*. 1995;10:1928–35.
39. Vitt UA, Nayudu PL, Rose UM, Kloosterboer HJ. Embryonic development after follicle culture is influenced by follicle-stimulating hormone isoelectric point range. *Biol Reprod*. 2001;65:1542–7.
40. Jefferis R. Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action. *Trends Pharmacol Sci*. 2009;30:356–62.
41. Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. *Reprod Biomed Online*. 2006;12:608–15.
42. Assou S, Haouzi D, De Vos J, Hamamah S. Human cumulus cells as biomarkers for embryo and pregnancy outcomes. *Mol Hum Reprod*. 29 Apr 2010. [Epub ahead of print].
43. Singh R, Sinclair KD. Metabolomics: approaches to assessing oocyte and embryo quality. *Theriogenology*. 2007;68(1):S56–62.
44. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. *Hum Reprod*. 2006;21: 3217–27.
45. Balasch J, Fabregues F, Creus M, Puerto B, Penarrubia J, Vanrell JA. Follicular development and hormone concentrations following recombinant FSH administration for an ovulation associated with polycystic ovarian syndrome: prospective, randomized comparison between low-dose step-up and modified step-down regimens. *Hum Reprod*. 2001;16:652–6.
46. Bergh C, Howles CM, Borg K, et al. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F®) versus highly purified urinary FSH (Metrodin HP®): results of a randomized comparative study in women undergoing assisted reproductive techniques. *Hum Reprod*. 1997;12:2133–9.
47. Coelingh Bennink HJ, Fauser BC, Out HJ. Recombinant follicle-stimulating hormone (FSH; Puregon) is more efficient than urinary FSH (Metrodin) in women with clomiphene citrate-resistant, normogonadotropic, chronic an ovulation: a prospective, multicenter, assessor-blind, randomized, clinical trial. European Puregon Collaborative Anovulation Study Group. *Fertil Steri*. 1998;69:19–25.
48. Revelli A, Poso F, Gennarelli G, Moffa F, Grassi G, Massobrio M. Recombinant versus highly-purified, urinary follicle-stimulating hormone (r-FSH vs. HP-uFSH) in ovulation induction: a prospective, randomized study with cost-minimization analysis. *Reprod Biol Endocrinol*. 2006;4:38.
49. Matorras R, Recio V, Corcostegui B, Rodriguez-Escudero FJ. Recombinant human FSH versus highly purified urinary FSH: a randomized study in intrauterine insemination with husbands' spermatozoa. *Hum Reprod*. 2000; 15:1231–4.
50. Ludwig M, Rabe T, Bühler K, Diedrich K, Felberbaum R. Efficacy of recombinant human FSH in comparison to urinary hMG following a long down-regulation protocol—an analysis of 24,764 ART cycles in Germany. *J Reproduktions med Endokrinol*. 2004;1:284–8.
51. Balen A, Platteau P, Andersen AN, Devroey P, Helmgaard L, Arce JC. Highly purified FSH is as efficacious as recombinant FSH for ovulation induction in women with WHO Group II an ovulatory infertility: a randomized controlled non-inferiority trial. *Hum Reprod*. 2007;22:1816–23.
52. Hugues JN, Bstandig B, Bry-Gauillard H, Uzan M, Cedrin-Durnerin I. Comparison of the effectiveness of recombinant and urinary FSH preparations in the achievement of follicular selection in chronic an ovulation. *Reprod Biomed Online*. 2001;3:195–8.
53. Balasch J, Fabregues F, Penarrubia J, et al. Follicular development and hormonal levels following highly purified or recombinant follicle-stimulating hormone administration in ovulatory women and WHO group II an ovulatory infertile patients. *J Assist Reprod Genet*. 1998;15:552–9.
54. Fulghesu AM, Apa R, Belosi C, et al. Recombinant versus urinary follicle-stimulating hormone in the low-dose regimen in an ovulatory patients with polycystic ovary syndrome: a safer and more effective treatment. *Horm Res*. 2001;55:224–8.
55. Isaza V, Requena A, Garcia-Velasco JA, Remohi J, Pellicer A, Simon C. Recombinant vs. urinary follicle-stimulating hormone in couples undergoing intrauterine insemination. A randomized study. *J Reprod Med*. 2003;48:112–8.
56. Gerli S, Casini ML, Unfer V, Costabile L, Mignosa M, Di Renzo GC. Ovulation induction with urinary FSH or recombinant FSH in polycystic ovary syndrome patients: a prospective randomized analysis of cost-effectiveness. *Reprod Biomed Online*. 2004;9:494–9.
57. Demirol A, Gurgan T. Comparison of different gonadotrophin preparations in intrauterine insemination cycles for the treatment of unexplained infertility: a prospective, randomized study. *Hum Reprod*. 2007;22:97–100.
58. Gerli S, Casini ML, Unfer V, Costabile L, Bini V, Di Renzo GC. Recombinant versus urinary follicle-stimulating hormone in intrauterine insemination cycles: a prospective, randomized analysis of cost effectiveness. *Fertil Steril*. 2004;82:573–8.
59. Trew GH, Brown AP, Gillard S, et al. In vitro fertilisation with recombinant follicle stimulating hormone requires less IU usage compared with highly purified human menopausal gonadotrophin: results from a European retrospective observational chart review. *Reprod Biol Endocrinol*. 2010;8:137.

60. Van Wely M, Westergaard LG, Bossuyt PM, van der Veen F. Effectiveness of human menopausal gonadotropin versus recombinant follicle-stimulating hormone for controlled ovarian hyper stimulation in assisted reproductive cycles: a meta-analysis. *Fertil Steril*. 2003;80:1086–93.
61. Coomarasamy A, Afnan M, Cheema D, van der Veen F, Bossuyt PMM, van Wely M. Urinary hMG versus recombinant FSH for controlled ovarian hyper stimulation following an agonist long down-regulation protocol in IVF or ICSI treatment: a systematic review and meta-analysis. *Hum Reprod* 2008;23:310–315.
62. Al-Inany HG, Abou-Setta AM, Aboulghar MA, Mansour RT, Serour GI. Highly purified hMG achieves better pregnancy rates in IVF cycles but not ICSI cycles compared with recombinant FSH: a meta-analysis. *Gynecol Endocrinol*. 2009;25:372–8.
63. Al-Inany HG, Abou-Setta AM, Aboulghar MA, Mansour RT, Serour GI. Efficacy and safety of human menopausal gonadotrophins versus recombinant FSH: a meta-analysis. *Reprod Biomed Online*. 2008;16:81–88.
64. Westergaard LW, Bossuyt PMM, Van der Veen F, van Wely M. Human menopausal gonadotropin versus recombinant follicle stimulating hormone for ovarian stimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2003;1:CD003973.
65. Lehert P, Schertz J, Ezcurra D. Recombinant human follicle-stimulating hormone produces more oocytes with a lower total dose per cycle in assisted reproductive technologies compared with highly purified human menopausal gonadotrophin: a meta-analysis. *Reprod Bio Endocrinol*. 2010;8:112.