

Comparison of pharmacokinetic and safety profiles between Bemfola[®] and Gonal-f[®] after subcutaneous application

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Abstract Recombinant human follicle stimulating hormone (r-hFSH) is effective and safe for controlled ovarian stimulation. Bemfola[®] (Finiox AG, Burgdorf, Switzerland), a new biosimilar r-hFSH, has proven comparable non-clinical pharmacological profiles to those of the widely used Gonal-f[®] (Serono Pharma S.p.A., Bari, Italy). The objective of this study was to show that Bemfola[®] yields comparable clinical pharmacokinetic (PK) and safety profiles to Gonal-f[®] in healthy female subjects. In this randomized, Phase I trial conducted in healthy female volunteers ($N = 32$), a 2-period, balanced 2-treatment crossover design was used. A single subcutaneous dose of 225 IU Bemfola[®] or Gonal-f[®] was administered in each treatment period per sequence. Blood was collected for pharmacokinetic analysis until 10 days after each r-hFSH treatment. For down-regulation of endogenous FSH subjects were given a depot injection with leuprolide acetate prior to the study drug in either sequence. Pharmacokinetic data was available for 23 subjects. No appreciable differences in key PK parameters were detected between the r-hFSH products as per non-compartmental PK analysis [i.e. for Bemfola[®] and Gonal-f[®] respectively AUC_{0-192} 424.90 and 432.75 IU h/L, C_{max} 0.98 and 0.95 IU/L, T_{max} 24.0 h (range 6.0–24.0) and 24.0 h (range 9.0–24.0), $t_{1/2}$

43.58 h [standard deviation (SD 14.17)] and 42.58 h (SD 16.47), and K_e 0.0075 1/h (SD 0.003) and 0.0077 1/h (SD 0.002)]. Subgroup analysis for estradiol (E2) response was similar for Bemfola[®] and Gonal-f[®] ($AUC_{(0-120)}$ $p = 0.21$ and C_{max} $p = 0.82$). No major safety issues were identified and no immunogenic reaction to r-hFSH was observed. The results of this study indicate that a single dose of Bemfola[®] exhibits pharmacokinetic and safety profiles comparable to Gonal-f[®] in healthy young women.

Keywords Pharmacokinetics · Biosimilar · Infertility · Follicle-stimulating hormone

1 Introduction

Bemfola[®] is a biological medicinal product similar to the licensed Gonal-f[®] (recombinant human follicle-stimulating hormone r-hFSH, INN Follitropin alfa). Follicle-stimulating hormone (FSH) is a pituitary glycoprotein hormone that plays a key role in regulating reproductive function in both males and females.

FSH is a heterodimeric hormone composed of two linked subunits. The alpha-subunit (92 amino acids) is common to other glycoprotein hormones and the beta-subunit (111 amino acids) is specific.

For clinical use either FSH extracted from the urine of postmenopausal women (u-hFSH) or recombinant human FSH (r-hFSH) is used.

For the production of Bemfola[®], a Chinese hamster ovary cell line transfected with the genes coding for the alpha and beta subunits of human FSH is used.

Gonal-f[®], which has been commercially available since 1995, is an example of a currently marketed r-hFSH product and is primarily used for treating infertility. In

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particular, Gonal-f® is used for ovulation induction in normogonadotrophic (WHO type II) anovulatory women and WHO type I anovulatory infertility in association with a luteinizing hormone (LH) preparation, as well as for controlled ovarian stimulation in patients undergoing assisted reproductive technology (ART).

High treatment costs restrict access to high-quality biological medicines (Schellekens and Moors 2010; Engelberg et al. 2009); in the case of r-hFSH biosimilar versions of this recombinant hormone have been developed in order to provide high-quality alternatives that are more economically attractive than their present r-hFSH counterparts. Biosimilars meet high standards for comparability to the originator medicine and are approved for use in the same indications (Weise et al. 2012).

Bemfola® is a r-hFSH biosimilar that has been demonstrated to have the same physicochemical properties (Gonal-f® SPC 2014; Bemfola® SPC 2014) and is marketed in the same therapeutic indications as Gonal-f®. Bemfola® has similar non-clinical pharmacological, pharmacokinetic and toxicological profiles to those of Gonal-f® (Gonal-f® SPC 2014; Bemfola® SPC 2014).

The current study was performed to examine the pharmacokinetic bioequivalence of Bemfola® to the marketed product Gonal-f® after single subcutaneous administration in healthy female volunteers. In addition, the safety profiles of Bemfola® and Gonal-f® were compared.

2 Materials and methods

Study FIN1001 was a Phase I, randomized, open-label, crossover trial conducted in healthy female volunteers between 18 and 38 years of age ($N = 32$) who were treated with a single subcutaneous (SC) dose of 225 IU Bemfola® and Gonal-f®.

The study was sponsored by Polymun Scientific GmbH and conducted in Austria at the Medical University of Vienna, Department of Clinical Pharmacology, between January and August 2009. All participants received a depot injection of leuprolide acetate (Enantone-Gyn® Depot, Dual chamber prefilled syringe containing 3.75 mg Leuprorelinacetat and 1 mL solvent, Takeda Italia Farmaceutici S.P.A., I-Cerano) for the down-regulation of endogenous FSH levels. Subjects were randomized to administration of Bemfola® or Gonal-f®, followed by a wash-out period and then crossed over to the alternate investigational product under study.

The study was approved by the Independent Ethics Committee of the Medical University of Vienna and was conducted in accordance with Good Clinical Practice (GCP), Declaration of Helsinki, the International Conference on Harmonization guidelines for Good Clinical

Practice CHMP/ICH/135/95, and local regulatory requirements/laws. The study is registered with the European Clinical Trials registry (EudraCT # 2008-006564-11). Following adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study, the investigator obtained written informed consent from each subject participating in this study.

2.1 Study population

Healthy female volunteers who fulfilled the following inclusion criteria were eligible: age between 18 and 38 years; body mass index (BMI) between 17 and 29 kg/m²; and regular menstrual cycles of 25–34 days before initiation of oral contraception. Other key inclusion criteria included (1) use of oral contraceptives for at least 3 months before study entry; (2) requirement to practice effective barrier methods for birth control; (3) normal findings in medical history as well as physical and gynecological examinations; and (4) presence of both ovaries.

Key exclusion criteria included: (1) polycystic ovary syndrome (PCOS); (2) history of FSH hypersensitivity [i.e., ovarian hyperstimulation syndrome (OHSS)]; (3) history of malignant disease; (4) presence of hepatic impairment [i.e., aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) >2 times the upper limit of normal (ULN)]; (5) excessive smoking habit (i.e., >5 cigarettes/day) or alcohol/drug consumption; (6) pregnancy or lactation period; and (7) other clinically relevant findings per physical, gynecological or laboratory examination or symptoms of a clinically relevant illness 3 weeks prior to the first dose of study drug.

2.2 Study design

A balanced 2-period, 2-treatment crossover design according to the recommendation for bioequivalence studies of the European Medicines Agency (“Note for guidance on the investigation of bioavailability and bioequivalence”; CPMP/EWP/QWP/1401/98) was implemented; hence, subjects were randomly allocated to the respective treatment sequence. This design minimizes the inter-subject variability and therefore lowers the required sample size. A validated software (RANCODE; idv-Datenanalyse und Versuchsplanung, Gauting, Germany) was used to generate the randomization schedule using block randomization with variable block sizes.

2.3 Treatment administration

Eligible subjects received a leuprolide acetate depot injection between cycle days 15–21 for the down-regulation of their endogenous FSH levels and discontinued oral

contraceptives on the same day. Ten days after leuprolide acetate administration (Study Day 1), blood was collected and serum 17 β -estradiol (E2) and FSH analyzed. Volunteers were eligible for the next study phase if they had E2 levels ≤ 50 pg/mL and FSH levels ≤ 4 IU/L, and were randomized to receive Bemfola[®] or Gonal-f[®] on the next study day. On Study Day 2, subjects received a single subcutaneous dose of 225 IU r-hFSH and stayed at the clinical site for 24 h for PK evaluation. Further blood collections were performed daily until Study Day 10 (i.e., 8 days after r-hFSH application). On Study Day 10, subjects received a second depot injection of leuprolide acetate. On Study Day 15, E2 and FSH were controlled, and the second cycle with application of the other r-hFSH preparation was initiated on Study Day 16, which followed an identical schedule. On Study Day 24, the last PK sample was collected, and physical and gynecological exam, vaginal ultrasound, and safety lab examinations were performed. A telephone follow-up was arranged to confirm that menstrual cycle had resumed.

2.4 Assessments

For the assessment of PK parameters [i.e., maximum concentration (C_{\max}), time to maximum concentration (T_{\max}), area under the curve between time 0 and 192 h (AUC_{0-192}), terminal half-life ($t_{1/2}$), and terminal elimination rate constant (K_e)] venous blood samples were collected at 0, 0.5, 1, 3, 6, 9, 12, 16, 24, 48, 72, 96, 120, 144, 168, and 192 h after each r-hFSH injection. Serum was prepared by centrifugation at room temperature (10 min, 3,000 rpm Hettich Rotana TRC centrifuge, $r = 194$ mm) and frozen at -70 °C until analysis. The serum concentrations of FSH and E2 were analyzed using the COBAS test kit for FSH and E2, respectively (Roche Diagnostics GmbH, Germany). Assays were performed using modular analytics E170 analyzer. The method was validated according ICH Guideline Q2R1 and ISO 5725.

Venous blood samples for immunogenicity testing were collected at Study Days 1, 15, and 24 and serum was prepared and frozen as described above. Analysis for anti FSH antibodies was performed with a validated Surface Plasmon Resonance method. The limit of quantification (LoQ) was 57.4 rel RU. All samples tested above the LoQ were tested in a competition based specificity assay.

For the assessment of safety, all adverse events (AEs) were reported from the first trial-related activity after informed consent was given until Study Day 24 (i.e., at least 192 h after the second r-hFSH injection) per relevant physical and laboratory examination.

2.5 Endpoints

The primary endpoint was the AUC_{0-192} of FSH. Secondary endpoints included other secondary PK parameters (i.e., C_{\max} , $t_{1/2}$, T_{\max} , K_e) and safety (i.e., AEs, biochemical monitoring including liver/kidney function, sexual hormones, gynecological examination, ultrasound scans for solid ovarian cysts, and antibody formation against FSH).

2.6 Statistical methods

The PK parameters were assessed by non-compartmental analysis. Bioequivalence was investigated by determining the 90 % confidence limits for the log-transformed ratio (test product/reference product) for the parameters $AUC_{(0-192)}$ and C_{\max} . The null and alternative hypotheses for determining bioequivalence were tested by using the difference method implemented in the used validated statistics program TESTIMATE. This method was equivalent to fitting an analysis of variance (ANOVA) to the primary endpoint, where the factors formulation, period, sequence, and subject nested within sequence were used to explain the overall variability in the observations. The sample size was calculated to ensure an adequate evaluation of the primary endpoint (equal to the primary objective) and based on a one-sided significance level of 5 % and a power of 80 %. The intra-subject variability was estimated to be 15 % based on published PK data of a bioequivalence study between liquid and freeze-dried formulation of Gonal-f[®]. Twenty-four randomized subjects were evaluable at the end of the study, which was within the estimated attrition rate of 15 %. Values for $t_{1/2}$ and K_e were calculated using the program R, Version 2.9.1 with the PK package by Jaki and Wolfsegger (Estimation of pharmacokinetic parameters using non-compartmental theory, Windows Binary PK 1.01). The primary efficacy analysis was based on the per protocol (PP) population, which consisted of all exposed subjects, who completed the trial and who did not violate the inclusion/exclusion criteria or other aspects of the protocol considered to potentially affect the PK results. The analysis of adverse events, laboratory data, vital signs, physical examination findings, and other safety evaluations was performed on the intent-to-treat (ITT), which included all patients who received at least one dose of r-hFSH.

Subgroup analysis for E2 increase was performed after amendment to the study protocol. $AUC_{(0-120)}$ and C_{\max} of the serum E2 concentration curves were compared with paired samples t test.

3 Results

A total of 32 subjects were enrolled in this trial. Seven subjects had to be excluded after screening (Fig. 1). A total of 25 subjects were randomized, one of these subjects was adversely randomized as the E2 level of ≤ 50 pg/mL was not reached, and consequently, 24 subjects received study treatment. The mean age of subjects in this study that received study treatment was 23 years (median 23 years, range 18–28 years). One subject withdrew from the Bemfola®-Gonal-f® treatment sequence due to an AE (i.e., abnormal ultrasound scan). As a result, 23 subjects completed this study.

3.1 Comparison of $AUC_{(0-192)}$ of Bemfola® to the reference product (Gonal-f®)

Mean AUC_{0-192} after administration of Bemfola® and Gonal-f® were 424.90 and 432.75 IU h/L, respectively (Fig. 2; Table 1). The 90 % confidence interval for AUC_{0-192} ranged from 0.85 to 1.14 (Table 1) and was within the bioequivalence acceptance criteria of 0.80–1.25. No period effect was detectable for AUC_{0-192} ($p = 0.252$).

3.2 Comparison of secondary PK parameters between Bemfola® and Gonal-f®

No appreciable difference in secondary PK parameters (i.e., C_{max} , $t_{1/2}$, T_{max} , K_e) was observed between Bemfola® and Gonal-f® (Table 1). Mean C_{max} for Bemfola® was observed at 5.69 IU/L and for Gonal-f® at 6.01 IU/L, with a confidence interval of 0.89 to 1.01. In particular, the median T_{max} was identical after FSH application for Bemfola® and Gonal-f® [Bemfola®: 24.0 h (range 6.0–24.0); Gonal-f®: 24.0 h (range 9.0–24.0)]. In addition, mean $t_{1/2}$ values were comparable between Bemfola® and Gonal-f® [43.58 h (standard deviation 14.17) vs. 42.58 h (standard deviation 16.47), respectively]. Similarity was observed for the elimination rate constant K_e , with 0.0075 1/h calculated for Bemfola® and 0.0077 1/h for Gonal-f®. Lastly, no period effect was seen for C_{max} ($p = 0.876$).

3.3 Comparison of E2 concentration between Bemfola® and Gonal-f®

The original study protocol was amended to add an additional secondary study objective, to assess the increase in

Fig. 1 Subject disposition (per CONSORT)

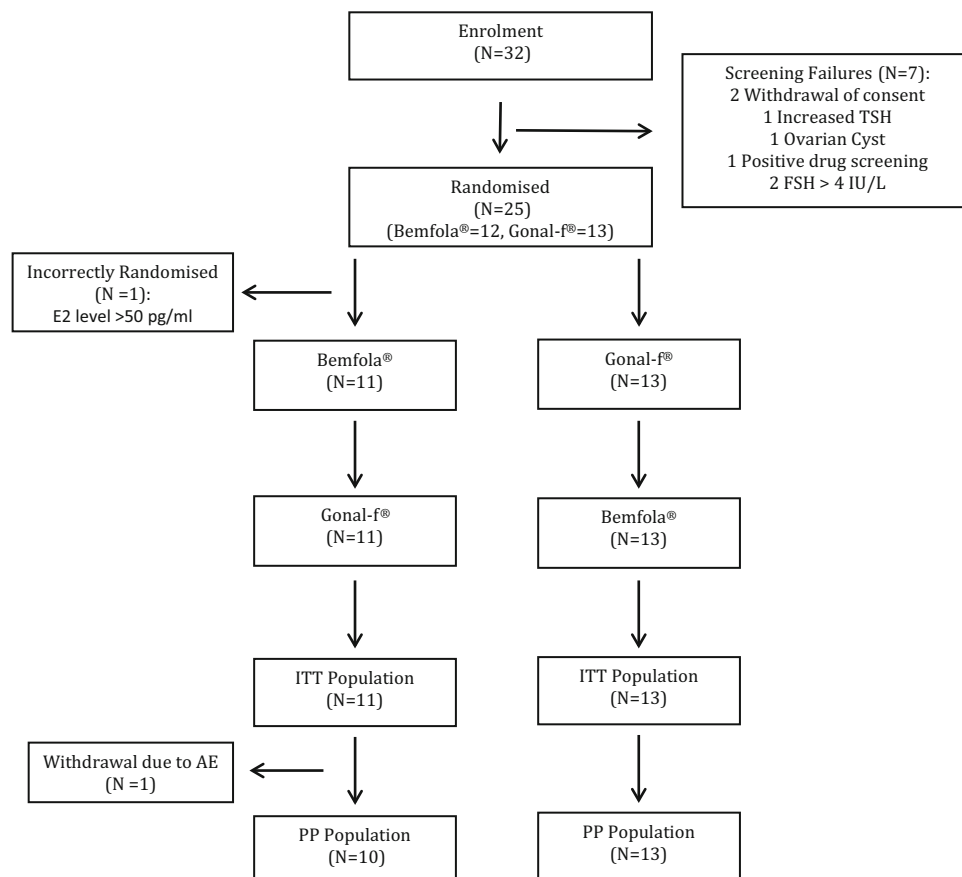


Table 1 Pharmacokinetic parameters and comparative bioavailability confidence intervals (CIs)

Parameter	Treatment		
	Bemfola [®] (<i>n</i> = 24)	Gonal-f [®] (<i>n</i> = 23)	90 % CI (Bemfola [®] vs. Gonal-f [®])*
<i>r</i> -hFSH			
<i>C</i> _{max} (IU/L)	5.69*	6.01*	94.7 % (89.2 %, 100.6 %)
<i>T</i> _{max} (h)	24.0 (6.0–24.0)**	24.0 (9.0–24.0)**	–
AUC _{0–192} (IU h/L)	424.90*	432.75*	98.2 % (84.7 %, 113.9 %)
<i>T</i> _{1/2} (h)	43.58 (14.17)***	42.58 (16.47)***	–
<i>K</i> _e (1/h)	0.0075 (0.003)***	0.0077 (0.002)***	–

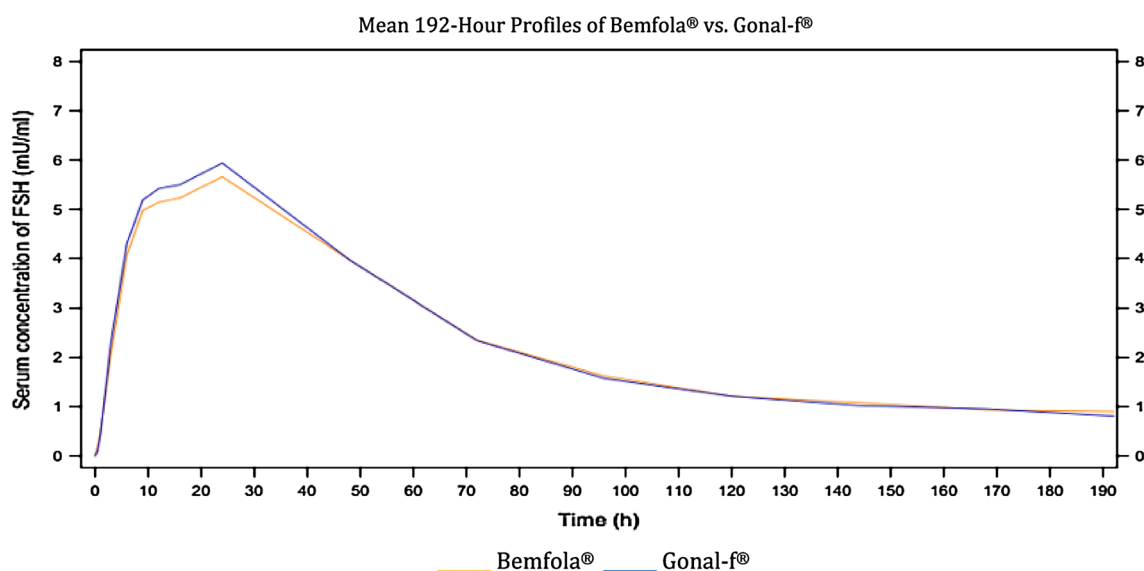
*C*_{max} maximum drug concentration, *T*_{max} time to reach maximum drug concentration, AUC_{0–192h}, area under the concentration curve from time 0 to 192 h, *t*_{1/2} elimination half-life, *K*_e elimination rate constant

* Geometric mean

** Median (range)

*** Arithmetic mean (SD)

**** Parametric means

**Fig. 2** Mean 192-h corrected profiles of serum concentration of FSH (mIU/mL)—Bemfola[®] vs. Gonal-f[®]

E2 serum concentration (AUC) after single application of Bemfola[®] and Gonal-f[®].

At this point, the study was already terminated, but spare serum aliquots reserved for PK analysis were still available and were used for analysis of E2 serum concentration. Altogether, 17 out of 24 subjects gave a written consent for further investigation of their blood samples.

Background for this amendment was the expectation of detecting an increase in E2 levels even after single application of *r*-hFSH to pituitary down-regulated subjects. This increase could be rated as marker for pharmacodynamic activity of Bemfola[®] and Gonal-f[®]. The rise in E2 serum concentration was expected to be low but detectable with a maximum approximately 24 h after *r*-hFSH application. Therefore sample collection times used for determination of FSH serum concentration were also appropriate for investigating E2.

A low but detectable rise and plateau by 24–48 h in mean E2 serum concentration was observed after a single subcutaneous dose of either Bemfola[®] or Gonal-f[®]. The application of Bemfola[®] induced a stronger increase in the mean E2 levels than Gonal-f[®], with a mean AUC_{0–120} of 3304.3 vs. 2624.3 IU h/L and mean *C*_{max} of 37.72 vs. 30.90 pg/mL, respectively (Fig. 3). The observed differences were not significant (AUC *p* = 0.21, *C*_{max} *p* = 0.82).

3.4 Adverse event profile

In total, 121 AEs occurred during the course of the study. 94 AEs were mild (78 %), 27 (22 %) were moderate, and none was severe or serious. 35 AEs (29 %) were drug unrelated. 86 AEs (71 %) were related to a study drug, with

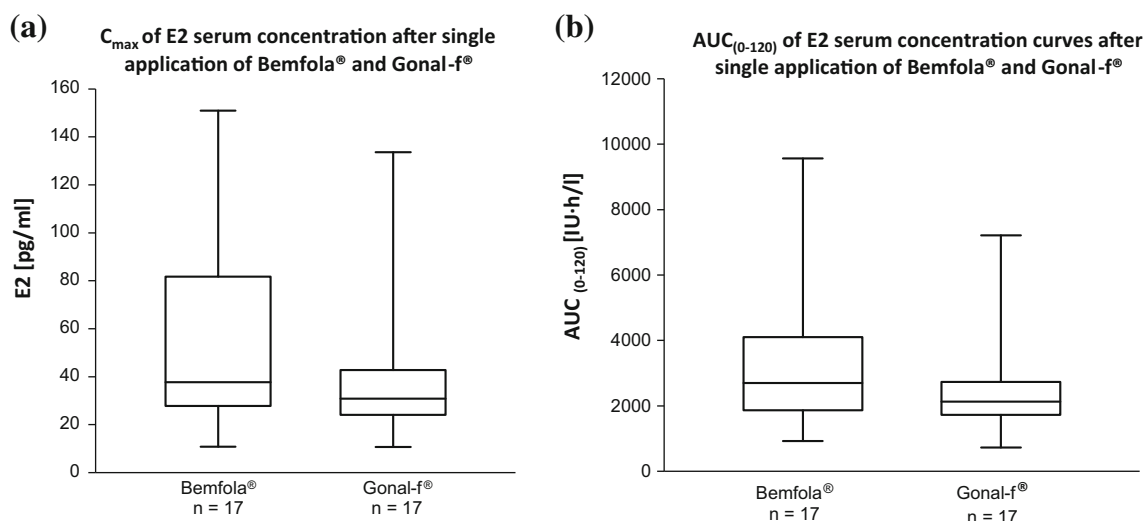


Fig. 3 E2 response after single application of Bemfola® and Gonal-f®. Subgroup analysis for 17 subjects. *Boxplots* show median, 25 and 75 quartiles and minimal and maximal value. **a** C_{max} of E2 (pg/mL),

b $AUC_{(0-120)}$ of the E2 serum concentration curve (IU h/L). The difference in AUC and C_{max} is not significant ($p = 0.21$ and 0.82 , respectively)

82 related to leuprolide acetate, 3 AEs were related to both leuprolide acetate and Bemfola®, and 1 to Bemfola® (mild local pain after the application of Bemfola®). The AEs occurring most frequently were headache (63 % of subjects) and hot flushes (46 % of subjects). One subject was withdrawn because of an erroneous ultrasound assessment. All but one subject recovered from their AEs, with the majority of AEs being mild in nature and requiring minimal or no therapeutic intervention for resolution. The one subject not recovered during the frame of the study was reported with acne of mild intensity; however, this was suspected by the investigator to be related to the leuprolide acetate depot treatment. Results of ECG, vital signs and physical examinations gave no reason for clinical concern. No continuous and relevant changes of the safety laboratory parameters were documented throughout the study.

3.5 Immunogenicity profile

No specific antibodies to FSH were detected after r-hFSH administration. In 64 of 72 samples, response signals were below the limit of Quantification (LoQ). 8 of 72 samples were above the LoQ (i.e., in the very low range of the standard curve). Results from a specificity assay demonstrated that these were background signals unspecific to FSH. In samples collected at Study Days 15 and 24, the detected response was similar to the baseline value for all subjects, and no significant increase was detected (data not shown).

4 Discussion

The results of Study FIN1001 indicate that 225 IU Bemfola® and the reference product Gonal-f® administered as a single dose are bioequivalent based on the systemic exposure as demonstrated by $AUC_{(0-192)}$. In support of this key finding, no appreciable differences in C_{max} , T_{max} , $t_{1/2}$, and K_e for r-hFSH were detected as per non-compartmental PK analysis. The PK and safety findings presented here therefore support the assumption that Bemfola® may be an acceptable alternative to Gonal-f® in the treatment of infertility.

As a marker for Pharmacodynamic activity of Bemfola® and Gonal-f®, a post hoc analysis was conducted, measuring whether an increase in E2 levels could be detected after a single application of r-hFSH to the pituitary down-regulated subjects. Both r-hFSH preparations induced a measurable increase in E2 levels. While there was a tendency to a stronger response to Bemfola®, the difference in AUC and C_{max} was not statistically different.

No significant safety issues were reported in this safety assessment; most AEs were mild in nature and resolved in response to minimal or no therapeutic intervention. One subject was withdrawn because of an erroneous ultrasound assessment. No clinically relevant findings as per ECG, vital-sign, physical, and laboratory examination were observed. Immunogenicity to FSH was not evident in this trial; however, the clinical impact of this observation is not clear given that it is based on the administration of a single SC dose.

The results of this study indicate that a single dose of Bemfola[®] exhibits pharmacokinetic and safety profiles comparable to Gonal-f[®] in healthy young women.

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Conflict of interest Michael Wolzt, Ghazaleh Gouya and Michael Sator have nothing to declare. Thomas Hemetsberger and Brigitta Vcelar are employed by Polymun Scientific Immunbiologische Forschung GmbH. Charlotte Irps and Manfred Rettenbacher are employed by Finox AG.

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