Retrospective analysis of the use of two recombinant follitropin alfa injections in patients undergoing in vitro fertilization treatment with the gonadotropin-releasing hormone antagonist protocol

Šprem Goldštajn, Marina; Dumančić, Stipe; Mikuš, Mislav

Source / Izvornik: **Journal of Obstetrics and Gynaecology Research, 2021, 47, 992 - 1001**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1111/jog.14626

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:804880

Efficacy comparison of the biosimilar and the reference follitropin alfa used within the GnRH antagonist protocol of the controlled ovarian hyperstimulation for IVF/ICSI technique

Prof. Marina Šprem Goldštajn[1,2], MD., PhD, Stipe Dumančić[2], MD., Mislav Mikuš[1,2], MD.

[1]Department of Obstetrics and Gynecology, University Hospital Center Zagreb, Croatia

[2]School of Medicine, University of Zagreb, Croatia

Corresponding author:

Mislav Mikuš, Department of Obstetrics and Gynecology, University Hospital Center Zagreb, Croatia, Petrova 13, Zagreb 10000; e-mail: m.mikus19@gmail.com

ORCID: 0000-0002-1365-8704

Contributions: All three authors contribute equally according to the criteria for scientific authorship of the International Committee of Medical Journal Editors (ICMJE): 1. Substantial contributions to conception and design, data collection or analysis, and interpretation of data; 2. Writing of the article or critical review of the intellectual content; 3. Final approval of the version to be published; and 4. Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Work was carried out at University Hospital Center Zagreb on Department of Obstetrics and Gynecology, Petrova 13, 10 000 Zagreb.

Declarations of interest: none.

Funding: none.

Abstract

Aim: To evaluate the clinical efficacy of biosimilar (Ovaleap®) compared with the referenced follitropin alfa (Gonal-f®), within the context of antagonistic multiple doses protocol of controlled ovarian hyperstimulation (COH) for in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) techniques.

Methods: A retrospective, monocentric study included 229 infertile women aged 22 to 43 years who underwent their first cycle of COH for the purpose of the IVF or ICSI during the period of 2017. Eligible patients underwent ovarian stimulation with either Ovaleap® (N = 152) or Gonal-f® (N = 77) starting at Cycle Day 2 and were receiving gonadotropin-releasing hormone (GnRH) antagonist in either fixed or flexible antagonist protocol manner.

Results: Ovaleap®-treatment resulted in fewer number of oocytes retrieved in regard to Gonal-f®-treatment, with the median of 7 oocytes retrieved in the Ovaleap® group versus 9 in the Gonal-f® group (U = 5369.5, P = 0.3079). Clinical pregnancy rate was 24.3% in the overall study sample and 31.9% in women with embryo transfer, in the Ovaleap group. Similarly, in the Gonal-f group these rates were 25.0% and 34.5%, respectively. Only 4 patients experienced ovarian hyperstimulation syndrome (OHSS), with 1 case in Ovaleap®-treatment group and 3 cases in Gonal-f®-treatment group.

Conclusions: While the clinical efficacy profile favoured using Gonal-f® formulation of follitropin alfa, this analysis showed that there is no significant difference in the number of oocytes retrieved between Ovaleap® and Gonal-f® follitropin alfa formulations, used within GnRH antagonist protocols of COH.

Keywords: assisted reproductive technology, follitropin alfa, GnRH antagonist, infertility, oocytes retreived.

INTRODUCTION

In the field of human reproduction two biosimilars of original recombinant follitropin alfa product (Gonal-f®) are currently approved (Bemfola® and Ovaleap®) [1]. These medications are used as stimulants of multifollicular development in controlled ovarian hyperstimulation (COH) protocols for the purposes of the assisted reproductive technology (ART), among several indications. Following generic approach, biosimilars or follow-on biologics (FOB) can be developed as a copy of the original, reference biological drug after the market protection of the reference product has expired and are approved for the use in all indications of the reference biologic [1].

Differences in the structure of biosimilars to the reference follitropin alfa were thoroughly researched in pre-clinical studies. These differences did not show any influence on the biological activity and were considered acceptable according to the concept of biosimilarity [2-4]. Phase I clinical trials have demonstrated the pharmacokinetic bioequivalence of both biosimilars according to the reference product in population of healthy voluntary women [5-7]. Phase III clinical trials have demonstrated comparable therapeutic efficacy of biosimilars and the reference follitropin alfa by primary endpoint, the number of retrieved oocytes, with comparable secondary endpoints and adverse event profile in women undergoing COH for the ART treatment [8-10]. The primary purpose of developing biosimilars, due to the potentially shorter production process, is to lower the price of biological therapies through bidding process of biosimilars with original products [1].

Aforementioned phase III clinical studies used formulations of follitropin alfa within gonadotropin-releasing hormone (GnRH) agonist protocol of ovarian stimulation for the pituitary down-regulation of endogenous levels of follicle-stimulating hormone (FSH) [8, 9]. Contrary, GnRH antagonists (GnRH-ant) were only used in minor proportion of patients in

the follow-up study of randomized controlled trial of Ovaleap®, and in one non-interventional study [10, 11].

The advantages of the use of GnRH antagonists are numerous. First, long pre-treatment for the pituitary down-regulation is not required, which is accompanied with the rarity of hypoestrogenic symptoms. Shorter period of ovarian stimulation is required contrary to GnRH agonists. Consequently, with the lower total doses of gonadotropins needed within antagonist protocols, a significantly lower incidence of ovarian hyperstimulation syndrome (OHSS) with GnRH antagonists was found, contrary to GnRH agonists [12, 13].

Given this background, the aim of this comparative analysis was to evaluate the clinical efficacy of Ovaleap® compared with the referenced follitropin alfa (Gonal-f®), within the context of antagonistic multiple doses protocol of COH for in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) techniques.

PATIENTS AND METHODS

Participants and data

A retrospective, monocentric study included 229 infertile women aged 22 to 43 years, with the body mass index (BMI) between 17 and 32 kg/m^2 and who underwent their first cycle of controlled ovarian hyperstimulation for the purpose of the assisted reproductive technique (ART) therapy (IVF/ICSI). Other inclusion criteria were: 1) basal FSH concentration less than 10 IU/L on the Cycle Day 2; 2) infertility that resulted from tubal factor, male factor, endometriosis, anovulation, idiopathic infertility and from a combination of causes; 3) presence of both normal ovaries and uterine cavity confirmed by transvaginal ultrasound before inclusion in the study. The data were obtained from the EHR database of the Department of Obstetrics and Gynaecology of UHC Zagreb, from women who were indicated with the controlled ovarian hyperstimulation (COH) and ART treatment of infertility during

the period of 2017, with the therapy outcomes (pregnancy loss, stillbirth or live birth) in the following year period. The study was approved by the local ethics committee, and the requirement to obtain informed consent was waived.

Treatment scheme

Eligible patients underwent ovarian stimulation with either Ovaleap® (N = 152) or Gonal-f® (N = 77) starting at Cycle Day 2 and were receiving GnRH antagonist in either fixed or flexible antagonist protocol manner (Figure 1). Starting doses with the dose adaptation of follitropin alfa in cases of risk of OHSS or other safety concerns, and the administration of hCG agent to induce follicular maturation and trigger ovulation were decided according to local practice (Figure 1). Oocyte retrieval took place 32 to 34 h after hCG administration. Elected ART procedure (IVF or ICSI) was carried out according to Centre's standards, and a developed embryos or blastocysts were transferred (maximum of two) 2-5 days following oocyte retrieval or were cryopreserved.

Endpoints and statistical analysis

The aim of this study was primarily to compare number of oocytes retrieved between Gonal-f® and Ovaleap® follitropin alfa products, which is the primary endpoint of randomized controlled trials (RCTs) for demonstrating clinical comparability regarding efficacy between the biosimilar and the reference biological medicinal products containing r-hFSH, as stipulated by the European Medical Agency (EMA). Additional parameters analysed were baseline demographic and clinical characteristics, stimulation phase factors, embryo and pregnancy assessment with adverse event profile. Study parameters' findings were descriptively reported and evaluated using the Mann-Whitney *U*, chi-squared or Fisher's

exact test, as applicable. Furthermore, Spearman correlation was carried out to measure association between variables (age level, AMH value, and stimulation phase parameters with the number of retrieved oocytes). The *P* value <.05 was considered to indicate statistical significance. Oocyte assessment was based on the nuclear maturity of retrieved oocytes, while the grading of transferred embryos was assessed based on the developmental stage of embryo/blastocyts obtained. The analysis was performed using Rstudio IDE/R software package (Version 1.1.463 © 2009-2018).

RESULTS

Baseline characteristics of study population

Demographic and baseline characteristics were comparable between treatment groups. A total of 229 patients were analysed in this study, i.e. 152 patients in Ovaleap®-treatment group and 77 patients in Gonal-f®-treatment group. In total, most patients (>80 %) were 30 years or older. While there wasn't a broad difference in the age distribution of patients with the flexible protocol, the majority of women under fixed antagonistic protocol were between 30-34 years. Similarly, BMI (body mass index) and duration of infertility were also comparable (Table 1).

There was a slightly higher FSH baseline level in the Gonal-f®-treatment group, contrary to the higher mean AMH level in the Ovaleap®-treatment group. Furthermore, there was a higher percentage of women having AMH level above 24 pmol/L, that was defined by Lee and associates as cut-off level predictor for evaluation of high responders [14]. In addition, there was a lack of data regarding the AMH level in patients of the Gonal-f®-treatment group (Table 1).

For the majority of women, the cause of infertility was found to be a male factor, and moreover combination of male factor and other infertility causal factors. In the Ovaleap®-

treatment group there were slightly more patients with endometriosis and anovulatory cycles, or cause has not been diagnosed.

Number of oocytes retrieved

Overall, at least one oocyte was obtained in 227/229 (99.1%) women, i.e. with one patient per treatment group who discontinued further ART procedure due to negative oocyte retrieval. Most patients (>80 %) had retrieval of 4 or more oocytes in both treatment groups (Table 2, Figure 2).

The number of oocytes retrieved per patient was similar between treatment groups. Ovaleap®-treatment resulted in fewer number of oocytes retrieved in regard to Gonal-f®-treatment, with the median of 7 oocytes retrieved in the Ovaleap® group versus 9 in the Gonal-f® group (U = 5369.5, P = 0.3079). Similar differences between treatment groups were also found by observing oocyte number in regard to subgroups of patients treated with fixed and flexible multiple doses GnRH-ant protocols (Table 2, Figure 3).

Evaluation of the stimulation phase

Characteristics of the GnRH antagonist administration were similar between GnRH-ant protocols (Table 3). The mean duration of GnRH-ant was comparable observing GnRH-ant protocol subgroups, with 4.5 days within fixed (U = 1507.5, P = 0.898), and circa 3.5 days within flexible GnRH-ant protocol (U = 1511.5, P = 0.2242). Patients started receiving GnRH-ant on 6^{th} day of stimulation within fixed GnRH-ant protocol in both treatment groups (U = 1285, P = 0.07361), while it was 8^{th} (Ovaleap®) and 7^{th} (Gonal-f®) day of stimulation regarding flexible GnRH-ant protocol (U = 1146.5, P = 0.1956).

The duration of stimulation with r-hFSH was comparable observing GnRH-ant protocol subgroups, with the mean of > 8 days of r-FSH stimulation within fixed protocol (U = 1205.5, P = 0.1616), and > 9 days within flexible protocol (U = 1361.5, P = 0.5321). Overall, patients prescribed with fixed GnRH-ant protocol received lower total dose of r-hFSH (*median* = 1800 IU in both groups; U = 1492, P = 0.8329), in regard to patients with flexible protocol.

However, total r-hFSH dose differed slightly between treatment groups in patients prescribed with flexible GnRH-ant protocol (Ovaleap®, *median* = 1950 IU; Gonal-f®, *median* = 1800 IU), which was not statistically significant (U = 1401.5, P = 0.657) (Table 3).

The most common starting doses were 150, 225 and 300 IU in both treatment groups (Figure 4). There was higher percentage of patients in the Gonal-f® group who required dose adjustment, with 36 patients (46.7%) versus 40 patients (26.7%) in the Ovaleap® group, which was statistically significant difference (U = 7200, P = 0.001143). Furthermore, more patients needed dose reduction than dose elevation (Table 3, Figure 4).

Patients prescribed with fixed GnRH-ant protocol in both treatment groups had higher median serum concentrations of estradiol (E_2) on the day of hCG administration (Ovaleap®, E2 = 1527 pmol/L; Gonal-f®, E2 = 1790 pmol/L), than patients with flexible GnRH-ant protocol (Ovaleap®, E2 = 1156 pmol/L; Gonal-f®, E2 = 1195 pmol/L). Furthermore, E_2 concentrations differed slightly between groups within fixed protocol (U = 967, P = 0.5872), which was not statistically significant (Table 3).

Embryo assessment

ICSI technique was performed in higher percentage of women treated with Ovaleap®. Most oocytes in the majority of patients in both groups were in the metaphase II stage of nuclear development (Table 4). Treatment groups were equable regarding embryo and blastocysts obtained after conducted ART procedure, with the mean of 2.5 embryos (median = 2) obtained per group. Regarding numbers and quality of transferred embryos/blastocysts, similar findings were revealed. Both treatment groups had the mean number of 1.2 transferred embryos, with closely similar proportions of embryo stages (Table 4).

Pregnancy and live births

Similar proportion of patients in both treatment groups underwent embryo transfer procedure and cryopreserved their embryos. Clinical pregnancy rate was 24.3% (37/152) in the overall study sample and 31.9% (37/116) in women with embryo transfer, in the Ovaleap group. Similarly, in the Gonal-f group these rates were 25.0% (20/77) and 34.5% (20/58), respectively (Table 5).

In the Ovaleap®-treatment group 75.7 % of patients with clinical pregnancy had live birth versus 90.0 % of patients in the Gonal-f®-treatment group with live birth. Take-home baby rate, which is defined as the percentage of patients with live birth divided by the number of randomized patients, was 18.4 % in the Ovaleap®-treatment group versus 23.4 % in the Gonal-f®-treatment group (Table 5).

Despite the higher percentages of patients with liveborn children rates in the Gonal-f®-treatment group, there were 52 live births overall with 32 live births in the Ovaleap®-treatment group and 20 in the Gonal-f®-treatment group. Four women in the Ovaleap®-treatment group and 2 women the Gonal-f®-treatment group had twins (Table 5).

Adverse event profile

Cases of OHSS and pregnancy loss were documented. Only 4 patients experienced OHSS, with 1 case in Ovaleap®-treatment group and 3 cases in Gonal-f®-treatment group, respectively (P = 0.1117). Cases of OHSS could not be classified by the data from medical records provided, but they led to the cancelling of embryo transfer in affected patients. There were 11 pregnancy loss events, with 9 cases in Ovaleap®-treatment group and 2 cases in Gonal-f®-treatment group (P = 0.3424), respectively, but the study was not designed to detect if they are related to treatment drugs (Table 6).

DISCUSSION

Our study found that Ovaleap® and Gonal-f® formulations are equivalent by observing the number of oocytes retrieved in a population of infertile women undergoing controlled ovarian hyperstimulation for the purpose of assisted reproduction techniques [15]. Infertile women included in this study were representative of the patient population usually treated with the COH for the purpose of the ART procedures, and in regard to the mean age, BMI and baseline FSH concentration similar to the population of previous studies that investigated effectiveness and safety of Ovaleap® follitropin alfa [9-11]. However, our patient population had higher AMH values contrary to the study population of Howles et al. [11]. Since the previous trial of Strowitzki et al. was designed by using long GnRH agonist protocol of COH [9], this study examined the clinical efficacy of these formulations within fixed or flexible GnRH antagonist multiple doses protocols.

Ovaleap® treatment resulted in the mean number of 8.57 retrieved oocytes, while the mean of 9.38 oocytes was retrieved in women treated with Gonal-f®. A similar oocyte count was found in subgroups treated with either fixed or flexible GnRH antagonist protocols of ovarian hyperstimulation. Overall, differences in oocytes numbers between subgroups were found to be nonsignificant. Our therapy regimen did not yield the number of retrieved oocytes that has been shown in the previous studies [9, 11], which could be explained with the absence of the comparable study protocol design.

Using Spearman correlation test, we investigated an impact of different variables, including the age and AMH level, as well as the duration and dose of r-hFSH, on the number of oocytes retrieved [16]. As expected, the number of retrieved oocytes decreased with age, shown by statistically significant weak negative correlation in both treatment groups (Ovaleap®, r_s = -0.3018, P = 0.0001576; Gonal-f®, r_s = -0.2925053, P = 0.009838). Contrary, the number of retrieved oocytes and AMH values showed statistically significant weak (Ovaleap®, r_s =

0.388, P = 0.00000697) and moderate (Gonal-f®, r_s = 0.6257985, P = 0.0000121) positive correlations. Along with findings of AMH values, including the proportion of potential high responders (Table 1) in regard to AMH, and baseline FSH levels in both groups, we can assume that the majority of our study population were normoresponding patients, with the expected number of retrieved oocytes shown by our study, despite cases of OHSS shown. There was no relationship with the duration of r-hFSH stimulation (Ovaleap®: r_s = 0.1946836, P = 0.01624; Gonal-f®: r_s = 0.09193821, P = 0.4265), or total r-hFSH dose (Ovaleap®: r_s = -0.1175292, P = 0.1493; Gonal-f®: r_s = -0.2420643, P = 0.03392) in both treatment groups.

Even though the analysis did not investigate differences in pregnancy rates, clinical pregnancy rates were favourable of Gonal-f®, with slightly more day-5 blastocyst transfers in the Gonal-f® treatment group. However, our study yielded lower pregnancy rates in comparison to studies of Strowitzki et al. and Howles et al. [9, 11].

Flexible approach to the use of the multiple dose GnRH antagonist protocol was found to have shorter administration period and consequent use of less vials of GnRH antagonist, contrary to fixed approach [17, 18]. Tailoring the start of GnRH antagonist in an ovarian stimulation by the size of the leading follicle could improve the outcome of these cycles [18]. Our study demonstrated shorter administration period and consequently using fewer vials of GnRH antagonist within flexible GnRH-ant protocol. Furthermore, while the parameters regarding stimulation with r-hFSH (total dose, starting doses and duration of stimulation) were similar between treatment groups, there was significantly lower proportion of patients with the dose adjustment of r-hFSH in the Ovaleap® treatment group (26.7%) versus Gonal-f® group (46.7%, P = 0.001143) with flexible GnRH antagonist protocol, contrary to the previous study of Strowitzki et al., which used long GnRH agonist protocol [9].

Both the usage of GnRH antagonist protocol, in regard to long GnRH agonist protocol, and flexible GnRH antagonist protocol of COH were found to be associated with the lower incidence of OHSS [13, 19]. Even though this study did not investigate safety profiles of r-hFSH agents with the GnRH antagonist co-treatment, there was lower OHSS rate in the Ovaleap® treatment group (0.7%). Furthermore, this finding was also in contrary to the previous studies, that yield higher OHSS rates with Ovaleap® treatment [9, 11].

While the clinical efficacy profile favoured using Gonal-f® formulation of follitropin alfa, this analysis showed that there is no significant difference in the number of oocytes retrieved between Ovaleap® and Gonal-f® follitropin alfa formulations, used within GnRH antagonist protocols of COH. This study further expands evidence of equivalent clinical profiles of the biosimilar and the reference biological medicinal products containing r-hFSH. Furthermore, results show that the use of Ovaleap® within the flexible GnRH antagonist protocol could reduce the need of dose adjustment of r-hFSH in controlled ovarian stimulation compared to Gonal-f®, with potentially lower OHSS rates.

Acknowledgments

None.

Disclosure

The authors have no conflicts of interests to declare in relation to this article.

References

1. Orvieto R, Seifer DB. Biosimilar FSH preparations – are they identical twins or just siblings. Reprod Biol Endocrinol. 2016;14:32. doi: 10.1186/s12958-016-0167-8

2. Mastrangeli R, Satwekar A, Cutillo F, Ciampolillo C, Palinsky W, Longobardi S. In-vivo biological activity and glycosylation analysis of a biosimilar recombinant human follicle-stimulating hormone product (Bemfola) compared with its reference medicinal product (GONAL-f). PLoS ONE 12(9): e0184139. https://doi.org/10.1371/journal.pone.0184139

3. Winstel R, Wieland J, Gertz B, Mueller A, Allgaier H. Manufacturing of Recombinant Human Follicle-Stimulating Hormone Ovaleap (XM17), Comparability with Gonal-f, and Performance/Consistency. Drugs R D. 2017;17:305–312.

4. Lispi M, Mastrangeli R, Galano E, Palmese A, Cutillo F, Casarini L, Riccetti L, Longobardi S, Simoni M. Comparative Analysis of Structural Differences of Ovaleap and GONAL-f: Site-specific Glycosylation Mapping and Cell Signalling Activation. Poster presented at: 34th Annual Meeting of the European Society of Human Reproduction and Embryology; 2018 Jul 1-4; Barcelona, Spain.

5. Wolzt M, Gouya G, Sator M, Hemetsberger T, Irps C, Rettenbacher M, Vcelar B. Comparison of pharmacokinetic and safety profiles between Bemfola and Gonal-f after subcutaneous application. Eur J Drug Metab Pharmacokinet. 2016;41:259–265.

6. Lammerich A, Bias P, Gertz B. Phase 1 safety, tolerability, and pharmacokinetic study of single ascending doses of XM17 (recombinant human follicle-stimulating hormone) in downregulated healthy women. Int J Womens Health. 2015;7:707–716.
7. Lammerich A, Mueller A, Bias P. Phase I, two-way, crossover study to demonstrate bioequivalence and to compare safety and tolerability of single-dose XM17 vs Gonal-f® in healthy women after follicle-stimulating hormone downregulation. Reprod Biol Endocrin. 2015;13:130. Doi: https://doi.org/10.1186/s12958-015-0124-y
8. Rettenbacher M, Andersen AN, Garcia-Velasco AJ, Sator M, Barri P, Lindenberg S, et al. A multi-centre phase 3 study comparing efficacy and safety of Bemfola® versus Gonal-f® in women undergoing ovarian stimulation for IVF. Reprod Biomed Online. 2015;30(5):504-513. doi: https://doi.org/10.1016/j.rbmo.2015.01.005
9. Strowitzki T, Kuczynski W, Mueller A, Bias P. Randomized, active-controlled, comparative phase 3 efficacy and safety equivalence trial of Ovaleap® (recombinant human follicle-stimulating hormone) in infertile women using assisted reproduction technology (ART). Reprod Biol Endocrin. 2016;14:1. doi: 10.1186/s12958-015-0135-8
10. Strowitzki T, Kuczynski W, Mueller A, Bias P. Safety and efficacy of Ovaleap® (recombinant human follicle-stimulating hormone) for up to 3 cycles in infertile women using assisted reproductive technology: a phase 3 open-label follow-up to Main Study. Reprod Biol Endocrin. 2016;14(1):31. DOI: 10.1186/s12958-016-0164-y
11. Howles C, German Ovaleap non-interventional study group (Sydow P, Gmeinwieser N, Pribbernow K, Keck C, Wiegratz I). Effectiveness and safety of biosimilar follitropin alfa in women undergoing routine ovarian stimulation with a GnRH

antagonist: Results from a German multi-centre non-interventional study (abstract). Fertil Steril. 2019;112(3, Suppl):e195.

12. Devroey P, Aboulghar M, Garcia-Velasco J et al. Improving the patient's experience of IVF/ICSI: A proposal for an ovarian stimulation protocol with GnRH antagonist co-treatment. Hum Reprod. 2009;24:764–74.
13. Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and metaanalysis. Hum Reprod Update. 2006;12(6):651–671.
14. Lee TH, Liu CH, Huang CC, Wu YL, Shih YT, Ho HN, et al. Serum anti-Mullerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproduction technology cycles. Hum Reprod. 2008;23(1):160-167.
15. European Medicines Agency (EMA): Note for guidance on Specifications: Test procedures and acceptance criteria for Biotechnological/Biological Products, CPMP/ICH/365/96 ICH Q6B. [accessed: 10.08.2020.] Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf
16. Olivennes F, Howles CM, Borini A, Germond M, Trew G, Wikland M, et al. Individualizing FSH dose for assisted reproduction using a novel algorithm: the CONSORT study. Reprod Biomed Online. 2009;18(2):195-204.
17. Escudero E, Bosch E, Crespo J, Simon C, Remohi J, Pellicer A. Comparison of two different starting multiple dose gonadotropin-releasing hormone antagonist protocols in a selected group of in vitro fertilization-embryo transfer patients. Fertil Steril. 2004;81(3):562-566.

18. Ludwig M, Katalinic A, Banz C, Schroder AK, Loning M, Weiss JM, Diedrich K. Tailoring the GnRH antagonist cetrorelix acetat to individual patients' needs in ovarian stimulation for IVF: results of a prospective, randomized study. Hum Reprod. 2002;17(11):2842-2845.
19. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2016 [accessed: 03.07.2020.] Available at: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD001750.pub4/fullxs

Table 1. Baseline demographic and clinical characteristics.

	Ovaleap® *N* = 152	***Gonal-f®*** *N* = 77	***Test statistic***[b]	***P value***[b]
Age, mean (SD), years	34.3 (4.0)	34.1 (4.1)	*U* = 5637	0.6496
Age distribution, n/N[a] (%), years				
Fixed protocol				
< 30 years	9/90 (10.0)	8/34 (23.53)	*U* = 1737	0.0522
30 – 34 years	46/90 (51.1)	18/34 (52.94)	*U* = 1570	0.7974
> 34 years	35/90 (38.9)	8/34 (23.53)	*U* = 1283	0.1053
Flexible protocol				
< 30 years	8/62 (12.9)	3/43 (6.98)	*U* = 1254	0.3349
30 – 34 years	26/62 (41.9)	20/43 (46.51)	*U* = 1377.5	0.729
> 34 years	28/62 (45.2)	20/43 (46.51)	*U* = 1367.5	0.7974
BMI, mean (SD), kg/m^2	22.8 (2.7)	22.9 (3.2)	*U* = 2537	0.9312
Duration of infertility, median (min-max), years	3 (1-13)	3 (2-12)	*U* = 2345	0.202
Causes of infertility, n (%)				
Tubal factor	12 (7.9)	8 (10.4)	X^2 = 0.14748	0.701
Male factor	38 (25.0)	30 (40.0)	X^2 = 4.1264	0.04222
Endometriosis	7 (4.6)	1 (1.3)	X^2 = 0.82177	0.3647
Anovulation	14 (9.2)	2 (2.6)	X^2 = 2.4971	0.1141
Idiopathic	10 (6.6)	3 (3.9)	X^2 =	0.5985

Combination	40 (26.3)	17 (22.1)	0.27733 X^2 = 0.29046	0.5899
No data	31 (20.4)	16 (20.8)		
FSH baseline levels, mean (SD), IU/L	6.1 (1.05)	7.0 (2.10)	*U* = 4115.5	0.5082
AMH levels, median (min-max), pmol/L	15 (1.1-316)	15 (0.8-71)	*U* = 2627	0.9323
AMH levels > 24 pmol/L, n (%)	44 (28.9)	14 (18.2)	*U* = 291.5	0.7712

SD standard deviation, *BMI* body mass index, *FSH* follicle-stimulating hormone, *AMH* anti-Müllerian hormone, *ART* assisted reproductive technology.

[a]Number divided by the number of patients prescribed with GnRH-ant protocol (See Table 2).

[b]Calculated using Mann-Whitney *U* or Pearson's chi-squared test (X^2), as shown.

Table 2. **Number of retrieved oocytes.**

	Ovaleap® *N* = 152	***Gonal-f®*** *N* = 77	***Test statistic***	***P value***
Patients per GnRH-ant protocol, n (%)				
Fixed protocol	90 (59.2)	34 (44.2)		
Flexible protocol	62 (40.8)	43 (55.8)		
Patients with oocyte retrieval, n (%)	151 (99.3)	76 (98.7)		
Patients with good response[a], n (%)	127 (84.0)	63 (82.0)		

Number of retrieved oocytes				
Mean (SD)	8.57	9.38	*U* = 5369.5	0.3079
Median (min-max)	(5.18)	(5.78)		
	7 (0-28)	9 (0-22)		
Number of retrieved oocytes per GnRH-ant protocol	8 (0-22)	9.5 (2-20)	*U* = 1776	0.1681
Fixed protocol, median (min-max)				
Flexible protocol, median (min-max)	7 (1-22)	8 (0-28)	*U* = 1362.5	0.8498

GnRH-ant gonadotropin-releasing hormone antagonist, *SD* standard deviation.

[a]Patients with an oocyte retrieval of four or more oocytes.

Table 3. **Assessment of the ovarian stimulation phase.**

	Ovaleap®	***Gonal-f®***	***Test statistic***	***P value***
GnRH-ant duration per protocol, mean (SD), days				
Fixed protocol	4.5 (1.2)	4.5 (1.2)	*U* = 1507.5	0.898
Flexible protocol	3.6 (0.95)	3.4 (1.4)	*U* = 1511.5	0.2242
Start of GnRH-ant per protocol**,** median (min-max), day	6 (6-7)	6 (6-7)	*U* = 1285	0.07361
Fixed protocol	8 (7-11)	7 (7-13)		0.1956
Flexible protocol			*U* = 1146.5	
r-hFSH dose per protocol, median (min-max), IU				
Fixed protocol	1800 (1050-3000)	1800 (1200-4125)	*U* = 1492	0.8329
Flexible protocol	1950 (1200-3000)	1800 (900-3600)	*U* = 1401.5	0.657
Duration of r-hFSH stimulation, mean				

(SD), days				
Fixed protocol	8.5 (1.3)	8.9 (1.3)	*U* = 1205.5	0.1616
Flexible protocol	9.3 (1.1)	9.6 (2.2)	*U* = 1361.5	0.5321
Starting dose of r-hFSH, IU				
Fixed protocol				
Mean (SD)	215.9 (51.9)	217.7 (43.2)	*U* = 1757	0.1518
Median (min-max)	225.0 (150-300)	225.0 (150-300)		
Flexible protocol				
Mean (SD)	222.4 (39.9)	226.1 (56.2)	*U* = 1265.5	0.6213
Median (min-max)	225.0 (150-300)	225.0 (75-375)		
r-FSH dose adaptation, n (%)				
Total	40 (26.7)	36 (46.7)	*U* = 7200	0.001143
Dose increase[a]	9 (22.5)	7 (19.4)		
Dose decrease[a]	31 (77.5)	29 (80.6)		
Estradiol serum levels[b], median (min-max), pg/mL	1527 (156-4636)	1790 (576-3877)	*U* = 967	0.5872
Fixed protocol	1156 (179-4544)	1195 (409-5085)	*U* = 699	0.8885
Flexible protocol				

GnRH-ant gonadotropin-releasing hormone antagonist, *r-FSH* recombinant FSH.

[a]Diveded by the number of patients required with dose adaptation of r-FSH.

[b]E2 serum concentration measured on the day of hCG administration.

Table 4. Oocytes and embryo assessment.

	Ovaleap®	**Gonal-f®**	***Test statistic***	***P value***
ART procedure, n (%)				
IVF	70 (46.0)	49 (63.6)	X^2 = 2.0972	0.1476
TESE-ICSI	80 (52.6)	27 (35.1)	X^2 = 2.0887	0.1484
Nihil	2 (1.4)	1 (1.3)		

Nuclear maturity of retrieved oocytes, n (%)	134 (10.3)	57 (7.9)	*U* = 5392	0.2631
Germinal vesicle	143 (11.0)	74 (10.2)	*U* = 5967.5	0.7927
Metaphase I	975 (74.8)	564 (78.0)	*U* = 6403.5	0.243
Metaphase II				
Number of obtained embryos/blastocysts				
Total	371	191	*U* = 5771	0.8576
Median (min-max)	2 (0-6)	2 (0-7)		
Number of transferred embryos/blastocysts	186 (50.1)	92 (48.2)	*U* = 5968	0.793
Total (%)[a]	1.2 (0.8)	1.2 (0.8)		
Mean (SD)				
Developmental stage of transferred embryos, n (%)	69 (37.1)	36 (39.1)	*U* = 5977.5	0.6874
Expanded blastocyst	56 (30.1)	29 (31.5)	*U* = 5828	0.9497
Blastocyst	61 (32.8)	27 (29.3)	*U* = 5630	0.5282
Embryo (cleavage)				

ART assisted reproductive technology, SD standard deviation.

[a]Proportion of the number of obtained embryos/blastocysts.

Table 5. **Clinical efficacy outcomes.**

	Ovaleap®	**Gonal-f®**	***Test statistic***	***P value***
Patients with embryo transfer, n/N (%)	116/152 (76.3)	58/77 (75.3)		
Patients with cryopreservation, n/N (%)	64/152 (43.1)	35/77 (45.4)		
Biochemical pregnancy rates, n/N (%)	50/152 (32.9)	22/77 (28.6)	$U = 5599$	0.5074
All patients	50/116 (43.1)	22/58 (37.9)	$U = 3219$	0.5879
Patients with embryo transfer				
Clinical pregnancy rates, n/N (%)	37/152 (24.3)	20/77 (26.0)	$U = 5933$	0.8215
All patients	37/116 (31.9)	20/58 (34.5)	$U = 3206$	0.6887
Patients with embryo transfer				
Take-home baby rates[a], n/N (%)				
All patients	28/152 (18.4)	18/77 (23.4)	$U = 6134$	0.3931
Patients with embryo transfer	28/116 (24.1)	18/58 (31.0)	$U = 3616$	0.2923
Number of live births, n	32	20		0.5825[b]

N/A not applicable.

[a]Take-home baby rate equals the percentage of patients with live births divided by the number of patients.

[b]Calculated using Fisher's exact test.

Table 6. **Adverse events.**

	Ovaleap®	**Gonal-f®**	***P value*[a]**
OHSS, n (%)	1 (0.7)	3 (3.9)	0.1117
Pregnancy loss, n	9 (5.9)	2 (2.6)	0.3424

OHSS ovarian hyperstimulation syndrome.

[a]Calculated using Fisher's exact test.

Figure legends:

Figure 1. Study treatment scheme with patient disposition.

Figure 2. Distribution of the number of oocytes retrieved in patients treated with follitropin alfa agents for controlled ovarian hyperstimulation (COH).

Figure 3. Presentation of the number of oocytes retrieved in patients using proposed stimulating agents within GnRH-antagonist protocol types.

Figure 4. Frequency of starting doses of follitropin alfa and the percentage of patients with dose adaptation.