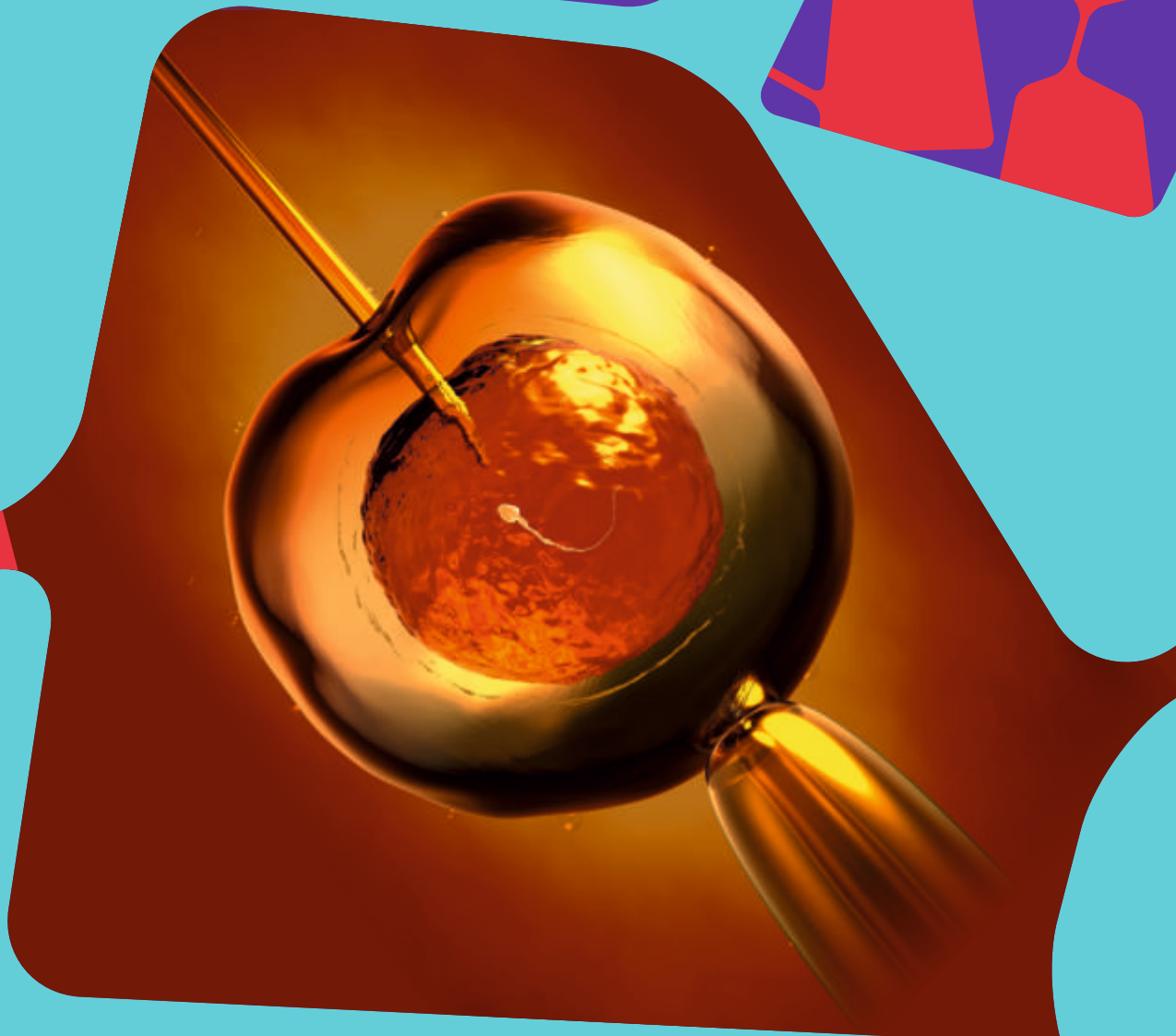
ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

Issue 9



Follitropin alfa originator vs. biosimilars: difference beyond RCTs

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expert insights

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Controlled ovarian stimulation (COS) with follicle stimulating hormone (FSH) injections is a pivotal step in the IVF and ICSI procedures which is in practice the past five decades.

Currently we are using the fourth-generation gonadotropin, Recombinant human follicle-stimulating hormone Follitropin alfa (a recombinant FSH (rFSH)) which is a biologic product produced by complex cutting-edge process. However, as the patent of the Biologics expire, the biosimilars which are copies of reference biological drugs are developed to replicate an original biological medicine.

Biosimilar is a biological product developed using a step-wise approach and approved based on a showing that it is highly similar to an already approved reference Biologic with no clinically meaningful differences from the reference biologic in terms of safety, purity, and potency of the product.

However, biosimilars are considered to be 'Similar but not the same' as an approved reference Biologic. This issue outlines the comparison of Follitropin Alfa Originator vs biosimilars, their manufacturing processes, discusses why the biosimilars are not identical to reference biological drugs, the variations in activity, structural differences, bioactivity and immunogenicity of the biosimilars vs. Originator and also compares the outcomes of the use of Follitropin Alfa Originator vs biosimilars in couples undergoing ART.

Follitropin alfa originator vs biosimilars: difference beyond RCTs

what is follitropin alfa originator?

1. *Xue W, Lloyd A, Falla E, Roeder C et al. A cost-effectiveness evaluation of the originator follitropin alpha compared to the biosimilars for assisted reproduction in Germany. Int J Womens Health. 2019 May13;11:319-331.*
2. *Schwarze JE, Venetis C, Iniesta S et al. Originator recombinant human follitropin alfa versus recombinant human follitropin alfa biosimilars in Spain: A cost-effectiveness analysis of assisted reproductive technology related to fresh embryo transfers. Best Pract Res Clin Obstet Gynaecol. 2022 Feb 8:S1521-6934(22)00020-7.*
3. *Declerck P, Danesi R, Petersel D et al. The Language of Biosimilars: Clarification, Definitions, and Regulatory Aspects. Drugs. 2017;77(6):671-677.*

Controlled ovarian stimulation (COS) with follicle stimulating hormone (FSH) injections is a pivotal step in the IVF and ICSI procedures. This strategy of stimulating ovaries, with gonadotropins is well-established with the first generation of gonadotropins, produced from the urine of menopausal women, on the market since the 1970's.

Recombinant human follicle-stimulating hormone follitropin alfa or **Follitropin Alfa Originator** (originator r-hFSH-alfa) is a fourth-generation gonadotropin and a recombinant FSH (rFSH). It was first approved in Europe in 1995 and in 1997 in the USA.

It has a well-established portfolio of published efficacy, safety and clinical real-world post-marketing evidence and experience.

Treatment with originator r-hFSH-alfa has resulted in the birth of more than 4 million babies across the world.

In a nutshell, Follitropin Alfa Originator is a biologic product which is defined as an approved product composed of proteins, nucleic acids, or combinations of these, or living entities such as cells and tissues, which is isolated from natural sources (including humans, animals, and microorganisms) and produced by biotechnology methods and other cutting-edge technologies.

what are biosimilars?

1. *Gámez-Belmonte R, Hernández-Chirlaque C, Arredondo-Amador M et al. Biosimilars: Concepts and controversies. Pharmacol Res. 2018 Jul;133:251-264.*
2. *Orvieto R, Seifer DB. Biosimilar FSH preparations- are they identical twins or just siblings? [published correction appears in Reprod Biol Endocrinol. 2016;14(1):59]. Reprod Biol Endocrinol. 2016;14(1):32.*
3. *Declerck P, Danesi R, Petersel D et al. The Language of Biosimilars: Clarification, Definitions, and Regulatory Aspects. Drugs. 2017;77(6):671-677.*
4. *Schwarze JE, Venetis C, Iniesta S et al. Originator recombinant human follitropin alfa versus recombinant human follitropin alfa biosimilars in Spain: A cost-effectiveness analysis of assisted reproductive technology related to fresh embryo transfers. Best Pract Res Clin Obstet Gynaecol. 2022 Feb 8:S1521-6934(22)00020-7.*

The EMA defines a biosimilar, or similar biological medicinal product, as 'a biological medicinal product that contains a version of the active substance of an already authorized original biological medicinal product (reference medicinal product)', with similarity established 'in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise.

The FDA describes biosimilars as Biologic products that are “highly similar to the reference product not with-standing minor differences in clinically inactive components and that there are no clinically meaningful differences between the biologic product and the reference product in terms of safety, purity, and potency of the product”.

According to this definition, it is very clear that the biosimilars are not identical molecules or “generics” for Biologic agents.

'**Similar but not the same**' constitutes a catch phrase as widely used in the biosimilar field. The phrase gives the impression of an attempt falling short in some way (since 'similar' is not quite the same as 'identical').
Biosimilars have variation in strength, purity and may contain different composition of isoforms and/or various glycosylation profiles, with consequent alterations in clinical efficacy or safety.
This necessitates the manufacturer of the biosimilars to conduct detailed analytical and functional studies, phase III randomized controlled trials and demonstrate with relevant results that the changes in the biosimilars do not adversely affect the identity, purity, or potency of the potentially approved biologic product.
In terms of their development and regulatory approval, biosimilars are different from both originator biologic products and generic small molecule drugs. Biosimilars undergo a rigorous evaluation using the criteria defined in the European Medicines Agency (EMEA), FDA, or WHO biosimilar guidelines before regulatory approval. Also, the biosimilars cannot be considered generic equivalents to the originator.
In short, biosimilar preparations are required to be biologically and clinically 'non-inferior' to the originator product.

Manufacturing process of originator vs. Biosimilars

1. *Kabir ER, Moreino SS, Sharif Siam MK. The Breakthrough of Biosimilars: A Twist in the Narrative of Biological Therapy. Biomolecules. 2019;9(9):410.*

A complex multi-step process is involved in the manufacturing of biologics and biosimilars as they utilize mammalian and microbial cell cultures to manufacture therapeutic proteins. **"The process is the product"** is a long existing paradigm of the biologic manufacturing process, which means that any variations in the production process could significantly alter the product's safety and efficacy profile.

Manufacturing process of the originator

1. *Camacho LH, Frost CP, Abella E et al. Biosimilars 101: considerations for U.S. oncologists in clinical practice. Cancer Med. 2014;3(4):889-899.*
2. *Kabir ER, Moreino SS, Sharif Siam MK. The Breakthrough of Biosimilars: A Twist in the Narrative of Biological Therapy. Biomolecules. 2019;9(9):410.*

The manufacturing process for biologics is a complex process requiring multiple steps for cloning; selecting, maintaining, and expanding the cell line; and isolating, purifying, and characterizing the product.
A combination of both analytical and process development methods allows for the assessment of the scale up process while ensuring that it maintains the adequate productivity of a quality product.
Different manufacturers use/develop different cell lines and production processes for production of Biologics thus making it a challenge for different manufacturers of biosimilars to develop identical copies of biologics.
Figure 1 outlines the different manufacturing process for Biologics.

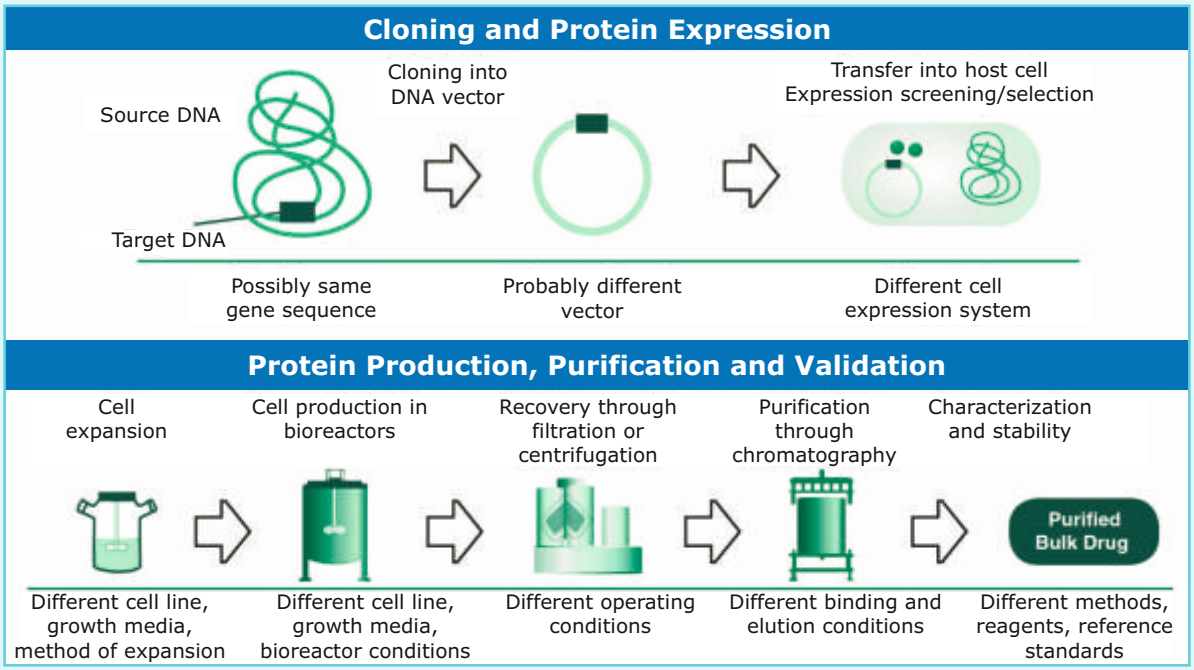


Figure adapted from Cancer Med. 2014;3(4):889-899.

Figure 1. Biologic manufacturing includes multiple steps that may vary between manufacturers, potentially leading to differences between a biosimilar and its reference product that cannot be fully characterized with available analytical methods

Owing to the process sensitivity, the manufacturing process of Biologics should involve a constant check of the impurities like the host cell proteins (HCPs), cell debris, cell culture medium serum proteins, immunoglobulin affinity ligands, protein A or protein G affinity ligands, viruses, endotoxin, DNA, and non-protein cell-wall constituents.

In addition, other parameters like pH, flow rate, temperature, media, equipment cleanliness, purification and sterilization processes should be closely monitored and maintained.

Manufacturing process of Biosimilar

1. *Kabir ER, Moreino SS, Sharif Siam MK. The Breakthrough of Biosimilars: A Twist in the Narrative of Biological Therapy. Biomolecules. 2019;9(9):410.*
2. *Misra M. Biosimilars: current perspectives and future implications. Indian J Pharmacol. 2012;44(1):12-14.*
3. *Mellstedt H, Niederwieser D, Ludwig H. The challenge of biosimilars. Ann Oncol. 2008 Mar;19(3):411-419.*
4. *Vulto AG, Jaquez OA. The process defines the product: what really matters in biosimilar design and production? Rheumatology (Oxford). 2017;56(suppl_4):iv14-iv29.*

On the contrary, the manufacturing process of biosimilars needs to be carefully designed and closely monitored. This process comprises of selecting the appropriate originator biologic agent, detecting its critical molecular characteristics, and tailoring the process to match these traits.

As the innovator products are a proprietary knowledge, biosimilar manufacturers will not have access to the manufacturing process of innovator products and thus will not be able to replicate any protein product.

The biosimilar manufacturing process thus seems more complicated as the developer is faced with several constraints at the start of development itself.

To begin with, the development exercise must begin with defining the originator fingerprint for

dozens of quality attributes of the biosimilar.
Next, as the manufacturing process for the originator molecule is unknown, a new process must be engineered to ensure that the biosimilar matches the originator fingerprint as closely as possible. Thus, the entire development process of a biosimilar drug is typically strategized via the following approach as outlined in figures 1 and 2.

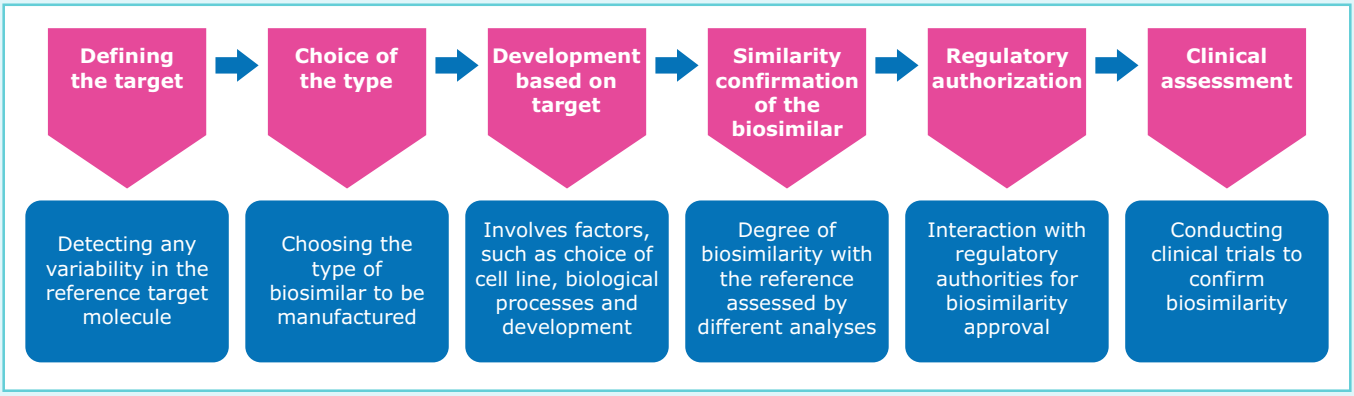


Figure adapted from Kabir ER et al. Biomolecules. 2019;9(9):410.

Figure 1. Development process of a Biosimilar drug

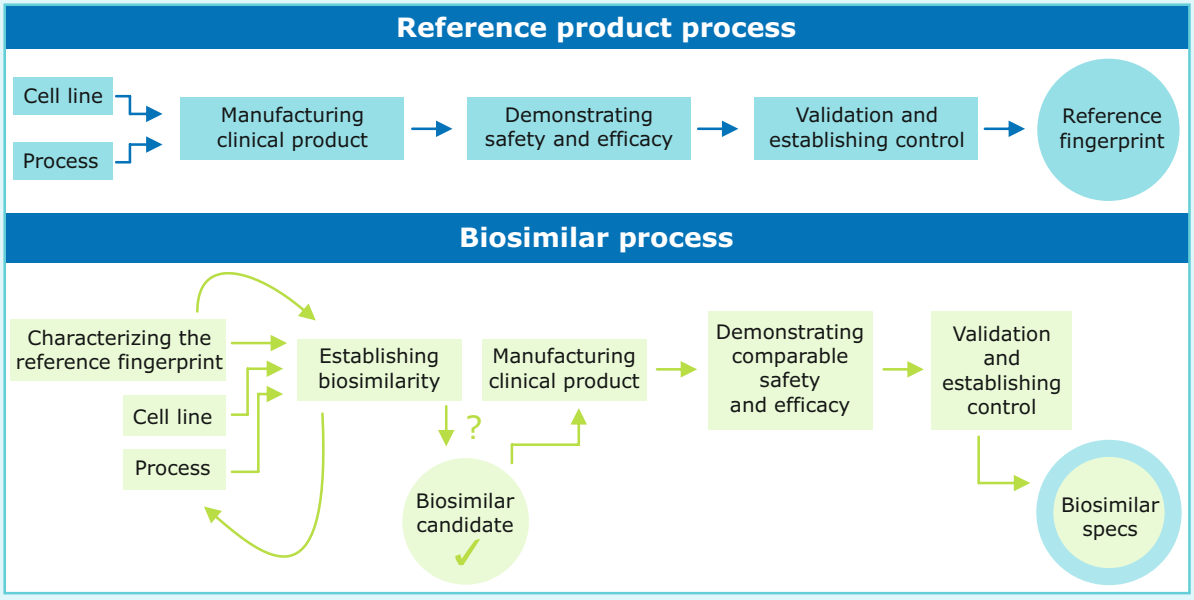


Figure adapted from Rheumatology (Oxford). 2017;56(suppl_4):iv14-iv29.

Figure 2. Comparison of the developmental processes for a reference (Originator) product and a Biosimilar

Why Biosimilars are not identical to reference biological drugs?

1. *Kabir ER, Moreino SS, Sharif Siam MK. The Breakthrough of Biosimilars: A Twist in the Narrative of Biological Therapy. Biomolecules. 2019;9(9):410.*
2. *Halimi V, Daci A, Ancevska Netkovska K et al. Clinical and Regulatory Concerns of Biosimilars: A Review of Literature. Int J Environ Res Public Health. 2020;17(16):5800.*

3. Biosimilar Development, Review, and Approval. Accessed from the website https://www.fda.gov/drugs/biosimilars/biosimilar-development-review-and-approval as on 19.05.2022.
4. Declerck P, Danesi R, Petersel D et al. The Language of Biosimilars: Clarification, Definitions, and Regulatory Aspects. Drugs. 2017;77(6):671-677.
5. Gámez-Belmonte R, Hernández-Chirlaque C, Arredondo-Amador M et al. Biosimilars: Concepts and controversies. Pharmacol Res. 2018 Jul;133:251-264.
6. Rumore MM, Randy Vogenberg F. Biosimilars: Still Not Quite Ready for Prime Time. P T. 2016;41(6):366-375.
7. Camacho LH, Frost CP, Abella E et al. Biosimilars 101: considerations for U.S. oncologists in clinical practice. Cancer Med. 2014;3(4):889-899. (figure ref)
8. Sekhon B, Saluja V. Biosimilars: an overview. Biosimilars. 2011;1:1-11.

Biologic medications (biologics) are complex macromolecular drugs that are manufactured via living systems (i.e., from living cells or organisms).

Originator Biologics are novel medicines manufactured through biotechnology, using complex system cells and recombinant DNA technology. They are also costly due to their lengthy and risky development process. Nonetheless, novel biologics enjoy two mechanisms of market protection: patents (which usually last up to 20 years), and a period of data exclusivity and market exclusivity (for up to 11-12 years).

However, as defined by the US FDA, a biosimilar is a biological product developed using a step-wise approach and approved based on a showing that it is highly similar to an already approved reference Biologic with no clinically meaningful differences from the reference biologic in terms of safety, purity and potency of the product.

Only after the originator biologic reaches expiry of all patents, a biosimilar is released to the market and be available in the clinics.

The following points enable us to note that biosimilars cannot be identical to originators or reference drugs:

1. Biosimilars are copies of reference biological drugs, developed as the patents for original biologicals expire
2. Biosimilars are thus developed to replicate an original biological medicine
3. Unlike chemical drugs, molecular identity cannot generally be established for any two biological drugs
4. Biosimilars have reverse engineering manufacturing process compared to that of originators
5. Manufacturing differences between a biosimilar and its reference product can lead to differences in molecular structure (e.g., glycosylation), content (e.g., isoforms, impurities, and aggregates), biological activity, and immunogenicity.
6. The pharmacological properties of biosimilars cannot be the same as originator owing to complexity of the production and to the presence of minor natural variations in the molecular structure (known as microheterogeneity)
7. Biosimilars cannot have increased immunogenicity [generally meaning higher anti-drug antibodies (ADA) incidence] vs. the reference drug as per regulatory standards
8. Biosimilars cannot have extrapolation of indication like originators which can have more than one indication

Thus, the biosimilars can only be "SIMILAR" but not "SAME/IDENTICAL" to originators.

Table 1 compares the different aspects of innovator, biosimilar and generic products.

Table 1. Comparison of different aspects between the innovator, biosimilar and generic products

Process	Innovator	Biosimilar	Generic
Manufacturing	Produced by biological process in host cell lines Sensitive to production process changes – expensive and specialized production facilities Reproducibility difficult to establish	Produced by biological process in host cell lines Sensitive to production process changes – expensive and specialized production facilities Reproducibility difficult to establish	Produced by using chemical synthesis Less sensitive to production process changes Reproducibility easy to establish
Clinical development	Extensive clinical studies, including Phase I–III Pharmacovigilance and periodic safety updates needed	Extensive clinical studies, including Phase I–III Pharmacovigilance and periodic safety updates needed	Often only Phase I studies Short timeline for approval
Regulation	Needs to demonstrate "comparability" Regulatory pathway defined by Europe (EMEA) Currently no automatic substitution intended	Needs to demonstrate "similarity" Regulatory pathway defined by Europe (EMEA) No automatic substitution allowed	Needs to show bioequivalence Abbreviated registration procedures in Europe and US Automatic substitution allowed

Abbreviation: EMEA, European Medicines Agency.

Table adapted from Biosimilars. 2011;1:1-11.

variations in activity and immunogenicity of biosimilars

1. Schellekens H. The first biosimilar epoetin: but how similar is it? Clin J Am Soc Nephrol. 2008;3(1):174-178.
2. Schellekens H. Biosimilar therapeutics-what do we need to consider? NDT Plus. 2009;2(Suppl_1):i27-i36.
3. Misra M. Biosimilars: current perspectives and future implications. Indian J Pharmacol. 2012;44(1):12-14.

The most important safety issue of protein drugs is their potential immunogenicity. Episodes of variations in activity and immunogenicity in biosimilars compared to their respective innovator products have made the world look biosimilars with caution.

In one case, three non-innovator products for epoetin alfa manufactured in Korea were shown to differ from the reference epoetin alfa product (Originator) with variations in the activity, concentration and isoforms of the products. An *in vitro* bioassay showed that both biosimilars had a higher bioactivity than was listed on their respective labels. In addition, both of these products had higher concentrations of epoetin alfa than stated on the labels when estimated by an enzyme linked immunosorbent assay.

In another case, a subtle change in the manufacturing process led to a concern regarding immunogenicity with upsurge of life-threatening pure red cell anemia (PCRA) caused by antibodies induced by epoetin alpha. The PRCA was associated with a formulation change when human serum albumin (HSA) as a protein stabilizer was exchanged with polysorbate 80. Although the mechanism of PCRA was not fully understood, it was believed that Polysorbate 80 was supposed to have increased the immunogenicity by eliciting the formation of epoetin-containing micelles or by interacting with leachates released by the uncoated rubber stoppers of prefilled syringes.

This case illustrated how a difference in the manufacturing process can alter product characteristics.

A case of PRCA in a hemodialysis patient with Chronic Kidney Disease (CKD) ~6 months after treatment with the follow-on epoetin alfa was also reported in India. Although the precise mechanism for the development of PRCA in this patient could not be elucidated, researchers suggest that the increase in immunogenicity could have been due to problems in the manufacturing and storage of the product.

In a study comparing 11 epoetin alfa products from four different countries (Korea, Argentina, China, India), the isoform distribution among these products had variability.
Significant diversions from specification for *in vivo* bioactivity were observed and the *in vivo* bioactivity ranged from 71 to 226%, with 5 products failing to fulfill their own specification.

Comparative analysis of structural differences of originator follitropin alfa and Bemfola (biosimilar) in vivo bioactivity and site-specific glycosylation mapping

1. *Mastrangeli R, Satwekar A, Cutillo F et al. 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. 2017;12(9):e0184139.*
2. *Meher BR, Dixit A, Bousfield GR et al. Glycosylation Effects on FSH-FSHR Interaction Dynamics: A Case Study of Different FSH Glycoforms by Molecular Dynamics Simulations. PLoS ONE.2015;10(9): e0137897.*

Recombinant human follicle-stimulating hormone (r-hFSH) is widely used in fertility treatment. Biosimilar versions of r-hFSH (follitropin alfa) are also currently in the market.
Highly purified preparations of recombinant-human FSH (r-hFSH; follitropin alfa) is available with high batch-to-batch consistency in FSH content, isoform profile and specific activity. Follitropin alfa can be filled by mass (FbM), providing very low batch-to-batch variability (< 2%) and enabling more precise dosing. This reduced variability might improve both convenience and effectiveness during stimulation cycles.
As both originator and biosimilars are available in the market, it is important to thoroughly evaluate a biosimilar in comparison with the reference product as there is difference in structural complexity and manufacturing process.
A significant portion of the functional diversity of proteins is derived from their glycosylation states.
The *in vivo* potency and the biological activity of FSH molecules are influenced by the differences in glycosylation. The α chain of FSH is glycosylated at asparagine 52 (Asn52) and Asn78, while the β subunit can be glycosylated at Asn7 and Asn24, with the glycosylation profile of each subunit playing a critical role in the activity and clearance of FSH.
Glycosylation of the α chain at Asn52 has been shown to play a pivotal role in FSHR activation/signalling. Glycosylation at this site is therefore considered to be essential for bioactivity.

A study by Mastrangeli et al., compared the site-specific glycosylation profile and batch-to-batch variability of the *in vivo* bioactivity of Bemfola, a biosimilar follitropin alfa, with its reference medicinal product **Originator follitropin alfa**. The focus of this analysis was the site-specific glycosylation at asparagine (Asn 52).
The study results were as follows:

N-glycan distribution at Asn52 (α chain)

The N-glycan distribution at Asn52 was consistent across batches of both **biosimilar Bemfola and Originator follitropin alfa** (Table 1).

Table 1. Antennarity, fucoslylation and sialylation at α chain Asn52						
		199F005 Fol.alfa.org	199F049 Fol.alfa.org	199F051 Fol.alfa.org	PPS30403 Bemfola	PNS30226 Bemfola
Antennarity	Bi-antennary	76.2	76.9	77.8	53.9	52.7
	Tri-antennary	22.9	22.6	22.2	40.7	41.8
	Tetra-antennary	0.9	0.5	0.0	5.3	5.5
Fucosylation	A-fucosylated	97.8	97.8	97.6	98.8	98.7
	Fucosylated	2.2	2.2	2.4	1.2	1.3
Sialylation	Mono-sialylated	2.6	2.7	2.4	6.2	6.8
	Di-sialylated	84.0	85.5	85.7	72.8	71.8
	Tri-sialylated	13.4	11.8	11.9	21.0	21.4
	Tetra-sialylated	0.0	0.0	0.0	0.8	1.0

Table adapted from PLoS One. 2017;12(9):e0184139.

A different glycan profile was observed at Asn52 in Bemfola compared with Follitropin Alfa originator (a lower proportion of bi-antennary structures [~53% vs ~77%], and a higher proportion of tri-antennary [~41% vs ~23%] and tetra-antennary structures [~5% vs <1%]). These differences in the Asn52 glycan profile might potentially lead to differences in FSHR activation. Differences were observed between Bemfola and Follitropin Alfa originator in the distribution of glycans at this site (Figures 1 and 2). Differences in the distribution of mono-, di-, tri- and tetra-sialylated species were observed between the two products (Figure 2B).

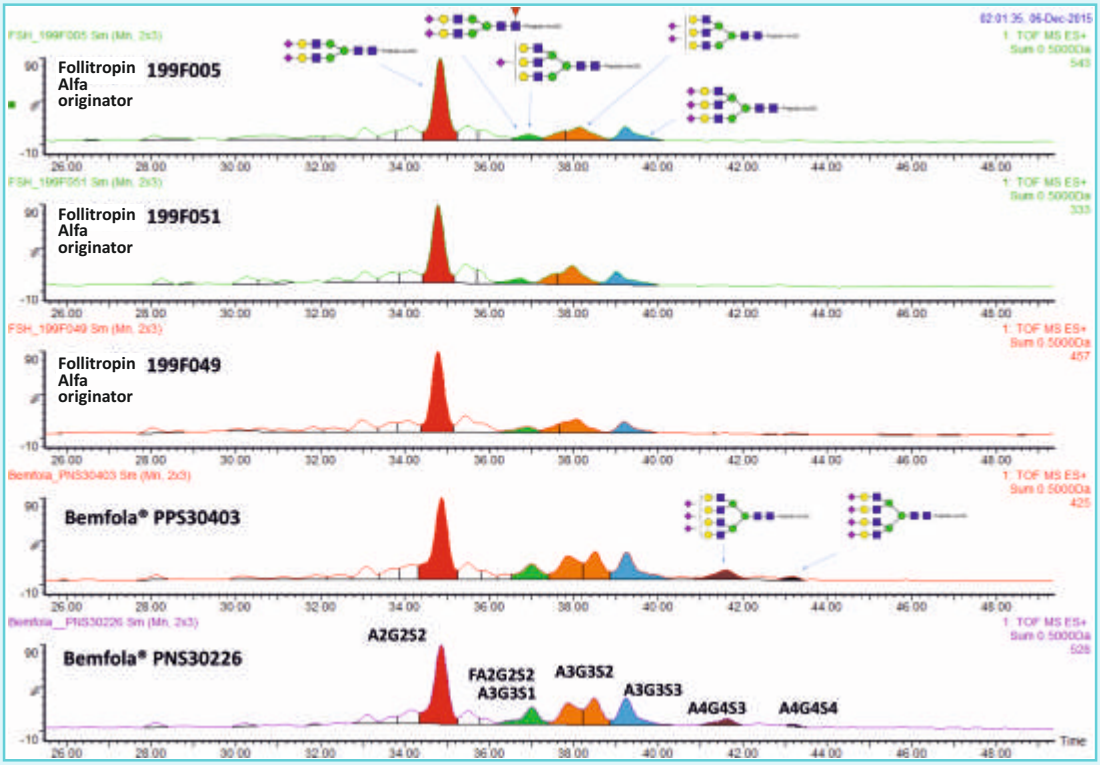
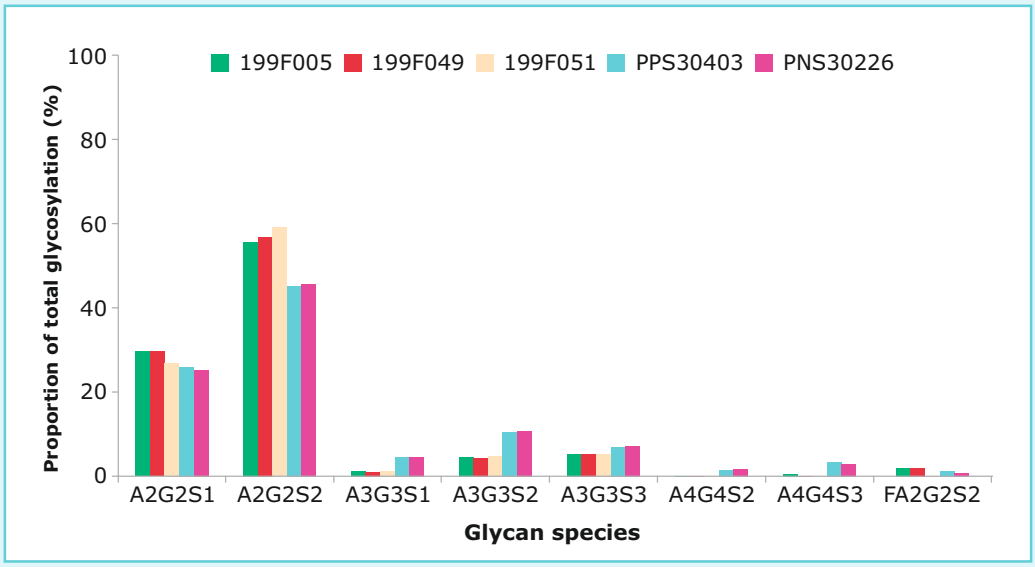


Figure 1. Extracted ion chromatograms of the N-glycan distribution at Asn52 for Bemfola and Follitropin Alfa originator

Asn, asparagine. Blue square, GlcNAc. Green circle, mannose. Yellow circle, galactose. Red triangle. fucose. Purple diamond, sialic acid NeuNAc. Glycan naming: F at the start of the abbreviation indicates a core a(1-6) fucose linked to the inner GlcNAc. Ax indicates the number of antenna (GlcNAc) on trimannosyl core. A2 indicates bi-antennary with both GlcNAcs as b(1-2) linked. A3 indicates tri-antennary with a GlcNAc linked b1-2 to both mannose and a third GlcNAc linked b(1-4) to the a(1-3) linked mannose. A4 indicates tetra-antennary with GlcNAcs linked as A3 with additional GlcNAc b(1-6) linked to a(1-6) mannose.
Gx indicates the number (x) of b1-4 linked galactose on the antenna. Sx indicates the number (x) of sialic acids linked to galactose

A



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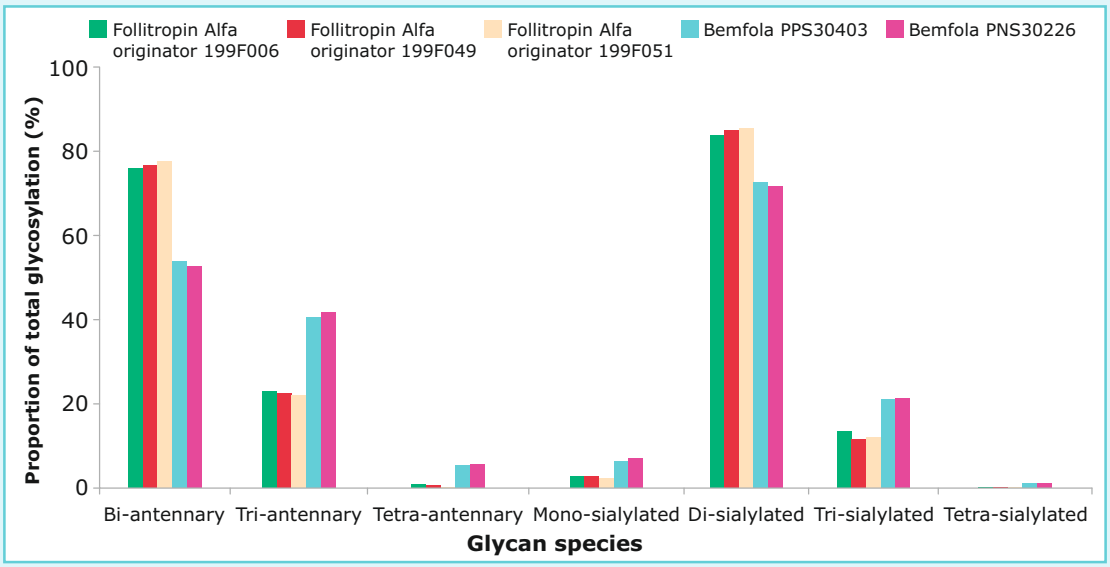


Figure adapted from PLoS One. 2017;12(9):e0184139.

Figure 2. Glycan and antennarity and sialylation distribution at Asn52

A) Comparison of glycan distribution at Asn52 between Bemfola and Follitropin Alfa originator (individual species)
B) Comparison of antennarity and sialylation at Asn52 between Bemfola and Follitropin Alfa originator. Asn, asparagine

Overall, there were bulkier glycan structures and greater sialylation in Bemfola than Follitropin Alfa originator.

Bioactivity

The average bioactivity observed for Bemfola was within the range stated on the product label (14,403 IU/mg [105.6% of the nominal value]).

However, most of the observed bioactivity values were higher than stated on the label and were also higher than the average bioactivity values of Follitropin Alfa originator (13,270 IU/mg [97.3% of the nominal value].

A higher batch-to-batch variability was observed for Bemfola (coefficient of variation, CV 8.3%) compared with Follitropin Alfa originator (CV 5.8%; Table 2).

Table 2. Summary statistics for relative % nominal value					
Site	**Count**	**Average**	**Median**	**Standard deviation**	**CV%**
Bemfola	8	105.625	106.5	8.71677	**8.25257**
Follitropin Alfa originator	22	97.3182	96.5	5.66011	**5.81608**
Total	30	99.5333	98.5	7.44976	7.48468

Table adapted from PLoS One. 2017;12(9):e0184139.

In conclusion, there were differences in the glycosylation profile at the Asn52 site of the α-chain with Bemfola and Follitropin Alfa originator. Overall, Bemfola showed higher antennarity, higher sialylation and higher batch-to-batch variability in activity compared with Follitropin Alfa originator. These elements could partly explain the differences in clinical outcomes between Bemfola and Follitropin Alfa originator reported in the literature.

comparison of outcomes with biosimilar recombinant follitropin alfa preparations versus the reference product in couples undergoing assisted reproductive technology treatment

1. *Chua SJ, Mol BW, Longobardi S et al. Biosimilar recombinant follitropin alfa preparations versus the reference product (Gonal-F®) in couples undergoing assisted reproductive technology treatment: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2021;19(1):51.*

In the current scenario, the success of infertility treatment is increasingly measured by live birth. There is also growing consensus that ongoing pregnancy is usually well correlated with live birth.
Previous analyses comparing biosimilar preparations of follitropin alfa versus the reference product have had insufficient power to detect differences in clinically meaningful outcomes such as live birth.
A meta-analysis was conducted by Chua et al., to investigate whether there were any differences in live birth, clinical and ongoing pregnancy rates between biosimilar preparations of follitropin alfa and the reference product.
About 17 studies were included in the systematic review out of the 292 unique publications initially identified.
The primary endpoint of the meta-analysis was live birth rate per randomized patient.
Secondary outcomes included clinical pregnancy rate, ongoing pregnancy rate, total dose of gonadotrophins, duration of ovarian stimulation, number of oocytes retrieved per aspirated cycle and number of embryos obtained per aspirated cycle, moderate or severe ovarian hyperstimulation syndrome (OHSS) rate, miscarriage rate, ectopic pregnancy rate, multiple pregnancy rate and immunogenicity.

The study results were as follows:

primary endpoint:

The Live Birth Rate was significantly lower with biosimilar preparations versus the reference product (RR 0.83, 95% CI 0.71, 0.97; 4 RCTs, n = 1881, I^2 = 0%, moderate quality evidence) (Figure 1).

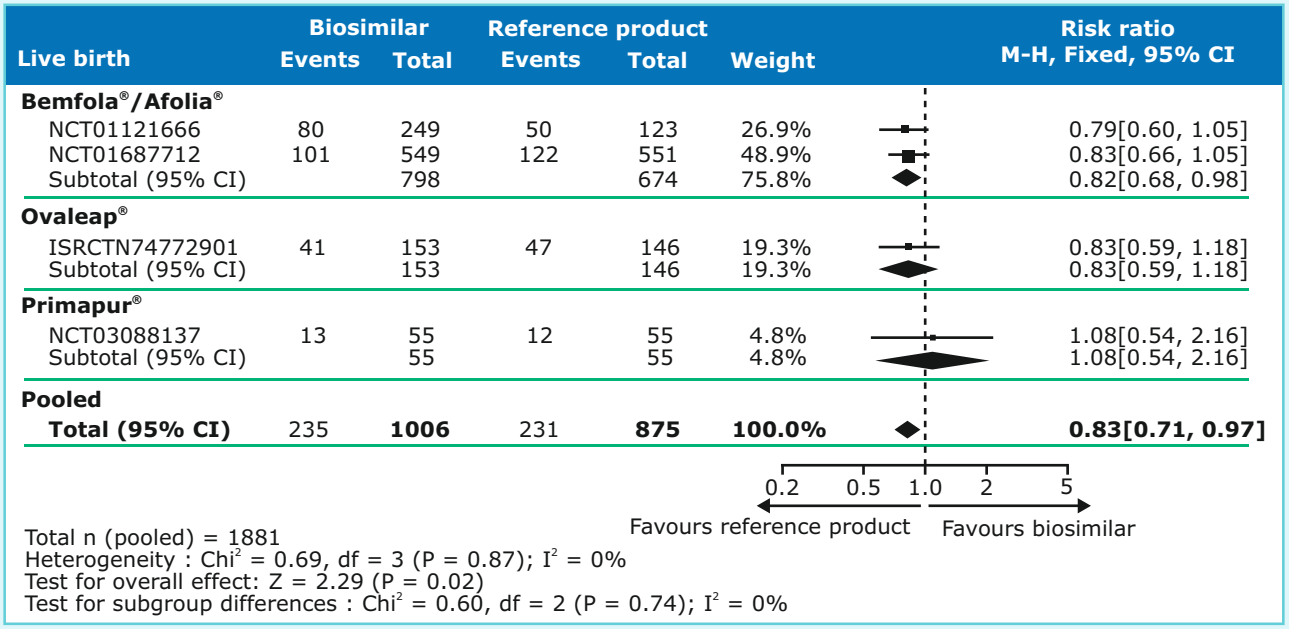


Figure adapted from Reprod Biol Endocrinol. 2021;19(1):51.

Figure 1. Relative risk for live birth rate with biosimilar preparations of follitropin alfa versus the reference product

The sensitivity analysis, which excluded the RCT with an unclear method of randomization, did not alter the effect size, however, it increased the uncertainty around this estimate resulting in a non-statistically significant finding (RR 0.83, 95% CI 0.68, 1.03; 3 RCTs, n = 781, I^2 = 0%, moderate quality evidence).

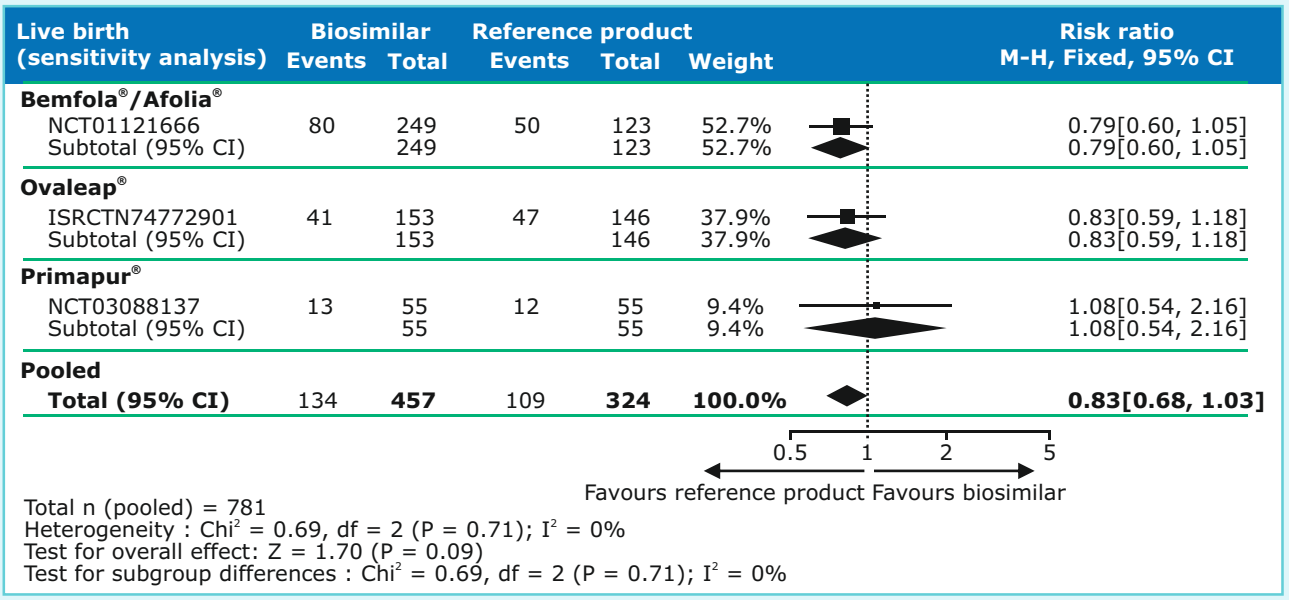
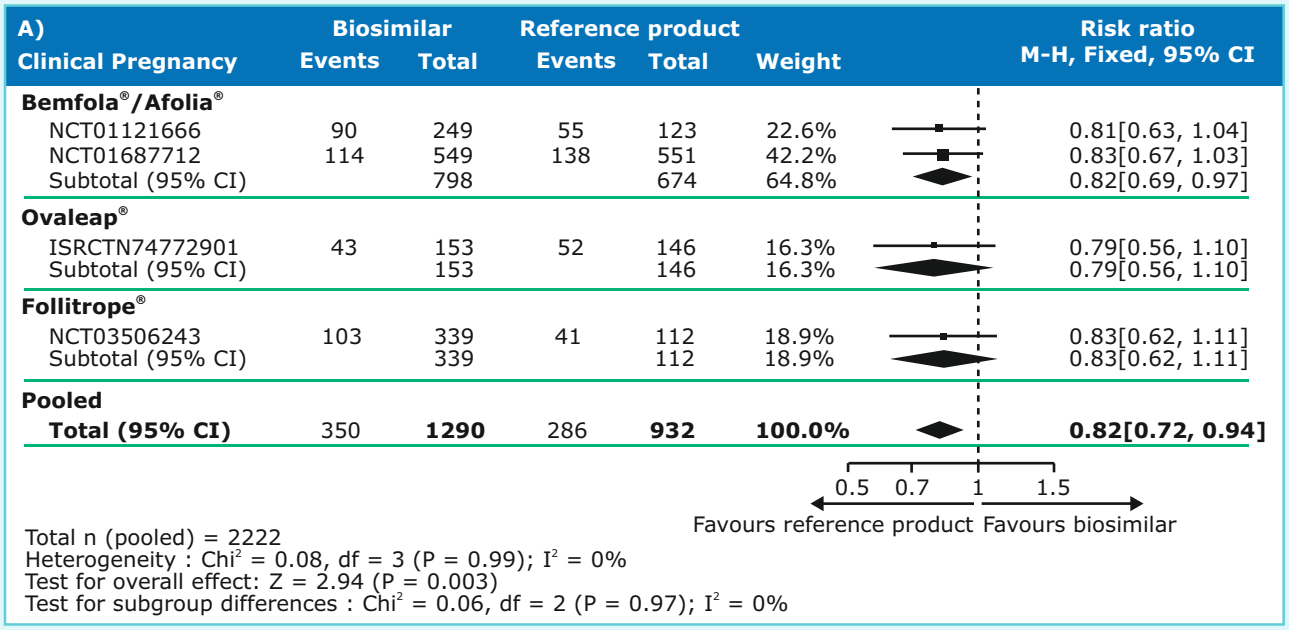


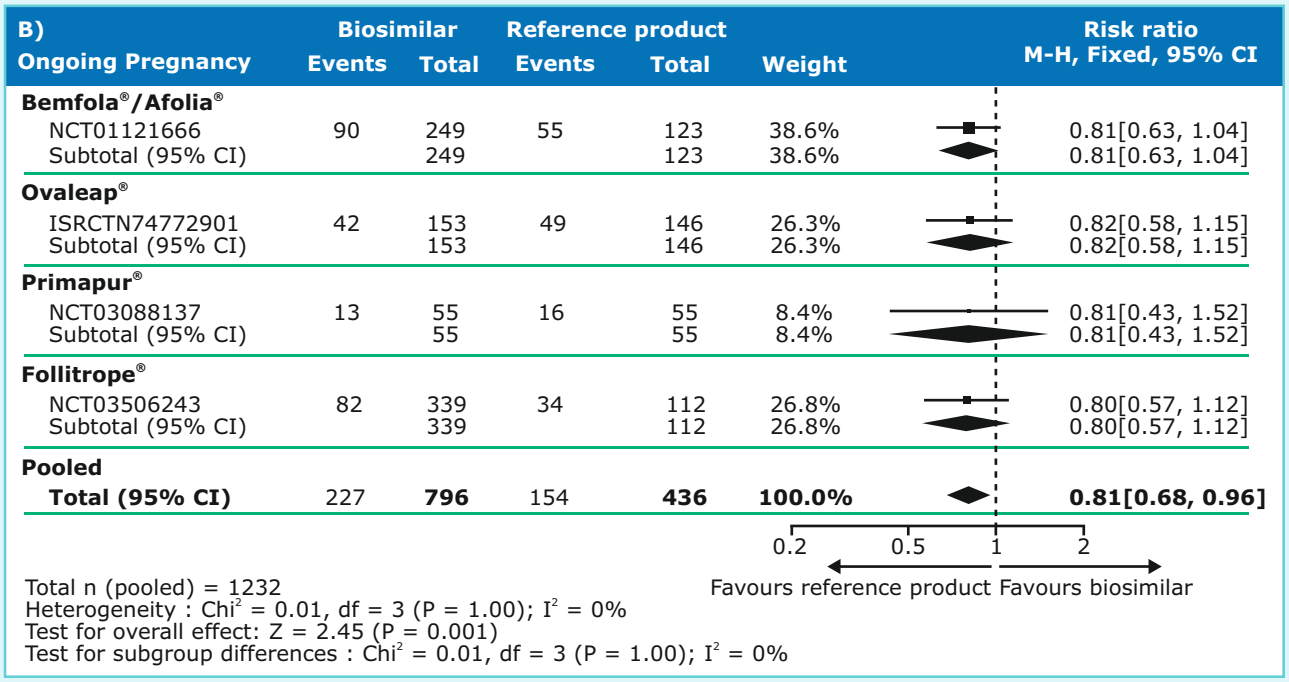
Figure adapted from Reprod Biol Endocrinol. 2021;19(1):51.

Figure 2. Relative risk for live birth rate with biosimilar preparations of follitropin alfa versus reference product (sensitivity analysis excluding the study with an unclear method of randomization)

secondary endpoints:

There was a significantly lower clinical pregnancy rate and ongoing pregnancy rate observed with combined data of biosimilar follitropin alfa preparations vs. the reference product (Figures 3 A and B).





Figures adapted from Reprod Biol Endocrinol. 2021;19(1):51.

Figure 3. Relative risk for clinical pregnancy rate (A), ongoing pregnancy rate (B) with biosimilar preparations of follitropin alfa versus the reference product

However, there was an inconclusive result for the OHSS rate (Figure 4).

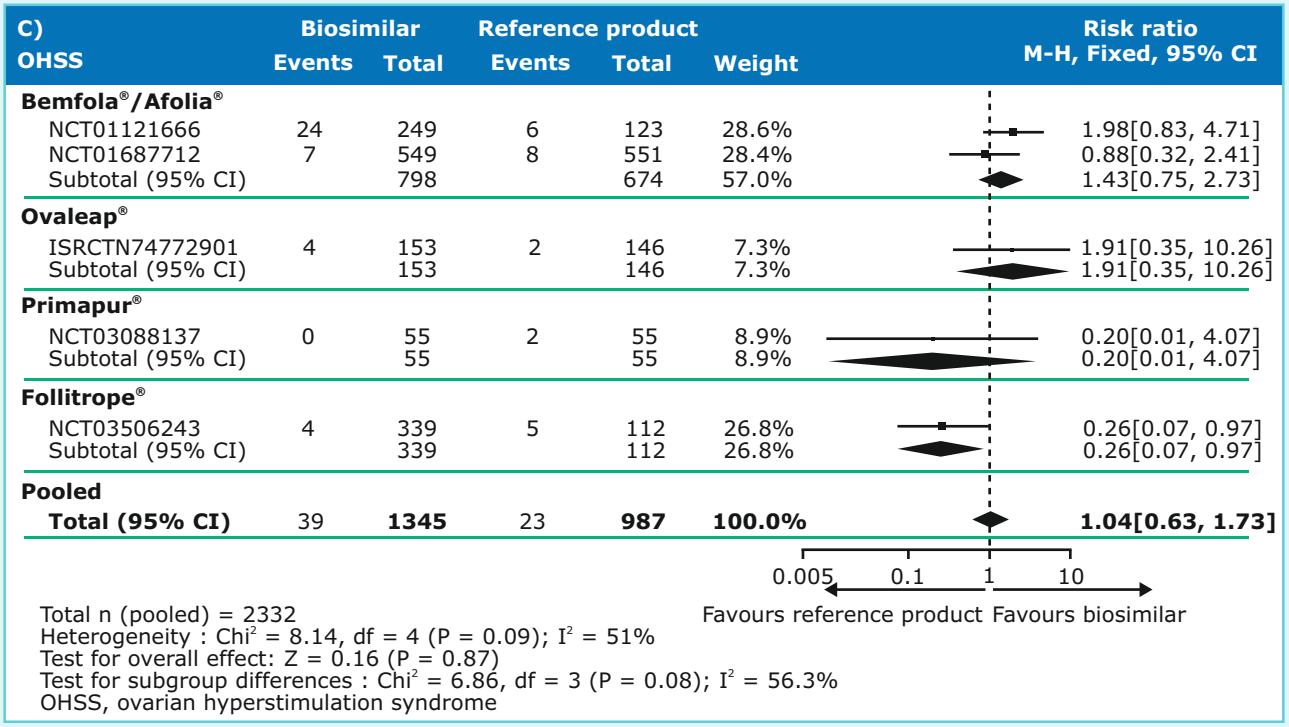
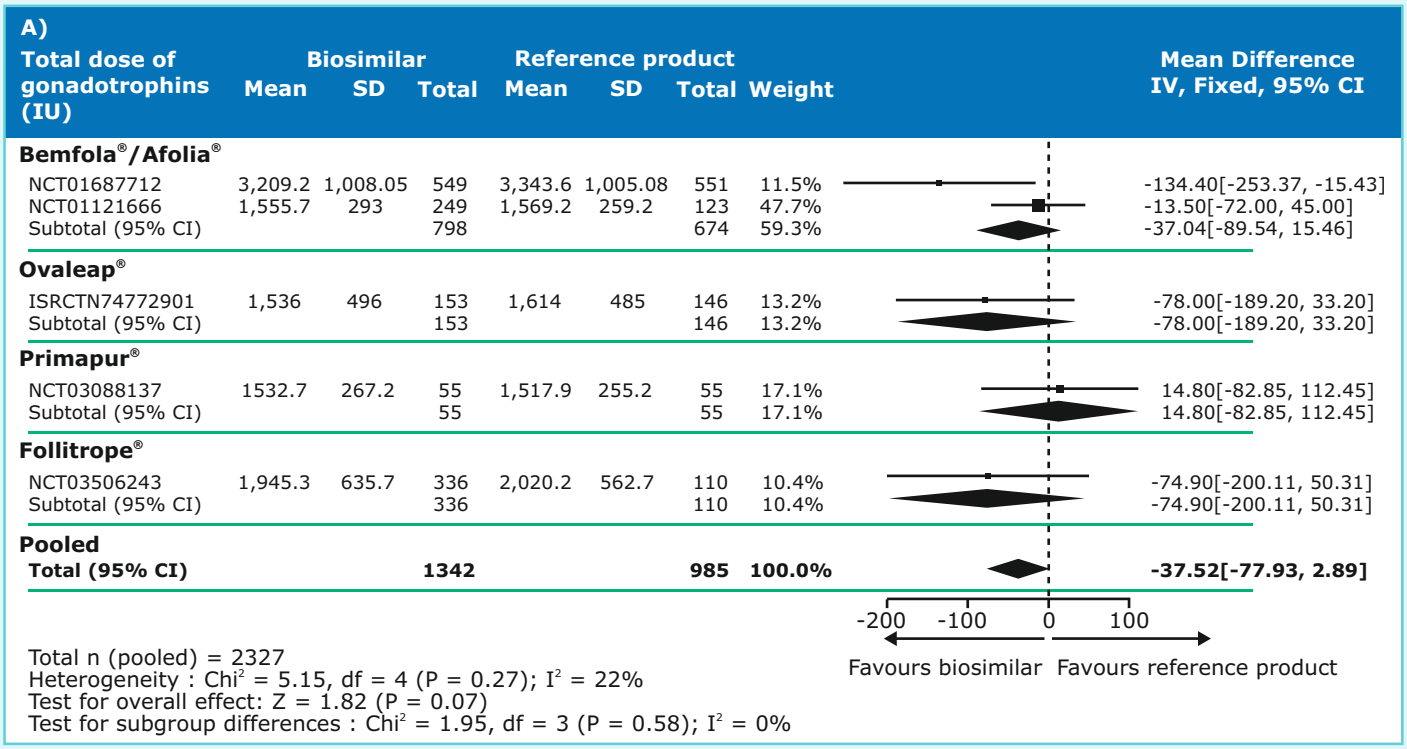


Figure adapted from Reprod Biol Endocrinol. 2021;19(1):51.

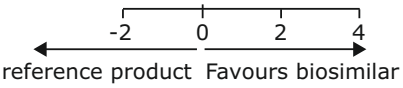
Figure 4. Relative risk for ovarian hyperstimulation syndrome with biosimilar preparations of follitropin alfa versus the reference product

There was insufficient evidence for a difference in the total dose of gonadotrophins. However, usage of biosimilar preparations resulted in retrieval of significantly higher number of oocytes and a significantly shorter duration of ovarian stimulation compared to the reference product (Figure 5).



B)

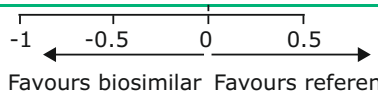
Number of oocytes retrieved	Biosimilar Mean	SD	Total	Reference product Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Bemfola®/Afolia®								
NCT01121666	10.7	5.62	249	10.4	6.14	123	1.7%	0.30[-0.99, 1.59]
NCT01687712	11.3	6.76	513	11.2	6.63	517	4.3%	0.10[-0.72, 0.92]
Subtotal (95% CI)			762			640	6.0%	0.16[-0.53, 0.85]
Ovaleap®								
ISRCTN74772901	12.2	6.8	153	11.9	6.9	146	1.2%	0.30[-1.25, 1.85]
Subtotal (95% CI)			153			146	1.2%	0.30[-1.25, 1.85]
Primapur®								
NCT03088137	12.16	7.28	55	11.62	6.29	55	0.4%	0.54[-2.00, 3.08]
Subtotal (95% CI)			55			55	0.4%	0.54[-2.00, 3.08]
Follitrope®								
NCT03506243	14.9	0.5	336	12.8	0.9	110	92.3%	2.10[1.92, 2.28]
Subtotal (95% CI)			336			110	92.3%	2.10[1.92, 2.28]
Pooled								
Total (95% CI)			**1306**			**951**	**100.0%**	**1.95[1.78, 2.12]**



Total n (pooled) = 2257
Heterogeneity : Chi^2 = 34.23, df = 4 (P < 0.00001); I^2 = 88%
Test for overall effect: Z = 22.59 (P < 0.00001)
Test for subgroup differences : Chi^2 = 34.16, df = 3 (P < 0.00001); I^2 = 91.2%

C)

Duration of ovarian stimulation (days)	Biosimilar Mean	SD	Total	Reference product Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Bemfola®/Afolia®								
NCT01121666	10.6	1.91	249	10.7	1.72	123	12.2%	-0.10[-0.49, 0.29]
NCT01687712	10.8	1.72	549	11.0	1.67	551	45.3%	-0.20[-0.40, 0.00]
Subtotal (95% CI)			798			674	57.5%	-0.18[-0.36, -0.00]
Ovaleap®								
ISRCTN74772901	9.3	1.8	153	9.7	1.6	146	12.2%	-0.40[-0.79, -0.01]
Subtotal (95% CI)			153			146	12.2%	-0.40[-0.79, -0.01]
Primapur®								
NCT03088137	9.75	1.08	55	9.73	1.03	55	11.7%	0.02[-0.37, 0.41]
Subtotal (95% CI)			55			55	11.7%	0.02[-0.37, 0.41]
Follitrope®								
NCT03506243	10.7	1.6	336	11.1	1.4	110	18.6%	-0.40[-0.71, -0.09]
Subtotal (95% CI)			336			110	18.6%	-0.40[-0.71, -0.09]
Pooled								
Total (95% CI)			**1342**			**985**	**100.0%**	**-0.22[-0.36, -0.09]**



Total n (pooled) = 2327
Heterogeneity : Chi^2 = 3.94, df = 4 (P = 0.41); I^2 = 0%
Test for overall effect: Z = 3.25 (P = 0.001)
Test for subgroup differences : Chi^2 = 3.74, df = 3 (P = 0.29); I^2 = 19.7%

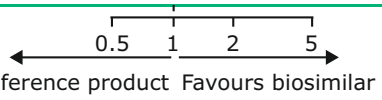
Figures adapted from Reprod Biol Endocrinol. 2021;19(1):51.

Figure 5. Mean difference in total dose of gonadotrophins (A), number of oocytes retrieved (B) and duration of ovarian stimulation (C) with biosimilar preparations of follitropin alfa versus the reference product

Cumulative data analysis showed a lower cumulative live birth rate and clinical pregnancy rate with biosimilar follitropin alfa preparations versus the reference product, while there was insufficient evidence for a difference in cumulative ongoing pregnancy rate (Figure 6).
There was inconclusive evidence on ectopic pregnancy rate (RR 1.16, 95% CI 0.39, 3.43; 3 RCTs, n = 1509, I^2 = 0%, moderate quality evidence) and multiple pregnancy rate (RR 1.34, 95% CI 0.61, 2.94; 2 RCTs, n = 409, I^2 = 0%, moderate quality evidence).
Estimation of miscarriage rate was difficult as data of pregnancy up to 22 weeks was not reported in all of the studies.

A)

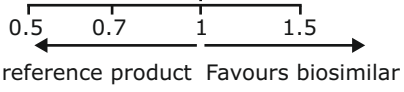
Cumulative live birth	Biosimilar Events	Biosimilar Total	Reference product Events	Reference product Total	Weight	Risk ratio M-H, Fixed, 95% CI
Bemfola®/Afolia®						
NCT01121666	102	249	59	123	27.6%	0.85[0.67, 1.08]
NCT01687712	121	549	147	551	51.3%	0.83[0.67, 1.02]
Subtotal (95% CI)		798		674	79.0%	0.84[0.71, 0.98]
Ovaleap®						
ISRCTN74772901	41	153	47	146	16.8%	0.83[0.59, 1.18]
Subtotal (95% CI)		153		146	16.8%	0.83[0.59, 1.18]
Primapur®						
NCT03088137	13	55	12	55	4.2%	1.08[0.54, 2.16]
Subtotal (95% CI)		55		55	4.2%	1.08[0.54, 2.16]
Pooled						
Total (95% CI)	277	**1006**	265	**875**	**100.0%**	**0.85[0.73, 0.97]**



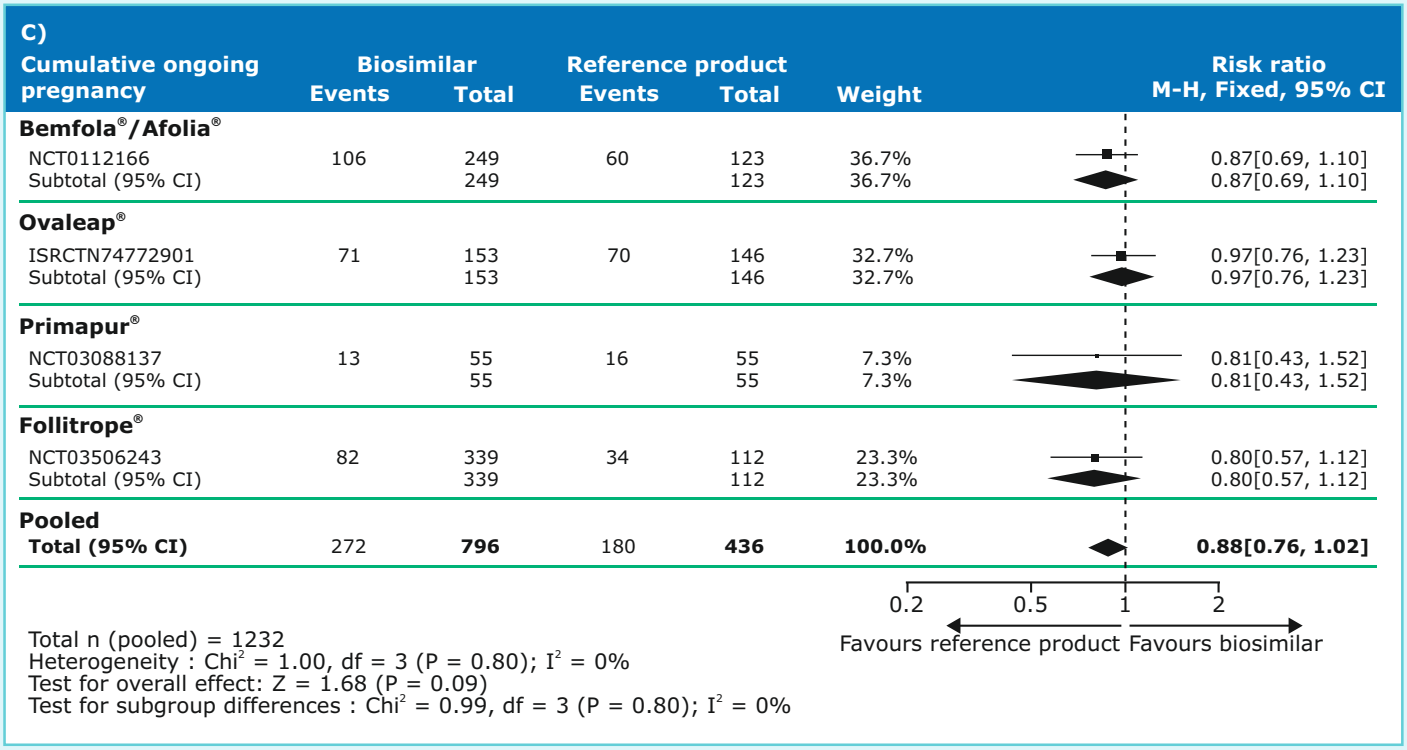
Total n (pooled) = 1881
Heterogeneity : Chi² = 0.56, df = 3 (P = 0.91); I^2 = 0%
Test for overall effect: Z = 2.31 (P = 0.02)
Test for subgroup differences : Chi² = 0.52, df = 2 (P = 0.77); I^2 = 0%

B)

Cumulative Clinical pregnancy	Biosimilar Events	Biosimilar Total	Reference product Events	Reference product Total	Weight	Risk ratio M-H, Fixed, 95% CI
Bemfola®/Afolia®						
NCT01121666	115	249	65	123	23.8%	0.87[0.71, 1.08]
NCT01687712	136	549	164	551	44.8%	0.83[0.69, 1.01]
Subtotal (95% CI)		798		674	68.6%	0.85[0.73, 0.98]
Ovaleap®						
ISRCTN74772901	43	153	52	146	14.6%	0.79[0.56, 1.10]
Subtotal (95% CI)		153		146	14.6%	0.79[0.56, 1.10]
Follitrope®						
NCT03506243	103	339	41	112	16.9%	0.83[0.62, 1.11]
Subtotal (95% CI)		339		112	16.9%	0.83[0.62, 1.11]
Pooled						
Total (95% CI)	397	**1290**	322	**932**	**100.0%**	**0.84[0.74, 0.94]**



Total n (pooled) = 2222
Heterogeneity : Chi² = 0.29, df = 3 (P = 0.96); I^2 = 0%
Test for overall effect: Z = 2.89 (P = 0.004)
Test for subgroup differences : Chi² = 0.15, df = 2 (P = 0.93); I^2 = 0%



Figures adapted from Reprod Biol Endocrinol. 2021;19(1):51.

Figure 6. Relative risk for cumulative live birth rate* (A), cumulative clinical pregnancy rate (B) and cumulative ongoing pregnancy rate (C) with biosimilar preparations of follitropin alfa versus the reference product

*For the cumulative live birth, only data from the first cycle could be used for the RCT investigating Ovaleap® as all participants crossed over to the exclusive use of Ovaleap® in subsequent cycles

The results of this meta-analysis suggests that treatment with biosimilar preparations of follitropin alfa was likely to result in lower probability of live birth, clinical and ongoing pregnancy compared with the reference product.

Biosimilar preparations carried a similar risk of OHSS, ectopic pregnancy and multiple pregnancy compared with the reference product when safety of the preparations were concerned.

The researchers opined that more head-to-head RCTs as well as real-world studies were required to ascertain clinically relevant fertility outcomes, including cumulative pregnancy and live birth rates.

Real-world evidence of clinical outcomes in patients receiving biosimilar follitropin alfa compared to originator follitropin alfa

1.Patel N, Bhadarka H, Patel N et al. Clinical outcomes in patients receiving follitropin alfa biosimilar to originator follitropin alfa (Gonal-f®) in real-world clinical practice. Data on File.

A retrospective study conducted by Patel et al., in a tertiary assisted conception unit compared the pregnancy outcome, and live birth rate (LBR) in patients undergoing ART using originator follitropin alfa or the biosimilar.

The study population included 174 and 190 women (aged between 30-32.5 years) who were administered originator follitropin alfa and biosimilar, respectively.

The main outcome measures were clinical pregnancy rate, miscarriage rate, healthy baby (term live birth with appropriate weight and no congenital anomaly) and preterm birth (< 37 weeks). AMH (anti Mullerian hormone - 4.20 ng/ml vs. 5.15 ng/ml), LH levels (4.57 IU/L vs. 5.28 IU/L) and Gonadotropin dose (2870.20 IU vs. 2606.95 IU) were as follows for originator follitropin alfa vs biosimilar respectively.

The results showed comparable number of oocytes retrieved from both groups (13.41 vs 14.59).

There was significant difference (originator follitropin alfa vs. biosimilar group) found in number of embryos (both cleavage and blastocyst stage) on day 6 (4.34 vs. 3.46; Figure 1); clinical pregnancy rate (55.88% vs. 40.43%; Figure 2); full term live birth rate (38.82% vs. 27.32%; figure 3) and higher percentage women having good quality embryos (both cleavage and blastocyst stage) in originator follitropin alfa group (83.33% vs. 69.47%, p=0.002; Figure 4).

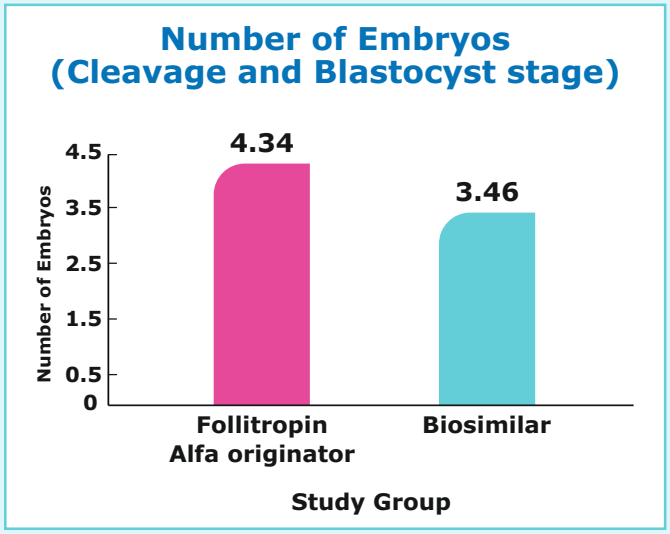


Figure adapted from Data on File.

Figure 1. Higher number of embryos in originator follitropin alfa vs biosimilar

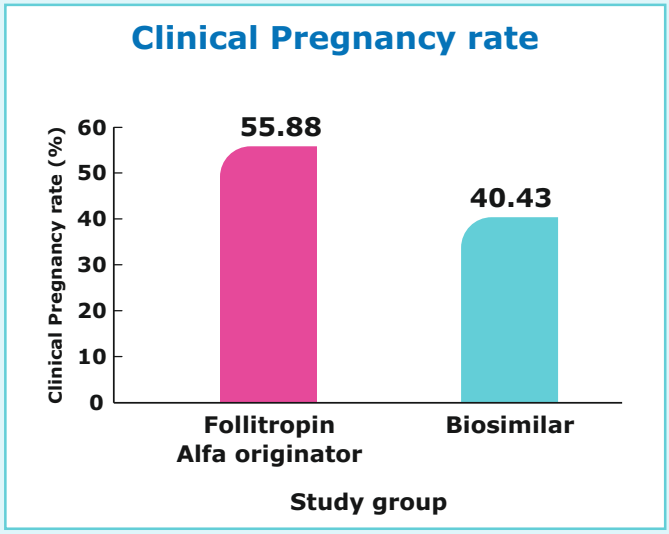


Figure adapted from Data on File.

Figure 2. Improved clinical pregnancy rate in originator follitropin alfa vs biosimilar

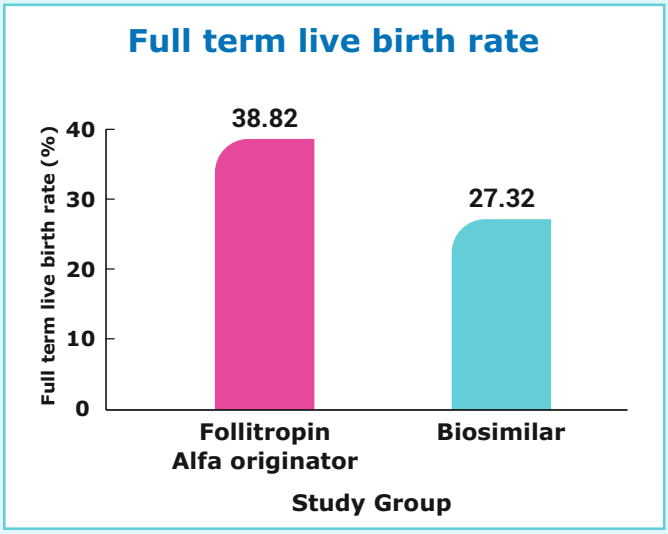


Figure adapted from Data on File.

Figure 3. Better full-term live birth rate in originator follitropin alfa vs biosimilar

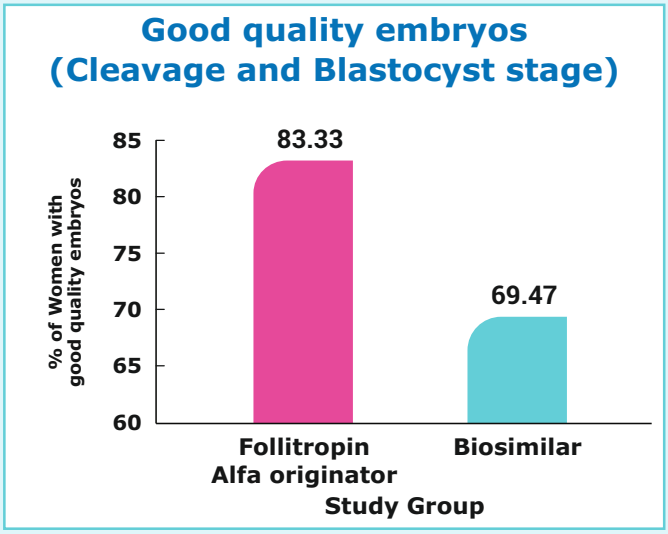


Figure adapted from Data on File.

Figure 4. Higher percentage women having good quality embryos (both cleavage and blastocyst stage) in originator follitropin alfa group vs biosimilar

Clinical miscarriage rate was comparable in both the groups (7.64% vs. 7.65%).
Subgroup analysis was done based on age: Group A (age<35 years) vs Group B (age>35 years). The results of Group A are shown in Table 1. No significant difference was found in group B.
The study results demonstrated the effectiveness (clinical pregnancy rate and live birth rate) of originator follitropin alfa treatment over its biosimilar.
Even after having the similar number of oocytes, pregnancy rate and the live birth rate was higher in the originator group. There was an increased benefit seen in the younger age group (<35 years) from originator treatment in this study.

Table 1. Level of significance in Group A

Group A	Follitropin Alfa originator (n=118)	Biosimilar (n=166)
No. of embryo (cleavage/blastocyst) on Day 6	4.38 + 2.60	3.16 + 2.84
Number of embryo (cleavage/blastocyst) transferred	2.28 + 1.11	2.01 + 0.58
Embryo (cleavage/ blastocyst) quality, number of patients	Good=97/118 (82.20%)	Good=114/166 (68.67%)
Clinical Pregnancy rate	Positive=65/115 (55.08%)	Positive=65/160 (40.62%)
Live birth rate	Full term=47/115 (40.86%)	Full term= 42/160 (26.25%)

Table adapted from Data on File.

Cost effectiveness of Follitropin Alfa originator vs. biosimilars

1. Schwarze JE, Venetis C, Iniesta S et al. Originator recombinant human follitropin alfa versus recombinant human follitropin alfa biosimilars in Spain: A cost-effectiveness analysis of assisted reproductive technology related to fresh embryo transfers. Best Pract Res Clin Obstet Gynaecol. 2022 Feb 8:S1521-6934(22)00020-7.
2. Xue W, Lloyd A, Falla E et al. A cost-effectiveness evaluation of the originator follitropin alpha compared to the biosimilars for assisted reproduction in Germany. Int J Womens Health. 2019;11:319-331.
3. Gizzo S, Garcia-Velasco JA, Heiman F et al. A cost-effectiveness evaluation comparing originator follitropin alfa to the biosimilar for the treatment of infertility. Int J Womens Health. 2016;8:683-689.

Several studies conducted across the world have proved the cost-effectiveness of Follitropin Alfa Originator vs. biosimilars for ovarian stimulation.

A Spanish study compared the cost per live birth and cost-effectiveness of the originator r-hFSH-alfa and r-hFSH-alfa biosimilars for ovarian stimulation prior to ART treatment.
The study was based on development of a decision tree model comprising pregnancy and live birth for one treatment cycle with fresh embryo transfer.
The study results showed that costs per live birth were lower with originator r-hFSH-alfa (€18,138) versus r-hFSH-alfa biosimilars (€20,377; Table 1).
However, the total cost (source costs multiplied by the probabilities in the decision tree) were higher for originator r-hFSH-alfa than for r-hFSH-alfa biosimilars, which was due to the higher proportion of pregnancies and live births with originator r-hFSH-alfa compared with r-hFSH-alfa biosimilars (Table 1).

Table 1. Cost outputs and costs per live birth

	Originator r-hFSH-alfa	r-hFSH-alfa biosimilars	Incremental
Live birth rate	26.4%	21.9%	4.5%
Total costs (source costs multiplied by decision tree probabilities)[a]	€4789	€4465	€323
Cost per live birth	€18,138	€20,377	

[a] Higher total costs for originator r-hFSH-alfa due to its higher rate of pregnancy and live birth and conclusively more costs for these.
r-hFSH-alfa, recombinant human follicle-stimulating hormone follitropin alfa.

Table adapted from Best Pract Res Clin Obstet Gynaecol. 2022 Feb 8:S1521-6934(22)00020-7.

The incremental cost-effectiveness ratio was €7208 for originator r-hFSH-alfa versus biosimilars.
This study results suggest that originator r-hFSH-alfa is associated with lower costs per live birth compared with r-hFSH-alfa biosimilars in the Spanish setting.

Xue et al., conducted a study in the German setting to evaluate the cost-effectiveness of the recombinant FSH originator per live birth in comparison to 2 biosimilars of follitropin alfa, (biosimilar 1) and (biosimilar 2). Here also a decision tree model was developed, based on one cycle of assisted reproduction, to compare the original product to biosimilars.
The study results demonstrated a higher live birth rate for the originator compared to biosimilar 1 (40.7% vs. 32.1% respectively), and biosimilar 2 (32.2% vs. 26.8%).
Treatment with originator resulted in lower cost per live birth compared to the 2 biosimilars and also pooled biosimilars; originator vs. biosimilar 1 (€10,510 vs €12,192), originator vs biosimilar 2 (€12,590 vs €13,606) and originator vs. pooled biosimilars (€ 11,676 vs. € 12,547; Figure 1).

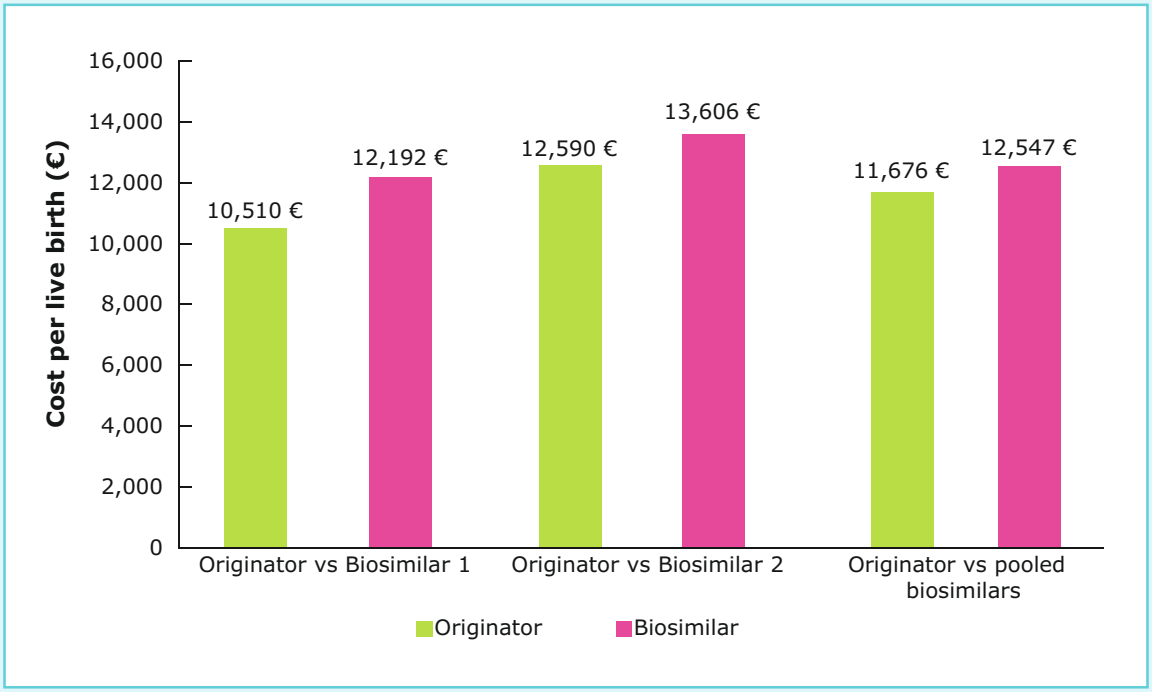


Figure adapted from Int J Womens Health. 2019;11:319-331.

Figure 1. Cost per live birth for Originator versus biosimilar 1, biosimilar 2 and pooled biosimilars

The analysis also found that the originator is associated with an incremental cost-effectiveness of €4,168 and €7,540 per additional live birth versus biosimilar 1 and biosimilar 2 respectively. This study results suggested that treatment with the originator could result in a lower cost per live birth in comparison to biosimilars.

Gizzo et al., conducted a study to evaluate the cost-effectiveness of the originator follitropin alfa to the biosimilar in the Italian and Spanish contexts, with an assessment of the German and UK backgrounds. A cost-effectiveness model was developed in the Italian and Spanish contexts. According to the study results, the cost of originator FSH was €3,663 and €6,387 in Italy and Spain, respectively, whereas biosimilar FSH costs were €3,483 and €6,342. The average cost per live birth was estimated to be €7,044 and €12,283 for the originator FSH and €7,411 and €13,494 for the biosimilar for Italy and Spain, respectively (Table 2).

Table 2. Results of Cost-effective analysis in Italian and Spanish contexts

Strategy	Cost (€)	Incremental cost (€)	Efficacy	Incremental efficacy	ICER[a] (€)	Cost per live birth (€)
Italy						
Biosimilar FSH	3,483	–	0.47	–	–	7,411
Originator FSH	3,663	180	0.52	0.05	3,600	7,044
Spain						
Biosimilar FSH	6,342		0.47			13,494
Originator FSH	6,387	45	0.52	0.05	900	12,283

Abbreviations: FSH, follicle-stimulating hormone; [a]ICER, incremental cost-effectiveness ratio.

Table adapted from Int J Womens Health. 2016;8:683-689.

The efficacy was found to be 0.52 for the originator and 0.47 for the biosimilar.
The originator FSH generated an incremental cost-effectiveness ratio of €3,600 for Italy and €900 for Spain compared to the biosimilar.
This study results indicated that the originator FSH was a cost-efficient treatment strategy for Italian and Spanish context compared to the biosimilars.

Time to asses FSH activity by Methods other than the Steelman-Pohley assay

1. *Orvieto R, Seifer DB. Biosimilar FSH preparations- are they identical twins or just siblings? [published correction appears in Reprod Biol Endocrinol. 2016;14(1):59]. Reprod Biol Endocrinol. 2016;14(1):32.*

Follitropin alpha is the FSH innovator product that all the recently biosimilar products are referred and compared to.
However, studies have shown that two r-hFSH preparations have apparently identical polypeptide chains but a somewhat different glycosylation pattern.
In such cases, it becomes necessary for the manufacturer of the biologic product to conduct a complex and comparability studies to demonstrate that the changes do not adversely affect the purity, potency or the identity of the product.
Researchers now believe that it is now necessary that the measurement of the biological activity of FSH in humans should require other methods than the Steelman-Pohley assay, such as the determination of dose-response curves for well characterized patient populations for well-defined outcomes during COH in preparation for ART.

Interchangeability of originator and Biosimilar Follitropin alfa preparations

1. *Orvieto R, Seifer DB. Biosimilar FSH preparations- are they identical twins or just siblings? [published correction appears in Reprod Biol Endocrinol. 2016;14(1):59]. Reprod Biol Endocrinol. 2016;14(1):32.*
2. *Schellekens H. The first biosimilar epoetin: but how similar is it? Clin J Am Soc Nephrol. 2008 Jan;3(1):174-178.*
3. *Declerck P, Danesi R, Petersel D et al. The Language of Biosimilars: Clarification, Definitions, and Regulatory Aspects. Drugs. 2017;77(6):671-677.*

Biosimilars are actually a regulatory synonym, facilitating a fast-track introduction of a FSH preparation to the COH armamentarium. The researchers recommend against interchanging or substituting innovator and biosimilar agents in clinical practice.
The decision whether to use originator Follitropin alfa or a biosimilar product, should be reserved to the discretion of the treating physician.
It is for this reason that the biosimilars undergo rigorous evaluation parameters defined by the regulatory authorities before approval. The approval of these biosimilar products does not substantiate interchangeability with reference products.

summary

Controlled ovarian stimulation (COS) with follicle stimulating hormone (FSH) injections is a pivotal step in the IVF and ICSI procedures. Recombinant Follitropin Alfa originator (originator r-hFSH-alfa) is a fourth-generation gonadotropin.

It has a well-established portfolio of published efficacy, safety and clinical real-world post-marketing evidence and experience. Treatment with originator r-hFSH-alfa has resulted in the birth of more than 4 million babies across the world.

The expiration of patent and exclusivity of originator biologics has opened up a new window of opportunity for the development and approval of biosimilars.

Biosimilars are biological products approved based on a showing that it is highly similar to an already approved reference biologic with no clinically meaningful differences from the reference biologic in terms of safety, purity, and potency of the product.

However, several factors like manufacturing process, molecular structure, content, biological activity and immunogenicity of biosimilars make them similar but not same/identical to originator biologics. Moreover, episodes of variations in activity and immunogenicity in biosimilars compared to their respective innovator products have made the world look biosimilars with caution.

Comparative analysis of *in vivo* bioactivity and site-specific glycosylation mapping of biosimilar follitropin alfa and originator follitropin alfa has demonstrated that the originator had lower antennarity, sialylation and batch-to-batch variability vs. the biosimilar.

Results of meta-analysis suggests that treatment with biosimilar follitropin alfa was likely to result in lower probability of live birth, clinical and ongoing pregnancy compared with the reference product.

Real world evidence study results have demonstrated the effectiveness (clinical pregnancy rate and live birth rate) of originator follitropin alfa treatment over its biosimilar with an increased benefit seen in the younger age group (< 35 years) from originator treatment.

Cost effectiveness studies of Follitropin Alfa originator vs. biosimilars across the world have shown that the Follitropin Alfa Originator offers lower costs per live birth compared to its biosimilars.

Overall, despite going off-patent and with the competition from the biosimilars in the market, the originator Follitropin Alfa is here to stay for long due to the better outcomes in the ART process.

Feedback form

ALIVE
ART LEARNING INITIATIVES FOR EXPERTS

Issue 9 | November 2022

Thank you for going through the contents of **ALIVE Newsletter Issue 9.** To ensure that future issues will be of interest to you, we would greatly appreciate your feedback on the format and content of this issue.

Name:_____

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Satisfaction Score for ALIVE Newsletter - Follitropin Alfa Originator vs. Biosimilars: Difference beyond RCTs : Issue 9; November 2022

Rating Scale	Poor ----------Excellent (Please circle the appropriate rating)									
Scientific content	1	2	3	4	5	6	7	8	9	10
Relevance of the topic	1	2	3	4	5	6	7	8	9	10
Impact on my daily practice	1	2	3	4	5	6	7	8	9	10
Innovation	1	2	3	4	5	6	7	8	9	10
Overall level of satisfaction	1	2	3	4	5	6	7	8	9	10

What aspects of the Newsletter issue 9 did you find particularly interesting and/or informative?

_____

Please suggest topics/areas that you would like to be covered in future issues of the Alive Newsletter?

_____

How can the subsequent Newsletter issues be improved?

_____

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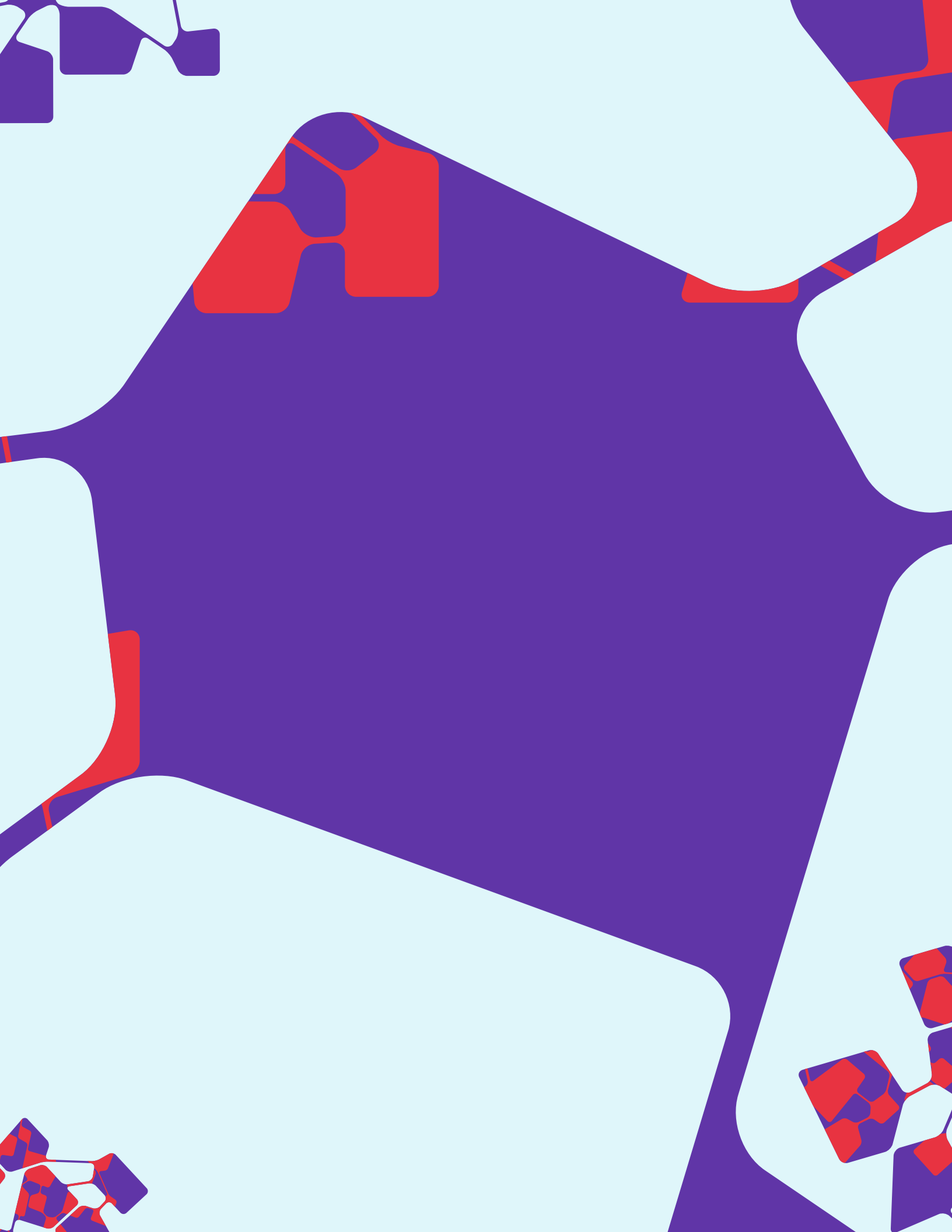

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