RBMO

ARTICLE

Effectiveness of recombinant human FSH: recombinant human LH combination treatment versus recombinant human FSH alone for assisted reproductive technology in women aged 35–40 years

BIOGRAPHY

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KEY MESSAGE

In this non-interventional study, women aged 35–40 years (advanced maternal age), with five to 14 oocytes retrieved (suggesting normal ovarian response) had higher clinical pregnancy and live birth rates after treatment with combined recombinant human FSH (r-hFSH) recombinant human LH than after treatment with r-hFSH alone.

ABSTRACT

Research question: According to real-world data, is recombinant human FSH (r-hFSH) combined with recombinant human LH (r-hLH) or r-hFSH alone more effective for women of advanced maternal age (AMA) in terms of live birth?

Design: Non-interventional study comparing the effectiveness of r-hFSH and recombinant r-hLH (2:1 ratio) versus r-hFSH alone for ovarian stimulation during ART treatment in women aged 35–40 years, using real-world data from the Deutsches IVF-Register.

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Declaration: APB has received honoraria for lectures from Merck Healthcare KGaA, Darmstadt, Germany, and affiliates; JES, DC, ML and TDH are employees of Merck Healthcare, Darmstadt, Germany; PV was an employee of Merck Healthcare KGaA, Darmstadt, Germany at the time of the study; RF has received honoraria for lectures and an advisory board on the role of LH from Merck Healthcare KGaA, Darmstadt, Germany, and affiliates; BH was an employee of EMD Serono, Rockland, MA, USA, an affiliate of Merck KGaA, at the time of the study; and JK has received honoraria for lectures from Merck Healthcare KGaA, Darmstadt, Germany, and affiliates.

KEYWORDS

advanced maternal age
clinical pregnancy
live birth
ovarian stimulation
real-word data
r-hFSH: r-hLH

Results: : Overall clinical pregnancy (29.8%, 95% CI 28.2 to 31.6 versus 27.8%, 95% CI 26.5 to 29.2) and live birth (20.3%, 95% CI 18.7 to 21.8 versus 18.0%, 95% CI 16.6 to 19.4) rates were not significantly different between the combined r-hFSH and r-hLH group and the r-hFSH alone group ($P = 0.269$ and $P = 0.092$, respectively). Treatment effect was significantly higher for combined r-hFSH and r-hLH compared with r-hFSH alone for clinical pregnancy (33.1%, 95% CI 31.0 to 35.0 versus 28.5%, 95% CI 26.6 to 30.4; $P = 0.001$, not adjusted for multiplicity) and live birth (22.5%, 95% CI 20.5 to 24.2 versus 19.4%, 95% CI 17.6 to 20.9; $P = 0.014$, not adjusted for multiplicity) in a post-hoc analysis of women with five to 14 oocytes retrieved (used as a surrogate for normal ovarian reserve), highlighting the potential benefits of combined r-hFSH and r-hLH for ovarian stimulation in women aged 35–40 years with normal ovarian reserve.

Conclusions: : Women of AMA with normal ovarian response benefit from treatment with combined r-hFSH and r-hLH in a 2:1 ratio versus r-hFSH alone in terms of live birth rate. The effectiveness of treatments is best assessed by RCTs; however, real-world data are valuable for examining the effectiveness of fertility treatment, especially among patient groups that are not well represented in clinical trials.

INTRODUCTION

Reduced follicle stimulating hormone (FSH) and luteinising hormone (LH) efficacy, caused by reduced circulating levels or a reduction in their bioactivity (severe LH and FSH deficiency), can be the cause of infertility (*Zegers-Hochschild et al., 2017*). As such, the importance of LH and FSH deficiency has been highlighted by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) in their definition of hypogonadotropic hypogonadism: which gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production caused by reduced gonadotrophin production or action (*Zegers-Hochschild et al., 2017*; *Bosch et al., 2021*).

The causes of LH and FSH deficiency are numerous, including congenital or acquired conditions, and can be functional or organic in nature. Congenital deficiency can be caused by chromosomal abnormalities and single gene mutations, (e.g. Kallmann syndrome (*Bosch et al., 2021*)), whereas acquired deficiencies can be precipitated by lifestyle factors (e.g. weight loss and stress (*Laughlin et al., 1998*)), and chronic conditions, (e.g. brain tumours, brain damage, and end-stage renal disease (*Hayes et al. 2000*)), therapeutics, (e.g. contraceptives) or both (*Hayes et al., 2000*). In addition, women of advanced maternal age (AMA), defined as women aged over 35 years, may have a 'hidden' functional FSH and LH deficiency that may impair the success of IVF, and, ultimately, the chance of live birth (*Ata et al., 2012*; *Behre et al., 2015*; *Bosch et al., 2021*).

Fecundity begins to decrease in women in their early thirties, with a steep decline occurring after the age of 35 years (*Steiner and Jukic, 2016*). This can be attributed to a decrease in oocyte quality in terms of euploid rate (*Ata et al., 2012*) and a decrease in ovarian reserve, as demonstrated by reduced antral follicle count (AFC) and anti-Müllerian hormone (AMH) levels (*Broekmans et al., 2009*). Increasing age is also associated with a progressive increase in FSH levels (*McTavish et al., 2007*), as well as decreased bioactivity of FSH and LH. In the case of FSH, the decrease in bioactivity may be caused by a decline in the presence of partially glycosylated (hypoglycosylated) FSH variants, which have greater affinity for the FSH receptor than fully glycosylated FSH glycoforms (*Casarini et al., 2016*; *Bousfield et al., 2018*). Decreased LH bioactivity impairs androgen production, leading to the distinct decline of circulating androgens that is observed in older women compared with younger women of reproductive age (*Bosch et al., 2021*). The use of gonadotrophin-releasing hormone (GnRH) analogue regimens (agonists and antagonists) for ovarian stimulation during assisted reproductive technology (ART) can result in transient dose-dependent LH suppression (*Bosch et al., 2011*; *Bosch et al., 2021*); however, the severity of LH and FSH deficiency caused by GnRH analogue treatment is not related to the absolute LH serum levels, but rather to the magnitude of suppression over time versus baseline (*Kol and Homburg, 2008*; *Di Segni et al., 2022*; *Zhang et al., 2022*). GnRH analogue downregulation protocols may also exacerbate age-related decreases in LH and FSH activity (reduced gametogenesis and reduced gonadal steroid production caused by reduced gonadotrophin production or action) (*Marrama et al., 1984*; *Mitchell et al., 1995*; *Vihko et al., 1996*; *Hurwitz and Santoro, 2004*; *Davison et al., 2005*; *Zegers-Hochschild et al., 2017*). Although treatment with exogenous FSH is typically sufficient for ovarian stimulation in normo-responder women (those with between five and 15 oocytes retrieved) (*La Marca and Sunkara, 2014*), combined treatment with recombinant human FSH (r-hFSH) and recombinant human LH (r-hLH) has proven more beneficial for women with FSH and LH deficiency (*Carone et al., 2012*; *Conforti et al., 2019*; *Bosch et al., 2021*; *Conforti et al., 2021*; *Casarini et al., 2022*).

As the average maternal age at first birth is rising globally, the proportion of women of AMA undergoing IVF treatment is increasing; therefore, the need for effective treatment options, such as combined r-hFSH and r-hLH, is particularly relevant for this population (*Tan et al., 2014*; *Beaujouan and Toulemon, 2021*; *Conforti et al., 2021*). As a result, more women of AMA are seeking treatment for infertility. Data from the European Society of Human Reproduction and Embryology (ESHRE) Registry (2002–2014) indicate that the percentage of fertility treatments, such as IVF and intracytoplasmic sperm injection (ICSI), has increased from 48.4% to 56.7% for women aged 35 years or older, and from 13.2 to 19.7% for women aged 40 years and older (*Andersen et al., 2006b*; *De Geyter et al., 2018*).

The benefit of combined treatment with r-hFSH and r-hLH in women of AMA is supported by a recent meta-analysis (*Conforti et al., 2021*), which reported a higher implantation rate (OR 1.49, 95% CI 1.10 to 2.01, $I^2 = 13\%$, $P = 0.01$) and clinical pregnancy rate (OR 1.45, 95% CI 1.05 to 2.00, $I^2 = 0\%$, $P = 0.03$) in women treated with combinebd r-hFSH and r-hLH versus r-hFSH alone. This suggests that combined treatment may improve these outcomes for women aged 35–40 years undergoing ovarian stimulation for ART; however, no difference in live birth rate was found between the two treatment groups (*Conforti et al., 2021*). Nevertheless, as this

meta-analysis was based on 12 randomized controlled trials (RCTs) rather than data captured from routine clinical practice, further evidence is needed to inform effective and safe treatments for women of AMA, a population that is often excluded from highly selective RCTs.

Most RCTs are powered to compare the number of oocytes or embryo blastulation rates but are commonly underpowered to detect clinically meaningful differences among treatment arms for key clinical outcomes, such as ongoing pregnancy and live birth, which are usually only reported as secondary outcomes (*Esteves et al., 2021*). Although live birth is the preferred outcome, clinical and ongoing pregnancy are also considered in line with clinical practice, and are often used as surrogate outcomes for ART, with treatment effect-based conclusions potentially comparable with live birth as long as miscarriage rates are considered, to account for the difference between clinical and ongoing pregnancy and live birth rate (*Clarke et al., 2010*; *Mol et al., 2018*). Accordingly, non-interventional studies can complement RCTs with evidence from routine clinical practice to provide comprehensive data on clinically relevant outcomes from a routine care perspective (*Blonde et al., 2018*; *Mulder et al., 2018*), with the advantage of more diverse populations and larger samples sizes compared with RCTs (*Kim et al., 2018*; *Sunkara et al., 2020*). The validity of the evidence derived from the analysis of real-world data is dependent on the availability and access to large, good-quality, real-world data sources, e.g. clinical and national registries. For this study, data were obtained from the Deutsches IVF-Register, which has been publishing an annual report on the use of ART in Germany since 1982 (*Kadi and Wiesing, 2016*). Importantly, the Deutsches IVF-Register receives prospective data from almost all IVF centres in Germany and allows for the comparison of different treatment strategies across diverse patient groups, which is essential to fully understand the factors influencing treatment-specific clinical outcomes (*Bühler, 2013*; *Hershkop et al., 2017*).

In line with this, the aim of this present study was to compare the effectiveness of combined r-hFSH and r-hLH combination in a 2:1 ratio, hereafter referred to as r-hFSH and r-hLH, versus r-hFSH alone in women aged 35–40 years undergoing their first cycle of ovarian stimulation for IVF or ICSI within the study period, in terms of clinical pregnancy and live birth, using data from the Deutsches IVF-Register.

MATERIALS AND METHODS

Study design

This was a non-interventional study based on secondary use of data obtained from the Deutsches IVF-Register from women undergoing ovarian stimulation for IVF/ICSI cycles initiated from 1 October 2017 to 31 December 2019, with follow-up to capture outcomes up to 31 December 2020. The follow-up period was defined as the time since the date of the first exposure to combined r-hFSH and r-hLH in a 2:1 ratio or r-hFSH alone, until whichever came first from: live birth, end of the study period or the woman was lost to follow-up.

Ethical approval

Approval of this protocol research by an independent ethics committee or an institutional review board was not required as the study did not extract data directly from clinics, but rather from an existing registry that has its own constituted governance board. As such, Ethics Committee approval was not needed. All data used in the study were de-identified.

Data source

The Deutsches IVF-Register provides information on IVF and related methods and their success rates across all regions in Germany, which are published in annual reports. Data were collected from 134 centres in 2017 and 131 IVF centres in the years 2018 and 2019, representing close to 100% of all fertility centres in Germany (*Blumenauer et al., 2018*; *Blumenauer et al., 2019*; *Blumenauer et al., 2020*). Data quality in the Deutsches IVF-Register is ensured via a three-step procedure: data management by the registry principal investigator; approval by the three-member Deutsches IVF-Register Board; and approval by the seven-member Board of Trustees.

Study population

Data were collected from women who received treatment with either combined r-hFSH and r-hLH or r-hFSH monotherapy for ovarian stimulation during their first stimulation cycle of ART within the study inclusion period. None of the patients underwent preimplantation genetic testing for aneuploidy (PGT-A) as this is not permitted in Germany.

Inclusion criteria

Women identified according to the following inclusion criteria were included: aged 35–40 years at the start of the first ovarian stimulation cycle, for whom the first stimulation cycle of ART treatment (IVF, ICSI, or both) was started between 1 October 2017 and 31 December 2019; ovarian stimulation was carried out with combined r-hFSH and r-hLH or with r-hFSH alone; and where a GnRH long agonist or GnRH antagonist downregulation protocol was used to prevent premature ovulation during ovarian stimulaton.

Exclusion criteria

Women were not eligible for this study if they fulfilled any of the following exclusion criteria: history of recurrent miscarriage, defined as three or more miscarriages; use of non-ejaculated spermatozoa for oocyte insemination; women whose first treatment recorded during the study period was a frozen embryo transfer, as this implies a previous stimulation cycle; women with ovarian stimulatoin followed by total oocyte/embryo freezing and without frozen embryo transfer within 6 months of oocyte retrieval; and current untreated hypothyroidism.

Outcomes

The outcomes for the first cycle of stimulation were clinical pregnancy (defined as the presence of an embryo with cardiac activity between 6–12 weeks of gestation); live birth (defined as the complete expulsion or extraction from a woman of a product of fertilization, after 22 completed weeks of gestational age, which, after such separation, breathes or shows any other evidence of life, such as heartbeat, umbilical cord pulsation or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is still attached or not); oocytes retrieved (defined as the number of oocytes retrieved from an aspiration after ovarian stimulation); and miscarriage (defined as the spontaneous loss of a clinical pregnancy before 24 completed weeks of gestational age, in which embryos or fetuses are non-viable and are not spontaneously absorbed or expelled from the uterus).

Exploratory safety analysis

Ovarian hyperstimulation syndrome (OHSS) defined as a diagnosis of OHSS (WHO Grade II, Grade III or hospitalization) during a stimulation cycle,

and multiple pregnancy (pregnancy with two or more embryos) were investigated as exploratory outcomes.

Statistical analysis

Study size

The estimated sample size needed to meet the study objectives was based on the assumption that clinical pregnancy rate for the first cycle of ovarian stimulation would be 5% higher in women treated with combined r-hFSH and r-hLH compared with r-hFSH alone, based on data from a meta-analysis reporting this difference in women with a normal ovarian reserve aged 35–40 years (*Conforti et al., 2021*). As such, assuming an expected clinical pregnancy rate per ovarian stimulation cycle of 25% with r-hFSH and 30% with combined r-hFSH and r-hLH, an estimated 1713 women per treatment group were needed to demonstrate an absolute increase in clinical pregnancy rate of 5% or greater with 90% power and a 5% two-sided type-1 error rate.

Outcomes

All outcomes were first reported descriptively. Categorical variables were reported as number and proportion, and continuous variables were reported as mean, SD, range, median and lower and upper quartiles. Clinical pregnancy and live birth rates (from the first stimulation and at most one fresh embryo transfer), as well as cumulative clinical pregnancy and cumulative live birth rates (first stimulation and all transfers of fresh and frozen–thawed embryos) were reported as the number of outcomes per 100 initiated cycles. Results were analysed according to treatment cohort and differences were expressed as absolute differences and also as relative risks.

Propensity score matching

Because of the non-interventional nature of this study, propensity score matching (1:1) was applied to minimize the effect of potential confounding factors related to exposure and outcomes of interest. Propensity scores were generated using a logistic regression model to estimate the predicted probability of receiving combinbed r-hFSH and r-hLH or r-hFSH alone, as a function of the variables recorded by Deutsches IVF-Register centres. For this study, the covariates included in the statistical models were chosen by the clinician co-authors, based on variables that have been reported/validated to predict either live birth or cumulative live birth after ovarian stimulation for ART treatment (*Leijdekkers et al., 2018*; *Ratna et al., 2020*; *McLernon et al., 2022*) and on data that were routinely available in the Deutsches IVF-Register database.

These covariates were as follows: age, body mass index (BMI), previous pregnancies (primary versus secondary infertility), infertility diagnosis, sterility indicators for the woman, her partner, or both, other conditions at baseline (obesity, hypertension, disease of the inner genitals, nicotine use or thyroid conditions), downregulation protocol (GnRH long agonist versus antagonist), planned fertilization type (IVF with or without ICSI) and year of first stimulation cycle. A list of all covariates is presented in Supplementary Table 1, and more background on the selection of covariates is presented in the Discussion section.

For matching, a greedy nearest neighbour algorithm was applied to the propensity scores. Standardized mean differences (SMD) were calculated to assess the balance of confounders between the treatment groups before and after matching; a threshold SMD within −0.1 and 0.1 indicated well-balanced treatment groups. The propensity score model and matching were finalized before running the analysis on the outcomes.

Outcomes analysis methods

Live birth rate and clinical pregnancy rate were analysed using logistic regression, with adjustment on age, total dose of r-hFSH and post-gonadotrophin treatment covariates that could influence the outcomes of interest (GnRH agonist trigger [yes/no], progesterone luteal phase support [yes/no], embryo transfer on day 5 or later (yes/no), and number of embryos transferred). The number of oocytes retrieved was assessed using a linear regression model with adjustment on age and total dose of r-hFSH. *P*-values were adjusted using the Benjamini and Hochberg method to account for multiple comparisons and control for false positive rates (*Benjamini and Hochberg, 1995*). Missing data were not imputed.

Post-hoc analysis

As the initial results highlighted a difference in the number of oocytes retrieved between the two cohorts, and considering the lack of registered ovarian reserve biomarkers in the Deutsches IVF-Register, a stratified post-hoc analysis was conducted. Because of the lack of ovarian reserve data in the Deutsches IVF-Register, this analysis defined strata according to ovarian response (number of oocytes retrieved) as a surrogate of ovarian reserve (four or fewer oocytes retrieved, five to 14 oocytes retrieved and 15 oocytes or more retrieved) based on the rationale provided by *Polyzos and Sunkara (2015)*. The surrogate range for normal ovarian response, five to 14 oocytes retrieved, was chosen as the range that lies between the definition of poor ovarian response defined by ICMART (*Zegers-Hochschild et al., 2017*), the Bologna criteria (*Ferraretti et al., 2011*) and the POSEIDON criteria (*Esteves et al., 2021*) as four or fewer oocytes retrieved, and the definition of a high response defined as more than 15 oocytes retrieved (*Feferkorn et al. 2023*). The post-hoc analysis was carried out by repeating the original analysis with the addition of an interaction term for normal ovarian response to test whether there was a uniform treatment effect across three different strata of ovarian reserve: four or fewer oocytes retrieved, five to 14 oocytes retrieved and 15 or more oocytes retrieved. If the treatment effect differed, clinical pregnancy and live birth outcomes within the group in which five to 14 oocytes were retrieved were estimated. Additionally, as studies have shown that GnRH antagonists are preferentially used in women of AMA, only antagonist cycles in women undergoing the first cycle of stimulation were included (*Bühler et al., 2021*), resulting in a more homogenous study population.

RESULTS

Study population

A total of 14,486 women undergoing their first cycle of ovarian stimulation for ART were included in the study, of whom 4250 women received treatment with combined r-hFSH and r-hLH and 10,236 women received r-hFSH alone. Demographics, medical history and baseline characteristics of all included women (before and after matching) are presented in TABLE 1. Few differences between the two treatment groups were observed before matching. After 1:1 matching, 4238 women were included in each treatment group. There was a large overlap of the propensity score distributions between treatment groups (FIGURE 1), with all SMDs after matching within ±0.1, confirming adequate matching between the two cohorts

TABLE 1 BASELINE CHARACTERISTICS

Characteristic	Before matching			After matching		
	r-hFSH and r-hLH	r-hFSH	SMD	r-hFSH and r-hLH	r-hFSH	SMD
Women, *n*	4250	10,236		4238	4238	
Age,[a] mean ± SD (minimum, Q1, median, Q3, maximum)	37.4 ± 1.6 (35, 36, 38, 39, 40)	37.0 ± 1.6 (35, 36, 37, 38, 40)	0.250	37.4 ± 1.6 (35, 36, 38, 39, 40)	37.5 ± 1.6 (35, 36, 38, 39, 40)	−0.010
BMI, mean ± SD (minimum, Q1, median, Q3, maximum)	24.8 ± 5.7 (14.4, 21, 23.2, 26.9, 63.7)	24.7 ± 5.5 (15.4, 21.1, 23.3, 26.7, 64.9)	0.018	24.8 ± 5.7 (14.4, 21, 23.2, 26.9, 63.7)	24.7 ± 5.5 (15.8, 21.1, 23.3, 26.6, 64.9)	0.019
Year of first ovarian stimulation cycle, *n* (%)						
2017	416 (9.8)	1006 (9.8)	0.015	416 (9.8)	392 (9.2)	0.019
2018	1873 (44.1)	4625 (45.2)	−0.022	1871 (44.1)	1910 (45.1)	−0.019
2019	1961 (46.1)	4605 (45.0)	0.023	1951 (46.0)	1936 (45.7)	0.007
Women who had previously been pregnant, *n* (%)	1168 (27.5)	2928 (28.6)	−0.025	1166 (27.5)	1191 (28.1)	−0.027
Women who had previously miscarried,[b] *n* (%)	663 (15.6)	1643 (16.1)	NA	663 (15.6)	648 (15.3)	NA
Women who previously had live births, *n* (%)	620 (14.6)	1646 (16.1)	NA	619 (14.6)	686 (16.2)	NA
Treatment indication, *n* (%)						
Female	964 (22.7)	2285 (22.3)	0.009	964 (22.7)	968 (22.8)	0.005
Male	2142 (50.4)	4255 (41.6)	0.178	2131 (50.3)	2144 (50.6)	0.000
Both	689 (16.2)	2231 (21.8)	NA	688 (16.2)	674 (15.9)	NA
Idiopathic	369 (8.7)	1029 (10.1)	−0.047	369 (8.7)	370 (8.7)	−0.001
Other	86 (2.0)	436 (4.3)	−0.128	86 (2.0)	82 (1.9)	0.007
Sterility factor, *n* (%)						
Endometriosis	448 (10.5)	867 (8.5)	0.071	442 (10.4)	447 (10.5)	−0.004
Hyperandrogenaemia or polycystic ovaryies	107 (2.5)	523 (5.1)	−0.136	107 (2.5)	103 (2.4)	0.006
Alteration of the cycle	244 (5.7)	930 (9.1)	−0.128	243 (5.7)	265 (6.3)	−0.022
Tubal pathology	443 (10.4)	1077 (10.5)	−0.003	442 (10.4)	434 (10.2)	0.006
Uterine pathology	150 (3.5)	360 (3.5)	0.001	147 (3.5)	144 (3.4)	0.004
Other	1370 (32.2)	3269 (31.9)	NA	1367 (32.3)	1341 (31.6)	NA
Partner sterility factor, *n* (%)						
Azoospermia	146 (3.4)	381 (3.7)	−0.015	146 (3.4)	152 (3.6)	−0.008
Restricted spermiogram[c]	1645 (38.7)	4243 (41.5)	−0.056	1644 (38.8)	1661 (39.2)	−0.008
Other/missing	637 (15.0)	1697 (16.6)	NA	633 (14.9)	774 (18.3)	NA
Pre-conditions, *n* (%)						
Obesity (BMI >30 kg/m^2)	282 (6.6)	780 (7.6)	−0.038	281 (6.6)	252 (5.9)	0.028
Allergies	24 (0.6)	58 (0.6)	NA	24 (0.6)	20 (0.5)	NA
Diabetes mellitus	26 (0.6)	97 (0.9)	NA	26 (0.6)	36 (0.8)	NA
Hypertension	46 (1.1)	148 (1.4)	−0.033	46 (1.1)	45 (1.1)	0.002
Disease of inner genitals	80 (1.9)	86 (0.8)	0.090	73 (1.7)	64 (1.5)	0.017
Malignancy	31 (0.7)	47 (0.5)	0.035	31 (0.7)	19 (0.4)	0.037
Mental illness	41 (1.0)	102 (1.0)	−0.003	41 (1.0)	45 (1.1)	−0.009
Nicotine consumption	266 (6.3)	888 (8.7)	−0.092	266 (6.3)	263 (6.2)	0.003
Thrombolic embolism	30 (0.7)	55 (0.5)	0.021	30 (0.7)	19 (0.4)	0.034
Thyroid disease	314 (7.4)	1105 (10.8)	0.318	314 (7.4)	325 (7.7)	−0.010

[a] Characteristic used in regression models.

[b] Miscarriage was defined as a spontaneous loss of a clinical pregnancy before 22 completed weeks of gestational age.

[c] Defined according to the sixth edition of the WHO Manual for the Laboratory Examination and Processing of Human Semen criteria as either a concentration below a sperm concentration of 16 mio/ml, or progressive motility lower the 32%, or normal morphology of less than 4%, or a combination of those parameters (*WHO, 2021*).

BMI, body mass index; NA, not applicable (variable not included in the calculation of the propensity score); Q1, first quartile; Q3, third quartile; r-hFSH, recombinant human FSH; r-hLH, recombinant human LH; SMD, standardized mean difference (within −0.1 and 0.1 are considered well balanced; if SMD was provided, the variable was included in the calculation of the propensity score).

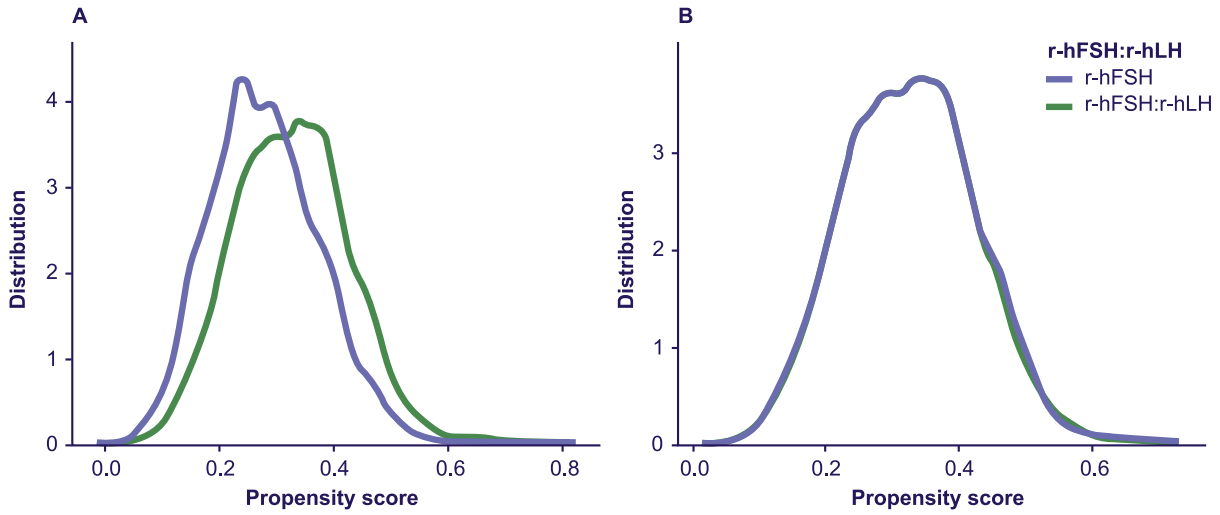

FIGURE 1 Propensity score distribution by treatment (A) before matching and (B) after matching.
All standardized mean differences after matching were below 0.1. r-hFSH, recombinant human FSH; r-hLH, recombinant human LH; r-hFSH:r-hLH, combined r-hFSH and r-hLH.

(TABLE 1). Numerical differences in therapy indication, sterility factor and comorbidities, however, were observed between the two cohorts.

Among treatment-related characteristics before matching, most cycles (91.1% [13,190/14,486]) included GnRH-antagonist downregulation; the proportion was similar after matching (89.3% [*n* = 3785] versus 88.8% [*n* = 3763]) for the r-hFSH alone group versus the combined r-hFSH and r-hLH group, respectively (TABLE 2).

Although matching was carried out to minimize the differences, numerical differences in certain treatment-related characteristics remained between the two cohorts. The mean daily dose of r-hFSH was numerically lower (232.9 IU [SD 64.9] versus 265.4 IU [SD 71.3]) for those treated with r-hFSH alone (*n* = 4238) compared with those treated with combined r-hFSH and r-hLH (*n* = 4238). Additionally, a numerical difference was observed between the drugs used to trigger oocyte maturation; a lower proportion of those treated with r-hFSH alone received a GnRH agonist (5.3% [*n* = 225] versus 14.0% [*n* = 594] treated with combined r-hFSH and r-hLH) to trigger final oocyte maturation, but a higher proportion received HCG for oocyte maturation (82.9% [*n* = 3512] versus 70.6% [*n* = 2991] treated with combined r-hFSH and r-hLH). Furthermore, a lower proportion of women treated with r-hFSH alone received HCG for luteal phase support compared with women treated with r-hFSH and r-hLH (11.4% [*n* = 484] versus 19.0% [*n* = 805]), but a higher proportion received progesterone 60.9% [*n* = 2581] versus 47.4% [*n* = 2007]) or oestrogen (8.7% [*n* = 369] versus 3.4% [*n* = 142]). Lastly, the proportion of women who underwent fresh transfers of two or more embryos was higher in those treated with r-hFSH alone (53.0% [*n* = 2245]) compared with those treated with combined r-hFSH and r-hLH (46.2% [*n* = 1956]), and a higher proportion of women treated with r-hFSH alone (35.2% [*n* = 1,492]) received an embryo transfer on day 5 or later after retrieval compared with those treated with combined r-hFSH and r-hLH (29.3% [*n* = 1241]) (TABLE 2).

Effectiveness

Clinical pregnancy rate and live birth rate

Overall, the clinical pregnancy rate was 29.8% (95% CI 28.2 to 31.6%) with combined r-hFSH and r-hLH and 27.8% (95% CI 26.5, 29.2%) with r-hFSH alone. The absolute difference between groups (2.0%, 95% CI −0.1 to 4.1) did not reach statistical significance (*P* = 0.269) (TABLE 3). The live birth rate was 20.3% (95% CI 18.7 to 21.8%) with combined r-hFSH and r-hLH compared with 18.0% (95% CI 16.6, 19.4%) with r-hFSH alone. The absolute difference between groups (2.3%, 95% CI 0.2 to 3.9) did not reach statistical significance (*P* = 0.092) (TABLE 3) No significant difference in cumulative clinical pregnancy rate or cumulative live birth rate was found between the two treatment groups (TABLE 3).

Number of oocytes retrieved

Overall, the mean number of oocytes retrieved was significantly lower with combined r-hFSH and r-hLH (7.66 [SD 5.81]) compared with r-hFSH alone (9.49 [SD 6.48]) (TABLE 3), with an adjusted mean difference of −1.74 (95% CI −2.00 to −1.48; *P* < 0.0001). The distribution of oocytes retrieved differed numerically between the two treatment groups. A higher proportion of women treated with combined r-hFSH and r-hLH had four or fewer oocytes retrieved compared with those treated with r-hFSH alone (34.2% versus 22.2%, respectively); however, a higher proportion of women treated with r-hFSH alone had 15 or more oocytes retrieved compared with those treated with combined r-hFSH and r-hLH (18.5% versus 11.9%, respectively) (TABLE 3).

Miscarriage

No difference in miscarriage rate was observed between the two treatment groups (TABLE 3).

Exploratory safety analyses

The incidence of OHSS was 0.7% (95% CI 0.5 to 1.1) with combined r-hFSH and r-hLH and 1.2% (95% CI 0.9, 1.6) with r-FSH alone (Supplementary Table 2). The absolute difference between groups (−0.5%, 95% CI −1.0 to −0.1]) did not reach statistical significance (*P* = 0.092). No significant difference in incidence of multiple pregnancy was found between the two treatment groups (Supplementary Table 2).

TABLE 2 TREATMENT-RELATED CHARACTERISTICS

Characteristic	Before matching		After matching	
	r-hFSH: and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH
Women, *n*	4250	10236	4238	4238
Downregulation protocol,[a] *n* (%) GnRHa antagonist GnRHa long agonist	 3768 (88.7) 482 (11.3)	 9422 (92.0) 814 (8.0)	 3763 (88.8) 475 (11.2)	 3785 (89.3) 453 (10.7)
Stimulation, days mean ± SD (minimum, Q1, median, Q3, maximum)	8.8 ± 2.0 (1, 8, 9, 10, 20)	8.8 ± 2.0 (1, 8, 9, 10, 20)	8.8 ± 2.0 (1, 8, 9, 10, 20)	8.8 ± 1.9 (1, 8, 9, 10, 20)
Total dose of r-hFSH, IU,[b,c] mean ± SD (minimum, Q1, median, Q3, maximum)	2325.4 ± 801.6 (150, 1787.5, 2250, 2775, 5000)	1990.2 ± 702.1 (150, 1500, 1875, 2400, 5000)	2323.6 ± 800.8 (150, 1775, 2250, 2775, 5000)	2025.3 ± 699.9 (150, 1500, 1950, 2425, 5000)
Daily dose of r-hFSH, IU mean ± SD (minimum, Q1, median, Q3, maximum)	265.5 ± 71.2 (84.4, 215.6, 257.1, 330, 600)	228.3 ± 65.6 (12.8, 171.4, 225, 260, 555.6)	265.4 ± 71.3 (84.4, 215.6, 257.1, 330, 600)	232.9 ± 64.9 (12.8, 175, 232.5, 264.8, 555.6)
Total dose of r-hLH, IU mean ± SD (minimum, Q1, median, Q3, maximum)	1165.2 ± 411.2 (150, 893.8, 1125, 1387.5, 5000)	NA	1164.3 ± 410.7 (150, 887.5, 1125, 1387.5, 5000)	NA
Daily dose of r-hLH, IU mean ± SD (minimum, Q1, median, Q3, maximum)	132.9 ± 35.8 (42.2, 107.8, 128.6, 165, 300)	NA	132.8 ± 35.8 (42.2, 107.8, 128.6, 165, 300)	NA
Drug used to induce final oocyte maturation: hCG (urinary or recombinant or r-hLH) (yes/no), *n* (%)[c,d]	2996 (70.5)	8337 (81.4)	2991 (70.6)	3512 (82.9)
Drug used to induce final oocyte maturation: GnRH agonist, yes/no, *n* (%)[d]	597 (14.0)	647 (6.3)	594 (14.0)	225 (5.3)
Women with freeze-all, *n* (%)	297 (7.0)	1072 (10.5)	297 (7.0)	385 (9.1)
Luteal phase support[c]				
Progesterone	2010 (47.3)	6048 (59.1)	2007 (47.4)	2581 (60.9)
hCG	808 (19.0)	1183 (11.6)	805 (19.0)	484 (11.4)
Oestrogen	143 (3.4)	920 (9.0)	142 (3.4)	369 (8.7)
Other	643 (15.1)	1519 (14.8)	641 (15.1)	601 (14.2)
Days between retrieval and fresh transfer Mean ± SD (minimum, Q1, median, Q3, maximum)[c]	3.6 ± 1.2 (2, 3, 3, 5, 7)	3.8 ± 1.2 (0, 3, 4, 5, 7)	3.6 ± 1.2 (2, 3, 3, 5, 7)	3.8 ± 1.2 (0, 3, 4, 5, 7)
1–4 days	2005 (47.2)	4330 (42.3)	2001 (47.2)	1853 (43.7)
5 or more days	1243 (29.2)	3612 (35.3)	1241 (29.3)	1492 (35.2)
No fresh transfer/unknown	1002 (23.6)	2294 (22.4)[e]	996 (23.5)	893 (21.1)[e]
Women with fresh embryo transfer, *n* (%) Number of embryos per transfer, *n* (%)[c]	3248 (76.4)	7943 (77.6)	3242 (76.5)	3346 (79.0)
One embryo	1289 (30.3)	2626 (25.7)	1286 (30.3)	1101 (26.0)
Two or more embryos	1959 (46.1)	5317 (51.9)	1956 (46.2)	2245 (53.0)
Frozen embryo transfers[f]				
Women with 0 FET, *n* (%)	802 (18.9)	1575 (15.4)	796 (18.8)	618 (14.6)
Women with 1 FET, *n* (%)	2742 (64.5)	6519 (63.7)	2736 (64.6)	2739 (64.6)
Women with 2 FET, *n* (%)	491 (11.6)	1436 (14.0)	491 (11.6)	603 (14.2)
Women with 3 or more FET, *n* (%)	214 (5.0)	706 (6.9)	214 (5.1)	278 (6.6)

[a] Included in the calculation of the propensity score (SMD = −0.115 before matching and SMD = −0.017 after matching).

[b] Included in models as an ordinal variable (0, 1, 2, 3), not binary.

[c] Characteristic used in regression analysis.

[d] The triggering data were recorded as one of four categories: gonadotrophin releasing hormone (GnRH) agonist, hCG, not specified and none. The 'not specified' and 'none' categories combined account for the remainder of the triggering data.

[e] One case of a fresh transfer with an unknown or implausible day was recorded for the transfer.

[f] Data on frozen embryo transfers were not available for one women in the r-hFSH and r-hLH group.

FET, frozen embryo transfer; GnRHa, gonadotropin releasing hormone analogue; Q1, lower interquartile value; Q3, upper interquartile value; r-hFSH, recombinant human FSH; r-hLH, recombinant human LH; SMD, standardized mean differences.

TABLE 3 ADJUSTED STIMULATION CYCLE OUTCOMES

Outcomes	r-hFSH and r-hLH (*n* = 4238)	r-hFSH alone (*n* = 4238)	Treatment comparison	*P*-values[a]
Clinical pregnancy rate, % (95% CI)[b]	29.8 (28.2 to 31.6)	27.8 (26.5 to 29.2)		
Absolute difference			2.0 (−0.1 to 4.1)	0.269
Relative risk			1.07 (1.00 to 1.15)	
Live birth rate, % (95% CI)[b]	20.3 (18.7 to 21.8)	18.0 (16.6 to 19.4)		
Absolute difference			2.3 (0.2 to 3.9)	0.092[c]
Relative risk			1.12 (1.01 to 1.23)	
Cumulative clinical pregnancy rate, % (95% CI)	34.7 (33.0 to 36.0)	36.0 (34.3 to 37.6)		
Absolute difference			−1.2 (−3.4 to 0.9)	0.551
Relative risk			0.97 (0.91 to 1.03)	
Cumulative live birth rate, % (95% CI)	23.9 (22.5 to 25.6)	24.0 (22.5 to 25.6)		
Absolute difference			−0.04 (−2.1 to 1.8)	0.966
Relative risk			1.00 (0.91 to 1.08)	
Number of oocytes retrieved, mean (SD), *n* (%)	7.66 (5.81)	9.49 (6.48)	−1.74 (−2.00, −1.48)	<0.0001
0–4	1450 (34.2)	939 (22.2)	NA	
5–14	2283 (53.9)	2517 (59.4)	NA	
15 or more	505 (11.9)	782 (18.5)	NA	
Clinical pregnancies, *n*	1049	1092	NA	
Miscarriage rate, % (95% CI)	26.2 (24.0 to 29.0)	26.7 (24.2 to 29.4)		
Absolute difference			−0.5 (−4.4 to 3.3)	0.943
Relative risk			0.98 (0.85 to 1.13)	

[a] *P*-values are for the effect of treatment from the adjusted outcome models and corrected for multiple comparisons using the method by Benjamini and Hochberg.

[b] The number of initiated cycles was used as a denominator (*n* = 4238).

[c] *P*-value without correction for multiple comparisons: 0.0206.

r-hFSH, recombinant human FSH; r-hLH, recombinant human LH.

Post-hoc analysis

To account for ovarian reserve, which is information not directly recorded in the Deutsches IVF-Register, clinical pregnancy and live birth rates for women treated with a GnRH antagonist protocol were assessed according to three different strata of ovarian reserve (number of oocytes retrieved). Importantly, propensity score matching was applied to the redefined population before stratification by number of oocytes retrieved, explaining the difference observed in the number of women per treatment group after stratification.

Overall, a total of 7502 women (3751 per treatment group) were matched; all SMDs after matching were between −0.1 and 0.1. The demographics, medical history, baseline and treatment-related characteristics are presented in Supplementary Tables 3 and 4; clinical pregnancy and live birth rates for all women included in the post-hoc analysis are shown in TABLE 4 and TABLE 5. The proportion of women with five to 14 oocytes retrieved was 54.0% (*n* = 2024) for combined r-hFSH and r-hLH and 59.0% (*n* = 2213) for r-hFSH alone (Supplementary Table 4).

In the subgroup of women with five to 14 oocytes retrieved, a higher clinical pregnancy rate and a higher live birth rate were observed with combined r-hFSH and r-hLH compared with r-hFSH alone (TABLES 4 and 5). The clinical pregnancy rate was significantly higher with combined r-hFSH and r-hLH (33.1%, 95% CI 31.0 to 35.0%) versus r-hFSH alone (28.5%, 95% CI 26.6 to 30.4) (*P* = 0.001, not adjusted for multiplicity), with an absolute difference of 4.6%, (95% CI 1.6 to 6.9; *P* = 0.001). This absolute difference of 4.6% translates into a 16% (95% CI 5 to 26%) higher chance (*P* = 0.001) of achieving clinical pregnancy for women with five to 14 oocytes retrieved treated with combined combined r-hFSH and r-hLH compared with those treated with r-hFSH alone (TABLE 4). Similarly, the live birth rate was significantly higher with combined r-hFSH and r-hLH (22.5%, 95% CI 20.5 to 24.2%]) versus r-hFSH alone (19.4%, 95% CI 17.6 to 20.9%) (*P* = 0.014, not adjusted for multiplicity), with an absolute difference of 3.1% (95% CI 0.6 to 5.5). This absolute difference of 3.1% translates into a 16% (95% CI 3 to 31%) higher chance (*P* = 0.014) of achieving live birth for women with five to 14 oocytes retrieved treated with combined r-hFSH and r-hLH compared with those treated with r-hFSH alone (TABLE 5).

DISCUSSION

This is the first non-interventional study to directly compare the effectiveness of combined r-hFSH and r-hLH combination in a 2:1 ratio versus r-hFSH alone for ovarian stimulation during ART in women of AMA. Overall, compared with stimulation with r-hFSH alone, stimulation with combined r-hFSH ad r-hLH resulted in a significantly higher clinical pregnancy rate and live birth rate in women aged 35–40 years with five to 14 oocytes retrieved. No difference in miscarriage, incidence of OHSS or incidence of

TABLE 4 POST-HOC ANALYSIS: CLINICAL PREGNANCY

	Women with first cycle GnRH antagonist protocol							
	All women		0–4 oocytes retrieved		5–14 oocytes retrieved		15 or more oocytes retrieved	
	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH
Pregnancy, % (95% CI)	24.7 (22.6 to 26.3)	24.8 (23.3 to 26.6)	14.6 (12.7 to 16.2)	16.2 (13.6 to 18.5)	33.1 (31.0 to 35.0)	28.5 (26.6 to 30.4)	21.3 (17.5 to 24.9)	19.7 (17.4 to 22.1)
Difference (r-hFSH and r-hLH minus r-hFSH alone) (95% CI)	−0.1 (−3.6 to 2.6)		−1.5 (−4.6 to 1.9)		4.6 (1.6 to 6.9)		1.6 (−3.4 to 5.4)	
Relative difference, % (95% CI)	No difference		No difference		1.16 (1.05 to 1.26)		No difference	
P-value (r-hFSH and r-hLH versus r-hFSH alone, not adjusted for multiplicity)	*P* = 0.942		*P* = 0.329		*P* = 0.001		*P* = 0.494	
P-value for interaction between treatment and category of oocytes retrieved	P=0.0373							

The treatment effect of combined r-hFSH and r-hLH versus r-hFSH alone did not differ significantly for live birth rate across the defined subgroups (0–4, 5–14 and 15 or more oocytes retrieved), with the live birth rate consistently numerically higher for r-hFSH and r-hLH for all three subgroups of oocytes retrieved.

GnRH, gonadotrophin-releasing hormone; r-hFSH, recombinant human FSH; r-hLH, recombinant human LH.

multiple pregnancies was found between the two groups.

To ensure validity and comparability of results, variables known and validated to predict live birth and cumulative live birth based on the available models and published research were included in the propensity score calculation, as previously mentioned in the Materials and methods section (*Arvis et al., 2013*; *Luke et al., 2014*; *McLernon et al., 2016*; *Leijdekkers et al. 2018*). These baseline factors included age (*Luke et al., 2014*; *McLernon et al., 2016*; *Leijdekkers et al., 2018*; *Arvis et al., 2021*), BMI (*Luke et al., 2014*; *Leijdekkers et al., 2018*), previous pregnancies (*McLernon et al., 2016*; *Leijdekkers et al., 2018*), cause of infertility (*Luke et al., 2014*; *McLernon et al., 2016*; *Leijdekkers et al., 2018*), other pre-conditions, e.g. obesity, smoking, thyroid disease), type of downregulation protocol (GnRH long agonist versus GnRH antagonist), type of fertilization (IVF with or without ICSI) (*McLernon et al., 2016*; *Leijdekkers et al., 2018*) and year of ovarian stimulation for ART (*McLernon et al., 2016*; *Leijdekkers et al., 2018*) (Supplementary Table 1). As cumulative live birth rate is affected by variations in treatment characteristics, the following additional post-treatment variables were incorporated into the final outcome model to adjust for potential confounders after propensity score matching: total r-hFSH dose, drug to trigger final oocyte maturation, type of luteal phase support, transfer of blastocyst(s) and number of embryos transferred (*Luke et al., 2014*; *McLernon et al., 2016*; *Leijdekkers et al., 2018*) (Supplementary Table 1). Further more, as age is considered the most important predictor of ovarian reserve, it was used in the propensity score model and the outcome analysis model. Other predictors of ovarian reserve, such as AMH and AFC, were not recorded in the Deutsches IVF-Register database and have shown limited ability to predict live birth (*Leijdekkers et al., 2018*).

Overall, our data may suggest that physician preference plays a key role in treatment decisions, with combined r-hFSH and r-hLH more commonly used in women with lower ovarian reserve, and r-hFSH alone more commonly used in women with higher ovarian reserve. First, more women treated with combined r-hFSH and r-hLH had four or fewer oocytes retrieved (likely representing lower ovarian reserve) compared with those treated with

TABLE 5 POST-HOC ANALYSIS: LIVE BIRTH

	Women with first cycle GnRH antagonist protocol							
	All women		0–4 oocytes retrieved		5–14 oocytes retrieved		≥15 oocytes retrieved	
	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH	r-hFSH and r-hLH	r-hFSH
Live birth, % (95% CI)	18.0 (16.1 to 19.7)	15.7 (14.1 to 17.4)	10.4 (8.5 to 12.2)	10.0 (8.3 to 11.8)	22.5 (20.5 to 24.2)	19.4 (17.6 to 20.9)	16.0 (12.8 to 19.6)	12.4 (10.2, 14.5)
Difference (r-hFSH and r-hLH minus r-hFSH alone) (95% CI)	2.3 (−0.1 to 5.4)		0.4 (−2.6 to 3.2)		3.1 (0.6 to 5.5)		3.6 (0.1 to 8.0)	
Relative difference, % (95% CI)	No difference		No difference		1.16 (1.03, 1.31)		No difference	
P-value (r-hFSH and r-hLH versus r-hFSH alone, not adjusted for multiplicity)	*P* = 0.1214		*P* = 0.742		*P* = 0.014		*P* = 0.073	
P-value for interaction between treatment and category of oocytes retrieved	*P* = 0.7825							

GnRH, GnRH, gonadotrophin-releasing hormone; r-hFSH, recombinant human FSH; r-hLH, recombinant human LH.

r-hFSH alone, and fewer women treated with combined r-hFSH and r-hLH had 15 or more oocytes retrieved (likely representing higher ovarian reserve) compared with those treated with r-hFSH alone. Second, although not significant, after matching (TABLE 2), a trend towards higher total FSH dose and higher daily FSH dose along with a numerically lower number of cycles with fresh transfers of two or more embryos were observed in women treated with combined r-hFSH and r-hLH compared with those treated with r-hFSH alone, likely related to lower ovarian reserve. Third, we are not able to comment on the exact reason why a specific oocyte maturation trigger was used, as the registry lacks information regarding the specific indications for GnRH agonist use to induce final oocyte maturation, i.e. whether to prevent OHSS, to manage cases of diminished ovarian reserve, or for dual triggering in women with diminished ovarian response or as part of standard clinical protocols. Despite having a lower prognosis, as outlined above, women treated with combined r-hFSH and r-hLH showed a non-significant trend towards higher clinical pregnancy and live birth rates compared with those treated with r-hFSH only in the overall study population and the difference was statistically significant in women with a normal ovarian response (four to 15 oocytes retrieved), as explained below.

As the Deutsches IVF-Register does not collect information about ovarian reserve biomarkers directly, any possible imbalance in ovarian reserve between both groups, which could explain the observations above, could not be assessed and adjusted for in our study. With this limitation in mind, we conducted a post-hoc analysis by stratifying patients based on the number of oocytes retrieved (with the stratum five to 14 oocytes retrieved' as a surrogate for normal ovarian reserve). This analysis of women expected to have normal ovarian reserve (five to 14 oocytes retrieved) included more than 50% of the study population (54.0% of all women treated with combined r-hFSH and r-hLH versus 59.0% of all women treated with r-hFSH alone) and showed that treatment with combined r-hFSH and r-hLH resulted in a higher clinical pregnancy and live birth rates compared with r-hFSH alone.

As shown by our post-hoc analysis, women of AMA with suspected normal ovarian reserve represent a group of patients who may benefit the most from treatment with

combined r-hFSH and r-hLH. Most participants in RCTs are women aged between 18 and 38 years (*Hershkop et al., 2017*). Consequently, the outcomes observed reflect an amalgamation of effects seen in both younger and older women. In the present study, however, a specific approach is taken by concentrating exclusively on women aged 35–40 years. This exclusive focus enables us to furnish clinicians with novel and tailored evidence, facilitating them in crafting personalized treatment strategies for this particular age group. This is in line with results from a meta-analysis of five RCTs in women of AMA between the ages of 35 and 40 years with normal ovarian reserve undergoing IVF/ICSI, which showed that treatment with combined r-hFSH and r-hLH was associated with higher clinical pregnancy rates (OR 1.45, 95% CI 1.05 to 2.00, $I^2 = 0\%$, $P = 0.03$) and implantation rates (OR 1.49, CI 95% 1.10 to 2.01, $I^2 = 13\%$, $P = 0.01$) than r-hFSH alone; however, this beneficial effect was lost when women aged 35 years or older, and those with reduced ovarian reserve, were included in the analysis (*Conforti et al., 2021*). Another study suggests that the timing of combined r-hFSH ad r-hLH treatment may also affect clinical outcomes, with higher implantation rates (24.7% versus 13.3%) and clinical pregnancy rates per embryo transfer (34.4% versus 18.9%) noted with initiation on day 1 of ovarian stimulation compared with day 6 of ovarian stimulation (*Behre et al. 2015*). As such, the use of combined r-hFSH and r-hLH in women of AMA represents an increasing practice in most countries today, including Germany.

Overall, the present data (including the post-hoc analysis) and the meta-analysis by *Conforti* et al. *(2021)* suggest that women aged between 35 and 40 years with normal ovarian reserve may benefit from combined r-hFSH and r-hLH treatment compared with treatment with r-hFSH alone, in terms of pregnancy and live birth rates, overcoming the effect of AMA and related LH deficiency on oocyte quality.

Oocyte quality, with direct effect on blastocyst euploidy (*Esteves et al., 2019*), is age-dependent, multifactorial, and potentially associated with 'relative' deficiency of FSH and LH, which can be attributed to reduced cell signalling at the level of the FSH and LH receptors, potentially caused by changes in gonadotrophin glycoform composition. Indeed, in women of AMA, despite normal to high FSH and LH levels, a reduction in ovarian follicular growth, maturation and androgen production is observed (*Alviggi et al. 2009*; *Andersen and Ezcurra, 2014*), resulting in lower levels of circulating androgens compared with younger women (*Mushayandebvu et al., 1996*; *Davison et al., 2005*; *Alviggi et al., 2009*). Female ageing is also associated with an increase of fully glycosylated FSH variants, which display lower affinity for the FSH receptor compared with the most common glycoforms found in younger women (*Anobile et al., 1998*; *Ulloa- Bousfield et al., 2018*; *Aguirre et al., 2001*), and a decrease in LH bioactivity, i.e. there are more sialylated and fewer sulfonated glycoforms (*Wide et al., 2007*). Lastly, in-vitro models have demonstrated that r-hLH in combination with r-hFSH exerts an anti-apoptotic effect on cumulus cells and promotes the paracrine signalling involved in cell expansion and oocyte maturation during folliculogenesis (*Casarini et al., 2016*; *Casarini et al., 2018*; *Sperduti et al., 2022*). On the basis of the above, we postulate that the effect of age-related FSH and LH deficiency on oocyte quality can be mitigated by co-treatment with combined r-hFSH and r-hLH rather than r-hFSH alone. This results in better oocyte quality (*Ruvolo et al., 2007*; *Revelli et al., 2015*) and related embryo quality (and a potential positive effect on endometrial receptivity) (*Revelli et al., 2015*), leading to higher implantation, pregnancy and live birth rates in women of AMA (*The European Recombinant Human LH Study Group 1998*).

Women of AMA with normal ovarian reserve are not the only group of patients who may benefit from r-hFSH and r-hLH combination treatment. Several clinical studies and meta-analyses have found that treatment with r-hFSH and r-hLH improves pregnancy outcomes in various ART populations versus r-hFSH alone, including women with a hypogonadotropic hypogonadal condition defined according to ICMART 2017, which refers to both gonadotrophin levels and their bioactivity (*Zegers-Hochschild et al., 2017*). Therefore, in addition to the potential reduction in gonadotrophin bioactivity caused by hypogonadotropic hypogonadism, women presenting with profoundly suppressed LH levels caused by GnRH agonist or antagonist treatment during ART displayed improved clinical outcomes in terms of oocyte quality and implantation rate after co-administration of r-hFSH and r-hLH during ovarian stimulation (*Lisi et al., 2002*; *Humaidan et al., 2004*; *Pezzuto et al., 2010*; *Lehert et al., 2014*). A further subgroup of women, those with a hypo-response to exogenous gonadotrophins, were also shown to benefit from combined treatment with r-hFSH and r-hLH, as summarized in the systematic review and meta-analysis by *Conforti* et al. *(2019)*. Combined treatment with r-hFSH and r-hLH also resulted in higher implantation rates and live birth rates compared with r-hFSH alone in women hyporesponsive to FSH (*Ferraretti et al., 2004*; *Conforti et al., 2019*). Finally, evidence suggests that combined treatment with r-hFSH and r-hLH may improve oocyte quality in women with poor ovarian response (POR) (*Lehert et al., 2014*; *Humaidan et al., 2017*; *Levi-Setti et al., 2019*; *Arvis et al., 2021*; *Koloda et al., 2022*); however, the definition of POR depends on the criteria used, e.g., ESHRE Bologna POR criteria, the PROsPeR classification or the POSEIDON group classification (*Ferraretti et al., 2011*; *Poseidon et al.. 2016*; *Lehert et al., 2018*), with the benefit of r-hFSH and r-hLH treatment inconsistently reported.

Although RCTs are the best way to assess the efficacy of a treatment, the recognized limitations of RCTs make it difficult to generalize findings to larger, more inclusive populations of patients, providers, and health care delivery systems or settings that reflect actual use in practice. (*Sherman et al., 2016*). Moreover, infertility RCTs are often too small to detect all treatment effects between the treatment arms that are relevant from the patient or physician perspectives, or the reporting of these treatment effects may be limited (*Wilkinson et al., 2022*); for example, RCTs were not powered to demonstrate superiority in terms of pregnancy/live birth outcomes between r-hFSH and urinary products (*Andersen et al., 2006a*). In a landscape in which resources and funding for conducting RCTs are limited, our findings, using real-world data, are particularly important considering that childbirth in women of AMA is increasing in high-income countries owing to lifestyle choices, underlying subfertility, or both (*Guedes and Canavarro, 2014*). For example, in the USA, the proportion of live births in women of AMA increased from 4.9% to 14.2% between 1908 and 2009 (*National Center for Health Statistics, 1984*; *Martin et al., 2011*). In the European Union, the percentage of live births to mothers aged 40 years or older has increased from 2.4% in 2001 to 5.5% in

2020 (*Eurostat, 2022*). In this respect, real-world data can complement RCT data by providing further evidence on the effect of treatment strategies regarding clinically relevant fertility outcomes, while also providing the added benefits of larger sample sizes and increased representativeness of the studied populations (*Hershkop et al., 2017*).

A particular strength of this study is the use of Deutsches IVF-Register as source of real-world data. The Deutsches IVF-Register is a comprehensive database that reflects the daily practice of German practitioners, who have almost 15 years of experience using combined r-hFSH and r-hLH for ART. The use of nationwide data and large sample sizes with limited inclusion and exclusion criteria minimizes the risk of selection bias and ensures a high level of generalizability of the observed results. This study also had limitations that warrant discussion. Firstly, matching with propensity score and, thereafter, use of a regression model can only balance and adjust for measured variables. As some critical variables in this context, e.g. ovarian reserve biomarkers, were not directly reported in the Deutsches IVF-Register, imbalance for some of these variables between both treatment groups is possible. This is supported by the fact that more women undergoing treatment with combined r-hFSH and r-hLH had four or fewer oocytes retrieved compared with those treated with r-hFSH alone, whereas numerically higher daily and total r-hFSH doses were used. Additionally, potential differences in treatment policies between centres, e.g. transfer policy, use of combined r-hFSH and r-hLH versus r-hFSH alone, number of embryos per transfer, may affect patient outcomes. Lastly, the addition of r-hLH may alter the endometrium compared with r-hFSH alone, which may explain why an improvement was noted in clinical pregnancy and live birth rates but not in cumulative outcomes. Unfortunately, owing to the use of aggregated anonymized data, a separate analysis of only the frozen embryo transfer cycles, which may have further clarified the issue, was not possible.

In the present study, we show that women of AMA with normal ovarian response benefit from treatment with combined r-hFSH and r-hLH combination in a 2:1 ratio versus r-hFSH alone in terms of live birth rate. Additionally, we highlight the value of real-world data when examining effectiveness of fertility treatment during routine clinical practice among patient groups that may not be included in clinical trials.

DATA AVAILABILITY

Data will be made available on request.

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DATA AVAILABILITY STATEMENT

Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to Merck KGaA's Data Sharing Policy. All requests should be submitted in writing to Merck KGaA's data sharing portal (https://www.merckgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html). When Merck KGaA has a co-research, co-development, or co-marketing or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck KGaA will endeavour to gain agreement to share data in response to requests.

AUTHORS' ROLES

PV was involved in the design of the study, interpretation of the results and writing of the manuscript; BH was involved in the design of the study, analysis of the data, interpretation of the results and writing of the manuscript; RF contributed treatment data to Recdate/ Deutsches IVF-Register and was involved in the interpretation of the data and writing of the manuscript;

ML, TDH, JES and DC were involved in the concept and design of the study, clinical interpretation of data and writing of the manuscript; JK and APB were involved in interpretation of the data and writing of the manuscript. All authors approved the final article.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2023.103725.

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