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ASSISTED REPRODUCTION TECHNOLOGIES

Utilization of in vitro maturation in cases with a FSH receptor mutation

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Abstract

Purpose To identify the FSH receptor (*FSHR*) variant and efficacy of in vitro maturation (IVM) in a 28-year-old woman with secondary amenorrhea, primary infertility, and ovarian resistance to FSH, and to analyze the genotype-to-phenotype relationship in cases of *FSHR* mutation for the development of an IVM algorithm for use in patients with gonadotropin resistance syndrome (GRS).

Methods Oocytes retrieved after menstruation induction with norethisterone, followed by daily estrogen and an ovulatory trigger, underwent IVM, ICSI, and culture in a time-lapse (TL) incubator. Embryo transfers were performed on day 2, and after thawing on day 5. Genes associated with disorders of sex development were sequenced for both the patient and her parents. All reported cases of *FSHR* mutation were analyzed to investigate genotype/phenotypic relationships.

Results After ovum pickup, seven of 16 oocytes matured and all fertilized. After unsuccessful day 2 transfer, our patient delivered with a thawed day 5 blastocyst, the sole embryo without abnormal TL phenotypes. Genetic analysis revealed a new composite heterozygous FSHR variant. Analysis of our patient case with published cases of GRS revealed associations among FSHR variant genotype, location on the FSHR, functionality of tested variants, and type of amenorrhea. An algorithm for application of IVM for GRS patients was developed.

Conclusions We report two novel variants of the *FSHR*. Although IVM successfully matured some oocytes, only one resulted in an embryo with normal TL phenotypes. We recommend FSHR genetic testing in GRS patients, which will help guide their suitability for IVM.

Keywords FSH resistance syndrome · FSH receptor variant/mutation · IVM · Time lapse · ART

Introduction

Gonadotropin resistance syndrome (GRS) of the ovaries is a rare pathology with an unknown prevalence, but its management by reproductive endocrinologists represents a significant medical challenge. This syndrome, which was first described about 50 years ago [1], is often caused by a variant of the follicle-stimulating hormone receptor (FSHR). Abnormal clinical and hormonal patient profiles typical of this syndrome include amenorrhea, increased serum FSH levels, normal and/or increased anti-Mullerian hormone (AMH) levels, and often high antral follicle counts [1, 2].

The FSHR is exclusively present in the granulosa cells of follicles [3]. The binding of FSH to its receptor induces stimulation of the granulosa cells which results in the growth of these cells, their secretion of estradiol, and the resumption of oocyte maturation under the influence of the pre-ovulatory luteinizing hormone (LH) surge [4, 5]. FSH receptors, like LH or TSH receptors, are part of the large family of G protein-coupled receptor family. Their protein structure comprises the extracellular (EC) ligand-binding domain, seven transmembrane domains, three short intracellular (IC) loops, three EC loops, and an IC domain [6] (see Fig. 1). A highly conserved structure shared by the G protein-coupled receptor family is the transmembrane domain. FSH binds to the EC

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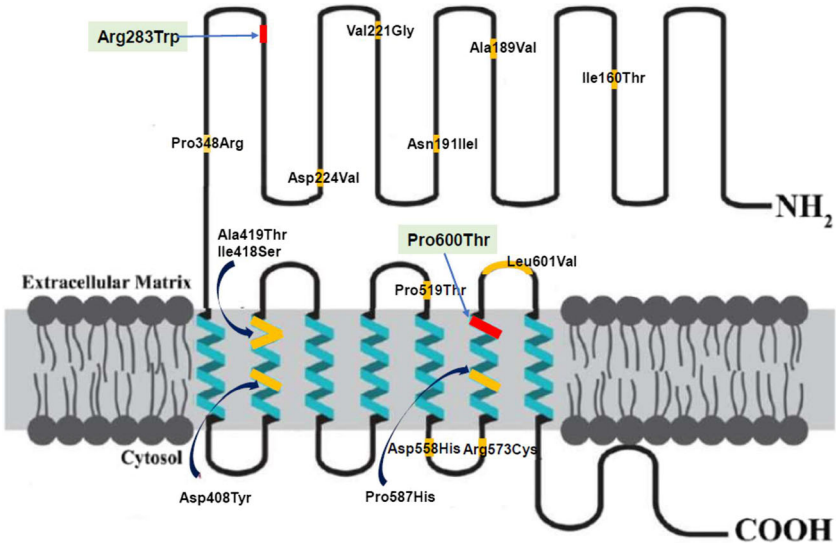

Fig. 1 Structure of the FSH receptor. The diagram shows the extracellular N-terminal and intracellular C-terminal domains and the 7 transmembrane domains, connected by intracellular or extracellular helices. The two amino acid substitutions identified in our patient are highlighted in green, compared to all the receptor-inactivating substitutions previously described in women. Adapted from Bramble et al. [7]

domain of FSHR, which triggers a conformational change in FSHR and subsequently activates G protein and adenylyl cyclase, resulting in increased cyclic adenosine monophosphate (cAMP) production [6].

The FSHR gene consists of 10 exons, the first nine encode the EC domain, whereas the transmembrane and IC domains are encoded by the terminal exon 10 [8, 9], which corresponds to almost 50% of the protein sequence. Several mutations in the FSHR have been described in women, all of whom experienced infertility.

The first description of a mutation in the FSHR gene was reported by Aittomäki and colleagues [10] in 1995. A C566T transition in exon 7 of FSHR was found, predicting an alanine-to-valine substitution at residue 189 (p.Ala189Val) in Finnish women, located in the EC domain of FSHR, resulting in disruption of membrane targeting and marked impairment of FSHR function in vitro.

To our knowledge, since this initial report, there have been eleven other reports of patients with variants of the FSHR with their associated amino acid substitutions and pathogenicity [7, 11–20]. Regardless of the mutation, all these patients lacked a response to ovarian stimulation (OS). This condition, therefore, makes classical assisted reproductive treatment (ART) with controlled ovarian stimulation with exogenous gonadotropins followed by in vitro fertilization (IVF) impossible.

In vitro maturation (IVM) of immature oocytes retrieved without previous ovarian stimulation has been proposed for these patients to obtain mature, developmentally competent oocytes, and embryos capable of supporting viable pregnancies [2]. Historically, IVM was proposed as an approach to rescue immature oocytes the day of oocyte retrieval after ovarian stimulation [21]. It was then proposed for patients with a contraindication to ovarian stimulation such as severe ovarian hyperstimulation syndrome in case of polycystic ovary syndrome (PCOS) [22, 23], in the absence of FSH stimulation or in conjunction with light FSH priming [24, 25]. More recently, IVM has been proposed for cases of hormone-sensitive cancers before preserving fertility [26]. Some teams have also proposed this as an alternative procedure for patients with low ovarian reserve [27] or for patients with a low yield of mature oocytes after conventional IVF [28, 29].

Here, we describe the case of a 28-year-old woman with FSHR resistance syndrome in whom two variants, never described previously, were identified. IVM was conducted, and a live birth was obtained after a frozen-thawed blastocyst transfer. In addition, as no previous study has reported embryo development after IVM for this indication, we describe the phenotypic and morphokinetic parameters of the embryos obtained. Finally, with now thirteen FSHR variant cases of this rare condition reported, we assess whether any relationship exists between the genotype and phenotype of the women involved.

Materials and methods

Patient consent to publish and ethics approval

The patient consented to publication of her case. This case report was approved by the Foch Hospital Ethics Committee, IRB: IRB00012437 (Approval number: 21-05-02).

Patient presentation and work-up

A 28-year-old patient with secondary amenorrhea was referred to our center after 3 years of primary infertility. After menarche at age 12, and menstrual cycles every 30 to 37 days until she was 16 years of age, the patient experienced spaniomenorrhea with menstruation every 6 months until she was 20 years, followed by secondary amenorrhea. Her

secondary sexual characteristics were normal, and she presented with no clinical hyperandrogenism and had no particular personal or family history of menstrual disorders. Her karyotype and screening for *FMR1* gene premutation were both normal, and no auto-antibodies (antithyroid peroxidase and antithyroglobulin) were identified.

Ultrasonography showed a normal uterus size (63 × 34 × 38 mm), two normal sized ovaries (30 × 18 mm and 28 × 20 mm), and an antral follicle count (AFC) of 45. Hormonal testing revealed elevated FSH, LH, and AMH, but all other hormones were in the normal range.

The patient underwent two failed follicular stimulations with exogenous gonadotropins and no ovarian response at another IVF center. Due to this history and her physical and hormone testing, we performed a genetic analysis to determine her karyotype and to investigate whether she carried a FSHR mutation. The genetic composition of her FSHR was characterized using a target sequencing approach of 59 genes for which variants have been previously associated with disorders of sex development. The analysis was performed using next-generation sequencing (Supplemental Material).

Patient index cycle

Cycle management for oocyte retrieval

To induce menstruation, 10 days of norethisterone (Primolut-Nor 10 mg; Bayer HealthCare, Loos, France) was administered and continuous treatment with 2 mg estrogen (E2) twice a day was started the first day of menstruation (Provames 2 mg; Sanofi-Aventis, Paris, France). The first ultrasound, undertaken on day 12 (D12) using a 5.0–9.0-MHz multifrequency transvaginal probe (Voluson™ S8 system, GE Healthcare), showed the presence of 6 follicles of 4 mm and 6 follicles of 3 mm on the right ovary and 4 follicles of 5 mm and 5 follicles of 3 mm on the left ovary. Endometrial thickness was measured at 7.5 mm. Three days later, on D15, an ultrasound was performed and showed the same follicular count and sizes and an endometrial thickness of 8.4 mm. Two days later, priming with 250 IU of choriogonadotropin alfa was performed (Ovitrelle®, Merck Pharmaceuticals) with egg retrieval 36 h later using a 19-gauge needle (Cook Medical) linked to a pump with a negative pressure at 80 mmHg.

In vitro maturation protocol

Collected cumulus-oocyte complexes (COCs) were placed in 4-mL IVM medium (MediCult IVM System; CooperSurgical, France) supplemented with 20% heat-inactivated patient serum and 10 IU/mL hMG (Menopur; Ferring Pharmaceuticals, France), overlain with mineral oil (Ovoil®, Vitrolife). COCs were incubated at 37 °C in an atmosphere of 6.5% CO_2 and 5.0% O_2 balanced with N_2 in a humid atmosphere (Panasonic MCO5M, France). After 27 h, cumulus cells were removed with hyaluronidase (Vitrolife, France) to assess oocyte maturity.

Handling and culture of oocytes post-IVM

Mature oocytes were micro-injected with the partner's spermatozoa. Immature oocytes were replaced in IVM medium for an additional day, after which maturity was reassessed.

Oocytes exhibiting 2PN at the fertilization check were cultured in a shared volume of 80 μL (filling the 16 micro-wells) of one-step culture media (Sage OneStep®, CooperSurgical) under mineral oil in a time-lapse imaging dish designed for Geri® (Geri-dish®, Genea Biomedx) in a controlled atmospheric condition (37 °C; 6.0% CO_2, 5.0% O_2 balanced with N_2 in a dry atmosphere).

Conventional embryo evaluation and analysis by time-lapse imaging

Embryos were evaluated, and their developmental fate (transfer, cryopreservation, or discard) was determined using conventional morphology according to international morphological criteria [30] and the Gardner classification [31].

Retrospective analysis of time-lapse videos was undertaken to assess the morphokinetics and any phenotypic aberrations of the embryos. Images, captured every 5 min through eleven focal planes, were processed by the software (Geri Connect®), with manual annotation of the images also performed [32].

Vitrification and warming protocols

Those embryos kept in extended culture that met our criteria for freezing (at least B3 expansion stage, with a minimum quality of B for the trophectoderm or inner cell mass according to Gardner's classification [31]) were vitrified on day 5 (D5) in a closed system (HS straws, CBS®) using the manufacturer's protocol (Freeze-Kit Irvine®, Biocare). Vitrified embryos were warmed according to the manufacturer's protocol (Thaw-Kit Irvine®, Biocare) and transferred 5 h post-warming.

Uterine preparation and embryo transfer

For fresh embryo transfer on D2, the patient started 200 mg vaginal progesterone (Utrogestan®; Besin Pharma, Paris, France) and 25 mg/day subcutaneous injections of progesterone (Progiron; IBSA, France) on the day of egg retrieval, in combination with 2 mg E2 treatment twice a day until her pregnancy test on day 14 post-embryo transfer.

Cryoembryo transfer was undertaken on D6 of progesterone (200 mg vaginal progesterone [Utrogestan®; Besin

Pharma, Paris, France] and 25 mg/day subcutaneous injections of progesterone [Progiron; IBSA, France]) as previously described [33]. Daily estrogen (2 mg twice a day) and progesterone administration was continued until the pregnancy test. If pregnancy was achieved, the same estrogen/progesterone regimen was continued until the expected luteo-placental shift, at 8 weeks of gestation. Embryo transfer was performed using a Frydman catheter (JCD, France).

Assessment of genotype-to-phenotype relationship in cases of FSHR mutation

To assess the impact of variants on protein function, we combined the prediction for pathogenicity obtained using Varsome software (https://varsome.com/), Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/), gnomAD (https://gnomad.broadinstitute.org/), and GERP (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html) conservation scores.

Varsome combined the data obtained from 11 prediction software (CADD, DANN, FATHMM-MKL, MVP, MutationTaster, SIFT, BayesDel_addAF, EIGEN, LIST-S2, M-CAP, and PrimateAI). These software programs evaluate the potential impact of missense variants. Variants are classified as pathological, likely pathological, variant of uncertain significance (VUS), likely benign, or benign.

ClinVar software reports the clinical descriptions for missense variants and interpretation. Variant classification is similar to that of Varsome, except that if a variant has never previously been described and so is not in the database, the classification is considered as unknown.

gnomAD software reports the frequency of a variant in the general population. The lower the frequency, the higher the probability of pathogenicity.

GERP compares a sequence among a large number of animal species. The higher the conservation, the higher the probability for variants in this region to be pathologic.

Combining all these data and adding functional studies when available, we propose an interpretation for all variants, both those previously described and for our case.

Results

Patient diagnosis

As shown in Table 1, the patient had elevated levels of FSH (34.75 mIU/mL), LH (20.0 mIU/mL), and AMH (6.5 ng/mL). However, all other hormones assessed were within the normal range.

The patient karyotype was normal, and auto-antibody testing was negative, as also was her FMR1 screening. Investigation of the panel of genes revealed two *FSHR* missense variants, both of which were inherited from her parents with no associated phenotype. One variant, c.847C>T; p.(Arg283Trp), was considered VUS, while the other, c.1798C>A p.(Pro600Thr) was classified as pathological. As the patient was compound heterozygous for *FSHR* gene variants and her clinical profile was consistent with FSHR dysfunction, the diagnosis of GRS was confirmed.

IVF laboratory results

A total of 16 oocytes were retrieved. After 27 h in IVM medium, 7 oocytes were mature, 3 were at metaphase I, and 6 remained at the germinal vesicle stage. No additional oocytes were mature the following day.

After ICSI, all seven mature oocytes fertilized normally, exhibiting two pronuclei (100% fertilization rate). Table 2 shows the development, time-lapse morphokinetics, phenotypes, and fate of each zygote.

Of the 7 zygotes, 6 underwent at least one cleavage division, giving a cleavage rate of 85.7%. Two embryos were transferred on D2 (E N° 1 and N° 4). After extended embryo culture of the four remaining embryos, three blastocysts were obtained on D5 (75% blastocyst rate/per cleaved embryo), with one embryo arrested at the 5-cell stage (E N° 5). Of the three blastocysts, one was discarded (E N° 2) because of its low morphological grade (B4CC) and the other two were frozen (E N° 3 and N° 7).

Retrospective evaluation of the time-lapse videos revealed that embryos N° 1 and N° 4 both exhibited abnormal developmental progression, one showing direct cleavage and the other having chaotic cleavage. Embryo N° 2, which produced a low-grade blastocyst not eligible for freezing, exhibited vacuoles as early as the 15th hour post-insemination and also showed a direct cleavage. Embryo No. 3, which produced a moderate-grade blastocyst, was frozen. Embryo N° 5, which showed a long S2 (t4–t3 cells) and two reverse cleavages, became blocked in development. Embryo No. 6 failed to cleave, and while E No. 7 showed delayed development in the first three cleavage divisions (to 2, 3, and 4 cells); no further remarkable qualitative morphokinetic or phenotypic events were identified in this embryo (Table 2).

Embryo transfer outcomes and relationship to TL findings

Embryo N° 1 and N° 4 were transferred together fresh on D2; as noted above, both exhibited abnormal phenotypes and no pregnancy resulted. A frozen blastocyst transfer (FBT) of E N° 7 was performed 1 month later, which resulted in delivery of a healthy, full-term baby. Of note, this embryo presented with a cc2 (t3–t2 cells; 11.1 h) and so was considered as good prognosis by Meseguer criteria [34]. A second FBT was performed 2 years later with the same protocol, but no pregnancy

Table 1 Patient hormone levels with reference values

Hormone testing	Patient value	Normal range
Follicle-stimulating hormone (FSH)	34.8 mIU/mL	1–7 mIU/mL
Luteinizing hormone (LH)	20.0 mIU/mL	3–10 mIU/mL
Anti-Mullerian hormone (AMH)	AMH at 6.5 ng/mL	N > 1.5 ng/mL
Thyroid-stimulating hormone (TSH)	1.5 mIU/mL	0.4–4.4 mIU/mL
Prolactin	6.2 ng/mL	N < 25 ng/mL
Estradiol (E2)	27 pg/mL	N < 50 pg/mL
Testosterone	0.4 ng/mL	0.1–0.6 ng/mL
Inhibin B	55 pg/mL	N/A
TEBG sex-binding protein SHBG	69.8 nmol/mL	32.4–128.0 nmol/mL
Androstenedione-delta 4	1.5 ng/mL	0.4–2.8 ng/mL
17-Alpha-hydroxy progesterone	0.9 ng/mL	0.3–1.5 ng/mL

Table 2 Time-lapse morphokinetic and phenotypic characteristics of embryos

Events times (h)	E N° 1	E N° 2	E N° 3	E N° 4	E N° 5	E N° 6	E N° 7
PN appearance	10.6	8.6	7.3	7.6	13.8	8.75	9.3
PN disappearance	27.7	27.6	21.4	24.8	27.2	N/A	28.1
2 cells	32.1	N/A	24.0	N/A	30.9		37.0
3 cells	34.8	39.4	24.8		64.5		48.1
4 cells	N/A	43.9	26.7		99.9		49.4
5 cells		46.5	51.8	29.5	100.2		62.3
8 cells		63.9	77.3	N/A	N/A		82.3
Morula		77.8	N/I				NI
compaction		83.8	84.5				82.6
B1		102.3	100.8				108.5
B2		116.8	102.3				111.3
B3		120.7	106.1				113.7
B4		138.5	115.1				114.1
Grade at the time of transfer/ freezing		B4CC	B4BC				B4BA
Direct cleavage	Yes*	39.4	Yes*		N/A		NA
Chaotic cleavage	N/A	N/A	N/A	29.5	N/A		
Reverse cleavage	N/A	N/A	N/A	N/A	43.7		
					45.6		
Vacuoles	N/A	15.1	N/A	N/A	16		
Embryo fate	Fresh D2 Transferred	Discarded	Frozen/thawed and transferred	Fresh D5 Transferred	Discarded		Frozen/thawed and transferred
S2 = t4–t3	N/A	4.5	1.9	N/A	35.4		1.3
cc2 = t3–t2	2.8	N/A	0.8	N/A	33.6		11.1
Result after ET	No pregnancy	N/A	No pregnancy	No pregnancy	N/A		Live birth

Embryos were obtained after microinjection of in vitro matured oocytes and culture in the time-lapse incubator. Retrospective annotation was manually performed. t3, t4, t5, and t8 indicate the appearance time of the 3rd, 4th, 5th, and 8th cells, respectively. The time of 5 cells (stage and (t5) formulae for S2 and cc2 are those described by Meseguer et al. [34] to predict implantation (good prognosis when: t5 = [48.8–56.6 h], s2 ≤ 0.76 h, cc2 ≤ 11.9 h). Blastocysts were graded according to Gardner's classification [31]

N/A not applicable due to either the absence of cell stage because of abnormal cleavage or the arrest or termination of culture, *N/I* stage not identifiable

*Direct cleavage was defined as t3–t2 < 5 h

was achieved after transfer of the remaining blastocyst (E N° 3), despite it having good prognosis based on the t5 (51.8 h).

Discussion

Our patient case

We report here the case of a woman presenting with secondary amenorrhea associated with high plasma levels of gonadotropins (especially FSH), elevated AMH level, but normal sized ovaries with numerous small antral follicles. Although the diagnosis of premature ovarian failure (POF) was initially suspected, the discordance between her hormonal profiles (increased FSH in favor of POF, and the AFC and AMH in favor of PCOS) and the absence of response to exogenous FSH stimulation led to the diagnosis of GRS. After karyotype analysis, a genetic test with a panel of genes was performed, which revealed 2 missense *FSHR* variants, never previously described. These variants, both of which were inherited from her parents, led to the substitution of two amino acids in the EC domain (Arg283Trp) and in the third EC loop (Pro600Thr) of the FSHR. Collectively, these findings likely explain the GRS and the patient's FSHR deficiency.

Following this diagnosis and with her history of failed response to ovarian stimulation with FSH, we concluded that a third cycle of conventional IVF would be futile. We therefore opted to use IVM of retrieved immature oocytes, a proven protocol for cycle management of patients with GRS [2, 19, 28, 35].

Although less than 50% (7/16) of the patient's oocytes matured after IVM, all mature oocytes fertilized and one achieved full developmental competency after transfer of a blastocyst, as evidenced by live birth. Nevertheless, the remaining 6 mature oocytes of our patient were all associated with embryos exhibiting abnormal time-lapse morphokinetics and/or phenotypes.

Only a few previous studies have reported TL morphometry of embryos resulting from IVM, and results are conflicting. While some have shown that the atypical phenotypes described in TL were more frequently observed with IVM embryos [36], others found no difference [37]. However, these studies differ from our case because they used IVM with FSH priming and the clinical reason to perform IVM was not GRS but hyperstimulation syndrome risk. When evaluating retrospectively the atypical embryo phenotypes events in our patient cohort, we found that all three transferred embryos failing to achieve a pregnancy (N° 1, N° 3, and N° 4) showed events of poor prognosis. These events included direct cleavage (defined as the division of a blastomere into 3 daughter cells or the time between 3 and 2 cell stages of less than 5 h) or chaotic cleavage (appearance of a disordered cleavage between the zygote stage and the 4-cell stage). All these abnormal events have been associated with decreased implantation rates [38–40]. In contrast, the embryo that resulted in a live birth (N° 7) exhibited no abnormal phenotype.

Whether embryos derived from IVM in patients with GRS typically exhibit a similarly high incidence of abnormal developmental events as found in our patient remains to be determined. Furthermore, the possibility exists that the most recent advances in IVM technology, using a biphasic approach [41], may result in a higher yield of developmentally competent mature oocytes in this unusual population of patients.

Assessment of genotype-to-phenotype relationship in cases of FSHR mutation

Table 3 summarizes the *FSHR* variants with associated amino acid substitutions, pathogenicity, and phenotypes in the twelve published reports and in our patient. As shown, aside from the two novel variants we reported for our patient, 14 variants have been described [7, 10–20].

In the following discussion, we first address the relationship between the location of the variant in the FSHR and the functionality of the receptor. We then consider any association between the variant genotype and the patient phenotype. Finally, we assess the potential application of IVM in cases of FSHR mutations, specifically considering the results of tests that give insight into the severity of the pathology.

Relationship between the variant location in the FSHR and the functionality of the receptor

Excluding our patient case, six of the 14 reported variants are in the EC domain of which five have been tested for functionality (Ala189Val, Asn191Ile, Ile160Thr, Asp224Val, Pro348Arg) [10–13, 16]. All five showed absent or decreased signal transduction, 3 showed altered ligand binding (Ile160Thr, Asp224Val, Pro348Arg), and 3 showed altered receptor expression at the membrane level (Ala189Val, Ile160Thr, Pro348Arg). Collectively, these observations demonstrate that EC location is associated with severe downstream effects, as evident from the phenotypes of the patients involved, all of which we classified as pathological.

The EC variant that was not tested was Val221Gly as reported by Nakamura et al. [20]. This variant resulted from a heterozygous mutation and was present with the polymorphism variants Ala307Thr and Ser680Asn, which are known to be non-deleterious and sometimes associated with the PCOS phenotype [42, 43].

The second types of variant are those present on the EC loop and IC loop or in the transmembrane region, of which eight have been reported (Arg573Cys, Leu601Val, Ala419Thr, Pro519Thr, Pro587His, Ile418Ser, Asp408Tyr, Asn558His) [7, 12–15, 17–19]. Given these locations, it is logical to suggest that these variants may impact signal

Table 3 Summary of the different FSHR variants previously described

Publication	Genotype	Variant description		Variant frequency	Variant pathogenicity prediction		Variant conservation	Localization
		Exon	AA substitution	gnomAD	ClinVar	Varsome	GERP	
Aittomäki et al. [10], 1995	Homozygote	7	Ala189Val	0.0007	Pathological	VUS (10/1)	Yes	EC domain
Gromoll et al. [11], 1996	Heterozygote	7	Asn191Ile	Unknown	Unknown	VUS (10/1)	Yes	EC domain
Beau et al. [12], 1998	Heterozygote composite	6	Ile160Thr	0.00004	VUS	VUS (10/1)	Yes	EC domain
		10	Arg573Cys	0.00001	Pathological	VUS (9/2)	Yes	IC loop
Touraine et al. [13], 1999	Heterozygote composite	9	Asp224Val	0.00003	Likely pathological	VUS (10/1)	Yes	EC domain
		10	Leu601Val	0.00004	Likely pathological	VUS (10/1)	Yes	EC loop
Doherty et al. [14], 2002	Heterozygote composite	7	Ala189Val	0.0007	Pathological	VUS (10/1)	Yes	EC domain
		10	Ala419Thr	0.000004	Pathological	VUS (10/1)	Yes	Transmembrane
Meduri et al. [15], 1996	Homozygote	10	Pro519Thr	0.000004	Pathological	VUS (9/2)	Yes	EC loop
Allen et al. [16], 2003	Homozygote or heterozygote composite with a FSHR microdeletion	10	Pro348Arg	0.000004	Likely pathological	VUS (10/1)	Yes	EC domain
Kuechler et al. [17], 2010	Heterozygote composite with a FSHR microdeletion due to an unbalanced t(2;8)(p16.3or21;p23.1)mat	10	Pro587His	Unknown	Likely pathological	Likely pathological (10/1)	Yes	Transmembrane
Katari et al. [18], 2015	Homozygote	10	Ile418Ser	Unknown	Unknown	VUS (11/0)	Yes	Transmembrane
Bramble et al. [7], 2016	Homozygote	10	Asp408Tyr	Unknown	Unknown	VUS (11/0)	Yes	Transmembrane
Flageole et al. [19], 2019	Heterozygote composite	6	Ile160Thr	0.00004	VUS	VUS (10/1)	Yes	EC domain
		10	Asn558His	0.00002	VUS	VUS (7/4)	Yes	EC loop
Nakamura et al. [20], 2008	Heterozygous mutation with 2 polymorphisms	8	Val221Gly	Unknown	Likely pathological	VUS (5/6)	Neutral	EC domain
		10	Ala307Thr Ser680Asn	Up to 0.2	Benign	Benign (9/0)	Neutral	IC domain
Our case report	*Heterozygote composite*	*9*	*Arg283Trp*	*0.0006*	*Unknown*	*VUS (6/5)*	*Neutral*	*EC domain*
		10	*Pro600Thr*	*Unknown*	*Unknown*	*VUS (10/1)*	*Yes*	*EC loop*

Publication	Activity comparison between missense variant and wild type			Interpretation	Clinical description		Hormonal levels		
	Level of expression of membrane receptor	Extent of ligand binding affinity	Transduction signal compared to wild type		Type of amenorrhea	Follicular appearance	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)
Aittomäki et al. [10], 1995	Lower	Normal	Absent	Pathological	Primary	Unknown	Unknown	Unknown	Unknown
Gromoll et al. [11], 1996	Unknown	Unknown	Absent	Pathological	No amenorrhea	Normal	Normal	Normal	Normal

Table 3 (continued)

Beau et al. [12], 1998	Absent Normal	Lower Normal	Lower Lower	Pathological Pathological	Secondary oligomenorrhea	5 mm	108.0	80.5	20–40
Touraine et al. [13], 1999	Absent Normal	Absent Normal	Absent Lower	Pathological Pathological	Primary	3 mm	63.0	26.0	40.0–80.0
Doherty et al. [14], 2002	Lower Normal	Normal Normal	Absent Absent	Pathological Pathological	Primary	Very small	91	38	0.1
Meduri et al. [15], 1996	Absent	Absent	Absent	Pathological	Primary	Primordial follicles	67.0	21.0	<10
Allen et al. [16], 2003	Unknown	Absent	Absent	Pathological	Primary	No follicles	105.0	36.3	76
Kuechler et al. [17], 2010	Unknown	Unknown	Absent	Likely pathological	Primary	Primordial follicles	121.8	47.8	13.3–10.7
Katari et al. [18], 2015	Unknown	Unknown	Unknown	Likely pathological	Primary	Small follicles	104.6	37.1	22–20
Bramble et al. [7], 2016	Lower	Unknown	Lower	Pathological	Primary	No or only primordial follicles	67.5	29.6	9.5
Flageole et al. [19], 2019	Absent Normal	Unknown Unknown	Absent Normal	Pathological VUS	Spaniomenorrhea	Abnormally large follicles	55.0	33.0	Unknown
Nakamura et al. [20], 2008	Unknown Normal	Unknown Normal	Unknown Normal	VUS Benign	Primary	Small antral follicles ≤8 mm	11.0	14.2	199.6
Our case report	*Unknown* *Unknown*	*Unknown* *Unknown*	*Unknown* *Unknown*	*VUS* *Likely pathological*	*Secondary*	*Antral follicles*	*34.8*	*20.0*	*27*

The different FSHR variants previously described, with the resulting amino acid substitution, their pathogenicity (determined by Varsome, ClinVar, and gnomAD software programs and/or by heterologous cellular models), and the patient's phenotypes. The numbers in parentheses for the Varsome pathogenicity prediction indicate the numbers of software programs predicting the pathogenicity of the total number of software programs that analyzed the data. The "Interpretation" column corresponds to our interpretation obtained by combining the predictive prognoses of the different software programs and functional studies carried out on heterologous cell models when available. There are four patients with a homozygous substitution, four other patients with heterozygote composite, one heterozygote with no fertility or cycle disorders, and three other patients associating a heterozygote substitution to another genetic defect. Our patient's case is presented in italics

AA amino acid, *EC* extracellular, *IC* intracellular, *VUS* variant of unknown significance

transduction, and not ligand binding since the binding site of the FSHR to its ligand is in the EC domain [6]. This suggestion is supported by the observation that signal transduction was altered for 6 of the 7 variants tested (Arg573Cys, Leu601Val, Ala419Thr, Pro519Thr, Pro587His, Asp408Tyr), the exception being the Asn558His variant [7, 12–15, 17]. Receptor expression was normal for 4 of the 6 tested (Arg573Cys, Leu601Val, Ala419Thr, Asn558His), and ligand affinity was normal for 3 out of the 4 tested (Arg573Cys, Leu601Val, Ala419Thr). The abnormal ligand binding in one of the four variants tested (Pro519Thr) was not due to altered affinity, but rather to alteration of cell surface targeting of the mutated receptor, such that the receptor remained trapped intracellularly [15]. Overall, the functionality of the 7 variants tested showed an alteration.

Association between the variant genotype and the patient phenotype

To simplify this analysis, we included only those patients either homozygous or heterozygous composite for *FSHR* variants or with neither classified as benign. Accordingly, two cases were excluded [11, 20]. In the case reported by Gromoll et al. [11], only one heterozygous variant was found (Asn191Ile); in the case reported by Nakamura et al. [20], one variant (Val221Gly) was classified as VUS and the other two (Ala307Thr and Ser680Asn) as benign.

Of note, the Ala307Thr and Ser680Asn variants in the case reported by Nakamura et al. are considered to be in genetic disequilibrium, frequently in cis, and on the same allele and are conserved at high frequency (under 0.2%) in the general population. It is therefore unlikely that this patient's primary infertility is associated with the combination of all 3 variants. However, a possible negative impact of a pathogenic Val221Gly variant on the wild-type allele cannot be ruled out, which led us to classify this variant as VUS, and which raises the question whether a homozygous mutation involving Val221Gly substitution may result in a more severe phenotype of GRS.

Of the eleven cases retained for our primary analysis of any association between FSHR variant genotype and phenotype, eight of them presented with primary amenorrhea, with absent or small follicles as the most frequent phenotype [7, 10, 13–18]. Six of these are homozygote [7, 10, 15, 18] or considered as homozygote because the other variant involved a *FSHR* gene deletion [16, 17]. The other two patients with primary amenorrhea [13, 14] exhibited two heterozygote composite variants considered to be pathologic, as largely predicted by ClinVar and by Varsome, which made this prediction from 10 of the 11 software programs used.

For the three patients without primary amenorrhea, all carry a FSHR heterozygote composite mutation ([12, 19] and our case). In both the cases reported by Beau et al. [12] and Flageole et al. [19], the same variant classified as pathologic was present (Ile160Thr), although this was associated with a different variant (Arg573Cys and Asn558His, respectively). However, in the case reported by Beau et al. [12], FSHR signal transduction for the Arg573Cys variant was identified as lower, but not absent, and with normal membrane expression and ligand-binding affinity. Thus, despite this variant being considered pathologic, these collective observations may explain the presentation of the patient with secondary oligomenorrhea. In the case reported by Flageole et al. [19], the Asn558His variant showed normal receptor activity and was classified as VUS, which is consistent with her clinical profile of spaniomenorrhea and abnormally large follicles. Similarly, in our patient case, one of her two variants (Arg283Trp) was classified as VUS, which may also explain her presentation of secondary amenorrhea.

Possible application of IVM

Based on our analyses above, we propose an algorithm for identifying patients with the various FSHR variants and phenotypes who are suitable candidates for use of IVM (Table 4). For patients with primary amenorrhea and a homozygote or heterozygote composite for a pathologic or likely pathologic variant, IVM in the management of their primary infertility is unlikely to be successful. In contrast, for patients with secondary amenorrhea or spaniomenorrhea [19] and/or a FSHR heterozygote composite mutation for at least one VUS (our patient case and [44]) and/or the presence of partial FSHR signal transduction [19], even if very low [12], IVM should be attempted. Furthermore, in patients with primary amenorrhea with gonadotropin levels in the menopausal range but AMH and AFC values both in the normal range and who are unresponsive to exogenous gonadotropin stimulation, IVM may be beneficial even when no FSHR variant is identified [2]. Similarly, IVM may be beneficial in the setting of secondary amenorrhea and normal AMH and AFC in combination with failure to respond to follicular stimulation, when either no FSHR genetic analysis is performed or when no variant is identified [28].

Conclusion

In conclusion, our case report describes a composite heterozygous variant of the FSHR that has not previously been described, which was associated with GRS. Our management strategy including IVM and the transfer of a frozen-thawed blastocyst in a hormonal replacement therapy cycle led to the birth of a healthy baby. That this embryo was the only one of seven not exhibiting abnormal phenotypes raises the possibility that IVM in this patient population may require further advancements, perhaps including a biphasic approach or other

Table 4 Proposed decision algorithm for use of IVM in the management of infertile patients with GRS

Type of amenorrhea	FSHR genotype	Interpretation of variant	IVM candidate
Primary	Homozygous	Pathologic or likely pathologic	No
	Heterozygous composite	Pathologic or likely pathologic	
	Heterozygous composite	Pathologic or likely pathologic, but with presence of residual FSHR signal transduction	Yes
	Homozygous	VUS or benign	
	Heterozygous composite	At least 1 VUS or benign	
	No variant found	N/A	
	Unknown	N/A	
Secondary	Any	Any	
Spaniomenorrhea	Any	Any	

N/A not applicable

fine-tuning. Analysis of our patient case with the twelve cases of GRS reported in the literature has allowed us to associate FSHR variant genotype with location on the FSHR, functionality of the tested variants, and their association with the type of amenorrhea. We propose an algorithm for application of IVM for patients exhibiting GRS. Further research is required to confirm the utility of this IVM algorithm in cases of this rare pathology.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Renato Fanchin, Meryem Filali Baba, Catherine Racowsky, François Vialard, Paul Pirtea, Camille Fossard, Jessica Vandame, Jean Marc Ayoubi, and Marine Poulain. The first draft of the manuscript was written by Achraf Benammar, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval The study was approved by the Ethics Committee of Hôpital Foch, with the Institutional Review Board Code of 00012437.

Consent for publication The patient consented to publication of her case.

Competing interests The authors declare no competing interests.

References

1. Jones GS, De Moraes-Ruehsen M. A new syndrome of amenorrhae in association with hypergonadotropism and apparently normal ovarian follicular apparatus. Am J Obstet Gynecol. 1969;104:597–600.
2. Grynberg M, Peltoketo H, Christin-Maître S, Poulain M, Bouchard P, Fanchin R. First birth achieved after in vitro maturation of oocytes from a woman endowed with multiple antral follicles unresponsive to follicle-stimulating hormone. J Clin Endocrinol Metab. 2013;98(11):4493–8.
3. Richards JS. Hormonal control of gene expression in the ovary. Endocr Rev. 1994;15(6):725–51.
4. LaPolt PS, Tilly JL, Aihara T, Nishimori K, Hsueh AJ. Gonadotropin-induced up- and down-regulation of ovarian follicle-stimulating hormone (FSH) receptor gene expression in immature rats: effects of pregnant mare's serum gonadotropin, human chorionic gonadotropin, and recombinant FSH. Endocrinology. 1992;130(3):1289–95.
5. Tilly JL, LaPolt PS, Hsueh AJ. Hormonal regulation of follicle-stimulating hormone receptor messenger ribonucleic acid levels in cultured rat granulosa cells. Endocrinology. 1992;130(3):1296–302.
6. Jiang X, Liu H, Chen X, Chen PH, Fischer D, Sriraman V, et al. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. Proc Natl Acad Sci U S A. 2012;109(31):12491–6.
7. Bramble MS, Goldstein EH, Lipson A, Ngun T, Eskin A, Gosschalk JE, et al. A novel follicle-stimulating hormone receptor mutation causing primary ovarian failure: a fertility application of whole exome sequencing. Hum Reprod. 2016;31(4):905–14.
8. Sprengel R, Braun T, Nikolics K, Segaloff DL, Seeburg PH. The testicular receptor for follicle-stimulating hormone: structure and functional expression of cloned cDNA. Mol Endocrinol. 1990;4:525–30.
9. Heckert LL, Daley IJ, Griswold G. Structural organization of the follicle-stimulating hormone receptor gene. Mol Endocrinol. 1992;6:70–80.
10. Aittomäki K, Lucena JLD, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, et al. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell. 1995;82:959–68.
11. Gromoll J, Simoni M, Nordhoff V, Behre HM, De Geyter C, Nieschlag E. Functional and clinical consequences of mutations in the FSH receptor. Mol Cell Endocrinol. 1996;125(1-2):177–82.
12. Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. J Clin Invest. 1998;102:1352–9.
13. Touraine P, Beau I, Gougeon A, Meduri G, Desroches A, Pichard C, et al. New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. Mol Endocrinol. 1999;13:1844–54.
14. Doherty E, Pakarinen P, Tiitinen A, Kiilavuori A, Huhtaniemi I, Forrest S, et al. A novel mutation in the FSH receptor inhibiting

signal transduction and causing primary ovarian failure. J Clin Endocrinol Metab. 2002;87(3):1151–5.
15. Meduri G, Hai MTV, Jolivet A, Takemori S, Kominami S, Driancourt MA, et al. Comparison of cellular distribution of LH receptors and steroidogenic enzymes in the porcine ovary. J Endocrinol. 1996;148:435–46.
16. Allen LA, Achermann JC, Pakarinen P, Kotlar TJ, Huhtaniemi IT, Jameson JL, et al. A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics. Hum Reprod. 2003;18(2): 251–6.
17. Kuechler A, Hauffa BP, Köninger A, Kleinau G, Albrecht B, Horsthemke B, et al. An unbalanced translocation unmasks a recessive mutation in the follicle-stimulating hormone receptor (FSHR) gene and causes FSH resistance. Eur J Hum Genet. 2010;18(6): 656–61.
18. Katari S, Wood-Trageser MA, Jiang H, Kalynchuk E, Muzumdar R, Yatsenko SA, et al. Novel inactivating mutation of the FSH receptor in two siblings of Indian origin with premature ovarian failure. J Clin Endocrinol Metab. 2015;100(6):2154–7.
19. Flageole C, Toufaily C, Bernard DJ, Ates S, Blais V, Chénier S, et al. Successful in vitro maturation of oocytes in a woman with gonadotropin-resistant ovary syndrome associated with a novel combination of FSH receptor gene variants: a case report. J Assist Reprod Genet. 2019;36(3):425–32.
20. Nakamura Y, Maekawa R, Yamagata Y, Tamura I, Sugino N. A novel mutation in exon8 of the follicle-stimulating hormone receptor in a woman with primary amenorrhea. Gynecol Endocrinol. 2008;24(12):708–12.
21. Veeck LL, Wortham JW Jr, Witmyer J, Sandow BA, Acosta AA, Garcia JE, et al. Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. Fertil Steril. 1983;39:594–602.
22. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod. 2005;20:420–4.
23. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertil Steril. 2001;76:936–42.
24. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod. 1999;14:1847–51.
25. Wynn P, Picton HM, Krapez JA, Rutherford AJ, Balen AH, Gosden RG. Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by in-vitro maturation. Hum Reprod. 1998;13:3132–8.
26. Grynberg M, Poulain M, le Parco S, Sifer C, Fanchin R, Frydman N. Similar in vitro maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum Reprod. 2016;31(3): 623–9.
27. Liu J, Lu G, Qian Y, Mao Y, Ding W. Pregnancies and births achieved from in vitro matured oocytes retrieved from poor responders under undergoing stimulation in in vitro fertilization cycles. Fertil Steril. 2003;80:447–9.
28. Galvão A, Segers I, Smitz J, Tournaye H, De Vos M. In vitro maturation (IVM) of oocytes in patients with resistant ovary syndrome and in patients with repeated deficient oocyte maturation. J Assist Reprod Genet. 2018;35(12):2161–71.
29. Hourvitz A, Maman E, Brengauz M, Machtinger R, Dor J. In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities". Fertil Steril. 2010;94(2):496–501.
30. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270–83.
31. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond 1999. Carnforth: Parthenon Press; 1999. p. 378–88.
32. ESHRE, Working group on Time-lapse technology, Apter S, Ebner T, Freour T, Guns Y, et al. Good practice recommendations for the use of time-lapse technology†. Hum Reprod Open. 2020;2: hoaa008.
33. Ramos NN, Pirtea P, Benammar A, Ziegler D, Jolly E, Frydman R, et al. Is there a link between plasma progesterone 1-2 days before frozen embryo transfers (FET) and ART outcomes in frozen blastocyst transfers? Gynecol Endocrinol. 2020;30:1–4.
34. Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26(10):2658–71.
35. Li Y, Pan P, Yuan P, Qiu Q, Yang D. Successful live birth in a woman with resistant ovary syndrome following in vitro maturation of oocytes. J Ovarian Res. 2016;9(1):54.
36. Roesner S, Dietrich JE, Weigert J, Montag M, Toth B, Strowitzki T. Time-lapse imaging reveals differences in growth dynamics of embryos after in vitro maturation compared with conventional stimulation. Fertil Steril. 2017;107(3):606–612.e3.
37. Dal Canto M, Novara PV, Coticchio G, Mignini Renzini M, Brambillasca F, Brigante C, et al. Morphokinetics of embryos developed from oocytes matured in vitro. J Assist Reprod Genet. 2016;33(2):247–53.
38. Liu Y, Chapple V, Roberts P, Matson P. Prevalence, consequence, and significance of reverse cleavage by human embryos viewed with the use of the Embryoscope time-lapse video system. Fertil Steril. 2014;102:1295–300.e2.
39. Athayde Wirka K, Chen AA, Conaghan J, Ivani K, Gvakharia M, Behr B, et al. Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development. Fertil Steril. 2014;101(6):1637–1648.e1-5.
40. Barrie A, Homburg R, McDowell G, Brown J, Kingsland C, Troup S. Preliminary investigation of the prevalence and implantation potential of abnormal embryonic phenotypes assessed using time-lapse imaging. Reprod BioMed Online. 2017;34(5):455–62.
41. Sanchez F, Le AH, Ho VNA, Romero S, Van Ranst H, De Vos M, et al. Biphasic in vitro maturation (CAPA-IVM) specifically improves the developmental capacity of oocytes from small antral follicles. J Assist Reprod Genet. 2019;36(10):2135–44.
42. d'Alva CB, Serafini P, Motta E, Kohek MB, Latronico AC, Mendonca BB. Absence of follicle-stimulating hormone receptor activating mutations in women with iatrogenic ovarian hyperstimulation syndrome. Fertil Steril. 2005;83(6):1695–9.
43. Du J, Zhang W, Guo L, Zhang Z, Shi H, Wang J, et al. Two FSHR variants, haplotypes and meta-analysis in Chinese women with premature ovarian failure and polycystic ovary syndrome. Mol Genet Metab. 2010;100(3):292–5.
44. Kornilov NV, Pavlova MN, Yakovlev PP. The live birth in a woman with resistant ovary syndrome after in vitro oocyte maturation and preimplantation genetic testing for aneuploidy. J Assist Reprod Genet. 2021 Jan;26.