Clinical research

Follicle-stimulating hormone receptor gene polymorphism in Albanian women

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Abstract

Introduction: Several parameters have been postulated as predictors of ovarian response (inhibin B, 17β-estradiol and anti-Müllerian hormone. Consequently, the variants of FSHR were explored and they may be involved in the role of FSH receptor in mediated signal transduction and with ovarian response in infertile women submitted to ovarian stimulation. The aim of the study was to investigate association of Asn680Ser FSHR polymorphism with the ovarian response in 104 women of Albanian ethnic population enrolled in ICSI program.
Material and methods: Analysis of the Asn680Ser polymorphism was performed using TaqMan® SNP Genotyping Assay. Clinical and endocrinologic parameters were analyzed based on the genotype, age, body mass index (BMI), oocyte yield, number of transferred embryos and pregnancy rate.
Results: The frequencies of genotypes were: Asn/Asn 22.1%, Asn/Ser 47.1%, and Ser/Ser 30.8%. Body mass index was significantly higher in the Ser/Ser as compared to the Asn/Ser (p = 0.0152) or the Asn/Asn group (p = 0.0014). Basal estradiol (bE2) levels showed statistically significant difference (0.0308) between the genotype variants. Correlation analysis showed statistically significant (p < 0.0001) negative correlation of oocyte retrieval number in respect to age, bFSH (basal FSH) stimulation length and gonadotropin dose.
Conclusions: The results in the present study provide new addition to the understanding of the impact of FSHR genotype variants on controlled ovarian stimulation. FSH receptor polymorphism is associated with different ovarian response to controlled ovarian stimulation (COS), but is not an important factor in increasing the degree of pregnancy.

Key words: follicle stimulating hormone, polymorphism, Asn680Ser, controlled ovarian stimulation.

Introduction

Despite attempts to standardize controlled ovarian stimulation (COH) regimens for women undergoing assisted reproduction programs (ART), they commonly experience either poor ovarian responses or ovarian hyperstimulation syndrome (OHSS) [1]. The patient response is individualized and the ovarian response to intense gonadotrophin stimulation is difficult to predict even in those with similar endocrine profiles. Therefore, determining the dose of exogenous gonadotropin to attain the optimum patient response thus avoiding a serious and potentially life-threatening complication of OHSS or insufficient stimulation and cy-

cle cancelation in poor responders is one of the ongoing challenges in the field of infertility management [1]. Follicle-stimulating hormone (FSH) (a key marker of ovarian reserve and the best-known predictor of COH response) plays a pivotal role in ovarian function, where its main effects are related to granulosa cell proliferation, oocyte maturation and estrogen synthesis via activation of the aromatase gene [1]. Since the secretion of FSH is in a negative feedback loop with the action of FSHR, the basal day 3 serum FSH levels (bFSH, one of the best predictive markers of ovarian reserve) are often indicative of the function of its receptor and could vary depending on the patient FSHR genotype background. This has led to the investigation of their potential value as predictors of ovarian response to an exogenous stimulation. Since the first report on the polymorphism of the FSHR gene in 1995, numerous activating and inactivating mutations and SNPs in coding (8 SNPs) and non-coding (> 1300 SNPs) regions of the FSHR have been described in both men and women of different ethnic backgrounds [1–6]. Two very common SNPs present at coding positions p.Thr307Ala (rs6165) and p.Asn680Ser (rs6166) in exon 10 are currently most extensively studied to assess the response of the FSHR receptor to FSH stimulation [1–6]. Because the Thr307 allele is almost always in linkage disequilibrium with Asn680 (Thr307-Asn680), and Ala307 almost always with Ser680 (Ala307-Ser680), and the frequency distribution of these isoforms in various populations is predominant, most studies are focused solely on p.Asn680Ser (rs6166) [1–6]. Several reports have shown that these two SNPs are associated with ovarian response in IVF but the findings are conflicting [1–6]. Some authors have reported predictability of the ovarian response to FSH stimulation in patients with different alleles, while others have refuted this finding [1–6]. One study suggested that the effects of the FSHR polymorphism are independent of ethnic background [5]. In order to verify this, in the present study we examined, for the first time, the prevalence of Asn680Ser genotype variants in a population of Albanian women from the Kosovo Dukagjin region who participated in an IVF/ICSI program. Furthermore, we investigated the association between the receptor polymorphism and subjects' hormonal profiles and correlated the variants with clinical characteristics and ovarian response in "poor responder" (PR) and "good responder" (GR) patient groups.

Material and methods

Subjects

The present study included 104 prospectively recruited female patients of an Albanian ethnic population from the Kosovo Dukagjin region enrolled in ICSI procedures at the Polyclinic – IVF Center, Peje, Republic of Kosovo, in the period from January 2010 to February 2012. All patients were otherwise healthy women with functioning ovulation and no history of endocrine diseases or known family history of inherited diseases. The cause of infertility in all cases was identified as the male factor. Informed consent was obtained from all the participants, and the study was approved by the local Medical Ethics Review Committee at the Faculty of Medicine, University of Prishtina, Republic of Kosovo.

Hormonal assays

Serum levels (day 3 of the menstrual cycle) of FSH, LH, estradiol (E2), prolactin, progesterone, and thyroid-stimulating hormone (TSH) were measured by enzyme-linked fluorescent assay (ELFA) using a bioMerieux Mini Vidas Automated Immunoassay Analyzer (bioMérieux S.A. 69280 Marcy l'Etoile, France). The peak E2 levels were measured on the day of human choriogonadotropin (HCG) administration.

Treatment

In all cases, controlled ovarian stimulation was performed according to the standard long protocol procedure as previously described. In general, transvaginal ultrasound (US) was performed to ascertain ovarian quiescence on the first 3 days of menses, and controlled ovarian stimulation was then initiated. Follicular development was monitored by transvaginal sonography, after 5 days of stimulation and then every other day. Exogenous FSH (Gonal F) was applied in a daily dosage of from three to six vials (225–450 IU), depending on the patient's previous or anticipated response, age, bFSH, basic luteinizing hormone (bLH), antral follicle count (AFC) and body mass index. The gonadotropin-releasing hormone (GnRH) antagonist ganirelix 0.25 mg (Orgalutran, NV Organon, The Netherlands) was administered daily from the day the leading follicle reached a diameter of 14 mm to prevent premature luteinizing hormone rises and luteinization. Ovulation was triggered by the application of 10,000 IU of HCG (Pregnyl, NV Organon, The Netherlands), when at least two follicles scored 17–18 mm in diameter and transvaginal ultrasound was performed to measure endometrial thickness. Oocyte retrieval was performed 34–36 h after HCG injection by transvaginal ultrasound-guided follicle aspiration under mild sedation and analgesia. Luteal support was provided by progesterone (Utrogestan; Besins International, Montrouge, France), which was introduced after oocyte aspiration and extended to 8 weeks, or

until the initiation of menses. Sperm and oocyte preparation, intra-cytoplasmic sperm injection (ICSI) fertilization, embryo culture, and transfer were performed according to the conventional IVF laboratory guidelines. The clinical pregnancy was confirmed 1–2 weeks later by transvaginal ultrasound detection of the gestational sac. Complete pregnancy follow-up of patients was performed by their private obstetricians in the majority of cases.

DNA isolation and genotyping

Genomic DNA was extracted from 250 µl of whole blood using a PureLink Genomic DNA Mini Kit (Invitrogen, Life Technologies Corporation, Carlsbad, California, USA) according to the manufacturer's instructions. Analysis of the FSHR gene polymorphism at position 680 was carried out using a predesigned TaqMan SNP Genotyping Assay (rs6166; Life Technologies Corporation, Carlsbad, California, USA). Real-time PCR was performed using the TaqMan Universal master mix II and Applied Biosystems 7500 Real-Time PCR System (Life Technologies Corporation, Carlsbad, California, USA) in accordance with the manufacturer's instructions. The analysis was carried out in accordance with the instructions for the device used.

Statistical analysis

Before statistical analysis, data were tested for normal distribution using the D'Agostino-Pearson omnibus test. Variables are presented as the mean ± standard deviation (SD) if distributed normally or as the median and range for nonparametrically distributed variables. Genotype and allele frequencies were analyzed by the chi-square (χ^2) test. Group comparisons were performed by the unpaired *t* test and one-way ANOVA (analysis of variance) or nonparametric Kruskal-Wallis and Mann-Whitney test for unpaired data. Correlation between two variables was ascertained by linear regression analysis and Pearson correlation coefficient calculation for parametrically distributed variables, while Spearman's correlation test was used for nonparametrically distributed variables. The influence of two different variables on oocyte retrieval number was determined by two-way ANOVA. Statistical analysis was performed by applying a commercially available software package (GraphPad Prism, GraphPad Software, San Diego, CA). All statistical tests were two-sided and a conventional value of $p < 0.05$ was used to represent statistical significance.

Results

A total of 104 patients underwent the ICSI procedure and were genotyped in this study. The frequencies of the Asn680Ser genotype variants were as follows: Asn/Asn 22.1%, Asn/Ser 47.1%, and Ser/Ser 30.8% (Table I). Clinical and endocrinologic parameters were analyzed based on the genotype variants (Table II), age (Table III), BMI (Table IV) and oocyte retrieval number (Table V). The mean ages of the three genotype groups were similar, and bFSH, bLH, bFSH/bLH, bE2, prolactin, progesterone, TSH level as well as peak E2 levels also showed no differences between the FSHR genotype variants (Table II). No difference was also found between the genotype groups either in terms of AFC, amount of the FSH required for ovulation induction, stimulation period days, number of dominant follicles, oocyte retrieval number or endometrial thickness (Table II). Body mass index was significantly higher in the Ser/Ser group as compared to the Asn/Ser ($p = 0.0152$) or the Asn/Asn group ($p = 0.0014$) (Table II). However, apart from significantly ($p = 0.0404$) higher prolactin levels in the overweight (BMI ≥ 25 kg/m^2) group, no statistically significant differences were found in any other clinical and endocrinologic parameter between the two BMI groups (Table IV). Although the overall analysis of bE2 levels between the three genotype variants showed a slight but statistically significant difference ($p = 0.0308$), Dunn's multiple comparison test resulted in non-significant *p*-values (Table II). No statistically significant difference was found between the analyzed age groups, in terms of BMI, bFSH, bLH, bFSH/bLH, prolactin, bE2, progesterone, TSH levels or number of dominant follicles (Table III). The AFC, amount of the FSH required for ovulation induction, stimulation length, number of dominant follicles and the peak E2 levels as well as oocyte retrieval and embryo transfer number were significantly different among analyzed age groups (Table III). Group III showed a statistically significant decrease in AFC compared to group I ($p = 0.0012$). The amount of FSH required for ovu-

Table I. SNP genotyping of Asn680Ser FSH gene in 104 patients in the study

SNP	Allele frequency % (*n*)		Genotype frequency % (*n*)		Clinical pregnancy % (*n*)
Ser680Asn	A (N)	45.67 (95)	AA (NN)	22.12 (23)	33.3 (10)
	G (S)	54.33 (113)	AG (NS)	47.12 (49)	43.3 (13)
			GG (SS)	30.77 (32)	23.3 (7)

n – number, A (N) – asparagine, G (S) – serine, AA (NN) – asparagine/asparagine, AG (NS) – asparagine/serine, GG (SS) – serine/serine.

Table II. Clinical and endocrine characteristics of patients with respect to the SNP Asn680Ser FSHR gene

Clinical and endocrinologic parameters	NN (*N* = 23)	NS (*N* = 49)	SS (*N* = 32)	*P*-value
Age [years]	33.65 ±6.278	34.06 ±5.528	32.38 ±5.707	0.4308
BMI [kg/m^2]	22.00 (20.60–24.70)*	22.30 (20.20–25.10)**	26.10 (22.88–27.70)*,**	0.0010
bFSH [mIU/ml]	10.01 (7.60–12.16)	10.20 (8.58–11.92)	10.28 (8.32–12.18)	0.8530
bLH [mIU/ml]	5.11 (4.56–5.82)	5.56 (4.755–6.370)	5.415 (4.553–5.878)	0.3606
bFSH/b LH	1.976 (1.720–2.256)	1.863 (1.666–2.181)	1.961 (1.754–2.111)	0.6532
bProlactin [ng/ml]	14.50 (7.20–20.00)	17.40 (12.02–20.75)	16.41 (9.50–20.58)	0.2403
bEstradiol [pmol/l]	46.88 (36.75–55.09)	42.90 (34.5–61.20)	33.18 (26.25–45.53)	0.0308
bProgesterone [ng/ml]	0.740 (0.610–1.080)	1.0 (0.780–1.205)	0.840 (0.6755–1.035))	0.0855
bTSH [µIU/ml]	2.030 (1.14–2.67)	1.76 (1.055–2.43)	1.780 (1.183–2.313)	0.7393
AFC	7.043 ±1.965	7.122 ±1.822	6.625 ±2.379	0.5474
FSH amount needed for ovulation induction [IU]	2482 ±467.8	2441 ±532.7	2466 ±481.5	0.9444
Length of stimulation [days]	12.57 ±0.6624	12.51 ±0.8447	12.66 ±0.8654	0.7336
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	4.565 ±1.674	4.551 ±1.542	3.813 ±1.693	0.1020
Estradiol on day of HCG administration [pg/l]	1746 ±811.0	1782 ±681.0	1467 ±627.3	0.1234
Endometrial thickness on day of HCG administration [mm]	8.50 (8.00–8.80)	8.40 (8.00–9.00)	8.30 (7.725–8.875)	0.6474
Number of oocytes retrieved	4.174 ±1.946	4.633 ±1.845	3.906 ±1.653	0.1997
Number of transferred embryos	2 (1–3)	3 (2–3)**	2 (1–2)**	0.0101
Pregnancy rate	43.48 (10/23)	26.53 (13/49)	21.875 (7/32)	0.19398

**, ** Values that are significantly different between the groups (Dunn's test).*

Table III. Clinical and endocrinologic parameters in relation to age of patients

Clinical and endocrinologic parameters	Group I (< 32) *N* = 37	Group II (32–36) *N* = 35	Group III (> 36) *N* = 32	*P*-value
BMI [kg/m^2]	23.83 ±3.283	24.00 ±3.20	23.11 ±3.634	0.5235
bFSH [mIU/ml]	9.870 (7.965–11.15)	10.02 (8.20–12.03)	10.65 (9.805–12.55)	0.0969
bLH [mIU/ml]	5.120 (4.270–5.810)	5.410 (4.80–6.950)	5.590 (4.890–6.163)	0.2038
bFSH/b LH	1.959 (1.7908–2.242)	1.779 (1.558–2.097)	1.946 (1.761–2.232)	0.0843
bProlactin [ng/ml]	15.30 (11.05–22.10)	17.40 (8.30–20.70)	15.60 (10.25–20.05)	0.7304
bEstradiol [pmol/l]	39.10 (28.75–48.51)	41.70 (30.20–57.20)	47.00 (30.70–67.78)	0.3673
bProgesterone [ng/ml]	0.820 (0.650–1.080)	0.910 (0.70–1.120)	0.90 (0.780–1.225)	0.6779
bTSH [µIU/ml]	1.637 ±0.7061	2.049 ±0.8150	1.775 ±0.8099	0.0789 ANOVA Tukey
AFC	7.703 ±2.308**	7.029 ±1.902	6.00 ±1.391**	0.0018
FSH amount needed for ovulation induction [IU]	2250 (1763–2625)****/*	2400 (2400–3000)*	2775 (2400–3000)****	< 0.0001
Length of stimulation [days]	12.24 ±0.8946	12.60 ±0.6945**	12.91 ±0.6891**	0.0024
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	4.514 ±1.592	4.571 ±1.754	3.844 ±1.505	0.1328
Estradiol on day of HCG administration [pg/l]	1864 ±651.8**	1796 ±710.6*	1331 ±645.8**/*	0.0028
Endometrial thickness [mm]	8.70 (8.20–9.450)**	8.50 (8.00–9.00)	8.150 (7.80–8.50)**	0.0090
Number of oocytes retrieved	4.919 ±1.862***	4.514 ±1.704*	3.375 ±1.561***/*	0.0011
Number of transferred embryos	2.514 ±0.6065***	2.314 ±0.7581*	1.844 ±0.7233***/*	0.0005
Pregnancy rate	35.14% (13/37)	34.29% (12/35)	15.625% (5/32)	0.139457

//* Values are significantly different between groups (Dunn's test or Tukey test).*

Table IV. Clinical and endocrine characteristics of patients with respect to BMI

Clinical and endocrinologic parameters	BMI ≤ 25 kg/m² *N* = 69	BMI > 25 kg/m² *N* = 35	*P*-value
Age [years]	34 (28–39)	33 (27–36)	0.3994
bFSH [mIU/ml]	10.20 (8.730–12.13)	10.22 (8.320–11.81)	0.9386
bLH [mIU/ml]	5.320 (4.695–5.905)	5.440 (4.620–6.710)	0.5097
bFSH/bLH	1.911 (1.732–2.193)	1.956 (1.657–2.229)	0.8556
bProlactin [ng/ml]	14.58 (10.15–19.86)	19.00 (11.40–22.40)	0.0404
bEstradiol [pmol/l]	40.90 (30.90–57.38)	40.64 (28.70–57.20)	0.7987
bProgesterone [ng/ml]	0.8600 (0.6750–1–155)	0.950 (0.770–1.20)	0.3683
bTSH [μIU/ml]	1.813 ±0.8185	1.828 ±0.7365	0.9291
AFC	6.826 ±2.10	7.200 ±1.891	0.3618
FSH amount needed for ovulation induction [IU]	2471 ±497.2	2430 ±508.8	0.6942
Length of stimulation [days]	12.65 ±0.7826	12.40 ±0.8471	0.1462
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	4.304 ±1.683	4.371 ±1.573	0.8415
Estradiol on day of HCG administration [pg/l]	1650 (1016–2108)	1948 (1092–2150)	0.3468
Endometrial thickness	8.50 (8.00–8.80)	8.40 (7.90–9.10)	0.9658
Number of oocytes retrieved	4.174 ±1.806	4.571 ±1.852	0.2955
Number of transferred embryos	2 (2–3)	2 (2–3)	0.6335
Pregnancy rate	28.985% (20/69)	28.57% (10/35)	0.96487

Table V. Clinical and hormonal parameters of subjects with respect to number of oocytes retrieved

Clinical and endocrinologic parameters	Low responders (≤ 5) *N* = 76	Normal responders (6–10) *N* = 28	*P*-value
Age [years]	34 (30–39.75)	31.50 (27–35)	0.0075
BMI [kg/m²]	23.10 (20.70–26.10)	24.10 (20.68–26.25)	0.6872
Period of infertility [years]	10.51 (8.872–12.19)	9.250 (7.278– 10.40)	0.0069
bFSH [mIU/ml]	5.440 (4.820–5.998)	5.160 (4.245–6.350)	0.5011
bLH [mIU/ml]	1.930 (1.754–2.207)	1.866 (1.566–2.218)	0.1620
FSH/LH	14.89 (10.13–20.68)	17.42 (13.48–20.20)	0.2259
bProlactin [ng/ml]	39.80 (28.96–56.40)	41.89 (29.95–76.45)	0.1852
bEstradiol [pmol/l]	0.8450 (0.6725–1.163)	0.910 (0.780–1.193)	0.2645
bProgesterone [ng/ml]	1.80 (1.123–2.435)	1.750 (1.005–2.608)	0.7693
bTSH [μIU/ml]	119.8 (98.75–170.2)	139.5 (102.9–177.0)	0.6659
AFC	2625 (2400–3000)	1950 (1725–2400)	< 0.0001
FSH amount needed for ovulation induction [IU]	12.79 ±0.7177	11.96 ±0.7445	< 0.0001
Length of stimulation [days]	3.855 ±1.439	5.607 ±1.474	< 0.0001
Estradiol on day of HCG administration [pg/l]	1505 (906.5–1905)	2179 (2025–2638)	< 0.0001
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	8.20 (7.90–8.675)	9.0 (8.50–9.80)	< 0.0001
Number of transferred embryos	2 (1.250–3)	3 (3–3)	< 0.0001
Pregnancy rate	18.42% (14/76)	57.14% (16/28)	0.000111

lation induction was significantly higher in group III than in group II ($p < 0.0001$) or group I ($p = 0.0396$), while the stimulation length was significantly longer in group III than group I ($p = 0.0016$). In addition, peak E2 levels were significantly lower in group III than in group I ($p = 0.0039$) or group II ($p = 0.0152$). Furthermore, endometrium thickness was significantly lower in group III than in group I ($p = 0.0065$), while oocyte retrieval number was significantly lower in group III than in group I ($p = 0.0010$) and group II ($p = 0.0215$). Considering the oocyte retrieval number, a statistically significant difference between PR and GR groups was found regarding patient age, BMI, bFSH, AFC, amount of FSH required for ovulation induction, stimulation period length and embryo transfer number as well as number of dominant follicles, peak EII levels and endometrium thickness (Table IV). Frequencies of the allelic variants in analyzed age groups were equally distributed ($\chi 2$ $p = 0.4061$) (Table V). The three genotype variants were also equally distributed in the PR and GR group as well (χ^2 $p = 0.73124991$) (Table VI). Two-way ANOVA analysis did not reveal significant interaction of FSHR genotype variants with age, BMI, bFSH or bFSH/bLH with respect to oocyte retrieval number. However, when observed independently, age ($p = 0.0010$), BMI ($p = 0.0232$), bFSH ($p = 0.0007$) and bFSH/bLH (0.0175) all exhibited a statistically significant influence on oocyte retrieval number (Table VII). The mean oocyte retrieval number was the highest among the Asn/Asn subjects with BMI > 25 kg/m^2 and lowest among Ser/Ser subjects with bFSH/bLH > 2.5, while with respect to age the mean oocyte retrieval number was the lowest in Asn/Asn subjects above 36 years of age. Correlation analysis of clinical and endocrinologic parameters with oocyte retrieval number showed a statistically highly significant ($p < 0.0001$) negative correlation with respect to age, bFSH, stimulation period days and amount of FSH required for ovulation induction. On the other hand, the AFC and embryo transfer number as well as number of dominant follicles, peak EII level and endometrium thickness all showed a statistically highly significant ($p < 0.0001$) positive correlation with oocyte retrieval number (Table VIII). The statistically significant negative correlation of age with oocyte retriev-

Table VI. Frequency SNP 680 FSHR alleles and genotypic variants with respect to age group and number of oocytes retrieved

Genotype	Grupa I (< 32) *n* (%)	Grupa II (32–36) *n* (%)	Grupa III (> 36) *n* (%)	*P* (χ^2 test)	PR *n* (%)	GR *n* (%)	*P* (χ^2 test)
NN	9 (24.33)	5 (14.29)	9 (28.125)		16 (21.05)	7 (25)	
NS	14 (37.84)	20 (57.14)	15 (46.875)		35 (46.05)	14 (50)	
SS	14 (37.84)	10 (28.57)	8 (25)		25 (32.89)	7 (25)	
Total	*N* = 37	*N* = 35	*N* = 32	0.4061	*N* = 76	*N* = 28	0.73124991

χ^2 analysis. P < 0.05 statistically significant.

Table VII. Two-way ANOVA – impact of FSHR genotype compared to BMI, bFSH, bFSH/BLH and resulting number of oocytes

Genotype	NN			NS			SS		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Age [years]:									
< 32	5.111	1.616	9	5.429	1.989	14	4.286	1.816	14
32–36	5.00	1.225	5	4.88	1.666	25	3.60	1.506	10
> 37	2.778	1.856	9	3.600	1.352	15	3.625	1.598	8
BMI [kg/m^2]:									
≤ 25	3.850	1.814	20	4.528	1.781	36	3.692	1.797	13
> 25	6.333	1.528	3	4.923	2.060	13	4.053	1.580	19
bFSH:									
≤ 10	4.818	1.888	11	5.130	1.984	23	4.786	1.578	14
> 10	3.583	1.881	12	4.192	1.625	26	3.222	1.396	18
bFSH/BLH:									
≤ 2.5	4.19	2.015	21	4.761	1.779	46	4.308	1.490	26
> 2.5	4.00	1.414	2	2.667	2.082	3	2.167	1.169	6

χ^2 analysis. P < 0.05 statistically significant.

al number was the most pronounced in Asn/Ser genotype variant and to a lesser degree in Asn/Asn subjects. On the other hand, bFSH exhibited a negative correlation with oocyte retrieval number in Ser/Ser subjects, and the same, although less statistically significant, was true for bLH and bFSH/bLH (Table IX). Contrary to that, AFC, the amount of FSH required for ovulation induction, stimulation length and embryo transfer number as well as the number of dominant follicles, peak EII levels and endometrium thickness all showed a statistically highly significant correlation in all genotype variants correspondingly (Table IX).

Discussion

Pharmacogenetic studies have revealed a series of genetic markers involved in the COH response, markers of ovarian reserve and reproductive lifespan [1, 7]. Among them, FSHR gene-associated SNPs, including the Asn680Ser missense variant, are the most promising genetic markers available to date. In the present study we investigated the association of Asn680Ser FSHR polymorphisms with the clinical and endocrinologic parameters of Albanian women from the Kosovo Dukagjin region. The study revealed the dominant frequency distribution of the Asn/Ser genotype consistent with its highest rates found in other ethnic groups [2–6]. However, contrary to other ethnic population examined so far, the Ser/Ser variant in our population was represented in higher frequency compared to Asn/Asn homozygotes. As already known, female fecundity decreases with increasing age, accompanied by a gradual decrease in both the quantity and the quality of the oocytes, thus substantially influencing the possible outcome of IVF procedures [8, 9]. Consistent with reported data, the present study also showed lower values for bFSH, and significantly higher AFC, peak E2 levels, and oocyte retrieval number in the youngest age group [10–14]. It has been reported that bFSH levels differ significantly among the Asn680Ser genotype variants with carriers of the Ser/Ser genotype, having slightly higher bFSH levels and requiring a significantly higher gonadotropin dose to induce ovulation [1, 2, 4, 15–18]. These studies suggest that the SS FSHR genotype variant is a factor of relative resistance to exogenous FSH stimulation. However, in our population the examined genotype variants were not significantly associated with either of these two parameters. These findings are in agreement with some previous studies reporting no difference in bFSH and/or gonadotropin dose among the different genotype variants [5, 6, 19, 20]. Nevertheless, the gonadotropin dose and the bFSH levels did show a statistically significant correlation in the overall studied population

Table VIII. Correlation of clinical and endocrine parameters obtained with number of oocytes

Clinical and endocrinologic parameters	Number of oocytes	
	r	*p*
Age [years]	–0.3904 Spearman	< 0.0001
BMI [kg/m^2]	0.1010 Spearman	0.3078
Period of infertility [years]	–0.4037 Spearman	< 0.0001
bFSH [mIU/ml]	–0.1940 Spearman	0.0484
bLH [mIU/ml]	–0.2484 Spearman	0.0114
FSH/LH	0.2386 Spearman	0.0147
bProlactin [ng/ml]	0.2722 Spearman	0.0052
bEstradiol [pmol/l]	0.2941 Spearman	0.0024
bProgesterone [ng/ml]	–0.04629 Spearman	0.648
bTSH [µIU/ml]	0.005915 Spearman	0.9525
AFC	–0.6369 Pearson	< 0.0001
FSH amount needed for ovulation induction [IU]	–0.6918 Pearson	< 0.0001
Length of stimulation [days]	0.7263 Pearson	< 0.0001
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	0.8092 Spearman	< 0.0001
Estradiol on day of HCG administration [pg/l]	0.6305 Spearman	< 0.0001
Endometrial thickness	0.7664 Spearman	< 0.0001

(Spearman $r = 0.3466$, $p = 0.0003$) and in Asn/Ser ($r = 0.3947$, $p = 0.0050$) and Ser/Ser ($r = 0.4345$, $p = 0.0130$) groups. Similar results were obtained by Loutradis *et al.*, but in their study a statistically significant correlation between gonadotropin dose and bFSH was also observed for the Asn/Asn group [16]. In agreement with previous reports, in the present study the peak E2 levels, number of follicles, and the number of oocytes retrieved, as well as the clinical pregnancy rates, were not significantly different among the genotype variants, suggesting that the treatment was equally successful, independent of the FSHR isoform [6, 20–23]. However, the lowest values for AFC, the peak E2 levels, number of dominant follicles and oocyte retrieval number in our studied population were observed in the Ser/Ser genotype group. Regarding the ovarian response, some studies report that

Table IX. Correlation of clinical and endocrine parameters with number of oocytes retrieved in relation to SNP Asn680Ser genotypic variants of FSHR gene

Clinical and endocrinologic parameters	NN (*N* = 23)		NS (*N* = 49)		SS (*N* = 32)	
	r	*p*	*r*	*p*	*r*	*p*
Age [years]	–0.5175 Pearson	0.0044	–0.4614 Pearson	0.0008	–0.2731 Pearson	0.1304
BMI [kg/m^2]	–0.04402 Spearman	0.8419	0.1905 Pearson	0.1899	0.1334 Pearson	0.4667
Period of infertility [years]	–0.3822 Spearman	0.0719	–0.2778 Spearman	0.0533	–0.6047 Spearman	0.0002
bFSH [mIU/ml]	–0.2240 Spearman	0.3024	–0.03079 Spearman	0.8337	–0.4798 Spearman	0.0055
bLH [mIU/ml]	–0.08661 Pearson	0.6944	–0.1694 Spearman	0.2447	–0.4972 Spearman	0.0044
FSH/LH	0.4685 Spearman	0.0241	0.04528 Spearman	0.7574	0.2966 Pearson	0.0933
bProlactin [ng/ml]	0.4532 Spearman	0.0299	0.05245 Pearson	0.7204	0.5537 Spearman	0.0010
bEstradiol [pmol/l]	0.3595 Spearman	0.0921	0.06386 Pearson	0.6629	0.5709 Spearman	0.0006
bProgesterone [ng/ml]	–0.1371 Spearman	0.5326	–0.004856 Pearson	0.9736	0.01506 Pearson	0.9348
bTSH [μIU/ml]	0.04413 Pearson	0.8415	0.07726 Spearman	0.5977	–0.1786 Spearman	0.3281
AFC	–0.5706 Pearson	0.0045	–0.6851 Pearson	< 0.0001	–0.6273 Pearson	0.0001
FSH amount needed for ovulation induction [IU]	–0.6792 Pearson	0.0004	–0.7061 Pearson	< 0.0001	–0.6997 Pearson	< 0.0001
Length of stimulation [days]	0.8336 Pearson	< 0.0001	0.6731 Pearson	< 0.0001	0.7196 Pearson	< 0.0001
Number of dominant follicles ($d \geq 17$ mm) on day of HCG administration	0.7928 Pearson	< 0.0001	0.7517 Pearson	< 0.0001	0.9016 Pearson	< 0.0001
Estradiol on day of HCG administration [pg/l]	0.7403 Pearson	< 0.0001	0.5372 Spearman	< 0.0001	0.6867 Pearson	< 0.0001
Endometrial thickness	0.8817 Spearman	< 0.0001	0.6467 Pearson	< 0.0001	0.8129 Pearson	< 0.0001

Ser/Ser subjects in comparison to Asn/Asn or heterogeneous genotype variants have higher AFC, peak E2 levels and oocyte retrieval number and thus greater risk for OHSS [16, 24–27]. Others report a smaller oocyte retrieval number in Ser/Ser vs. Asn/Asn and/or Asn/Ser subjects, thus suggesting quite contrarily that the Ser/Ser genotype variant may be associated with a reduced ovarian response to COH [4, 18, 28, 29]. In yet another report the three genotype variants were equally distributed in the PR group while patients in the GR group had a statistically significant tendency to carry the NS genotype variant [16]. The GR group members in our study showed significantly lower basal FSH levels and required a smaller gonadotropin dose for ovulation induction. Furthermore, they exhibited significantly higher AFC, higher number of dominant follicles, elevated peak E2 levels, and shorter stimulation period length as well as higher endometrium thickness and frequency of clinical pregnancy rate per embryo transfer when compared to the PR group. Nevertheless, the three genotype variants were equally distributed in both responder groups with an apparent but statistically non-significant tendency toward the Asn/Ser genotype variant in both groups. In the present study Asn680Ser polymorphism was significantly associated with bE2 levels in the overall studied population, with the highest median level found among Asn/Asn and the lowest level in the Ser/Ser group. In addition, values of bE2 were highest in the GR group, but this difference was not statistically significant. Nevertheless, Spearman's correlation revealed a statistically significant association with oocyte retrieval number in the overall studied population and spe-

cifically in Asn/Asn, and more pronounced in the Ser/Ser genotype variant. In contrast, peak E2 levels were equally distributed among genotype variants in the overall population. Their values statistically differed with respect to age and oocyte retrieval number, with the highest value recorded in the youngest patient group as well as the GR group and the smallest in the oldest patient subgroup. In addition, peak E2 levels were significantly correlated with oocyte retrieval number both in the overall studied population and all three genotype variants, with the highest Spearman's correlation coefficient in Ser/Ser genotype. However, the mean number of embryos transferred and the pregnancy rate were the same in all examined groups, thus suggesting that elevated E2 levels do not affect pregnancy outcome. These findings are in agreement with observations from previous studies that also indicated that elevated bE2 levels result in a greater number of oocytes and embryos for selection at the time of embryo transfer or cryopreservation without affecting pregnancy outcome [30–36]. Body mass index was also significantly associated with genotype variant subgroups in the present study, with the Ser/Ser group showing the highest and the Asn/Asn group the lowest BMI index. Interestingly, the members of the PR group showed lower (statistically nonsignificant) BMI values. A recently published study compared the obese non-PCOS population with the non-obese population and did not observe differences in the distribution of Ser680Asn FSHR genotype variants [37]. Therefore, to the best of our knowledge this is the first study showing a statistically significant association of BMI and Asn680Ser FSHR polymorphism. Nevertheless, the two-way ANOVA analysis did not confirm a significant interaction effect of genotype variants and BMI on oocyte retrieval numbers. It is already known that increased BMI may alter hormone metabolism and clearance in several complex ways [38]. Raised BMI was recently associated with adverse pregnancy outcome in women undergoing IVF/ICSI treatment. This effect was present in overweight (BMI $\geq$ 25 kg/m^2) as well as obese women [39, 40]. Some studies demonstrate that women with an elevated BMI produce more follicles, stimulate quicker, and require less gonadotropin during IVF, but without a significant effect on pregnancy outcome rates [41, 42]. However, in other studies overweight was associated with impaired response to ovarian stimulation, with overweight women requiring a higher gonadotrophin dose and showing a statistically significant decrease in the number of follicles and a lower oocyte retrieval number [43, 44]. Apart from significantly higher prolactin levels in the overweight (BMI $\geq$ 25 kg/m^2) group, we did not find any statistically significant difference between clinical and endocrinologic parameters in the two BMI groups or a statistically significant correlation of BMI with other examined parameters in the overall population. The ovarian AFC has emerged as a useful predictor of ovarian response and stimulation quality in assisted reproductive technologies [45–49]. The antral follicle count decreases over the years, is a predictor of the number of retrieved oocytes and can predict the likelihood of the IVF success [45, 50]. The set of candidate genes that are associated with AFC and other markers of ovarian reserve and reproductive lifespan has already been described in the past [7, 51]. As expected, in the present study antral follicle count showed a statistically significant age-related difference in the overall studied population, with the lowest values in the oldest patients and the PR subgroup. A positive significant correlation between antral follicle count and number of retrieved oocytes and a negative correlation between antral follicle count and age were also observed in previous reports [52–57]. Although AFC values showed a statistically significant correlation with oocyte retrieval number equally in the overall population and all three genotype variants, we found no statistically significant associations between the polymorphisms and this marker of ovarian reserve. No associations of FSHR genotypes with AFC were also described in a previous study [6]. It should be noted that there is a great heterogeneity in the results that concern the efficacy of the Asn680Ser FSHR genetic marker for the prediction of the ovarian response and pregnancy outcome. Therefore, these findings should be confirmed in larger studies, which will probably reveal more comprehensive results. Nevertheless, the results in the present study add to the understanding of the impact of FSHR genotype variants on controlled ovarian stimulation. As such, they may be useful for better tailoring of IVF treatment protocols to patient individual genotype background and thus for optimizing drug dose for more successful outcome rates and clinical benefit of the ART procedure.

Conflict of interest

The authors declare no conflict of interest.

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