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Combined Effects of the *FSHR* 2039 A/G and *FSHR* -29 G/A Polymorphisms on Male Reproductive Parameters

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Purpose: The aim of this study was to evaluate the combined effect of *FSHR* 2039 A/G and *FSHR* -29 G/A single nucleotide polymorphisms (SNPs) on the male reproductive function in a cohort of Sicilian men.
Materials and Methods: One-hundred thirty Sicilian men were enrolled and underwent blood withdrawal for hormone measurement and *FSHR* 2039 A/G and *FSHR* -29 G/A SNP genotyping, testicular volume evaluation by ultrasound scan, and semen analysis. A meta-analysis of the *FSHR* -29 G/A SNP, evaluated in a previous study of the Sicilian population was done.
Results: No genotype of the *FSHR* 2039 A/G SNP correlated with serum follicle-stimulating hormone (FSH) levels, testicular volume, sperm concentration, and total sperm count. In contrast, normozoospermic men with *FSHR* -29 GG and *FSHR* -29 GA genotypes had significantly lower sperm concentrations compared to men with the *FSHR* -29 AA genotype. The other sperm parameters did not show any significant difference. The meta-analysis showed no significant difference in serum FSH levels, testicular volume, sperm concentration, and total sperm count between *FSHR* -29 GG and *FSHR* -29 AA in Sicilian men. No difference was found even when the two SNPs were evaluated in combination. However, this combination was present, as expected, only in a low proportion (3.8%) of the men studied.
Conclusions: The SNPs *FSHR* 2039 A/G and *FSHR* -29 G/A in combination did not seem to have any effect on male reproductive function in a cohort of Sicilian men. The effect of these SNPs has only been studied in granulosa cells so far. Further studies on their role in Sertoli cells are needed.

Keywords: Follicle stimulating hormone; Oligozoospermia; Single nucleotide polymorphism; Spermatozoa

INTRODUCTION

Male reproductive health represents an issue of extreme actuality in the modern society that deserves further investigation [1]. Follicle-stimulating hormone (FSH), a glycoprotein made up of an α and a β subunit, plays a pivotal role for the reproductive function. In men, FSH stimulates spermatogenesis and increases the testicular volume by interacting with its receptor (FSHR) expressed in testicular Sertoli cells [2]. There-

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fore, any factor capable of interfering with the FSH-FSHR signaling pathway can alter the reproductive function [3,4].

The HapMap (http://hapmap.ncbi.nlm.nih.gov) database recognized 900 different single nucleotide polymorphisms (SNPs) for the *FSHR* gene and 24 SNPs for the *FSH* β *chain* (*FSH*β) gene, which are organized in linkage disequilibrium blocks [5]. The clinical research has mainly focused on *FSH*β -211 G/T (rs 10835638), *FSHR* -29 G/A (rs 1394205), *FSHR* c. 2039 A/G (p. Asp680Ser) (rs 6166), and *FSHR* c. 919 A/G (p. Thr307Ala) (rs6165) [5]. The *FSHR* 2039 A/G maps on exon 10 and changes the amino acid 680 from Asparagine to Serine, in the transmembrane domain of the FSHR. This SNP is known to influence the efficiency of signal transduction. Studies on granulosa cells have shown that the Ser660Ser (genotype GG) leads to the synthesis of a FSHR more resistant to FSH compared to the Asp680Asp (genotype AA) [6]. The *FSHR* -29 G/A maps on the *FSHR* gene promoter and influences the gene expression. According to data on granulosa cells, the A allele is associated with a lower transcription activity of the promoter (by 56%) [7]. Hence, hypothetically, the combination 2039 GG/-29 AA may be associated with worse male reproductive parameters by interfering with the FSH-FSHR signal pathway.

The results of previous studies on these SNPs are somewhat contrasting and, therefore, the real role of their role on male reproductive parameters is still matter of debate. The most shared vision addresses to a combination of different SNPs, more than to a single SNP, a possible role on serum FSH levels and testicular volume, as reported for *FSH*β -211 G/T and *FSHR* c. 2039 A/G haplotypes in a Baltic population [8]. This meta-analysis shows that the *FSH*β -211 TT - *FSHR* c. 2039 GG haplotype is associated with lower testicular volume, sperm count and higher serum FSH levels compared to other haplotypes [8]. However, the race may likely impact on the results of the various studies and this may, at least partially, explain the discrepancy existing in literature.

Some difference in allelic distribution of the *FSHR* SNPs has been reported in an ethnicity-dependent manner [5]. The role of *FSHR* haplotypes (c. 2039 A/G, c. 919 A/G and -29 G/A), investigated by various authors, seems to support the lack of effect of the *FSHR* haplotype in Turkish [9], Chinese [10], and Northern Italian [11] men. Contrasting conclusions have been reached in Estonian men [12,13]. We have previously shown the effect of the *FSHR* -29 G/A [14] SNP on the gonadal function in Sicilian men, reporting an influence on both FSH and luteinizing hormone (LH) serum levels in normozoospermic men. The aim of the present study was to assess the combined effect of *FSHR* 2039 A/G and *FSHR* -29 G/A SNPs in a cohort of Sicilian men. To accomplish this, 130 Caucasian men from Eastern Sicily were evaluated for *FSHR* 2039 A/G and *FSHR* -29 G/A SNPs genotyping, hormone levels, testicular volume, and conventional sperm parameters. To further understand the effects of these SNPs on Sicilian men, we have also meta-analyzed the data of the present cohort with those a previous study on Eastern Sicilian men [14].

MATERIALS AND METHODS

1. Patient selection

The study was carried out in male patients referring to the Division of Andrology and Endocrinology, University of Catania for andrological screening, couple infertility, follow-up for history of cryptorchidism, varicocele, or post-varicocelectomy follow-up. A detailed medical history was collected and physical examination was performed in each man. The presence of azoospermia or infertility in the family history was also investigated. The following exclusion criteria were used: genetic abnormalities (*e.g.*, Klinefelter syndrome, Y chromosome microdeletions, *CFTR* gene mutations), testicular tumor, trauma or torsion, obstructive azoospermia, use of gonadotoxic drugs, hypogonadism, testosterone replacement therapy, major comorbidities, not otherwise explained azoospermia. Taking all this into account, 130 Caucasian men from Eastern Sicily were finally included in the study. They underwent hormone evaluation, testicular volume assessment by scrotal ultrasound, sperm analysis, and *FSHR* 2039 A/G and *FSHR* -29 G/A SNP genotyping.

2. Hormone analysis

Each man underwent blood testing for the measurement of FSH, LH, total testosterone (TT) serum levels. The blood sample was collected in the fasting state between 8:00 and 10:00 a.m. Hormone evaluation was performed by electro chemiluminescence (Hitachi-Roche equipment, Cobas 6000; Roche Diagnostics, Indianapolis, IN, USA). Reference values were as follows: FSH

0.95–11.95 IU/L, LH 1.14–8.75 IU/L, TT 2.65–9.8 ng/mL.

3. Scrotal ultrasound evaluation

The ultrasound examination was performed with a GX Megas Esaote (Esaote SpA, Genoa, Italy) device, equipped with linear, high-resolution, and high-frequency (7.5 to 14 MHz) probes dedicated to the study of soft body areas, with color Doppler for detecting slow flow and a scanning surface of at least 5 cm. The testicular volume was calculated using the ellipsoid formula (length×width×thickness×0.52). The testis was considered normal in size when it had a volume between 15 and 25 cm^3, low-normal when it had a volume between 12 and 14.9 cm^3, and hypotrophic when it had a volume lower than 12 cm^3 [15]. Testicular volume was evaluated by ultrasound since this technique is the gold standard and shows the best accuracy [16].

4. Sperm analysis

Semen samples were collected by masturbation into a sterile container after 2–7 days of sexual abstinence and were analyzed immediately after liquefaction. According to the 2010 WHO guidelines, each sample was evaluated for seminal volume, pH, sperm count, progressive motility, morphology and round cell concentration [17]. Normozoospermia was considered for men who had sperm concentration >15 $\times 10^6$/mL and total sperm count >39 $\times 10^6$/ejaculate [17].

5. Follicle-stimulating hormone receptor analysis

Genomic DNA for the assessment of both SNPs was extracted from a unique blood sample using the PureLink® Genomic DNA Kits (Invitrogen Catalog Numbers K1821-04; Invitrogen, Carlsbad, CA, USA) for purification of genomic DNA according to the manufacturer's instructions. The concentration and the quality of the DNA was determined using a ND-1000 spectrophotometer (NanoDrop; Thermo Scientific, Wilmington, DE, USA). Allelic Discrimination was performed with TaqMan assay in order to show a different genotyping distribution of *FSHR* polymorphism. Probes and primers for *FSHR* 2039 A/G and *FSHR* -29 A/G polymorphisms were chosen on https://www.thermofisher.com/it/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html?SID=fr-taqman-2. The reaction was carried out according to manufacturer's instructions (cod 4371355; Applied Biosystems, CA, USA). Each DNA sample was analyzed in triplicate [18]. Allelic Discrimination real-time polymerase chain reaction analysis was performed using LightCycler® 480 System (Roche Molecular Systems, Inc., Pleasanton, CA, USA).

6. Statistical analysis

The distribution of each variable was evaluated using the Shapiro–Wilk test. Descriptive statistical results have been reported as mean±standard deviation for variables with a normal distribution and median and interquartile range for skewed ones. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test, with the Wilcoxon signed rank test and the chi-squared test, using IBM SPSS ver. 22.0 for Windows (IBM Corp., Armonk, NY, USA). A p-value less than 0.05 was accepted as statistically significant.

For meta-analysis, the mean difference (MD) for continuous variables was used for data pooling. For each outcome, the 95% confidence interval (CI) was calculated. The Cochran-Q and I^2 statistics were used in the assessment of statistical heterogeneity. Specifically, statistical heterogeneity was tested using the chi-squared test. If $I^2 \leq 50\%$, the variation of the studies was considered to be homogenous, and the fixed effect model was adopted. If $I^2 > 50\%$, there was significant heterogeneity between studies, and the random effects model was used. All $p \leq 0.05$ were considered statistically significant. The analysis was performed using RevMan software ver. 5.3 (Cochrane Collaboration, Oxford, UK). All $p < 0.05$ were considered statistically significant.

7. Ethical approval

This study was approved by the Institutional Review Board of the Teaching University Hospital "G. Rodolico" (Reg. No. n. 178/2017/PO). An informed written consent was obtained from each participant after full explanation of the purpose and the nature of all procedures used. The study has been carried out in accordance with the principles expressed in the Declaration of Helsinki.

RESULTS

Men included in this study had a mean age of 36.5±8.7 years and their body mass index was 27.0±4.9 kg/m^2. The *FSHR* allelic frequencies and the estimat-

Table 1. Percentage of abnormal andrological parameters in patients divided according to the *FSHR* 2039 A/G or the *FSHR* -29 A/G single nucleotide polymorphisms of the FSH receptor gene

Parameter	*FSHR* 2039 A/G			*FSHR* -29 G/A		
	AA (%)	AG (%)	GG (%)	AA (%)	AG (%)	GG (%)
No. of subject	33/130 (25.4)	65/130 (50.0)[a]	32/130 (24.6)[b]	14/130 (10.8)	38/130 (29.2)[a]	78/130 (60.0)[a,b]
FSH≥8 IU/L	4/29 (13.8)	6/55 (10.9)	1/26 (3.8)	0/13 (0.0)	3/33 (9.1)	6/64 (9.4)
Mean testicular volume ≤12 mL	8/28 (28.6)	18/48 (37.5)	9/24 (37.5)	4/13 (30.8)	10/29 (34.5)	20/58 (34.5)
Sperm concentration ≤15 mL/mL	8/28 (28.6)	26/54 (48.1)	8/27 (29.6)	4/13 (30.8)	15/33 (45.5)	24/63 (38.1)
Total sperm number ≤39 ×10^6/ejaculate	7/28 (25.0)	26/54 (48.1)[a]	8/27 (29.6)	5/13 (38.5)	13/33 (39.4)	24/63 (38.1)

Values are presented as number/total number (%).
FSH: follicle-stimulating hormone.
The p-values were calculated using the chi-squared test ([a]p<0.05 *vs*. AA. [b]p<0.05 *vs*. AA and AG).

ed *FSHR* haplotype frequencies found in the cohort of men analyzed in the present study were consistent with previously published data from unrelated Caucasian populations [14,19-21].

1. *FSHR* 2039 A/G

Men enrolled in this study had the following genotypes: 33 (25.4%) AA, 65 (50.0%) AG, and 32 (24.6%) GG (Supplement Table 1). The frequency of the genotype AG was significantly higher than those of AA and GG genotypes. The AG carriers had higher frequency of oligozoospermia than AA homozygotes men. The frequency of men with FSH>8 IU/mL, oligozoospermia, and low testicular volume (<12 mL) was not different in AA *vs*. GG homozygotes and in GG homozygotes *vs*. A allele carries (Table 1).

2. *FSHR* -29 G/A

Overall, men enrolled in this study had the following genotypes: 14 (10.8%) AA, 38 (29.2%) AG, and 78 (60.0%) GG (Supplement Table 1). The frequency of the genotype AA was significantly lower than the AG and GG genotypes. GG homozygotes were more frequent then A allele carries. The frequency of patients with FSH>8 IU/mL, oligozoospermia, and low testicular volume (<12 mL) did not differ in AA *vs*. GG homozygotes and in GG homozygotes *vs*. carries of the A allele (Table 1).

The analysis of data in normozoospermic men and patients with oligozoospermia is shown in Fig. 1, Supplement Tables 2 and 3. FSH serum levels, testicular volume, sperm concentration, and total sperm count did not differ in normozoospermic men or in patients with oligozoospermia who were carriers of the FSHR 2039 AA, AG, and GG genotypes. Normozoospermic men with the AG genotype had a significantly lower percentage of spermatozoa with normal forms compared to normozoospermic men with the AA genotype. Normozoospermic men or oligozoospermic patients who were carriers of the *FSHR* -29 AA, AG, and GG genotype did not show any difference in FSH, testicular volume, and total sperm count. In contrast, sperm concentration was significantly lower in normozoospermic men carriers of AG and GG genotypes compared to those homozygotes for AA (Fig. 1B). Sperm concentration did not differ in a statistically significant manner in patients with oligozoospermia carriers of AA, AG, and GG genotypes.

3. Analysis of *FSHR* 2039 A/G and *FSHR* -29 G/A haplotypes

Data were then analyzed taking into account concomitantly both *FSHR* 2039 A/G and *FSHR* -29 G/A haplotypes. Overall, the sample included 5 (3.8%) *FSHR* 2039AA/-29 AA, 28 (21.5%) 2039GG/-29 GG, 16 (12.3%) 2039AA/-29 GG, and 2 (1.5%) 2039GG/-29 AA carries. The 2039GG/-29 GG haplotype was significantly more frequent than the 2039AA/-29 AA. The 2039GG/-29 AA haplotype was significantly less frequent than the 2039AA/-29 GG. No differences in the frequency of FSH >8 IU/mL, oligozoospermia, and low testicular volume was observed among the four haplotypes (Table 2). Only one men carrier of the 2039AA/-29 AA and one carrier of the 2039GG/-29 AA had oligozoospermia. Therefore, no further analysis was done. No difference in hormone serum levels, testicular volume, and conventional sperm parameters was found in 2039AA/-29 AA *vs*. 2039GG/-29 GG men with normozoospermia (Supplement Table 4). Data stratified by both *FSHR* 2039 A/G and *FSH* -29 G/A haplotypes are shown in Fig. 2. No difference among the groups

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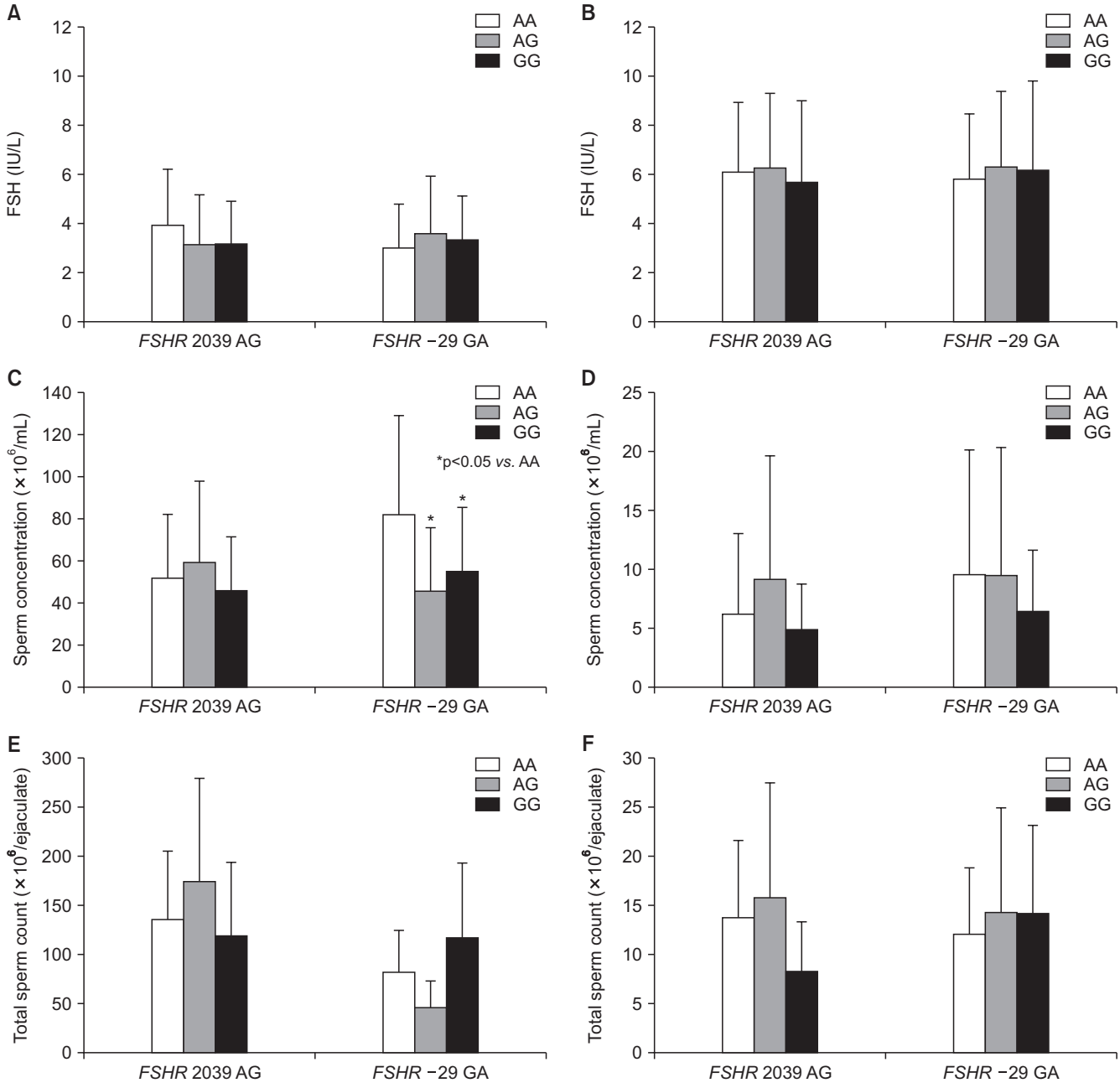

Fig. 1. Follicle stimulating hormone (FSH) serum levels, sperm concentration, and total sperm count in normozoospermic men and oligozoospermic patients divided according to the *FSHR* 2039 A/G or the *FSHR* -29 G/A genotype. FSH levels in normozoospermic men (A) and oligozoospermic patients (B). Sperm concentration in normozoospermic men (C) and oligozoospermic patients (D). Total sperm count in normozoospermic men (E) and oligozoospermic patients (F).

was observed for FSH values, testicular volume, and conventional sperm parameters. In the *FSHR* 2039 GG group, the FSHR -29 A allele was associated with a non-significant trend towards higher sperm concentration and total sperm count (Fig. 2C, 2D).

4. Meta-analysis of *FSHR* -29 G/A including previous studies in Sicilian population

To increase the statistic power of the analysis, the main andrological parameters genotyped for the *FSHR* -29 G/A in a previous study in Sicilian population [14] were meta-analyzed with those of the present study. This allowed us to compare 185 men with the GG genotype with 27 men with AA. Overall, FSH serum levels

Table 2. Percentage of abnormal andrological parameters in patients divided according to both *FSHR* 2039 A/G and *FSHR* -29 A/G single nucleotide polymorphisms of the FSH receptor gene

Parameter	*FSHR* 2039 A/G - *FSHR* -29 A/G haplotype			
	2039AA/-29 AA (%)	2039GG/-29 GG (%)	2039AA/-29 GG (%)	2039GG/-29 AA (%)
No. of subject	5/130 (3.8)	28/130 (21.5)[a]	16/130 (12.3)	2/130 (1.5)[b]
FSH≥8 IU/L	0/4 (0.0)	1/23 (4.3)	3/14 (21.4)	0/2 (0.0)
Mean testicular volume ≤12 mL	2/4 (50.0)	9/21 (42.9)	5/14 (35.7)	0/2 (0.0)
Sperm concentration ≤15 mL/mL	1/5 (20.0)	6/25 (24.0)	6/12 (50.0)	1/2 (50.0)
Total sperm number ≤39 ×10^6/ejaculate	1/5 (20.0)	6/25 (24.0)	6/12 (50.0)	1/2 (50.0)
Azoospermia	0/5 (0.0)	1/25 (4.0)	0/12 (0.0)	0/2 (0.0)

Values are presented as number/total number (%).
FSH: follicle-stimulating hormone.
The p-values were calculated using the chi-squared test ([a]p<0.05 *vs*. 2039AA/-29 AA. [b]p<0.05 *vs*. 2039AA/-29 GG).

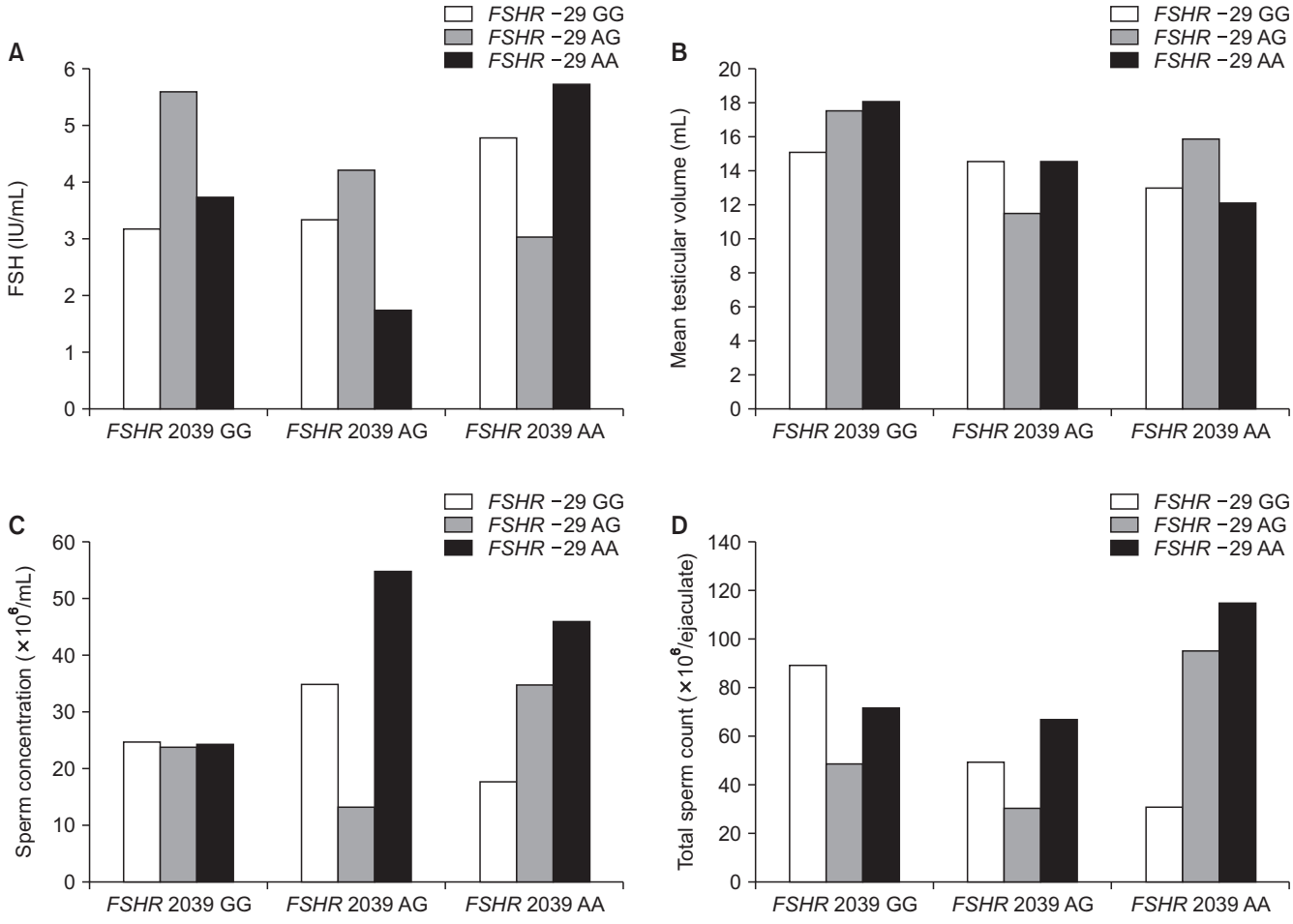

Fig. 2. Effects of *FSHR* 2039 A/G and *FSHR* -29 G/A haplotype on male reproductive function. Follicle stimulating hormone (FSH) levels (A), mean testicular volume (B), sperm concentration (C), and total sperm count (D) of the overall sample divided according to the *FSHR* 2039 A/G - *FSHR* -29 G/A haplotype. The bars report the median values.

(MD=-0.74 IU/mL, 95% CI=-3.58–2.11; p=0.61) (Fig. 3A), testicular volume (MD=0.82 mL, 95% CI=-5.19–6.83; p=0.79) (Fig. 3B), sperm concentration (MD=-3.31×10^6/mL, 95% CI=-29.93–-23.31; p=0.81) (Fig. 3C), and total sperm count (MD=3.16×10^6/ejaculate, 95% CI=-44.81–51.14; p=0.90) (Fig. 3D) did not significantly differ between carriers of GG or AA genotypes.

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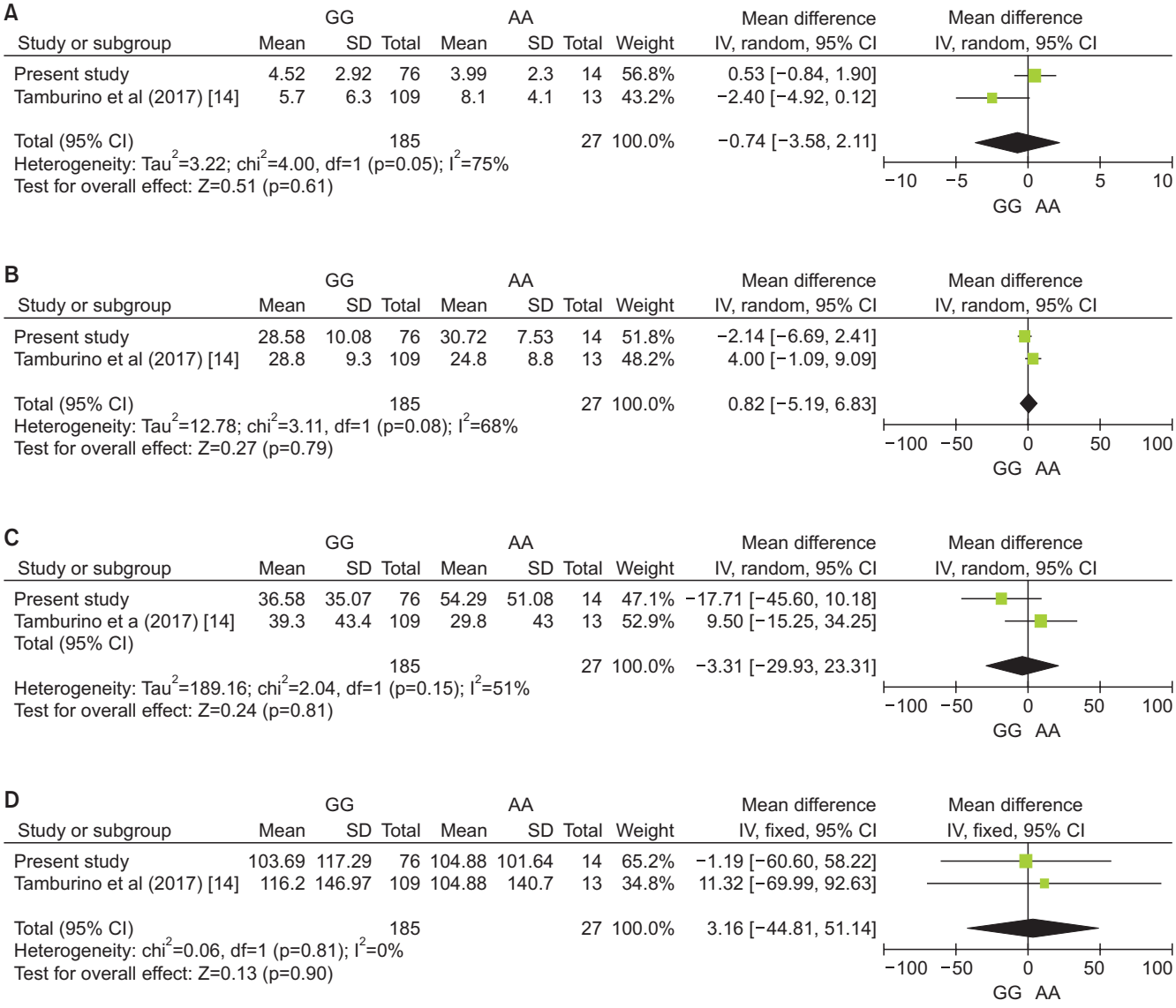

Fig. 3. Effects of the *FSHR* -29 G/A single nucleotide polymorphism on male reproductive parameters. Patients with the GG genotype did not show significantly different follicle stimulating hormone levels (A), testicular volume (B), sperm concentration (C), and total sperm count (D) compared to the AA genotype. SD: standard deviation, CI: confidence interval, DF: degree of freedoms.

DISCUSSION

The single and combined effect of the *FSHR* 2039 A/G and *FSHR* -29 G/A SNPs in a cohort of Sicilian men has been reported in this study. The exclusion criteria used for the present study retrace those utilized in previous studies on the role of *FSH* SNPs on male reproductive function [22]. In line with previous studies [5,8-14], obesity was not considered among the exclusion criteria, since the role of obesity in the pathogenesis of abnormal sperm parameters is still matter of debate [23]. Furthermore, only a low number of the men included in this study were obese.

Although we have previously shown higher FSH serum levels in normozoospermic A carries compared to normozoospermic GG Sicilian men [14], the present study did not confirm this finding. Furthermore, meta-analysis of data from Sicilian men showed the normozoospermic men with the GG genotype (n=185) did not have significantly different serum FSH levels, testicular volume, sperm concentration, and total sperm count compared to 27 men with normozoospermia carrier of the AA genotype. We have also found a significantly lower sperm concentration in normozoospermic men with the *FSHR* -29 GG and *FSHR* -29 GA genotypes compared to the *FSHR* -29 AA genotype, but no differ-

ence in total sperm count was observed, thus suggesting no real effect on this SNP on the sperm output. No influence of the single *FSHR* 2039 A/G SNP on serum FSH levels, testicular volume, sperm concentration, and total count was found, as well for the combination of the two SNPs.

These findings are in line with those of previous reports, thus neglecting the role of *FSHR* SNPs in the modulation of the reproductive function in men. Indeed, a meta-analysis including 700 patients and 600 controls reported no association between the *FSHR* 2039 A/G polymorphism and male infertility [24]. A subsequent meta-analysis on seven case-control studies (1644 infertile patients *vs.* 1,748 controls) confirmed no association between both the *FSHR* 2039 A/G and *FSHR* -29 G/A SNPs and spermatogenic impairment [25]. In the Han-Chinese population, an analysis on 364 patients with idiopathic infertility and 281 controls found that the FSHR 2039 A/G, the 919 A/G and the -29 G/A SNPs did not influence male reproductive parameters and an association between the *FSHR* haplotype and idiopathic male infertility was not observed [10]. Likewise, an Italian study on 544 patients reached the same conclusion [11]. In contrast, other studies support the relevance of the *FSHR* SNPs in male reproductive function. A single study on 240 men carried out among a Southeast Turkey population, reported no influence of the *FSHR* haplotype (2039 A/G and -29 G/A) on FSH serum levels, although a different distribution of the haplotype was observed in proven fathers compared to infertile patients [9]. Similarly, a distinct distribution of the *FSHR* haplotype as also been reported between azoospermic patients and controls [26]. Lastly, only few studies have reported an influence of the *FSHR* -29 G/A SNP on the FSH serum levels [13,14], while the majority of the available data fail to support a role of this polymorphism.

Therefore, the results of the present study add controversy to the possible role of the *FSHR* SNPs in modulating male reproductive function. This topic is still unresolved. The available molecular evidence showing the impact of *FSHR* 2039 A/G and *FSHR* -29 G/A SNPs on *FSHR* gene transcription and protein signal transduction comes from studies carried out in granulosa cells. The *FSHR* 2039 A/G influences the amino acid at position 680 of the FSHR. An *in-vitro* study, performed in human granulosa cells, reported a lower intracellular cAMP production in Ser680 homozygotes, as well as a decreased FSH-induced ERK1/2 phosphorylation, CREB activation, and *AREG* and *STARD1* gene expressions, after incubation with FSH. This suggests a worse signal transduction capability of the Ser680Ser *FSHR* gene SNP [6], although this outcome was not supported by other studies [20,27-30]. Similarly, *in-vitro* evidence on transfected Chinese Hamster ovary (CHO) cells reported a decrease of the *FSHR* gene promoter activity by the ~56% of the *FSHR* -29 A allele compared to the G allele [6].

So far, the molecular evidence gathered from studies on granulosa cells have been extended to Sertoli cell function: Hence, as the *FSHR* SNPs has been shown to influence female fertility, it has been hypothesized to also impact on male reproductive function. However, the same study assessing the impact of the *FSHR* -29 A/G SNP on CHO cells has also shown the significantly higher prevalence of the A allele in female patients with essential hypertension compared to normotensive women, but not in men [7], highlighting the presence of a difference between the two sexes. The findings obtained in granulosa cells may not necessarily applied to Sertoli cells, as these cells have a different biology.

Sertoli cells are committed to support spermatogenesis in the testis. Until puberty, they show an immature and proliferative appearance, and have the ability to secrete anti-Müllerian hormone (AMH). During puberty, these cells maturate, loss the capability to proliferate and to secrete AMH, and acquire the spermatogenetic function under the control of FSH [31,32]. These properties are not shared by granulosa cells, which are selected and mature at each ovarian cycle. Furthermore, Sertoli cells express only the FSHR, while the LH receptor (LHR) is expressed only by testicular Leydig cells. On the contrary, granulosa cells concomitantly express both the FSHR and the LHR, which undergo to dimerization upon hormone stimulation [33]. This mechanism is completely different in Sertoli cells, where no dimerization occurs. Furthermore, a high and not fully understood complexity of the FSH signaling pathway has been reported in Sertoli cells [34]. However, this knowledge mainly refers to immature Sertoli cells, as adult Sertoli cells cannot be cultured *in-vitro*. As a result, the FSH signaling of mature Sertoli cells of the adulthood is unknown and molecular studies assessing the impact of the *FSHR* SNPs on FSH responsiveness in adult Sertoli cells cannot be performed. Therefore, it is not clear whether such SNPs could

impact on the responsiveness of mature Sertoli cells to FSH as they do in granulosa cells. The effects of these SNPs may deserve to be studied at least in transfected pre-pubertal Sertoli cells.

CONCLUSIONS

In conclusion, we found that *FSHR* 2039 A/G and/or *FSHR* -29 G/A had any effect on male reproductive function in Sicilian men. This outcome is supported by the majority of the available studies in this sex in other populations. The evidence on the role of these SNPs on *FSHR* expression and signal transduction come from studies in granulosa cells. The known difference of FSH molecular mechanisms between granulosa and Sertoli cells and the lack of knowledge on the FSH signaling pathway in adult Sertoli cells, suggest that these *in-vitro* findings may not necessarily occur in Sertoli cells. Therefore, the effects of FSHR 2039 A/G and FSHR -29 G/A SNPs need to be reconsidered in Sertoli cells.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: RC, AEC. Data curation: MM. Formal analysis: NM. Methodology: RC, NM. Project administration: AEC. Supervision: RAC, SS, SLV. Writing – original draft: RC, NM. Writing – review & editing: AEC, SLV.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.200070.

Data Sharing Statement

The data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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