The effect of polymorphisms in FSHR and FSHB genes on ovarian response

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The effect of polymorphisms in *FSHR* and *FSHB* genes on ovarian response. A prospective multicenter multinational study in Europe and Asia.

Running title: Influence of *FSHR*/*FSHB* polymorphisms on ovarian response

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ABSTRACT

Study question:

Does the presence of SNPs in *FSHR and/*or *FSHB* influence ovarian response in predicted normal responders treated with rFSH?

Summary answer:

The presence of *FSHR* SNPs (rs6165, rs6166, rs1394205) has a statistically significant impact in ovarian response, although this effect is of minimal clinical relevance in predicted normal responders treated with a fixed dose of 150IU rFSH.

What is known already:

Ovarian reserve markers have been a breakthrough in response prediction following ovarian stimulation. However, a significant percentage of patients show a disproportionate lower ovarian response, as compared with their actual ovarian reserve. Studies on pharmacogenetics have demonstrated a relationship between *FSHR* or *FSHB* genotyping and drug response, suggesting a potential effect of individual genetic variability on ovarian stimulation. However, evidence from these studies is inconsistent, due to the inclusion of patients with variable ovarian reserve, use of different starting gonadotropin doses and allowance for dose adjustments during treatment. This highlights the necessity of a well-controlled prospective study, in a homogenous population treated with the same fixed protocol.

Study design, size, duration:

We conducted a multicenter multinational prospective study, including 368 patients from Vietnam, Belgium and Spain (168 from Europe and 200 from Asia), from November 2016 until June 2019. All patients underwent ovarian stimulation followed by oocyte retrieval in an antagonist protocol with fixed daily dose of 150IU rFSH until triggering. Blood sampling and

DNA extraction was performed prior to oocyte retrieval, followed by genotyping of 4 SNPs from *FSHR* (rs6165, rs6166, rs1394205) and *FSHB* (rs10835638).

Participants/materials, setting, methods:

Eligible were predicted normal responder women <38 years old undergoing their first or second ovarian stimulation cycle. Laboratory staff and clinicians were blinded to the clinical results and genotyping respectively. The prevalence of hypo-responders, the number of oocytes retrieved, the follicular output rate (FORT) and the follicle to oocyte index (FOI) were compared between different *FSHR* and *FSHB* SNPs genotypes.

Main results and the role of chance:

The prevalence of derived allele homozygous SNPs in the *FSHR* was: rs6166 (genotype G/G) 15.8%, rs6165 (genotype G/G) 34.8% and rs1394205 (genotype A/A) 14.1%, with significant differences between Caucasian and Asian women ($p<0.001$). *FSHB* variant rs10835638 (c.-211 G>T) was very rare (0.5%).

Genetic model analysis revealed that the presence of the G allele in *FSHR* variant rs6166 resulted in less oocytes retrieved when compared to the AA genotype (13.54 ± 0.46 vs. 14.81 ± 0.61, EMD -1.47 (95%CI -2.82 - -0.11)). In *FSHR* variant rs1394205, a significantly lower number of oocytes was retrieved in patients with an A allele when compared to G/G (13.33 ± 0.41 vs. 15.06 ± 0.68, EMD -1.69 (95%CI -3.06 - -0.31)). A significantly higher prevalence of hypo-responders was found in patients with the genotype A/G for *FSHR* variant rs6166 (55.9%, n=57) when compared to A/A (28.4%, n=29), ORadj 1.87 (95%CI 1.08-3.24). No significant differences were found regarding the FORT across the genotypes for *FSHR* variants rs6166, rs6165 or rs1394205. Regarding the FOI, the presence of the G allele for *FSHR* variant rs6166 resulted in a lower FOI when compared to the A/A genotype, EMD -13.47 (95%CI -22.69 - -4.24). Regarding *FSHR* variant rs6165, a lower FOI was reported for genotype A/G (79.75 ± 3.35) when compared to genotype A/A (92.08 ± 6.23), EMD -13.81 (95%CI -25.41 - -2.21).

Limitations, reasons for caution:

The study was performed in relatively young women with normal ovarian reserve to eliminate biases related to age-related fertility decline; thus, caution is needed when extrapolating results to older populations. In addition, no analysis was performed for *FSHB* variant rs10835638 due to the very low prevalence of the genotype T/T (n=2).

Wider implications of the findings:

Based on our results, genotyping *FSHR* SNPs rs6165, rs6166, rs1394205 and *FSHB* SNP rs10835638 prior to initiating an ovarian stimulation with rFSH in predicted normal responders should not be recommended taking into account the minimal clinical impact of such information in this population. Future research may focus on other populations and other genes related to folliculogenesis or steroidogenesis.

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Trial registration number: NCT03007043

KEY WORDS

Polymorphisms, FSH, controlled ovarian stimulation, gonadotropins, pharmacogenetics

INTRODUCTION

Introduction of ovarian reserve markers in everyday clinical practice has been a breakthrough in modern assisted reproduction techniques (ART), not only because they allowed a proper prediction of response following ovarian stimulation (Polyzos et al., 2012, 2013b, 2013a; Broer et al., 2013, 2014) but also because they have been the first step towards a patient-tailored individualized ovarian stimulation (La Marca and Sunkara, 2014). This personalized approach appears to be more relevant than ever today, considering that the number of oocytes retrieved following stimulation for IVF/ICSI is strongly associated with patients' safety, namely ovarian hyperstimulation syndrome (OHSS) incidence (Schirmer et al., 2020), and reproductive outcomes such as live birth (Sunkara et al., 2011) and cumulative live birth rates (Drakopoulos et al., 2016; Polyzos et al., 2018).

Nevertheless, despite the excellent ability of ovarian reserve markers to predict both low (Nelson, 2013) and high (Broer et al., 2011) responses to stimulation, their prognostic performance, in several cases, is far from perfect. Despite the pharmacological advances in ART (Racca *et al.*, 2020), a significant percentage of patients demonstrates a disproportionate lower ovarian response to stimulation, as compared with their actual ovarian reserve (Polyzos and Sunkara, 2015; Esteves et al., 2018). This highlights that ovarian reserve markers only reflect the number of antral and pre-antral follicles in the ovary and not their sensitivity to ovarian stimulation.

Over the years, significant research has been conducted in an attempt to identify other biomarkers that could be associated with ovarian sensitivity to gonadotropins. The vast bulk of evidence focused on the identification of biomarkers associated with the Follicle Stimulating Hormone (FSH) and its receptor (FSHR) which are two essential molecules for ovarian stimulation and function. Early reports have shown that common single nucleotide polymorphisms (SNPs) in the *FSHR* gene have been associated with FSH consumption (Perez Mayorga et al., 2000) whereas others have shown that variants in the *FSHR* gene may also influence the response to ovarian stimulation in terms of duration of stimulation and number of MII oocytes retrieved after IVF treatment (Alviggi et al., 2018b).

The FSHβ-subunit confers hormone-specific biological properties and is encoded by the *FSHB* gene. The effect of the SNP within the *FSHB* promoter region c.-211G>T (rs10835638) has also been studied in ART patients, with higher FSH levels in the follicular phase and decreased progesterone production in the luteal phase being reported in c.- 211G>T female carriers (Schuring et al., 2013), as well as a lower response to ovarian stimulation in patients carrying the GT genotype when compared to the GG (Trevisan et al., 2019).

Despite the fact that these studies demonstrated a relationship between *FSHR* or *FSHB* genotypes and ovarian response, suggesting a potential effect of individual genetic variability, evidence is inconsistent. This is mainly due to the heterogeneity of the inclusion criteria across the studies, involving women with variable ovarian reserve, use of different gonadotropin doses and allowance for dose adjustments during treatment. Furthermore, the effect size of this genetic variability appears to be rather small, given that the different variants in the *FSHR/FSHB* genes have demonstrated only a moderate reduction in ovarian response to stimulation (Casarini and Simoni, 2014; Tang et al., 2015).

Taking all the above into consideration we set out to perform a controlled multicenter multinational prospective study on *FSHR* or *FSHB* variants with adequate sample size and a fixed gonadotropin dose during the whole stimulation phase in order to evaluate the actual impact of the presence of *FSHR* or *FSHB* SNPs on ovarian response.

MATERIALS AND METHODS

Study design & setting

This is a prospective non-interventional study including patients of Caucasian and Asian ethnic origin undergoing ovarian stimulation for in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) between November 2016 and June 2019. Patients were recruited in 4 University affiliated tertiary IVF units in Spain, Belgium and Vietnam (Dexeus University Hospital Barcelona, SPAIN; Centre for Reproductive Medicine, UZ Brussel, BELGIUM;

Department of Obstetrics and Gynecology, University of Medicine and Pharmacy at HCMC, Ho Chi Minh City, VIETNAM; IVFMD, My Duc Hospital, Ho Chi Minh City, VIETNAM). The Ethics Committee of the hospitals involved approved the study and all participants gave their written informed consent for blood sampling and genetic investigations for this specific target.

Patient selection criteria

The study included patients <38 years old undergoing their $1^{st}/2^{nd}$ ovarian stimulation cycle with a predicted normal response, as defined by normal ovarian reserve markers (antimullerian hormone (AMH) or antral follicle count (AFC)). Patients were considered ineligible if they had an AFC <9, AMH < 1.1 ng/ml or polycystic ovarian syndrome (PCOS) according to the Rotterdam criteria (Teede et al., 2018). Furthermore, patients with untreated endocrine abnormalities or undergoing in-vitro maturation were also excluded.

Stimulation protocol

All patients underwent ovarian stimulation followed by oocyte retrieval in a Gonadotrophin-releasing hormone (GnRH) antagonist protocol. All women were treated with a fixed daily subcutaneous (SC) dose of 150IU rFSH initiated either on cycle day 2 or 3, or 5 days following discontinuation of an oral contraceptive pill (Montoya-Botero et al., 2020), followed by a daily dose of 0.25 mg of GnRH antagonist in a fixed protocol starting 6 days later. No dose adjustments were allowed until final oocyte maturation. As soon as 3 follicles ≥ 17-18 mm diameter were observed by ultrasound, human chorionic gonadotropin (hCG 5000/10000 IU or rhCG 250mg) was administered the same day or the day thereafter to induce final oocyte maturation. In case of excessive ovarian response (≥17 follicles >11mm on the day of final oocyte maturation), triggering with a GnRH agonist (triptorelin 0.2mg) was used for safety reasons. Oocyte retrieval was performed 34-36h thereafter.

Blood sampling and DNA sequencing

Blood sampling and DNA extraction was performed for all patients prior to oocyte retrieval and laboratory staff and clinicians were blinded to the clinical results and genotyping. Blood sampling was performed in accordance with the treatment protocol and no extra venepunctures were performed beyond the standard treatment.

All blood samples were collected and stored at −80°C until the time of genotyping. Genomic DNA was extracted from peripheral leucocytes using the DNeasy blood and tissue extraction kit of Qiagen. Alternatively, conventional phenol/chloroform extraction, followed by ethanol precipitation was used.

The genotyping of the SNPs was carried out using the predesigned TaqMan SNP assays of Life Technologies for three SNPs from *FSHR* (c.919A>G (rs6165, in the National Center for Biotechnology Information (NCBI) SNPs database); c.2039A>G (rs6166); c.-29G>A (rs1394205)) and one SNP from *FSHB* (c.-211G>T (rs10835638)).

Outcome measures

The primary outcome measure of the current study was to determine the differences in ovarian response to stimulation according to different genotypes in *FSHR* and *FSHB* genes. Therefore, we compared, for each polymorphism, the patients' genotypes according to the following outcomes:

- Percentage of patients categorized as hypo-responders, defined as a total number of oocytes retrieved < 10 (Polyzos and Sunkara, 2015)
- Total number of oocytes retrieved
- Follicular output rate (FORT), defined as the ratio between ratio between the number of follicles that reached pre-ovulatory maturation in response to COS (16-22 mm) and the number of antral follicles available at the start of stimulation (Genro et al., 2011)

- Follicle to Oocyte Index (FOI), defined as the ratio between the number of oocytes recovered in the end of COS and the number of antral follicles available at the start of stimulation (Alviggi et al., 2018a)

Sample size calculation

The sample size was calculated in order to estimate the common odds ratio (based on the Cochran-Mantel-Haenszel test (CMH)) for a hypo-response (<10 oocytes) associated with the presence of the *FSHR* SNP in position rs6166 c.2039A > G (rs6166), controlling by the origin of the patient. The study hypothesis was that the presence of c.2039A > G (rs6166) in homozygous state (GG) is associated with significantly higher incidence of hypo-response (<10 oocytes) in women with predicted normal ovarian response.

In this context, we calculated by using a two-sided test with a significance level set to 0.05 and power set to 0.8, that at least 365 patients would be required, 167 from Europe and 198 from Asia. The estimated proportions of hypo-response to detect were 55% in the 2039A > G (rs6166) in homozygous state (GG) and 35% in the AA genotype, respectively, and was calculated taking into account the different prevalence of rs6166 SNP among Asian (13.3%) and Caucasian (European) populations (22.7%).

Statistical methods for analysis

Continuous variables were described as means and standard deviations (SD), meanwhile categorical or nominal variables were described by percentages and frequencies.

The Hardy-Weinberg (H-W) Equilibrium for each polymorphism was tested using the Chi-Square test.

The analysis of the association between the different polymorphisms inheritance models and outcomes was evaluated using multivariable models. When the outcome was categorical a logistic model was used, and when the outcomes were continuous a linear model was used. In both cases, each model was adjusted by patient age, AMH levels, days of stimulation, and continent (Asia vs. Europe).

The polymorphisms inheritance genetic models used were: co-dominant (AA vs. aa and AA vs. Aa), dominant (AA vs. Aa+aa), recessive (aa vs. Aa+AA) and additive (each copy of a modifies the risk in an additive form 2aa+Aa vs. AA), with A being the ancestral allele and a the derived allele (Attia et al., 2003; Thakkinstian et al., 2005).

All analyses were performed using R software (R Core Team, 2019).

RESULTS

Patients´ baseline characteristics

Overall, 368 patients (168 Caucasian from Europe and 200 Asian from Vietnam) were included in the study and genotyped for the 3 variants in the *FSHR* gene (rs6165, rs6166 and rs1394205) and one variant in the *FSHB* gene (rs10835638).

Included patients had a mean age of 30.49±3.64 years, a body mass index (BMI) of 21.26± 2.23 Kg/m^2, a basal AMH of 3.8±1.84 ng/ml and an AFC of 17.16±5.31. Most of the patients were nulliparous (90,2%). The causes of infertility are displayed in Table I.

Genotyping and Polymorphisms analysis

The distribution of the studied SNPs across the different populations is described in Figure 1. Comparison between Caucasian and Asian women has demonstrated significant differences, with *FSHR* rs6166 G/G being more frequent in Caucasian than in Asian women (23.2% vs 9.5%, $p<0.001$), and *FSHR* rs6165 G/G and *FSHR* rs1394205 A/A being more frequent in Asian than in Caucasian women (44.5% vs 23.2% ,$p<0.001$, and 19.5% vs 7.7%, $p<0.001$, respectively).

Regarding the variant *FSHB* rs10835638 (c.-211 G>T) the presence of homozygous T/T was very rare, with a prevalence of 0.5% among the study population, whereas none of the Asian patients presented this genotype. Based on the scarcity of homozygous T/T or heterozygous patients for this variant, it has not been included in any of the analysis for the reported outcome measures.

Association between the different genotypes and ovarian response

Ovarian response category

Ovarian response was categorized in hypo-response (< 10 oocytes retrieved) and optimal (≥ 10 oocytes retrieved). Among the patients included, 102 (27.7%) were hypo-responders, while 266 (72.3%) presented an optimal response. Univariate analysis revealed no significant differences regarding the prevalence of hypo-response according to the different genotypes of *FSHR* rs6166, rs6165 and rs1394205 (p=0.096, p=0.145 and p=0.830, respectively). Also, the prevalence of hypo-responders was not significantly different between the 3 variants when data were analysed individually per continent (Figure 2).

Genetic model analysis is displayed in Table II. Analysis of the co-dominant model for *FSHR* variant rs6166 revealed a significantly higher prevalence of hypo-response in patients with the genotype A/G (55.9%, n=57) when compared to A/A (28.4%, n=29), ORadj 1.87 (95%CI 1.08-3.24). No significant differences were observed in ovarian response categories according to *FSHR* rs6165 nor rs1394205 genotypes.

Number of oocytes retrieved

Univariate analysis of the number of oocytes retrieved according to the genotypes of *FSHR* rs6166, rs6165 and rs1394205 is presented in Figure 3a.

Genetic model analysis revealed a statistically significant difference in both the co-dominant and dominant models for variants *FSHR* rs6166 and rs1394205 (Table III). Regarding the *FSHR* variant rs6166, the co-dominant model revealed a lower number of oocytes retrieved for the genotype A/G (13.49 ± 0.55) when compared to A/A (14.81 ± 0.61), estimated mean difference (EMD) -1.59 (95%CI -3.01 - -0.16). This was confirmed in the dominant model, in which the presence of the G allele in G/G and A/G genotypes resulted in less oocytes retrieved when compared to the AA genotype (13.54 ± 0.46 vs. 14.81 ± 0.61, EMD -1.47 (95%CI -2.82 - -0.11)). Analysis of the *FSHR* variant rs1394205 has also revealed a significantly lower number of oocytes retrieved for the genotype G/A (13.24 ± 0.45) when compared to G/G (15.06

± 0.68) in the co-dominant model, EMD -1.62 (95%CI -3.06 - -0.17). The dominant model confirmed a significantly lower number of oocytes retrieved in genotypes G/A and A/A when compared to G/G (13.33 ± 0.41 vs. 15.06 ± 0.68, EMD -1.69 (95%CI -3.06 - -0.31)).

No significant difference was found in the number of oocytes retrieved according to variant *FSHR* rs6165 genotype. Also, a similar oocyte yield was found when the 3 variants were compared individually per continent (Suppl. Figure 1a).

FORT and FOI

Univariate analysis of the FORT and FOI according to the genotypes of *FSHR* variants rs6166, rs6165 and rs1394205 is displayed in Figures 3b and 3c, respectively.

Genetic models' analysis revealed no significant differences regarding the FORT across the genotypes for *FSHR* variants rs6166, rs6165 or rs1394205 (Table IV).

Regarding the FOI, analysis of the co-dominant model for *FSHR* variant rs6166 revealed a significantly lower FOI for genotype A/G (79.48 ± 3.11) than for genotype A/A (92.79 ± 4.33), EMD -14.48 (95%CI -24.17 - -4.79) (Table V). In the dominant model, the presence of the G allele resulted in a combined lower FOI for the A/G and G/G genotypes (80.53 ± 2.65) when compared to the A/A genotype, EMD -13.47 (95%CI -22.69 - -4.24). Also, the additive model confirmed that each copy of the G allele reduced the FOI with an EMD -7.36 (95%CI -14.03 - -0.69).

Regarding *FSHR* variant rs6165, the co-dominant model revealed a lower FOI for genotype A/G 79.75 ± 3.35) than for genotype A/A (92.08 ± 6.23), EMD -13.81 (95%CI -25.41 - -2.21).

No statistically significant difference was observed for FOI according to *FSHR* variant rs1394205 genotypes.

When the 3 variants were compared either in Asian or European patients, no significant difference was found concerning FORT nor FOI (Suppl. Figures 1b and 1c, respectively).

DISCUSSION

According to our results, *FSHR* SNPs are associated with a statistically significant reduction in ovarian response to COS. However, this effect is of minor clinical relevance, since it resulted in only 1-2 less oocytes retrieved in a population of predicted normal responders. To our knowledge, this is the first prospective study on *FSHR* SNPs in which a fixed dose of 150 UI rFSH was administered to all patients throughout ovarian stimulation, with no dose-adjustments allowed, in order to provide an unbiased interpretation of the actual effect of the presence of these SNPs on ovarian response to stimulation. Furthermore, this is the first study to include a homogeneous cohort of good prognosis patients with normal ovarian reserve, involving 2 different ethnic populations in three different countries (Spain, Belgium and Vietnam).

The current study confirms previous reports demonstrating that the prevalence of SNPs in the *FSHR* is different between different ethnic groups and shows that the prevalence of the genotype T/T of the variant rs10835638 in the *FSHB* gene is extremely low in both Caucasian and Asian populations, as previously reported (Simoni and Casarini, 2014).

With regards to the ovarian response, we found a statistically significant lower number of oocytes retrieved in heterozygous patients for the *FSHR* variants rs6166 and rs1394205, a significantly higher rate of hypo-response in heterozygous patients for the *FSHR* variant rs6166 and a significantly lower FOI in heterozygous patients for the *FSHR* variants rs6166 and rs6165. Although homozygous patients for the minor allele of each variant also demonstrated a higher rate of hypo-response and a lower number of oocytes retrieved as compared to the major allele, results did not reach statistical significance. However, this does not mean the lack of effect of the presence of the allele, but it is probably associated with the low number of patients with these genotypes.

Several studies have been published up to date regarding the effect of *FSHR* polymorphisms on ovarian response yielding conflicting results. Perez Mayorga et al. first reported a significantly higher basal FSH and increased FSH requirement in patients with the genotype G/G for the variant rs6166 (Perez Mayorga et al., 2000). Since then, multiple original studies and meta-analysis have reported a higher consumption of FSH and reduced ovarian response during COS in patients with *FSHR* rs6166 and rs6165 G/G genotypes and rs1394205 A/A

352 genotype (Achrekar et al., 2009; La Marca et al., 2013; Trevisan et al., 2014; Alviggi et al.,
353 2018b; König et al., 2019; Song et al., 2019). However, most of those studies included a small
354 number of patients and heterogeneous treatment protocols, with different gonadotropin doses
355 or with dose adjustments during treatment. Although this definitely reflects clinical practice, it
356 doesn't allow a proper estimation of the true effect of SNPs on ovarian response.
357 The clinical relevance of our study relies on the fact that we were able to demonstrate a
358 statistically significant impact of *FSHR* polymorphisms in an ethnically diverse population of
359 predicted normal responders treated with a fixed dose of 150IU rFSH. However, this difference
360 is of minimal clinical relevance, since it resulted in a variation of 1-2 oocytes in a population
361 of normal responders. This finding is in line with two recent meta-analysis that reported a
362 similar mean difference in the number of oocytes retrieved (Tang et al., 2015; Alviggi et al.,
363 2018b).
364 The clinical implication of our findings is that *FSHR* polymorphisms should not be routinely
365 analyzed in predicted normal responders, given that such a small difference in number of
366 oocytes, albeit statistically significant, has minimal clinical relevance in a population of young
367 normal responders with a mean number of approximately 14 oocytes retrieved. Our findings
368 and conclusions do not mean that *FSHR* SNPs do not have a role in the future of
369 pharmacogenetics in ART; on the contrary, they may guide research towards different
370 directions in an attempt to find clinically meaningful differences. In this context, future
371 research is needed and should focus on other study populations such as hypo-responders, in
372 which an increase of 1 or 2 oocytes recovered does have a clinically significant impact on ART
373 outcomes (Polyzos and Popovic-Todorovic, 2020). Moreover, different SNPs or combined
374 SNPs in other genes involved in folliculogenesis, steroidogenesis and ovarian response should
375 be analyzed.
376 The major strengths of the current study reside in three main factors: a. the use of only one type
377 of gonadotropin (rFSH) and the maintenance of the same fixed dose of 150IU throughout the
378 whole stimulation phase; b. the strict inclusion criteria involving a good prognosis

homogeneous population selected based on ovarian reserve tests; c. the wide cover in the genetic sample, as it included Asian and Caucasian patients.

Nonetheless, despite its strengths, our prospective study has several limitations. First of all, we included only young good prognosis patients with normal ovarian reserve in order to eliminate biases related to age-related fertility decline. Although this was done because our aim was to identify a screening genetic biomarker that could be applied to all good prognosis patients in order to identify those needing a higher starting dose to avoid a hypo-response, caution is needed when extrapolating results to other populations, especially in women with worse prognosis. In addition, no analysis was performed for the variant in *FSHB* rs10835638 due the very low prevalence of homozygotes (n=2). Therefore, we cannot conclude about the effect of this variant on ovarian response. Still, its clinical significance appears to be minimal due to the low prevalence of the homozygous genotype.

In conclusion, genotyping of *FSHR* SNPs rs6165, rs6166, rs1394205 or *FSHB* SNP rs10835638 prior to initiating ovarian stimulation in predicted normal responders should not be routinely recommended taking into account the minimal clinical impact of such information in this population.

AUTHORS' ROLES

NPP conceptualized and designed the study, supervised the performance of the study, wrote the first draft of the manuscript, contributed substantially to the interpretation of the findings, critically revised the manuscript and accepted the final version. **ARN** contributed substantially to the interpretation of the findings, writing of the manuscript, critically revised it and accepted the final version. **PD** and **DS** contributed to the design of the study protocol, critically revised the manuscript and accepted the final version. **CS** collected the data and performed the experiments, contributed substantially to the interpretation of the findings, critically revised the manuscript and accepted the final version. **BAM** collected the data, wrote the first draft of the manuscript, contributed substantially to the interpretation of the findings, critically revised the manuscript and approved the final version. **SG** Performed the statistical analysis, contributed substantially to the interpretation of the findings, critically revised the

manuscript and approved the final version. **PQMM**, **LHL**, **MTH** and **NLV** collected the data, critically revised the manuscript and accepted the final version. **JM** collected the data and performed the experiments. **HT** critically revised the manuscript and accepted the final version.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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CONFLICT OF INTEREST

NPP reports grants and/or personal fees from MSD, Merck Serono, Roche Diagnostics, Ferring International, Besins Healthcare, Gedeon Richter, Theramex and IBSA (Institut Biochimique SA).

NLV, **MTH** report consultancy and conference fees from Merck, Ferring and MSD, outside the submitted work.

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ARN, **BAM, CS, MJ, LHL, PQMM, HT** and **SG** report no conflict of interests.

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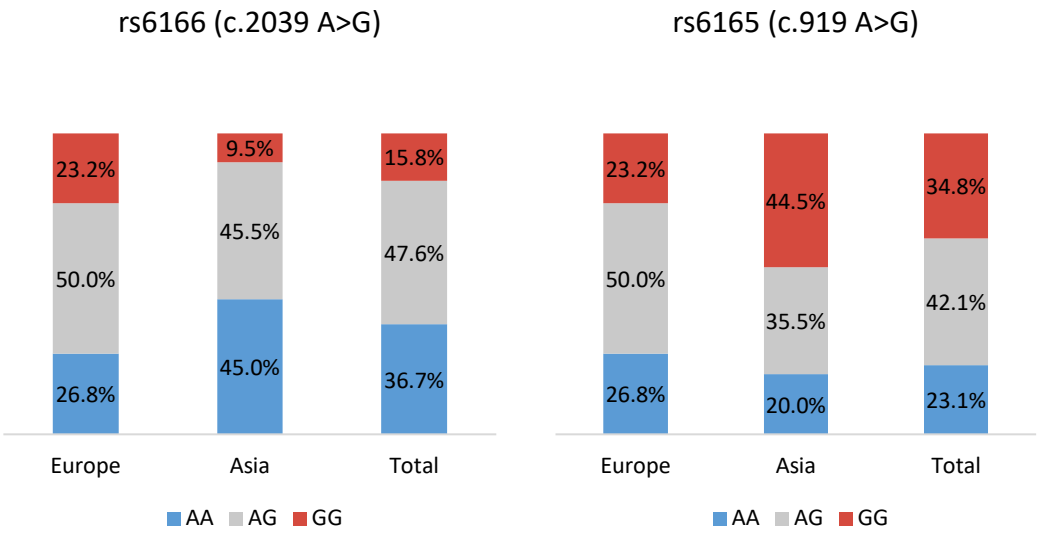
Figure 1. Prevalence (%) of the different genotypes per continent and total

Figure 2. Prevalence of hypo-response according to the genotypes of polymorphisms rs6166, rs6165 and rs1394205

Figure 3. Univariate analysis of the number of oocytes retrieved (a), FORT (b) and FOI (c) according to the genotypes of polymorphisms rs6166, rs6165 and rs1394205

Suppl Figure 1. Univariate analysis of the number of oocytes retrieved (a), FORT (b) and FOI (c) according to the genotypes of polymorphisms rs6166, rs6165 and rs1394205 in European and Asian patients



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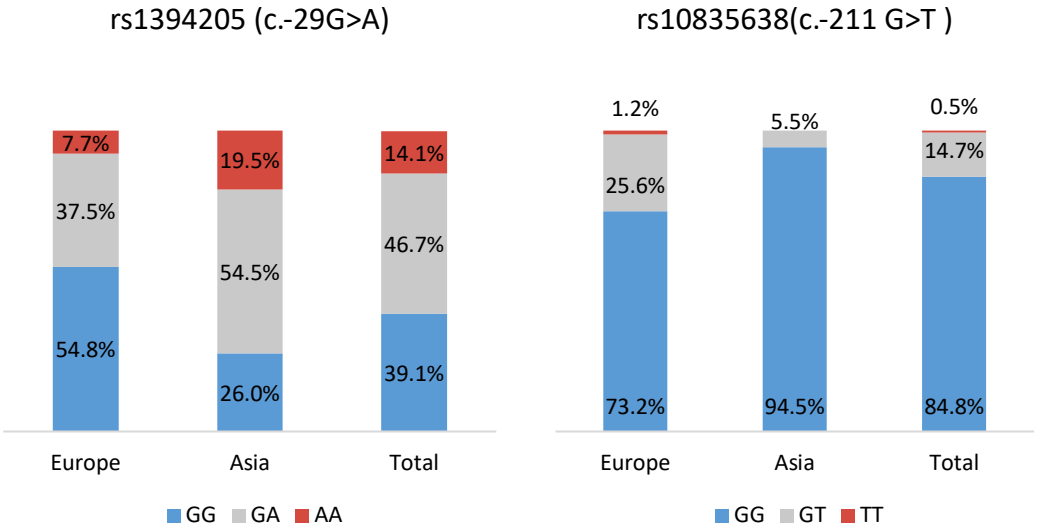

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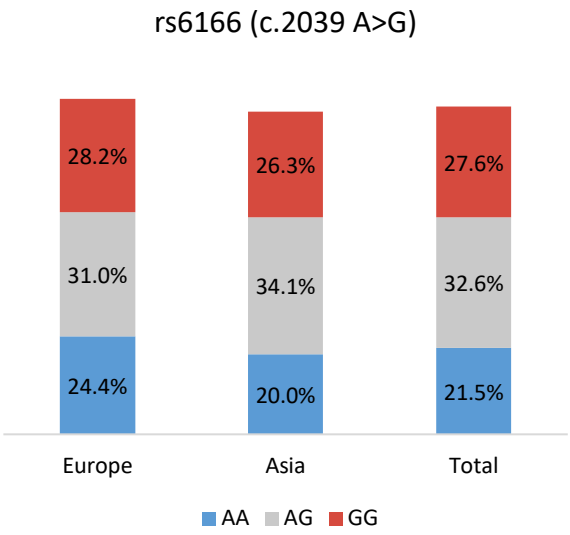

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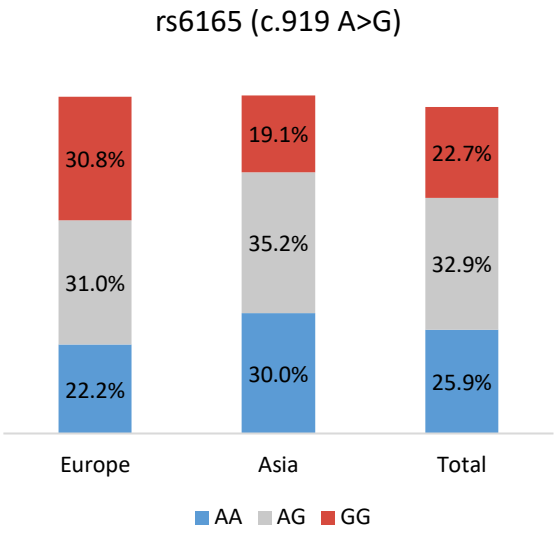

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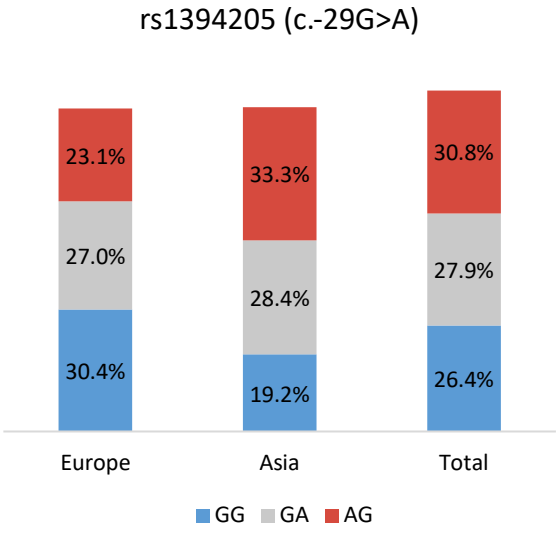

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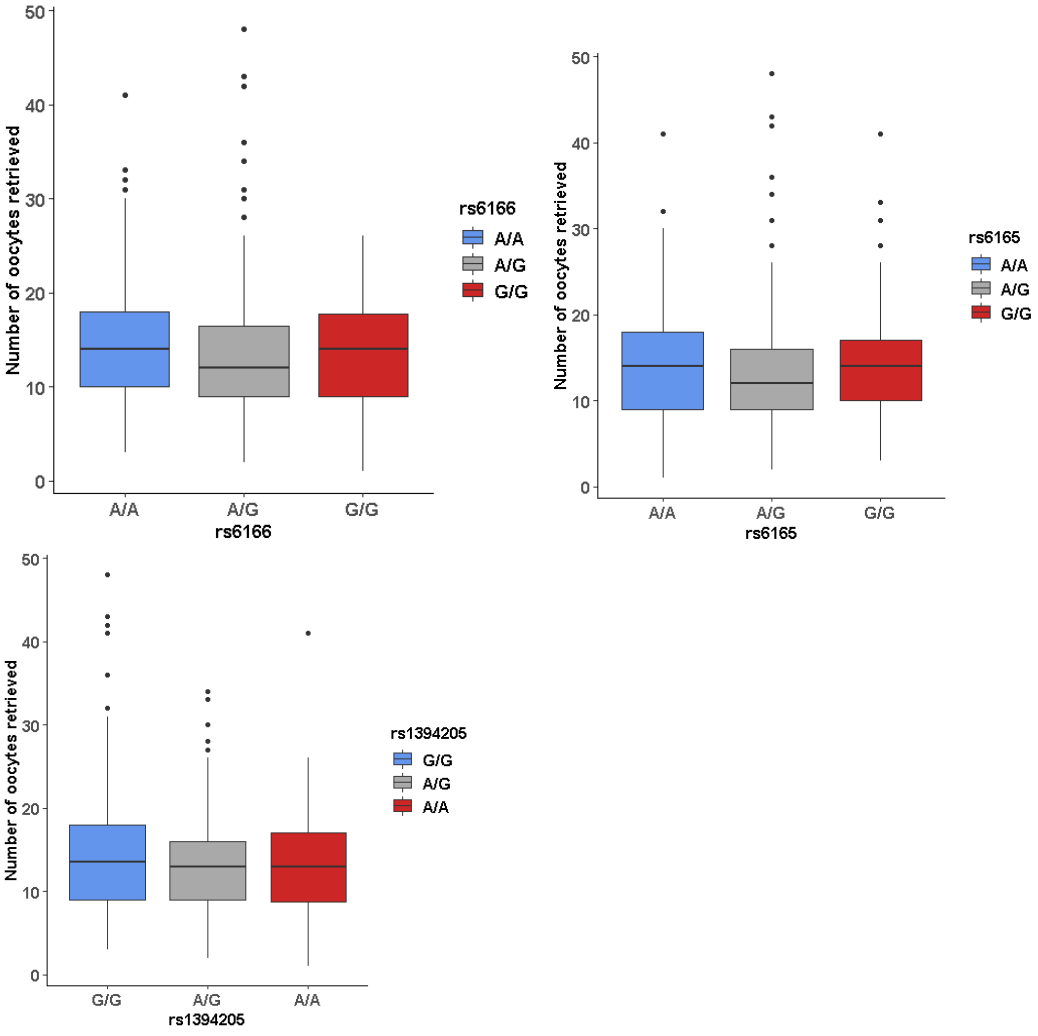
Number of oocytes retrieved
50
40
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20
10
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A/A
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G/G
rs6166
rs6166
A/A
A/G
G/G
Number of oocytes retrieved
rs6165
rs6165
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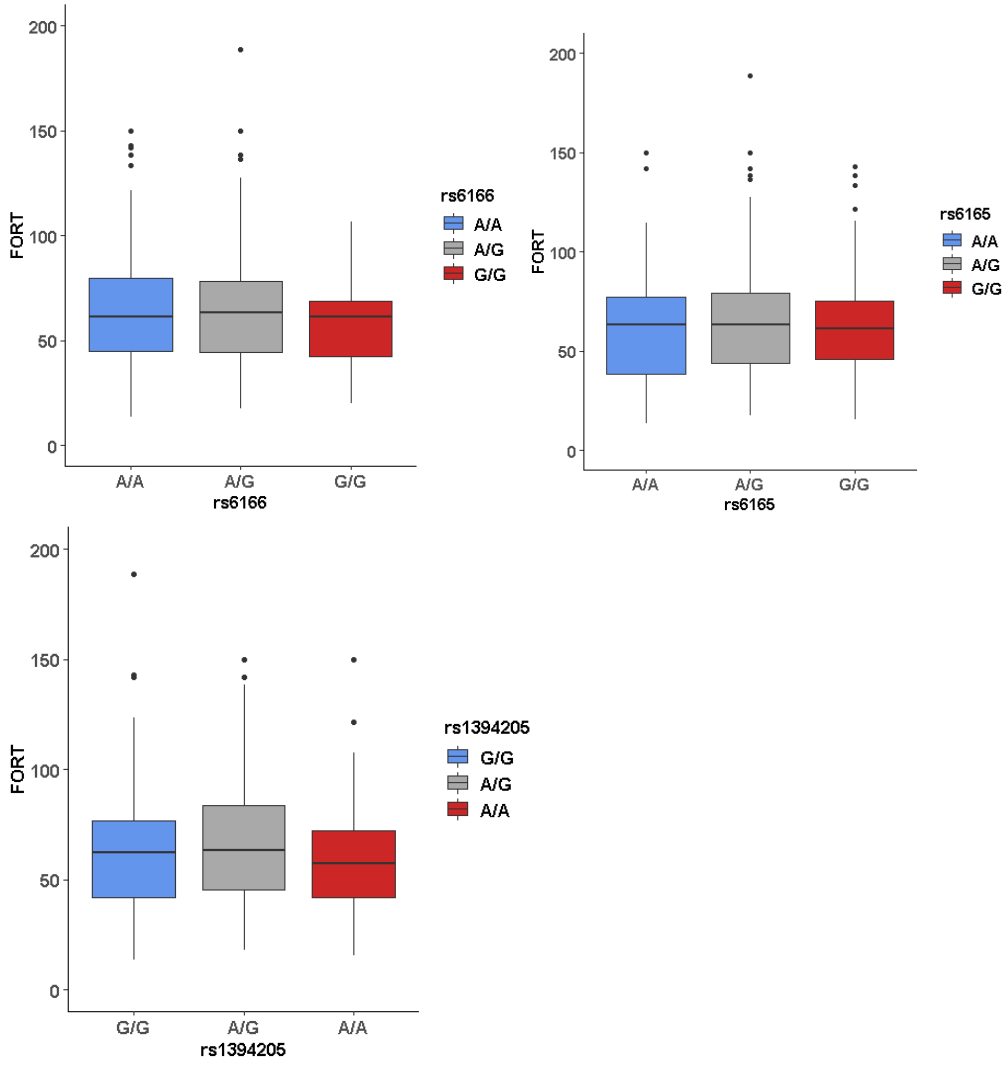
A/G
G/G
Number of oocytes retrieved
rs1394205
rs1394205
G/G
A/G
A/A

Figure 3a


FORT
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rs1394205
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A/G
A/A

Figure 3b.

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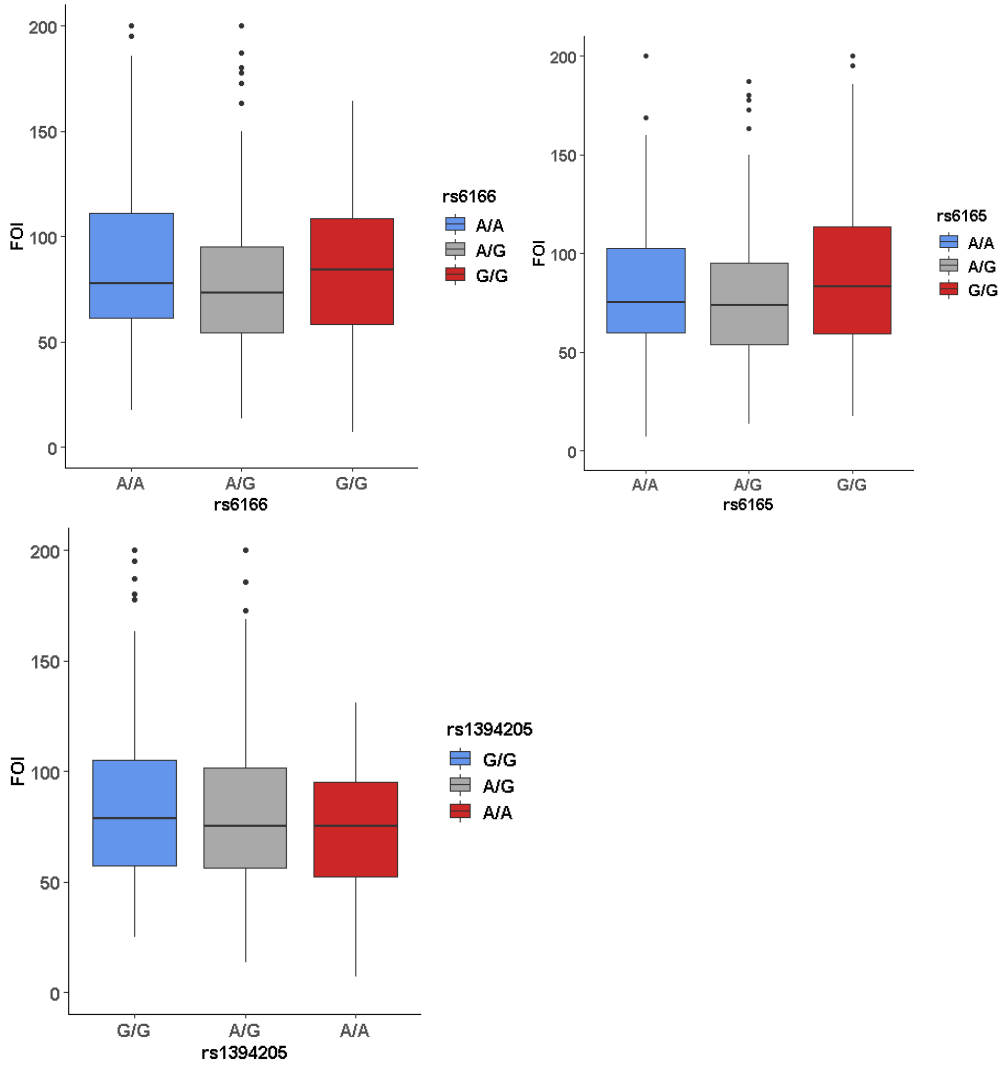

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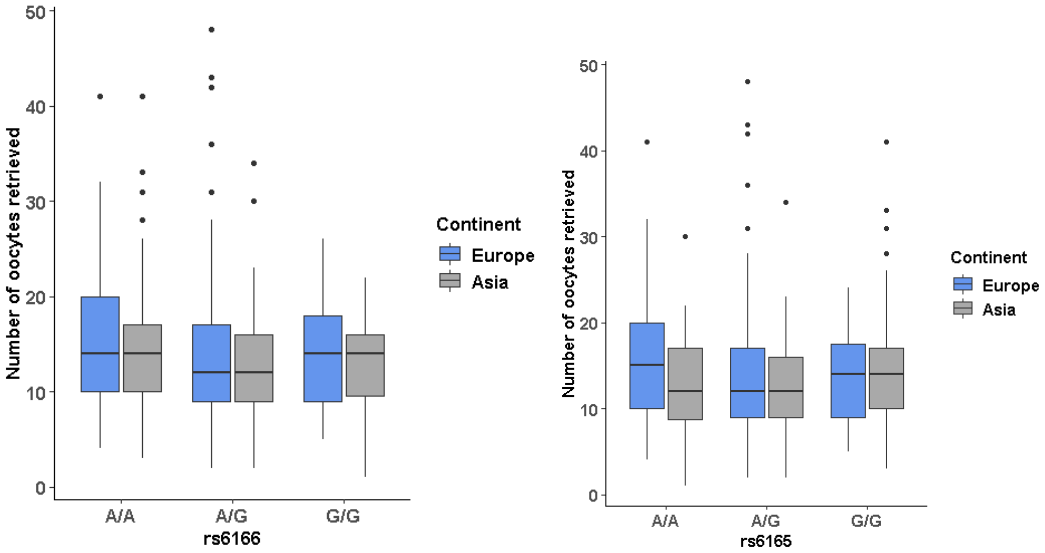

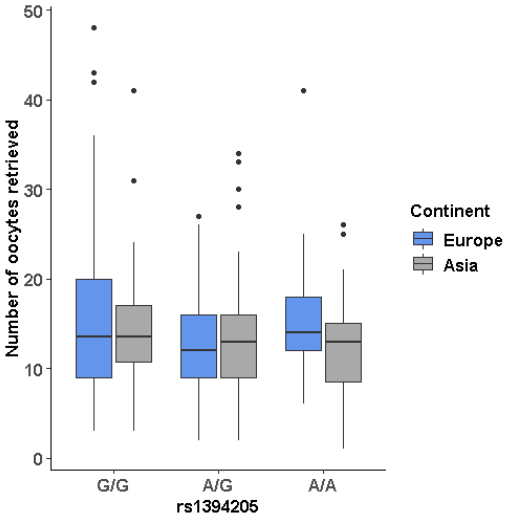

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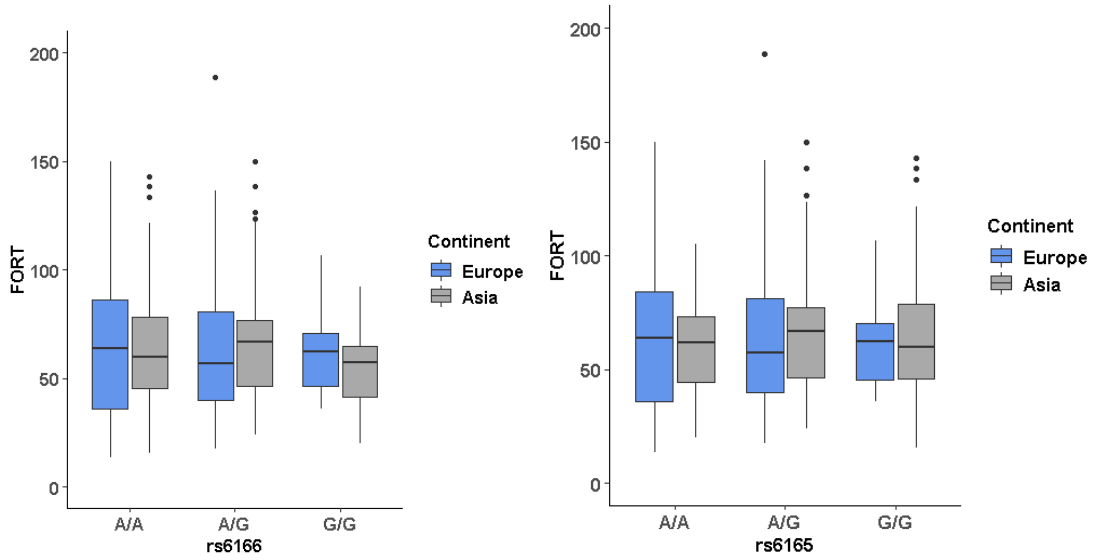
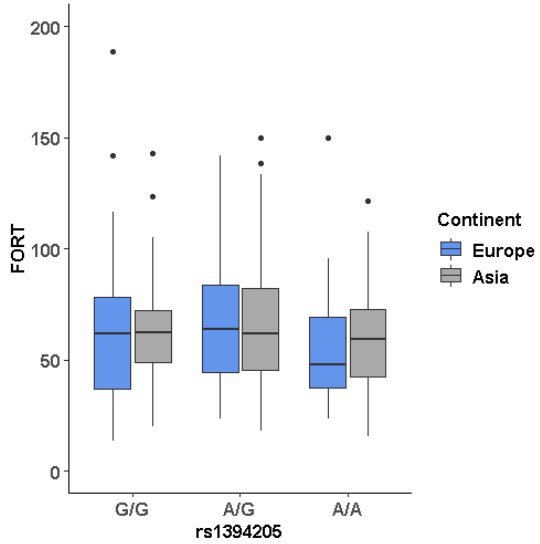
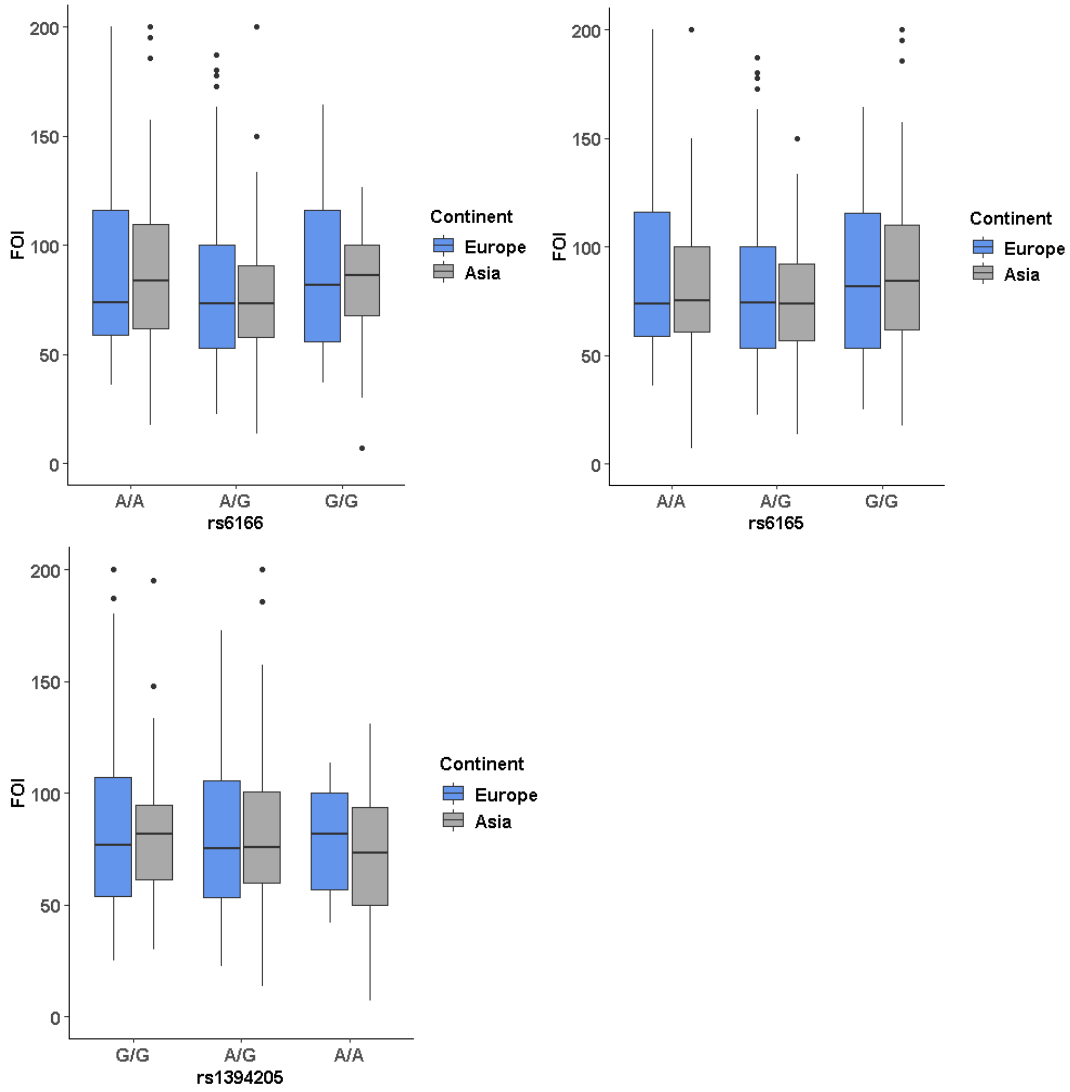




Supp Figure 1b.



Supp Figure 1c.

Table I. Causes of infertility

	Male factor	Idiopathic	Tubal	Others
Europe	66 (39.3%)	64 (38.1%)	8 (4.8%)	30 (17.9%)
Asia	106 (53%)	35 (17.5%)	44 (22%)	15 (7.5%)
Total	172 (46.7%)	99 (26.9%)	52 (14.1%)	45 (12.2%)

637 Table II. Association between FSHR polymorphisms and progesterone category.

rs6166				
Model	**Genotype**	**Normal (≤1.50 ng/ml)**	**High (>1.50 ng/ml)**	**OR (95% CI)**
Co-dominant	A/A			1.00
	A/G			1.02 (0.54-1.91)
	G/G			0.71 (0.27-1.83)
Dominant	A/A			1.00
	A/G-G/G			0.95 (0.52-1.73)
Recessive	A/A-A/G			1.00
	G/G			0.70 (0.29-1.67)
rs6165				
Model	**Genotype**	**Normal (≤1.50 ng/ml)**	**High (>1.50 ng/ml)**	**OR (95% CI)**
Co-dominant	A/A			1.00
	A/G			1.18 (0.56-2.46)
	G/G			00.9 (0.41-2.00)
Dominant	A/A			1.00
	A/G-G/G			1.05 (0.53-2.09)
Recessive	A/A-A/G			1.00
	G/G			0.81 (0.43-1.52)
rs1394205				
Model	**Genotype**	**Normal (≤1.50 ng/ml)**	**High (>1.50 ng/ml)**	**OR (95% CI)**
Co-dominant	G/G			1.00
	A/G			1.13 (0.59-2.16)
	A/A			1.25 (0.51-3.06)
Dominant	G/G			1.00
	A/G-A/A			1.15 (0.63-2.14)
Recessive	G/G-A/G			1.00
	A/A			1.16 (0.52-2.61)

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Table IV. Association between FSHR polymorphisms and FORT. CI, confidence interval; EMD, estimated mean difference, SD, standard deviation.

rs6166				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	A/A	128	70.6 (4.3)	0.00
	A/G	155	66.59 (2.48)	-4.62 (-13.36 - 4.12)
	G/G	46	59.98 (2.82)	-10.28 (-23.05 - 2.49)
Dominant	A/A	128	70.6 (4.3)	0.00
	A/G-G/G	201	65.08 (2.03)	-5.84 (-14.16 - 2.49)
Recessive	A/A-A/G	283	68.4 (2.37)	0.00
	G/G	46	59.98 (2.82)	-7.64 (-19.39 - 4.11)
Log-additive	---	---	---	-5.01 (-10.99 - 0.98)
rs6165				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	A/A	78	72.43 (6.73)	0.00
	A/G	134	66.35 (2.62)	-6.52 (-16.97 - 3.93)
	G/G	117	64.76 (2.29)	-6.02 (-17.00 - 4.97)
Dominant	A/A	78	72.43 (6.73)	0.00
	A/G-G/G	251	65.61 (1.76)	-6.30 (-15.87 - 3.27)
Recessive	A/A-A/G	212	68.59 (2.98)	0.00
	G/G	117	64.76 (2.29)	-1.85 (-10.59 - 6.88)
Log-additive	---	---	---	-2.77 (-8.22 - 2.67)
rs1394205				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	G/G	124	65.75 (3.31)	0.00
	A/G	157	70.63 (3.29)	6.81 (-2.27 - 15.89)
	A/A	48	59.91 (3.72)	-4.44 (-17.32 - 8.45)
Dominant	G/G	124	65.75 (3.31)	0.00
	A/G-A/A	205	68.12 (2.68)	4.39 (-4.34 - 13.12)
Recessive	G/G-A/G	281	68.47 (2.35)	0.00
	A/A	48	59.91 (3.72)	-8.64 (-20.26 - 2.99)
Log-additive	---	---	---	-0.23 (-6.43 - 5.96)

Table V. Association between FSHR polymorphisms and FOI. CI, confidence interval; EMD, estimated mean difference, SD, standard deviation.

rs6166				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	A/A	128	92.79 (4.33)	0.00
	A/G	155	79.48 (3.11)	-14.48 (-24.17 - -4.79)
	G/G	46	84.08 (4.89)	-9.78 (-23.94 - 4.37)
Dominant	A/A	128	92.79 (4.33)	0.00
	A/G-G/G	201	80.53 (2.65)	-13.47 (-22.69 - -4.24)
Recessive	A/A-A/G	283	85.5 (2.62)	0.00
	G/G	46	85.5 (2.62)	-1.51 (-14.69 - 11.67)
Log-additive	---	---	---	-7.36 (-14.03 - -0.69)
rs6165				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	A/A	78	92.08 (6.23)	0.00
	A/G	134	79.75 (3.35)	-13.81 (-25.41 - -2.21)
	G/G	117	87.14 (3.4)	-3.60 (-15.80 - 8.60)
Dominant	A/A	78	92.08 (6.23)	0.00
	A/G-G/G	251	83.2 (2.4)	-9.37 (-20.05 - 1.32)
Recessive	A/G-A/A	212	84.29 (3.14)	0.00
	G/G	117	87.14 (3.4)	5.23 (-4.54 - 14.99)
Log-additive	---	---	---	-0.99 (-7.09 - 5.10)
rs1394205				
Model	**Genotype**	**n**	**Mean (SD)**	**EMD (95% CI)**
Co-dominant	G/G	124	88.72 (4.03)	0.00
	A/G	157	85.3 (3.44)	-1.35 (-11.52 - 8.83)
	A/A	48	76.48 (5.02)	-12.33 (-26.77 - 2.11)
Dominant	G/G	124	88.72 (4.03)	0.00
	A/G-A/A	205	83.24 (2.89)	-3.71 (-13.48 - 6.06)
Recessive	G/G-A/G	281	86.81 (2.61)	0.00
	A/A	48	76.48 (5.02)	-11.50 (-24.49 - 1.49)
Log-additive	---	---	---	-5.11 (-12.01 - 1.80)