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Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche

Direttore: Prof. Lucio Annunziato

Tesi di Dottorato

Impact of Polymorphisms of Gonadotrophins and their receptors on controlled ovarian stimulation

Relatore
Ch.mo.
Prof. Carlo Alviggi

Candidato
Dott.
Alessandro Conforti

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Introduction

Genetic characteristics and impaired response to controlled ovarian stimulation

Standard *in vitro fertilization* (IVF) protocols are characterized by the administration of exogenous *follicle stimulation hormone* (FSH) which is widely adopted regimen for controlled ovarian stimulation (COS) in normogonadotrophic women. In daily clinical practice, the ovarian response to these protocols are optimal in about 85% of patients, with more than 3 mature oocytes recruited. In about 12-15% of cases, however, an initial low response is seen, leading to an increase in the daily dose of FSH, resulting in a higher total FSH consumption (e.g. >2500 IU) (Alviggi et al., 2013). These observations lead to

the development of the concept of "hypo-response" to COS to identify normogonadotrophic women who have normal estimated ovarian reserves but require high amounts of FSH to obtain an adequate number of oocytes retrieved (De Placido et al., 2005, Ferraretti et al., 2004, Devroey et al., 2009). These women seem to be distinct from classical poor responders because they have normal ovarian reserve, but show an unexpected sub-optimal response when stimulated with standard regimens. Conversely, specific adjustments of classical protocols seem to optimise ovarian response (De Placido et al., 2005).

On the basis of the current literature, it is possible to argue that hypo-response could be related to genetic characteristics. More specifically, several lines of evidence indicate that this clinical condition may be related to polymorphisms of the genes of gonadotropins and their

receptors (Alviggi et al., 2009b, Alviggi et al., 2013).

Doctoral research: objectives and sessions

The aim of this research project was to exploit the effects of polymorphisms of gonadotropins and their receptors on ovarian response:

- The Session A, developed in the first year, is devoted to the results of retrospective analysis concerning the role of FSH-R receptor polymorphism (rs6166). This findings were recently published in a peer review journal (Alviggi et al., 2016b).
- The Session B, developed in the second year, is devoted to the results of a prospective analysis in which eight polymorphism of gonadotropins and their receptors were evaluated. Our findings regarding

Italian population were recently published in a peer review journal. (Conforti et al., 2017). The preliminary results about all population included were recently published as a supplement in Human Reproduction Journal (Alviggi et al., 2016c).

- The Session C developed in the third year of research is devoted to a Systematic Review and Meta-analysis of available worldwide literature data regarding the effect of gonadotropin and their receptors polymorphism on COS. This analysis includes 33 studies and involves more than 4,000 observations. This systematic review was accepted for submission by Human Reproduction Update editorial board which represent the most eminent journal in Obstetrics and Gynaecology (Journal Citation Reports Thomson Reuters)

Section A: Unexpected "Hyporesponse" to Controlled Ovarian Stimulation is related to polymorphisms of FSH Receptor: a retrospective study.

Background

Recently, the European Society for Human Reproduction and Embryology (ESHRE) published criteria for definition of "poor ovarian response" to COS in IVF cycles. According to these criteria, at least two of the following characteristics should be present to meet the definition: advanced maternal age (>40 years) or any other risk factor for poor ovarian response; previous poor ovarian response (<3oocytes with a conventional stimulation protocol); and abnormal ovarian reserve test (Ferraretti et al., 2011). Nevertheless, a further subgroup of "low prognosis" patients who do not fit neither with these criteria nor with the classical "normal responder" profile can be identified. More specifically, it has been reported that 10% to 15% of young, normogonadotrophic women show suboptimal response to standard gonadotropin-releasing hormone

(GnRH)-a long protocol (Alviggi et al., 2013). These patients, despite an apparently normal ovarian response (namely, the recovery of at least 5 oocytes), require higher doses of exogenous FSH than expected based on age, anthropometric variables and ovarian reserve tests (Alviggi et al., 2011a, Alviggi et al., 2013). We suggest the definition of hypo-responders for these patients. Indeed, new lines of evidence indicate that this phenomenon could be associated with genetic characteristics (Huhtaniemi et al., 1999, Alviggi et al., 2015). We recently reported that the frequency of an allelic variant of the luteinizing hormone (LH) beta subunit is higher in women with a hypo-response to recombinant human FSH (r-hFSH) than in the general population (Alviggi et al., 2011b, Alviggi et al., 2009b, Alviggi et al., 2013).

This observation is consistent with clinical trials demonstrating that recombinant human LH significantly increases both ovarian response and implantation rate in hypo-responders to monotherapy with r-hFSH (Ferraretti et al., 2004, De Placido et al., 2005). However, the LH variant has been found in only 32% of IVF candidates with a

hypo-response profile. Thus, the pathogenetic mechanism underlying the hypo-response to FSH in about two-third of women remains to be established.

Two polymorphisms of the FSH receptor (FSH-R), Thr307/Asn680 and Ala307/Ser680, have been associated with a higher requirement of exogenous gonadotrophins during COS, (Perez Mayorga et al., 2000, Mohiyiddeen and Nardo, 2010, Yao et al., 2011), suggesting higher ovarian threshold compared with wild type.

The FSH-R is a member of G-protein-coupled receptor which mediates FSH intracellular signals through cyclic adenosinemonophosphate pathway (Simoni et al., 1997).

In addition, FSH-R might explain inter-individual differences in menstrual pattern. In fact, in homozygotes Ser680/Ser680 of FSH-R, a higher ovarian threshold to FSH, a decreased negative feedback of luteal secretion to the pituitaryduring the intercycle transition, and longer menstrual cycles have been described (Greb et al., 2005b).

The aim of this study is to explore FSH-R allelic frequency in a cohort of young normogonadotrophic patients expected to be "good

prognosis" based on ESHRE criteria, by stratifying the population according to r-hFSH consumption and focusing on the impact that these polymorphisms may have on COS outcome.

Material and Methods

This study was conducted at the Outpatient Fertility Unit of the Federico II University in Naples, Italy, from October 2011 to April 2015.

The clinical management of women included in this study was not modified by the investigators, and no adjunctive interventions were necessary. In accordance with our internal protocol, upon admission, we obtained written informed consent from all patients for the use of their data in agreement with privacy protection laws (Italian Law 675/96).

We retrospectively selected 17 normoresponder young patients undergoing a standard IVF/ICSI cycle, with at least 5 oocytes recovered, who required a cumulative dose of r-FSH >2500 UI (group A). A control group was selected (ratio 1:2) among

normoresponder young patients undergoing a standard IVF/ICSI cycle, with at least 5 oocytes recovered, who required a cumulative dose of r-FSH <2500 UI (group B). The enrolment phase was concluded when a total number of 42 patients was reached.

Inclusion criteria were: age <37 years; menstrual cycle lasting 24 to 35 days (intra-individual variability + 3 days) 6 months before the onset of IVF cycle; FSH <11 IU/L, LH <8 IU/L, and prolactin <30 ng/mL (measured from the 2nd to 4th day of spontaneous menstrual cycle); and at least 5 oocytes retrieved for each patient. We excluded from the study patients meeting one or more of the following criteria: polycystic ovary syndrome (Rotterdam, 2004); stage III to IV endometriosis or elevated CA125 (ASRM, 1997, Patrelli et al., 2011); autoimmune disorders; and chromosomal abnormalities.

All patients received a standard GnRH agonist-long protocol using triptorelin (Decapeptyl 0.1; Ipsen, Italy) at the daily dose of 0.1 mg subcutaneously (SC) starting on the 21st day of the cycle preceding IVF treatment. Pituitary desensitization was confirmed by

transvaginal ultrasound (no evidence of ovarian activity, endometrial thickness < 5 mm) and circulating oestradiol assessment (<50 pg/mL). Patients with pituitary down-regulation started r-hFSH treatment (Gonal F; MerckSerono, Rome, Italy); a starting dose of 225 IU/day SC was administered for the first 4 days of stimulation. This dose was reduced to 150 IU/day in women with serum oestradiol >160 pg/mL on day 5 of stimulation. If the serum oestradiol was <100 pg/mL on day 5, the daily dose was increased to 300 IU. Otherwise, the daily dose remained unchanged.

In all patients, the ovulatory dose of 10,000 IU human chorionic gonadotrophin (hCG) was administered intramuscularly (im) in the presence of at least 1 follicle reaching a mean diameter of 17 mm. All patients received luteal phase supplementation with a daily dose of 50 mg progesterone im (Prontogest, IBSA Farmaceutici Italia S.r.l., Italy) from the day of oocyte retrieval. Ongoing pregnancy rate was defined as the presence of foetal heart activity detected at 12 weeks of gestation.

A blood sample was collected from each patient. Genomic DNA was

extracted from peripheral blood leukocytes with a Cell Culture DNA kit (QIAGEN, Dusseldorf, Germany) according to the manufacturer's instructions. A fragment of the FSH-R gene from exon 10 (from 10D to 10G) was amplified by polymerase chain reaction and analyzed by electrophoresis on 2% agarose gel. The segment was then extracted with phenol chloroform. The purified fragment was digested by Bsrl1 (Biolabs, Schwabach, Germany), and fragments were analysed by electrophoresis on 2.5% agarose gel. The unpurified fragment, indicating homozygosity for Asn, measured 755 bp. The purified fragment indicating homozygous Ser generated two fragments measuring 612 and 143 bp, respectively. The presence of all three fragments indicates the status of homozygosity.

The serum concentrations of oestradiol and LH were measured using an enzyme-linked fluorescent assay (Vidas oestradiol and Vidas LH II, respectively; Bio Me´rieux SA, Lyon, France). The sensitivity of the method, defined as the lowest concentration that is significantly different from zero with probability of 95%, was 0.03 pg/mL for oestradiol and 0.1 IU/L for LH. The coefficient of

variations (CVs) of intra- and inter-assay was <8% for both oestradiol and LH. Serum levels of FSH were determined by an immunoassay based on luminescence (Amerlite FSH Assay; Amersham International plc, Amersham).

Statistical Analysis

We performed a statistical analysis by SPSS software (Chicago, Illinois) version 19 for Windows, applying parametric and nonparametric tests where appropriate. The Kolmogorov-Smirnov test was used to assess the normality of distribution. Continuous variables were expressed as absolute numbers, average ± standard deviation; categorical variables were expressed as percentages. The Student t test was adopted to determine the effects of stimulation protocols on continuous variables and to evaluate the differences between the groups. The χ2 test was used to compare categorical data and to assess the Hardy-Weinberg equilibrium of FSH-R genotypes. Statistical

significance was defined as P values <0.05.

Results

We retrospectively reviewed the outcome of 42 cycles of IVF women. Demographic, anthropometric, and hormonal characteristics did not differ significantly between the two groups (Table 1A). The duration of infertility status was significantly different among patients with higher r-hFSH consumption versus normal responders (4.15 ± 1.2 years vs 3.2 ± 0.9, P = 0.0055). Indications for assisted reproduction were comparable in both groups (Table 1A).

Table 2A shows the outcome of assisted reproduction technology (ART) cycles in the two groups. The mean number of r-hFSH vials (36.3 ± 7.5 vs 28.6 ± 4.5, P = 0.0001) and number of days of stimulation (12.7 ± 2.4 days vs 10.8 ± 2.8, P =0.03) were significantly lower in the control group (group B). The number of oocytes retrieved was significantly lower in group A (7.1 ± 1.5 vs 9.6 ± 2.4; P = 0.0003). While the average number of embryos transferred was significantly higher in group B (2.7 ± 0.4 vs 2.1 ±

0.7; P = 0.001). There were no statistically significant differences regarding cumulative pregnancy rates, abortion rates, and rates of ongoing pregnancy. Serum levels of oestradiol, measured on the day of hCG administration, were significantly lower in group A (997.8 ± 384.9 pg/mL vs 1749.1 ± 644.4; P =0.0001).

In group A, the Ser/Ser genotype was identified in 10 (58.8%) patients, the Asn/Ser genotype in 4 (23.5%) patients, and the Asn/Asn genotype in 3 (17.6%) patients (Table 1A). In group B, the Ser/Ser genotype occurred in 5 (20%) patients, Asn/Ser genotype in 15 (60%) patients, and Asn/Asn genotype in 5 (20%) patients (Table 1A). The χ2 analysis revealed that the genotypes were in Hardy-Weinberg equilibrium.

The incidence of Ser/Ser genotype was higher in patients with higher r-hFSH consumption (group A) compared to control group (group B; P = 0.02). On the contrary, the Asn/Ser genotype was more frequent in group B (P = 0.04).

Discussion

This study confirms that the FSH-R genotype may interfere with physiological responsiveness of the target organ to FSH stimulation. The presence of the FSH-R Ser680 variant seems to result in a significant decrease in ovarian response to r-hFSH during ART cycles and, therefore, in a significant increase in drug consumption. More specifically, among patients requiring a higher cumulative dose of r-hFSH (group A), the expression of Ser/Ser genotype was significantly higher compared to the subgroups carrying variants Asn/Ser or Asn/Asn of FSH-R (Table 1A).

Interestingly, our results show that patients with higher r-hFSH consumption and FSH-R Ser680 variant carriers have a longer infertility condition (Table 1A). From the analysis of these data, we could assume that the increased resistance to endogenous FSH, observed in FSH-R Ser680 carriers may affect female fertility, delaying pregnancy occurrence. As a matter of fact, a higher basal level of FSH was detected in hypo-responder group (Table 2A). Nonetheless, this hypothesis needs to be confirmed by larger population studies.

The frequency of FSH-R polymorphism in our study population differed from what reported by other authors. We compared our results with those published by Perez-Mayorga et al., (Perez Mayorga et al., 2000) taking into consideration the ethnic group (Caucasian) and the number of patients involved (n = 161). As shown in Figure 1A, Perez-Mayorga et al. observed a prevalence of 26% for Ser/Ser genotype, 45% for heterozygous Asn/Ser, and 29% for homozygous Asn/Asn, whereas, in our study population, we observed a prevalence of 36%, 45%, and 19%, respectively. The difference observed in the frequency of FSH-R polymorphism could reflect a particular pattern of distribution in the Campania Region. Alternatively, it could be due to a major difference in the design of the two studies. More specifically, our recruitment was not randomized, and we chose patients from a selected pool of women affected by reproductive problems. Moreover, 17 of our 42 patients showed "resistance" to the protocols of ovarian stimulation, and 10 of 17 were homozygous carriers of the Ser680 variant. Therefore, the selection criteria used for group A patients (i.e., high

consumption of r-FSH) may have significantly affected the distribution of allelic frequency in the whole study population, which could account for the discrepancy between our results and the ones by Perez-Mayorga et al. Our results highlight the existence of a subgroup of patients (hypo-responders) who require a higher cumulative dose of r-hFSH to obtain a reproductive outcome compared to normal responders. As a matter of fact, in all our patients at least 3 oocytes were retrieved with peak oestradiol levels >500 pg/mL; therefore, on the basis of the new criteria, they were classified as "normal responders." However, when the groups A and B were analysed based on the cumulative dose of r-hFSH, the average number of oocytes retrieved, the serum oestradiol peak and the number of embryo replaced were statistically higher in the group receiving the lowest cumulative dose of gonadotropin. This observation suggests an "intermediate" category of patients which, although not fitting the criteria of "poor responders," still have a marked resistance to ovarian stimulation and a less favourable prognosis compared to "normal responders."

The association between FSH-R Ser680 variant and ovarian resistance to exogenous gonadotropins observed in our study complies with a recent meta-analysis that showed a higher consumption of exogenous gonadotropins in homozygous carriers of Ser680 (Ser/Ser) genotype compared to Asn/Asn variant carriers (Yao et al., 2011). The main differences between our study and other publications on this topic (Perez Mayorga et al., 2000, De Castro et al., 2003, Behre et al., 2005, Loutradis et al., 2006, Genro et al., 2012) concern the study design and the parameters evaluated.

In our study, we stratified the population on the basis of gonadotrophins consumption rather than on the FSH-R genotype expression, in order to identify the mechanism underlying the hyporesponse phenomenon. Thus, we were able to identify a significant difference in the frequency of Ser/Ser genotype between hyporesponder patients and control group (58.8% vs 20.0%).

Our results disagree with the findings of the Fanchin group (Genro et al., 2012), which indicate that follicle-stimulating hormone

receptor genotype does not influence antral follicle responsiveness to FSH. This important difference may be due to the elevated FSH doses (300 UI) used in the Fanchin study that could have overcome the lack of functionality of FSH receptor in women carriers of Ser680 variant. Whereas our decision to use a cumulative r-hFSH dose of 2500 IU as a cut-off to define the two profiles of response was based on our clinical experience, according to which young normogonadotrophic women with body mass index <27 kg/m^2 achieve an adequate ovarian response with cumulative doses of FSH not exceeding 2000 to 2225 IU.

Although none of the patients enrolled showed a poor responder profile, we observed a decreased number of retrieved oocytes (Figure 2A) and transferred embryos in the hypo-responder group, in which the incidence of FSH-R Ser680 variant was higher (58.8% of cases; Table 1A). A possible effect on ovarian stimulation outcome was argued by De Castro et al. (De Castro et al., 2003). Specifically, they reported a higher incidence of Ser/Ser carriers among poor responder patients in which the number of oocytes

retrieved was less than three. Interestingly, we found that the homozygous Ser680 variant seems to affect serum oestradiol (Figure 3A). In fact, during stimulation, oestradiol levels were lower in Ser680 variant carriers than in Asn/Asn ones. This observation is in line with another report showing lower serum oestradiol in Ser/Ser carriers at the time of hCG administration. As mentioned previously, another group of hypo-responder patients was identified among common LH variant carriers. This polymorphism is quite common with a prevalence estimated around 42% in some countries of Northern Europe and is characterized by a reduced in vivo bioactivity and low response to r-hFSH. Therapeutic behaviour of both v-beta LH and FSH-R polymorphism carriers seems to be comparable in terms of cumulative r-hFSH dosage and number of oocyte retrieved. Although our results are supported by rigorous methods and statistical analysis, they are not free from limitation potentially affecting the accuracy of evidences. The relative small sample size, in particular of case group, and the retrospective design of the study led us to recommend caution in the data

interpretation. Certainly, our results require further validation by large-scale randomized prospective trials. Although the reduced size of our study, 58% (10/17) of patients with a hypo-response profile during CO carried FSH-R homozygous Ser680 genotype. Thus, we can hypothesize an association between the investigated FSH-R polymorphisms and the risk of ovarian resistance to exogenous FSH. The fact that the FSH-R genotype affects the ovarian response to FSH has implications for the sub-stratification of patients, for the choice of stimulation protocol, and for the starting dose of gonadotropins. There is increasing evidence that the generic definition of the expected ovarian response (poor, normo, and high, according to the ovarian reserve test) does not cover all possible differences in the general population. Moreover, in the large pool of normoresponders patients, great differences remain in terms of ovarian responsiveness. In our opinion, the identification of a different cohort of patients with an increased or decreased ovarian sensitivity to FSH, based on unique genetic patterns (FSH receptor polymorphisms), could improve the cost-effectiveness of IVF

treatments. Indeed, in case of particular FSH-R polymorphisms associated with lower ovarian response, we suggest the choice of a higher gonadotropins starting dose and the combination of different gonadotropin preparations, for instance by adding recombinant LH to standard r-FSH stimulation. This already proved effective also in poor-responder patients (Alviggi et al., 2011a, Gizzo et al., 2015, Gizzo et al., 2016). It is conceivable that a "tailored" FSH therapy may be adopted on the basis of patient genetic profile, customizing not only the dosage but also the timing of stimulation. Other possible benefits could be the reduction in the stimulation duration and the amount of FSH needed. Moreover, the knowledge of the mechanisms regulating the ovarian sensitivity to FSH can be useful in the prevention of ovarian hyperstimulation syndrome. The immediate implications would be saving in costs and increased treatment acceptance.

Section B:

Background

Pharmacogenomic approach to ovarian stimulation is attracting an increasing interest in reproductive field (Altmäe et al., 2011). Currently, COS is guided by clinical history, demographic, anthropometric characteristics and ovarian reserve markers such as antral follicle count and anti-müllerian hormone. Several lines of evidence suggest that individual genotype profile could influence COS. In detail, most of researchers focused about the possible effect of specific polymorphisms in gonadotropins and gonadotropins receptor genes.

FSH-R polymorphism located in exon 10 in the amino acid position 680 (FSH-R A680G; rs6166) was the most widely investigated. Specifically, women homozygous for FSH-R S680 required higher amount of exogenous gonadotropin during COS (Behre et al., 2005, Sudo et al., 2002) and showed higher basal FSH levels (Yan et al., 2013, Perez Mayorga et al., 2000). Furthermore, some authors

reported an increased risk of ovarian hyperstimulation syndrome (OHSS) syndrome in FSHR S680 homozygous (Daelemans et al., 2004).

In addition polymorphism in 5' untranslated region of FSH-R (FSH-R-G29A, rs1394205) seem to also affect ovarian response to exogenous gonadotropin. In detail, AA homozygotes have a reduced number of oocytes retrieved and lower clinical pregnancy rate compared with other genotypes (Achrekar et al., 2009a). Another study seems to support these finding reporting also a reduced number of MII oocytes in subjects with AA genotype comparing with GG genotype (Desai et al., 2011). Nonetheless, Tohlob et al. reported higher live birth rate in women carrying A allele in a retrospective analysis of 603 women who underwent in vitro fertilization (Tohlob et al., 2016).

So far, an increased exogenous FSH consumption in carries of genetic variant of LH beta subunit (rs1800447) was also reported (Alviggi et al., 2011b, Alviggi et al., 2013). This polymorphism is characterized by a reduced half-life in vivo compared with wild type

form (Haavisto et al., 1995) and is widely expressed in Northern Europe population.

Luteinizing hormone/human chorionic receptor's (LHCG-R) polymorphisms were also recently investigated. In detail, in a large cross sectional study involving 384 IVF women higher pregnancy rate was observed in women carrying LHCG-R 312G polymorphism compared with A312 carriers (LHCG-R A312G, rs2293275). Furthermore, LHCG-R G homozygotes required higher doses of exogenous FSH for follicular recruitment versus A homozygotes. This polymorphism was also associated with polycystic ovarian syndrome (PCOS) with a 2.7-fold increased risk of AA homozygotes in Sardinian population (Capalbo et al., 2012) .

These aforementionated polymorphisms seem also to exert an effect on COS when combined. For instance, women homozygous for G in both FSH-R A680G and LHCG-R A312G polymorphism showed higher pregnancy rate compared with those homozygous for A (Lindgren et al., 2016). Furthermore, a retrospective analysis demonstrate how homozygotes of both AA FSH-R -29 and AA of

FSH-R A680g polymorphism have an increased risk of impaired ovarian response following ovarian stimulation (Desai et al., 2013). Moreover, presence of both FSH B 211 GT plus FSHR2039 AA genotype had a significant reduced day 3 FSH levels compared with FSHB-211 GG/FSHR2039 GG genotype.

Nonetheless, the majority of studies on that issue are based on retrospective analysis with relevant selection bias among trials. In addition, the heterogeneity in terms of IVF protocols adopted and patients recruited makes these results still debateable. Finally, to our knowledge the combined effect of polymorphisms was mainly studied in retrospective manner involving only a few number of polymorphisms.

The aim of the present multicentre prospective analysis is to evaluate the influence of multiple gonadotropin and their receptor polymorphisms in women undergoing COS for ART co-treated with a GnRHa long down-regulation protocol and fixed FSH starting dose.

Material and methods

Study population

Only Caucasian women have been included adopting the following inclusion criteria: Age between 20–35 years; Body Mass Index (BMI) between 20–27 Kg/m^2; Basal FSH ≤10 IU/l; Indication for IVF treatment; presence of both functional ovaries. Exclusion criteria were: anomalies of the uterine cavity on both ultrasound and hysteroscopy, endocrine, genetic or systemic inflammatory-immunological disorders, diagnosis of polycystic ovarian syndrome according Rotterdam criteria, endometriosis. In addition, women with history of more than two previous IVF cycles with normal ovarian response or previous stimulation cycle which had been cancelled for insufficient ovarian response or in which <4 oocytes had been retrieved was excluded. The study was approved by the ethical committee board Federico II University, Naples, Italy.

Stimulation protocol

All patients underwent a GnRH-a long down-regulation protocol with buserelin acetate (Suprefact) as follows: 0.5 mg s.c. daily from the

mid-luteal phase for 12-14 days, after which the dose was reduced to 0.2 mg. After 14 days, transvaginal-ultrasonographic (TV-USG) and biochemical evaluations were carried out: only women with serum oestradiol level <40 pg/ml, endometrial thickness <5 mm, and arrested follicular development were admitted for controlled ovarian stimulation. Women with delayed suppression (including subjects who develop ovarian cysts after the GnRH-a administration) were excluded. A fixed starting-daily dose of 150 of r-hFSH was established for all the participant (Gonal-F®; Merck Serono S.p.A, Rome, Italy). The starting gonadotropin dose was maintained for four days. Oestradiol serum levels was measured on day five of stimulation. On that day, the daily dose of gonadotropin was modified only in women having oestradiol concentration >180 pg/ml. Only in these cases, according standard clinical practice, a daily dose of r-hFSH of 112.5 IU was adopted. Follicular growth was evaluated by on day 8 of stimulation by TV-USG. Only patients who displayed at least 6 follicles ranging between 6 and 10 mm, but no follicle with a mean diameter >10 mm received an increase in the

daily gonadotropin dose. Specifically, the dose of FSH was increased by 150 IU per day of r-hFSH, giving a cumulative daily dose of 300 IU. Women who had their daily dose of gonadotropin reduced on the fifth day of stimulation and who required a new increase on day 8 was excluded from the observation. Analogously, women who required "coasting" for reducing the risk for OHSS was not included in this study. Oestradiol serum levels were measured on days 1, 5, 8 of stimulation and on the day of hCG administration. All the other determinations, including hormone measurements and polymorphism evaluation are described in Table 1. The ovulatory dose 10,000 IU of hCG or 250 mcg of recombinant hCG was administered in the presence of three follicles with a mean diameter of at least 17 mm according to clinical practice. Oocytes was retrieved by transvaginal ultrasound-guided aspiration 34-36 h after the hCG injection. Serum concentrations of LH was measured on the day of pituitary suppression assessment and on the eighth day of stimulation.

Sampling and polymorphisms analyses

Blood samples were collected for evaluating the presence of different polymorphisms. The venous blood (10 ml) was allowed to clot and centrifuged at 400 g for 10 min. Serum was separated, divided into a maximum of four aliquots and frozen. Pellets was also divided in four aliquots and stocked at -80°C to be successively evaluated. The PCR-based Custom TacMan® DNP Genotyping Assay (Applied Biosystems) was used to genotype the following eight single nucleotide polymorphisms (SNP): (i) FSHR 307 rs6165, (ii) FSHR 680 rs6166, (iii) FSHR-29 rs1394205, (iv) LHCGR intronic rs4073366, (v) LHCGR 291 rs12470652, (vi) LHCGR rs2293275, (vii) FSHB 2623 rs6169, (viii) v-LH rs1800447

Primary and secondary endpoints

Primary endpoint was the ratio of FSH/oocytes retrieved. Secondary endpoint(s): Estradiol levels on the day of hCG; cumulative dosage of r-hFSH, number of preovulatory follicles, mature oocyte retrieved (MII oocytes), percentage of mature oocytes; n. oocytes fertilized;

number of embryos transferred, days of stimulation, implantation rate, pregnancy rate per cycle, pregnancy rate per transfer, clinical pregnancy rate for started cycle (presence of embryo with heartbeat) clinical pregnancy rate per transfer (presence of embryo with heartbeat).

Statistical Analysis

Genotype frequencies of SNPs evaluated were obtained by direct computing, using SNPStats. Linkage disequilibrium was evaluated using SNPStat. Hardy-Weinberg equilibrium was evaluated by direct computing. Chi-square test was used to compare SNPs frequencies of enrolled patients to general population (http://hapmap.ncbi.nim.nih.gov). We created genetic models of inheritance, comparing the allele frequency to general population. Thus according to genotypes frequencies, four models were generated: codominant, dominant, recessive and overdominant. Dominance in genetics is a relationship between alleles of one gene, in which the effect on phenotype of one allele masks the

contribution of a second allele at the same locus. Codominance occurs when the contributions of both alleles are visible in the phenotype. Considering data available in the literature, we considered dominant the allele most frequent in the general population and we generated these four models starting from these observation.

Kolmogorov-Smirnov test was used for evaluation of variables distribution. Differences for continuous variables among groups were evaluated performing ANOVA univariate, for parametric variables, and Kruskal-Wallis or Mann-Whitney for non-parametric ones. Dunnet test was used as post-hoc test. Rho-Sperman's regression was used for correlation. Statistical analysis was performed using the 'Statistical Package for the Social Sciences' software for Macintosh (SPSS Inc version 20.0 USA, Chicago, IL).

A p value < 0.05 was considered as statistical significant.

Results

Ninety-four women with a mean age of 30.71 ± 2.61 years and a

mean BMI of 22.94 ± 2.35 kg/m^2, attending IVF/ICSI cycles were enrolled. Genotype distribution of SNPs was consistent with the Hardy-Weinberg equilibrium and no differences were seen comparing the allele frequencies in the study group to general population (Table 1B).

At baseline, the mean FSH serum levels were 6.75 ± 1.98 IU/L. All patients were treated with r-HFSH 150 IU daily, according to the study protocol and the mean cumulative r-hFSH dosage used was 1,725.33 ± 520.15 IU for an average duration of about 11.24 ±1.69 days (Table 1B). Only one cycles (1.1%) were interrupted for OHSS, whereas no cycles were interrupted for absent response to OS.

After OS, E2 serum levels reached the mean value of 1655.43 ± 895.59 pg/mL and women under went to IVF in 28.7% of cases (27 women) and to ICSI in 71.2% of cases (67 women) (Table 2B). Forty pregnancies (42.5%) were obtained through the βhCG measurement and 32 of these (34.0%) were confirmed at ultrasonography evaluation (Table 2B). No differences between pregnant and non-pregnant women were found for each parameter

considered in the study.

FSHR 307 (rs6165) and FSHR 680 (rs6166)

The number of total oocytes retrieved was not different considering both FSHR rs6165 (p=0.510) and FSHR rs6166 (p=0.170).

The ratio between total consumption and n of oocytes retrieved was significant different among three genotypes (p=0.050), with lower ratio in homozigotic A/A compared to homozigotic G/G and heterozigotic women.

The ratio between total consumption and number of oocytes retrieved was significant lower in homozigotic G/G compared to both homozigotic (A/A) and heterozigotic patients (A/G) (p=0.049).

Overall, no differences were found in both SNPs considering the total r-hFSH dosage used, the ratio between fertilized and inseminated oocytes, and other outcomes (Tables 3B and 4B).

FSHR -29 (rs1394205)

No significant differences with respect of treatment outcomes were

found among models generating according to genotypes frequencies (Table 5B).

LHCGR 291 (rs12470652)

LHCGR heterozygous women showed higher E2 levels at the day of hCG administration (p=0.005) compared to wild type (Table 6B). Similarly, higher number of total oocytes retrieved (p=0.035), MII (p=0.002), insemined (p=0.001), fertilised oocytes (p=0.001) and cryopreserved embryos (p=0.001) was detected in heterozygous compared to wild type (Table 6B). No significant differences among other variables were found (Table 6B).

LHCGR intronic (rs4073366), LHCGR 312 (rs2293275), FSHB 2623 (rs6169) and v-LH (rs1800447)

No significant differences were found for all parameters considering LHCGR rs4073366, LHCGR rs2293275, FSHB rs10835638 and v-LH rs1800447.

Multivariate analysis

The overall consideration of the eight SNPs evaluated in the study showed that the co-presence of allele G of FSHR -29 rs1394205 and allele C of LHCGR 291 rs12470652 was related to an increased ratio between cumulative r-hFSH dose and total number of oocytes retrieved (5.47, CI 95%:3.13-7.81, $p<0.001$) (Figure 1B). This effect of SNPs on these two genes was confirmed also considering the SNP FSHR rs6166. In particular, the co-presence of allele G of both FSHR -29 rs1394205 and FSHR rs6166 and allele C LHCGR 291 rs12470652 were related to an increased ratio between cumulative FSH dose and total number of oocytes retrieved (5.44, CI:3.18-7.71, $p<0.001$) (Figure 1B).

Discussion

For the first time, the simultaneous analysis of eight SNPs was carried out in 94 IVF women. By the analysis of our data, emerged a significant impact of several SNPs on female reproductive outcome. In particular two common SNPs of FSHR (FSHR rs6165 and rs6166)

seem to significantly influence FSH/oocytes ratio (Table 3B, 4B), whereas basal E2 levels seem to be associated with LHCGR 291. Furthermore, the expression of LHCGR 291 allele C is associated with higher number of oocyte retrieved and consequently more embryo cryopreserved (Table 6B).

In addition, multivariate analysis revealed the expression of allele C of FSHR -29 (rs1394205), LHCGR 291 (rs12470652) and FSH rs6166 have showed a significantly relation to the cumulative r-hFSH dosage and total number of mature oocyte.

The possible implication of LHCGR 291 (rs12470652) polymorphism on female reproduction was never reported so far. In contrast to Ackrekar et al. 2009 (Achrekar et al., 2009b), FSHR -29 polymorphism alone was not associated with impaired response alone. This discrepancy could be due to retrospective design, inclusion criteria and heterogeneous protocol adopted by Achrekar et al. Conversely, in our study the population included fulfilled strict inclusion criteria and was prospectively analysed with a standardized ovarian stimulation. Findings regarding polymorphism of FSH-R

rs6166 and rs6165 are consistent with those reported in literature (De Castro et al., 2003, Yan et al., 2013, Perez Mayorga et al., 2000). Resistance to FSH in GG carriers of FSHR rs6166 seems to be related to specific molecular characteristics (Casarini et al., 2014, Casarini et al., 2015). In opposite to our previous studies (Alviggi et al., 2011b, Alviggi et al., 2013), we did not observe an association between ovarian response and common LH beta polymorphism (rs1800447). This incongruity could be explained by the absence of homozygous carriers of the variant in this study and the limited sample size comparing with those reported in 2013 (Alviggi et al., 2013). Furthermore, the different study-design could also have been crucial for this discrepancy.

The strength points of our study resided in several aspects. Firstly, we have conducted a multicenter prospective analysis of data using strict inclusion criteria. Indeed, we have included only good prognosis women with established a fixed starting-daily dose without any anomalous response during ovarian stimulation. This decision was taken considering that the use of higher FSH dosage or

adjusting dosage during stimulation could in some mitigate the effect of genotype on IVF which represent a common bias reported in other studies (Genro et al., 2012). Furthermore we have also performed a multivariate analysis with the aim to established whether any interaction among different polymorphism is present and could influence ovarian response. So fa,r the most of published studies focused their attention in single polymorphism (De Castro et al., 2003, Achrekar et al., 2009b, Jun et al., 2006, Genro et al., 2012, Lazaros et al., 2012) and the most recent ones to at least two of them (Lindgren et al., 2016, Desai et al., 2013). Furthermore, to our knowledge for the first time we provide data about unexplored polymorphism such as LHCGR intronic (rs4073366), FSHB 2623 (rs10835638). The limitation of our analysis is essentially due to the relative small number of patients involved considering the amount of polymorphism analysed. Furthermore, we was not able to follow up patient until birth, however we provide data about ongoing pregnancy rate. As other trials, ours failed to found a significant association with pregnancy outcomes. Nonetheless, in our opinion

ART births did not represent the ideal parameter to measure the effect of polymorphism. Indeed, various factors such as embryo quality, maternal age, some of which occurring during the late stages of pregnancy like intrauterine growth restriction transcend the "physiological" effects of gonadotropins and their receptors. In other words, we sustained that the ovarian response in terms of number of oocytes and consumption of gonadotropin represent the most appropriate outcomes to address the effects of gonadotropins and their receptor polymorphisms in ART. Finally, we only considered women who underwent long analogue protocol so we cannot provide data about antagonist regimens.

In conclusion, our study confirmed that specific polymorphism might affect ovarian response to ovarian stimulation. In addition we demonstrated how a comprehensive evaluation of multiple polymorphism could provide useful information about COS response. Our data need to be corroborated by further investigations especially for other polymorphism in which no sufficient data are still present to drawn a definitive conclusion (such as LHCGR intronic

[rs4073366], FSHB 2623 [rs10835638]). The genotype assessment in ART could lead to an innovative and individual tailored pharmacogenomic approach to ovarian stimulation.

Session C. Clinical relevance of genetic variants of gonadotropins and their receptors in controlled ovarian stimulation: a systematic review and meta-analysis

Background

Ideally, the individual approach for COS in infertile patients who seek ART, would involve a comprehensive evaluation of the patient's characteristics, including genotype profile.

Pharmacogenomics evaluates how genes influence individual responses to medication. Pharmacogenomic approaches could be a cost-effective strategy in several medical fields (Patel et al., 2014, Mizzi et al., 2016). Data regarding the clinical utility of pharmacogenomics in ART are still scanty (Greb et al., 2005a). Nonetheless, increasing evidence indicates that specific genetic characteristics of gonadotropins and their receptors could influence ovarian response to exogenous gonadotropins. Specifically, a common single nucleotide polymorphism (SNP) of the follicle stimulating hormone receptor (FSHR, rs6166) was associated with increased FSH consumption during COS (Yao et al., 2011). This SNP

was also associated with increased FSH basal levels, which suggests an impaired response to both endogenous and exogenous gonadotropins (Perez Mayorga et al., 2000, Behre et al., 2005, Simoni and Casarini, 2014, Alviggi et al., 2016b). Moreover, the FSHR polymorphism at position -29 (FSHR, rs1394205) was associated with a poor ovarian response (Achrekar et al., 2009b). Similarly, a suboptimal response to in IVF was observed in SNP carriers of the gene encoding the LH beta subunit (Alviggi et al., 2011b, Alviggi et al., 2013). Recently, a possible effect of the LH receptor SNPs (LHCGR, rs2293275 and LHCGR, rs12470652) on COS and ART was also reported (O'Brien et al., 2013, Alviggi et al., 2016c, Lindgren et al., 2016). Based on this evidence, some authors have also hypothesized that a "hypo response" to gonadotropin therapy could be explained by specific genotype characteristics. Contrary to what is observed for poor-responders, "hypo-responders" are women with a good prognosis for ART in terms of basal characteristics and ovarian reserve but in which a higher than expected dose of gonadotropins and more prolonged stimulation are

required to obtain an adequate number of oocytes (Alviggi et al., 2013).

Given the steady increase in evidence that SNPs affect COS and ART outcomes, we conducted a systematic review and meta-analysis data in the attempt to summarize the clinical evidence regarding the impact of polymorphisms of gonadotropin and their receptors on the outcome of COS.

Materials and Methods

Protocol and registration

This study was exempt from institutional review board approval because it did not involve human intervention. We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The study protocol was registered at http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42016050402) on 31 October 2016, before starting the review process.

Eligibility criteria

The selection criteria are described according to PICO (Patients, Intervention, Comparison, and Outcomes). We only included women who underwent COS, and evaluated COS outcomes according to individual genotype expression.

Search strategy

We conducted a systematic search using the MEDLINE (PubMed), EMBASE, SCOPUS, and Cochrane Library databases to identify all relevant studies published before January 2017. Combinations of the following keywords and MESH search terms were used: "COH", "COS", "controlled ovarian stimulation" "ART", "IVF", "ICSI", "FIVET", "IUI", "intrauterine insemination", "ovulation induction", "polymorphism" OR "SNP" "luteinizing hormone/choriogonadotropin receptor" "LHCGR", "FSH Receptor", "FSHR", "FSH", "follicle

stimulating hormone", "follicle stimulating hormone, beta subunit", LH", "luteinizing hormone", "luteinizing hormone, beta subunit". No time or language restrictions were adopted, and queries were limited to human studies. The reference lists of relevant reviews and articles were also hand-searched.

Selection of studies

Titles and abstracts were independently evaluated. Duplications were removed using Endnote online software and also manually. Disagreements were resolved by discussion among authors, and if required, with the involvement of the most experienced authors. Only clinical trials published in peer-reviewed journals were evaluated. Case series, case reports, book chapters, congress abstracts, and grey literature were not included.

Data extraction

Data were extracted independently using predefined data fields, and study quality indicators. Discrepancies were resolved by discussion with the senior authors.

Risk of bias, summary measures and synthesis of the results

The risk of bias and quality assessment of the included studies were performed using the Newcastle-Ottawa Scale (NOS) (Wells et al., 2004). Two authors independently assessed the risk bias for each study. The senior authors resolved conflicts. The NOS score was used to evaluate the studies included, and judgement on each one was passed according to three issues: selection of the study group, comparability between groups, and ascertainment of exposed/not exposed cohorts.

The primary outcome was the number of oocytes retrieved. Secondary outcomes were: FSH consumption, stimulation duration (number of days of gonadotropin use for COS), ratio between FSH consumption (total dosage of exogenous gonadotropin used) and

the number of oocytes retrieved, the number of metaphase II oocytes, and ongoing pregnancy rate (OPR). The latter was defined as a pregnancy diagnosed by ultrasonographic visualization of at least one gestational sac. Bias across studies regarding the primary outcome was assessed using visual inspection of funnel plots, and the trim and fill method (Duval, 2006) and the Egger test (Egger et al., 1997).

Quantitative analysis

Statistical analysis was carried out using Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration). Categorical data were combined with a pooled odds ratio (OR) using the Mantel-Haenszel method. Continuous data were combined with weight mean differences (WMD) using the inverse variance method. Only when at least 3 or more studies were available, a meta-analysis was conducted using the fixed-effect-model (FEM) or the random effect model (REM). REM was used in the case of significant

heterogeneity among studies. Heterogeneity was assessed using the percentage of total variation in the estimated effect across studies (I2). An I2 value > 50% indicates substantial heterogeneity. P values < 0.05 were considered statistically significant. A *post-hoc* estimation of the ratio between FSH consumption and the number of oocytes retrieved was carried out when three or more studies were available.

Subgroup analysis

Subgroup analysis by type of exogenous FSH (i.e., recombinant versus urinary), was conducted to assess potential sources of heterogeneity for FSH/oocytes ratio and number of oocytes retrieved. Sensitivity analysis was carried out to assess the leverage of studies with low risk of bias (NOS ≥ 6) on the results.

Results

Study selection and study characteristics

A total 1,051 items were identified from the databases (Figure 1C). After removing 167 duplicates using Endnote software (EndNote X 6.0.1, California State University) and 45 duplicates manually, abstracts and titles of 839 papers were scrutinized. Hand search of reference lists of relevant reviews were used to complement database searches. Overall, 59 articles were assessed for eligibility. Fifteen articles were excluded because they did not fulfill inclusion criteria. Data extraction was not possible in 10 articles (Daelemans et al., 2004, De Castro et al., 2004, D'Alva et al., 2005, Livshyts et al., 2009, Lazaros et al., 2012, Boudjenah et al., 2014, Colognato et al., 2014, Almawi et al., 2015, Laisk-Podar et al., 2015, Valkenburg et al., 2015), because COS and ART were not evaluated on the basis of polymorphism genotype expression. Data duplication was detected in studies by Desai et al. (Desai et al., 2013, Desai et al., 2011) and by Mohiyiddeen and collaborators (Mohiyiddeen et al., 2013a, Mohiyiddeen et al., 2013b). The following studies were included in our analysis (Desai et al., 2011, Mohiyiddeen et al., 2013b). Of note, we only extracted data regarding MII oocytes from

Mohiyiddeen et al. 2013a that were not reported in their subsequent paper (Mohiyiddeen et al., 2013a). Thirty-three studies were included in our quantitative and qualitative analysis (Figure 1C and Table IC). Seven polymorphisms were reported in these studies: FSHR 919 G>A (rs6165), FSHR 2039 G>A (rs6166), FSHR -29 G>A (rs1394205), LHB 82 T>C (rs1800447), LHB 1502 G>A (rs1056917), LHCGR 935 A>G (rs2293275), LHCGR 3442-25260 A>G (rs13405728).

Risk of bias within studies

Bias assessment within studies is shown in Table IC. A high rate of agreement evaluated by k-Cohen calculation, was observed between the authors (k-Cohen = 0.83).

Summary of results

The results of the quantitative analysis of each outcome measure

according to genotype distribution are reported below and summarized in Table 2C.

FSH consumption

A meta-analytic approach was possible only for FSHR (rs6165), FSHR (rs6166), FSHR (rs1394205). No data were found regarding LHB (rs1056917).

Four studies (Laven et al., 2003, Achrekar et al., 2009a, Genro et al., 2012, Yan et al., 2013) for a total of 729 women, evaluated FSH consumption in relation to the FSHR (rs6165) genotype distribution. FSH consumption did not differ statistically among FSHR (rs6165) AA homozygotes, GG homozygotes (Random WMD: 227.64 IU, 95% CI: -452.95 to 908.22 IU, I2 = 96%), and AG heterozygotes (Random WMD: 110.24 IU, 95% CI: -323.57 to 544.05 IU, I2 = 93%). Similarly, FSH consumption did not differ between GG homozygotes and AG heterozygotes (Random WMD: 134.09 IU, 95% CI: -162.06 to 430.25, I2 = 81%).

Eighteen studies (Perez Mayorga et al., 2000, Sudo et al., 2002, Laven et al., 2003, Behre et al., 2005, Jun et al., 2006, Loutradis et al., 2006, Achrekar et al., 2009a, Huang et al., 2010, Nordhoff et al., 2011, Sheikhha et al., 2011, Anagnostou et al., 2012, Genro et al., 2012, Lledo et al., 2013, Mohiyiddeen et al., 2013b, Yan et al., 2013, Huang et al., 2015, Lindgren et al., 2016, Lledó et al., 2016), for a total of 4,094 women, evaluated FSH consumption according to FSHR (rs6166) genotype distribution. FSH consumption in FSHR AA homozygotes was comparable to that in GG homozygotes (Random WMD: -158.50 IU, 95% CI: -338.32 to 21.32 IU, I2 = 96%) and AG heterozygotes (Random WMD: 18.00 IU, 95% CI: -119.36 to 155.35 IU, I2 = 96%). Similarly, no differences were found between FSHR GG homozygotes and AG heterozygotes (Random WMD: -137.53 IU, 95% CI:-293.04 to 17.97 IU, I2 = 86%).

Three studies (Achrekar et al., 2009b, Desai et al., 2011, Tohlob et al., 2016) including 709 women, evaluated FSH consumption according to the FSHR (rs1394205) genotype. The

consumption of FSH was significantly lower in FSHR GG homozygotes than in FSHR AA homozygotes (Random WMD: -1294.61 IU, 95% CI: -1996.14 to -593.08, P < 0.001, I2 = 99%); however, no differences were observed between GG and AG heterozygotes (Random WMD: -277.84 IU, 95% CI: -1145.28 IU to 589.60, I2 = 100%). FSH consumption was lower in AG heterozygotes than in FSHR AA homozygotes (Random WMD: -1014.36 IU, 95% CI: -1664.61 to -364.11, P = 0.002, I2 = 99%) (Figure 2C).

Two studies (Alviggi et al., 2011b, Alviggi et al., 2013) reported FSH consumption according to LHB (rs1800447) genotype distribution. Both showed significantly higher FSH consumption in variant carriers compared with wild-type carriers.

One study (Lindgren et al., 2016) reported FSH consumption in relation to the distribution of the LHCGR SNP (rs2293275) genotype. No significant differences among genotypes were detected.

One study (Yin et al., 2015) reported FSH consumption in relation to

the distribution of the LHCGR (rs13405728) genotype. No significant differences among genotypes were reported.

The overall effect estimated by the analyses indicated that FSH consumption was only affected by the presence of FSHR (rs1394205), which was significantly higher in AA carriers. However, these results may be conservative given the high heterogeneity and the relatively small number of patients evaluated.

Stimulation duration

A meta-analytic approach was possible only for FSHR (rs6165), FSHR (rs6166), and FSHR (rs1394205). No data were found regarding the other polymorphisms.

Three studies (Laven et al., 2003, Genro et al., 2012, Yan et al., 2013) for a total of 679 patients, evaluated stimulation duration in relation to the distribution of the FSHR (rs6165) genotype. The length of stimulation did not differ between FSHR AA homozygotes

and GG homozygotes (Random WMD: -0.59, 95% CI: -1.24 to 0.05, I2 = 60%), however it was significantly shorter than in AG heterozygotes (Fixed WMD: -0.48, 95% CI: -0.87 to -0.10, P = 0.01, I2 = 44%). On the contrary, stimulation duration did not differ between FSHR GG homozygotes and AG heterozygotes (Fixed WMD -0.29, 95% CI: -0.95 to 0.37, I2 = 0%) (Figure 3C).

Fifteen studies (De Castro et al., 2003, Laven et al., 2003, Behre et al., 2005, Klinkert et al., 2006, Huang et al., 2010, Nordhoff et al., 2011, Genro et al., 2012, Lledo et al., 2013, Yan et al., 2013, Zalewski et al., 2013, Huang et al., 2015, Alviggi et al., 2016b, Loutradis et al., 2006) that included 3,069 women, evaluated stimulation duration in relation to the distribution of the FSHR (rs6166) genotype. The duration of stimulation did not differ among FSHR AA homozygotes, GG homozygotes (Fixed WMD: -0.01, 95% CI: -0.16 to 0.14 days, I2 = 17%) and AG heterozygotes (Random WMD: -0.01, 95% CI: -0.04 to 0.05, I2 = 27%). Lastly, no differences were observed between FSHR GG homozygotes and FSHR AG heterozygotes (Fixed WMD: -0.12, 95% CI: -0.29 to 0.04,

I2 = 2%).

In summary, the only difference in stimulation duration was a shorter duration in in FSHR (rs6165) AA homozygotes than in AG heterozygotes.

Number of oocytes retrieved

A meta-analytic approach was possible only for FSHR (rs6165), FSHR (rs6166), and FSHR (rs1394205). No data were found regarding LHCGR (rs2293275).

Five studies (Achrekar et al., 2009a, Genro et al., 2012, Lazaros et al., 2013, Yan et al., 2013, Trevisan et al., 2014) including 1,020 women, reported the number of oocytes retrieved in relation to the distribution of the FSHR (rs6165) genotype. The number of oocytes retrieved was significantly higher in AA homozygotes than in GG homozygotes (Fixed WMD: 1.85, 95% CI: 0.85 to 2.85, P < 0.001, I2 = 0%) and in AG heterozygotes

(Random WMD: 1.62, 95% CI: 0.28 to 2.95, P = 0.02, I2 = 56%). No difference was detected between GG homozygotes and AG heterozygotes (Fixed WMD: -0.37, 95% CI: -1.51 to 0.78, I2 = 18%) (Figure 4C).

Twenty-one studies (Perez Mayorga et al., 2000, Sudo et al., 2002, De Castro et al., 2003, Behre et al., 2005, Jun et al., 2006, Klinkert et al., 2006, Loutradis et al., 2006, Achrekar et al., 2009a, Huang et al., 2010, Nordhoff et al., 2011, Sheikhha et al., 2011, Genro et al., 2012, Lazaros et al., 2013, Lledo et al., 2013, Mohiyiddeen et al., 2013b, Yan et al., 2013, Zalewski et al., 2013, Trevisan et al., 2014, Huang et al., 2015, Alviggi et al., 2016b, Lledó et al., 2016) including 4,425 women, reported the number of oocytes retrieved in relation to the distribution of the FSHR (rs6166) genotype. The number of oocytes retrieved was significantly higher in AA homozygotes than in GG homozygotes (Random WMD: 0.84, 95% CI: 0.19 to 1.49, P = 0.01, I2 = 76%), but it was similar to AG heterozygotes (Random WMD: -0.18, 95% CI: -0.84 to 0.48, I2 = 85%). Significantly higher number of oocytes were found in AG

heterozygotes than in GG homozygotes (Random WMD: 0.92, 95% CI: 0.18 to 1.66, P = 0.020, I2 = 75%) (Figure 5C).

Three studies (Achrekar et al., 2009b, Desai et al., 2011, Tohlob et al., 2016) including 709 women, evaluated the number of oocytes retrieved in relation to the distribution of the FSHR (rs1394205) genotype. The number of oocytes retrieved was lower but not significantly different between FSHR (rs1394205) AA homozygotes and both GG homozygotes (Random WMD: -5.20, 95% CI: -11.22 to 0.82, I2 = 99%) and AG heterozygous (Random WMD: -3.88, 95% CI: -7.93 to 0.18, I2 = 98%). No differences were observed between GG homozygotes and AG heterozygotes (Random WMD: -1.29, 95% CI: -3.51 to 0.93, I2 = 97%).

Only two studies (Alviggi et al., 2011b, Alviggi et al., 2013) reported the number of oocytes retrieved considering the LHB (rs1800447) genotype. In both studies, the authors did not observed a significant difference regarding the number of oocytes retrieved among genotypes.

Only one study (Davar et al., 2014) reported the number of oocytes retrieved considering the LHB (rs1056917) and no significant difference among genotypes was observed.

Only one study (Yin et al., 2015) reported the number of oocytes retrieved according to LHCGR (rs13405728) genotype distribution; no significant differences among genotypes were detected.

The overall effect estimated by the analyses indicated that both the FSHR (rs6165) and FSHR (rs6166) genotypes impacted on the number of retrieved oocytes. In both polymorphisms, AA homozygote was associated with an increased number of oocytes retrieved, whereas GG homozygote had an opposite effect. Due to high heterogeneity, the effect size estimated for the FSHR (rs6166) may be conservative.

Number of metaphase II oocytes

A meta-analytic approach was possible only for FSHR (rs6166). No

data were found regarding LHB (rs1056917).

Only two studies (Genro et al., 2012, Trevisan et al., 2014) evaluated the number of MII oocytes retrieved considering FSHR (rs6165). In both studies, there was no difference in the number of MII oocytes among genotypes.

Five studies (Genro et al., 2012, Mohiyiddeen et al., 2013a, Trevisan et al., 2014, Lindgren et al., 2016) including 1,185 patients, reported the number of oocytes MII retrieved in relation to the distribution of the FSHR (rs6166) genotype. The number of MII oocytes was significantly higher in AA homozygotes than GG homozygotes (Fixed WMD: 1.03, 95% CI: 0.01 to 2.05, P = 0.050, I2 = 0%). On the contrary, no significant differences were observed between AA homozygotes and AG heterozygotes (Fixed WMD: 0.79, 95% CI: -0-05 to 1.62, I2 = 0%), or between GG homozygous and AG heterozygous (Fixed WMD: 0.34, 95% CI: -0.57 to 1.26, I2 = 49%) (Figure 6C).

Only two studies (Desai et al., 2011, Dan et al., 2015)

reported the MII oocytes number considering the FSHR (rs1394205). In detail, Dan et al. observed a significantly higher number of MII oocytes in GG comparing with AG/AA carriers. In the same line, findings by Desai et al. showed significantly higher number of MII oocytes in GG than AG and AA groups.

Only one study (Alviggi et al., 2013) reported the number of MII oocytes retrieved considering the LHB (rs1800447); no significant difference was found between wild-type and variant carriers.

Only one study (Lindgren et al., 2016) reported the number of MII oocytes retrieved considering the LHCGR (rs 2293275); likewise, no significant difference among haplotypes was identified.

Only one study, (Yin et al., 2015) reported number of MII oocytes retrieved according to LHCGR (rs13405728) genotype distribution; no significant differences among genotypes were detected.

The overall effect size estimated by the analyses indicates a possible negative influence of the FSHR (rs6166) GG homozygote genotype on the number of mature oocytes. Due to the limited number of

studies and the P levels of exactly 0.05, these results should be taken with caution.

Ratio between FSH consumption and number of oocytes retrieved

A meta-analytic approach was possible for FSHR (rs6165), FSHR (rs6166), FSHR (rs1394205). Calculation of FSH dosage/n. oocytes ratio was not carried out for LHB (rs1800447), LHB (rs1056917), LHCGR (rs2293275), and LHCGR (rs13405728).

In three studies (Achrekar et al., 2009a, Genro et al., 2012, Yan et al., 2013) including 581 women, we calculated the FSH consumption/oocyte ratio in relation to the distribution of the FSHR (rs6165) genotype. This ratio was significantly lower in AA homozygotes than GG homozygotes (Fixed WMD -24.06, 95% CI: -47.28 - 0.84, P = 0.040, I2 = 50%). On the contrary, no differences were found between AA homozygotes and AG heterozygotes (Random WMD: -24.31, 95% CI: -65.37 to 16.75, I2 = 58%), or between GG homozygotes and AG heterozygotes (Random WMD:

14.05, 95% CI: -39.59 to 67.69, I2 = 73%) (Figure 7C).

In 16 studies (Perez Mayorga et al., 2000, Sudo et al., 2002, Behre et al., 2005, Jun et al., 2006, Loutradis et al., 2006, Achrekar et al., 2009a, Huang et al., 2010, Nordhoff et al., 2011, Sheikhha et al., 2011, Genro et al., 2012, Lledo et al., 2013, Mohiyiddeen et al., 2013b, Yan et al., 2013, Huang et al., 2015, Alviggi et al., 2016b, Lledó et al., 2016) including 3,729 patients, we calculated the FSH consumption/oocyte ratio in relation to the distribution of the FSHR (rs6166) genotype. This ratio was significantly lower in AA homozygotes than GG homozygotes (Random WMD: -41.96, 95% CI: -82.90 to -1.03, P = 0.04, I2 = 93%). On the contrary, no difference was observed between AA homozygotes and AG heterozygous (Random WMD: -9.71, 95% CI: -37.41 to 17.99, I2 = 93%). The AG heterozygotes showed a significantly lower FSH/n. oocytes ratio than GG homozygotes (Random WMD: -34.75, 95% CI: -60.19 to -9.30, P = 0.007, I2 = 86%) (Figure 8C).

The same approach was used for FSHR (rs1394205) genotype distribution. This analysis included three studies (Achrekar et al., 2009b, Desai et al., 2011, Tohlob et al., 2016) and 709 patients. No difference was observed between AA homozygotes and both GG homozygotes (Random: WMD: 219.27, 95% CI: -66.11 to 504.65, I2 = 95%), and AG heterozygotes (Random: WMD: 217.72, 95% CI: -20.63 to 456.07, I2 = 92%). No significant differences were detected between GG homozygotes and AG heterozygotes (Random: WMD: -4.93, 95% CI: -78.01 to 68.15, I2 = 84%).

The overall effect estimated by the analyses indicated that both FSHR (rs6165) and FSHR (rs6166) genotype impacted on the ratio between FSH consumption and the number of retrieved oocytes. In both cases, the presence of GG haplotype is associated with ovarian resistance to exogenous FSH stimulation. Due to high heterogeneity, the effect size estimated for the FSHR (rs6166) may be conservative.

Ongoing pregnancy rate

A meta-analytic approach was possible only for FSHR (rs6166). No data were found regarding FSHR (rs6165), LHB (rs1056917) and LHCGR (rs13405728).

Seven studies (Jun et al., 2006, Sheikhha et al., 2011, Lledo et al., 2013, Mohiyiddeen et al., 2013b, Huang et al., 2015, Alviggi et al., 2016b, Lindgren et al., 2016) including 3,191 patients, evaluated OPR in relation to the distribution of the FSHR (rs6166) genotype. The overall OR was not different between AA homozygotes and both GG homozygotes (Fixed OR: 0.89, 95% CI: 0.70 to 1.12, I2 = 0%) and AG heterozygotes (Fixed OR: 0.97, 95% CI: 0.82 to 1.16, I2 = 29%). Moreover, no significant differences were observed comparing GG homozygotes and AG heterozygotes (Fixed OR: 0.95, 95% CI: 0.77 to 1.18, I2 = 0%).

Only two studies (Achrekar et al., 2009b, Tohlob et al., 2016)(Achrekar et al., 2009b, Tohlob et al., 2016) reported OPR considering FSHR (rs1394205). Achrekar et al. reported comparable

OPR among GG, AG and AA whereas Tohlob et al. observed that women carrying the A allele had higher OPR than G carriers (OR 1.32, 95% CI 1.01 – 1.74, P = 0.04), albeit this association was not significant considering the number of embryos transferred.

Only one study (Alviggi et al., 2013) reported OPR with regards to LHB (rs1800447); this study reported no differences between wild-type and variant carriers.

Only one study (Lindgren et al., 2016) reported OPR considering the LHCGR (rs2293275). Differences in terms of OPR were observed among haplotypes (AA: 18%; AG: 27%; GG: 31%, P = 0.037), with higher prevalence in GG carriers.

Risk of bias across studies

We found no significant risk of bias across studies regarding the primary outcome adopting Egger's test (P = 0.828 for FSHR rs6166; P = 0.27 for FSHR rs6166, and P = 0.12 for FSHR rs1394205),

visual inspection of the funnel plots, and trim and fill method (Figure 9C).

Subgroup and sensitivity analyses

We estimated ratio between FSH consumption and number of oocytes retrieved according to type of gonadotropin, namely recombinant versus urinary FSH (Figure 10-11C). We did not include papers in which both gonadotropin have been used for COS (Behre et al., 2005, Jun et al., 2006, Huang et al., 2010, Sheikhha et al., 2011, Mohiyiddeen et al., 2013b) or in which the formulation adopted was not clearly stated (Achrekar et al., 2009a).

The overall FSH/oocyte ratio was significantly lower in FSHR (rs6166) AA homozygotes than GG homozygotes (Recombinant Fixed WMD -44.32, 95% CI -65.14 to -23.49, P < 0.0001, I2 = 14%; extractive Fixed WMD -18.83, 95% CI -35.23 to -2.42, P = 0.02, I2 = 30%) regardless of the type of gonadotropin used. On the other hand, a higher number of oocytes retrieved was observed

in AA than GG carriers when recombinant FSH was used (Fixed WMD 1.13, 95% CI 0.51 to 1.75, P = 0.0003, I2 = 44%); this outcome was not different when extractive gonadotropin was adopted (Random WMD 0.68, 95% CI: -2.19 to 3.54; I2 = 86%).

Sensitivity analysis revealed that the observed pooled effect sizes were materially affected with regards to the number of retrieved oocytes between FSHR (rs6165) AA and AG carriers.

Discussion

We conducted this systematic review to unravel the role of gonadotropins and their receptors polymorphisms in the outcome of COS. We evaluated OPR rather than live-birth rate, because of the many confounders that may condition later stages of pregnancy, which in turns renders the impact of folliculogenesis-related polymorphisms questionable. Our findings indicate that FSH receptor polymorphisms affect the outcome of COS. In particular, FSH consumption was higher in A allele homozygous carriers of the FSHR (rs1394205) genotype. Furthermore, the number of oocytes

retrieved was significantly higher in FSHR (rs6165) AA carriers and, moreover, stimulation was significantly shorter in these patients than in GG and AG carriers. Along the same lines, FSHR (rs6166) AA homozygotes had a significantly higher number of both retrieved and mature oocytes than carriers of other haplotypes. Therefore, both FSHR (rs6165) and FSHR (rs6166) GG homozygotes seem to be less responsive to COS treatment than AA and AG carriers. Gonadotropin type did not seem to affect the FSH consumption/oocytes ratio in FSHR (rs6166) haplotypes, but could affect the number of oocytes retrieved. Notably, the number of oocytes retrieved was significantly higher in AA carriers than in GG carriers when recombinant FSH was used. The *FSHR* (rs6166) genotype did not significantly affect the ongoing pregnancies rate.

Our results are consistent with previous reviews (Altmäe et al., 2011) However, here we used a quantitative approach to determine the impact of polymorphisms of gonadotropins and their receptors on the main outcomes of COS. It remains to be determined whether a pharmacogenomic approach could counteract the effect of such

polymorphisms. Only one trial partially addressed this issue (Behre et al., 2005). In particular, in this study, normogonadotropic patients were stratified according the FSHR (rs6166) haplotype. The authors showed that an FSH daily dose of 150 IU resulted in significantly lower levels of estradiol in GG carriers than in AA carriers. Increasing the FSH dose from 150 to 225 IU/day counteracted the lower oestradiol levels in GG carriers.

Regarding LH polymorphisms, it has been reported that C allele carriers of the LHB (rs1800447) variant require higher FSH consumption (Alviggi et al., 2011b, Alviggi et al., 2013) than T carriers. Moreover, a higher ongoing pregnancy rate has been reported in G allele carriers of the LHCGR polymorphism (rs2293275) (Lindgren et al., 2016). However, given the paucity of data regarding the two aforementioned polymorphisms, we were unable to carry out a meta-analysis.

To sum up, we demonstrate that specific polymorphisms of gonadotropins and their receptors could modulate the ovarian

response to exogenous FSH. On the other hand, further studies are necessary to evaluate the impact on OPR and live birth rate. Nonetheless, it should be considered that ART births are strongly influenced by various factors, most of which occur during the late stages of pregnancy and transcend the "physiological" effects of gonadotropins and their receptors. In other words, we maintain that the effects of gonadotropins and their receptor polymorphisms in ART should be more thoroughly evaluated in terms of ovarian response and, more cautiously, at the early stages of pregnancy.

Interpretation of results and clinical considerations

Our findings could be related to the molecular characteristics of the genotypes associated with the COS response (Table III). The FSHR gene carries more than 2000 SNPs, although only FSHR (rs6165) and FSHR (rs6166) seem to play a prominent role in the COS response. Both SNPs cause an amino acid exchange: in FSHR (rs6166) asparagine is substituted by serine thereby introducing a

potential phosphorylation site whereas in FSHR (rs6165) threonine is substituted by alanine, which results in a change from a polar to a nonpolar hydrophobic amino acid and thereby removing a potential O-linked glycosylation site. These genotypes are in nearly complete linkage disequilibrium, except in some African populations (Simoni and Casarini, 2014, Casarini et al., 2015). In vitro studies conducted using human granulosa cells showed that GG carriers of the FSHR (rs6166) genotype have greater resistance to FSH than AA carriers (Casarini et al., 2014, Casarini et al., 2015). Our results corroborate these observations, since we found that GG carriers require higher doses of FSH per oocyte retrieved than AA carriers. Furthermore, these carriers showed also fewer oocytes at the end of stimulation compared with the other FSHR (rs6166) haplotypes. In these women, FSHR resistance to endogenous FSH was also reported (Mohiyiddeen and Nardo, 2010). This effect is modulated in both man and women by another polymorphism of the FSH beta subunit (FSHB, rs10835638) (Grigorova et al., 2010, Ferlin et al., 2011, La Marca et al., 2013) which is significantly correlated with the FSH

beta subunit transcriptional activity and metabolism (Hoogendoorn et al., 2003). There is also evidence that FSHR (rs6166) could interact with other polymorphisms that influence ART outcomes. Indeed, in a large cohort study, FSHR (rs6166) and LHCGR (rs2293275) allele G carriers had a 4-fold increased chance of pregnancy versus A carriers of both polymorphisms. Moreover, the number of mature oocytes was significantly higher in subjects with both FSHR (rs1394205) GG plus FSHR (rs6166) AA genotypes than in other genotype combinations of the same polymorphisms (Desai et al., 2013).

The FSHR (rs1394205) polymorphism located in the 50-untranslated region of the gene has been extensively studied in association with ovarian response. The transcription activity of the FSHR (rs1394205) A allele is lower than that of the G allele (Nakayama et al., 2006). Moreover, the expression of FSHR and protein levels is also significantly lower in FSHR (rs1394205) AA homozygotes than in other haplotypes, thus suggesting that A allele expression is associated with ovarian resistance to COS (Desai et al., 2011). This

means that FSHR (rs1394205) AA carriers are expected to have a higher FSH consumption than GG and AG haplotypes, and therefore, need more costly treatment to achieve a comparable number of oocytes.

Limitations and strengths

Like all meta-analyses, our study has several limitations. First, most of the studies included were observational and retrospective, and thus more prone to bias. Second, the number of studies evaluating COS outcomes in relation to the patient's gonadotropin receptor genotype, is relatively small. Third, high heterogeneity among the studies included was observed. This could be probably explained by the wide variation in terms of population and treatment strategies. Lastly, OPRs were inconsistently reported in the included studies, however, we were able to conduct a meta-analysis for OPR with regards to the FSHR (rs6166), involving an elevated number of observation (over 3,000 patients). We used several strategies to

overcome these limitations. First we applied REM to strengthen the validity of our results in case of substantial heterogeneity among trials. Furthermore, we conducted a sensitivity analysis in which we considered only papers with a low risk of bias, namely those with NOS score above 6. The observed pooled effect sizes did not differ significantly from the overall analysis except in a few cases. Hence, the consistency in the direction of our findings is reliable and the methods were applied rigorously.

Future research

The pharmacogenomic approach to medical care is becoming a reality in several fields, notably for patients at a high risk of adverse drug reactions (Sychev and Malova, 2015). In the ART setting, a pharmacogenomic approach to COS could lead to better standardization of treatments, thereby increasing the chance of ART success and reducing a potentially life-threatening excessive ovarian response.

Remarkably, no large randomized clinical trial has yet been conducted, notwithstanding the relatively high number of studies published over the last 20 years. Based on existing evidence, we believe that the pharmacogenomic approach to COS is still a neglected topic in the reproductive field. Furthermore, it should be considered that most of polymorphisms reported in our paper are widespread in the general population and in women with reproductive disorders (Nilsson et al., 1997, Alviggi et al., 2009a, Alviggi et al., 2011b, Simoni and Casarini, 2014, Alviggi et al., 2015), and that genotype analysis can now be provided at the same costs of other commonly used analyses (e.g. AMH, AFC).

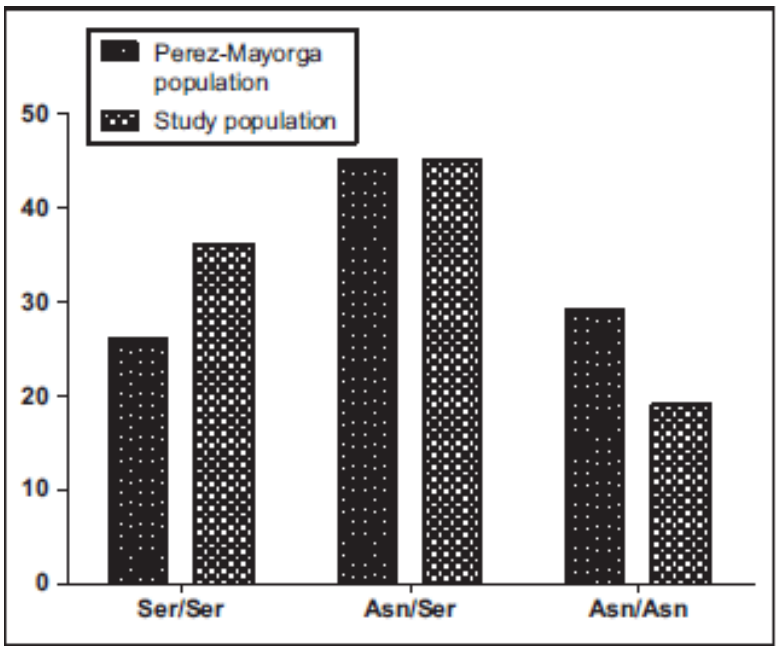
Conclusions

Our systematic review indicates that specific SNPs of gonadotropins and their receptors could influence ovarian stimulation outcomes. This evidence is supported by a large number of trials mainly focused on FSHR (rs6165) and FSHR (rs6166). Our analysis showed

that higher FSH consumption is expected in homozygotes for the A allele of FSHR (rs1394205) polymorphism than allele G carriers. Moreover, FSHR (rs6166) GG homozygotes seem to be less responsive to COS treatment; in fact, they have fewer oocytes and require larger FSH doses per oocyte than AA and AG carriers. Although LHB (rs1800447) and LHCGR (rs2293275) has been implicated in COS outcome, their role in clinical practice remains to be established. It was hypothesized that the effect of these polymorphism on COS may partially explain the phenomenon of "hypo-response" that was reported in 10-15% of normogonadotropic ART women (Alviggi et al., 2013). This peculiar ovarian response profile was recently included in the new classification of low prognosis women (Alviggi et al., 2016a, Humaidan et al., 2016). Given the overall effect of gonadotropin and their receptor SNPs on COS, further consideration of a pharmacogenomic approach to COS seems justified.

Figures

Figure 1A. Prevalence of follicle-stimulating hormone receptor (FSHR) polymorphisms: comparison between population study and data reported by Perez-Mayorga et al. (Perez Mayorga et al., 2000).



Figures 2A: Mean number of oocytes retrieved in hyporesponders (group A) and in controls (group B), P = .0003

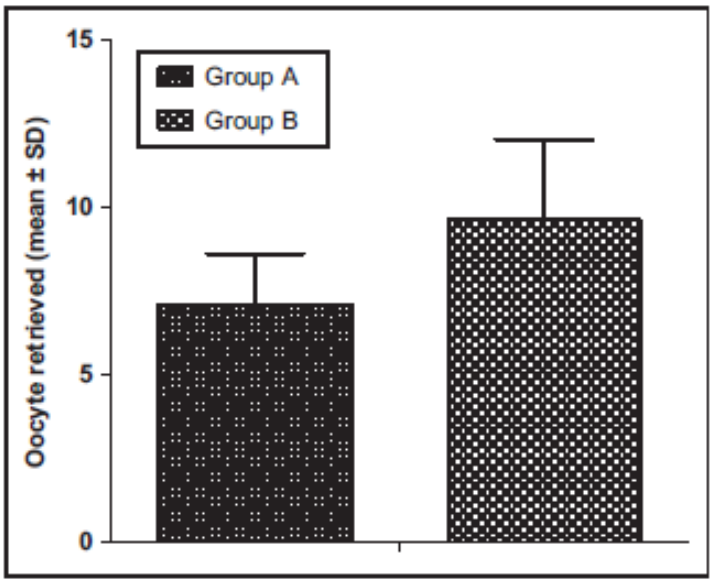


Figure 3A. Serum estradiol levels on the day of human chorionic gonadotrophin (hCG) administration in hyporesponders (group A) and in normal responders (group B). P ¼ .0001.

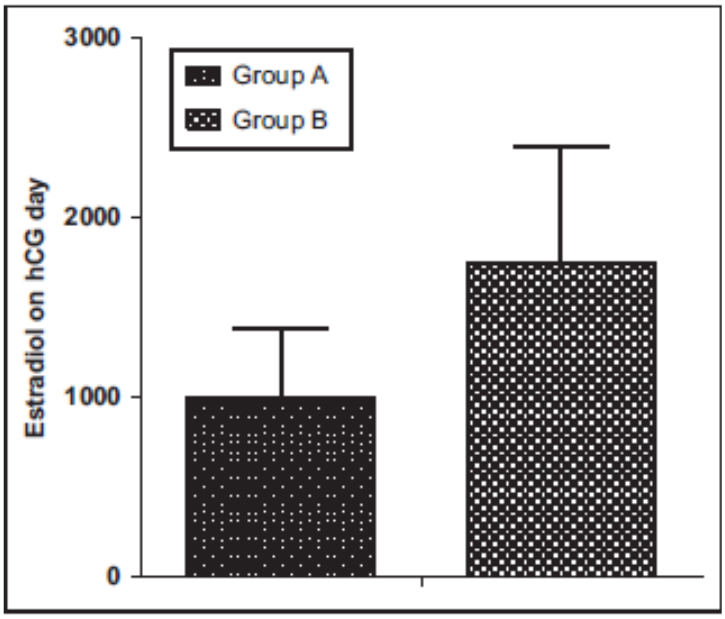

3000
2000
1000
0
Estradiol on hCG day
Group A
Group B

Figure 1B Allele C coexpression of FSHR -29; LHCGR 291 and cumulative and FSHR rs6166 r-hFSH dose/total number of oocytes or mature oocytes ratio.

	FSHR..29	LHCGR.291	Frequency	Difference (95% CI)	P-value
1	C	T	0.7287	0.00	---
2	T	T	0.2412	0.26 (-0.5 - 1.02)	0.5
3	G	C	0.0243	**5.47 (3.13 - 7.82)**	<0.0001
rare	*	*	0.0058	1.23 (-4.03 - 6.5)	0.65
Global haplotype association p-value: 0.65					

	FSHR.680	FSHR..29	LHCGR.291	Frequency	Difference (95% CI)	P-value
1	C	C	T	0.3739	0.00	---
2	T	C	T	0.3556	-0.5 (-1.43 - 0.42)	0.29
3	T	T	T	0.1498	0.08 (-0.96 - 1.12)	0.88
4	C	T	T	0.0905	0.07 (-1.16 - 1.3)	0.91
5	G	G	C	0.0235	**5.44 (3.18 - 7.71)**	<0.0001
rare	*	*	*	0.0066	1.36 (-3.65 - 6.37)	0.6
Global haplotype association p-value: 0.6						

Figure 1C. Study flow chart

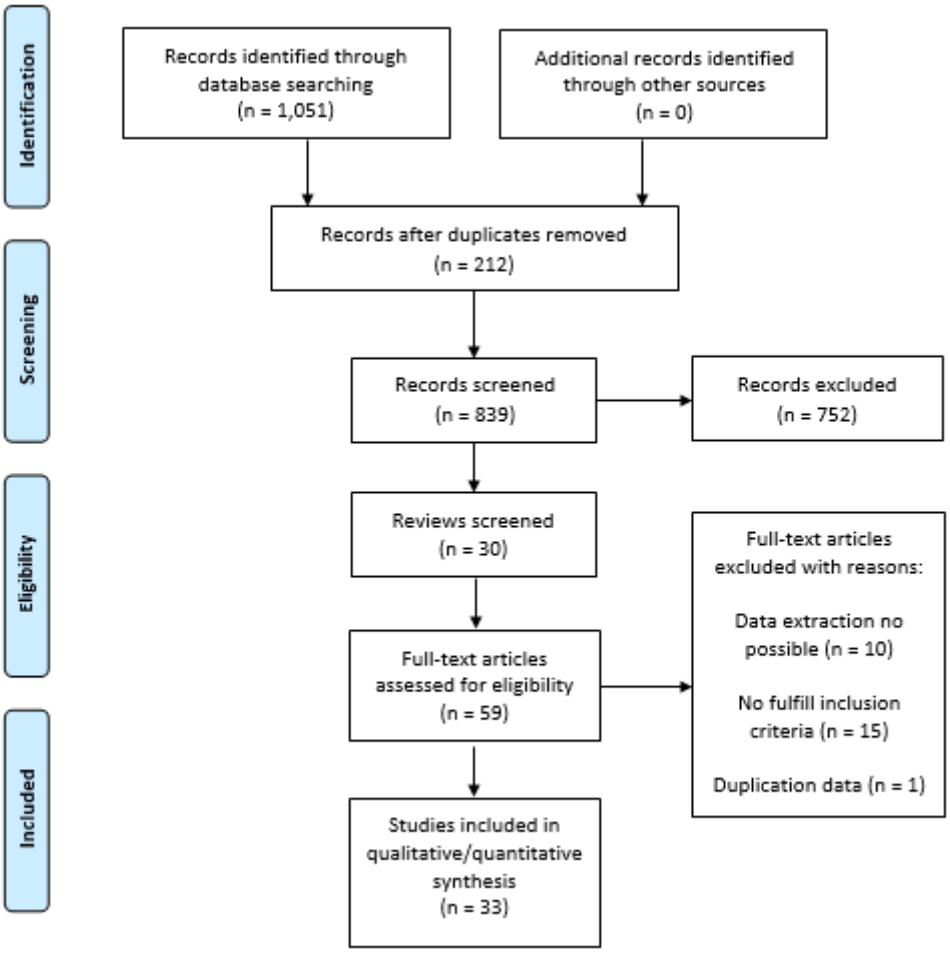
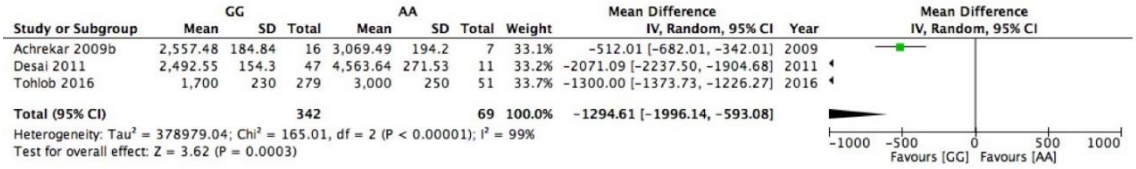
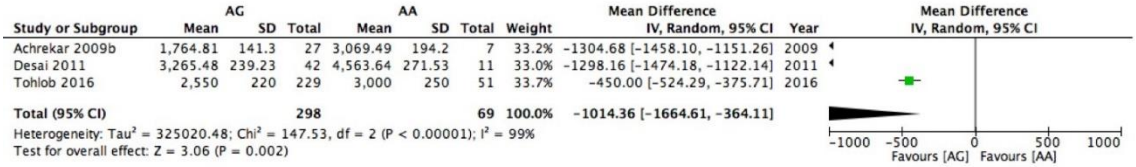


Figure 2C: Forest plots evaluating the overall differences among FSHR (rs1394205) genotypes carriers in relation to FSH consumption. (A) (rs1394205) G homozygous versus A homozygous, (B) (rs1394205) G homozygous versus heterozygous, (C) (rs1394205) heterozygous versus A homozygous.

A



B



C

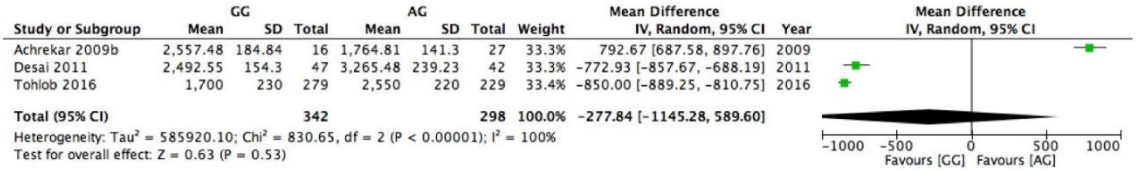
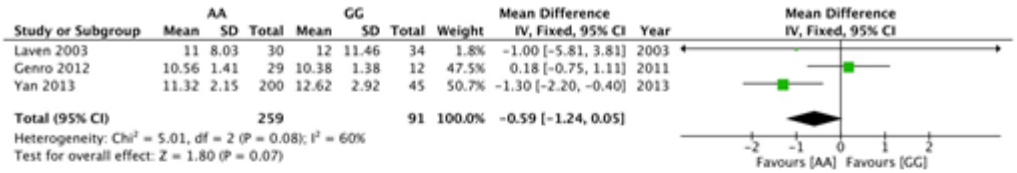
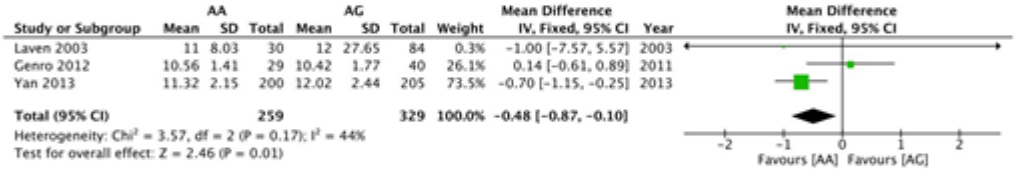


Figure 3C: Forest plots evaluating the differences among the FSHR (rs6165) genotype carriers in relation to stimulation duration. (A) (rs6165) A homozygous versus G homozygous. (B) (rs6165) A homozygous versus heterozygous, (C) (rs6165) heterozygous versus G homozygous.

A



B



C

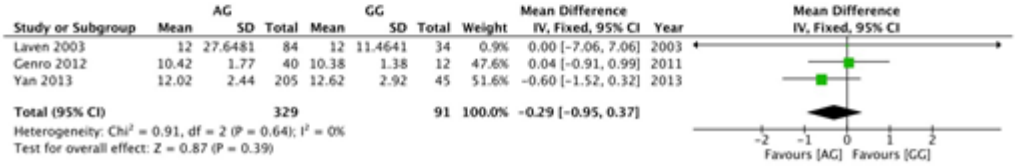
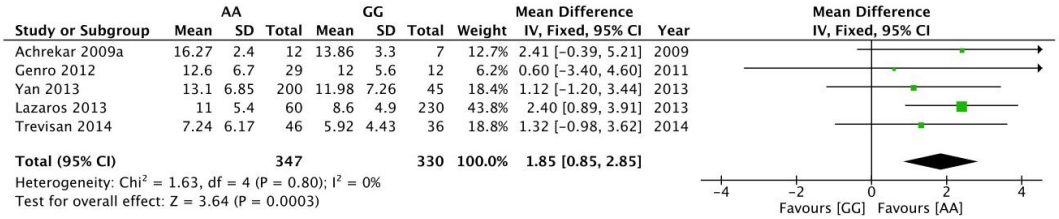
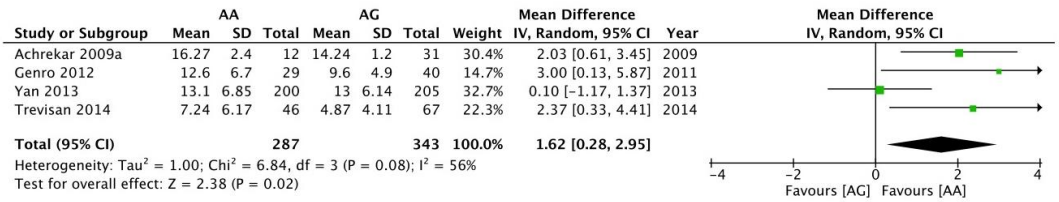


Figure 4C: Forest plots of the differences among FSHR (rs6165) genotype carriers in relation to the number of oocytes retrieved. (A) (rs6165) T (A) homozygous versus A (G) homozygous, (B) (rs6165) T (A) homozygous versus heterozygous, (C) (rs6165) heterozygous versus A (G) homozygous.

A



B



C

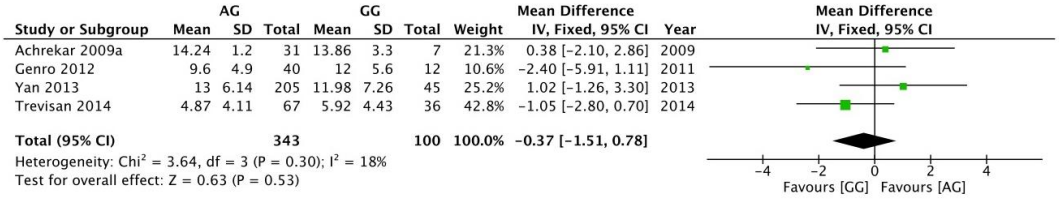
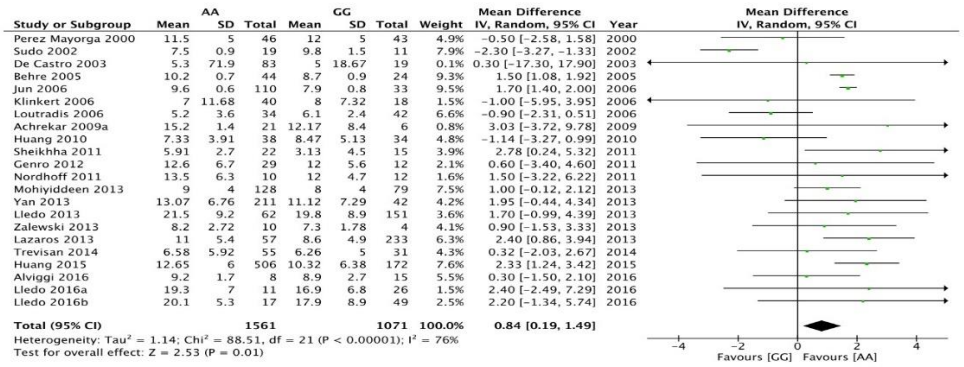
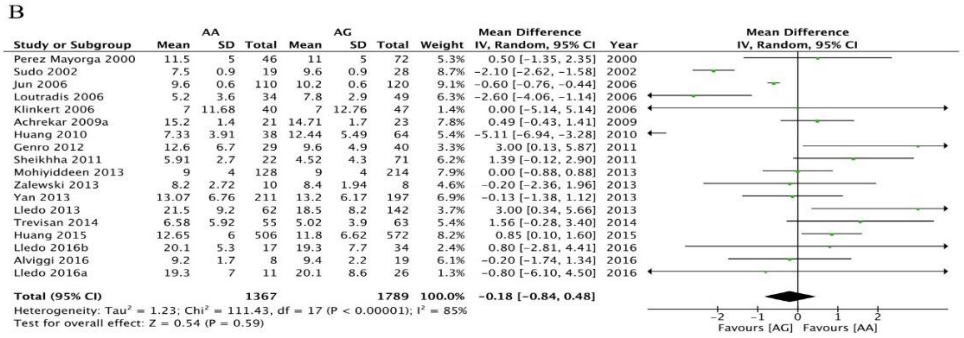


Figure 5C: Forest plots evaluating the overall differences among FSHR (rs6166) genotype carriers considering the total number of oocytes retrieved. (A) (rs6166) N (A) homozygous versus S (G) homozygous, (B) (rs6166) N (A) homozygous versus heterozygous, (C) (rs6166) heterozygous versus S (G) homozygous.

A



B



C

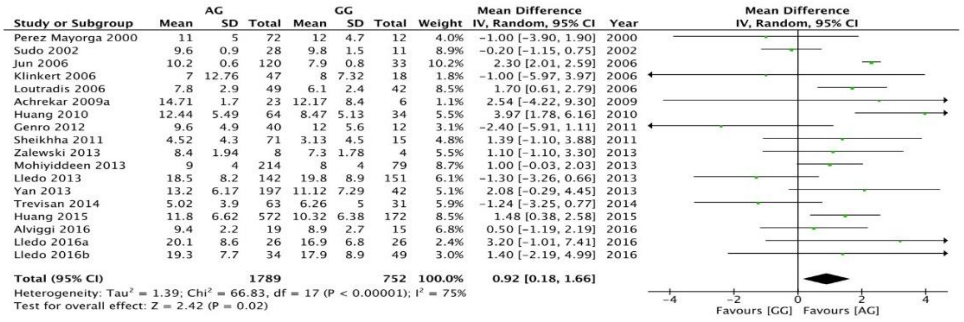
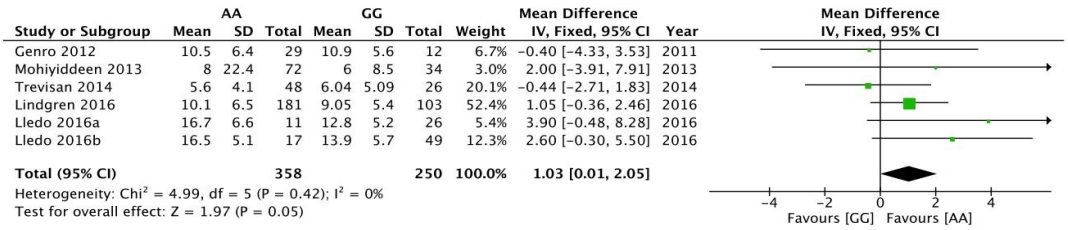
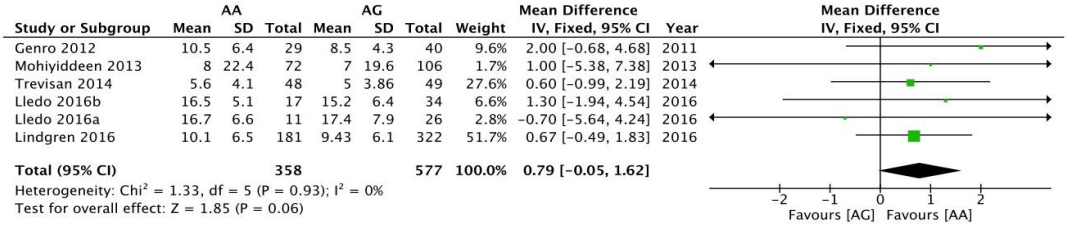


Figure 6C: Forest plots evaluating the overall differences among FSHR (rs6166) genotype carriers considering the total number of mature oocytes retrieved. (A) (rs6166) N (A) homozygous versus S (G) homozygous, (B) (rs6166) N (A) homozygous versus heterozygous, (C) (rs6166) heterozygous versus S (G) homozygous.

A



B



C

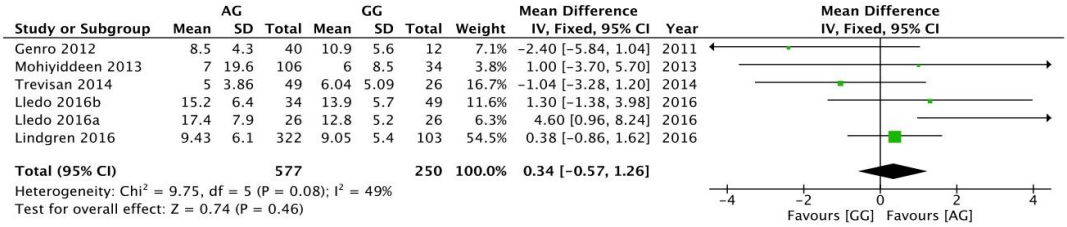
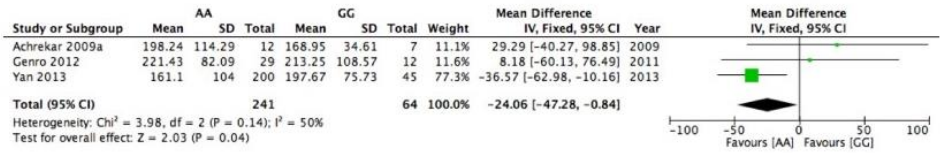


Figure 7C: Forest plots evaluating the overall differences among FSHR (rs6165) genotype carriers considering the ratio between the FSH consumption and the number of oocytes retrieved. (A) (rs6165) T (A) homozygous versus A (G) homozygous, (B) (rs6165) T (A) homozygous versus heterozygous, (C) (rs6165) heterozygous versus A (G) homozygous.

A



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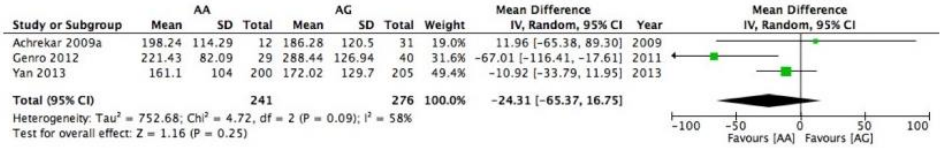


C

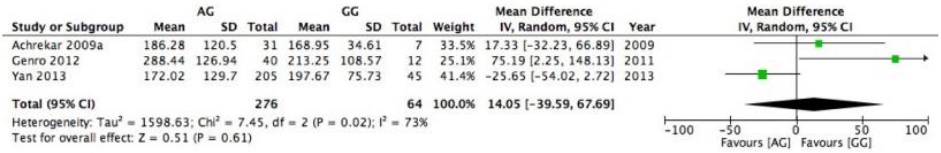
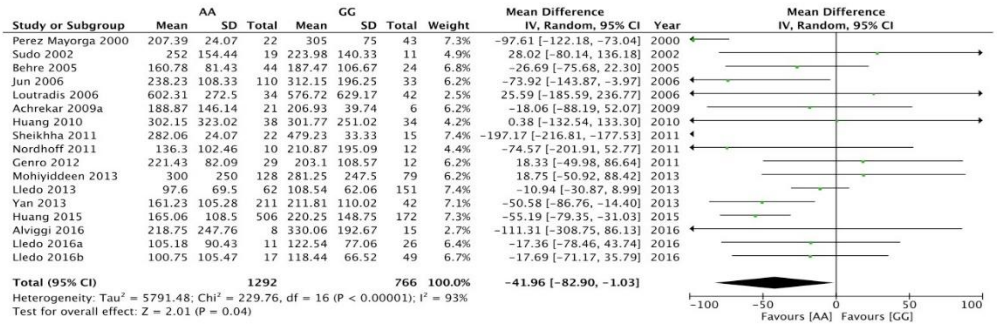


Figure 8C: Forest plots evaluating the overall differences among FSHR (rs6166) genotype carriers considering the ratio between the FSH consumption and the number of oocytes retrieved. (A) (rs6166) N (A) homozygous versus S (G) homozygous, (B) (rs6166) N (A) homozygous versus heterozygous, (C) (rs6166) heterozygous versus S (G) homozygous.

A



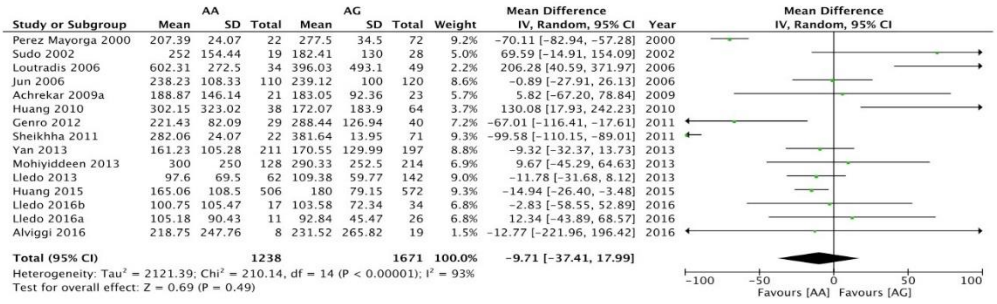
Study or Subgroup	AA Mean	AA SD	AA Total	GG Mean	GG SD	GG Total	Weight	Mean Difference IV, Random, 95% CI	Year
Perez Mayorga 2000	207.39	24.07	22	305	75	43	7.3%	−97.61 [−122.18, −73.04]	2000
Sudo 2002	252	154.44	19	223.98	140.33	11	4.9%	28.02 [−80.14, 136.18]	2002
Behre 2005	160.78	81.43	44	187.47	106.67	24	6.8%	−26.69 [−75.68, 22.30]	2005
Jun 2006	238.23	108.33	110	312.15	196.25	33	6.2%	−73.92 [−143.87, −3.97]	2006
Loutradis 2006	602.31	272.5	34	576.72	629.17	42	2.5%	25.59 [−185.59, 236.77]	2006
Achrekar 2009a	188.87	146.14	21	206.93	39.74	6	6.2%	−18.06 [−88.19, 52.07]	2009
Huang 2010	302.15	323.02	38	301.77	251.02	34	4.2%	0.38 [−132.54, 133.30]	2010
Sheikhha 2011	282.06	24.07	22	479.23	33.33	15	7.4%	−197.17 [−216.81, −177.53]	2011
Nordhoff 2011	136.3	102.46	10	210.87	195.09	12	4.4%	−74.57 [−201.91, 52.77]	2011
Genro 2012	221.43	82.09	29	203.1	108.57	12	6.2%	18.33 [−49.98, 86.64]	2011
Mohiyiddeen 2013	300	250	128	281.25	247.5	79	6.2%	18.75 [−50.92, 88.42]	2013
Lledo 2013	97.6	69.5	62	108.54	62.06	151	7.4%	−10.94 [−30.87, 8.99]	2013
Yan 2013	161.23	105.28	211	211.81	110.02	42	7.1%	−50.58 [−86.76, −14.40]	2013
Huang 2015	165.06	108.5	506	220.25	148.75	172	7.3%	−55.19 [−79.35, −31.03]	2015
Alviggi 2016	218.75	247.76	8	330.06	192.67	15	2.7%	−111.31 [−308.75, 86.13]	2016
Lledo 2016a	105.18	90.43	11	122.54	77.06	26	6.4%	−17.36 [−78.46, 43.74]	2016
Lledo 2016b	100.75	105.47	17	118.44	66.52	49	6.7%	−17.69 [−71.17, 35.79]	2016
Total (95% CI)			**1292**			**766**	**100.0%**	**−41.96 [−82.90, −1.03]**	

Heterogeneity: $Tau^2 = 5791.48$; $Chi^2 = 229.76$, df = 16 ($P < 0.00001$); $I^2 = 93\%$
Test for overall effect: Z = 2.01 (P = 0.04)

Mean Difference IV, Random, 95% CI

−100 −50 0 50 100
Favours [AA] Favours [GG]

B



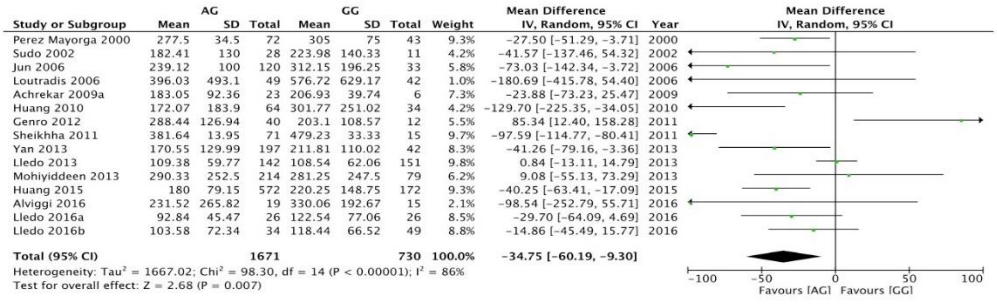
Study or Subgroup	AA Mean	AA SD	AA Total	AG Mean	AG SD	AG Total	Weight	Mean Difference IV, Random, 95% CI	Year
Perez Mayorga 2000	207.39	24.07	22	277.5	34.5	72	9.2%	−70.11 [−82.94, −57.28]	2000
Sudo 2002	252	154.44	19	182.41	130	28	5.0%	69.59 [−14.91, 154.09]	2002
Loutradis 2006	602.31	272.5	34	396.03	493.1	49	2.2%	206.28 [40.59, 371.97]	2006
Jun 2006	238.23	108.33	110	239.12	100	120	8.6%	−0.89 [−27.91, 26.13]	2006
Achrekar 2009a	188.87	146.14	21	183.05	92.36	23	5.7%	5.82 [−67.20, 78.84]	2009
Huang 2010	302.15	323.02	38	172.07	183.9	64	3.7%	130.08 [17.93, 242.23]	2010
Genro 2012	221.43	82.09	29	288.44	126.94	40	7.2%	−67.01 [−116.41, −17.61]	2011
Sheikhha 2011	282.06	24.07	22	381.64	13.95	71	9.3%	−99.58 [−110.15, −89.01]	2011
Yan 2013	161.23	105.28	211	170.55	129.99	197	8.8%	−9.32 [−32.37, 13.73]	2013
Mohiyiddeen 2013	300	250	128	290.33	252.5	214	6.9%	9.67 [−45.29, 64.63]	2013
Lledo 2013	97.6	69.5	62	109.38	59.77	142	9.0%	−11.78 [−31.68, 8.12]	2013
Huang 2015	165.06	108.5	506	180	79.15	572	9.3%	−14.94 [−26.40, −3.48]	2015
Lledo 2016b	100.75	105.47	17	103.58	72.34	34	6.8%	−2.83 [−58.55, 52.89]	2016
Lledo 2016a	105.18	90.43	11	92.84	45.47	26	6.8%	12.34 [−43.89, 68.57]	2016
Alviggi 2016	218.75	247.76	8	231.52	265.82	19	1.5%	−12.77 [−221.96, 196.42]	2016
Total (95% CI)			**1238**			**1671**	**100.0%**	**−9.71 [−37.41, 17.99]**	

Heterogeneity: $Tau^2 = 2121.39$; $Chi^2 = 210.14$, df = 14 ($P < 0.00001$); $I^2 = 93\%$
Test for overall effect: Z = 0.69 (P = 0.49)

Mean Difference IV, Random, 95% CI

−100 −50 0 50 100
Favours [AA] Favours [AG]

C



Study or Subgroup	AG Mean	AG SD	AG Total	GG Mean	GG SD	GG Total	Weight	Mean Difference IV, Random, 95% CI	Year
Perez Mayorga 2000	277.5	34.5	72	305	75	43	9.3%	−27.50 [−51.29, −3.71]	2000
Sudo 2002	182.41	130	28	223.98	140.33	11	4.2%	−41.57 [−137.46, 54.32]	2002
Jun 2006	239.12	100	120	312.15	196.25	33	5.8%	−73.03 [−142.34, −3.72]	2006
Loutradis 2006	396.03	493.1	49	576.72	629.17	42	1.0%	−180.69 [−415.78, 54.40]	2006
Achrekar 2009a	183.05	92.36	23	206.93	39.74	6	7.3%	−23.88 [−73.23, 25.47]	2009
Huang 2010	172.07	183.9	64	301.77	251.02	34	4.2%	−129.70 [−225.35, −34.05]	2010
Genro 2012	288.44	126.94	40	203.1	108.57	12	5.5%	85.34 [12.40, 158.28]	2011
Sheikhha 2011	381.64	13.95	71	479.23	33.33	15	9.7%	−97.59 [−114.77, −80.41]	2011
Yan 2013	170.55	129.99	197	211.81	110.02	42	8.3%	−41.26 [−79.16, −3.36]	2013
Lledo 2013	109.38	59.77	142	108.54	62.06	151	9.8%	0.84 [−13.11, 14.79]	2013
Mohiyiddeen 2013	290.33	252.5	214	281.25	247.5	79	6.2%	9.08 [−55.13, 73.29]	2013
Huang 2015	180	79.15	572	220.25	148.75	172	9.3%	−40.25 [−63.41, −17.09]	2015
Alviggi 2016	231.52	265.82	19	330.06	192.67	15	2.1%	−98.54 [−252.79, 55.71]	2016
Lledo 2016a	92.84	45.47	26	122.54	77.06	26	8.5%	−29.70 [−64.09, 4.69]	2016
Lledo 2016b	103.58	72.34	34	118.44	66.52	49	8.8%	−14.86 [−45.49, 15.77]	2016
Total (95% CI)			**1671**			**730**	**100.0%**	**−34.75 [−60.19, −9.30]**	

Heterogeneity: $Tau^2 = 1667.02$; $Chi^2 = 98.30$, df = 14 ($P < 0.00001$); $I^2 = 86\%$
Test for overall effect: Z = 2.68 (P = 0.007)

Mean Difference IV, Random, 95% CI

−100 −50 0 50 100
Favours [AG] Favours [GG]

Figure 9C: Funnel plots, trim and fill and Egger test results considering ongoing pregnancy rate (A) (rs6166), (B) (rs6165) and (C) (rs1394205)

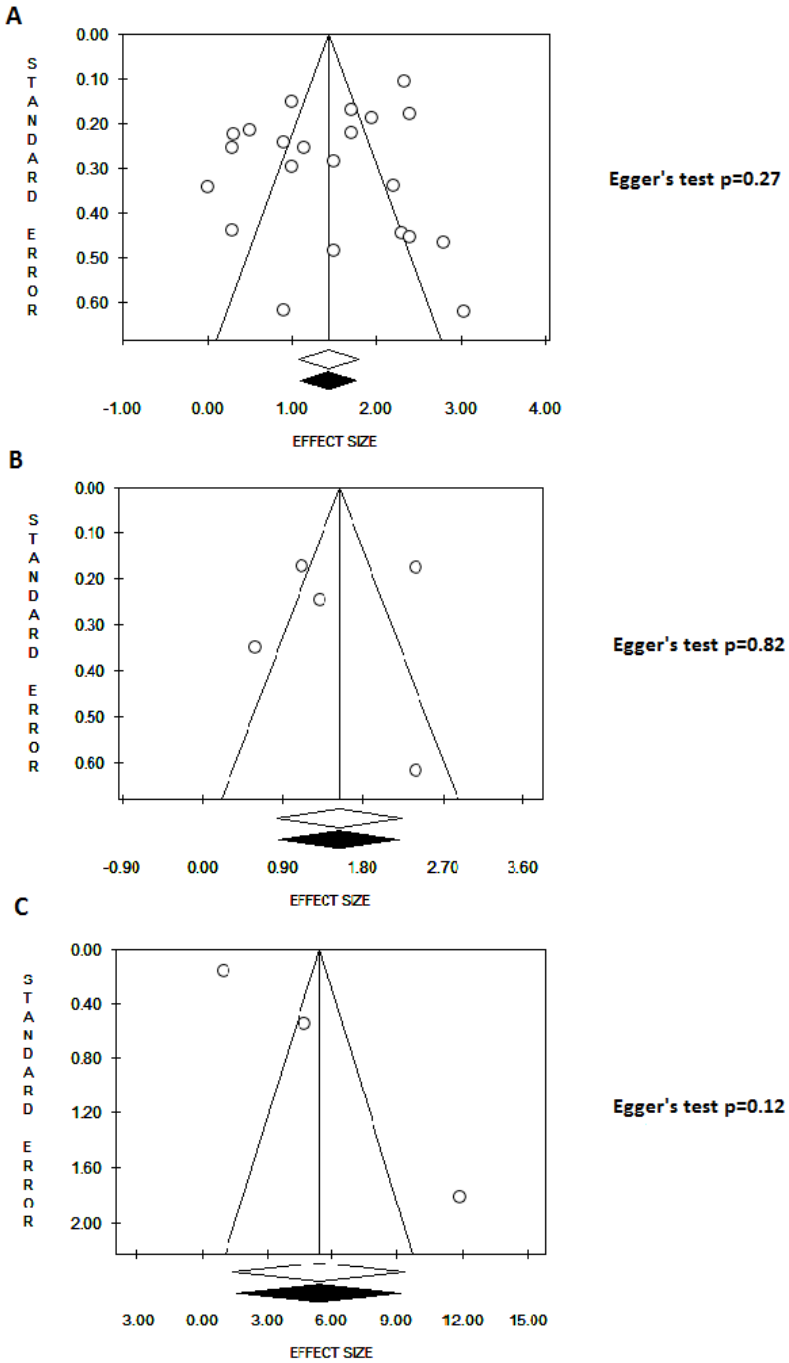
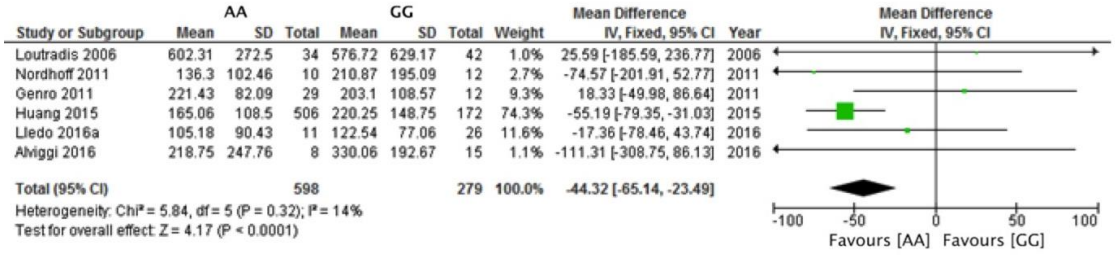


Figure 10C: Forest plots evaluating the overall differences between NN (AA) and SS (GG) carriers [FSHR (rs6166)] considering FSH/oocyte ratio. (A) recombinant gonadotropin (B) no-recombinant gonadotropin.

A



B

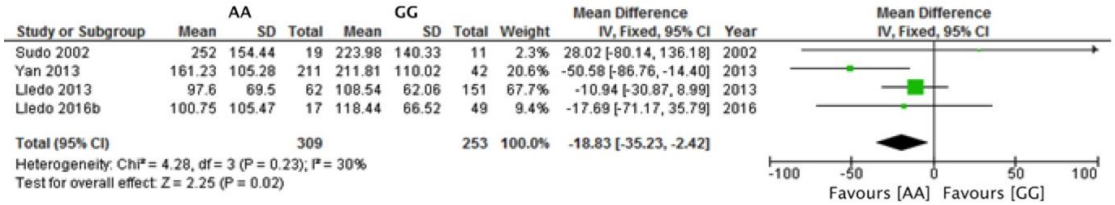
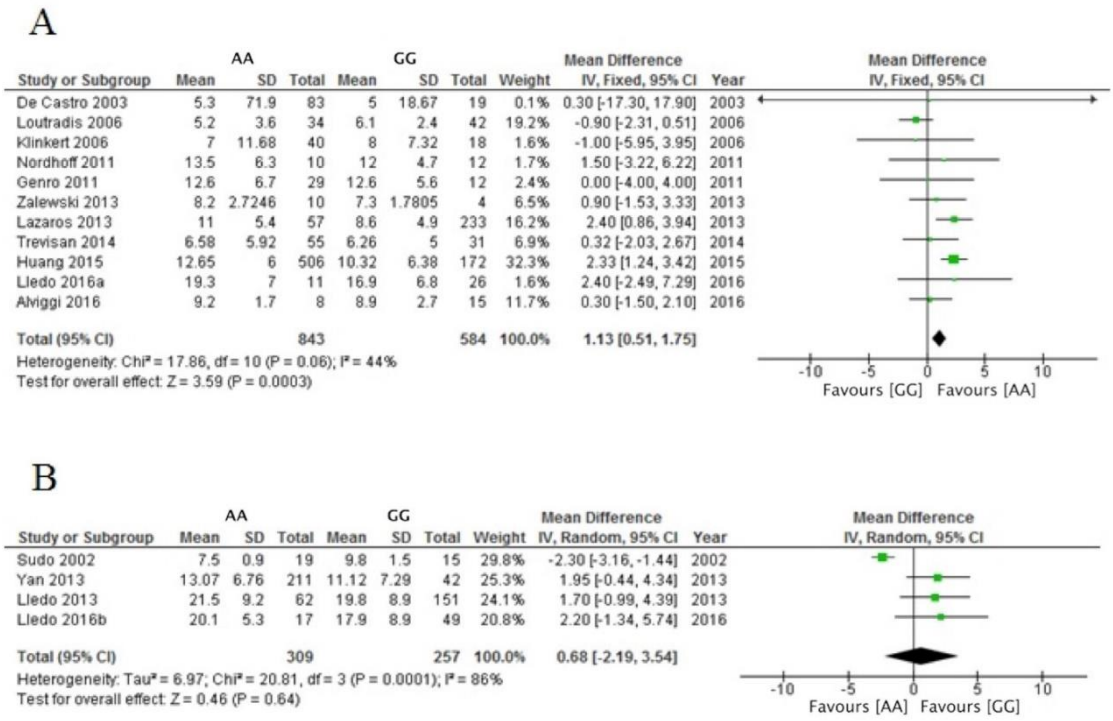


Figure 11C: Forest plots evaluating the overall differences between NN (AA) and SS (GG) carriers [FSHR (rs6166)] considering the number of oocytes retrieved oocytes retrieved (A) recombinant gonadotropin (B) no-recombinant gonadotropin**.**



Tables

Table 1A: Characteristics of Group A and Group B patients and Indications for *In Vitro* Fertilization.

Characteristics	Group A, Hyporesponders (N=17)	Group B, Controls (N=25)	P Value
Age, years	31.82 ± 4.08	29.32 ± 4.67	NS
BMI, kg/m^2	25.0 ± 3.4	23.6 ± 3.2	NS
Years of infertility	4.15 ± 1.2	3.2 ± 0.9	.0055
Baseline LH,IU/L	4.2 ± 1.2	4.1 ± 1.8	NS
Baseline estradiol, pg/mL	48.75 ± 16.9	43.11 ± 19.4	NS
Indications for IVF			
Tubal factor (%)	5 (29.4)	5 (20)	NS
Male factor (%)	7 (41.2)	8 (32)	NS
Combined (%)	3 (17.6)	5 (20)	NS
Other (%)	2 (11,8)	7 (28)	NS
Distribution of the FSH-R genotypes	10 (58.8)	5 (20)	.02
Ser/Ser (%)	4 (23.5)	15 (60)	.04
Asn/Ser (%)	3 (17.6)	5 (20)	NS
Asn/Asn (%)			

Abbreviations: NS, not significant; BMI, body mass index; LH, luteinizing hormone; IVF, in vitro fertilization; FSH-R, follicle-stimulating hormone receptor.
[a]Data are showed as means ± standard deviation.

Table 2A: Outcome of Cycles of Assisted Reproduction in Groups A and B.

Characteristics	Group A, Hyporesponders (N=17)	Group B, Controls (N=25)	P Value
Baseline FSH	6.9 ± 1.9	5.6 ± 1.9	.035
Baseline LH	4.2 ± 1.2	4.1 ± 1.8	NS
Duration of stimulation, days	12.7 ± 2.4	10.8 ± 2.8	.03
N. of r-hFSH vials	36.3 ± 7.5	28.6 ± 4.5	.0002
Estradiol on hCG day	997.8 ± 384.9	1749.1 ± 644.4	.0001
N. oocytes retrieved	7.1 ± 1.5	9.6 ± 2.4	.0005
N. embryo transferred	2.1 ± 0.7	2.7 ± 0.4	.001
Implantation rate, %	11.1	16.2	NS
Pregnancy rate, %	17.6	36.0	NS
Rate of ongoing pregnancies, %	11.7	32.0	NS

Abbreviations: NS, not significant; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotrophin; LH, luteinizing hormone; r-FSH: recombinant FSH; r-hFSH, recombinant human follicle-stimulating hormone.
[a]Data are showed as mean ± standard deviation.

Table 1B: Baseline characteristics of population study**.**

Basal characteristics	Values
Age (years)	30.71±2.61
BMI (kg/m^2)	22.94±2.35
AMH (ng/mL)	2.70±1.76
Antral follicle count	12.36±3.63
Basal FSH (IU/L)	6.73±1.98
Basal estradiol (pg/mL)	80.65±101.16

Table 2B: Treatment outcomes; continuous data are expressed as mean ± standard deviation; categorical data as percentage.

Treatment outcomes	Values
Total FSH doses (IU)	1725.33±520.15
Days of stimulation	11.24±1.69
Estradiol at the day of hCG (pg/mL)	1655.43±895.59
Follicles >10 mm	11.04±4.41
Follicles >16 mm	7.72±3.15
Oocytes number	9.51±3.82
Mature oocytes number	7.78±3.39
Oocytes inseminated	5.35±3.50
Oocytes fertilized	3.61±2.55
Oocytes cryopreserved	0.35±1.36
Embryos cryopreserved	6.73±1.98
Embryos transferred	1.65±0.80
Cycles cancelled for hyper-response	2 (2.1%)
OHSS	1 (1.1%)
Pregnancy rate (beta-hCG) per cycle	42.5%
Clinical pregnancy rate per cycle	34.0%
Miscarriage rate per cycle	9.4%

Table 3B: Treatment outcomes, stratifying patients according to the FSHR 307 (rs6165).

	homozygous A/A	**heterozygous A/G**	**homozygous G/G**	***p-value***
Total FSH doses (IU)	1781.23±568.45	1730.04±550.19	1647.17±383.58	*0.536*
FSH/oocytes	243.42±97.60	338.52±251.80	252.60±166.33	***0.050***
Days of stimulation	11.13±1.68	11.35±1.82	11.10±1.41	*0.769*
Endometrial thickness (mm)	9.70±1.15	10.38±2.00	10.28±2.09	*0.547*
Estradiol at the day of hCG (pg/mL)	1555.24±663.85	1607.54±906.21	1859.42±1092.75	*0.513*
Follicles ≥ 16mm hCG day hCG	7.63±2.72	7.73±3.26	7.80±3.50	*0.983*
Oocytes number	9.58±3.32	9.24±3.57	10.10±4.98	*0.685*
Mature oocytes number	8.13±2.72	7.50±3.71	8.06±3.47	*0.643*
Oocytes inseminated	6.08±3.26	5.18±3.60	4.90±3.55	*0.346*
Oocytes fertilized	3.92±2.53	3.60±2.66	3.25±2.38	*0.537*
Oocytes cryopreserved	0.21±1.02	0.36±1.44	0.50±1.54	*0.802*
Embryos cryopreserved	0.96±1.81	1.16±2.05	0.65±1.31	*0.534*
Embryos transferred	1.63±0.77	1.56±0.79	1.90±0.85	*0.236*
Implantation rate	10/39	23/77	10/38	*0.795*
Pregnancy rate per embryo transferred	12/39	24/77	11/38	*0.867*
Ongoing pregnancy rate per embryo transferred	9/39	21/77	9/38	*0.792*
Pregnancy rate per cycle	12/24	24/50	11/20	*0.930*
Ongoing pregnancy rate per cycle	9/24	21/50	9/20	*0.863*

Table 4B: Treatment outcomes, stratifying patients according to the 680 (rs6166).

	homozygous A/A	**heterozygous A/G**	**homozygous G/G**	***p-value***
Total FSH doses (IU)	1809.76±563.38	1725.25±554.53	1633.02±379.45	*0.698*
FSH/oocytes	248.80±96.34	333.44±250.88	252.60±166.33	***0.049***
Days of stimulation	11.42±1.72	11.23±1.81	11.50±1.40	*0.804*
Endometrial thickness (mm)	9.70±1.15	10.38±2.00	10.28±2.09	*0.547*
Estradiol at the day of hCG (pg/mL)	1624.09±722.40	1568.46±880.90	1859.42±1092.75	*0.514*
Follicles ≥ 16mm hCG day hCG	7.58±2.70	7.71±3.28	7.90±3.45	*0.944*
Oocytes number	9.67±3.33	9.20±3.59	10.05±4.86	*0.697*
Mature oocytes number	8.22±2.78	7.45±3.67	8.06±3.47	*0.725*
Oocytes inseminated	6.21±3.35	5.18±3.55	4.76±3.52	*0.476*
Oocytes fertilized	4.04±2.56	3.57±2.65	3.19±2.38	*0.694*
Oocytes cryopreserved	0.21±1.02	0.37±1.45	0.48±1.50	*0.779*
Embryos cryopreserved	1.00±1.82	1.16±2.06	0.62±1.28	*0.581*
Embryos transferred	1.63±0.77	1.55±0.79	1.90±0.83	*0.273*
Implantation rate	10/37	21/77	12/40	*0.844*
Pregnancy rate per embryo transferred	12/37	22/77	13/40	*0.839*
Ongoing pregnancy rate per embryo transferred	9/37	19/77	11/40	*0.848*
Pregnancy rate per cycle	12/23	23/50	13/21	*0.812*
Ongoing pregnancy rate per cycle	9/23	19/50	11/21	*0.867*

Table 5B: Treatment outcomes, stratifying patients according to the FSHR – 29 (rs1394205).

	homozygous G/G	**heterozygous G/A**	**homozygous A/A**	***p-value***
Total FSH doses (IU)	1730.04±554.55	1745.19±476.55	1601.71±487.46	*0.804*
FSH/oocytes	322.78±239.49	229.73±109.79	312.83±161.03	*0.186*
Days of stimulation	11.20±1.52	11.39±1.87	11.50±1.40	*0.733*
Endometrial thickness (mm)	10.29±2.00	9.91±1.55	10.47±1.36	*0.776*
Estradiol at the day of hCG (pg/mL)	1518.52±742.11	1832.04±1184.50	2039.14±706.30	*0.197*
Follicles ≥ 16mm hCG day hCG	8.07±3.23	6.90±2.83	8.50±3.50	*0.197*
Oocytes number	9.60±3.90	9.58±3.84	8.63±3.54	*0.794*
Mature oocytes number	7.67±3.65	8.46±2.90	6.50±2.83	*0.344*
Oocytes inseminated	5.22±3.49	5.90±3.67	4.13±2.70	*0.404*
Oocytes fertilized	3.47±2.67	3.97±2.60	3.13±1.36	*0.595*
Oocytes cryopreserved	0.38±1.38	0.19±1.08	0.75±2.12	*0.572*
Embryos cryopreserved	1.05±2.03	1.03±1.72	0.50±0.76	*0.730*
Embryos transferred	1.62±0.85	1.61±0.76	2.00±0.53	*0.435*
Implantation rate	18/88	21/50	4/16	*0.934*
Pregnancy rate per embryo transferred	22/88	21/50	4/16	*0.754*
Ongoing pregnancy rate per embryo transferred	16/88	19/50	4/16	*0.770*
Pregnancy rate per cycle	22/55	21/31	4/8	*0.879*
Ongoing pregnancy rate per cycle	16/55	19/31	4/8	*0.435*

Table 6B: Treatment outcomes, stratifying patients according to the LHCGR 291 (rs12470652).

	T/T	**C/T**	***p-value***
Total FSH doses (IU)	1736.38±534.53	1568.75±196.17	*0.449*
FSH/oocytes	305.86±208.66	147.65±46.77	*0.069*
Days of stimulation	11.21±1.70	11.57±1.62	*0.588*
Endometrial thickness (mm)	9.97±1.49	11.04±2.76	*0.146*
Estradiol at the day of hCG (pg/mL)	1580.60±860.03	2733.00±747.23	***0.005***
Follicles ≥ 16mm hCG day hCG	7.80±3.16	6.71±2.98	*0.382*
Oocytes number	9.28±3.81	12.43±2.82	***0.035***
Mature oocytes number	7.45±3.21	11.43±3.41	***0.002***
Oocytes inseminated	4.92±3.20	10.71±2.56	***0.001***
Oocytes fertilized	3.24±2.16	8.14±2.91	***0.001***
Oocytes cryopreserved	0.38±1.41	0.00±0.00	*0.480*
Embryos cryopreserved	0.75±1.47	4.14±3.08	***0.001***
Embryos transferred	1.68±0.81	1.29±0.49	*0.213*
Implantation rate	41/145	2/9	*0.992*
Pregnancy rate per embryo transferred	45/145	4/9	*0.639*
Ongoing pregnancy rate per embryo transferred	37/145	2/9	*0.861*
Pregnancy rate per cycle	45/87	4/7	*0.907*
Ongoing pregnancy rate per cycle	37/87	2/7	*0.747*

Table 1C: Characteristics of studies included in the analysis.

Authors	Year	SNPs evaluated	Country	Partecipants	Mean age	Study design	NOS score
Achrekar et al.	2009	*FSHR* (rs6165), *FSHR* (rs6166)	India	50	30.09 ± 1.50	Retrospective	7
Achrekar et al.	2009	*FSHR* (rs1394205)	India	150	NA	Retrospective	7
Alviggi et al.	2009	*LHB* (rs1800447)	Italy	60	30.81 ± 3.39	Retrospective	6
Alviggi et al.	2013	*LHB* (rs1800447)	Denmark	220	30.65 ± 3.95	Retrospective	6
Alviggi et al.	2016	*FSHR* (rs6165), *FSHR* (rs6166)	Italy	42	30.57 ± 4.37	Retrospective	6
Anagnostou et al.	2012	*FSHR* (rs6166)	Greece	109	35.00 ± 4.50	Prospective	6
Behre et al.	2005	*FSHR* (rs6166)	Germany	93	33.10 ± 0.64	Prospective	7
Dan et al.	2015	*FSHR* (rs1394205)	China	158	NA	Prospective	7
Davar et al.	2014	*LHB* (rs1056917)	Iran	220	29.94 ± 5.98	Prospective	7
De Castro et al.	2003	*FSHR* (rs6165)	Spain	102	33.70 ± 3.10	Retrospective	6
Desai et al.	2011	*FSHR* (rs1394205)	India	100	33.11 ± 0.82	Retrospective	8
Genro et al.	2012	*FSHR* (rs6165), *FSHR* (rs6166)	Brazil	124	34.95 ± 3.82	Prospective	8
Huang X et al.	2015	*FSHR* (rs6166)	China	1250	31.31 ± 3.34	Retrospective	6
Huang S et al.	2010	*FSHR* (rs6166)	China	136	30.33 ± 3.31	Prospective	6
Jun et al.	2006	*FSHR* (rs6166)	South Corea	263	32.60 ± 0.40	Prospective	7
Klinkert et al.	2006	*FSHR* (rs6166)	Netherlands	105	36.90 ± 5.10	Prospective	6
Laven et al.	2003	*FSHR*	Germany	148	28.20 ± 3.10	Prospective	6

		(rs6165), *FSHR* (rs6166)					
Lazaros et al.	2013	*FSHR* (rs6165), *FSHR* (rs6166)	Greece	604	NA	Retrospective	6
Lindgren et al.	2016	*LHCGR* (rs2293275)	Denmark	384	31.92 ± 2.90	Prospective	8
Lledo et al.	2013	*FSHR* (rs6166)	Spain	145	25.60 ± 3.80	Retrospective	6
Lledo et al.	2016	*FSHR* (rs6166)	Spain	191	25.60 ± 3.90	Retrospective	6
Loutradis et al.	2006	*FSHR* (rs6166)	Greece	125	30.30 ± 3.00	Retrospective	5
Mohiyiddeen et al. a	2013	*FSHR* (rs6166)	UK	212	33.17 ± 3.50	Prospective	7
Mohiyiddeen et al. b	2013	*FSHR* (rs6166)	UK	504	33.50 ± 3.70	Prospective	7
Nordhoff et al.	2011	*FSHR* (rs6166)	Germany	22	32.40 ± 3.35	Retrospective	3
Perez Mayorga et al.	2000	*FSHR* (rs6166)	Germany	161	32.60 ± 0.50	Prospective	6
Yin et al.	2015	*LHCGR* (rs13405728)	China	236	NA	Prospective	6
Sheikhha et al	2011	*FSHR* (rs6166)	Iran	108	29.63 ± 4.70	Retrospective	6
Sudo et al.	2002	*FSHR* (rs6166)	Japan	522	31.83 ± 0.77	Retrospective	5
Tohlob et al.	2016	*FSHR* (rs1394205)	UK	559	33.23 ± 5.1	Retrospective	6
Trevisan et al.	2014	*FSHR* (rs6165), *FSHR* (rs6166)	Italy	149	NA	Retrospective	5
Yan et al.	2013	*FSHR* (rs6165), *FSHR* (rs6166)	China	450	32.15 ± 4.96	Retrospective	6
Zalewski et al.	2013	*FSHR* (rs6166)	Poland	22	33.10 ± 5.00	Retrospective	3

Table 2C: Pooled effect estimates including only FSHR haplotypes with significant overall effect on ovarian stimulation outcomes.

FSHR variant	Comparison	Parameter	Effect size [95%CI]	I^2%	Test for overall effect (P value)
FSHR (rs6165)	AA *vs* GG	Stimulation duration	-0.59 [-1.24, 0.05]	60	0.35
		Number of oocytes	1.85 [0.85, 2.85]	0	**<0.01**
		FSH/oocytes ratio	-24.06 [-47.28, -0.84]	50	**0.04**
	AA *vs* GA	Stimulation duration	-0.48 [-0.87, -0.10]	44	**0.01**
		Number of oocytes	1.62 [0.28, 2.95]	56	**0.02**
		FSH/oocytes ratio	-24.31 [-65.37, 16.75]	58	0.61
	GA *vs* GG	Stimulation duration	-0.29 [-0.95, 0.37]	0	0.39
		Number of oocytes	-0.37 [-1.51, 0.78]	18	0.53
		FSH/oocytes ratio	14.05 [-39.59, 67.69	73	0.61
FSHR (rs6166)	AA *vs* GG	Number of oocytes	0.82 [0.13, 1.51]	81	**<0.01**
		Number of M2 oocytes	1.03 [0.01, 2.05]	0	**0.05**
		FSH/oocyte ratio	-45.24 [-86.62, -3.85]	93	**0.03**
	AA *vs* GA	Number of oocytes	0.18 [-0.84, 0.48]	85	0.59
		Number of M2 oocytes	0.79 [-0.05, 1.62]	0	0.06
		FSH/oocyte ratio	-14.84 [-42.13, 12.44]	93	0.29
	GA *vs* GG	Number of oocytes	0.89 [0.13, 1.66]	77	**0.01**
		Number of M2 oocytes	0.34 [-0.57, 1.26]	49	0.46
		FSH/oocyte ratio	-31.47 [-57.01, -5.93]	85	**0.02**
FSHR (rs1394205)	GG *vs* AA	FSH consumption	-1294.61 [593.08, 1996.14]	99	**<0.01**
	AA *vs* GA	FSH consumption	-1014.36 [364.11, 1664.61]	99	**<0.01**
	GA *vs* GG	FSH consumption	-277.84 [-589.60, 1145.28]	100	0.53

Table 3C Worldwide distribution, pathogenic mechanism and clinical effects of SNPs significantly related to COS outcome

Gene	*refSNP*	*Che*	*DNA nucleotide*	*Ancestral allele*	*Amino acid and allele*	*Worldwide distribution*	*Protein*	*Pathogenic mechanism*	*Clinical effect*
FSHR	rs6165	2	c.919 G>A	G	A = Asn = N G = Ser = S	G allele shows similar distribution of rs6166 with exception of African population (African ancestry in Southwest USA, Kenya, Nigeria)	T307A	Greater in vivo resistance to FSH activity	Higher FSH basal levels Highest amount of FSH required during COS
FSHR	rs6166	2	c.2039 G>A	A	A = Thr = T G = Ala = A	G allele is highly prevalent in North-Western Pakistan, Siberia, Mato Grosso, (Brazil) and Oceania	N680S	Greater in vivo resistance to FSH activity	Higher FSH basal levels Highest amount of FSH required

									during COS
FSHR	rs1394205	2	c.-29 G>A	G	/	A allele highly prevalent in African population and Japanise and Iberian population (Spain)	/	A allele showed reduced transcriptional activity compared with G allele	Higher amount of FSH required during COS In allele A carriers
LHB	rs1800447	19	c.82 T>C	T	T = Trp = W C = Arg = R	C allele highly prevalent in Australian aboriginal and Finnish populations	W8R	Shorter half-life than wild type form	Higher amount of exogenous FSH required during COS
LHCGR	rs2293275	2	c. 935 A>G	A	A = Asn = N G = Ser = S	G allele highly expressed in Asian and African population	N312S	Impaired second messenger (cAMP) pathway	Higher ongoing pregnancy rate in SS carriers

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