

Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: a systematic review and meta-analysis

Carlo Alviggi^{1,2}, Alessandro Conforti ^{1,*}, Daniele Santi ³, Sandro C. Esteves ⁴, Claus Yding Andersen⁵, Peter Humaidan⁶, Paolo Chiodini⁷, Giuseppe De Placido¹ and Manuela Simoni³

¹Department of Neuroscience, Reproductive Science and Odontostomatology, University of Naples Federico II, 80131, Italy ²Istituto per l'Endocrinologia e l'Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli 80131, Italy ³Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, and Azienda Ospedaliera-Universitaria di Modena, Italy 41126 ⁴Androfert, Andrology and Human Reproduction Clinic, and Department of Surgery (Division of Urology), University of Campinas (UNICAMP), Campinas, SP 13075-460, Brazil ⁵Laboratory of Reproductive Biology, University Hospital of Copenhagen, Faculty of Health and Medical Sciences, Copenhagen 2100, Denmark ⁶Fertility Clinic, Skive Regional Hospital, Skive, Denmark, and Faculty of Health, Aarhus University, Aarhus 7800, Denmark ⁷Medical Statistics Unit, University of Campania "Luigi Vanvitelli", Naples 80138, Italy

*Correspondence address. E-mail: confale@hotmail.it  orcid.org/0000-0002-5676-0055

Submitted on October 30, 2017; resubmitted on May 5, 2018; editorial decision on May 9, 2018; accepted on May 12, 2018

TABLE OF CONTENTS

- Introduction
 - Methods
 - Protocol and registration
 - Eligibility criteria
 - Search strategy
 - Selection of studies
 - Data extraction
 - Risk of bias, summary measures and synthesis of the results
 - Quantitative analysis
 - Results
 - Study selection and study characteristics
 - Risk of bias within studies
 - Summary of results
 - Risk of bias across studies
 - Subgroup and sensitivity analyses
 - Discussion
 - Summary of evidence
 - Interpretation of results and clinical considerations
 - Limitations and strengths
 - Future research
 - Conclusion
-

BACKGROUND: Genotype has been implicated in the outcome of ovarian stimulation. The analysis of patient-specific genotypes might lead to an individualized pharmacogenomic approach to controlled ovarian stimulation (COS). However, the validity of such an approach remains to be established.

OBJECTIVE AND RATIONALE: To define the impact of specific genotype profiles of follicle-stimulating hormone, luteinizing hormone and their receptors (FSHR, LHR and LHCGR) on ovarian stimulation outcome. Specifically, our aim was to identify polymorphisms that could be useful in clinical practice, and those that need further clinical investigation.

SEARCH METHODS: A systematic review followed by a meta-analysis was performed according to the Cochrane Collaboration and Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines without time restriction. We searched the PubMed/MEDLINE, Cochrane Library, SCOPUS and EMBASE databases to identify all relevant studies published before January 2017. Only clinical trials published as full-text articles in peer-reviewed journals were included. The primary outcome was the number of oocytes retrieved.

OUTCOMES: Fifty-seven studies were assessed for eligibility, 33 of which were included in the qualitative and quantitative analyses. Data were independently extracted using quality indicators. COS outcomes related to seven polymorphisms (*FSHR* [rs6165], *FSHR* [rs6166], *FSHR* [rs1394205], *LHB* [rs1800447], *LHB* [rs1056917], *LHCGR* [rs2293275] and *LHCGR* [rs13405728]) were evaluated. More oocytes were retrieved from *FSHR* (rs6165) AA homozygotes (five studies, 677 patients, weighted mean difference [WMD]: 1.85, 95% CI: 0.85–2.85, $P < 0.001$; $I^2 = 0\%$) than from GG homozygotes and AG heterozygotes (four studies, 630 patients, WMD: 1.62, 95% CI: 0.28–2.95, $P = 0.020$; $I^2 = 56\%$). Moreover, stimulation duration was shorter in *FSHR* (rs6165) AA homozygotes than in AG carriers (three studies, 588 patients, WMD -0.48 , 95% CI: -0.87 to -0.10 , $P = 0.010$, $I^2 = 44\%$). A higher number of oocytes (21 studies, 2632 patients WMD: 0.84, 95% CI: 0.19 to 1.49, $P = 0.01$, $I^2 = 76\%$) and metaphase II oocytes (five studies, 608 patients, WMD: 1.03, 95% CI: 0.01–2.05, $P = 0.050$, $I^2 = 0\%$) was observed in AA than in GG homozygote carriers. FSH consumption was significantly lower in *FSHR* (rs1394205) GG homozygotes (three studies, 411 patients, WMD: -1294.61 IU, 95% CI: -593.08 to -1996.14 IU, $P = 0.0003$, $I^2 = 99\%$) and AG heterozygotes (three studies, 367 patients, WMD: -1014.36 IU, 95% CI: -364.11 to -1664.61 IU, $P = 0.002$, $I^2 = 99\%$) than in AA homozygotes.

WIDER IMPLICATIONS: These results support the clinical relevance of specific genotype profiles on reproductive outcome. Further studies are required to determine their application in a pharmacogenomic approach to ovarian stimulation.

Key words: assisted reproduction / ovarian stimulation / polymorphisms / FSH / gonadotrophins / LH / pharmacogenomics

Introduction

Ideally, a tailored approach to controlled ovarian stimulation (COS) in infertile patients would involve a comprehensive evaluation of the patient's characteristics, including genotype profile. Pharmacogenomics evaluates how genes influence individual responses to medication. Pharmacogenomic approaches appeared to 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., 2005). Nonetheless, increasing evidence indicates that specific genetic characteristics of gonadotrophins and their receptors could influence the ovarian response to exogenous gonadotrophins. Specifically, a common single nucleotide polymorphism (SNP) of the FSH receptor (*FSHR*, rs6166) has been associated with increased FSH consumption during COS (Yao et al., 2011). It has also been associated with increased basal levels of FSH, which suggests an impaired response to both endogenous and exogenous gonadotrophins (Perez Mayorga et al., 2000; Behre et al., 2005; Simoni and Casarini, 2014; Alviggi et al., 2016a). Moreover, a *FSHR* polymorphism at position -29 (*FSHR*, rs1394205) was found to be associated with a poor ovarian response (Achrekar et al., 2009b). Similarly, a suboptimal response to IVF was observed in SNP carriers of the gene encoding the LH beta subunit (Alviggi et al., 2011, 2013). Recently, LH receptor SNPs (*LHCGR*, rs2293275 and *LHCGR*, rs12470652) were reported to affect COS and ART (O'Brien et al., 2013; Lindgren et al., 2016; Alviggi et al., 2016b). These findings prompted the hypothesis that a 'hypo-response' to gonadotrophin therapy could be related to specific genotype characteristics (Alviggi et al., 2016a).

Contrary to poor-responders, 'hypo-responders' have a good prognosis for ART in terms of basal characteristics and ovarian reserve, but require a higher-than-expected dose of gonadotrophins and more prolonged stimulation 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 gonadotrophins and their receptors on the outcome of COS.

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-analysis and provide its checklist in the Supplementary material. 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

We used the PICO (Patients, Intervention, Comparison and Outcomes) model to select our study population. We included only women who underwent COS, and evaluated COS outcomes according to individual genotype expression (Supplementary Table I).

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', 'intra-uterine 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' and '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

Two reviewers (A.C. and D.S.) independently evaluated titles and abstracts. Duplications were removed using Endnote online software and manually. Disagreements were resolved by discussion among authors, and if required, with the involvement of the most experienced authors (C.A., S.E., C.Y.A, P.H, G.D. and M.S.). Only clinical trials published in peer-reviewed journals were evaluated. Case series, case reports, book chapters, congress abstracts and gray literature were not included.

Data extraction

Data were extracted independently by two reviewers (A.C. and D.S.) using predefined data fields, and study quality indicators. Discrepancies were resolved by discussion with the senior authors (C.A., S.E., C.Y.A., P.H, G.D. and M.S.).

Risk of bias, summary measures and synthesis of the results

The risk of bias and the quality of the studies included in this meta-analysis were evaluated. Two authors (A.C. and D.S.) independently assessed the risk bias of each study. The senior authors (C.A., S.E., C.Y.A., P.H., G.D. and M.S.) resolved conflicts. The Newcastle–Ottawa scale (NOS) score was used to evaluate the studies included, and judgment on each one was passed according to three issues: selection of the study group, comparability between groups and ascertainment of exposed/not exposed cohorts (Wells *et al.*, 2004).

The primary outcome was the number of oocytes retrieved. Secondary outcomes were: FSH consumption, stimulation duration (number of days of gonadotrophin use for COS), the number of metaphase II (MII) oocytes and ongoing pregnancy rate (OPR). OPR 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, 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 weighted mean difference (WMD) using the inverse variance method. When at least three studies were available, a meta-analysis was conducted using the fixed-effect model. The random-effect model was used in case of significant heterogeneity among studies. Heterogeneity was assessed using the percentage of total

variation in the estimated effect across studies (I^2). An I^2 value $> 50\%$ indicates substantial heterogeneity. P -values < 0.05 were considered statistically significant. We applied Bonferroni correction in case of statistical significance. Subgroup analysis by type of exogenous FSH (i.e. recombinant versus urinary) was conducted to assess potential sources of heterogeneity in the number of oocytes retrieved. We also evaluated differences in FSH basal level in relation to *FSHR* 919 G>A (rs6165) and *FSHR* 2039 G>A (rs6166) genotype distribution. Sensitivity analysis was carried out to assess the leverage of studies with a low risk of bias ($NOS \geq 6$) on the results. For the primary outcome, an additive effect of genotypes was also tested. Using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria), we estimated Hedges' g and the corresponding SE for each study adopting a simulation approach based on a linear regression model (John *et al.*, 2018). In detail, to test the additive model pooled Hedges' g was computed with a fixed-effect model or random-effect model in case of significant heterogeneity among studies.

Results

Study selection and study characteristics

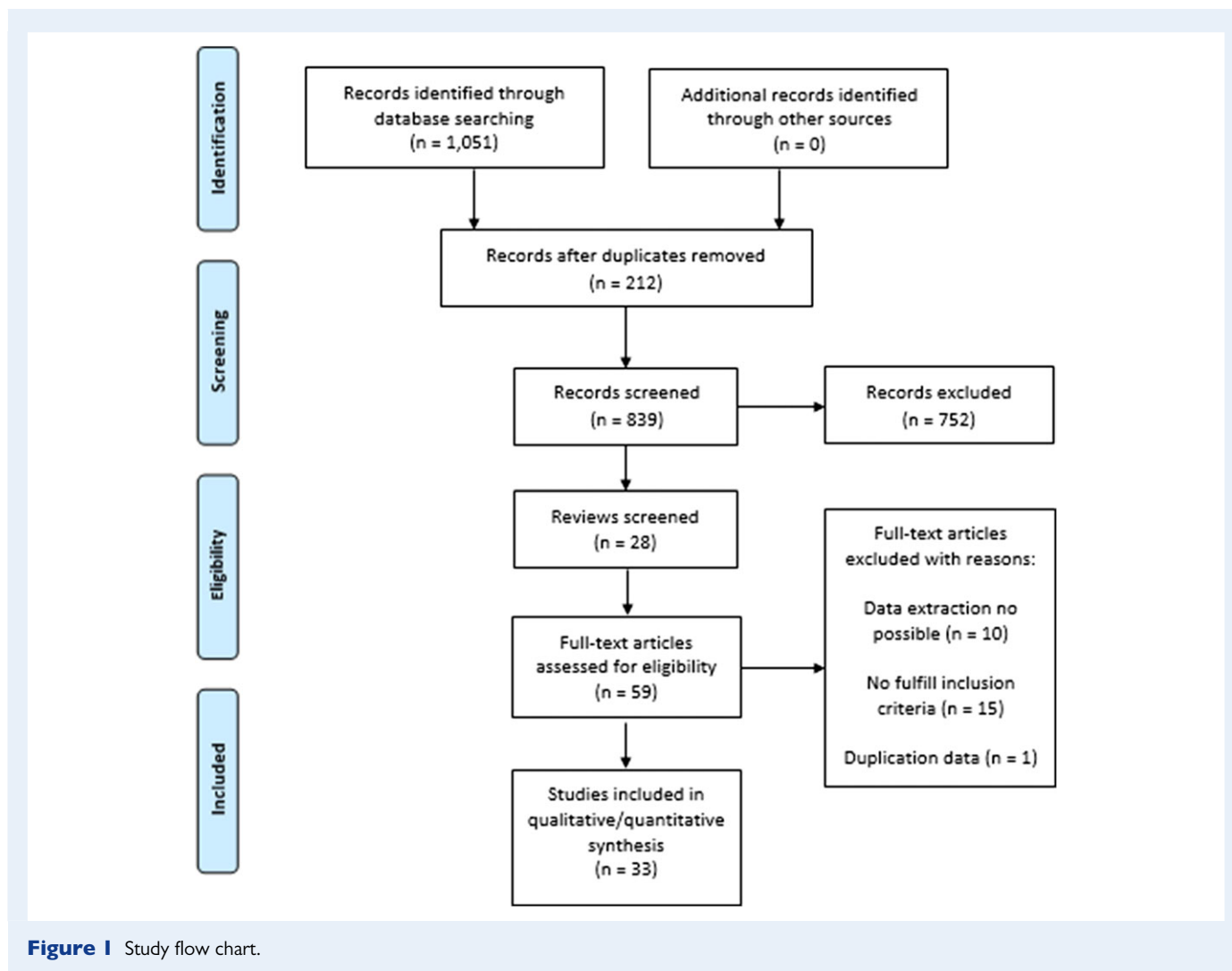
A total of 1051 items were identified (Fig. 1). After removing 167 duplicates using Endnote software (EndNote X 6.0.1, Thomson Reuters, USA, California State University) and 45 duplicates manually, the titles and abstracts of 839 paper were scrutinized. The reference lists of relevant reviews were hand-searched. Fifty-nine articles were assessed for eligibility. Fifteen articles were excluded because they did not fulfill the inclusion criteria. Data extraction was not possible in the case of 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 in relation to polymorphism genotype expression. Data duplication was detected in studies by Desai *et al.* (2011, 2013) and by Mohiyiddeen and collaborators (Mohiyiddeen *et al.*, 2013a; 2013b). Of these, we included two studies in our analysis (Desai *et al.*, 2011; Mohiyiddeen *et al.*, 2013b). We extracted data regarding MII oocytes from the Mohiyiddeen *et al.* (2013a) study that were not reported in their subsequent paper (Mohiyiddeen *et al.*, 2013b). Thirty-three studies were included in our quantitative and qualitative analysis (Fig. 1 and Table I). Seven polymorphisms were reported in these studies: *FSHR* 919 G>A (rs 6165), *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) and *LHCGR* 3442–25 260 A>G (rs13405728).

Risk of bias within studies

Bias assessment within studies is shown in Table I. A high rate of agreement, evaluated by k -Cohen calculation, was observed between the two authors (A.C. and D.S.; 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 II.



FSH consumption

A meta-analytic approach was possible only for *FSHR* (rs6165), *FSHR* (rs6166) and *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, $I^2 = 96\%$), and AG heterozygotes (Random WMD: 110.24 IU, 95% CI: -323.57 to 544.05 IU, $I^2 = 93\%$). Similarly, FSH consumption did not differ between GG homozygotes and AG heterozygotes (Random WMD: 134.09 IU, 95% CI: -162.06 to 430.25, $I^2 = 81\%$) (Supplementary Fig. S1).

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, 2015; Nordhoff et al., 2011; Sheikhha et al., 2011; Anagnostou et al., 2012; Genro et al., 2012; Lledo et al., 2013; Yan et al., 2013; Mohiyiddeen et al., 2013b; 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, $I^2 = 96\%$) and AG heterozygotes (Random WMD: 18.00 IU, 95% CI: -119.36 to 155.35 IU, $I^2 = 96\%$). 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, $I^2 = 86\%$) (Supplementary Fig. S2).

Three studies (Achrekar et al., 2009b; Desai et al., 2011; Tohlob et al., 2016), for a total of 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, $P < 0.001$, Bonferroni adjusted $P = 0.008$, $I^2 = 99\%$), whereas no differences were observed between GG and AG heterozygotes (Random WMD: -277.84 IU, 95% CI: -1145.28 IU to 589.60, $I^2 = 100\%$). FSH consumption was lower in AG heterozygotes than in *FSHR* AA homozygotes (Random WMD: -1014.36 IU, 95% CI: -1664.61 to -364.11, $P = 0.002$, Bonferroni adjusted $P = 0.006$, $I^2 = 99\%$) (Fig. 2).

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

Table 1 Characteristics and Newcastle–Ottawa scale score of studies included in a systematic review and meta-analysis of the impact of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation.

References	SNPs evaluated	Country	Number of patients	Mean age ± SD (years)	Study design	NOS score
Achrekar et al. (2009a)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	India	50	30.09 ± 1.50	Retrospective	7
Achrekar et al. (2009b)	<i>FSHR</i> (rs1394205)	India	150	NA	Retrospective	7
Alviggi et al. (2009)	<i>LHB</i> (rs1800447)	Italy	60	30.81 ± 3.39	Retrospective	6
Alviggi et al. (2013)	<i>LHB</i> (rs1800447)	Denmark	220	30.65 ± 3.95	Retrospective	6
Alviggi et al., 2016a	<i>FSHR</i> (rs6166)	Italy	42	30.57 ± 4.37	Retrospective	6
Anagnostou et al. (2012)	<i>FSHR</i> (rs6166)	Greece	109	35.00 ± 4.50	Prospective	6
Behre et al. (2005)	<i>FSHR</i> (rs6166)	Germany	93	33.10 ± 0.64	Prospective	7
Dan et al. (2015)	<i>FSHR</i> (rs1394205)	China	158	NA	Prospective	7
Davar et al. (2014)	<i>LHB</i> (rs1056917)	Iran	220	29.94 ± 5.98	Prospective	7
De Castro et al. (2003)	<i>FSHR</i> (rs6166)	Spain	102	33.70 ± 3.10	Retrospective	6
Desai et al. (2011)	<i>FSHR</i> (rs1394205)	India	100	33.11 ± 0.82	Retrospective	8
Genro et al. (2012)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	Brazil	124	34.95 ± 3.82	Prospective	8
Huang et al. (2015)	<i>FSHR</i> (rs6166)	China	1250	31.31 ± 3.34	Retrospective	6
Huang et al. (2010)	<i>FSHR</i> (rs6166)	China	136	30.33 ± 3.31	Prospective	6
Jun et al. (2006)	<i>FSHR</i> (rs6166)	South Korea	263	32.60 ± 0.40	Prospective	7
Klinkert et al. (2006)	<i>FSHR</i> (rs6166)	The Netherlands	105	36.90 ± 5.10	Prospective	6
Laven et al. (2003)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	Holland	148	28.20 ± 3.10	Prospective	6
Lazaros et al. (2013)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	Greece	604	NA	Retrospective	6
Lindgren et al. (2016)	<i>LHCGR</i> (rs2293275), <i>FSHR</i> (rs6166)	Denmark	384	31.92 ± 2.90	Prospective	8
Lledo et al. (2013)	<i>FSHR</i> (rs6166)	Spain	145	25.60 ± 3.80	Retrospective	6
Lledo et al. (2016)	<i>FSHR</i> (rs6166)	Spain	191	25.60 ± 3.90	Retrospective	6
Loutradis et al. (2006)	<i>FSHR</i> (rs6166)	Greece	125	30.30 ± 3.00	Retrospective	5
Mohiyiddeen et al. (2013a)	<i>FSHR</i> (rs6166)	UK	212	33.17 ± 3.50	Prospective	7
Mohiyiddeen et al. (2013b)	<i>FSHR</i> (rs6166)	UK	504	33.50 ± 3.70	Prospective	7
Nordhoff et al. (2011)	<i>FSHR</i> (rs6166)	Germany	22	32.40 ± 3.35	Retrospective	3
Perez Mayorga et al. (2000)	<i>FSHR</i> (rs6166)	Germany	161	32.60 ± 0.50	Prospective	6
Yin et al. (2015)	<i>LHCGR</i> (rs13405728)	China	236	NA	Prospective	6
Sheikhha et al. (2011)	<i>FSHR</i> (rs6166)	Iran	108	29.63 ± 4.70	Retrospective	6
Sudo et al. (2002)	<i>FSHR</i> (rs6166)	Japan	522	31.83 ± 0.77	Retrospective	5
Tohlob et al. (2016)	<i>FSHR</i> (rs1394205)	UK	559	33.23 ± 5.1	Retrospective	6
Trevisan et al. (2014)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	Italy	149	NA	Retrospective	5
Yan et al. (2013)	<i>FSHR</i> (rs6165), <i>FSHR</i> (rs6166)	China	450	32.15 ± 4.96	Retrospective	6
Zalewski et al. (2013)	<i>FSHR</i> (rs6166)	Poland	22	33.10 ± 5.00	Retrospective	3

NOS, Newcastle–Ottawa scale; *FSHR*, FSH receptor; *LHCGR*, LHCG receptor; SNP, single nucleotide polymorphism; SD, standard deviation; NA, not available.

One study (Lindgren et al., 2016) reported FSH consumption in relation to the distribution of the *LHCGR* (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). However, these results may be conservative given the high heterogeneity among trials and the relatively small number of patients evaluated.

Stimulation duration

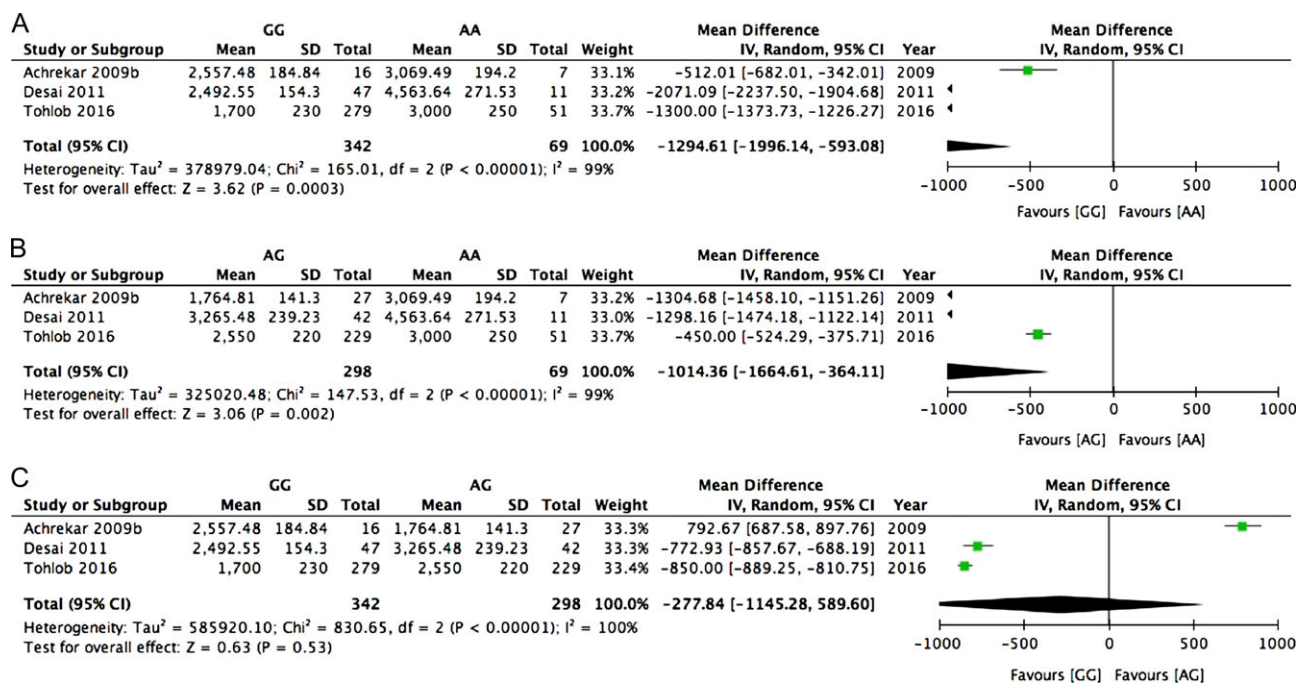
A meta-analytic approach regarding stimulation duration was possible only for *FSHR* (rs6165) and *FSHR* (rs6166). No data on the other polymorphisms were found.

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 duration of stimulation did not differ between *FSHR* AA homozygotes and GG homozygotes (Random WMD: −0.59, 95% CI: −1.24 to 0.05, $I^2 = 60\%$), however it was significantly shorter in AA than in AG heterozygotes (Fixed WMD: −0.48, 95% CI: −0.87 to −0.10,

Table II Pooled effect estimates including only FSH receptor 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 versus GG	Stimulation duration	-0.59 [-1.24, 0.05]	60	NS
		Number of oocytes	1.85 [0.85, 2.85]	0	0.008*
	AA versus AG	Stimulation duration	-0.48 [-0.87, -0.10]	44	0.04*
		Number of oocytes	1.62 [0.28, 2.95]	56	0.052*
	AG versus GG	Stimulation duration	-0.29 [-0.95, 0.37]	0	NS
		Number of oocytes	-0.37 [-1.51, 0.78]	18	NS
FSHR (rs6166)	AA versus GG	Number of oocytes	0.84 [0.19, 1.49]	76	0.03*
		Number of MII oocytes	1.03 [0.01, 2.05]	0	NS
	AA versus AG	Number of oocytes	0.18 [-0.84, 0.48]	85	NS
		Number of MII oocytes	0.79 [-0.05, 1.62]	0	NS
	AG versus GG	Number of oocytes	0.88 [0.12, 1.63]	76	0.04*
		Number of MII oocytes	0.34 [-0.57, 1.26]	49	NS
FSHR (rs1394205)	GG versus AA	FSH consumption	-1294.61 [-1996.14, -593.08]	99	0.008*
	AA versus AG	FSH consumption	-1014.36 [-1664.61, -364.11]	99	0.006*
	AG versus GG	FSH consumption	-277.84 [-1145.28, 589.60]	100	NS

NS, not significant; *Bonferroni adjusted P-value.

**Figure 2** Forest plots of differences among FSHR (rs1394205) genotype carriers in relation to FSH consumption. (A) (rs1394205) G homozygotes versus A homozygotes. (B) (rs1394205) heterozygotes versus A homozygotes. (C) (rs1394205) G homozygous versus heterozygotes.

$P = 0.01$, Bonferroni adjusted $P = 0.04$, $I^2 = 44\%$). 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 , $I^2 = 0\%$) (Fig. 3).

Fifteen studies (De Castro et al., 2003; Laven et al., 2003; Behre et al., 2005; Klinkert et al., 2006; Loutradis 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., 2016a) for a total of 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, $I^2 = 17\%$) and AG heterozygotes (Fixed WMD: 0.01 , 95% CI: -0.04 to 0.05 , $I^2 = 27\%$). Lastly, no differences were observed between FSHR GG homozygotes and FSHR AG heterozygotes (Fixed WMD: -0.12 , 95% CI: -0.29 to 0.04 , $I^2 = 2\%$) (Supplementary Fig. S3).

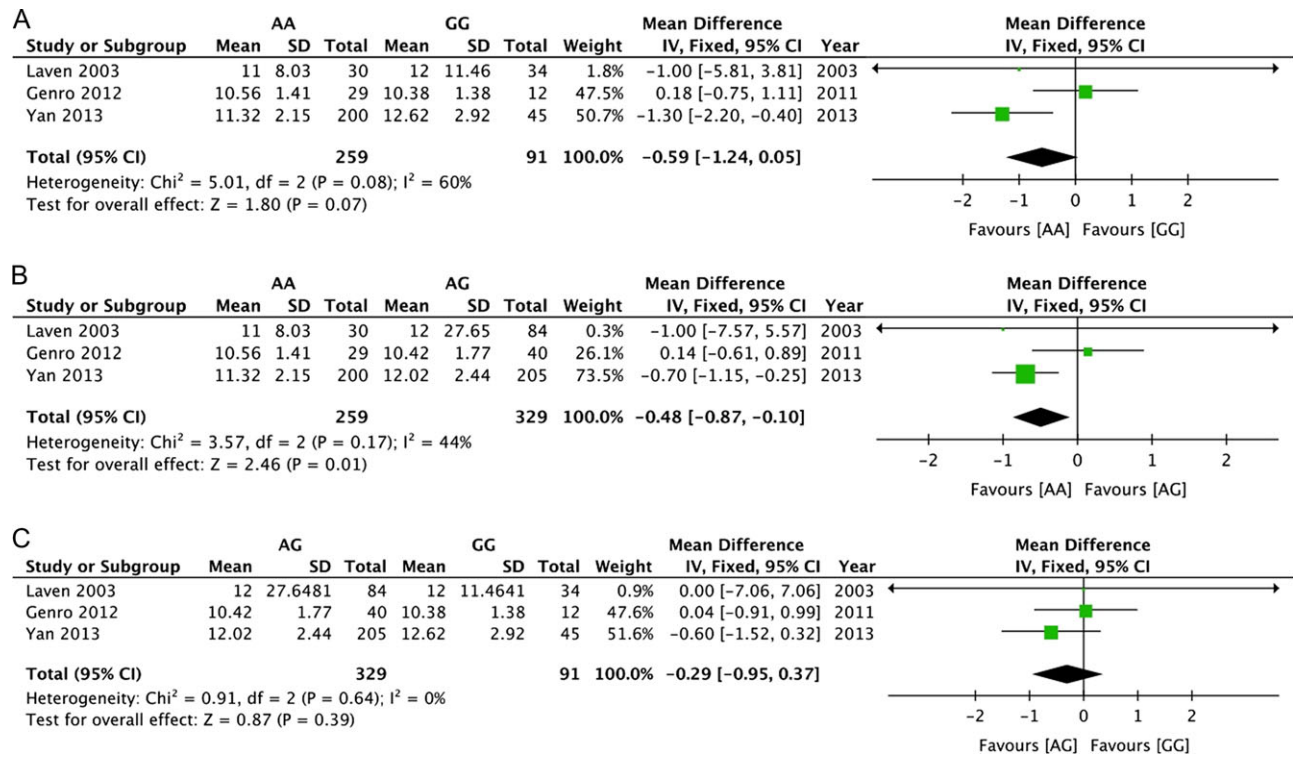


Figure 3 Forest plots of differences among *FSHR* (rs6165) genotype carriers in relation to stimulation duration. **(A)** (rs6165) A homozygotes versus G homozygotes. **(B)** (rs6165) A homozygotes versus heterozygotes. **(C)** (rs6165) heterozygous versus G homozygotes.

In summary, the only difference observed was a shorter duration of stimulation 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), for a total of 1020 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 either GG homozygotes (Fixed WMD: 1.85, 95% CI: 0.85–2.85, $P < 0.001$, Bonferroni adjusted $P = 0.008$, $I^2 = 0\%$). No difference was detected among AG heterozygotes, GG homozygotes (Fixed WMD: -0.37, 95% CI: -1.51 to 0.78, $I^2 = 18\%$) and AA homozygotes (Random WMD: 1.62, 95% CI: 0.28–2.95, $P = 0.02$, Bonferroni adjusted $P = 0.052$, $I^2 = 56\%$) (Fig. 4). The additive model was marginally significant in the association between *FSHR* (rs6165) and number of oocytes retrieved (pooled Hedges' $g = -0.129$; Fixed: 95% CI -0.258 to 0.000, $P = 0.05$, $I^2 = 43\%$).

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, 2015; Nordhoff *et al.*, 2011; Sheikhha *et al.*, 2011; Genro *et al.*, 2012; Lazaros *et al.*, 2013; Lledo *et al.*, 2013, 2016; Yan *et al.*, 2013; Zalewski *et al.*, 2013; Mohiyiddeen *et al.*, 2013b; Trevisan

et al., 2014; Alviggi *et al.*, 2016a) including 4425 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: 0.19–1.49, $P = 0.01$, Bonferroni adjusted $P = 0.03$, $I^2 = 76\%$), but it was similar to that retrieved from AG heterozygotes (Random WMD: -0.18, 95% CI: -0.84 to 0.48, $I^2 = 85\%$). The number of oocytes was significantly higher in AG heterozygotes than in GG homozygotes (Random WMD: 0.88, 95% CI: 0.12–1.63, $P = 0.02$, Bonferroni adjusted $P = 0.04$, $I^2 = 76\%$) (Fig. 5). The additive model was not significant for the association between *FSHR* (rs6165) and number of oocytes retrieved (pooled Hedges' $g = -0.072$, 95% CI -0.179 to 0.035, $P = 0.19$, $I^2 = 65\%$).

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, albeit not significantly lower, in *FSHR* (rs1394205) AA homozygotes than in either GG homozygotes (Random WMD: -5.20, 95% CI: -11.22 to 0.82, $I^2 = 99\%$) or AG heterozygotes (Random WMD: -3.88, 95% CI: -7.93 to 0.18, $I^2 = 98\%$). No differences were observed between GG homozygotes and AG heterozygotes (Random WMD: -1.29, 95% CI: -3.51 to 0.93, $I^2 = 97\%$) (Supplementary Fig. S4).

Two studies (Alviggi *et al.*, 2011; Alviggi *et al.*, 2013) reported the number of oocytes retrieved in relation to the distribution of the *LHB* (rs1800447) genotype. In both studies, the number of oocytes retrieved did not differ among genotypes.

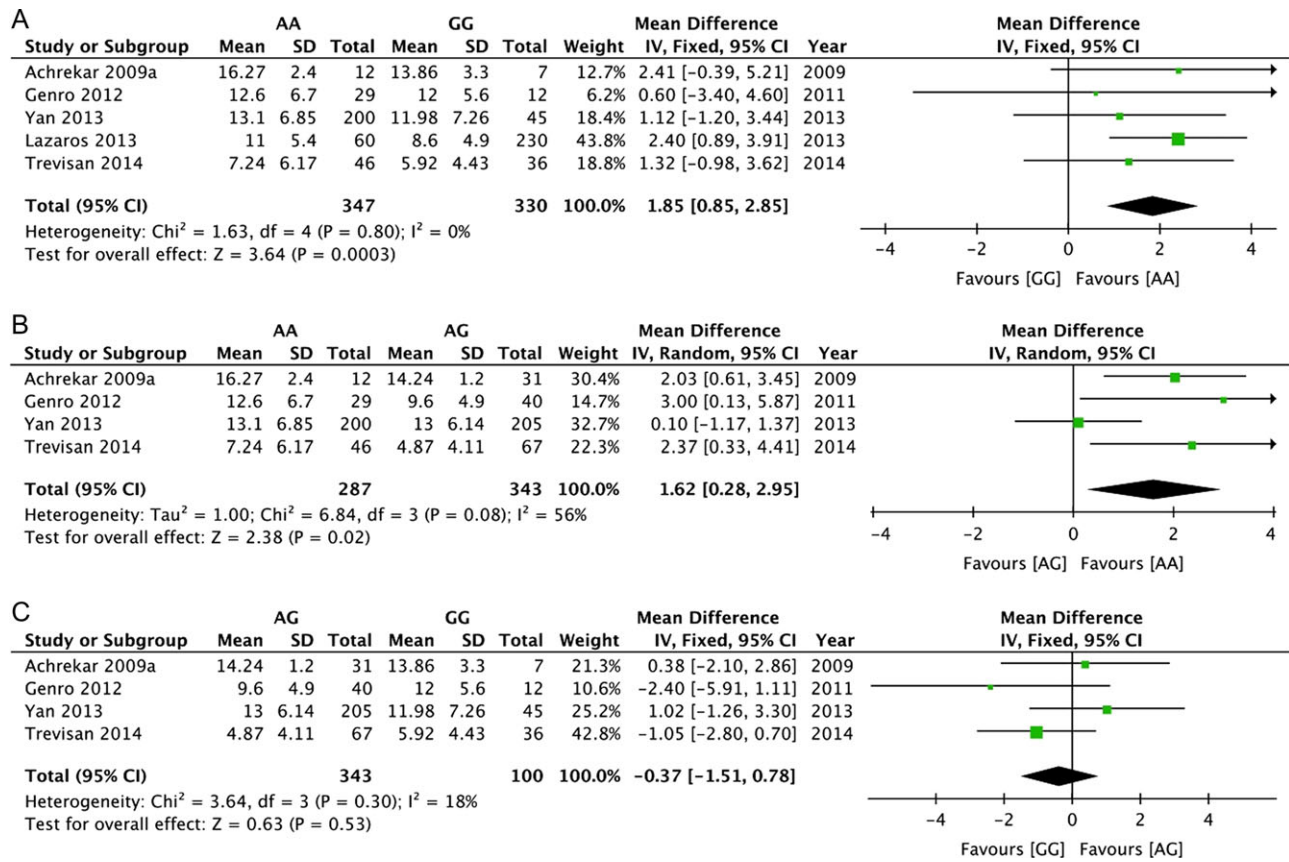


Figure 4 Forest plots of differences among *FSHR* (rs6165) genotype carriers in relation to the number of oocytes retrieved. (A) (rs6165) A homozygotes versus G homozygotes. (B) (rs6165) A homozygotes versus heterozygotes. (C) (rs6165) heterozygous versus G homozygotes.

Only one study (Davar et al., 2014) reported the number of oocytes retrieved in relation to the *LHB* (rs1056917) genotype, and no significant differences among genotypes were observed.

Similarly, only one study (Yin et al., 2015) reported the number of oocytes retrieved in relation to the distribution of the *LHCGR* (rs13405728) genotype, and there were no significant differences among genotypes.

The overall effect size indicates that the *FSHR* (rs6165) and *FSHR* (rs6166) genotypes impacted on the number of oocytes retrieved. In both cases, AA homozygosity was associated with a higher number of oocytes retrieved, whereas GG homozygotes exerted an opposite effect. The effect size estimated for the *FSHR* (rs6166) genotype may be conservative because of the high heterogeneity.

Number of MII oocytes

A meta-analytic approach was possible only in the case of *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 in relation to *FSHR* (rs6165). In both studies, the number of MII oocytes did not differ among genotypes.

Five studies (Genro et al., 2012; Mohiyiddeen et al., 2013a; Trevisan et al., 2014; Lindgren et al., 2016; Lledó et al., 2016) including 1185 patients, reported the number of MII oocytes retrieved in relation to the distribution of the *FSHR* (rs6166) genotype. The number of MII oocytes was higher in AA homozygotes than in GG homozygotes but the differences did not reach statistical significance after Bonferroni correction (Fixed WMD: 1.03, 95% CI: 0.01–2.05, $P = 0.05$, Bonferroni adjusted $P = 0.14$, $I^2 = 0\%$). No significant differences were observed between AA homozygotes and AG heterozygotes (Fixed WMD: 0.79, 95% CI: -0.05 to 1.62, $I^2 = 0\%$), or between GG homozygotes and AG heterozygotes (Fixed WMD: 0.34, 95% CI: -0.57 to 1.26, $I^2 = 49\%$) (Supplementary Fig. S5).

Only two studies (Desai et al., 2011; Dan et al., 2015) reported the number of MII oocytes in relation to the distribution of the *FSHR* (rs1394205) genotype. In detail, Dan et al. (2015) observed a significantly higher number of MII oocytes in GG carriers than in AG/AA carriers. Similarly, Desai et al. (2011) found a significantly higher number of MII oocytes in GG carriers than in AG and AA carriers.

The only study to report the number of MII oocytes retrieved in relation to the *LHB* (rs1800447) genotype did not find any difference between wild-type and variant carriers (Alviggi et al., 2013). Similarly, the only study to report the number of MII oocytes retrieved in

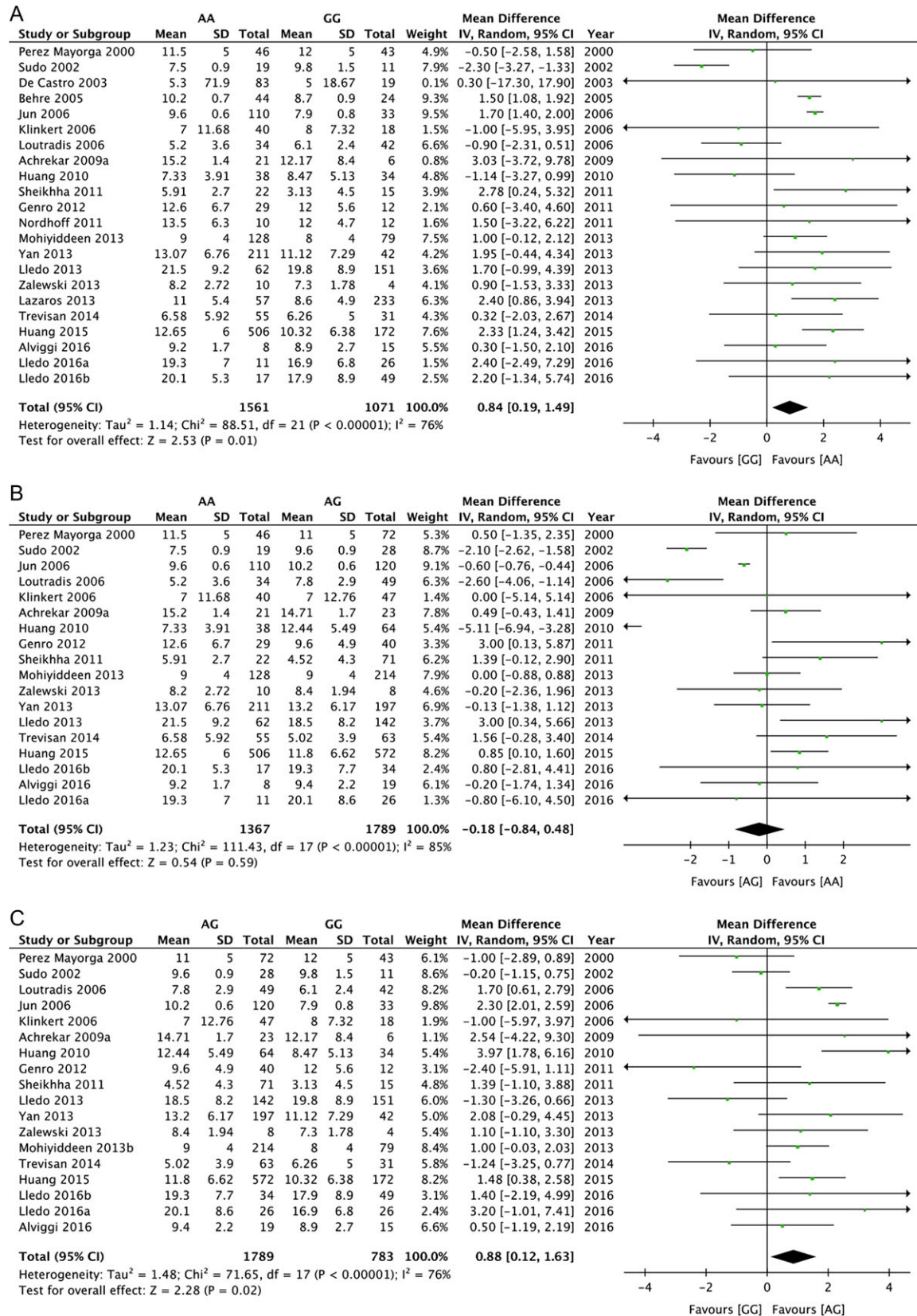


Figure 5 Forest plots of differences among *FSHR* (rs6166) genotype carriers in relation to the number of oocytes retrieved. **(A)** (rs6166) A homozygotes versus G homozygotes. **(B)** (rs6166) A homozygotes versus heterozygotes. **(C)** (rs6166) heterozygotes versus G homozygotes.

relation to the *LHCGR* (rs2293275) genotype did not find any difference among the haplotypes identified (Lindgren et al., 2016). The only study to report the number of MII oocytes retrieved in relation to the distribution of the *LHCGR* (rs13405728) genotype did not find any differences among genotypes (Yin et al., 2015).

The overall effect size indicates that the *FSHR* (rs6166) genotype did not significantly affect the number of mature oocytes retrieved. However, given the limited number of studies available, these observations should be viewed with caution.

OPR

A meta-analytic approach to OPR was possible only for *FSHR* (rs6166). No data were found regarding the *FSHR* (rs6165), *LHB* (rs1056917) or *LHCGR* (rs13405728) genotypes.

Seven studies (Jun et al., 2006; Sheikhha et al., 2011; Lledo et al., 2013; Mohiyiddeen et al., 2013b; Huang et al., 2015; Lindgren et al., 2016; Alviggi et al., 2016a) including 3191 patients, evaluated OPR in relation to the distribution of the *FSHR* (rs6166) genotype. The overall OR did not differ among AA homozygotes, GG homozygotes (Fixed OR: 0.89, 95% CI: 0.70–1.12, $I^2 = 0\%$) and AG heterozygotes (Fixed OR: 0.97, 95% CI: 0.82–1.16, $I^2 = 29\%$). Moreover, no significant differences were observed between GG homozygotes and AG heterozygotes (Fixed OR: 0.95, 95% CI: 0.77–1.18, $I^2 = 0\%$) (Supplementary Fig. S6).

Only two studies (Achrekar et al., 2009b; Tohlob et al., 2016) reported OPR in relation to *FSHR* (rs1394205). Achrekar et al. (2009b) reported a comparable OPR among GG, AG and AA carriers, whereas Tohlob et al. (2016) reported a higher OPR in women

carrying the A allele than in those carrying the G allele (crude OR 1.32, 95% CI 1.01–1.74, $P = 0.04$), but this association was not significant when adjusted for the number of embryos transferred.

The only study to report OPR in relation to *LHB* (rs1800447) did not find any difference between wild-type and variant carriers (Alviggi et al., 2013).

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

Risk of bias across studies

The risk of significant bias across studies regarding the primary outcome was rejected by Egger's test ($P = 0.828$ for *FSHR* rs6166; $P = 0.27$ for *FSHR* rs6165, and $P = 0.12$ for *FSHR* rs1394205), visual inspection of the funnel plots, and the trim and fill method (Supplementary Fig. S7).

Subgroup and sensitivity analyses

We estimated the number of oocytes retrieved according to type of gonadotrophin, namely recombinant versus urinary FSH (Fig. 6). We did not include papers in which both gonadotrophins were 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). While a higher number of oocytes were retrieved in AA *FSHR* (rs6166) carriers than in GG carriers when recombinant FSH was used (Fixed WMD 1.15, 95% CI 0.53–1.76, $P = 0.0003$, Bonferroni

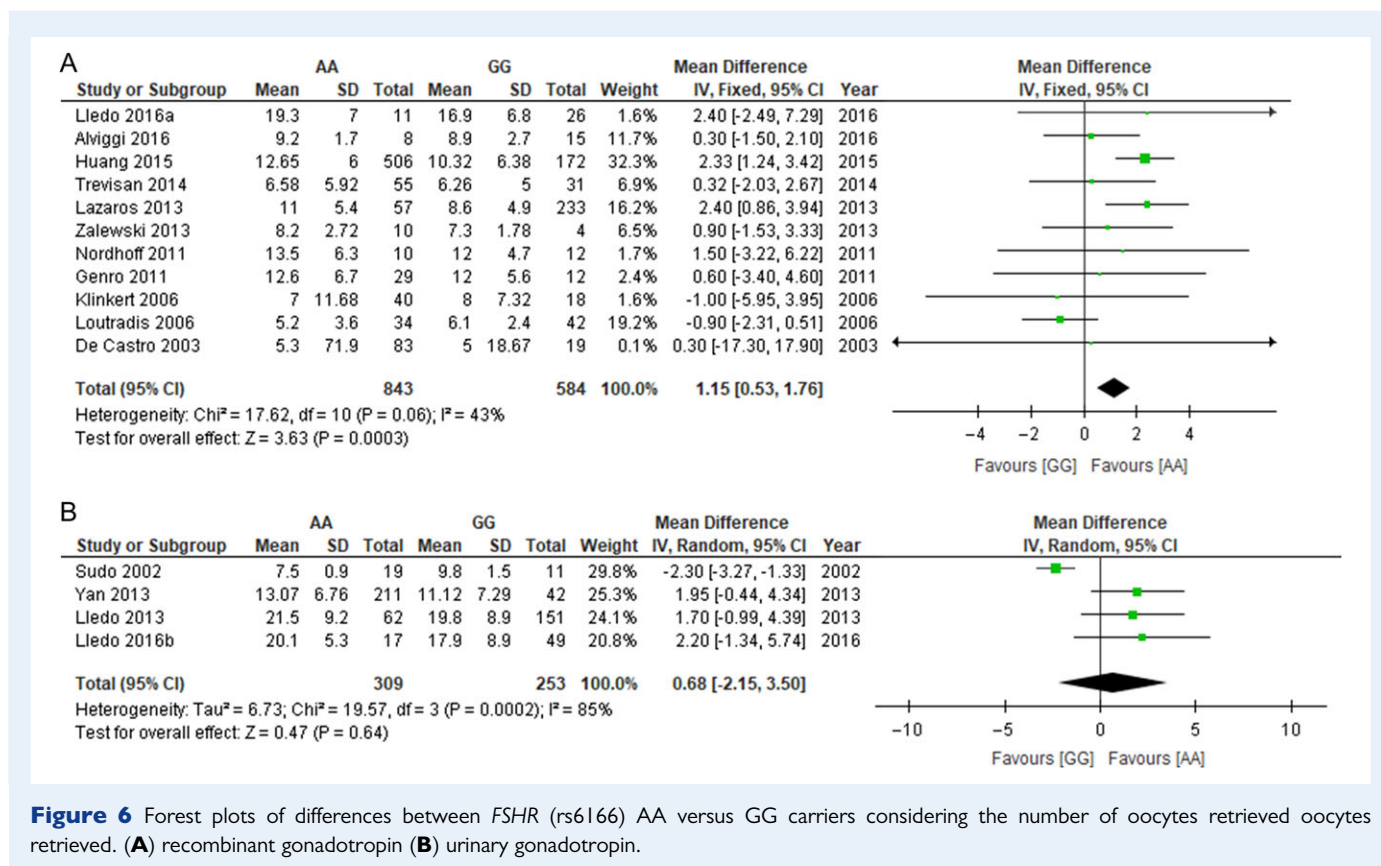


Figure 6 Forest plots of differences between *FSHR* (rs6166) AA versus GG carriers considering the number of oocytes retrieved oocytes retrieved. (A) recombinant gonadotropin (B) urinary gonadotropin.

adjusted 0.0009, $I^2 = 43\%$) no differences were observed when urinary gonadotrophin was used (Random WMD 0.68, 95% CI: -2.19 to 3.54 ; $I^2 = 86\%$). Concerning the *FSHR* (rs6165) genotype, FSH basal levels did not differ among haplotypes after Bonferroni correction (Supplementary Fig. S8). On the other hand, FSH basal levels were significantly lower in AA than in GG *FSHR* (rs6166) carriers (Fixed WMD -0.54 , 95% CI -0.72 -0.36 , $P < 0.00001$, Bonferroni adjusted $P < 0.0001$, $I^2 = 21\%$) (Fig. 7).

Lastly, sensitivity analysis revealed that the pooled effect sizes were affected only with regard to the number of retrieved oocytes between *FSHR* (rs6165) AA and AG carriers (Supplementary Table II).

Discussion

Summary of evidence

We conducted this systematic review in the attempt to unravel the role of gene polymorphisms of gonadotrophins and their receptors on the outcome of COS. We evaluated OPR rather than live birth rate because the many features that can affect the later stages of pregnancy may confound the impact of folliculogenesis-related polymorphisms. Our findings indicate that *FSHR* 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 ovarian stimulation was significantly shorter in these patients than in GG and AG carriers. Similarly, the number of oocytes retrieved was significantly higher in *FSHR* (rs6166) AA carriers than in GG carriers. Although neither polymorphism had an additive effect, a significant impact on the number of oocytes was observed under co-dominance (AA versus GG and AG versus GG) for *FSHR* rs6166 and only under homozygote models (AA versus GG) for *FSHR* rs6165. Therefore, both *FSHR* polymorphisms seem to influence responsiveness to COS treatment.

Gonadotrophin type seems to affect the number of oocytes retrieved in relation to *FSHR* (rs6166) genotype distribution. In fact, the number of oocytes retrieved was significantly higher in AA carriers than in GG carriers when recombinant FSH was used but not when urinary FSH was used. *FSHR* (rs6166) also affects endogenous levels of FSH as shown by the finding of higher plasma FSH values in GG carriers than in AA carriers. Lastly, the *FSHR* (rs6166) genotype did not significantly affect OPR.

The results of our review of gonadotrophins and their receptor polymorphisms conducted with a quantitative approach are consistent with those reported in qualitative reviews (Altmæ et al., 2011). However, it remains to be determined whether a pharmacogenomic approach could counteract the effect of such polymorphisms. Behre et al. (2005) partially addressed this issue by stratifying normogonadotrophic patients according to the distribution of the *FSHR* (rs6166) genotype, and found that increasing daily FSH dose from 150 to 225 IU/day counteracted the lower estradiol levels in GG carriers.

Given the paucity of data, we were unable to carry out a meta-analysis of remnant gonadotrophins and their receptor polymorphisms [LHB (rs1800447), LHB 1502 G>A (rs1056917), LHCGR 935 A>G (rs2293275) and LHCGR 3442–25 260 A>G (rs13405728)].

In summary, our meta-analysis demonstrates that specific polymorphisms of gonadotrophins and their receptors modulate the ovarian response to exogenous FSH. On the other hand, further studies are required to evaluate the impact of these polymorphisms on OPR and live birth rate. In this context, it is noteworthy that ART births are greatly influenced by various factors, most of which occur during the late stages of pregnancy and transcend the 'physiological' effects of gonadotrophins and their receptors. In other words, we maintain that the ovarian response is more reliable than pregnancy rate in determining the effect of gonadotrophins and their receptor polymorphisms on COS.

Interpretation of results and clinical considerations

Our findings can be related to the molecular characteristics of the genotypes associated with the response to COS (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 response to COS. 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 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 do AA carriers (Casarini et al., 2014, 2015). Our findings corroborate these previous observations. Indeed, we found that GG *FSHR* (rs6166) carriers had higher ovarian resistance to exogenous gonadotrophin and consequently had fewer oocytes compared with AA carriers. Moreover, we demonstrate that such *FSHR* resistance involves also endogenous FSH levels, as reported elsewhere (Fig. 7) (Mohiyiddeen and Nardo, 2010). These effects corroborate the potential impaired function of *FSHR* in G allele carriers and could explain why GG allele carriers have an impaired prognosis to COS as fewer oocytes are collected in these patients. *FSHR* function is controlled in both men and women by another FSH beta subunit (rs10835638) polymorphism (Grigорова et al., 2010; Ferlin et al., 2011; La Marca et al., 2013), which is significantly correlated with FSH beta subunit transcriptional activity and metabolism (Hoogendoorn et al., 2003). There is also evidence that *FSHR* (rs6166) could interact with 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 *FSHR* (rs1394205) GG plus *FSHR* (rs6166) AA genotypes than in other genotype combinations of these polymorphisms (Desai et al., 2013).

The finding of Borgbo et al. (2015) that *FSHR* (rs6166) and *FSHR* (rs6165) GG carriers had higher *LHCGR* gene expression but lower anti-Müllerian hormone (AMH) receptor-2 expression versus carriers of other haplotypes suggested that these polymorphisms could affect the protein expression of human antral follicles. Nonetheless, it remains to be established whether *FSHR* (rs6166) and *FSHR* (rs6165) affect *FSHR* protein expression.

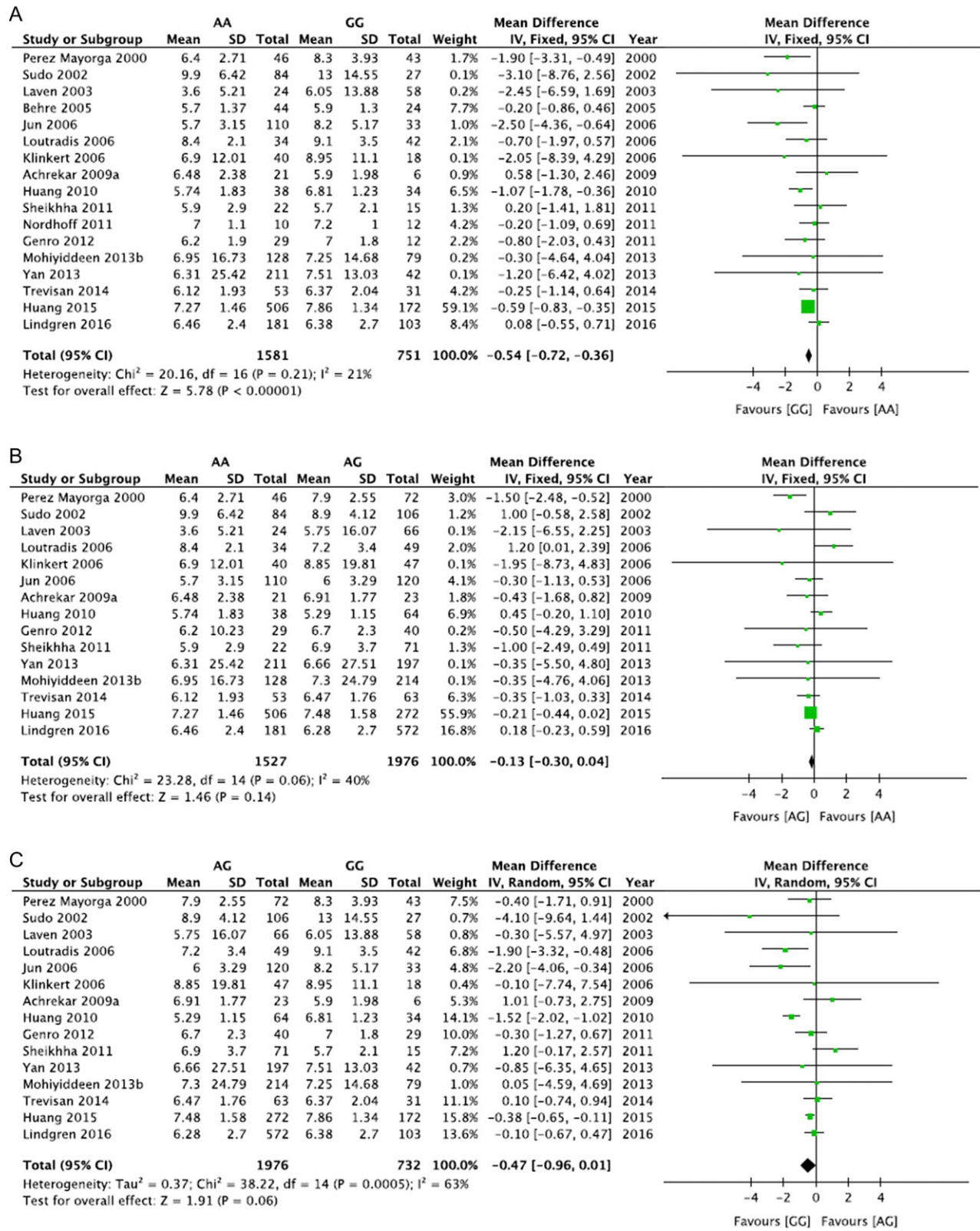


Figure 7 Forest plots of differences among *FSHR* (rs6166) genotype carriers considering FSH basal levels. **(A)** (rs6166) A homozygotes versus G homozygotes. **(B)** (rs6166) A homozygotes versus heterozygotes **(C)** (rs6166) heterozygotes versus G homozygotes.

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

Gene	refSNP	Chromosome	DNA nucleotide	Ancestral allele	Amino acid and allele	Worldwide distribution	Protein	Pathogenic mechanism	Clinical effect
FSHR	rs6166	2	c.919 G>A	G	A = Asn = N G = Ser = S	G allele is highly prevalent in North-Western Pakistan, Siberia, Mato Grosso, (Brazil) and Oceania (Simoni and Casarini, 2014)	N680S	Greater in vivo resistance to FSH activity Casarini et al. (2014)	Higher FSH basal levels in G carriers Perez Mayorga et al. (2000); Huang et al. (2015) Highest amount of FSH required during COS in G carriers
FSHR	rs6165	2	c.2039 G>A	A	A = Thr = T G = Ala = A	G allele shows a similar distribution of rs6166 with exception of African population (African ancestry in Southwest USA, Kenya, Nigeria) (Simoni and Casarini, 2014)	T307A	Greater in vivo resistance to FSH activity Simoni and Casarini (2014)	Higher FSH basal levels Yan et al. (2013) Highest amount of FSH required during COS in T carriers Achrekar et al. (2009a)
FSHR	rs1394205	2	c.-29 G>A	G	/	A allele highly prevalent in African population and Central South Asia population Simoni and Casarini (2014)	/	A allele showed reduced transcriptional activity compared with G allele (Nakayama et al., 2006)	Higher amount of FSH required during COS In allele A carriers Achrekar et al. (2009b)
LHB	rs1800447	19	c.82 T>C	T	T = Trp = W C = Arg = R	C allele highly prevalent in Australian aboriginal and Finnish populations (Nilsson et al., 1998)	W8R	Shorter half-life than wild-type form (Haavisto et al., 1995)	Higher amount of exogenous FSH required during COS Alviggi et al. (2011); Alviggi et al. (2013)
LHCGR	rs2293275	2	c. 935 A>G	A	A = Asn = N G = Ser = S	G allele highly expressed in Asian and Ethiopian population (ALFRED database https://alfred.med.yale.edu/alfred/mvograph.asp?siteuid=SI323604S)	N312S	Impaired second messenger (cAMP) pathway Lindgren et al. (2016)	Higher ongoing pregnancy rate in SS carriers Lindgren et al. (2016)

COS, controlled ovarian stimulation.

Despite the linkage disequilibrium between *FSHR* (rs6165) and *FSHR* (rs6166), we decided to report the two polymorphisms separately for two reasons. Firstly, as stated above, the linkage disequilibrium between them is not universal (Simoni and Casarini, 2014). Secondly, they seem to be associated with different COS outcomes. For instance, Achrekar et al. (2009a) detected significant differences in terms of total FSH consumption among *FSHR* (rs6165) genotypes but not among *FSHR* (rs6166) genotypes. In addition, Trevisan et al. (2014) observed that only the *FSHR* (rs6165) genotype influences the number of embryos produced. Thus, these studies suggest that, although in linkage disequilibrium, these two polymorphisms could influence COS outcome in a different way.

The *FSHR* (rs1394205) polymorphism located in the 5'-untranslated region of the gene has been extensively studied in association with ovarian response. In Chinese hamster ovary cells, the transcription activity of *FSHR* was significantly lower in A allele carriers than in G allele carriers (Nakayama et al., 2006). In another study, even the expression of *FSHR* was significantly lower in AA than in GG carriers (Desai et al., 2011). Furthermore, the relative level of protein expression and membrane receptor expression in cumulus cells was significantly lower in AA carriers than in carriers of other haplotypes (Desai et al., 2011). Consequently, basic science evidence suggests that the postulated role of *FSHR* (rs1394205) is more relevant in modulating *FSHR* protein function versus *FSHR* (rs6166) and *FSHR* (rs6165). At clinical level, we observed that *FSHR* (rs1394205) AA carriers have a higher FSH consumption in COS than carriers of the GG and AG haplotypes, and therefore, AA carriers may have an impaired response to ovarian stimulation.

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 gonadotrophin receptor genotype is relatively small. Third, it was not possible to evaluate the effect of alternate alleles on our findings because most trials did not evaluate more than one SNP simultaneously. Fourth, the studies included in our review were highly heterogeneous. This could be probably explained by the wide variation in terms of populations and treatment strategies. Lastly, OPRs were inconsistently reported in the included studies, however, we were able to conduct a meta-analysis for OPR with regard to the *FSHR* (rs6166), involving an elevated number of observations (over 3000 patients). We used several strategies to overcome these limitations. First, we applied random-effect model 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 a 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 treatment, thereby increasing the chance of ART success and reducing a potentially life-threatening excessive ovarian response.

Remarkably, no large randomized clinical trial on this topic has yet been conducted notwithstanding the relatively high number of studies published over the last 20 years. We believe that the pharmacogenomic approach to COS is still a largely neglected topic in the reproductive field. Furthermore, it is noteworthy that most of the polymorphisms reported in our review are widespread in the general population and in women with reproductive disorders (Nilsson et al., 1997; Alvigi et al., 2009, 2015; Simoni and Casarini, 2014), and that genotype analysis can now be provided at the same costs of other commonly used analyses (e.g. AMH, antral follicle count).

Conclusion

Our systematic review indicates that specific SNPs of the gonadotrophins and their receptors influence COS outcomes. This evidence is supported by a large number of trials mainly devoted to *FSHR* (rs6165) and *FSHR* (rs6166) polymorphisms. Our analysis shows that a higher FSH consumption is expected in homozygotes for the A allele of the *FSHR* (rs1394205) polymorphism than carriers of the G allele. Moreover, *FSHR* (rs6166) GG homozygotes seem to be less responsive to COS treatment. In fact, they have fewer oocytes than do AA and AG carriers. It is feasible that the effect of these polymorphisms on COS may partially explain the phenomenon of 'hypo-response' that has been reported in 10–15% of normogonadotrophic ART women (Alvigi et al., 2006; 2013). This peculiar ovarian response profile was recently included in the new classification of low prognosis women (Humaidan et al., 2016; Poseidon et al., 2016). Given the overall effect of gonadotrophins and their receptor SNPs on COS, a pharmacogenomic approach seems a promising strategy with which to improve the clinical management of infertile women candidates for COS.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Acknowledgements

The authors thank Jean Ann Gilder (Scientific Communication srl, Naples, Italy) for revising and editing the manuscript.

Authors' roles

C.A., A.C., D.S., S.E., C.Y.A., P.H., G.D. and M.S. participated in study design. C.A., A.C., D.S. and S.E. wrote the first draft of the manuscript; A.C., D.S. and P.C. performed the statistical analysis; all authors contributed to critical discussion and to final version of the paper.

Funding

None.

Conflict of interest

Prof. C.A. reports personal fees honoraria from Merck outside the submitted work. Prof. P.H. reports unrestricted research grants from MSD, Merck and Ferring as well as personal fees from honoraria for lectures from MSD, Merck and Finox, outside the submitted work. Prof. S.C.E. reports lecture fees from Merck, Besins and Lilly, outside the submitted work. Dr A.C., Dr D.S., Prof. C.Y.A., Prof. P.C. and M.S. have nothing to disclose.

References

- Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli RV, Mahale SD. Follicle-stimulating hormone receptor polymorphism (Thr307Ala) is associated with variable ovarian response and ovarian hyperstimulation syndrome in Indian women. *Fertil Steril* 2009a;**91**:432–439.
- Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli RV, Mahale SD. Poor ovarian response to gonadotrophin stimulation is associated with FSH receptor polymorphism. *Reprod Biomed Online* 2009b;**18**:509–515.
- Almawi WY, Hubail B, Arekat DZ, Al-Farsi SM, Al-Kindi SK, Arekat MR, Mahmood N, Madan S. Leutinizing hormone/choriogonadotropin receptor and follicle stimulating hormone receptor gene variants in polycystic ovary syndrome. *J Assist Reprod Genet* 2015;**32**:607–614.
- Altmae S, Hovatta O, Stavreus-Evers A, Salumets A. Genetic predictors of controlled ovarian hyperstimulation: where do we stand today? *Hum Reprod Update* 2011;**17**:813–828.
- Alvigi C, Mollo A, Clarizia R, De Placido G. Exploiting LH in ovarian stimulation. *Reprod Biomed Online* 2006;**12**:221–233.
- Alvigi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, Coppola M, Ranieri A, D'uva M, De Placido G. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. *Reprod Biomed Online* 2011;**22**:S67–S72.
- Alvigi C, Conforti A, Caprio F, Gizzo S, Noventa M, Strina I, Pagano T, De Rosa P, Carbone F, Colacurci N et al. In estimated good prognosis patients could unexpected 'Hyporesponse' to controlled ovarian stimulation be related to genetic polymorphisms of FSH receptor? *Reprod Sci* 2016a;**23**:1103–1108.
- Alvigi C, Conforti A, Cariati F, Alfano S, Strina I, Huhtaniemi I, Humaidan P. Abstracts of the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology. *Hum Reprod* 2016b;**31**:i1–i513.
- Alvigi C, Conforti A, Esteves SC. Impact of mutations and polymorphisms of gonadotrophins and their receptors on the outcome of controlled ovarian stimulation. In: Ghumman S (ed). *Principles and Practice of Controlled Ovarian Stimulation in ART*. New Delhi, India: Springer, 2015;147–156.
- Alvigi C, Conforti A, Fabozzi F, De Placido G. Ovarian stimulation for IVF/ICSI cycles: a pharmacogenomic approach. *MT médecine de la reproduction, gynécologie, endocrinologie* 2009;**11**:271–277.
- Alvigi C, Pettersson K, Longobardi S, Andersen CY, Conforti A, De Rosa P, Clarizia R, Strina I, Mollo A, De Placido G et al. A common polymorphic allele of the LH beta-subunit gene is associated with higher exogenous FSH consumption during controlled ovarian stimulation for assisted reproductive technology. *Reprod Biol Endocrinol* 2013;**11**:51.
- Anagnostou E, Mavrogianni D, Theofanakis C, Drakakis P, Bletsas R, Demiroglu A, Gurgan T, Antsaklis A, Loutradis D. ESR1, ESR2 and FSH receptor gene polymorphisms in combination: a useful genetic tool for the prediction of poor responders. *Curr Pharm Biotechnol* 2012;**13**:426–434.
- Behre HM, Greb RR, Mempel A, Sonntag B, Kiesel L, Kaltwasser P, Seliger E, Röpke F, Gromoll J, Nieschlag E et al. Significance of a common single nucleotide polymorphism in exon 10 of the follicle-stimulating hormone (FSH) receptor gene for the ovarian response to FSH: a pharmacogenetic approach to controlled ovarian hyperstimulation. *Pharmacogenet Genomics* 2005;**15**:451–456.
- Borgbo T, Jeppesen JV, Lindgren I, Lundberg Giwerzman Y, Hansen LL, Yding Andersen C. Effect of the FSH receptor single nucleotide polymorphisms (FSHR 307/680) on the follicular fluid hormone profile and the granulosa cell gene expression in human small antral follicles. *Mol Hum Reprod* 2015;**21**:255–261.
- Boudjenah R, Molina-Gomes D, Torre A, Boitrelle F, Taieb S, Dos Santos E, Wainer R, De Mazancourt P, Selva J, Vialard F. Associations between individual and combined polymorphisms of the TNF and VEGF genes and the embryo implantation rate in patients undergoing *in vitro* fertilization (IVF) programs. *PLoS One* 2014;**9**:e108287.
- Casarini L, Moriondo V, Marino M, Adversari F, Capodanno F, Grisolia C, La Marca A, La Sala GB, Simoni M. FSHR polymorphism p.N680S mediates different responses to FSH *in vitro*. *Mol Cell Endocrinol* 2014;**393**:83–91.
- Casarini L, Santi D, Marino M. Impact of gene polymorphisms of gonadotropins and their receptors on human reproductive success. *Reproduction* 2015;**150**:R175–R184.
- Cognato R, Aiello R, Dulcetti F, Ruggeri AM, De Toffol S, Marcato L, Irollo AM, Criscuolo C, Gangale MF, Maggi F et al. A pharmacogenetic-driven approach for controlled ovarian hyperstimulation by FSH treatment. *Minerva Ginecol* 2014;**66**:543–549.
- Daelemans C, Smits G, De Maertelaer V, Costagliola S, Englert Y, Vassart G, Delbaere A. Prediction of severity of symptoms in iatrogenic ovarian hyperstimulation syndrome by follicle-stimulating hormone receptor Ser680Asn polymorphism. *J Clin Endocrinol Metab* 2004;**89**:6310–6315.
- Dan W, Jing G, Liangbin X, Ting Z, Ying Z. Association of follicle stimulating hormone receptor promoter with ovarian response in IVF-ET patients. *Iran J Reprod Med* 2015;**13**:715–720.
- Davar R, Tabibnejad N, Kalantar SM, Sheikha MH. The luteinizing hormone beta-subunit exon 3 (Gly102Ser) gene mutation and ovarian responses to controlled ovarian hyperstimulation. *Iran J Reprod Med* 2014;**12**:667–672.
- De Castro F, Morón FJ, Montoro L, Galán JJ, Pérez-Hernández D, Sánchez-Casas Padilla E, Ramírez-Lorca R, Real LM, Ruiz A. Human controlled ovarian hyperstimulation outcome is a polygenic trait. *Pharmacogenetics* 2004;**14**:285–293.
- De Castro F, Ruiz R, Montoro L, Pérez-Hernández D, Sánchez-Casas Padilla E, Real LM, Ruiz A. Role of follicle-stimulating hormone receptor Ser680Asn polymorphism in the efficacy of follicle-stimulating hormone. *Fertil Steril* 2003;**80**:571–576.
- Desai SS, Achrekar SK, Paranjape SR, Desai SK, Mangoli VS, Mahale SD. Association of allelic combinations of FSHR gene polymorphisms with ovarian response. *Reprod Biomed Online* 2013;**27**:400–406.
- Desai SS, Achrekar SK, Pathak BR, Desai SK, Mangoli VS, Mangoli RV, Mahale SD. Follicle-stimulating hormone receptor polymorphism (G-29A) is associated with altered level of receptor expression in Granulosa cells. *J Clin Endocrinol Metab* 2011;**96**:2805–2812.
- Duval S. The Trim and Fill Method. In: Rothstein HR, Sutton AJ, Borenstein M (eds). *Publication Bias in Meta-Analysis*. The Atrium, Southern Gate, Chichester, West Sussex, England: John Wiley & Sons, Ltd, 2006;127–144.
- D'alva CB, Serafini P, Motta E, Kohek MB, Latronico AC, Mendonca BB. Absence of follicle-stimulating hormone receptor activating mutations in women with iatrogenic ovarian hyperstimulation syndrome. *Fertil Steril* 2005;**83**:1695–1699.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**:629–634.
- Ferlin A, Vianini C, Selice R, Garolla A, Frigo AC, Foresta C. Toward a pharmacogenetic approach to male infertility: polymorphism of follicle-stimulating hormone beta-subunit promoter. *Fertil Steril* 2011;**96**:1344–1349.e2.
- Genro VK, Matte U, De Conto E, Cunha-Filho JS, Fanchin R. Frequent polymorphisms of FSH receptor do not influence antral follicle responsiveness to follicle-stimulating hormone administration as assessed by the Follicular Output RaTe (FORT). *J Assist Reprod Genet* 2012;**29**:657–663.
- Greb RR, Behre HM, Simoni M. Pharmacogenetics in ovarian stimulation—current concepts and future options. *Reprod Biomed Online* 2005;**11**:589–600.
- Grigorova M, Punab M, Poolamets O, Kelgo P, Ausmees K, Korrovits P, Vihljajev V, Laan M. Increased prevalence of the -211 T allele of follicle stimulating hormone (FSH) beta subunit promoter polymorphism and lower serum FSH in infertile men. *J Clin Endocrinol Metab* 2010;**95**:100–108.
- Haaavisto AM, Pettersson K, Bergendahl M, Virkamaki A, Huhtaniemi I. Occurrence and biological properties of a common genetic variant of luteinizing hormone. *J Clin Endocrinol Metab* 1995;**80**:1257–1263.
- Hoogendoorn B, Coleman SL, Guy CA, Smith K, Bowen T, Buckland PR O'donovan MC. Functional analysis of human promoter polymorphisms. *Hum Mol Genet* 2003;**12**:2249–2254.
- Huang X, Li L, Hong L, Zhou W, Shi H, Zhang H, Zhang Z, Sun X, Du J. The Ser680Asn polymorphism in the follicle-stimulating hormone receptor gene is associated with the ovarian response in controlled ovarian hyperstimulation. *Clin Endocrinol (Oxf)* 2015;**82**:577–583.

- Huang S, Yang J, Yin T, Xing LI, Jie LI, Xu W. Association of gene polymorphism of follicle stimulating hormone receptor with ovarian response in IVF cycles. *Med J Wuhan Univ* 2010;**31**:334–338.
- Humaidan P, Alvigi C, Fischer R, Esteves SC. The novel POSEIDON stratification of 'Low prognosis patients in Assisted Reproductive Technology' and its proposed marker of successful outcome. *F1000Res* 2016;**5**:2911.
- John M, Lencz T, Malhotra AK, Correll CU, Zhang JP. A simulations approach for meta-analysis of genetic association studies based on additive genetic model. *Meta Gene* 2018;**16**:143–164.
- Jun JK, Yoon JS, Ku SY, Choi YM, Hwang KR, Park SY, Lee GH, Lee WD, Kim SH, Kim JG et al. Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. *J Hum Genet* 2006;**51**:665–670.
- Klinkert ER, Te Velde ER, Weima S, Van Zandvoort PM, Hanssen RG, Nilsson PR, De Jong FH, Looman CW, Broekmans FJ. FSH receptor genotype is associated with pregnancy but not with ovarian response in IVF. *Reprod Biomed Online* 2006;**13**:687–695.
- La Marca A, Papaleo E, Alvigi C, Ruvolo G, De Placido G, Candiani M, Cittadini E, De Michele F, Moriondo V, Catellani V et al. The combination of genetic variants of the FSHB and FSHR genes affects serum FSH in women of reproductive age. *Hum Reprod* 2013;**28**:1369–1374.
- Laisk-Podar T, Kaart T, Peters M, Salumets A. Genetic variants associated with female reproductive ageing—potential markers for assessing ovarian function and ovarian stimulation outcome. *Reprod Biomed Online* 2015;**31**:199–209.
- Laven JS, Mulders AG, Suryandari DA, Gromoll J, Nieschlag E, Fauser BC, Simoni M. Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility. *Fertil Steril* 2003;**80**:986–992.
- Lazaros LA, Hatzl EG, Pamporaki CE, Sakaloglou PI, Xita NV, Markoula SI, Stefanos TI, Zikopoulos KA, Georgiou IA. The ovarian response to standard gonadotropin stimulation depends on FSHR, SHBG and CYP19 gene synergism. *J Assist Reprod Genet* 2012;**29**:1185–1191.
- Lazaros L, Hatzl E, Xita N, Takenaka A, Sofikitis N, Zikopoulos K, Georgiou I. Influence of FSHR diplotypes on ovarian response to standard gonadotropin stimulation for IVF/ICSI. *J Reprod Med* 2013;**58**:395–401.
- Lindgren I, Baath M, Uvebrant K, Dejmek A, Kjaer L, Henic E, Bungum M, Bungum L, Cilio C, Leijonhufvud I et al. Combined assessment of polymorphisms in the LHCGR and FSHR genes predict chance of pregnancy after *in vitro* fertilization. *Hum Reprod* 2016;**31**:672–683.
- Livshyts G, Podlesnaja S, Kravchenko S, Sudoma I, Livshits L. A distribution of two SNPs in exon 10 of the FSHR gene among the women with a diminished ovarian reserve in Ukraine. *J Assist Reprod Genet* 2009;**26**:29–34.
- Lledo B, Guerrero J, Turienzo A, Ortiz JA, Morales R, Ten J, Llacer J, Bernabeu R. Effect of follicle-stimulating hormone receptor N680S polymorphism on the efficacy of follicle-stimulating hormone stimulation on donor ovarian response. *Pharmacogenomics* 2013;**23**:262–268.
- Lledó B, Dapena P, Ortiz JA, Morales R, Llacer J, Bernabeu R. Clinical efficacy of recombinant versus highly purified follicle-stimulating hormone according to follicle-stimulating hormone receptor genotype. *Pharmacogenomics* 2016;**26**:288–293.
- Loutradis D, Patsoula E, Minas V, Koussidis GA, Antsaklis A, Michalakis S, Makrigiannakis A. FSH receptor gene polymorphisms have a role for different ovarian response to stimulation in patients entering IVF/ICSI-ET programs. *J Assist Reprod Genet* 2006;**23**:177–184.
- Mizzi C, Dalabira E, Kumuthini J, Dzimirli N, Balogh I, Basak N, Bohm R, Borg J, Borgiani P, Bozina N et al. A European spectrum of pharmacogenomic biomarkers: implications for clinical pharmacogenomics. *PLoS One* 2016;**11**:e0162866.
- Mohiyiddeen L, Nardo LG. Single-nucleotide polymorphisms in the FSH receptor gene and ovarian performance: future role in IVF. *Hum Fertil (Camb)* 2010;**13**:72–78.
- Mohiyiddeen L, Newman WG, Cerra C, Home G, Mulugeta B, Byers H, Roberts SA, Nardo LG. FSH receptor genotype does not predict metaphase-II oocyte output or fertilization rates in ICSI patients. *Reprod Biomed Online* 2013a;**27**:305–309.
- Mohiyiddeen L, Newman WG, Cerra C, Mcburney H, Mulugeta B, Roberts SA, Nardo LG. A common Asn680Ser polymorphism in the follicle-stimulating hormone receptor gene is not associated with ovarian response to gonadotropin stimulation in patients undergoing *in vitro* fertilization. *Fertil Steril* 2013b;**99**:149–155.
- Nakayama T, Kuroi N, Sano M, Tabara Y, Katsuya T, Ogihara T, Makita Y, Hata A, Yamada M, Takahashi N et al. Mutation of the follicle-stimulating hormone receptor gene 5'-untranslated region associated with female hypertension. *Hypertension* 2006;**48**:512–518.
- Nilsson C, Pettersson K, Millar RP, Coerver KA, Matzuk MM, Huhtaniemi IT. Worldwide frequency of a common genetic variant of luteinizing hormone: an international collaborative research. International Collaborative Research Group. *Fertil Steril* 1997;**67**:998–1004.
- Nilsson C, Jiang M, Pettersson K, Iitia A, Makela M, Simonsen H, Eastaie S, Herrera RJ, Huhtaniemi I. Determination of a common genetic variant of luteinizing hormone using DNA hybridization and immunoassays. *Clin Endocrinol (Oxf)* 1998;**49**:369–376.
- Nordhoff V, Sonntag B, Von Tils D, Gotte M, Schuring AN, Gromoll J, Redmann K, Casarini L, Simoni M. Effects of the FSH receptor gene polymorphism p.N680S on cAMP and steroid production in cultured primary human granulosa cells. *Reprod Biomed Online* 2011;**23**:196–203.
- O'Brien TJ, Kalmin MM, Harralson AF, Clark AM, Gindoff I, Simmens SJ, Frankfurter D, Gindoff P. Association between the luteinizing hormone/chorionic gonadotropin receptor (LHCGR) rs4073366 polymorphism and ovarian hyperstimulation syndrome during controlled ovarian hyperstimulation. *Reprod Biol Endocrinol* 2013;**11**:71.
- Patel V, Lin FJ, Ojo O, Rao S, Yu S, Zhan L, Touchette DR. Cost-utility analysis of genotype-guided antiplatelet therapy in patients with moderate-to-high risk acute coronary syndrome and planned percutaneous coronary intervention. *Pharm Pract (Granada)* 2014;**12**:438.
- Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *J Clin Endocrinol Metab* 2000;**85**:3365–3369.
- Poseidon G, Alvigi C, Andersen CY, Buehler K, Conforti A, De Placido G, Esteves SC, Fischer R, Galliano D, Polyzos NP et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil Steril* 2016;**105**:1452–1453.
- Sheikhha MH, Eftekhar M, Kalantar SM. Investigating the association between polymorphism of follicle-stimulating hormone receptor gene and ovarian response in controlled ovarian hyperstimulation. *J Hum Reprod Sci* 2011;**4**:86–90.
- Simoni M, Casarini L. Mechanisms in endocrinology: genetics of FSH action: a 2014-and-beyond view. *Eur J Endocrinol* 2014;**170**:R91–R107.
- Sudo S, Kudo M, Wada S, Sato O, Hsueh AJ, Fujimoto S. Genetic and functional analyses of polymorphisms in the human FSH receptor gene. *Mol Hum Reprod* 2002;**8**:893–899.
- Sychev DA, Malova EU. Evidence-based pharmacogenetics: is it possible? *Int J Risk Saf Med* 2015;**27**:S97–S98.
- Tohlob D, Abo Hashem E, Ghareeb N, Ghanem M, Elfarahaty R, Byers H, Pemberton P, Roberts SA, Newman WG, Mohiyiddeen L. Association of a promoter polymorphism in FSHR with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment. *Reprod Biomed Online* 2016;**33**:391–397.
- Trevisan CM, Peluso C, Cordts EB, De Oliveira R, Christofolini DM, Barbosa CP, Bianco B. Ala307Thr and Asn680Ser polymorphisms of FSHR gene in human reproduction outcomes. *Cell Physiol Biochem* 2014;**34**:1527–1535.
- Valkenburg O, Van Santbrink EJ, Konig TE, Themmen AP, Uitterlinden AG, Fauser BC, Lambalk CB, Laven JS. Follicle-stimulating hormone receptor polymorphism affects the outcome of ovulation induction in normogonadotropic (World Health Organization class 2) anovulatory subfertility. *Fertil Steril* 2015;**103**:1081–1088.e3.
- Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. Quality Assessment Scales for Observational Studies. *Ottawa Health Research Institute* 2004.
- Yan Y, Gong Z, Zhang L, Li Y, Li X, Zhu L, Sun L. Association of follicle-stimulating hormone receptor polymorphisms with ovarian response in Chinese women: a prospective clinical study. *PLoS One* 2013;**8**:e78138.
- Yao Y, Ma CH, Tang HL, Hu YF. Influence of follicle-stimulating hormone receptor (FSHR) Ser680Asn polymorphism on ovarian function and *in vitro* fertilization outcome: a meta-analysis. *Mol Genet Metab* 2011;**103**:388–393.
- Yin Q, Li Y, Huang J, Yang D. [Association of rs13405728 polymorphism of LHR gene with low ovarian response]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2015;**32**:840–843.
- Zalewski G, Wolczynski S, Chyczewski L. Association of rs6166 polymorphism with FSH receptor transcript variants and steroid production in human granulosa cell cultures. *Syst Biol Reprod Med* 2013;**59**:191–198.