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Personalized medicine – what is the role of FSH, LH and Estrogen receptors polymorphisms in ovarian function?

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Review article

Title: *Personalized medicine - what is the role of follicle stimulating hormone (FSH), luteinising hormone (LH) and estrogen (ES) receptors polymorphisms in ovarian function?*

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Abstract

Controlled ovarian stimulation (COS) is used to induce an ovarian response of multiple follicles in assisted reproductive technology (ART). The precise management of ovarian response is determinant for the efficacy of the *in vitro* fertilization (IVF) treatments by affecting both pregnancy and abortion rate. Therefore, achieving an appropriate ovarian response is pivotal, but it is also a huge challenge in ART.

It is assumed that the ovarian response is dependent on age, ovarian reserve, endocrine profile and environmental factors, as well as on genetic factors. In such a scenario, the analysis of patient's genome, particularly gonadotropins receptors, constitutes a promising tool to guarantee the optimal ovarian response.

Single nucleotide polymorphisms (SNPs) are common genetic variations and its role in COS has already been studied, particularly polymorphisms of hormonal receptors involved in follicle recruitment and development. However, the association of these polymorphisms and ovarian response remains to be systematised.

Considering its influence in ovarian response to stimulation and in IVF success, the existing evidence on the impact of *follicle stimulating hormone receptor* (*FSHR)*, *luteinising hormone receptor* (*LHR)* and *estrogen receptor* (*ESR)* polymorphisms on the ovarian response was reviewed.

Three different databases were searched: PubMed, MEDLINE and TripDataBase, between May and November of 2022. We included studies concerning: 1) Women aged between 18 and 40 years old; 2) Polymorphisms of *FSHR*, *LHR* and *ESR;* 3) Human studies; 4) *In vitro* studies, case-control studies, cohort studies, randomized clinical trials; 5) Studies where response to COS were evaluated. From the initial number of articles (n=141), we selected 42 articles to include in this review.

This review shows that *FSHR* Asn680Ser polymorphism is the most studied predictor of the ovarian response and that Ser680 allele is mostly found to be associated with a worse outcome in COS, while Ans680 is defined as more favourable. *LHR* and *ESR* role, on the other hand, is not well documented. More studies are needed to sustain the applicability of pharmacogenetics in daily clinical practice.

Keywords: polymorphism, genetic; ovulation induction; receptors, gonadotropin; reproductive techniques, assisted.

Abbreviations

ART - assisted reproductive technology

IVF - *in vitro* fertilization

SNP - single nucleotide polymorphism

FSHR - follicle-stimulating hormone receptor

LHR – luteinising hormone receptor

ESR - estrogen receptor

COS - controlled ovarian stimulation

FSH - follicle stimulating hormone

OHSS - ovarian hyperstimulation syndrome

POI - premature ovarian insufficiency

r-hFSH - recombinant human follicle stimulating hormone

hMG - human menopausal gonadotropin

1. Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse (1). This condition is a global health problem affecting between 8-12% of couples of reproductive age (2). Since the success of assisted reproductive technology (ART) is closely related to the response to controlled ovarian stimulation (COS), multiple strategies have been discussed to define the most appropriate pharmacological protocol. Various treatment regimens have been suggested to increase the ovarian responsiveness and reduce the number of cancelled cycles (3). The demonstration of a genetic determination to COS has opened the opportunity to explore new ways of diagnosing and understanding women who have a poor response to the pharmacological stimulation protocol (4,5). Thus, the pharmacogenetics approach and the study of the influence of genotype on COS response has been proposed in the context of ART to personalize treatment and ensure a better ovarian response (5–7).

Genetic alterations in genes that code for hormonal receptors, i.e. polymorphisms of *follicle-stimulating hormone receptor* (*FSHR*), *luteinising hormone receptor* (*LHR*) and *estrogen receptors* (*ESR*), are able to influence the follicular recruitment and development, altering the ovarian response (8). For instance, single nucleotide polymorphisms (SNPs) are well described genetic alterations related to ovarian response (9) Nevertheless, the influence of these SNPs in ovarian function has contradictory findings.

Understanding how polymorphisms of *FSHR*, *LHR* and *ESR* influence ovarian function is fundamental to optimise an IVF treatment. The role of these polymorphisms in COS remains to be systematised, specifically their impact on the number of oocytes obtained, number of embryos, pregnancy rate and live-birth rate. Accordingly, it is imperative to deepen the knowledge in this field to allow a better advice and decision of the pharmacological protocol of ART.

Therefore, we carried out a review to summarize the existing clinical evidence on the impact of gonadotrophin receptors and ESR polymorphisms on the response to COS.

2. Methods

The goal of this narrative review was to analyse the role of *FSHR*, *LHR* and *ESR* polymorphisms in ovarian stimulation. A systematic approach was used for the study's identification and selection, aiming to increase the quality of the manuscript.

2.1. Bibliographical Research

For the literature search, three databases were selected: PubMed, MEDLINE and TripDataBase. The first search was conducted in May 2022, and all the resulting articles were selected and proved to be sufficient to conduct a review on the topic. The last search was conducted in November 2022, in which the most recent articles were added. No publication year limits were applied to the articles.

2.2. Search Terms

For the search strategy, on the PubMed database it was used the following search formula: "((((Estrogen receptor*)) AND ((ovulation Induction or ovulation stimulation)) AND ((Polymorphism*))) OR ((Receptor* FSH OR Follicle Stimulating Hormone) AND (ovulation Induction or ovulation stimulation) AND (Polymorphism*))) OR ((((Receptor* LH OR Luteinizing Hormone Receptor* OR Chorionic Gonadotropin Receptor)) AND ((ovulation Induction or ovulation stimulation)) AND ((Polymorphism*)))". For the MEDLINE database search, the Boolean operators AND (AND) and OR (OR) and the following MESH terms, in different combinations, were used: "Polymorphism, Genetic", "Receptors, FSH", "Ovulation Induction", "Receptors, LH", "Receptors, Estrogen", and "Ovarian reserve". For TripDataBase, it was used the PICO strategy: Patients - women of reproductive age with evaluation of *FSHR*, *LHR*, ESR; Intervention - Ovarian Stimulation; Comparison - women of reproductive age with different genotypes; Outcome - response to ovarian stimulation.

2.3. Eligibility Criteria

Studies that met the following criteria were included: 1) Women of childbearing age (aged between 18 and 40 years old); 2) Polymorphisms of FSH, LH and estrogen receptor genes; 3) Human studies; 4) *In vitro* studies, case-control studies, cohort studies, randomized clinical trials; 5) Studies where the purpose of this review is evaluated, namely the response to COS.

2.4. Exclusion Criteria

Articles were excluded if: 1) Women under 18 and over 40 years old; 2) Polymorphisms not within the scope of the study; 3) Species other than human; 4) Literature reviews, systematic reviews, meta-analyses, commentaries, guidelines; 5) Populations of women with polycystic ovarian syndrome.

The search led to 141 articles, after excluding duplicates. Articles were initially selected, according to the defined criteria, based on the title and abstract, by two independent researchers. After that, we selected 42 articles for full-text reading. In the end, 42 studies were included in this review: 26 on *FSHR*, 8 on *ESR*, 3 on *LHR* and 5 on *FSHR* and *ESR.* The process of paper selection in summarized in

Figure 1 and the main conclusions from the analysed studies is summarized in Table 1.

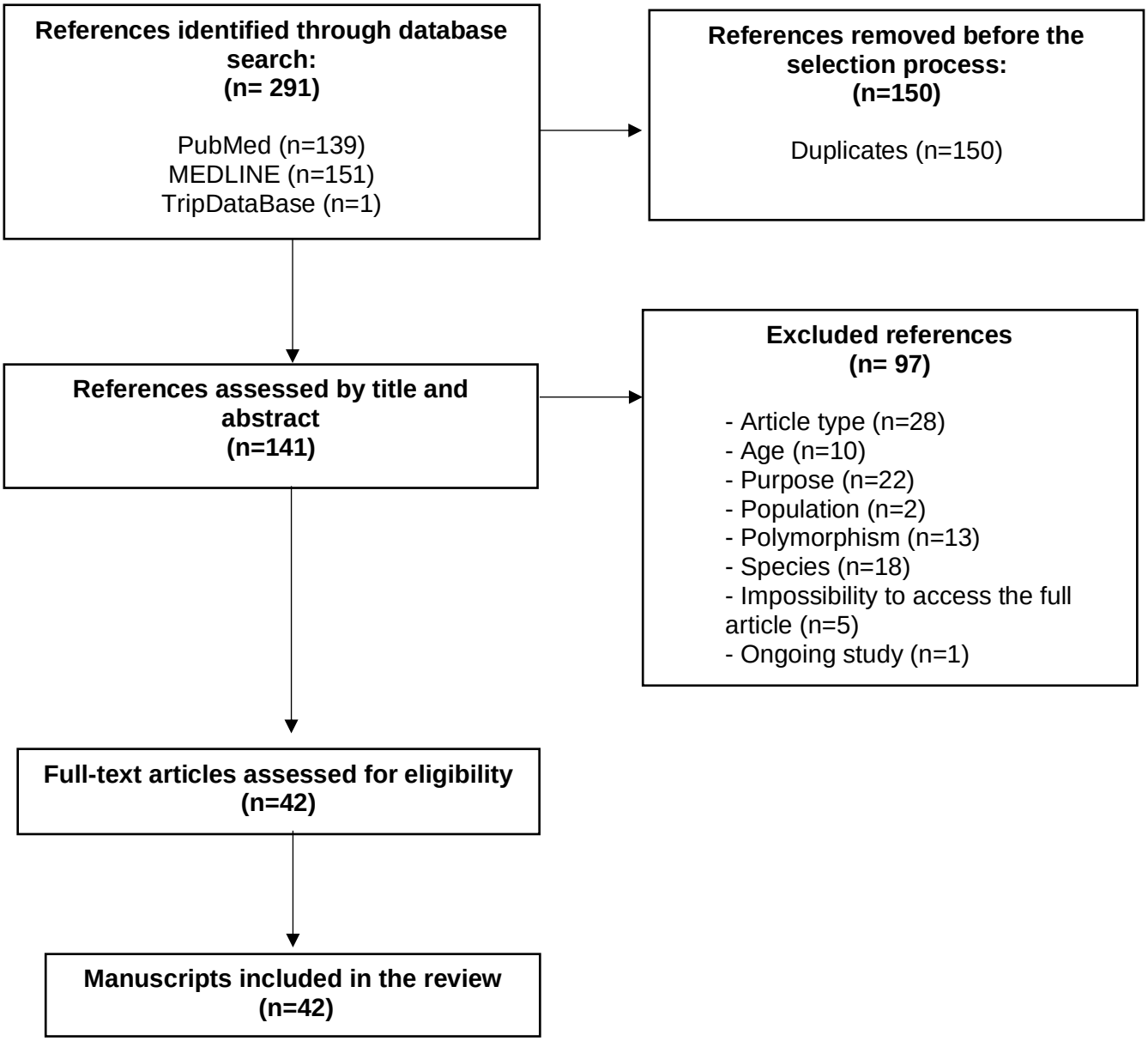

Figure 1. Flow diagram from initial search to final analysed articles included in this review.

3. Discussion

3.1. *FSHR* polymorphisms

Several studies evaluatied the role of *FSHR* SNPs on reproductive function (10). The first *FSHR* mutation identified was the substitution of Alanine to Valine at position 189 (Ala189Val), at the receptor glycosylation site. This mutation was described in women with ovarian dysgenesis and primary amenorrhea (11).

Other inactivating and activating alterations in *FSHR* were identified in women with primary amenorrhea, increased FSH levels, polycystic ovary syndrome (12) and ovarian hyperstimulation syndrome (OHSS) (13–15).

Particularly, two variants of *FSHR* SNPs have intensively been studied, both in exon 10. One at position 680, Asn680Ser, where Asparagine is replaced by Serine in the intracellular domain of the *FSHR* protein, and the other at position 307, Ala307Thr, causing the replacement of Threonine by Alanine. *FSHR* Asn680Ser Ser/Ser homozygous genotype was associated with higher FSH serum levels and dosage of FSH to get a similar ovarian response (16) suggesting that Ser/Ser homozygous variant is less sensitive to FSH (17).

Provided that, women homozygous for the serine (Ser/Ser) have been associated with a reduced response, higher concentrations of basal levels of FSH and higher requirement of gonadotrophins, when compared with women with the two other variants (18). For this reason, it could be hypothesized that *FSHR* phamacogenetic modulates the ovarian response (19,20).

Considering *FSHR* G-29A SNP (rs1394205), it has been suggested that A variant could be related to high serum FSH and low estradiol levels (21,22) but there is not a consensus about its influence on ovarian response to FSH (23,24).

The *FSHR* polymorphisms reviewed in the literature that are related to response to COS were: Asn680Ser (rs6166); Ala307Thr (rs6165); G-29A (rs1396205); Ala189Val (rs121909658); Ile160Thr (rs121909659) and Thr449Ile (rs28928870).

The Asn680Ser polymorphism was evaluated in 24 studies. Almost every study confirms that this polymorphism is correlated with the COS outcome, and the majority of them found that the 680Ser allele is associated with a worse outcome. Alviggi e*t al.* came to the conclusion, after analysing 42 Italian women, that this variant seems to result in a significant decrease in ovarian response to recombinant human follicle stimulating hormone (r-hFSH) during ART cycles and, therefore, in a significant increase in drug consumption (25). de Castro *et al.* studied a population of 102 Spanish women and also

concluded that patients who carry the Ser/Ser genotype seem to have an increased risk of cycle cancellation during recombinant FSH treatment (26). Lledó *et al.,* added the information that, in subjects with Ser/Ser genotype, the use of a corifollitropin alfa-based ovarian stimulation protocol may be linked to a lower number of oocytes and metaphase II oocytes retrieved (27). Valkenburg *et al.* corroborated this finding, concluding that homozygous carriers of the 680Ser allele have a 90% higher probability of clomiphene-resistant anovulation during ovulation induction with clomiphene citrate. With this, they could hypothesise that these patients are more effectively treated with exogenous gonadotropins (28). Beyond this, Sudo *et al.*, in a study with 522 Japanese women, observed that the difference in ovarian response to human menopausal gonadotropin (hMG) among these polymorphisms could be used not only for determination of its dose in an ovarian stimulating cycle, but also for prediction of OHSS (29).

Behre *et al.* studied 93 women from Germany and compared different FSH doses based on their genotype. The authors noticed that by increasing the daily dose from 150 to 225Ul, they were able to overcome the lower estradiol response in women with the Ser/Ser FSH receptor variant. Thus, they concluded that, in women with normal ovarian function undergoing ART, it might be possible to define the optimal FSH starting dose based on the simple determination of the FSH receptor genotype (30).

However, Binder *et al.,* that also performed a study in a German population of 259 women, concluded that this variation may not be a decisive factor for poor or low response to fertility treatment (31). The same conclusion was achieved by Laven *et al.*, in a population of 148 Dutch patients, where *FSHR* genotypes aren't related with altered ovarian sensitivity to exogenous FSH during COS in anovulatory patients (32).

Another important finding was reported by Nenonen *et al*., in a study that evaluated 586 Swedish women, where they observed that women with 680Asn allele are hyperresponsive to FSH and consequently are at increased risk for OHSS when undergoing IVF treatment. These observations allowed to conclude that genetic testing may be an additional OHSS predictor to identify these women (33).

Therefore, it can be concluded that *FSHR* gene polymorphism at position 680 is associated with altered ovarian responses to COS (34), namely that Ser/Ser variant influence negatively on COS outcome (35).

Regarding Ala307Thr polymorphism, it was studied by 14 authors. Kaviani *et al.* findings are consisted with most reported conclusions. They observed that this *FSHR* polymorphism showed a strong association with treatment response. Their results showed that the frequency of Ala/Ala genotype was significantly higher in the poor responders than in the good responders to IVF treatment, and that women with poor response showed a higher 307Ala frequency than good responders (36). Laven *et al.*

found that Ala/Thr genotype was significantly more prevalent among anovulatory women and that the Thr/Thr was significantly more prevalent in controls (30 healthy volunteers), despite concluding that *FSHR* genotypes are not associated with altered ovarian sensitivity to exogenous FSH during ovulation induction in anovulatory patients (32). Song *et al.* observed that, in a population of 705 Chinese women, the frequency of 307Ala was significantly higher in low responders than in good responders. Also, it was noticed that the risk of a low response increased with the number of 307Ala alleles present (37). The analysis performed by Monge-Ochoa *et al.* showed that COS response was not independent of Ala307Thr genotypes, since the Ala/Ala genotype was significantly increased in poor responder and underrepresented in the pregnancy group, concluding that Ala/Ala variant influence negatively on COS outcome (35). Motawi *et al*. found that, after analysing 216 Egyptian women, Ala/Ala variant was 2.5-fold higher in the poor responders group than in the good responders group (38).

d'Alva *et al.* reported a finding regarding OHSS related to this *FSHR* polymorphism. They studied a population of 29 Brazilian women who experienced moderate-to-severe OHSS during ovarian stimulation and came to the conclusion that the FSH receptor genotype did not play a significant role in the risk of iatrogenic OHSS, inferring that ovarian response to exogenous gonadotropin stimulation is still unpredictable (39).

Thus, it is corroborated the hypothesis that 307Ala allele is associated with worst COS outcomes (37).

The G-29A polymorphism was analysed in 7 studies. It is speculated by some authors that the AA genotype at position –29 of the FSH receptor gene is probably responsible for poor ovarian response. A study by Achrekar *et al.*, performed in 50 normogonadotrophic ovulatory females with male or tubal factor infertility , from Indian ethnicity, found that AA genotype is resistant to FSH treatment (40). These results indicate that the subjects with the AA genotype have a significantly lower rate of successful clinical pregnancy as compared with the GG and GA genotypes. The AA genotype appears to be associated with poor responder to gonadotrophin treatment based on endocrine and clinical parameters. However, Čuš *et al.* studied 60 women undergoing ovarian stimulation from Slovenia and concluded that the GG genotype is strongly linked with poor response on ovarian stimulation (41). Also, Zamaniara *et al*. observed that this polymorphism had no effects on poor responders, after comparing the genotypes with good responders (42). Tohlob *et al.* observed that women carrying the variant A allele were more likely to have a clinical pregnancy and a live birth following COS. However, these relationships did not retain significance when the analysis was adjusted for the number of embryos transferred, concluding that the results of their study do not provide enough evidence to support the use of genotyping *FSHR* G-29A in the

individualization of treatment for women undergoing IVF (43).

When analysed in combination, the Asn680Ser with the G-29A polymorphism, Desai *et al.* concluded that A/A–Asn/Asn genotype is associated with poor ovarian response and suggested that the 680Asn allele in combination with the 29A allele serves as a better marker to predict poor ovarian response (44). Furthermore, Livshyts *et al*. also analysed Asn680Ser in association with Ala307Thr*,* and deduced that the statistically significant prevalence of Ala307-Ser680 observed in the ovarian dysfunction and poor responders groups is an additional evidence that the polymorphisms in exon 10 are associated with diminished reserve (45).

Lastly, one study performed by Binder *et al.* analysed three less studied variants of *FSHR*, along with Asn680Ser: Ala189Val, Ile160Thr and Thr449Ile. They concluded that frequencies of these variants were not significantly different between the low responders and controls, hypothesizing that this variants may not be a decisive factor for poor or low response to COS (31).

In brief, among the studied *FSHR* polymorphisms, Ans680Ser stands out, being the most studied one and, consequently, is where most authors agree. Ser680 allele is mostly found to be associated with a worse outcome in COS, while Ans680 is defined as more favourable to achieve good response. The other *FSHR* polymorphisms, namely Ala307Thr and G-29A were also found to be related to COS outcome, but are less studied, and, therefore, it is more difficult to find consensus about the genotypes with better or worse response. Ala189Val, Ile160Thr and Thr449Ile were only reported in one study, where they hypothesized that these variants may not be a decisive factor for poor or low response to COS.

3.2. *LHR* polymorphisms

The expression of the *LHR* gene has been described to be increased in poor responders (46). *LHR* inactivating variants were associated with premature ovarian insufficiency (POI). Patients with this condition are characterized by high levels of LH (LH levels > FSH levels) leading to a compromised ovulation and oligomenorrhea (47,48) and a lower fertilization rate (49). The evidence about the relationship between the *LHR* mutations and the reproductive outcomes is scarce.

In this review, regarding *LHR* polymorphisms, two studies were found that analysed the N312S (rs2293275) polymorphism (50,51). Despite different populations (Indians and Iranians), both studies conclude that women with A/A genotype or A-allele demonstrate

a better outcome from COS, in particular a significant increase in total number of oocytes and metaphase II oocytes, compared to women that are homozygous and heterozygous for G allele. However, Ramaraju *et al.* found a significant increase in clinical pregnancy rate in women homozygous or heterozygous for G allele compared to women homozygous for A allele (50).

Other polymorphisms, namely insLQ (rs4539842) and rs4073366, were studied by O'Brien *et al..* They concluded that insLQ was not associated with patient response to COS, nor was it a predictor for OHSS. Carriers of the rs4073366 C allele exhibited a 3-fold increased risk of developing OHSS. This means that rs4073366 can be considered a potential predictor of OHSS risk, while the functional consequences of this polymorphism on *LHR* function still have to be elucidated (52).

In short, due to the scarcity of evidence, is more difficult to draw conclusions about their role in ovarian response to COS, but the results of these studies suggested that it would be interesting to perform further studies to analyse the usefulness of these polymorphisms to predict the response to COS.

3.3. *ESR* polymorphisms

ESR polymorphisms have been studied because of their role in the regulation of the menstrual cycle, low fecundity and PCOS (53–56). Additionally, *ESR* variants are expected to predict 10 to 15% of poor responders to r-hFSH in IVF treatments (57).

The two most common SNPs in *ESR* are rs2234693 and rs9340799, respectively, PvuII and XbaI, named accordingly to their detecting restriction enzyme. These SNPs are located in the first intron of *ESR* and have been associated with ovarian follicles senescence and POI (56,58,59) (60). Nevertheless, the results are still discrepant (56,58–60).

Other *ESR* polymorphisms were described in the literature, AluI (rs4986938), BstUI (rs12722) and RsaI (rs1256049).

There are eight studies analysing PvuII polymorphism (41,53,61–66). The majority of women studied are from Greece (four studies) but were also studied women from West Europe in two studies, from China in one study and from Brazil in one study. T allele was identified as having a negative outcome in COS in the most of them, while C allele was associated with a better outcome. However, Čuš *et al.* findings disagreed. They conclude that these SNPs did not show any association with COS outcome (41). Nevertheless, this study has the smallest population sample, with only 60 women analysed, what may

explain the lack of association found. Therefore, the recent studies corroborate the premise that Sundarrajan *et al* made in 1999, that we can conclude that this polymorphism down-regulates the *ESR* gene, interfering with the effective mediation of estrogen and its functions, on the follicle-oocyte unit (62).

XbaI polymorphism was analysed in five studies (42,53,61,63,66). Again, the most prevalent population is from Greece (two studies). The most common conclusion is that A allele is related with worst COS outcome than G allele. Altmäe *et al.* described that GG genotype was associated with higher estradiol levels achieved during COS (67). In contrast, Zamaniar *et al.* studied a population of 202 Iranian women and described a higher prevalence of A allele in good responders to COS (42). However, it can be hypothesized that women with XbaI AA genotype are possibly more resistant to FSH action and therefore need greater amounts of gonadotropin during COS to achieve follicular sizes as high as those observed in XbaI GG (63).

Another *ESR* polymorphism studied was AluI that is described in six studies (36,38,57,61,63,68). The population sample is more diverse, with women from Greece, China, Iran, Spain, Egypt and Brazil. Despite this, all studies observed that AA genotype is more resistant to COS, having worst outcomes, when compared to AG and GG genotypes. The exception is De Mattos *et al.* study that studied 136 Brazilian women and concluded that GG genotype had longer COS protocols, used a higher dose of r-hFSH and was also e more associated with OHSS (61).

Regarding BstUI polymorphism, it is reported only in two studies (62,64). Sundarrajan *et al* led a study among 200 Chinese women undergoing IVF and did not find any case with this polymorphism present (62). However, Georgiou *et al* found this polymorphism among 200 Greek women and concluded that it did not show an influence on the number of the follicles, oocytes and ratios of follicles to oocytes, when compared alone or in combination with PvuII (64).

Lastly, RsaI polymorphism is assessed in four studies (61,65,67,68). Once again, the population sample is diverse, including women from Estonia, Greece, China and Brazil. Only De Mattos *et al.* found that GG genotype is associated with higher doses of r-hFSH in IVF cycles and concluded that more studies with larger samples are needed to confirm their results (61). The other studies describe that this polymorphism is not associated with the parameters describing ovarian stimulation outcomes (65,67,68).

In summary, further studies are necessary to draw conclusions about the role of *ESR* polymorphisms in COS outcome and the advantage of using them for the prediction of ovarian response in ART protocols. For now, it only can be concluded that these

polymorphisms, other than BstUI, have been described as having a role in COS by most authors.

Table 1. Polymorphisms studied in relation with COS.

Gene	Polymorphism	Main Findings
FSHR	- Ans680Ser (rs6166)	Ans680Ser is the most studied and is correlated with COS outcome: the 680Ser allele is associated with a worse outcome and a significant increase in drug consumption during ART cycles.
	- Ala307Thr (rs6165)	Ala/Ala307 genotype is more usually found in poor responders to COS, and Ala307Thr is not associated with risk of iatrogenic OHSS.
	- G-29A (rs1396205)	Studies about G-29A don't report consensual results.
	- Ala189Val (rs121909658) - Ile160Thr (rs121909659) - Thr449Ile (rs28928870)	Ala189Val, Ile160Thr and Thr449Ile may not be a decisive factor for poor or low response to COS.
LHR	- N312S (rs2293275) - insLQ (rs4539842) - (rs4073366)	In all studies that an influence on COS outcome.
ERS1	- PvuII (rs2234693)	PvuII T allele was identified has having a negative outcome in COS.
	- XbaI (rs9340799)	XbaI, A allele was found to be related with worst COS outcome than G allele.
	- BstUI (rs12722)	BstUI has no influence on ovarian response.
ERS2	- AluI (rs4986938)	AA genotype in AluI is more resistant to COS, having worst outcomes, when compared to AG and GG genotypes.
	- RsaI (rs1256049)	RsaI has non-consensual results.

4. Conclusions and future perspectives

In this review, the polymorphisms of *FHSR*, *LHR* and *ESR* and their association with ovarian function were analysed.

Overall, *FSHR* Asn680Ser is by far the most studied genetic predictor and *ESR* and *LHR* variants are very common in general population, but its impact in the ovarian response is not well documented. Once the specificity and sensitivity of a single genetic marker would be too low to be employed as a predictive biomarker, it is crucial to provide further evidence about the role of *LHR* and *ESR* in ovarian response.

Notwithstanding, more studies regarding these polymorphisms are needed to predict more accurately the ovarian response to COS according to each genotype, in order to identify the poor responders' patients, the most suitable management strategy, develop the chances of a strong outcome and avoid obstacles related to ovarian stimulation.

In conclusion, given the overall effect of gonadotrophin receptors and *ESR* polymorphisms on COS, a pharmacogenomic approach seems a promising strategy to improve the clinical management of infertile women candidates for COS.

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