Ovarian stimulation for IVF/ICSI cycles: a pharmacogenomic approach

La stimulation ovarienne contrôlée des cycles FIV et ICSI : une approche pharmacogénomique

Abstract. Controlled ovarian stimulation (COS) is a crucial step in in vitro fertilisation (IVF) programs. In this context, GnRH agonist (GnRH-a) long protocol, in association with recombinant FSH (r-hFSH) still represents the most utilised strategy for normogonadotrophic patients. Such an approach leads to optimal follicular growth and steroidogenesis in about 85-90% of women. Conversely, in about 10-15% of patients the ovarian response to this protocol is sub-optimal or inadequate. The concept of “hypo-response” to OS has been proposed to identify those “good prognosis” normogonadotrophic women who require high amounts of r-hFSH to obtain an adequate number (i.e. > 4) of oocytes retrieved. The pathogenesis of this phenomenon is still unknown. Preliminary data has shown that woman with ovarian resistance to exogenous FSH may benefit from an LH supplementation. In addition, it has been found that hypo-response to FSH is associated with an increased frequency of a common and less bioactive LH polymorphism (v-LH). A polymorphic variant of the FSH receptor (FSH-R) in which aminoacid asparagine (Asn) at position 680 is replaced by Serine (Ser) has been associated with higher FSH basal levels and increased number of antral follicles during the early follicular phase. Recent studies have also proven that this common polymorphism is associated with a higher consumption of exogenous FSH during COS for IVF/ICSI cycles. These lines of research suggest that ovarian resistance (hypo-response) to exogenous FSH can be related to specific gene polymorphisms. In addition, these data support the idea of a tailored gonadotrophins administration based on a pharmacogenomic approach.

Key words: ART/IVF, pharmacogenomics, ovarian stimulation, poor responders

Résumé. La stimulation ovarienne contrôlée est une étape cruciale des programmes de FIV. Dans ce contexte, le protocole long utilisant les agonistes du GnRH en association avec la FSH recombinante est encore la stratégie la plus utilisée chez les patientes normogonadotropes. Une telle approche conduit à une croissance folliculaire et à une stéroïdogenèse satisfaisantes chez environ 85 à 90 % des patientes. Ainsi, dans environ 10 à 15 % des cas, la réponse ovarienne à ce protocole est suboptimale ou inadéquate. Cette observation a conduit à proposer le concept d’hypo-réponse mais de pronostic favorable car, malgré la nécessité d’utiliser des fortes doses de FSH, le nombre d’ovocytes recueillis est satisfaisant (> 4 ovocytes). Sa physiopathologie n’en est pas, pour autant, clairement établie. Des données préliminaires ont montré que ces patientes “résistantes à la FSH” peuvent bénéficier d’une supplémentation par la LH. De plus, il a été observé que l’hypo-réponse à la FSH est associée à une incidence élevée d’un polymorphisme de la molécule LH avec des variants dont l’activité biologique est réduite. Il avait déjà été mis en évidence qu’un des polymorphismes du récepteur de la FSH (pour lequel l’asparagine [Asn] en position 680 est remplacée par une sérine [Ser]) s’accompagne d’un tableau particulier caractérisé par une FSH plasmatique de base au-dessus de la norme et un nombre élevé de follicules antraux au début de la phase folliculaire. Des études plus récentes ont également montré que ce polymorphisme particulier est associé à un besoin de doses importantes de FSH exogène durant la stimulation pour FIV-ICSI. Cette recherche suggère que la “résistance ovarienne à la FSH exogène” peut être liée à des polymorphismes spécifiques. De plus, ces données plaident en faveur d’une administration ajustée des gonadotrophines sur la base de cette approche pharmacogénomique.

Mots clés : ART/FIV, pharmacogénomique, stimulation ovarienne, hypo-réponse
The concept of “hypo-response” to exogenous FSH

Controlled ovarian stimulation (COS) for in vitro fertilisation (IVF) techniques is usually performed in a low endogenous LH environment due to pituitary desensitisation with a GnRH agonist (GnRH-a). In our institution, COS in normogonadotrophic women is performed with a standard GnRH-a long protocol associated with recombinant FSH (r-hFSH). According to our experience, in about 85-90% of these patients, the ovarian response to this protocol is optimal, with more than 6 mature oocytes recruited. In about 10-15% of cases, however, an initial low response is seen, which in turn leads physicians to augment the daily dose of r-hFSH. The increase in the daily dose of FSH and in the stimulation length often results in a higher total FSH consumption (e.g. >2,500 IU). These observations lead to develop the concept of “hypo-response” to COS to identify those normogonadotrophic women who have normal estimated ovarian reserve but require high amounts of FSH to obtain an adequate number of oocytes retrieved [4-7, 9, 16]. Such women seem to be distinct from classical poor responders because they are normo-ovulatory and normogonadotrophic and present normal antral follicular count (AFC). In addition, there is evidence that supplementation with exogenous LH activity significantly improves their ART outcome.

The pathogenesis of hypo-response to FSH is unknown. Nevertheless, an increasing body of evidence leads researchers to focus on a possible role of LH and FSH receptor (FSH-R). This idea is based on both basic and clinical studies.

Hypo-response to FSH in light of revised “two cells–two gonadotrophins” model

The classical “two cells–two gonadotrophins” model highlighted the role of LH in promoting androgen production and release throughout folliculogenesis [10, 13]. According to this model, LH exerts its activity in theca cells, which express enzymatic pathways of androgen synthesis. Theca involucres surround the granulosa cells, whose activities and proliferation are directly regulated by FSH. This hormone induces the expression of the aromatase enzyme, which in turn converts theca-deriving androgens into estradiol (E2). This theory reinforced the notion that granulosa and theca cells are distinct compartments regulated by FSH and LH, respectively. This model has been recently revised. More specifically, it has been found that LH receptors are also detectable on the granulosa compartment at the intermediate follicular phase [8, 11, 13, 22]. Therefore, it appears that LH regulates both granulosa and theca cells.

FSH and LH cooperate in inducing the local production of the soluble molecule B inhibin, and growth factors. Among these, insulin growth factors (IGF)-I and II, which are expressed by both granulosa and theca cells throughout folliculogenesis, are important in promoting follicular maturation [14, 25]. Locally produced peptides, rather than estrogens, are known to be the key factor regulating primate follicle growth and development [17, 20, 21, 24]. In light of these findings, we can conclude that:
- both gonadotrophins contribute (via granulosa) to maintain the autocrine-paracrine system governing dominant/s follicle/s growth;
- LH is crucial in sustaining FSH activity in the granulosa during intermediate-late stages of folliculogenesis;

On these bases it is possible to argue that lack of each gonadotrophin can be counteracted by higher levels of the other. This hypothesis is consistent with the observation that FSH activity can be totally substituted by LH once granulosa cells express adequate amounts of LH receptors [11, 23]. Conversely, higher exogenous FSH doses during COS seem to be able to compensate GnRH-a related reduction of LH. Nevertheless, fall of LH concentration and/or activity below a hypothetical threshold may lead to impairment of granulosa paracrine activities, which in turn can determine higher requirement of FSH.

Finally, presence of less effective FSH and/or LH receptors can also lead to an impairment in gonadotrophins dependent mechanism and explain ovarian resistance to FSH in stimulated cycles.

LH and hypo-response to FSH

The idea that LH-dependent mechanism can affect ovarian response to FSH led to evaluate the efficacy of LH supplementation in women selected according to specific profiles of ovarian response to standard r-hFSH doses. In 2001, Lisi et al. [15] conducted a “self-control study” of the effect of recombinant LH (r-hLH) supplementation in 12 patients who, during previous stimulation with r-hFSH, required >3,000 IU to reach follicular maturity. Women had a mean basal FSH of 12.2 IU/l and a mean age of 36.1 years. Re-stimulation entailed the addition of 75 IU of r-hLH from day 57 to the standard r-hFSH regimen. As in the first cycle, all women received triptorelin 0.1 mg daily, from the mid-luteal phase (GnRH-a long protocol). There was no difference in the total consumption of r-hFSH, days of stimulation or number of M2 oocytes per patient between the two cycles of the study. However, fertilization (86.0% vs 60.9%) and clinical pregnancy rates (50.0% vs 5.9%) were significantly higher.
(P < 0.05) in r-hLH supplemented cycles. Despite small sample size and the methodological limits of not randomised trials, this study provided the first clinical evidence that a sub-optimal response to r-hFSH may be significantly improved by LH supplementation.

The role of LH supplementation in hypo-responders was evaluated in at least three controlled randomised trials.

De Placido et al. [4] suggested to recognize hyporesponse since initial phases of r-hFSH administration. The authors noticed that in about 10-12% of normogonadotropic patients, an initial response (i.e., at least five 2-9 mm follicles in each ovary) during the first days of stimulation is followed by a plateau in which there is no significant increase in follicular size or E2 production in the next 3-4 days of stimulation. This profile of initial ovarian response to r-hFSH usually leads physicians to increase the r-hFSH dose. On this basis, De Placido et al. [4] conducted a RCT to determine whether this clinical condition could be reverted by LH administration. Women (age < 37 years, basal FSH ≤ 10 IU/l) who had no follicle with a mean diameter superior to 10 mm and E2 serum levels inferior or equal to 180 pg/ml on day S8 were randomised to receive LH supplementation (N = 20) in the form of hMG (150 IU/day) or an increase in the r-hFSH daily dose (maximum daily dose of 375 IU; N = 23). In order not to modify the daily FSH administration, the r-hFSH dose was reduced to 150 IU in women of the hMG group. Forty women matched for age and body mass index (BMI) and with an initial adequate response to r-hFSH (i.e., a tripling of serum E2 concentration between days 5/8 in association with > 4 follicles > 10 mm on day S8) served as a not randomised control population. All women received triptorelin 3.75 mg (depot preparation) on the first day of spontaneous menstruation. After pituitary desensitisation, a starting dose of 300 IU of r-hFSH was administered. First dose adjustment was performed on day S5. The mean number of oocytes retrieved was significantly higher in women treated with hMG supplementation than in those who received r-hFSH “step up”. Moreover, the ovarian outcome of the hMG group was comparable with that observed in “normal responders”, suggesting that LH supplementation was able to “rescue” this apparently abnormal response to r-hFSH. Following these findings, De Placido et al. [5, 6] evaluated the efficacy of r-hLH supplementation in women displaying an initial ovarian resistance to r-hFSH. With this purpose, they performed a multicentre RCT [6] with the r-hFSH “step up protocol” as reference standard. A total of 229 IVF/ICSI cycles performed in seven Italian units were analysed. In all patients (age < 37 years, basal FSH ≤ 10 IU/l), triptorelin 3.75 mg (depot preparation) was administered on the first day of spontaneous menstruation. The starting dose of r-hFSH was 225 IU/day. Hypo-responders were identified on day 8 (E2 serum levels < 180 pg/ml and at least 6 follicles ranging between 6 and 10 mm, but no follicle with a mean diameter > 10 mm) and randomised to receive either an r-hLH supplementation of 150 IU/day (N = 59), or an increase of 150 IU in the daily r-hFSH dose (N = 58; r-hFSH “step-up” protocol). Also in this case, an age/BMI-matched population of “normal responders” (tripling E2 levels between days S5 and S8, more than 4 follicles > 10 mm on day S8) was selected as a control group (N = 112). The number of cumulus-oocyte complexes (primary end-point) and mature oocytes retrieved was significantly higher in women receiving r-hLH than in those treated with the r-hFSH step up protocol. Moreover, the mean number of mature oocytes of r-hLH group was similar to that observed in “normal responders”. Although power analysis was not performed for these categorical variables, it is noteworthy that also implantation and ongoing pregnancy rates were similar in “hypo-responders” treated with r-hLH and “normal responders” (14.2 and 32.5% vs 18.1 and 40.2%, respectively). Conversely, both parameters were significantly lower (P < 0.05) in “hypo-responders” treated with step-up r-hFSH (10.0 and 22.0%) than in “normal responders”. Ferraretti et al. [9] conducted a RCT on 184 patients (age < 38 years) undergoing the GnRH-a long protocol. Patients with normal initial follicular recruitment (> 10 antral follicles ≥ 8 mm in diameter and E2 ≥ 100 pg/ml) with the fixed starting dose of recombinant FSH (150-300 IU), but showing a plateau in follicular growth between days S7 and S10 (no increase in follicle size or in E2 level) were randomised as follows: group A (N = 54) received an increase in the daily dose of r-hFSH; group B (N = 54) received 75-150 IU of r-hLH in addition to the increased dose of FSH; group C (N = 26) received hMG; group D consisted of 54 age-matched patients with an optimal response (no need to increase the FSH dose). The mean number of oocytes retrieved was significantly lower in group A (8.2) vs the other groups (11.1, 10.9, 9.8 in groups B, C, and D, respectively). Furthermore, the pregnancy-per-embryo transfer and implantation rates were significantly higher in group B than in groups A and C, and did not differ from normal responders.

Taken together, these studies reinforced the idea that hypo-responders benefit from LH supplementation.

Interestingly, all RCTs [4, 6, 9], clearly showed that hypo-responders who benefited from LH activity had endogenous levels of LH in the normal range. In addition, endogenous LH concentrations of these patients during early phases of COS were always comparable with those observed in women who had optimal response to FSH and who did not require any change of FSH dose during stimulation. This observation led to the hypothesis that hypo-response to r-hFSH is associated with a less bioactive LH. We have recently performed an observational trial [1] aimed to evaluate whether the presence of a com-
mon polymorphism of LH (v-LH), which has been shown to have altered in vitro and in vivo activities, is associated with different profiles of ovarian response to r-hFSH. V-LH was initially discovered by Petterson and Söderholm [19] as an immunologically anomalous form of LH. V-LH is due to two point mutations in the β subunit gene, both altering the aminoacid sequence (Trp8Arg and Ile15Thr). V-LH has elevated bioactivity in vitro but significantly shorter (5-9 min) half life in circulation when compared with the wild type LH (wt-LH) (12-22 min). As the pulse frequency of the v-LH is normal, this results in an overall LH action that is more potent at the receptor site, but shorter in duration in vivo.

The v-LH is common worldwide, with carrier frequency varying from 0 to 52% in various ethnic groups (figure 1). Its incidence in Italy ranges between 12-13%. The v-LH differs functionally from wt-LH, and it seems to predispose its carrier, both men and women, to mild aberrations of reproductive function. In our observational trial, sixty normogonadotrophic patients undergoing a GnRH-a long down-regulation plus r-hFSH for IVF/ICSI, and in whom at least 5 oocytes were retrieved were divided into three groups: 22 women requiring a cumulative dose of r-hFSH > 3,500 IU constituted group A, 15 patients requiring 2,000-3,500 IU were included in the group B, 23 women requiring < 2,000 IU severed as control group (group C). The presence of the v-LH was evaluated using specific immunoassays. Group A (table 1) showed a significantly lower (P < 0.05) number of oocytes retrieved when compared with group B and C (7.3 ± 1.5, 11.7 ± 2.4 and 14.7 ± 4.1 in the three groups, respectively). Seven carriers (32%) of v-LH were found in group A whereas only one variant (7%) was observed in group B; no variant was detected in group C. This study suggested, for the first time, an association between a less bioactive LH and a higher FSH requirement. In addition, it supports the idea that hypo-responders represent a specific sub-group of patients. In fact, all women requiring > 3,500 IU of FSH had at least 5 oocytes retrieved and showed peak E2 > 500 pg/ml. These findings would have lead physician to classify them as normal responders. Nevertheless, they had a statistically significant reduction of the number of oocytes retrieved and E2 levels when compared with woman requiring lower FSH doses.

In light of these finding we further investigated into the relationship between v-LH and ovarian response to FSH [2]. A total of 204 normogonadotrophic patients undergoing GnRH-a long protocol and r-hFSH administration were retrospectively considered. IFMA assays were performed in each patient to find out the presence of v-LH.

Figure 1. Worldwide occurrence of the common v-LH (by Pettersson and Huhtaniemi, modified).
The following variables were considered for analysis in an ANOVA model, having the condition of v-LH carrier as independent variable: basal levels of FSH, LH and E2; day 8 levels of FSH and LH; total r-hFSH consumption; number of oocytes retrieved and number of embryos transferred. Among the 204 patients considered, a total of 24 v-LH carriers (21 [10.2%] in heterozygosis and 3 [1.4%] in homozygosis) were found. The r-hFSH consumption was significantly higher ($P < 0.001$) in v-LH carriers (3,558 ± 970 IU in homozygosis carriers; 2,267 ± 824 IU in heterozygosis carriers) than wild type LH homozygotes (1,959 ± 736 IU). These results confirmed that v-LH is associated with ovarian resistance to r-hFSH.

FSH-R polymorphism: possible explanation for hypo-response to exogenous FSH

A polymorphic variant of the FSH receptor (FSH-R) where aminoacid asparagine (Asn), at position 680, is replaced by Serine (Ser), has been associated with higher FSH basal levels and increased number of antral follicles during the early follicular phase [12] (figure 2). In an observational trial, Perez-Mayorga et al. [18] evaluated the relationship between the presence of the Ser/680 FSH-R variant and the ovarian response to COS in 161 norm-ovulatory women undergoing IVF. All women were below 40 years. The distribution of genotypes in the study population was 29% for the Asn/Asn, 45% for the Asn/Ser, and 26% for the Ser/Ser FSH-R variant. E2 levels at the day of human chorionic gonadotrophin (hCG) and number of retrieved oocytes were similar in the three groups. Conversely, basal FSH levels were significantly different among the three groups (6.4 ± 0.4 IU/l, 7.9 ± 0.3 IU/l, and 8.3 ± 0.6 IU/l for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $P < 0.01$). In addition, the mean number of FSH ampoules required for successful stimulation was significantly different among groups (31.8 ± 2.4, 40.7 ± 2.3, and 46.8 ± 5.0 for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $P < 0.05$). These clinical findings demonstrated that the ovarian response to FSH stimulation depends on the FSH-R genotype. Following these observations, Behre et al. [3] tested whether the same daily dose of FSH results in lower levels of oestradiol in women homozygous for the Ser/Ser, and whether the difference can be overcome by higher FSH doses. Fifty-nine women undergoing COS for in IVF or ICSI and homozygous for the FSH-R polymorphism Ser/680, were randomly allocated in the Group I (N = 24), to receive a daily FSH dose of 150 IU/day, or group II (N = 25), to receive an FSH dose of 225 IU/day. In the group III (Asn/Asn, N = 44), the FSH dose was 150 U/day. Age and basal FSH levels were not different between groups. Total FSH doses were comparable in group I (1,631 ± 96 IU) and group III (1,640 ± 57 IU) but significantly higher in group II (2,421 ± 112 IU).

### Table 1. Outcome of ovarian stimulation in 60 young normogonadotrophic women down-regulated with a GnRH-a long protocol and stimulated with r-hFSH, divided according to cumulative r-hFSH consumption

<table>
<thead>
<tr>
<th>Group</th>
<th>FSH dose</th>
<th>N. of V-LH carriers (%)</th>
<th>Suppressed LH (IU/l)</th>
<th>Staring FSH dose (IU/l)</th>
<th>Duration of stimulation (days)</th>
<th>N. of r-hFSH ampoules</th>
<th>Day 5 E2 concentration (pg/ml)</th>
<th>Day 8 E2 concentration (pg/ml)</th>
<th>E2 at hCG day (pg/ml)</th>
<th>N. of oocytes retrieved</th>
<th>Implantation Rate (%)</th>
<th>Pregnancy rate (%)</th>
<th>Abortion rate (%)</th>
<th>Ongoing PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (N = 22)</td>
<td>&gt; 3,500 IU</td>
<td>7 (31.8)</td>
<td>NS</td>
<td>0.85 ± 0.3</td>
<td>201.1 ± 34.9</td>
<td>13.7 ± 1.0</td>
<td>51.9 ± 6.3</td>
<td>104.3 ± 85.6</td>
<td>636.0 ± 514.9</td>
<td>7.3 ± 1.5</td>
<td>11.1</td>
<td>(7) 31.8</td>
<td>(2) 9%</td>
<td>(5) 22.7</td>
</tr>
<tr>
<td>B (N = 15)</td>
<td>2,000-3,500 IU</td>
<td>1 (6.6)</td>
<td>NS</td>
<td>1.2 ± 0.4</td>
<td>195.0 ± 36.7</td>
<td>&lt; 0.05</td>
<td>34.0 ± 5.5</td>
<td>245.3 ± 173.7</td>
<td>987.4 ± 579.5</td>
<td>&lt; 0.05</td>
<td>11.7 ± 2.4</td>
<td>(6) 40.0%</td>
<td>(1) 6.6%</td>
<td>(5) 33.3%</td>
</tr>
<tr>
<td>C (N = 23)</td>
<td>&lt; 2,000 IU</td>
<td>0</td>
<td>NS</td>
<td>1.6 ± 0.7</td>
<td>192.4 ± 37.2</td>
<td>NS</td>
<td>18.9 ± 3.1</td>
<td>234.6 ± 250.6</td>
<td>1,549.7 ± 1,110.6</td>
<td>&lt; 0.05</td>
<td>14.7 ± 4.1</td>
<td>&lt; 0.001</td>
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</table>

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### FSH-R polymorphism: possible explanation for hypo-response to exogenous FSH

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(P < 0.001). Peak E2 levels were significantly lower in group I (5,680 ± 675 pmol/l) compared to group III (8,679 ± 804 pmol/l) (P < 0.05). Increasing the FSH dose from 150 to 225 IU/day overcame the lower E2 response in women with Ser/Ser (group II, 7,804 ± 983 pmol/l). The authors concluded that the Ser/Ser FSH-R results in lower E2 levels following FSH stimulation. This lower FSH receptor sensitivity can be overcome by higher FSH doses. The study represented the first case of pharmaco-genomic approach to COS.

Recently, we have evaluated the occurrence of the Ser680Asn FSH-R variant among women classified as “hypo-responders” (Alviggi et al., unpublished data). Forty-two normogonadotrophic patients undergoing a GnRH-a long down-regulation protocol followed by stimulation with r-hFSH for IVF/ICSI, and in whom at least 5 oocytes were retrieved were retrospectively included. On the basis of the total r-hFSH consumption, patients were divided into two groups: 17 women requiring a cumulative dose of r-hFSH > 2,500 IU constituted group A, whereas 25 patients requiring < 2,500 IU severed as control group (B). DNA was analysed to determine the FSH receptor genotype. E2 peak levels were significantly lower in group A (997 ± 385 pg/ml) when compared with group B (1,749 ± 644; P < 0.001). Group A also had a significantly lower (P < 0.001) number of oocytes retrieved (7.1 ± 1.5 vs 9.6 ± 2.4). Homozygous Ser/Ser receptor variant at codon 680 was observed in 47.0% of women of the group A and in 28.0% of women of the control group. The homozygous Asn/Asn receptor variant was found in 23.6% and 20.0% of patients in the two groups, respectively. Heterozygosis Ser/Asn was detected in 29.4% of patients of the group A and in 52.0% of patients of the group B. These preliminary results reinforced that the Ser 680/variant of the FSH-R is more frequent in women with hypo-response to r-hFSH.

Concluding remarks

Recent clinical data clearly identified a subset of normogonadotrophic “good prognosis” women who show sub-optimal response to the classical association of the GnRH-a long protocol with r-hFSH monotherapy. These
women cannot be classified neither as “poor responders”, because they have normal follicular reserve and usually have at least five oocytes retrieved, nor as “normal responders”, because they require high cumulative dose of r-hFSH during COS. These patients also show a sub-optimal outcome of IVF and seem to benefit from r-hLH administration and/or higher FSH doses. These patients have been recently defined “hypo-responders” [1, 7]. There are clinical observational trials suggesting that hypo-response is a genetically determined condition. More specifically, ovarian resistance to exogenous FSH has been associated with the presence of at least two common polymorphisms, including v-LH-and FSH-R Ser/680 variant. These data strongly support the idea of a tailored gonadotrophins administration based on a pharmacogenomic approach. More specifically, presence of v-LH may lead to consider LH supplementation, whereas timely identification of Ser/680 FSH-R variant may represent an indication to employ higher doses of FSH.

References