Association of FSHR, LH, LHR, BMP15, GDF9, AMH, and AMHR polymorphisms with poor ovarian response in patients undergoing in vitro fertilization

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ABSTRACT
Objective: This paper aimed to assess the correlation between LH, LHR, GDF9, FSHR, AMH, AMHR2, and BMP15 polymorphisms, which are related to follicular development, and decreased ovarian response in women undergoing controlled ovarian hyperstimulation (COH) for IVF.

Methods: This age-matched case-control study included three or four controls per woman undergoing COH. Controls were women with normal ovarian response (NOR) and cases were women with poor ovarian response (POR) in oocyte retrieval (three or fewer oocytes). DNA was extracted from peripheral blood and potential associations with gene polymorphisms related to follicular development (LH, LHR, GDF9, FSHR, AMH, AMHR2, and BMP15) were analyzed.

Results: Sixty-six patients were included, 52 in the NOR and 14 in the POR group. Two GDF9 polymorphisms were associated with follicular response after COH, one associated with POR - the presence of a mutant polymorphism in heterozygosis and homozygosis of the GDF9 398-39 (C to G) [23% NOR versus 68% POR (OR 4.01, CI 1.52-10.6, p=0.005)] - and another associated with protective response - the presence of normal homozygosis of GDF9 (C447T) [19.2% NOR versus 50% POR (OR 0.34, IC 0.14-0.84, p=0.019)]. No additional associations were found between the other analyzed polymorphisms and POR.

Conclusions: This study found that GDF9 appears to play an important role in follicular development, whereas polymorphisms in its DNA chain may negatively affect ovarian reserve, such as 398-39 (C to G), or positively, as seen in C447T.

Keywords: poor ovarian response, polymorphisms, ovarian reserve, IVF, SNPs

INTRODUCTION
Approximately 10% of women seeking fertility treatment have diminished ovarian reserve (DOR), defined as a decreased number or quality oocytes (Ferraretti et al., 2011). Poor ovarian response (POR) is defined as meeting two or three of the following criteria: age greater than 39 years; prior POR to conventional stimulation protocols (less than three oocytes retrieved); and abnormal ovarian reserve testing (Ferraretti et al., 2011). DOR can have multiple etiologies, including autoimmune, idiopathic, iatrogenic, and genetic causes, and single nucleotide polymorphisms (SNPs) (Goswami & Conway, 2005; Greene et al., 2014). The impact of mutations and polymorphic variant genes involved in folliculogenesis is still uncertain.

Polymorphisms exist within genes and are a source of variation between individuals. Gene association studies have identified a number of SNPs that affect gonadotropin, steroid, and transforming growth factor beta (TGFβ) pathways, which include TGF-β, anti-Müllerian hormone (AMH), activins, inhibins, bone morphogenetic proteins (BMPs), and growth differentiation factors (GDFs) involved in ovarian response (Greene et al., 2014). Most of them affect mRNA levels or the protein sequence, and thus lead to quantitative functional protein variations that may account for the observed inter-individual variability in controlled ovarian hyperstimulation (COH) (Levi et al., 2001; Greene et al., 2014).

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP-15), members of the TGFβ superfamily, are potent regulators of folliculogenesis and ovulation and are both expressed in oocytes from early stage follicles (Aaitonen et al., 1999; Chang et al., 2016; Sanfins et al., 2018). With respect to COH phenotypes, GDF9 and BMP15 alleles have been associated with stimulation outcome (Moron et al., 2006; Wang et al. 2010a; Hanevik et al., 2011; Bilbio et al., 2020). Several genetic variants of GDF9 have been identified, and their correlation with POR has been noted, suggesting that these variants contribute to aberrant follicular development and oocyte loss (Di Pasquale et al., 2004; Shimizu et al., 2004; Abir et al., 2008; Wang et al., 2010a;b). Other variant alleles of BMP15 were associated with increased follicle production in COH, such as the 9C>G polymorphism, which was associated with high response to ovarian stimulation (Moron et al., 2006; Hanevik et al., 2011).

The follicle stimulating hormone receptor (FSHR) carries more than two thousand SNPs, and one polymorphism, Asn608Ser, was found to have an important impact on ovarian response (Livshyts et al., 2009; Sheikha et al., 2011; Pabalan et al., 2014). Homozygous mutant women with this polymorphism have higher baseline FSH levels, require higher FSH doses during COS, and present lower estradiol levels than heterozygous and normal homozygous women. Another study showed that women homozygous for the Ser680 variant had a greater number of mature oocytes than women homozygous for the Asn680 variant (Lawson et al., 2003; Ferrarini et al., 2013).

AMH (Ile49Ser) and AMHR polymorphisms (482A>G) have been associated with variations in estradiol levels and may modulate FSH sensitivity (de Boer et al., 2002). In regard to LH polymorphisms, variant 8Arg-15Thr in women undergoing IVF is more frequently seen among poor
responders to rFSH and women with ovarian resistance to rFSH who, despite requiring higher rFSH dosages, have fewer oocytes retrieved (Alviggi et al., 2009).

This paper aimed to assess the correlation between LH, LHR, GDF9, FSHR, AMH, AMHR2, and BMP15 polymorphisms, which are related to follicular development, and decreased ovarian response in women undergoing COH for IVF.

MATERIALS AND METHODS

This age-matched case-control study included three or four controls per case involving women undergoing COH for IVF. Controls were women with normal ovarian response (NOR) and cases were women with POR in oocyte retrieval (three or fewer oocytes). The study was carried out at the Pronatus Assisted Reproduction Center and at the Federal University of Rio Grande do Sul. The study was approved by the National Ethics and Research Committee on Human Beings and granted certificate no. 25525413.0.0000.5327 by the Ethics Committee of the Hospital de Clínicas de Porto Alegre (an Institutional Review Board equivalent).

Sixty-six patients were included in the study. The inclusion criteria were as follows: age between 30 and 39 years; presence of both ovaries; diagnosis of infertility (more than one year) by male factor, tubal factor (confirmed by hysterosalpingography or videolaparoscopy), or unexplained causes; and no prior IVF treatment. Three or four controls were matched by age to each case. The exclusion criteria were as follows: endocrine disorders; polycystic ovary syndrome; prior chemotherapy; abnormal karyotype; and other factors affecting ovarian function, such as ovarian surgery and endometrioma.

The patients underwent antral follicle counts on Day 2 or 3 of the menstrual cycle and were tested for FSH, LH, estradiol, and anti-Müllerian hormone (AMH) levels. Ovarian stimulation was initiated on Day 3 of the menstrual cycle with recombinant FSH (Puregon, Organon) and LHCG, and, following denudation, the oocytes were categorized as metaphase II (MII), metaphase I (MI), or prophase I (PI) stage. After oocyte retrieval, the patients were split into two groups: NOR (four or more oocytes) or POR (three or fewer oocytes).

Whole blood samples were collected and aliquots of 350 μL from each sample were used for genomic DNA extraction using the Easy DNA kit according to manufacturer’s instructions (Invitrogen, UK). Concentration and purity were determined by spectrophotometry NanoDrop ND1000 (NanoDrop Technologies Inc, USA). DNA was used for analysis of polymorphisms in the LHβ, LHR, GDF9, FSHR, AMH, AMHR2, and BMP15 genes (Table 1).

Polymerase chain reaction (PCR) was performed to amplify the regions of interest in the LHβ, LHR, FSHR and GDF9 genes (Table 1). Forward and reverse primers delimited the region where the polymorphisms were located, as described in Table 1. Amplification of DNA at a concentration of 200 ng was performed in a thermocycler (Veriti® 96-Well Thermal Cycler, Applied Biosystems, USA) with reagent Invitrogen (UK). The following annealing temperatures were applied: -63°C, LHR; -64°C, GDF9; -63°C, FSHR; and LH, 60°C. PCR products were stained with SYBR® Gold (Life Technologies, USA) and verified by electrophoresis on 1.5% agarose gel, and the DNA fragments obtained had the following sizes: LHβ - 662 base pairs, LHR - 350 pairs bases, GDF9 - 491 base pairs and FSHR - 520 base pairs.

Table 1. Studied gene polymorphisms

| Gene       | Chromosome | Polymorphism | Molecular biology techniques | Primer |
|------------|------------|--------------|------------------------------|--------|
| GDF9b      | 5          | c.398-39 C>G (intron) | Direct sequencing            | Forward: 5' TTGACTTGACTGCCTTGTG 3' |
|            |            | p. Thr147Thr (c.447T>G) |                              | Reverse: 5' AGCTTGAGACTTGTG 3' |
|            |            | p. Glu186Glu (c.546G>A) |                              |        |
|            |            | p. Val121Met (c.646G>A) |                              |        |
| FSHRc      | 2          | p. Asn680Ser | RFLP - restriction enzyme BsrI | Forward: 5' TTGGTGCTATCGTGCG 3' |
|            |            |              |                              | Reverse: 5' AAAGCAAGAGACTGATTAC 3' |
| LHβd       | 19         | p. Trp8Arg   | Direct sequencing            | Forward: 5' GAAGCAGTGTCCTGTTCCA 3' |
|            |            | p. Ile15Thr  |                              | Reverse: 5' GAAGAGAGGGCGTAGAGTTG 3' |
| LHCGRe     | 2          | 18InsLQ     | Direct sequencing            | Forward: 5' CACTCGAGGCGCTCCAAG 3' |
| AMHf       | 19         | p. Ile49Ser (c.146T>G) | Taqman                       | Assay ID: C_25599842_10 |
| AMHR2g     | 12         | c.-482A>G   | Taqman                       | Assay ID: C_1673084_10 |
| BMP15h     | X          | c.-9G>C     | Taqman                       | Assay ID: C_27504454_10 |

*a: cytosine, T: thymine, A: adenine, G: guanine
*Human growth differentiation factor 9
*Follicle-stimulating hormone receptor
*Bone morphogenetic protein 15

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The PCR products (detection of polymorphisms of LHβ, LHR and GDF9 - direct sequencing) were purified using the purification protocol with PEG8000 and 2.5% NaCl and subjected to direct sequencing by the Sanger method on an automated sequencer ABI 3500 Genetic Analyzer (Applied Biosystems, USA). Assays were performed at the Unit of Molecular and Protein Analysis of Hospital de Clínicas de Porto Alegre (UAMP/HCPA). Primers for sequencing were used at a concentration of 4 pmol/μL. The results were compared with NCBI reference sequences LHβ - NM_000894.2, LHR - NM_000233.3, and GDF9 - NM_005260.3 (Table 1).

The product of the PCR for the FSHR gene underwent RFLP using restriction enzyme BSRI for four hours at 65 °C. After digestion, the samples were stained with SYBR ® Gold (Life Technologies, USA) and the results obtained were determined by electrophoresis on 2.5% agarose gel: NN (680Asn/Asn) fragments of 520 base pairs; SS (680Ser/Ser) fragments of 413 and 107 base pairs; and NS (680Asn/Ser) fragments of 520, 413 and 107 base pairs. The results were compared with the NCBI reference sequence NM_000145.3 (Table 1).

The polymorphisms in the AMH, AMHR2, and BMP15 genes were determined by TaqMan Allelic Discrimination - Real-Time PCR from DNA at a concentration of 10-20 ng. The tests shown in Table 1 (Applied Biosystems, USA) were performed on a StepOne ™ Real-Time PCR System (Applied Biosystems, USA) at the Unit of Analysis Molecular and Proteins Hospital de Clínicas de Porto Alegre (UAMP/HCPA).

Observed numbers for each genotype were compared against expected values to test whether the samples were in Hardy-Weinberg equilibrium using the Chi-Squared test with one degree of freedom. Data were tested for normality using the Kolmogorov-Smirnov test, and data with a normal distribution, as determined by the t-test, were used to compare means. The Kruskal-Wallis test was used for nonparametric data. Qualitative variables were analyzed with the Chi-squared test. Statistical analyses to determine the association between the polymorphisms were performed using the Chi-squared test and the Monte Carlo method for multiple simulations assuming that the null hypothesis was correct with fixed marginal values. Marginal values were determined from experimental data. This method determines an empirical P value for the observed data. Moreover, we utilized advanced statistical analysis to evaluate the effect of polymorphisms using logistic regression to predict the effect on POR. Statistical significance was set at p<0.05. Statistical tests were performed on the Statistical Package for the Social Sciences 23 (SPSS Inc., Chicago, IL).

**RESULTS**

The clinical characteristics of patients undergoing COH for IVF are presented in Table 2. Mean age was similar (34.6 NOR versus 35.5 POR, p=0.271), and the women with NOR were heavier than the individuals with POR (65.9 NOR versus 56.4 POR, p=0.001).

Ovarian and hormonal characteristics of patients submitted to COH for IVF are presented in Table 3. Antral follicle count (12.3 NOR versus 4.2 POR, p<0.001), number of follicles greater than 17 mm at rhCG day (7.1 NOR versus 2.0 POR, p<0.001), MII oocyte count (9.8 NOR versus 1.7 POR, p<0.001), and AMH (2.0 NOR versus 0.6 POR, p=0.017) were lower in the POR group.

The test for Hardy-Weinberg equilibrium was performed via the Chi-squared test, and the following results were found: p=0.51 for GDF-9 398; p=0.33 for GDF-9 C447T; p=0.74 for GDF-9 546; p=0.51 for FSHR (Asn680Ser); p=0.60 for LH (Trp8Arg); p=0.60 for LH (Ile15Thr); p=0.99 for LHR (Ile18isnLQ); p=0.16 for AMH (Ile49Ser); p=0.91 for AMHR2 (482A>G); and p=0.98 for BMP15 (9C>G) polymorphisms.

The genotype and allele frequencies of polymorphisms in the NOR and POR groups are demonstrated in Table 4. Two polymorphisms of GDF9 were associated with follicular response after COH. One was associated with POR: the presence of a mutant polymorphism in heterozygosis and homozygosis of the GDF9 398-39 (C to G) (23% NOR versus 68% POR, OR 4.01, IC 1.52-10.6, p<0.001); and the presence of normal homozygosis of GDF9 (C447T) were associated with follicular response (19.2% NOR versus 68% POR, OR 4.01, IC 1.52-10.6, p<0.001), MII oocyte count (9.8 NOR versus 1.7 POR, p<0.001), and AMH (2.0 NOR versus 0.6 POR, p=0.017) were lower in the POR group.

When we evaluated our data with logistic regression to investigate the influence of polymorphisms, only the GDF9 398 polymorphism was associated with POR after controlling for bias from other polymorphisms (Table 5). The logistic regression with other parameters of poor responders showed that AMH, gonadotropin, and the GDF9 C447T polymorphism were associated with POR.

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**Table 2.** Clinical characteristics of patients submitted to controlled ovarian hyperstimulation for *in vitro* fertilization (mean±SD)

| Parameter                  | Normal ovarian response n = 52 | Poor ovarian response n = 14 | p value* |
|----------------------------|--------------------------------|-----------------------------|----------|
| Age (years)                | 34.6±3.3                       | 35.5±3.7                    | 0.271    |
| Infertility (years)        | 3.9±2.68                       | 5.3±4.7                     | 0.208    |
| Menstrual cycles (days)    | 29.1±3.98                      | 28.3±2.25                   | 0.504    |
| Ethnicity                  |                                 |                             |          |
| -Caucasian                 | 25                             | 4                           | 0.341b   |
| -African-American          | 1                              | 0                           |          |
| -Latin American            | 26                             | 10                          |          |
| Pregnancy (n)              | 0.08±0.33                      | 0.21±0.57                   | 0.093    |
| BMIc (kg/m²)               | 24.6±3.18                      | 22.0±3.10                   | 0.005    |
| Weight (kg)                | 65.9±9.38                      | 56.4±8.13                   | 0.001    |
| Height (m)                 | 1.6±0.06                       | 1.5±0.06                    | 0.117    |

*aT-test

*bChi-squared test

cBMI: Body mass index
GDF9 also stimulates granulosa cell proliferation (Vitt et al., 1996), cumulus cell expansion (Yan et al., 2001), follicular apoptosis inhibition (Orisaka et al., 2006), and enhancement of oocyte and embryo development (Hussein et al., 2006; Yeo et al., 2008). In vitro studies using recombinant GDF9 protein have clarified the biological roles and the importance of GDF9 in follicular growth and development in all stages of folliculogenesis. In the pre-antral stage, GDF9 effectively stimulated the growth of in vitro cultured preantral follicles (Hayashi et al., 1999). GDF9 also promotes early preantral follicular growth in human ovaries (Hreinsson et al., 2002). In the transition to the antral stage, it appears that GDF9 promotes follicular survival by suppressing granulosa cell apoptosis and follicular atresia (Orisaka et al., 2006). This may be achieved in part by GDF9 stimulation of FSHR expression, since adequate FSRH levels in granulosa cells are essential for FSH-dependent antral follicle growth.

GDF9 also plays an important role during the final stages of follicular growth prior to ovulation. Prior to LH surge, cumulus cells require GDF9 to support metabolic cascades such as glycolysis and sterol biosynthesis (Sugiura et al., 2000), cumulus cell expansion (Hussein et al., 2006; Yeo et al., 2008). In vitro studies, and in vivo studies, and humans studies have revealed the role of GDF9 in regulating follicular development, little is known about it in human ovarian function, since studies have demonstrated a strong correlation between GDF9 polymorphism in women with DOR and poor ovarian response followed by poor IVF outcomes, indicating that GDF9 plays an important role in determining ovarian reserve status and function (Wang et al., 2010b; 2013; Sanfins et al., 2018; Bilibio et al., 2020). In this study, we saw that the number of antral follicles, total

**Table 3. Ovarian and hormonal characteristics of patients submitted to controlled ovarian hyperstimulation for in vitro fertilization (mean±SD)**

| Parameter | Normal ovarian response | Poor ovarian response | p value* |
|-----------|-------------------------|-----------------------|----------|
| Antral follicle count (n) | 12.3±4.6 | 4.2±1.5 | <0.001 |
| Induction length (days) | 9.7±1.6 | 8.7±2.2 | 0.005 |
| Gonadotropin administration (RFSH) (UI) | 1586±281 | 1573±405 | 0.270 |
| Endometrial thickness (mm) | 9.9±1.8 | 8.9±2.1 | 0.095 |
| Follicles > 17 mm on rhCG Day (n) | 7.1±3.3 | 2.0±0.6 | <0.001 |
| Follicles between 14-16 mm on rhCG day (n) | 3.3±2.0 | 1.0±0.8 | <0.001 |
| Follicles between 12-14 mm on rhCG day (n) | 2.5±1.7 | 0.4±0.8 | <0.001 |
| Total oocytes (n) | 12.4±7.4 | 2.1±0.9 | <0.001 |
| MII oocytes (n) | 9.8±6.2 | 1.7±0.8 | <0.001 |
| FSH Day 3 of menstrual cycle | 5.0±3.5 | 6.9±6.0 | 0.185 |
| LH Day 3 of menstrual cycle | 50.9±58.3 | 56.4±41.2 | 0.850 |
| AMH | 4.5±3.3 | 4.2±1.5 | 0.721 |
| LH day rhCG | 2.0±2.4 | 0.6±0.5 | 0.017 |
| Progesterone day rhCG | 1.6±1.9 | 1.4±1.1 | 0.071 |
| E2 day rhCG | 0.9±1.1 | 0.7±1.3 | 0.717 |
| Prolactin (ng/ml) | 1370.8±778.2 | 568.3±306.0 | 0.001 |
| E2 day rhCG | 21.3±17.6 | 18.2±19.2 | 0.694 |

*Chi-squared test
*BMI: Body mass index
*T-test

**DISCUSSION**

The present study revealed that GDF9 polymorphisms were associated with follicular response after COH for IVF. These polymorphisms can have a negative impact, i.e., the presence the allele mutant genotype in heterozygosity or homozygosis of GDF9 398-39 (C to G) was found in 69% of the individuals in the POR group and in only 23% of the women in the NOR group (OR 4.01). They may also have a protective response, i.e., the presence of normal homozygosis of GDF9 (C447T) was associated with POR (19.2% versus 50% POR).

Multivariate analysis showed the possible influence of polymorphisms of genes regulating follicular growth and oocyte development (FSHR, LH, LHR, AMH, AMH2, BMP15 and GDF9). Response to administration of gonadotropins and FSH (Elvin et al., 1999), but not without FSH (Dragovic et al., 2008). Unlike our study, other authors described associations between the FSHR (Livshyts et al., 2009; Sheikhha et al., 2011) and LHGR polymorphisms (Skiadas et al., 2012) and POR. A possible cause for this difference is the variety of ethnic groups within the populations included in each study.

GDF9 plays a pivotal role during early folliculogenesis, and deletion of GDF9 in mice causes follicular arrest at the primary stage and infertility (Dong et al., 1996). GDF9 also stimulates granulosa cell proliferation (Vitt et al., 2000), cumulus cell expansion (Yan et al., 2001), follicular apoptosis inhibition (Orisaka et al., 2006), and enhancement of oocyte and embryo development (Hussein et al., 2006; Yeo et al., 2008). In vitro studies using recombinant GDF9 protein have clarified the biological roles and the importance of GDF9 in follicular growth and development in all stages of folliculogenesis. In the pre-antral stage, GDF9 effectively stimulated the growth of in vitro cultured preantral follicles (Hayashi et al., 1999). GDF9 also promotes early preantral follicular growth in human ovaries (Hreinsson et al., 2002). In the transition to the antral stage, it appears that GDF9 promotes follicular survival by suppressing granulosa cell apoptosis and follicular atresia (Orisaka et al., 2006). This may be achieved in part by GDF9 stimulation of FSHR expression, since adequate FSRH levels in granulosa cells are essential for FSH-dependent antral follicle growth.
Polymorphisms and ovarian reserve - Meireles, AJC.

### Table 4. Genotype and allele frequencies of polymorphisms in women submitted controlled ovarian hyperstimulation for IVF with normal ovarian response (NOR) and poor ovarian response (POR).

| Polymorphism       | Population studied | N   | Genotypes | p value | Alleles | OR (95% CI) | p value |
|--------------------|--------------------|-----|-----------|---------|---------|-------------|---------|
|                   |                    |     | n (%)     | n (%)   | n (%)   | n (%)       |         |
|                   |                    | S2  | CC        | 40 (76.9%) | 10 (19.2%) | 2 (3.8%) | 90 (86.5%) | 14 (13.5%) | 4.01 (1.52-10.60) | 0.005 |
| GDP9 (398-39; C to G) | NOR                | 52  | CC        | 7 (13.8%) | 24 (46.2%) | 18 (34.6%) | 44 (42.3%) | 60 (57.7%) | 0.34 (0.14-0.84) | 0.019 |
|                   | POR                | 13  | CT        | 5 (35.7%) | 2 (14.3%)  |           | 19 (67.9%) | 9 (32.1%)   |               |       |
| GDP9 (C447T)       | NOR                | 52  | TT        | 2 (14.3%) | 15 (28.8%) | 2 (14.3%) | 89 (85.6%) | 17 (16.4%) | 0.87 (0.26-2.83) | 0.821 |
|                   | POR                | 14  | AA        | 2 (14.3%) | 2 (14.3%)  |           | 24 (85.7%) | 4 (14.3%)   |               |       |
| GDF9 (G546A)       | NOR                | 52  | GG        | 11 (78.6%) | 11 (78.6%) | 2 (14.3%) | 89 (85.6%) | 17 (16.4%) | 0.87 (0.26-2.83) | 0.821 |
|                   | POR                | 14  | AG        | -         | 2 (14.3%)  |           | 24 (85.7%) | 4 (14.3%)   |               |       |
| FSHR (Asen680Ser)  | NOR                | 52  | NN        | 18 (34.6%) | 25 (48.1%) | 9 (17.3%) | 61 (58.7%) | 43 (41.3%) | 1.41 (0.61-3.27) | 0.410 |
|                   | POR                | 14  | NS        | 2 (14.3%) | 10 (71.4%) | 2 (14.3%) | 14 (50.0%) | 14 (50.0%) |               |       |
| LH (Tyr8Arg)       | NOR                | 52  | TT        | 45 (86.5%) | 7 (13.5%)  | 0 (0.0%) | 97 (93.3%) | 7 (6.7%)   | 0.51 (0.06-4.35) | 0.541 |
|                   | POR                | 14  | TC        | 13 (29.2%) | 7 (17.1%)  | 0 (0.0%) | 27 (59.6%) | 13 (29.2%) |               |       |
| LH (Ile13Thr)      | NOR                | 52  | TT        | 45 (86.5%) | 7 (13.5%)  | 0 (0.0%) | 97 (93.3%) | 7 (6.7%)   | 0.51 (0.06-4.35) | 0.541 |
|                   | POR                | 14  | TC        | 13 (29.2%) | 7 (17.1%)  | 0 (0.0%) | 27 (59.6%) | 13 (29.2%) |               |       |
| LHR (18insLQ)      | NOR                | 52  | NN        | 31 (61.5%) | 18 (34.6%) | 3 (5.8%) | 80 (78.4%) | 22 (21.6%) | 0.82 (0.28-2.43) | 0.729 |
|                   | POR                | 14  | NV        | 9 (16.5%)  | 4 (28.6%)  | 2 (3.8%) | 22 (78.5%) | 5 (21.5%)   |               |       |
| AMH (Ile49Ser)     | NOR                | 52  | TT        | 30 (57.7%) | 21 (40.4%) | 1 (1.9%) | 81 (77.9%) | 23 (22.1%) | 1.95 (0.79-4.81) | 0.144 |
|                   | POR                | 14  | TG        | 5 (35.7%)  | 8 (57.1%)  | 1 (1.9%) | 18 (64.3%) | 10 (35.7%) |               |       |
| AMH-R2 (A to G)    | NOR                | 52  | AA        | 41 (78.8%) | 11 (21.1%) | 0 (0.0%) | 93 (89.4%) | 6 (10.6%)  | 2.30 (0.76-6.91) | 0.136 |
|                   | POR                | 14  | AG        | 9 (63.4%)  | 4 (28.6%)  | 1 (1.9%) | 22 (78.5%) | 6 (21.5%)   |               |       |
| BMP15 (9>C)        | NOR                | 52  | CC        | 33 (63.5%) | 16 (30.8%) | 3 (5.8%) | 82 (78.9%) | 22 (21.1%) | 1.01 (0.36-2.81) | 0.975 |
|                   | POR                | 14  | CC        | 8 (57.1%)  | 6 (42.9%)  | 0 (0.0%) | 82 (78.9%) | 22 (21.1%) | 1.01 (0.36-2.81) | 0.975 |

*a Monte Carlo test  
*b: C: cytosine, T: thymine, A: adenine, G: guanine  
*c: No statistics are computed because GDF9 G646A is a constant  
*d: There was one less case in this group due to failure of DNA sequencing.
Table 5. Logistic regression - influence of polymorphisms on ovarian response after controlled ovarian hyperstimulation for IVF

| Unstandardized Coefficients B | Standardized Coefficients | Beta   | 95% Confidence Interval for B |
|-------------------------------|---------------------------|--------|-----------------------------|
| Constant                      | 3.087                     | 0.393  | 21.916                      |
| GDF9 (398-39; C to G)         | -1.361                    | 0.039  | 0.257 (0.070-0.935)         |
| GDF9 (C447T)                  | 0.841                     | 0.113  | 2.318 (0.820-6.555)         |
| GDF9 (G546A)                  | 0.675                     | 0.296  | 1.964 (0.554-6.968)         |
| FSHR (Asn680Ser)              | -0.937                    | 0.077  | 0.392 (0.139-1.107)         |
| LH (Trp8Arg/Ile15Thr)         | 0.263                     | 0.678  | 1.301 (0.376-4.498)         |
| LHR (18insLQ)                 | 0.344                     | 0.631  | 1.410 (0.346-5.730)         |
| AMH (Ile49Ser)                | -0.371                    | 0.371  | 0.690 (0.306-1.555)         |
| AMH R2 (A to G)               | 0.110                     | 0.810  | 1.116 (0.457-2.723)         |
| BMP15*(9C>G)                  | 0.275                     | 0.675  | 1.316 (0.364-4.759)         |

*Bone morphogenetic protein 15

Correction and treatment to improve the number of oocytes retrieved in patients with this polymorphism.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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