A chromosome 19 locus positively influences the number of retrieved oocytes during stimulated cycles in Brazilian women

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Abstract

Purpose To evaluate if several genetic loci that are associated with variation in normal menopause age and early menopause can account for a poor response to controlled ovarian stimulation.

Methods A total of 71 patients age ≤ 35 years old who were undergoing intracytoplasmic sperm injection were genotyped for four genetic variants that are associated with normal variation in menopausal age and early menopause. The patients were divided into two groups based upon treatment response: a poor responder group (PR group, $n=21$) and a normal responder group (NR group, $n=50$). The genetic variants *rs244715*, *rs9379896*, *rs4806660* and *rs16991615* were analyzed.

Results There was no significant difference in the incidence of the genetic variants between the NR and PR group. The risk allele for the chromosome 19 variant (*rs4806660*) demonstrated a protective effect for a poor ovarian response. The presence of a risk allele was associated with an increased response to COS, which resulted in an elevated number of follicles (Coef: 2.54, $P=0.041$) and retrieved oocytes (Coef: 1.41, $P=0.041$).

Conclusions The genetic variants *rs244715*, *rs9379896*, *rs4806660* and *rs16991615* are not risk factors for poor ovarian response in Brazilian women. In contrast, *rs4806660* is associated with higher number of follicles and retrieved oocytes. *rs4806660* may be associated with an increased response to gonadotrophin stimulation in this population.

Capsule *rs4806660* may be associated with an increased response to gonadotrophin stimulation in Brazilian women.

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Introduction

Modern trends show that women have been postponing childbirth in recent years. This trend has consequently led to an increase in age-related infertility, which has increased the application of assisted reproductive technologies [5, 27, 58]. However, premature reduction in the ovarian follicle number has been demonstrated to significantly affect the success of in vitro fertilization (IVF), despite the age of the woman [42, 50], and is thus a challenge for these patients who undergo ART [43].

Despite recent advances in assisted conception, estimates suggest that 9–24% of patients who undergo IVF treatment have a poor ovarian response [17]. Therefore, poor ovarian

response to gonadotrophin stimulation remains a significant problem in modern fertility treatment, especially with in vitro fertilization (IVF), where a large number of oocytes is desirable.

A poor response to IVF may be an early sign of ovarian ageing and reduced ovarian reserve [11]. This phenomenon was initially described by Garcia et al. [12], who defined this condition as patients who have a peak E2 level <300 pg/mL after standard stimulation with human menopausal gonadotrophin. Poor responders have been defined based on the presence of mature oocytes [23], an elevated early follicular phase of the FSH peak [10], the number of basal antral follicles [28], the number of follicles at the end of ovarian stimulation [53], the plasma oestradiol concentration [49] or the number of oocytes retrieved [20]. A novel consensus defined poor responders as patients who have an advanced maternal age or any other risk factor for poor response in combination with a previous poor response and an abnormal ovarian reserve test prediction [11].

A large number of clinical parameters for predicting menopause age rely upon detecting peri-menopausal changes in the oocyte number and are therefore poor long-term predictors [11, 22]. Hormonal serum levels change prior to menopause, including the basal follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH) and inhibin B levels. Of these endocrine markers, AMH seems to be the best long-term predictor because AMH levels decrease approximately 10 years prior to menopause [6, 21, 37, 57]. Other additional markers of ovarian reserve are the antral follicle count (AFC) and ovarian volume [22]. Although the relationship between the test results and true ovarian reserve is unknown, these may predict in a moderate or good way quantitative parameters, but not accurately predict qualitative parameters. In conclusion, both AMH and AFC are currently considered to be the most reliable and accurate markers of ovarian reserve [6, 21]. Apart from the maternal age, endometriosis and prior pelvic surgery can also be used as predictors for a poor ovarian response [38].

In contrast to transient parameters, genetic predictors of menopausal age are present from birth. Genetic markers may therefore provide reproductive lifespan information much earlier than traditional markers and allow women to make informed reproductive choices at earlier ages. Current estimates suggest that the heritability of menopausal age ranges from 31% to 87% [8, 33, 34, 47, 52, 55], where variability among family members is attributed to common environmental factors [33].

Numerical and structural chromosomal aberrations as well as mutations or variability in specific genes that play a role in reproductive ageing may influence reduced ovarian reserves [9]. Genetic variation occurs naturally in the human genome both as a result of errors or mistakes during cell division and DNA replication as well as due to viral and

chemical mutagenesis [19]. The most common type of inter-individual genetic variation in the human genome is a single nucleotide polymorphism (SNP), which is present in at least 1% of any population, while the frequency of point mutations is less than 1% [7, 15, 19, 44].

Recent genome-wide association studies (GWAS) have successfully identified genetic loci for numerous complex traits. For example, several osteoporosis susceptibility SNPs have been described that influence the age of natural menopause in Chinese women [62], and multigenic interactions formed by three SNPs related to oestrogen signalling pathways are correlated with the normal menopause age in a Spanish population [31]. Two independent large-scale studies identified loci on chromosomes 5, 6, 19 and 20 that are associated with variation in the normal menopause age [15, 48]. Moreover, Murray et al. [35] demonstrated that these four variants increase the odds of having early menopause. However, whether these variants can account for a poor response to controlled ovarian stimulation in IVF patients is still unknown. Thus, the goal of this study was to determine if these four previously identified SNPs are correlated with a poor response in IVF patients.

Materials and methods

Patients

A total of 71 Caucasian patients undergoing intracytoplasmic sperm injection (ICSI) were included in this study. All of the patients were 35 years old or younger. The patients were divided into two groups according to their response to Controlled Ovarian Stimulation (Garcia et al.): a poor responder group (PR group, patients with number of retrieved oocytes ≤ 4 , $n=21$) and a normal responder group (NR group, patients with a number of retrieved oocytes from 5 to 15, $n=50$). For all of the patients, we analyzed four newly identified genetic variants that have been associated with normal variation in menopausal age and early menopause: *rs244715*, *rs9379896*, *rs4806660* and *rs16991615* (chromosomes 5, 6, 19 and 20, respectively).

This study was approved by the local Institute review board, and written informed consent was obtained. All of the patients agreed to share the outcome of their own cycles for research purposes.

Controlled ovarian stimulation and oocyte retrieval

The patients started recFSH treatment (Gonal-F®, Serono, Geneva, Switzerland) daily from the third day of their menstrual cycles. The first ultrasound control and the E2 plasma dosage tests were performed at the seventh cycle day. Depending on the response of each patient, controlled by

ultrasound monitoring of the follicles size, the dose of recFSH was adjusted. GnRH antagonist was administered when the dominant follicle was 14 mm in mean diameter. Oocyte retrieval was performed 35 h after the administration of recombinant human chorionic gonadotrophin (rhCG, Ovidrel™, Serono, Geneva, Switzerland) through transvaginal ultrasonography.

Preparation of oocytes and morphology assessment

Retrieved oocytes were maintained in human tubal cultured medium (HTF, Irvine Scientific, Santa Ana, USA) supplemented with 10% Human Synthetic Albumin (HSA, Irvine Scientific, Santa Ana, USA), covered with mineral oil (Ovoil™, Vitrolife) and incubated for 2–3 h before cumulus cell removal. The surrounding cumulus cells were removed via incubation with a HEPES buffered-medium containing hyaluronidase (80 IU/mL, Irvine Scientific, Santa Ana, USA). The remaining cumulus cells were then mechanically removed by gentle pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Oocyte morphology was assessed with an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under 400× magnification just prior to sperm injection (3–4 h after retrieval).

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection (ICSI) was performed with all of the MII oocytes according to the technique described by Palermo et al. [39]. The oocytes were transferred into a micro-injection dish, which was prepared with 4-μL droplets of buffered medium (HEPES, Irvine Scientific, Santa Ana, USA), covered with mineral oil and placed on the heated stage ($37.0 \pm 0.5^\circ\text{C}$) of an inverted microscope. Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and extrusion of the second polar body. The embryos were maintained in a 50-μL drop of HTF medium supplemented with 10% HSA, covered with mineral oil and kept in a humidified atmosphere (6% CO₂ in air) at 37°C until transfer on day 3 of development. High-quality embryos were defined as having the following characteristics: 8–10 cells on the third day of development, less than 15% fragmentation, symmetric blastomeres and the absence of multinucleation and zona pellucida dysmorphisms.

Blood sample collection

Blood samples were collected from all of the patients prior to the beginning of infertility treatment. The blood samples

were centrifuged, and the white cell buttons were removed and cryopreserved with liquid nitrogen. The cells were subsequently thawed and lysed to obtain the cellular DNA.

Genotyping

All of the samples were genotyped for the following four SNPs: *rs244715*, *rs9379896*, *rs4806660* and *rs16991615* on chromosomes 5, 6, 19 and 20, respectively. The genomic DNA was isolated with a QIAamp blood reagent kit (Qiagen) according to the manufacturer's instruction. Genotype analysis was performed with a real-time PCR equipment (7500 Real Time PCR System, Applied Biosystems, Foster City, CA, USA), and TaqMan system (Applied Biosystems, Foster City, CA, USA) was used to detect the amplification product [24, 26].

Each PCR reaction was set up in a final volume of 10 μL and was comprised of 5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM of each primer, 150 nM of each TaqMan Minor Groove Binder (MGB) probe, and 2 μL of the extracted DNA. The PCR cycle consisted of an initial 2-min incubation at 50°C (to allow for AmpErase uracil N-glycosylase cleavage of contaminant PCR products from previous reactions), denaturation at 95°C for 10 min, and amplification with denaturation (95°C, 15 s) followed by annealing (60°C, 60 s) for 45 cycles. The fluorescence was continually monitored during the annealing step for each sample. The assays were analyzed in separate reactions and in duplicate. Positive and negative controls were used in each SNP reaction.

Statistical analysis

The incidence of risk alleles was compared between the PR and NR patients. Furthermore, the influence of the individual risk allele frequency on the response to COS (total dose of FSH administered, number of follicles, number of retrieved oocytes, oocyte recovery rate and metaphase II (MII) oocyte rate) was evaluated. The results are expressed as the mean $\pm$ standard deviation (SD) for numeric continuous variables or proportions (%) for the categorical variables. The mean values were compared with Student's *t* or Mann-Whitney test as appropriate, and proportions were compared with the chi-squared test. Linear regression models were used to determine the effects of the risk allele for each SNP on the variables associated with COS response. The results were expressed as regression coefficients (Coef), R^2 and *P* values. The influence of the risk allele frequency on the odds of being a case was assessed through binary logistic regression, and the results are expressed as odds ratios (OR), 95% confidence intervals (CI) and *P* values. *P* values less than 0.05 were considered to be statistically significant. The data analysis was performed

Table 1 General cycle characteristics and the risk allele frequencies

Variable	PR group (n=21)	NR group (n=50)	P value
Female age (years)	32.4±2.6	32.5±2.5	0.1191
Dose of FSH administered (IU)	2206.5±700.7	2276.8±669.7	0.9059
Follicles	5.6±2.1	15.5±7.1	0.0002
Retrieved oocytes	3.1±0.8	10.2±3.3	<0.0001
Oocyte recovery rate (%)	60.9	71.8	0.2180
MII oocyte rate (%)	70.2	74.8	0.5950
Risk allele frequency for rs244715 (%)	71.4	78.0	0.5537
Risk allele frequency for rs9379896 (%)	90.5	96.0	0.5764
Risk allele frequency for rs4806660 (%)	42.8	52.8	0.4819
Risk allele frequency for rs16991615 (%)	100	100	–

The values are the means ± standard deviation, unless otherwise noted. PR poor responder; NR normoresponder; IU international units

with the Minitab (version 14) Statistical Program (Minitab Inc., USA).

Results

All of the individuals were successfully genotyped for all four of the SNPs. The general characteristics of the patients' cycles and the risk allele frequency for the SNPs are shown in Table 1. None of the four variants was a determinant of the odds of being a case (Table 2).

The influence of the risk allele on the response to COS (total dose of FSH administered, number of follicles, number of retrieved oocytes, oocyte recovery rate and metaphase II (MII) oocyte rate) is shown in Table 3. Patients that possess risk allele rs4806660 showed a significant protective effect related to an increased response to COS, as was demonstrated by a higher number of follicles and retrieved oocytes.

Of the 71 patients, 4 (5.6%) did not possess the risk allele for rs9379896 (2 in the NR group and 2 in the PR group); 17 patients (23.9%) did not possess any risk allele for rs244715 (11 in the NR group and 6 in the PR group) and 36 patients (50.7%) did not possess any risk allele for rs4806660 (24 in the NR group and 12 in the PR group). Conversely, all of the

patients and controls (100%) possessed the risk allele for rs16991615.

The homozygosity and heterozygosity incidences for the risk alleles in both groups are shown in Table 4. When the risk alleles for all four of the SNPs are combined, the number of risk alleles has no influence on the odds of being in the case group (OR: 0.65, CI: 0.40–1.04, $P=0.066$). None of the patients had clinical manifestations of ovarian hyperstimulation syndrome.

Discussion

Although ART relies on COS for the production of a high number of oocytes, a reasonable percentage of women undergoing IVF do not respond adequately to exogenous stimulation [14, 17, 42]. The gold standard in COS is to achieve an adequate number of good quality oocytes while minimizing adverse drug response and cycle cancellation rates [27]. Poor response to COS has been associated with premature ovarian failure (POF), is an early sign of ovarian ageing [11, 36] and has a negative impact on the ART success rate [17, 42]. Because menopause drastically influences women's fertility and health, there has been considerable interest recently regarding the mechanisms that control ovarian ageing and the timing of natural menopause [58].

Recently, four genetic loci on chromosomes 5, 6, 19 and 20 that are associated with variation in the normal menopause age and early menopause were identified in large-scale studies [15, 35, 48]. Chromosome 5 locus (rs244715) is a genetic variant at 5q35.2, in an intronic region of ZNF346 gene, with sequences of 294 amino acids (AA). The protein encoded by this gene is a zinc finger protein that preferentially binds to double-stranded RNA or RNA/DNA hybrids, rather than DNA alone, and may be involved in apoptosis. Another analyzed genetic variant (rs9379896) is at 6p24.2, in a promoter region of SYCP2L gene (synaptonemal complex protein 2-like), a nucleolar protein of 812 AA. Variation on chromosome 19 (rs4806660) is localized in an intronic region

Table 2 The influence of the genetic variants on the odds of ovarian response to COS

SNP	Chromosome	Risk allele frequency	OR (95% CI)	P value
rs244715	5	74.7	0.68 (0.36–1.30)	0.242
rs9379896	6	93.2	0.78 (0.34–1.81)	0.567
rs4806660	19	47.4	0.81 (0.40–1.66)	0.566
rs16991615	20	100	*	0.088

SNP single nucleotide polymorphism; COS controlled ovarian stimulation; OR odds ratio; CI confidence interval

* This value could not be calculated because all of the patients possessed this risk allele

Table 3 The influence of the risk alleles on COS response

Variable	<i>rs244715</i>			<i>rs9379896</i>			<i>rs4806660</i>			<i>rs16991615</i>		
	Coef	R ² (%)	P	Coef	R ² (%)	P	Coef	R ² (%)	P	Coef	R ² (%)	P
FSH	−76.8	0.8	0.458	137.0	1.5	0.320	−46.3	0.3	0.676	58.2	0.0	0.885
N° follicles	−0.09	0.0	0.936	1.02	0.6	0.517	2.54	5.9	0.041	−0.69	0.0	0.863
N° oocytes	−0.258	0.2	0.692	0.324	0.2	0.711	1.41	5.9	0.041	−2.53	1.9	0.254
Recovery (%)	−1.95	0.5	0.561	−2.26	0.4	0.616	−0.21	0.0	0.953	−10.11	1.1	0.376
MII oocyte (%)	0.594	0.1	0.852	4.16	1.4	0.329	−2.89	1.0	0.397	−6.51	0.5	0.549

COS controlled ovarian stimulation; *Coef* coefficient; *R*² coefficient of determination; *P* *P* value; *MII* metaphase II

of TMEM150B gene (transmembrane protein 150B), which encodes a protein of 233 AA. This protein belongs to the DRAM/TMEM150 family, involved in apoptosis pathway. The last analyzed variant on 20p12.3 (*rs16991615*) is the only SNP in an exonic region, at MCM8 gene (minichromosome maintenance complex component 8). The 840 AA protein encoded by this gene is one of the highly conserved minichromosome maintenance proteins, which binds chromatin throughout the cell cycle and is essential for DNA replication and cell proliferation [16, 54]. In this study, we sought to determine if these four loci are also associated with a poor ovarian response in patients undergoing IVF.

Genetic studies facilitate our understanding of disease pathogenesis and may eventually allow us to personalise treatment for patients [44, 46]. Several SNPs alone or in epistatic interactions have been shown to affect the timing of natural menopause in postmenopausal Chinese and Spanish women as well as in white populations [14, 25, 29, 31, 62]. In contrast, oestrogen receptor-1 genetic polymorphisms, although related to POF, were not associated with early menopause in a Korean research study [60]. Microdeletions and microduplications at SNPs in autosomal chromosomes have been associated with POF, although these variants have not been reported to be significant factors compared to the X chromosome [18, 30]. The role of these genomic imbalances in human disorders is not well understood because there is no animal model data or the data are difficult to interpret

Table 4 The homozygosity and heterozygosity incidences of the risk alleles in the NR and PR groups

SNP	Homozygous for risk allele			Heterozygous for risk allele		
	NR (%)	PR (%)	<i>P</i> value	NR (%)	PR (%)	<i>P</i> value
<i>rs244715</i>	46.0	28.6	0.1727	32.0	42.8	0.3820
<i>rs9379896</i>	70.0	66.7	0.7816	26.0	23.8	0.8465
<i>rs4806660</i>	16.0	14.3	1.000	36.0	28.6	0.5949
<i>rs16991615</i>	92.0	100	0.3116	8.0	0.0	0.3116

SNP single nucleotide polymorphism; *NR* normoresponder group; *PR* poor responder group

[30]. A recently published systematic review identified 25 genes and several SNPs associated with the natural menopause age as well as the majority of genes that belong to steroid pathways and vascular-function-related genes. However, when the overall results of previously published studies are compared, the results are rather disappointing [58].

Several studies have investigated possible associations between SNPs and ovarian stimulation outcome [2, 13, 14, 27, 45, 46, 56, 59]. These studies have reported that SNPs in FSH and FSH-receptor genes may be related to adverse outcomes with COS [45, 46, 59]. Additionally, SNPs in genes that encode for other proteins that play a role in follicular development are currently under investigation. Previous studies have reported that oestrogen receptors [2], bone morphogenetic protein 15 (BMP15) [14, 32], LH hormone [3], 5,10-methylenetetrahydrofolate reductase (MTHFR) gene [51], and anti-Müllerian hormone (AMH) and its receptor AMHR2 [13] play a role in follicular development. Although a majority of these studies reported statistically significant results, these findings need to be confirmed in additional study populations.

Our data suggest that the genetic variation associated with normal menopausal age and early menopause are not significant risk factors for a poor response to COS in IVF cycles in young Brazilian patients. For all four of the SNPs that were tested, there was no evidence that the odds of being a PR case, per risk allele, was different from the odds of being a NR case. Another novel study utilised GWASs to look for SNPs in patients undergoing ICSI cycles, and these authors did not observe any correlation between the SNPs and ovarian response, embryo quality or pregnancy rate [56].

We acknowledge that this study is limited by its retrospective design and lack of sample size calculation. Nevertheless, our results indicate that the risk allele for chromosome 19 variant (*rs4806660*) demonstrates a protective effect for poor ovarian response. The presence of the risk allele in either in homozygous or heterozygous women was associated with an increased response to COS, which resulted in an elevated number of follicles and retrieved oocytes.

Because inherited variations in the DNA sequence between individuals can have a major impact on drug response, we hypothesise that *rs4806660* is associated with an increased response to gonadotrophin stimulation in Brazilian patients undergoing ART [19, 44].

Several different genetic studies regarding menopausal age have had difficulty identifying genetic loci that are associated with this complex trait. When the different types of studies are compared, there is almost no overlap in the genes that are associated with menopausal age [46, 58]. Single or combined SNPs, nevertheless, are able to modify endocrine feedback systems and hormone function, thereby establishing inter-individual reproductive performance that can range from high rates of fertility to infertility [45]. However, it is currently unclear how SNPs alone determine the susceptibility of an individual to complex diseases or to adverse drug interactions as most common traits and phenotypes result from long-term interactions between genetic and environmental factors [19, 44].

Another reason that the identified genes may differ from study to study may be related to differences in ethnicity due to genetic heterogeneity. Ethnic-specific variation in the distribution of polymorphisms has been previously demonstrated [1, 4, 14, 46], and the difficulty in correlating any genetic variability with ethnicity has been previously discussed [40, 61]. Studies in Brazilian populations are even more complicated because the population is miscegenated [41]. Nevertheless, the identification of risk alleles in patients previous to COS may help predict poor or normal ovarian response and prevent ovarian hyperstimulation syndrome.

The genetic variants *rs244715*, *rs9379896*, *rs4806660* and *rs16991615* are not risk factors for a poor ovarian response in Caucasian Brazilian women undergoing ART. Instead, *rs4806660* is associated with a higher number of follicles and retrieved oocytes. We hypothesise that the genetic variant *rs4806660* is associated with an increased response to gonadotrophin stimulation in this population.

References

- Allocco DJ, Song Q, Gibbons GH, Ramoni MF, Kohane IS. Geography and genography: prediction of continental origin using randomly selected single nucleotide polymorphisms. *BMC Genomics*. 2007;8:68.
- Altmae S, Haller K, Peters M, Hovatta O, Stavreus-Evers A, Karro H, et al. Allelic estrogen receptor 1 (ESR1) gene variants predict the outcome of ovarian stimulation in in vitro fertilization. *Mol Hum Reprod*. 2007;13:521–6.
- Alviggi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, et al. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. *Reprod Biomed Online*. 2009;18:9–14.
- Barbosa AA, Pedrazzoli M, Koike BD, Tufik S. Do Caucasian and Asian clocks tick differently? *Braz J Med Biol Res*. 2010;43:96–9.
- Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev*. 2009;30:465–93.
- Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimüllerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. *Fertil Steril*. 2009;91:705–14.
- Cordell HJ, Clayton DG. Genetic association studies. *Lancet*. 2005;366:1121–31.
- de Bruin JP, Bovenhuis H, van Noord PA, Pearson PL, van Arendonk JA, te Velde ER, et al. The role of genetic factors in age at natural menopause. *Hum Reprod*. 2001;16:2014–8.
- De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet*. 2010;376:911–21.
- Espósito MA, Coutifaris C, Barnhart KT. A moderately elevated day 3 FSH concentration has limited predictive value, especially in younger women. *Hum Reprod*. 2002;17:118–23.
- Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of ‘poor response’ to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod*. 2011;26:1616–24.
- Garcia JE, Jones GS, Acosta AA, Wright Jr G. Human menopausal gonadotropin/human chorionic gonadotropin follicular maturation for oocyte aspiration: phase II, 1981. *Fertil Steril*. 1983;39:174–9.
- Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA. Single nucleotide polymorphisms in the anti-Müllerian hormone signalling pathway do not determine high or low response to ovarian stimulation. *Reprod Biomed Online*. 2010;21:616–23.
- Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA. A single nucleotide polymorphism in BMP15 is associated with high response to ovarian stimulation. *Reprod Biomed*. 2011; Online
- He C, Kraft P, Chasman DI, Buring JE, Chen C, Hankinson SE, et al. A large-scale candidate gene association study of age at menarche and age at natural menopause. *Hum Genet*. 2010;128:515–27.
- Institute EB, Bioinformatics SSIO, Resource PI. Universal Protein Resource (UniProt), 2002.
- Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. *Br J Obstet Gynaecol*. 1997;104:521–7.
- Knauff EA, Blauw HM, Pearson PL, Kok K, Wijmenga C, Veldink JH, et al. Copy number variants on the X chromosome in women with primary ovarian insufficiency. *Fertil Steril*. 2011;95(1584–1588):e1581.
- Ku CS, Loy EY, Salim A, Pawitan Y, Chia KS. The discovery of human genetic variations and their use as disease markers: past, present and future. *J Hum Genet*. 2010;55:403–15.
- Kumbak B, Ulug U, Erzik B, Akbas H, Bahceci M. Early clinical pregnancy loss rate in poor responder patients does not change compared to age-matched normoresponders. *Fertil Steril*. 2009;91:106–9.
- La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update*. 2010;16:113–30.
- Lambalk CB, van Disseldorp J, de Koning CH, Broekmans FJ. Testing ovarian reserve to predict age at menopause. *Maturitas*. 2009;63:280–91.
- Lashen H, Ledger W, Lopez-Bernal A, Barlow D. Poor responders to ovulation induction: is proceeding to in-vitro fertilization worthwhile? *Hum Reprod*. 1999;14:964–9.
- Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Res*. 1993;21:3761–6.
- Liu P, Lu Y, Recker RR, Deng HW, Dvornyk V. ALOX12 gene is associated with the onset of natural menopause in white women. *Menopause*. 2010;17:152–6.

26. Livak KJ, Flood SJ, Marmaro J, Giusti W, Deetz K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* 1995;4:357–62.
27. Loutradis D, Theofanakis C, Anagnostou E, Mavrogianni D, Partsiavelos GA. Genetic Profile of SNP(s) and Ovulation Induction. *Curr Pharm Biotechnol.*; 2011.
28. Loverro G, Nappi L, Mei L, Giacomoantonio L, Carriero C, Tartagni M. Evaluation of functional ovarian reserve in 60 patients. *Reprod Biomed Online.* 2003;7:200–4.
29. Lu Y, Liu P, Recker RR, Deng HW, Dvornyk V. TNFRSF11A and TNFSF11 are associated with age at menarche and natural menopause in white women. *Menopause.* 2010;17:1048–54.
30. McGuire MM, Bowden W, Engel NJ, Ahn HW, Kovanci E, Rajkovic A. Genomic analysis using high-resolution single-nucleotide polymorphism arrays reveals novel microdeletions associated with premature ovarian failure. *Fertil Steril.* 2011;95:1595–600.
31. Mendoza N, Sanchez-Borrego R, Galiano D, Salamanca A, Mozas J, Quereda F, et al. Multigenic combination of estrogen-related genes is associated with age at natural menopause in a Spanish population. *Menopause Int.* 2009;15:150–6.
32. Moron FJ, de Castro F, Royo JL, Montoro L, Mira E, Saez ME, et al. Bone morphogenetic protein 15 (BMP15) alleles predict over-response to recombinant follicle stimulation hormone and iatrogenic ovarian hyperstimulation syndrome (OHSS). *Pharmacogenet Genomics.* 2006;16:485–95.
33. Morris DH, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Familial concordance for age at natural menopause: results from the Breakthrough Generations Study. *Menopause*; 2011.
34. Murabito JM, Yang Q, Fox C, Wilson PW, Cupples LA. Heritability of age at natural menopause in the Framingham Heart Study. *J Clin Endocrinol Metab.* 2005;90:3427–30.
35. Murray A, Bennett CE, Perry JR, Weedon MN, Jacobs PA, Morris DH, et al. Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study. *Hum Mol Genet.* 2010;20:186–92.
36. Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure? *Hum Reprod.* 2002;17:1106–11.
37. Onland-Moret NC, Peeters PH, van Gils CH, Clavel-Chapelon F, Key T, Tjonneland A, et al. Age at menarche in relation to adult height: the EPIC study. *Am J Epidemiol.* 2005;162:623–32.
38. Padhy N, Gupta S, Mahla A, Latha M, Varma T. Demographic characteristics and clinical profile of poor responders in IVF/ICSI: a comparative study. *J Hum Reprod Sci.* 2010;3:91–4.
39. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet.* 1992;340:17–8.
40. Pena SD. Reasons for banishing the concept of race from Brazilian medicine. *Hist Cienc Saude Manguinhos.* 2005;12:321–46.
41. Ribeiro Jr CL, Arruda JT, Silva CT, Moura KK. Analysis of p53 codon 72 gene polymorphism in Brazilian patients with endometriosis. *Genet Mol Res.* 2009;8:494–9.
42. Saldeen P, Kallen K, Sundstrom P. The probability of successful IVF outcome after poor ovarian response. *Acta Obstet Gynecol Scand.* 2007;86:457–61.
43. Setti AS, Braga DP, Figueira RD, Azevedo MD, Iaconelli Jr A, Borges Jr E. Are poor responders patients at higher risk for producing aneuploid embryos in vitro? *J Assist Reprod Genet.* 2011;28:399–404.
44. Shastri BS. SNP alleles in human disease and evolution. *J Hum Genet.* 2002;47:561–6.
45. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. *Hum Reprod Update.* 2002;8:413–21.
46. Simoni M, Tempfer CB, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: Part I: Polycystic ovary syndrome and ovarian response. *Hum Reprod Update.* 2008;14:459–84.
47. Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin Endocrinol Metab.* 1998;83:1875–80.
48. Stolk L, Zhai G, van Meurs JB, Verbiest MM, Visser JA, Estrada K, et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. *Nat Genet.* 2009;41:645–7.
49. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril.* 1998;69:419–24.
50. Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. *Lancet.* 1996;348:1402–6.
51. Thaler CJ, Budiman H, Ruebsamen H, Nagel D, Lohse P. Effects of the common 677C>T mutation of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene on ovarian responsiveness to recombinant follicle-stimulating hormone. *Am J Reprod Immunol.* 2006;55:251–8.
52. Torgerson DJ, Thomas RE, Reid DM. Mothers and daughters menopausal ages: is there a link? *Eur J Obstet Gynecol Reprod Biol.* 1997;74:63–6.
53. Ulug U, Ben-Shlomo I, Turan E, Erden HF, Akman MA, Bahceci M. Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. *Reprod Biomed Online.* 2003;6:439–43.
54. University S. Stanford On Line Universal resource for Clones and ESTs (SOURCE); 2000.
55. van Asselt KM, Kok HS, van der Schouw YT, Peeters PH, Pearson PL, Grobbee DE. Role of genetic analyses in cardiology: part II: heritability estimation for gene searching in multifactorial diseases. *Circulation.* 2006;113:1136–9.
56. van Disseldorp J, Franke L, Eijkemans R, Broekmans F, Macklon N, Wijmenga C, et al. Genome-wide analysis shows no genomic predictors of ovarian response to stimulation by exogenous FSH for IVF. *Reprod Biomed Online.* 2011;22:382–8.
57. van Rooij IA, Tonkelaar I, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, et al. Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause.* 2004;11:601–6.
58. Voorhuis M, Onland-Moret NC, van der Schouw YT, Fauser BC, Broekmans FJ. Human studies on genetics of the age at natural menopause: a systematic review. *Hum Reprod Update.* 2010;16:364–77.
59. Wunsch A, Ahda Y, Banaz-Yasar F, Sonntag B, Nieschlag E, Simoni M, et al. Single-nucleotide polymorphisms in the promoter region influence the expression of the human follicle-stimulating hormone receptor. *Fertil Steril.* 2005;84:446–53.
60. Yang JJ, Cho LY, Lim YJ, Ko KP, Lee KS, Kim H, et al. Estrogen receptor-1 genetic polymorphisms for the risk of premature ovarian failure and early menopause. *J Womens Health (Larchmt).* 2010;19:297–304.
61. Yeh E, Kimura L, Errera FIV, Angeli CB, Mingroni-Netto RC, Silva MER, et al. Association of polymorphisms at the ADIPOR1 regulatory region with type 2 diabetes and body mass index in a Brazilian population with European or African ancestry. *Braz J Med Biol Res.* 2008;41:468–72.
62. Zhao L, Cui B, Liu JM, Zhang MJ, Zhao HY, Sun LH, Tao B, Zhang LZ, Ning G. Interactions of osteoporosis candidate genes for age at menarche, age at natural menopause, and maximal height in Han Chinese women. *Menopause*; 2011.