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ARTICLE

Androgen receptor CAG repeat length is associated with ovarian reserve but not with ovarian response

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Abstract The human androgen receptor (AR) gene contains a highly polymorphic CAG repeat sequence within exon 1. In-vitro studies have shown a relationship between CAG repeats in the AR gene and its transactivation potential. This variation in length may play a role in anovulatory infertility. The objective of this study was to investigate whether CAG polymorphism of the AR gene has a predictive value for ovarian reserve, response and cycle outcome in an egg donor programme. CAG length of the AR gene was determined in 147 oocyte donors. All donors underwent ovarian stimulation with a gonadotrophin-releasing hormone antagonist protocol ($n = 355$). No differences were reported in days of stimulation, gonadotrophin doses, and number of oocytes retrieved. Clinical outcomes were not affected by the CAG repeat length of the AR gene; the primary end-point, antral follicle count, was significantly affected ($P < 0.05$). In conclusion, in a population of fertile egg donors AR gene CAG polymorphism does not affect ovarian response to gonadotrophins. Antral follicle count was associated with the CAG polymorphism genotype. This suggests that genetic factors may increase susceptibility to poor ovarian reserve, and that AR gene genotype could play a role in the natural ovarian ageing process.

KEYWORDS: androgen receptor, CAG polymorphism, oocyte donor, ovarian reserve, ovarian stimulation

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Introduction

Androgens play an important role in male reproductive function, and the role of testosterone as an oestradiol precursor in women is well understood (Simpson, 2002). The direct involvement of androgens in female reproductive physiology, however, remains controversial (Walters et al., 2008).

Recent experiments in androgen receptor (AR) knockout mice have shown the importance of androgens. Female knockout-AR mice, although fertile, display a diminished ovarian reserve (Sen and Hammes, 2010). Human clinical experience seems to support the importance of appropriate androgen levels in female fertility (Gleicher et al., 2011).

Ovarian stimulation is a largely used strategy in assisted reproductive techniques. Ovarian response, however, varies widely in women undergoing ovarian stimulation (Oehninger, 2011). Recently, the Bologna criteria standardized the definition of poor ovarian response in a simple and reproducible manner. Poor ovarian response to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes. It has been recognized that, in order to define poor response in IVF, at least two of the following three features must be present: advanced maternal age or any other risk factor for poor ovarian response; a previous poor ovarian response; and an abnormal ovarian reserve test (Ferraretti et al., 2011).

About 9–24% of women undergoing IVF respond more poorly than expected to the ovarian stimulation protocol prescribed in accordance with their clinical characteristics (Oehninger, 2011). Experience in primates has shown an increase in the number of growing follicles after the stimulation of the AR with the administration of testosterone or 5-dihidrotestosterone (Vendola et al., 1998). In recent years, some strategies have been developed to increase androgen concentrations in the ovarian milieu in order to increase ovarian response (Kyrou et al., 2009).

The biological effect of androgens is mediated through the activation of the AR and, therefore, their action does not depend solely on serum androgen levels. The human AR is encoded by a single copy gene on the X-chromosome at Xq12 (Brown et al., 1989). In women, AR is expressed in the ovary, mainly in the granulosa cells, suggesting involvement in folliculogenesis (Kimura et al., 2007). Similarly, AR protein has been localized to the theca cells of preantral follicles (Suzuki et al., 1994), granulosa cells of antral follicles (Chadha et al., 1994) and most intensely in granulosa cells and theca cells of dominant follicles (Horie et al., 1992). Androgen activated AR exerts its biological effects by stimulating target genes via a sequence of processes, including ligand binding, homodimerization, nuclear translocation, DNA binding and a complex formation with co-regulators and general transcription factors (Walters et al., 2008).

The human androgen receptor gene contains a highly polymorphic CAG repeat sequence within exon 1. In-vitro studies showed a relationship between the number of CAG repeats in the AR and its transactivation potential (Ferk et al., 2008). Expansion of the CAG tract to beyond the polymorphic range (>40) results in a fatal neuromuscular disorder, spinal and bulbal muscular atrophy (La Spada et al., 1991). Recently, variations of the AR-CAG tract, while still within the normal polymorphic range (11–38), have been linked to an increase in the severity of different diseases associated with low or high androgenic activity (Mifsud et al., 2000).

Although within the normal polymorphic range, some studies have shown that relatively long tracts are associated with an increased risk of male infertility caused by impaired sperm production (Yong et al., 2003). In men, it has also been reported that serum androgen levels are modulated by AR activity based upon the length of its polyglutmine tract (Davis-Dao et al., 2007). Data on the functional importance of the CAG repeat in women is sparse, but conditions associated with androgen insensitivity, including hirsutism, androgenic alopecia and breast cancer have been studied (Kim et al., 2008). Because AR-CAG length can be correlated to androgenicity and androgen-regulated diseases, it is possible that variations in this length may play a role in the cause of anovulatory infertility (Mifsud et al., 2000). Different studies have shown an association between CAG repeats in the AR gene and polycystic ovaries (Kim et al., 2008). Moreover, recent publications reported this association with premature ovarian failure patients (Chatterjee et al., 2009; Sugawa et al., 2009).

To our knowledge nothing is known about the potential hazardous effect of CAG repeats in AR gene and ovarian stimulation. To investigate the possibility of a correlation between CAG polymorphism in the AR gene and ovarian stimulation, we evaluated ovarian stimulation in a non-confounding model in women participating in an egg donation programme. Egg donors are young and fertile women with normal ovulation; therefore, there is minimal variability in oocyte and embryo quality. Moreover, data have been published showing a relationship between CAG repeats and ovarian reserve in infertile patients, as cited above, but no results have been published that include fertile patients. The aim of this study was to investigate whether CAG polymorphism in the AR gene had a predictive value for ovarian response to stimulation and ovarian reserve in oocyte donors as a model of normoovulatory women.

Materials and methods

Study population

Egg donation is the best model to evaluate the determinants of implantation for several reasons. First, oocyte and embryo quality vary minimally, as donors are young women with normal ovulation. Second, the preparation of the endometrium is similar, as all recipients receive the same hormone replacement protocol.

The selection and recruitment of donors was carried out in our clinic following strict quality criteria, including an extensive chromosomal and genetic evaluation. All donors studied were Mediterranean and met the legal requirements in Spain (Spanish Law 14/2006). All women included in the Instituto Bernabeu's egg donation programme have to pass a psychological evaluation. They were aged between 18 and 33 years, healthy, with no family history of hereditary diseases and with an antral follicle count (AFC) more than 8. Preferably, they had previous proven fertility (previous ongoing pregnancies). The donors underwent a complete gynaecological examination, karyotyping, and screening for infectious diseases, such as human immunodeficiency virus, hepatitis B and C, gonoccocia, and syphilis. In addition to the legal

requirements, genetic screening was carried out for cystic fibrosis, fragile X syndrome and alpha and beta thalasaemia. Furthermore, guidelines from both the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology for oocyte donors were followed (ESHRE Task Force, 2002; Pfeifer et al., 2013).

In this study, the results of CAG polymorphism in the AR gene from 147 oocyte donors were included. These donors performed 355 COH cycles, and the results from stimulation and cycle outcome were included in the present research. The average number of COH cycles per donor is 2.6 ± 2.1.

All the participants included in the study gave their informed consent to collect peripheral blood samples suitable for molecular analysis. This study involved only retrospective analysis of anonymous medical records, and was approved by the Instituto Bernabeu Institutional Review Board (reference number 08/2012; 8 Febraury 2012).

Genotyping

DNA was isolated from peripheral blood lymphocytes according to manufacturer instructions (Wizard® Genomic DNA Purification Kit, Promega, USA) and stored at 4°C. CAG repeat of exon 1 in the AR gene was amplified from the genomic DNA using the TaKaRa LA Taq kit (Takara Bio Inc, Shiga, Japan) and primers flanking the CAG repeat region (Cram et al., 2000). The forward primer was 5′-CAGAATCTGTTCCAGAGCGTGC-3′ and labeled at 5′ with the dye 6FAM and the reserve primer was 5′-AAGGTTGCTGTTCCTCATCCAG-3′. Polymerase chain reaction was carried out under the following conditions: initial denaturation 95°C for 5 min, followed by five cycles of denaturation at 95°C, anneling at 55°C and polymerization at 72°C for 45 s with a final extension at 72°C for 5 min. For genotyping 1.0 μl of polymerase chain reaction product was mixed with 0.3 μl of LIZ 500 and 9.7 μl of Hi-Di formamide and analysed on ABI 310 DNA analyzer (Applied Biosystems, Madrid, Spain) using Gene Mapper software (Applied Biosystems) to ascertain the size of AR alleles and the number of repeats. The number of CAG repeats was calculated in relation to a series of standards obtained by direct sequencing.

Ovarian stimulation and oocyte retrieval

In accordance with Spanish Fertility Act requirements, all patients underwent an ovarian stimulation protocol with tailored doses of urinary FSH (Fostipur; Angelini, Barcelona. Spain). Gonadotrophin stimulation started from day 2 of the menstrual cycle, with doses varying between 150 and 300 IU/day depending on the age of the donor, body mass index and antral follicle count. The gonadotrophin-releasing hormone antagonist, cetrorelix 0.25 mg/day (Cetrotide; Merck-Serono, Geneva) was introduced according to a multiple-dose, flexible protocol. In all cases, triggering was exclusively performed with 0.4 mg of subcutaneous triptorelin (Decapeptyl; Ipsen Pharma, Paris). Ovarian response was monitored by transvaginal ultrasound and plasma oestradiol concentrations. Oocytes were aspirated 36 h after analogue administration by transvaginal, ultrasound-guided needle aspiration under sedation. Sperm and oocyte preparation, fertilization, embryo culture and transfer were carried out according to IVF laboratory guidelines.

Recipient protocol

A total of 355 recipient women underwent a standard protocol as previously reported (Bernabeu et al., 2006). The number of previous IVF cycles with donor eggs per recipient was on average 1.5 ± 0.8. Patients with ovarian activity received either birth control pills (Yasmin; Bayer Hispania, Spain) or analogue depot (Decapeptyl depot 3.75; Ipsen Pharma, Paris) in the luteal phase of the previous cycle. Menopausal patients were treated with a sequential regimen of oestrogen and progesterone the month before the transfer. Oral oestradiol valerate (Progynova, Schering) or oestradiol patches releasing 50 μg daily (Dermestril 50; Rottapharm-Madaus) were used in increasing doses for the endometrial preparation. From day 12 of treatment, patients received up to 6 mg oestradiol valerate per day or three patches every other day and the duration of the treatment varied in accordance with the availability of a phenotypically matched donor, ranging from 14 to 24 days. After 13 days of oestradiol valerate administration, endometrial thickness and pattern were tested. If a trilaminar pattern was observed in a ≥7 mm endometrium, the aforementioned dose of oestradiol therapy was continued at least until the pregnancy test was carried out 2 weeks later. If the endometrium was not seen to be sufficiently developed, doses of oestradiol valerate were increased to 8 mg/day or four patches. From the day of oocyte retrieval until the pregnancy test, 600 mg of micronized progesterone (Utrogestan; Seid, Paris) were administered vaginally daily.

Statistical analysis

Values are presented as mean ± SD, and medians and range for continuous data and percentages for categorical variables. Data were analysed with Statistical Package for the Social Sciences (SPSS) software (version 20.0, SPSS, IBM Corp, Armonk, NY, USA). For CAG repeat length, we considered the biallelic mean, as in previous reports (Mifsud et al., 2000). The primary end-point was AFC and the secondary endpoints were gonadotrophin consumption, stimulation length and total number of oocytes retrieved in donors. Linear regression was applied for AFC adjusting for age, previous fertility and smoking status as possible confounding factors, as these have been reported to affect ovarian reserve. Linear regression was applied to evaluate donor ovarian stimulation parameters adjusting for age, AFC, previous fertility and smoking status as possible confounding factors, as these have been reported to affect ovarian response. Clinical pregnancy was diagnosed as a pregnancy with ultrasound visualization of a gestational sac with fetal heartbeat at 8 weeks gestation. The implantation rate was defined as the number of gestational sacs observed divided by the number of embryos transferred. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 weeks gestation. Logistic regression was carried out to evaluate the association between number of CAG repeats and categorical variables (positive beta-HCG, clinical pregnancy,

implantation and spontaneous abortion rates). $P < 0.05$ was considered significant.

Results

Clinical characteristics

The clinical characteristics of the egg donors are detailed in **Table 1**. Donor age varied significantly ($P = 0.002$) between CAG repeat length of the AR gene, suggesting that the population was not homogenously distributed. Age was therefore used as a confounding variable in later statistical analyses. No significant differences were observed in body mass index, proven fertility or the number of smokers. The mean antral follicle count was 15 ± 5 (range 8–26) (**Table 2**). Linear regression analysis indicated that AFC was significantly associated with CAG repeat length (uncorrected $P = 0.013$; age-adjusted $P < 0.001$).

CAG repeat length genotyping

For this study, we included the results of the AR-CAG repeat counts obtained from 147 oocyte donors. The AR-CAG repeat numbers were in the normal polymorphic range in all women. The frequency of the CAG repeat length in the two alleles and the biallelic mean is represented in **Figure 1**. In the 147 oocyte donors analysed, the biallelic mean of CAG repeats ranged from 17 to 29. The shortest repeat lengths ranged from 11 to 27. In the longer of the two alleles, repeat lengths ranged from 20 to 34. The 21 and 25 CAG repeat alleles were found to be at the highest frequency for allele 1 and 2, respectively. The median CAG repeat in exon 1 of AR gene was found to be 23.

Table 1 Donor characteristics.

Characteristic	n = *147*
Number of CAG repeats[a]	23 ± 2.3 (17–29)
Body mass index[a]	22.0 ± 3.06 (15.9–29.3)
Donor age (years)[a]	25.6 ± 3.84 (18–33)[b]
Proven fertility (%)	90.1
Smoker (%)	65.4

Linear regression model adjusting for number of CAG repeats.
[a]Mean ± SD (range).
[b]$P < 0.01$ (r = –0.175) in relation to CAG repeat length of the androgen receptor gene.

Table 2 Egg donor antral follicle count in relation to CAG repeats length on androgen receptor gene.

Total (147)	*Antral follicle count*
Mean ± SD	15 ± 4.5
Median	14
Range	8–26
P unadjusted	0.013 (r = 0.140)
P age-adjusted	<0.001 (r = 0.260)

Linear model adjusting for number of CAG repeats.

Ovarian stimulation and cycle outcome

The 147 patients included in this study underwent 355 ovarian stimulation cycles. The ovarian stimulation parameters in the 355 ovarian stimulation cycles are presented in **Table 3**. No significant differences were reported in the number of oocytes retrieved, the gonadotropin doses or the days of stimulation.

Egg donation treatment outcomes are presented in **Table 3**. No significant differences in cycle outcome were observed in relation to CAG repeat length of the androgen receptor. No differences were observed in recipient age, endometrial thickness, days of hormone replacement therapy, number of oocyte received, fertilization rates for conventional IVF and ICSI, fertilization technique or the day of embryo transfer (data not shown). Also, no significant associations were found in biochemical pregnancy, clinical pregnancy rate, spontaneous abortion rate and implantation rate.

Discussion

Our data suggest that AFC is associated with CAG polymorphism of the AR gene but ovarian stimulation is not. Moreover, the clinical outcome is not associated with this polymorphism. To the best of our knowledge, these data show for the first time the relationship between, CAG repeat length in the AR gene and ovarian reserve and stimulation using a non-confounding model of egg donation.

Oocyte donation is the best model to evaluate the determinants of stimulation and embryo implantation potential. Donors are young women of similar age with normal ovarian function and, in our egg donation programme, with up to 90% of previous proven fertility (**Table 1**). Overall, no differences were reported in length of CAG repeats in relation to ovarian response and cycle outcome in our population. This is not surprising, because androgen-mediated actions occur in the early stages of follicular development. Recent approaches, however, have been developed with the ultimate aim of increasing serum androgens to deliver high androgen concentrations to growing follicles (Kyrou et al., 2009) and increase ovarian response to ovarian stimulation in patients with poor ovarian response. Two studies whose common goal was to increase androgen levels, one using testosterone (Fabregues et al., 2009) and the second using dehydroepiandrosterone (Gleicher et al., 2011), had different outcomes according to embryo quality and pregnancy rates. The data in the current study indicate that these strategies could improve the ovarian response by increasing the number of small antral follicles. This increase would be mediated by androgens instead of via a direct action on ovarian stimulation, because the current study found an association between CAG repeat number in the androgen receptor gene and ovarian reserve but not with ovarian response.

Success of assisted reproductive technology depends on the selection of a correct protocol for ovarian stimulation. This can be decided mainly by a proper assessment of ovarian

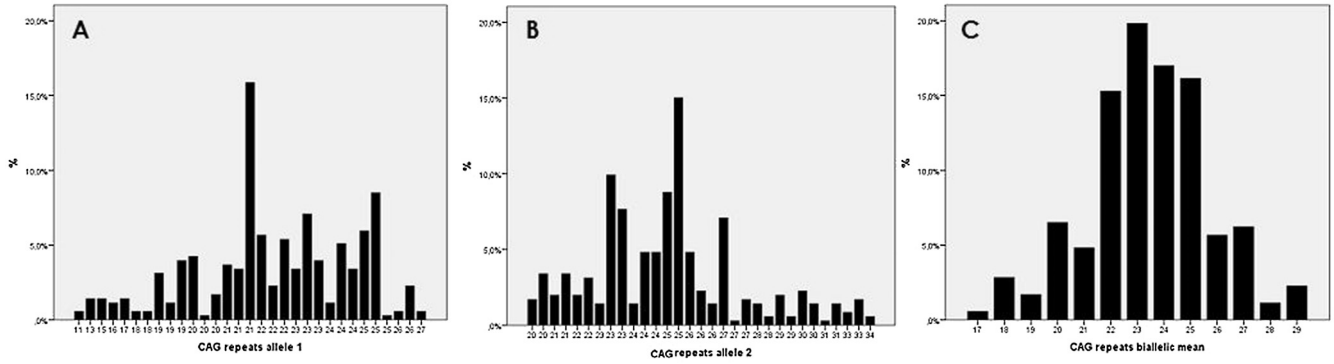

Figure 1 Distribution of CAG repeats numbers in exon 1 of androgen receptor gene in egg donor. (A) Allele 1 is the shortest CAG repeat of the allele pair; (B) allele 2 is the longest CAG repeat of the allele pair; (C) Biallelic mean.

Table 3 Egg donation cycle outcomes.

	Total (n = 355)	
	Average ± SD (range)	*Correlation coefficient Pearson (r)*
Stimulation length (days)	11.6 ± 1.56 (7–18)	0.161
Gonadotropin used (IU)	2090.2 ± 546.51 (600–3900)	0.371
Number of retrieved oocytes	19.6 ± 8.74 (6–29)	0.166
Recipient age (years)	40.8 ± 4.39 (24–51)	−0.039
Follicular phase length (days)	18.8 ± 4.07 (9–32)	0.001
Recipient endometrial thickness (mm)	8.7 ± 1.63 (9–13)	−0.140
Number of oocytes received	12.9 ± 3.50 (9–17)	−0.008
Two pronuclei conventional IVF	7.4 ± 3.83 (1–16)	−0.052
Two pronuclei ICSI	8.3 ± 3.12 (1–17)	0.044
Transferred embryos	1.8 ± 0.47 (1–3)	−0.018
Positive beta-HCG (%)	245 (69.0)	
Clinical pregnancy rate (%)	192 (54.0)	
Implantation rate (%)	252/639 (39.4)	
Spontaneous abortion rate (%)	22 (11.5)	

Tests performed for statistical analysis were logistic regression for categorical variables and linear regression for continuous variables. No statistically significant differences were found in the above parameters in relation to androgen receptor repeat numbers as indicated by linear regression analysis adjusting for age, antral follicle count, previous fertility and smoking status as possible confounding factors.

reserve before the ovarian stimulation. Ovarian reserve is a major factor in determining success rates after IVF. Ovarian reserve relates to secondary, pre-antral and antral ovarian follicle pool. Various methods have been proposed to assess ovarian reserve. The most common are serum FSH, anti-Müllerian hormone (AMH) and AFC, the latter assessed by ultrasound (Ledger, 2010). Compared with FSH, AMH has lately been found to be a reliable and more accurate parameter assessing ovarian reserve (Panchal and Nagori, 2012). AFC provides a direct measure of ovarian reserve, whereas AMH, inhibin B and oestradiol are released from growing follicles and so their levels reflect the size of the developing follicle cohort (Hendricks et al., 2007). AFC and AMH are the most significant predictors of ovarian reserve. Both parameters alone, or in combination, have a similar predictive power (Jayaprakasan et al., 2010). AFC alone may be sufficient for the estimation of ovarian reserve. Gonadotrophin doses for ovarian stimulation usually increase with decreasing ovarian reserve. A recent meta-analysis, however, has shown that markers of ovarian reserve have only modest value in predicting the response to gonadotrophins (Broekmans et al., 2006). From this assumption, recent studies have attempted to show an association between different genotypes and ovarian response (Lledó et al., 2013).

The present study has shown a positive direct association between CAG repeat length in the AR gene and AFC. The average length of the CAG repeat in the normal fertile population is 22.1 ± 2 (range 14–31) (Davis-Dao et al., 2007). Similar results (23 ± 1, range, 17–29) in our population. Previous studies have indicated a relationship between the CAG repeat number on AR gene and AR function (Zitzmann and Nieschlang, 2003). This normal but variable CAG repeat size may alter the

transactivation function (Tut et al., 1997), because these repeats are located in the N-terminal region, altering the interaction with co-regulator proteins. Alteration of the transactivation function caused by loss of a co-regulator protein has been associated with androgen insensitivity (Adachi et al., 2000). Furthermore, premature ovarian failure may actually be associated with long CAG repeats (Chatterjee et al., 2009) with a relatively low AR function in ovarian follicular cells, because premature ovarian failure is found in female mice lacking AR (Shiina et al., 2005). The explanation of this apparent disagreement could be found in the different population characteristics in different studies and in the effect of CAG repeats on the AR function. The present work seems to be the first to study a fertile population of egg donors with a range of shorter repeat lengths than patients with premature ovarian failure who carry longer CAG repeats; therefore, the results compare populations with a different range of repeats. On the other hand, the AR action depends on the CAG repeat length. Recent work supports previous evidence published by Ding et al. (2004), showing that CAG repeat number is not inversely associated with androgen receptor activity *in vitro* (Neonen et al., 2010). The highest AR activity was confined to that with 22 CAG repeats, the most frequent in normal population. This may at least be able to explain the discrepancy in data aiming to link CAG repeat length and physiological conditions.

Neonen et al. (2010) also showed that the method used for the measurement of transactivation efficiency may highly influence the final conclusion. Enzyme-linked immunosorbent assay experiments might be a more efficient way to obtain these data (Sharp et al., 2000). A poliglutamine tract of about 22 glutamine residues would represent the baseline activation status of the AR. A higher or lower number of glutamine residues would increase the repression of the receptor, leading to a reduction in its transactivation function and, consequently to a lower activation of the androgren-regulated genes (Neonen et al., 2010).

The AR gene is X-linked and is known to undergo X-inactivation. This process is essentially random in normal women. As great variations in the degree of X-inactivation between tissues have been identified (Sharp et al., 2000), future epigenetic studies might provide more unequivocal answers regarding the relative expression of CAG alleles. It is important to investigate tissue specific X chromosome inactivation patterns in future studies. Although in this study we have not investigated the pattern of X-inactivation, it is plausible that effects of repeat length at the functional level may be further amplified in the event of the shorter allele being preferentially inactivated. In the present study, however, and in a previous study (Chatterjee et al., 2009), the association between CAG repeats lengths and ovarian reserve is only reported when the results are analysed from the biallelic mean. This suggests a random AR gene inactivation. These data agree with previous work reporting random inactivation (Aruna et al., 2011).

Finally, advance identification of patients who will be affected by premature ovarian failure would be of great clinical advantage for such patients. Various predictive markers have been proposed such as AMH and AFC, but these are age related and depend on when the tests are performed (Jayaprakasan et al., 2010). Besides these parameters, genetic variability also seems to be an important factor. In conclusion, this research reveals that, in a population of fertile egg donors, CAG repeat length on AR gene is associated with different ovarian reserve but not with different ovarian response to ovarian stimulation. AR gene genotype could be an important factor for determining the natural ovarian ageing process, because the genotype does not change throughout life and so is independent of the time at which it is determined. AR gene genotype may help to predict ovarian behaviour with age. Egg cryopreservation could be an important option to preserve the fertility in at risk patients. Genotyping CAG in AR gene together with some additional markers may therefore provide a tool for identifying patients who will suffer lower ovarian reserve in the near future.

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References

Adachi, M., Takayanagi, R., Tomura, A., Imasaki, K., Kato, S., Goto, K., Yanase, T., Ikuyama, S., Nawata, H., 2000. Androgen-insensitivity syndrome as a possible coactivator disease. N. Engl. J. Med. 343, 856–862.

Aruna, M., Dasgupta, S., Sirisha, P.V., Andal Bhaskar, S., Tarakeswari, S., Singh, L., Reddy, B.M., 2011. Role of androgen receptor CAG repeat polymorphism and X-inactivation in the manifestation of recurrent spontaneous abortions in Indian women. PLoS ONE 6, e17718.

Bernabeu, R., Roca, M., Torres, A., Ten, J., 2006. Indomethacin effect on implantation rates in oocyte recipients. Hum. Reprod. 21, 364–369.

Broekmans, F.J., Kwee, J., Hendriks, D.J., Mol, B.W., Lambalk, C.B., 2006. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum. Reprod. Update 12, 685–718.

Brown, C.J., Goss, S.J., Lubahn, D.B., Joseph, D.R., Wilson, E.M., French, F.S., Willard, H.F., 1989. Androgen receptor locus on the human X chromosome: regional localization to Xq11-12 and description of a DNA polymorphism. Am. J. Hum. Genet. 44, 264–269.

Chadha, S., Pache, T.D., Huikeshoven, J.M., Brikmann, A.O., van der Kwast, T.H., 1994. Androgen receptor expression in human ovarian and uterine tissue of long-term androgen-treated transsexual women. Hum. Pathol. 25, 1198–1204.

Chatterjee, S., Singh, R., Kadam, S., Maitra, A., Thangaraj, K., Meherji, P., Modi, D., 2009. Longer CAG repeat length in the androgen receptor gene is associated with premature ovarian failure. Hum. Reprod. 24, 3230–3235.

Cram, D.S., Song, B., McLachlan, R.I., Trounson, A.O., 2000. CAG trinucleotide repeats in the androgen receptor gene of infertile men exhibit stable inheritance in female offspring conceived after ICSI. Mol. Hum. Reprod. 6, 861–866.

Davis-Dao, C.A., Tuazon, E.D., Sokol, R.Z., Cortessis, V.K., 2007. Male infertility and variation in CAG repeat length in the androgen

receptor gene: a meta-analysis. J. Clin. Endocrinol. Metab. 92, 4319–4326.
Ding, D., Xu, L., Menon, M., Veer Reedy, G.P., Barrack, E.R., 2004. Effect of a short CAG repeat on human androgren receotir function. Prostate 58, 23–32.
ESHRE Task Force, 2002. ESHRE Task Force on Ethics and Law. III. Gamete and embryo donation. Hum. Reprod. 17, 1407–1408.
Fabregues, F., Penarrubia, J., Creus, M., Manau, D., Casals, G., Carmona, F., Balasch, J., 2009. Trasndermal testosterone may improve ovarian esponser to gonadotropins in low-responser IVF patients: a randomized, clinical trial. Hum. Reprod. 4, 349–359.
Ferk, P., Perme, M.P., Teran, N., Gersak, K., 2008. Androgen receptor gene (CAG)n polymorphism in patients with polycystic ovary syndrome. Fertil. Steril. 90, 860–863.
Ferraretti, A.P., La Marca, A., Fauser, B.C., Tarlatzis, B., Nargund, G., Gianaroli, L., 2011. ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum. Reprod. 26, 1616–1624.
Gleicher, N., Weghofer, A., Barad, D.H., 2011. The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment? Reprod. Biol. Endocrinol. 9, 116.
Hendricks, D.J., Kwee, J., Mol, B.W., te Velde, E.R., Broekmans, F.J., 2007. Ultrasonography as a tool for prediction of outcome in IVF patients: a comparative meta-analysis of ovarian volume and antral follicle count. Fertil. Steril. 87, 764–775.
Horie, K., Takakura, K., Fujiwara, H., Suginami, H., Liao, S., Mori, T., 1992. Immunohistochemical localization of androgen receptor in the human ovary throughout the menstrual cycle in relation to oestrogen and progesterone receptor expression. Hum. Reprod. 7, 184–190.
Jayaprakasan, K., Campbell, B., Hopkisson, J., Johnson, I., Rainne-Fenning, N., 2010. A prospective, comparative analysis of anti-mullerian hormone, inhibin-B, and three dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertil. Steril. 93, 30–39.
Kim, J.J., Choung, S.H., Choi, Y.M., Yoon, S.H., Kim, S.H., Moon, S.Y., 2008. Androgen receptor gene CAG repeat polymorphism in women with polycystic ovary syndrome. Fertil. Steril. 90, 2318–2323.
Kimura, S., Matsumoto, T., Matsuyama, R., Shiina, H., Sato, T., Takeyama, K., Kato, S., 2007. Androgen receptor function in folliculogenesis and its clinical implication in premature ovarian failure. Trends Endocrinol. Metab. 18, 183–189.
Kyrou, D., Kolibianakis, E.M., Venetis, C.A., Papanikolaou, E.G., Bontis, J., Tarlatzis, B.C., 2009. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil. Steril. 91, 749–766.
La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E., Fischbeck, K.H., 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352, 77–79.
Ledger, W.L., 2010. Clinical utility of measurement of anti-mullerian hormone in reproductive endocrinology. J. Clin. Endocrinol. Metab. 95, 5144–5154.
Lledó, B., Guerrero, J., Turienzo, A., Ortiz, J.A., Morales, R., Ten, J., Llacer, J., Bernabeu, R., 2013. Effect of follicle-stimulating hormone receptor N680S polymorphism on the efficacy of follicle-stimulating hormone stimulation on donor ovarian response. Pharmacogenet. Genomics 23, 262–268.
Mifsud, A., Ramirez, S., Yong, E.L., 2000. Androgen receptor gene CAG trinucleotide repeats in anovulatory infertility and polycystic ovaries. J. Clin. Endocrinol. Metab. 35, 3484–3488.
Neonen, H., Björk, C., Skjaerpe, P.A., Giwercman, A., Rylander, L., Svartberg, J., Lundberg Giwercman, Y., 2010. CAG repeat length is not inversely associated with androgen receptor activity in vitro. Mol. Hum. Reprod. 16, 153–157.
Oehninger, S., 2011. Ovulation induction in IVF. Minerva Ginecol. 63, 137–156.
Panchal, S., Nagori, C., 2012. Comparison of anti-mullerian hormone and antral follicle count for assessment of ovarian reserve. J. Hum. Reprod. Sci. 5, 274–278.
Pfeifer, S., Goldberg, J., McClure, R., Lobo, R., Thomas, M., Widra, E., Licht, M., Collins, J., Cedars, M., Racowsky, C., Vernon, M., Davis, O., Gracia, C., Catherino, W., Thornton, K., Rebar, R., La Barbera, A., 2013. Recommendations for gamete and embryo donation: a committee opinion. Fertil. Steril. 99, 47–62.
Sen, A., Hammes, S.R., 2010. Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. Mol. Endocrinol. 24, 1393–1403.
Sharp, A., Robinson, D., Jacobs, P., 2000. Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. Hum. Genet. 107, 343–349.
Shiina, H., Marsumoto, T., Sato, T., Igarashi, K., Myamoto, J., Takemasa, S., Sakari, M., Takada, I., Nakamura, T., Metzger, D., Chambon, P., Kanno, J., Yoshikawa, H., Kato, S., 2005. Premature ovarian failure in androgen receptor-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 13, 224–229.
Simpson, E.R., 2002. Aromatization of androgens in women: current concepts and findings. Fertil. Steril. 77, S6–S10.
Sugawa, F., Wada, Y., Maruyama, T., Uchida, H., Ishizuka, B., Ogata, T., 2009. Premature ovarian failure and androgen receptor gene CAG repeat lengths weighted by X chromosome inactivation patterns. Fertil. Steril. 9, 649–652.
Suzuki, T., Sasano, H., Kimura, N., Tamura, M., Fukaya, T., Yajima, A., Natura, H., 1994. Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. Hum. Reprod. 9, 1589–1595.
Tut, T.G., Ghadessy, F.J., Trifiro, M.A., Pinsky, L., Yong, E.L., 1997. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J. Clin. Endocrinol. Metab. 82, 3777–3782.
Vendola, K.A., Zhou, J., Adesanya, O.O., Weil, S.J., Bondy, C.A., 1998. Androgens stimulate early stages of follicular growth in the primate ovary. J. Clin. Invest. 101, 2622–2629.
Walters, K.A., Allan, C.M., Handelsman, D.J., 2008. Androgen actions and the ovary. Biol. Reprod. 78, 380–389.
Yong, E.L., Loy, C.J., Sim, K.S., 2003. Androgen receptor gene and male infertility. Hum. Reprod. Update 9, 1–7.
Zitzmann, M., Nieschlang, E., 2003. The CAG repeat polymorphism within the androgen receptor gene and maleness. Int. J. Androl. 6, 76–83.

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