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Very low anti-müllerian hormone concentrations are not an independent predictor of embryo quality and pregnancy rate

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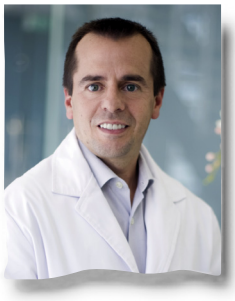

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KEY MESSAGE
Anti-Müllerian hormone (AMH) levels cannot be used as an independent predictor of reproductive outcome. In cases of low AMH levels, the probability of achieving pregnancy is reasonable if the patient's age is not very advanced.

A B S T R A C T

Research question: Is anti-Müllerian hormone (AMH) serum concentration a useful tool to predict the outcome of assisted reproductive treatment?
Design: Retrospective cohort study involving 2971 patients who underwent 5570 IVF cycles. Patients were classified into six groups according to their AMH levels and analysed for associations with reproductive outcome. Several parameters of ovarian response and clinical outcome were compared between groups.
Results: Cancellation rate and clinical pregnancy rate varied by AMH group, with highest cancellation rates (32.8%, $P = 0.021$) and lowest clinical pregnancy rates (9.8%, $P < 0.001$) in the group with lowest AMH. When these patients achieved embryo transfer, the implantation rate (30.5%) did not significantly differ from the other groups, and retained a low, but reasonable, clinical pregnancy rate per transfer (45.9%). When this group was classified into three female age groups, the clinical pregnancy rate was found to be significantly higher in the patients younger than 37 years (58.1%) compared with patients aged between 37 and 39 years (48.9%) and those aged over 39 years (27%, $P < 0.001$).
Conclusions: Although significant differences in pregnancy rates were observed among the different AMH groups, even in the lowest AMH level group, the probability of achieving pregnancy was reasonable, especially if the patient's age is not very advanced.

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Introduction

Managing patient expectations is always a challenge. Although different prognostic markers for ovarian response have been identified (Bancsi et al., 2002), currently age is still the best predictor to evaluate the chances of achieving pregnancy through IVF. Yet, ovarian response may suggest different probabilities of pregnancy, even for the same age groups, according to the number of oocytes or embryos obtained. Nowadays, AMH seems the most robust ovarian response marker (Fleming et al., 2015), similarly to antral follicle count (AFC) and, given their low intercycle variability, outweigh the so-called 'classical' day 3 FSH and oestradiol.

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a glycoprotein dimer that belongs to the transforming growth factor beta (TGFβ superfamily (Dewailly et al., 2014). This hormone is produced exclusively in gonads by granulosa cells and plays an important role in folliculogenesis by acting in the modulation of follicular recruitment in granulosa cells to limit the number of recruited oocytes, and to regulate the number of growing follicles and their selection for ovulation. Compared with other hormonal markers, AMH begins to gradually decline earlier in life and its levels are not influenced by menstrual cycle day (La Marca et al., 2006).

It is also well established that AMH is strongly associated with, and could therefore be capable of, predicting ovarian response (Brodin et al., 2015; Fleming et al., 2015). Although its role as a good predictor of poor or excessive ovarian response after ovarian stimulation has been well established (Iliodromiti and Nelson, 2015), its potential value in predicting the likelihood of pregnancy after assisted reproductive treatments has been contentious. Several meta-analyses (Iliodromiti et al., 2014; Tal et al., 2015) and individual studies (Brodin et al., 2013; Gleicher et al., 2010; Weghofer et al., 2011) have suggested that low AMH is correlated with lower clinical pregnancy rates; contrary to the aforementioned findings, some other groups have questioned the utility of AMH as a predictor of pregnancy outcomes after assisted reproductive technology (ART) (Reichman et al., 2014).

Consequently, the counselling and management of women with low AMH pose a significant challenge as a poor response is anticipated and, in some units, even these patients may not be offered the possibility to be treated. Therefore, we designed this study to determine if AMH serum concentration is a useful tool to predict the outcome of ART.

Materials and methods

Study population and design

Between 2008 and 2014, 2971 women who underwent 5570 cycles of ART treatment at IVI, Madrid, Spain, were included in this non-interventional, retrospective, multicentre cohort study of patients who were submitted to routine clinical examinations and procedures. Only first or second cycles were analysed. All the procedures were approved by an Institutional Review Board (1405-MAD-020-JG) on 27 July 2015 and complied with Spanish Law on Assisted Reproductive Technologies (14/2006). The diagnosis of polycystic ovary syndrome was made after the ultrasound standards described in the Rotterdam criteria (Broekmans et al., 2006a).

All the patients began ovarian stimulation on day 5 after taking an oral contraceptive pill, day 2 after oestrogen, or on cycle day 3 after a transvaginal ultrasound confirmed ovarian quiescence (Cruz et al., 2017). Although we mainly use gonadotrophin releasing hormone (GnRH) antagonists at our centre, two different protocols were used for pituitary suppression during ovarian stimulation. The specific distribution was 14.9% ($n = 832$) with GnRH agonist, and 85.1% ($n = 4738$) with GnRH antagonist. Starting doses of gonadotrophins were calculated according to the patient's AMH concentration, AFC, age, and body mass index. Doses were adjusted according to ovarian response, which was monitored by vaginal scans and oestradiol determinations. If patients underwent a second cycle, taking into consideration that the target response was eight to 15 oocytes, if she obtained more than 15 metaphase II oocytes, dose was reduced by 75 IU of recombinant FSH, and if she produced less than eight metaphase II oocytes, dose was increased by 75 IU of highly purified human menopausal gonadotrophin. Final oocyte maturation was achieved by administering 250 µg of recombinant HCG (Ovitrelle, Merck-Serono) or 0.2 ml of GnRH agonist (Decapeptyl, Ipsen-Pharma) when at least one follicle reached a mean diameter of 17 mm. Transvaginal oocyte retrieval was carried out 36 h later. Oocytes were fertilized by IVF (2.5%, 142/5570) or by intracytoplasmic sperm injection (72.4%, 4034/5570); finally, in 25.0% (1394/5570) of cycles the oocytes were cryopreserved. In all cases with suitable embryos available, fresh embryo transfer was carried out and the surplus embryos were vitrified. Day 3 embryos were categorized into four grades from high quality to low quality depending on the number of blastomeres, fragmentation, multinucleation and symmetry; blastocysts were evaluated according to inner mass cell, trophoectoderm and blastococel expansion (Gardner, 1999). Embryo transfer was carried out on day 3 or day 5 according to the clinical criteria and embryo development. Single embryo transfer was carried out in 31.4% cycles (650/2070). Luteal phase support consisted of administering micronized progesterone (400 mg/day, vaginally), which commenced on the day of oocyte retrieval.

A serum beta-HCG analysis was carried out 12 days after embryo transfer, and clinical pregnancy was confirmed when a gestational sac with a fetal heart beat was visible by ultrasound determination. The implantation rate was calculated as the number of intrauterine gestational sacs observed by transvaginal ultrasonography, divided by the number of transferred embryos. The miscarriage rate refers to the number of pregnancies lost before 24 weeks of gestation, divided by the number of positive beta-HCG. Finally, ongoing pregnancy was characterized by the presence of a developing embryo at over 12 weeks of gestation.

Hormonal measurements

Blood samples were obtained by venipuncture. The collected blood samples were allowed to clot for 20 min and were then centrifuged for 10 min at 6000 g. Serum samples were analysed by chemiluminescence with the Architect Analyser (Abbott Diagnostics). The analytical sensitivity of the oestradiol assay was less than 12 pg/ml and the coefficient of variation was lower than 7.7%. The analytical sensitivity of the progesterone assay was <0.1 pg/ml and the coefficient of variation was lower than 7%. The analytical sensitivity of the FSH assay was less than 0.37 pg/ml and the coefficient of variation was lower than 6.8%.

AMH measurement

AMH was evaluated in serum on days 2–5 of a previous menstrual cycle, and no oral contraceptive pill was taken at that time. Samples were frozen at –80°C until assayed. For AMH evaluations, we used

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