Original article

Serum antimüllerian hormone is not predictive of oocyte quality in vitro fertilization

L'hormone antimüllérienne sérique n'est pas prédictive de la qualité ovocytaire en fécondation in vitro

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Résumé

Objectif. – L'évaluation de la réserve ovarienne est indispensable chez les femmes prises en charge en assistance médicale à la procréation. L'hormone antimüllérienne (AMH), produite par les cellules de la granulosa des follicules préantraux et antraux précoces est un marqueur prometteur de cette réserve ovarienne. Cependant, peu d'études se sont intéressées à la valeur prédictive de l'AMH quant à la qualité ovocytaire. Matériels et méthodes. – Une étude rétrospective a été menée au CHRU de Tours sur 559 femmes prises en charge en fécondation in vitro entre janvier et décembre 2007. Pour toutes ces patientes, un dosage d'AMH sérique a été réalisé avec le même kit Elisa ultrasensible. Les paramètres mesurés étaient le nombre total d'ovocytes, le taux d'ovocytes matures, le taux de fécondation, la qualité embryonnaire et le taux de grossesse clinique. Résultats. – Si une AMH basse est associée à un nombre réduit d'ovocytes à la ponction, elle ne prédit pas la maturité nucléaire des ovocytes recueillis, la fécondation et la qualité des embryons précoces. Une AMH basse n'exclut pas l'initiation d'une grossesse clinique en fécondation in vitro. Conclusion. – À l'heure actuelle, l'AMH sérique est un marqueur relativement prédictif de la réserve ovarienne sur le plan quantitatif mais pas sur le plan qualitatif. Par ailleurs, il n'est pas encore possible de définir une valeur seuil excluant l'initiation d'une grossesse en fécondation in vitro.

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Mots clés : Hormone antimüllérienne ; Réserve ovarienne ; Maturité ovocytaire ; Qualité embryonnaire ; Grossesse clinique

Abstract

Objectives. – The assessment of the ovarian reserve is mandatory in women undergoing assisted reproduction. Antimüllerian hormone (AMH) produced by granulosa cells from preantral and early antral follicles, is a promising indicator of ovarian reserve. However, few studies have evaluated the predictive value of AMH on oocyte quality. Material and methods. – A retrospective study was undertaken at the Bretonneau University Hospital of Tours. A total of 559 women undergoing in vitro fertilization treatment between January 2007 and December 2007 were included in the study. Serum AMH levels were determined by using an ultrasensitive ELISA test. Total number of oocytes, rate of mature oocytes, fertilization rate, embryo quality and clinical pregnancy rate were recorded. Results. – Serum AMH was significantly lower in groups of patients with few oocytes collected. However, serum AMH was not predictive of nuclear maturity of oocytes, fertilization rate and quality of early embryos. Additionally, low levels of AMH do not preclude clinical pregnancy, in vitro fertilization. Conclusion. – At the moment, serum AMH is a relatively predictive indicator of the ovarian reserve, in terms of quantity but not in terms of quality. Moreover, it is still not possible to determine serum AMH cut-off value to predict clinical pregnancy in IVF programmes.

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Keywords: Antimüllerian hormone; Ovarian reserve; Oocyte maturity; Embryo quality; Clinical pregnancy

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1. Introduction

Antimüllerian hormone (AMH) is a glycoprotein that belongs to the Transforming Growth Factor β superfamily (TGFβ). AMH expression was assessed using immunohistochemistry in ovarian sections [1]. The highest level of AMH expression was present in the granulosa cells of secondary, preantral and small antral follicles less than 4 mm in diameter. In larger (4–8 mm) antral follicles, AMH expression gradually disappeared. In vitro and in vivo studies have shown that AMH has two sites of action during progressing stages of folliculogenesis. It inhibits initial follicle recruitment [2–4], FSH-dependant growth and selection of preantral and small antral follicles [5,6].

The number of primordial follicles that are left in the ovary is an important parameter for ovarian reserve. Ultrasonography is not able to count those latter follicles because of their small size; only growing follicles may be counted; these are called antral follicle count (AFC). However, it appears that the number of growing follicles is correlated to the size of primordial follicle stock from which they are recruited [7]. In addition, many studies have shown that there was a good correlation between AFC and serum AMH levels [8–10].

Preliminary assessment of ovarian reserve in women undergoing assisted reproduction is essential. The status of ovarian reserve will supply reliable information to optimize the treatment protocol. With ovarian aging, the stock of primordial follicles decreases steadily. Some studies have shown that AMH concentrations decreased significantly over time whereas levels of FSH and inhibin B did not change [8,11]. In this way, many studies have investigated the relationship between serum AMH and number of oocytes collected after ovarian stimulation for couples undergoing in vitro fertilization (IVF) procedures [9,12–20]. All studies observed that mean serum AMH concentration decreased with the number of oocytes retrieved. Regarding embryo characteristics, most studies did not reveal any significant correlation between AMH and embryo quality [18,20–22]. Some authors have suggested a threshold under which the probability of becoming pregnant was significantly reduced [16,23–24]; the cut-off values ranging from 1.1 to 2.7 ng/mL.

The primary outcome of this retrospective study including 559 couples undergoing IVF cycles was to determine if AMH concentration could contribute to predict oocyte quality (defined by nuclear maturity), fertilization rate and embryo quality on Day 2. Secondary outcomes included the value of AMH concentration to predict both the quantitative status of ovarian reserve (defined by the number of oocytes retrieved) and clinical pregnancy.

2. Patients

A retrospective study analyzing the value of AMH concentration to predict oocyte quality was conducted at the Unit of Medicine and Reproductive Biology of the University Hospital, Tours. Between January and December 2007, 559 couples (n = 559 cycles) were included in the study. The main characteristics of women included in the study were as following: mean age = 32.0 ± 4.3 years, primary infertility = 64%, duration of infertility = 4.5 ± 2.8 years. Female etiologies of infertility were tubal abnormalities (7%), endometriosis (3%), ovulation disorder (45%), mixed (30%) and unexplained infertility (15%). Patients with polycystic ovary syndrome (PCOS) were excluded from the study. Rotterdam criteria were used to establish the diagnosis of PCOS [25]. The main characteristics of men were as follows: mean age = 34.3 ± 5.0 years, sperm concentration = 40.4 ± 47.1 millions/mL, a+b sperm motility = 20 ± 13%, normal shapes = 8 ± 9%, ICSI rate = 70%.

3. Methods

The ovarian stimulation protocol and the IVF procedures used (classical IVF or intracytoplasmic sperm injection [ICSI]) have already been described elsewhere [26].

3.1. Antimüllerian hormone measurement

All patients included in the study had their AMH levels measured during routine explorations (about six months before IVF attempt). All serum AMH levels were determined using an ultrasensitive enzyme-linked immunosorbet assay (ELISA) (Beckman-Coulter). During the study, the reference values provided by the laboratory performing AMH measurements were 2.2–6.8 ng/ml on Day 3 of the menstrual cycle for women with regular ovulatory menstrual cycles. Interassay and intra-assay coefficients of variation were less than 10%.

The quantitative status of the ovarian reserve was evaluated by the total number of oocytes collected whatever their nuclear quality. It means that both mature (metaphase II) and immature oocytes (prophase I and metaphase I) were counted.

Oocyte quality was evaluated by the rate of mature oocytes (= number of metaphase II oocytes divided by the total number of oocytes collected).

Fertilization rate was calculated by dividing the number of embryos on Day 2 by the number of mature oocytes.

Embryo quality was evaluated by the ratio of “top quality” embryos on Day 2 (= number of top quality embryos on Day 2 divided by the total number of embryos on Day 2). A “top quality” embryo on Day 2 was defined as having four mononuclear similar blastomeres and less than 20% fragmentation.

The outcome of IVF attempts was determined by the clinical pregnancy rate per oocyte retrieval. Clinical pregnancy was defined as the presence of a gestational sac with foetal heart activity on ultrasound examination five weeks after oocyte retrieval.

3.2. Statistical analysis

Data were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed using Statview 4.1 software (Abacus Concepts, Berkeley, CA, USA). Quantitative variables were compared using Student’s t-test and Spearman’s correlation coefficients. Qualitative data were compared using contingency tables (χ² test). Differences were considered significant if p is less than 0.05.
4. Results

Regarding IVF attempts, the main biological and clinical characteristics of the 559 couples were the following: total number of mature oocytes = 7.7 ± 4.6, rate of mature oocytes = 72 ± 25%, fertilization rate = 59 ± 32%, rate of “top quality” embryos available on Day 2 = 16 ± 25%, mean number of embryos transferred = 1.4 ± 0.5, clinical pregnancy rate per oocyte retrieval = 26%.

4.1. Antimüllerian hormone and female age

Increased female age was associated with low serum AMH levels (r = 0.23, p < 0.0001) (Fig. 1). In women aged equal or over 39 years old, AMH concentrations were therefore twice as lower compared to those women aged less than or equal to 29 years old (2.7 ± 2.3 ng/mL vs. 5.5 ± 4.8 ng/mL, respectively, p < 0.05).

4.2. Antimüllerian hormone and oocyte quality

There was a significant but weak correlation (r = 0.32, p < 0.0001) between AMH and mean number of total oocytes (Fig. 2). In patients achieving poor oocyte retrieval, the mean serum AMH concentration was significantly lower compared to patients with more oocytes retrieved ([≤ 5 oocytes, AMH = 3.2 ± 3.4 ng/mL], [6 to 9 oocytes, AMH = 4.0 ± 3.5 ng/mL], [≥ 10 oocytes, AMH = 5.6 ± 4.7 ng/mL], p < 0.0001]. However, the rate of mature oocytes did not differ according to AMH concentrations (Fig. 3). Additionally, fertilization rates did not differ whatever AMH concentrations were (Fig. 4).

4.3. Antimüllerian hormone and embryos on Day 2

Serum AMH concentration decreased with the mean number of embryos at Day 2. However, the rate of “top quality” embryos available on Day 2 did not differ according to serum AMH concentrations (Fig. 5). The correlation between AMH concentration and mean number of “top quality” embryos available on Day 2 was therefore markedly low (r = 0.1, p < 0.005).
4.4. Antimüllerian hormone and clinical pregnancy after IVF attempt

In our study, patients initiating a clinical pregnancy after IVF attempts had similar serum AMH levels compared to patients with implantation failure after embryo transfer (4.6 ± 4.3 ng/mL vs. 4.0 ± 3.8 ng/mL, respectively). Clinical pregnancy rates according to AMH concentration classes are reported in Table 1. Two patients with very low levels of AMH concentration (<0.5 ng/mL) achieved a clinical pregnancy. Consequently, we could not report any threshold value of serum AMH concentration that would be predictive of low probability in achieving a pregnancy after IVF attempt.

5. Discussion

The aim of this study was to determine the usefulness of AMH measurement to predict quantitative and qualitative ovarian response to gonadotrophins as well as the achievement of a clinical pregnancy in couples undergoing IVF attempts. It has been shown that serum AMH concentration was correlated with the quantitative statement of ovarian reserve (mean number of total oocytes retrieved). However, regarding qualitative aspect, AMH measurements do not seem to provide any information on oocyte nuclear maturity or developmental potential.

Infertility assessment procedures are essential before the initiation of an Assisted Reproduction Technology (ART) programme. In women, it is obviously not possible to count directly into the ovary the number of oocytes and evaluate oocyte quality. Only indirect investigations may be performed such as AFC and hormone measurements. Among the hormonal markers that have been evaluated to predict ovarian reserve, FSH is considered as a good marker of ovarian reserve, whereas inhibin B test did not reach the promising initial results [7]. Serum AMH measurement has been recently added as a marker of ovarian reserve. Two commercial ultra sensitive kits measurement are mainly used: AMH Beckman Coulter ELISA kit and Diagnostic System Laboratories (DSL) ELISA kit. Despite a close linear relationship between the two kits [27], AMH levels are about fivefold lower with the DSL kit than with the Beckman Coulter kit [27–29]. In order to homogenize the data, all AMH measurements carried out in our study were performed with the Beckman Coulter kit. The goal of infertility assessment procedures is first to determine the causes of patient’s infertility but also to help clinicians to predict the future ovarian response after folliculogenesis stimulating treatment. Two studies including 70 to 80 patients reported on significantly lower mean AMH levels in women who cancelled their treatment cycle due to poor response to gonadotrophin stimulation [13,30]. This indicates that the decline of ovarian reserve is associated with a decrease in AMH levels. In women undergoing IVF attempts, the mean number of oocytes retrieved is also a good parameter to evaluate the ovarian response to the hormonal treatment. Analysis of previous reported data highlights the lack of consensus to define “poor responders” [9,12,15–16]. We observed low levels of serum AMH when the number of oocytes retrieved was less than or equal to five. In our study, the relationship between those two parameters was weak (0.31) as in a previous study including as many patients as we did (r = 0.30 and n = 276) [20]. Only one recent publication did not report any correlation between both parameters [22]. Thus, the majority of studies observed that serum AMH measurement seemed to be predictive of quantitative ovarian response to the stimulating IVF treatment. For clinicians, it is therefore important to get this information to counsel patients with low AMH levels about their poor prognosis in terms of ovarian response. Moreover, this may help them to schedule a higher dose of gonadotrophins for the further treatment in such profiles of patients. However, to our knowledge, no consensus determining a threshold value of serum AMH concentration to define a “poor responder” is available.

Similarly to the number of oocytes retrieved, oocyte quality (defined in terms of nuclear and cytoplasmic maturity) is also an important parameter. Do women with low AMH levels produce oocytes with poor quality regarding to nuclear maturity? Some studies reported on high correlations ranging from 0.33 to 0.89 [19,24]. Our results showed a low correlation (r = 0.26) between serum AMH concentration and mean number of mature oocytes. As a consequence, it means that in our study, AMH was not predictive of oocyte quality in terms of nuclear maturity. One study investigated oocyte cytoplasm appearance and reported higher frequency of morphological abnormalities (dark central granulations, aggregation of smooth endoplasmic reticulum) when AMH concentration was less than 1.66 ng/mL [21]. However, similarly to our results, fertilization rate was not different according to AMH levels. In other words, low AMH concentration does not preclude a normal fertilization to happen, which represents comforting information for infertile couples.

Nowadays, most of the studies focusing on the association between AMH and embryo quality did not observe any correlation between both parameters [18,20–22]. Only one study reported on a significant but extremely weak relationship (r = 0.14) between AMH level and embryo quality [24]. Such information is also important for clinicians when counselling patients with low AMH levels. Decline of the ovarian reserve is not systematically associated with decreased oocyte or embryo quality.

Some studies tried to establish a cut-off value of AMH level under which the probability of achieving a pregnancy would be reduced (1.1 ng/mL [23], 1.4 ng/mL [16] and 2.7 ng/mL [24]). In our study including 559 couples, we did not observe any difference between serum AMH levels for couples achieving...
a clinical pregnancy or for unsuccessful couples. In addition, two couples achieved a clinical pregnancy with serum AMH concentrations less than 0.5 ng/mL.

6. Conclusion

In conclusion, our study including more than 500 women confirmed the main previously published biological and clinical data related to AMH measurement: AMH concentration decreases significantly over time with ovarian aging. Moreover, regarding the quantitative status of the ovarian reserve (expressed in the present study by the total number of oocytes retrieved), a relationship with AMH level was observed. On the other hand, regarding the qualitative status of the ovarian reserve, AMH is not predictive of both oocyte nuclear maturity and developmental potential. Moreover, embryo quality is not associated with AMH concentration. Finally at the moment, no cut-off value seems able to predict further implantation in IVF programmes. As a consequence, AMH is an interesting marker to investigate a part of the ovarian reserve but has some limitations as previous markers. Further studies are needed to define more accurately the optimal power of serum AMH measurement in infertile women.

References