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ORIGINAL ARTICLE *Reproductive endocrinology*

Serum anti-Müllerian hormone levels are negatively related to Follicular Output RaTe (FORT) in normo-cycling women undergoing controlled ovarian hyperstimulation

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BACKGROUND: Since in rodents anti-Müllerian hormone (AMH) has been shown to inhibit antral follicle responsiveness to FSH, we aimed at verifying whether a relationship exists between serum AMH levels and antral follicle responsiveness to exogenous FSH in normo-cycling women.

METHODS: Serum AMH, estradiol (E_2) and FSH levels were prospectively measured on cycle day 3 in patients undergoing controlled ovarian hyperstimulation (COH) with a time-release GnRH agonist and standardized FSH doses. In 162 patients, follicles were counted after pituitary suppression and before FSH administration (baseline; small antral follicles; 3–8 mm), and on the day of hCG (dhCG; pre-ovulatory follicles; 16–22 mm). Antral follicle responsiveness to FSH was estimated by the Follicular Output RaTe (FORT), determined by the ratio pre-ovulatory follicle count on dhCG × 100/small antral follicle count at baseline.

RESULTS: Serum AMH levels were positively correlated with the number of small antral follicles at baseline ($r = 0.59$; $P < 0.0001$) and pre-ovulatory follicles on dhCG ($r = 0.17$; $P < 0.04$). Overall, FORT was 47.5 ± 1.4% and failed to be influenced by the woman's age, BMI or basal E_2 and FSH level. Conversely, multiple regression analysis showed that FORT was negatively correlated with AMH levels ($r = -0.30$; $P < 0.001$), irrespective of duration of COH and total FSH dose.

CONCLUSIONS: The percentage of follicles that effectively respond to FSH by reaching pre-ovulatory maturation is negatively and independently related to serum AMH levels. Although the mechanisms underlying this finding remain unclear, it is in keeping with the hypothesis that AMH inhibits follicle sensitivity to FSH.

Key words: anti-Müllerian hormone / Müllerian-inhibiting substance / FSH / controlled ovarian hyperstimulation / IVF–ET

Introduction

Whereas the precise physiological role of anti-Müllerian hormone (AMH), a peptide exclusively produced by the granulosa cells of ovarian follicles (Vigier *et al.*, 1984), remains to be established in adult women, evidence exists that this glycoprotein may exert pivotal regulatory effects on folliculogenesis. Experiments conducted in mice have suggested that AMH not only inhibits growth initiation of primordial follicles (Durlinger *et al.*, 1999, 2002), but also partakes in the regulation of the growth of preantral and small antral follicles by inhibiting their sensitivity to FSH. In support of this latter hypothesis, AMH has been shown to inhibit the expected development of preantral mouse follicles cultured for 4 or 5 days in the presence of FSH, presumably by decreasing the rate of granulosa cell proliferation (Durlinger *et al.*, 2001). Although these results could not be duplicated in another study that followed a slightly different methodology (McGee *et al.*, 2001), they are in keeping with previous experiments showing that AMH inhibits basal and epidermal growth-factor-stimulated proliferation of human granulosa cells (Kim *et al.*, 1992), and follicle differentiation, as reflected by FSH-regulated aromatase and LH receptor

expression (Di Clemente et al., 1994). The possible inhibitory effect of AMH on the acquirement of sensitivity to FSH by follicles at later stages of folliculogenesis was corroborated by *in vivo* studies using AMH null mice. In the presence of low serum FSH levels resulting from GnRH-antagonist treatment, AMH null mice showed more large preantral and small antral follicles than those from wild-type mice, a phenomenon that could be attenuated by the eventual rise in serum FSH levels (Durlinger et al., 2001). Together, these data add weight to the hypothesis that AMH may be implicated in the regulation of cyclic follicle recruitment during the luteal-follicular transition of the menstrual cycle.

In adult women, documentation of the possible inhibitory role of AMH on the sensitivity of preantral and antral follicles to FSH still is lacking. The reported observation of a negative relationship between AMH and FSH levels on cycle day 3 does not help in clarifying this question since both parameters are influenced by a common variable (i.e. the number of small antral follicles). A helpful clinical model to indirectly address this issue is the ovarian response to exogenous FSH during pituitary-desensitized controlled ovarian hyperstimulation (COH). Yet, it has been exhaustively shown that women with elevated serum AMH levels respond strongly to COH (Seifer et al., 2002; Hazout et al., 2004; Fanchin et al., 2005a; La Marca et al., 2007; Broer et al., 2009), data that apparently stands in contradiction to the hypothesis that AMH attenuates antral follicle responsiveness to FSH. It is, however, noteworthy that the intensity of the ovarian response to COH is a function not only of the inherent sensitivity of antral follicles to FSH but also of the pretreatment size of the antral follicle pool. Indeed, women with a large number of small antral follicles tend to produce, in response to FSH, more mature follicles and fertilizable eggs than those having a reduced count of small antral follicles. The quantitative relationship between AMH levels and the number of mature follicles and fertilizable eggs (Seifer et al., 2002; Hazout et al., 2004; Fanchin et al., 2005a; La Marca et al., 2007; Broer et al., 2009) may merely result from the putative positive correlation between peripheral AMH levels and the pretreatment number of small antral follicles (De Vet et al., 2002; van Rooij et al., 2002; Pigny et al., 2003; Fanchin et al., 2003a, 2005b), and does not itself attest to the status of responsiveness of antral follicles to FSH treatment.

Hence, in an effort to evaluate the follicle responsiveness to exogenous FSH, we elected to use as a primary endpoint the ratio between the number of follicles that reach pre-ovulatory maturation in response to FSH and the available pool of FSH-sensitive follicles. We called this innovative endpoint Follicular Output RaTe (FORT). This measure benefits from being independent of the size of pretreatment cohort of small antral follicles, contrary to the absolute number of mature follicles and oocytes obtained at the end of COH. Therefore, the present investigation aimed at studying the possible relationship between serum AMH levels and the efficiency of COH cycles, expressed by FORT, in normo-cycling women.

Materials and Methods

Subjects

We prospectively studied 179 IVF–ET (IVF–embryo transfer) candidates, 24–43 years of age. All of them met the following inclusion criteria: (i) both ovaries present, with no morphological abnormalities (such as cysts, endometriomas, etc.), no history of past surgery and adequately visualized in transvaginal ultrasound scans; (ii) regular menstrual cycle lengths ranging between 25 and 35 days; (iii) no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion; (iv) no clinical signs of hyperandrogenism; (v) BMI ranging from 16 to 30 kg/m^2. Incidentally, indications for IVF–ET were male factor (35.8%), tubal factor (14.8%) or idiopathic infertility (36.4%); in the remaining 13.0% of cases, IVF–ET was performed for PGD. As the present study was merely observational and included only analysis of data from routine measurements, it did not require submission to our Institutional Review Board.

Protocol

Our study protocol is summarized in Fig. 1. Serum AMH, estradiol (E_2) and FSH levels were measured in blood samples taken on cycle day 3. On cycle days 1–3 of a subsequent cycle, women received a single-dose, time-release GnRH agonist, triptorelin (3 mg, i.m., Decapeptyl, Beaufour Ipsen Pharma, Paris, France). The interval between AMH measurement and the GnRH agonist administration never exceeded 3 months. Three weeks later, complete pituitary desensitization was confirmed by the detection of low serum levels of progesterone, E_2 and LH (baseline). Patients also underwent a conventional ultrasound examination to exclude ovarian cysts and to verify that endometrial thickness was <5 mm at baseline. Recombinant FSH therapy (Gonal-F, Serono Pharmaceuticals, Boulogne, France) was then initiated, at a dosage of 300 IU/day for at least 5 days, and continued until the day of hCG (dhCG; Gonadotrophine Chorionique 'Endo', Organon Pharmaceuticals, Saint-Denis, France, 10 000 IU, i.m.) administration. From the 6th day of recombinant FSH therapy onwards, daily FSH doses were adjusted according to E_2 levels and/or the number of growing follicles. During the last days of COH, patients had frequent visits to our Institution for ultrasonographic and hormonal examinations to define the proper timing for hCG administration. Administration of hCG (dhCG administration) was performed as soon as ≥ 4 pre-ovulatory follicles (16–22 mm in diameter) were observed and E_2 levels per pre-ovulatory follicle were >200 pg/ml. Women whose response to COH did not meet these criteria, or for whom the analysis of growing follicle number precluded the achievement of ≥ 4 pre-ovulatory follicles, had their IVF–ET attempt canceled ($n = 29$). Some of them ($n = 12$) who preferred to complete their COH anyway and, therefore, achieved only 1–3 pre-ovulatory follicles, eventually underwent hCG administration followed by intrauterine insemination. Since the number of 16–22 mm follicles could be effectively determined in these patients, they were maintained in the study. The remaining cancelled patients ($n = 17$), who preferred to discontinue the treatment before any follicle had reached pre-ovulatory maturation, were excluded from the analysis because their number of 16–22 mm follicles could not be determined. Therefore, the population actually included in the analysis was 162 patients. These patients underwent IVF–ET ($n = 150$) or intrauterine insemination ($n = 12$), according to routine procedures.

Hormone and follicle measurements

Serum AMH levels were determined using a 'second generation' enzyme-linked immunosorbent assay (reference A16507; Immunotech Beckman Coulter Laboratories, Villepinte, France). Intra- and inter-assay coefficients of variation (CV) were <6 and $<10\%$, respectively, lower detection limit at 0.13 ng/ml, and linearity up to 21 ng/ml for AMH. Serum progesterone, E_2 and LH levels were determined by an automated multi-analysis system using a chemiluminescence technique (Advia-Centaur, Bayer Diagnostics, Puteaux, France). For progesterone, lower detection limit was 0.10 ng/ml, linearity up to 60 ng/ml and intra- and inter-assay CV were 8 and

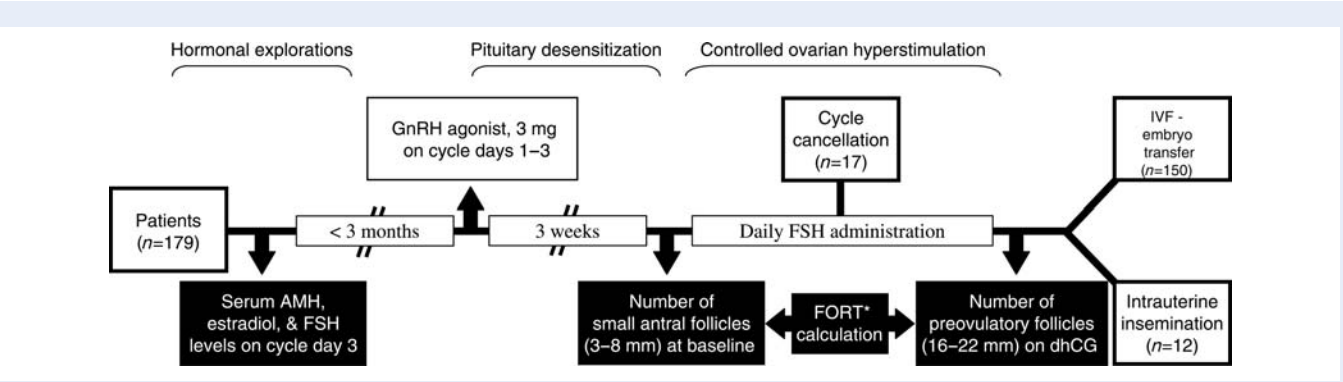

Figure 1 Flow chart of the study of FORT and serum AMH levels in normo-cycling women undergoing COH. *FORT: ratio of pre-ovulatory follicle count on dhCG × 100/small antral follicle count at baseline.

9%, respectively. For E_2, lower detection limit was 30 pg/ml, linearity up to 1000 pg/ml, and intra- and inter-assay CV were 8 and 9%, respectively. For LH lower detection limit was 0.1 mIU/ml and intra- and inter-assay CV were 3 and 5%, respectively.

At baseline and dhCG, ovarian ultrasound scans were performed using a 5.0–9.0 MHz multi-frequency transvaginal probe (Voluson 730 Expert, General Electric Medical Systems, Paris, France) to evaluate the number and sizes of ovarian antral follicles. We determined, at baseline, the number of all follicles with diameters ranging from 3 to 8 mm in both ovaries and, on dhCG, the number of all follicles with diameters ranging from 16 to 22 mm. On dhCG, follicles measuring 16–22 mm in diameter were considered pre-ovulatory follicles (Dubey *et al*., 1995).

FORT was calculated as the ratio of pre-ovulatory follicle (16–22 mm in diameter) count on dhCG × 100/small antral follicle (3–8 mm in diameter) count at baseline. The choice of considering only 16–22 mm follicles for the calculation of FORT was arbitrary and represented a methodological attempt for discriminating, among the cohort of small antral follicles, those that were the most FSH-responsive.

Finally, the reported lack of cycle-to-cycle variation in serum AMH levels (Fanchin *et al*., 2005b) and their robustness to the influence of pharmacological suppression of endogenous gonadotrophins (Mohamed *et al*., 2006) allowed us to evaluate reliably the possible relationship between serum AMH levels before COH and FORT values.

Definition of AMH groups

To make the interpretation of the possible relationship between serum AMH and follicle responsiveness to COH easier, we decided to sort our population into three distinct groups. The three groups were arbitrarily chosen according to whether pre-COH serum AMH levels were under the 25th percentile (<1.69 ng/ml, low-AMH group; $n = 41$), between the 25th and the 75th percentile (1.69–3.20 ng/ml, average AMH group; $n = 82$) or above the 75th percentile (>3.20 ng/ml, high-AMH group; $n = 39$) of value distribution.

Statistics

The measure of central tendency used was the mean and the measure of variability was the SE. Medians and minimum–maximum values were used when normality of data distribution could not be ascertained. Comparisons of continuous variables from the low, average and high-AMH groups were performed using analysis of variance. Categorical variables in the three groups were compared using the two-sided Pearson χ^2 test. Relationship between two continuous variables was assessed by correlation when they were independent of each other and by simple regression when there was a dependency relationship. The Spearman's test was used to determine if coefficients of correlation (r) were significantly different from zero. The relationship between serum AMH levels and FORT was adjusted for possible covariates, such as women's age, BMI value, serum E_2 and FSH levels, and total dose of recombinant FSH used for COH, by running a stepwise multiple regression model. According to this model, in case of strict correlation between two variables, one of them was excluded from the analysis to avoid multicolinearity. Since variables included in the stepwise multiple regression analysis followed a parametric distribution, prior logarithmic transformation was not required. A $P < 0.05$ was considered statistically significant.

Results

Overall population and COH characteristics

At the time of inclusion, women were aged 34.6 ± 0.3 years and presented with BMI values at 21.9 ± 0.2 kg/m^2. On cycle day 3, serum AMH, E_2 and FSH levels were 2.52 ± 0.08 ng/ml, 34.0 ± 1.8 pg/ml and 6.59 ± 0.14 mIU/ml, respectively. At baseline, women had 14.8 ± 0.3 antral follicles and serum progesterone, E_2 and LH levels at 0.19 ± 0.01 ng/ml, 30.3 ± 0.3 pg/ml and 1.53 ± 0.05 mIU/ml, respectively. On dhCG, the total number of pre-ovulatory follicles obtained was 6.9 ± 0.2 and serum progesterone, E_2 and LH levels were 0.98 ± 0.05 ng/ml, 2444 ± 94 pg/ml and 1.54 ± 0.16 mIU/ml, respectively. COH required a total dose of 3225 ± 42 IU of recombinant FSH and lasted 12.1 ± 0.1 days. Overall, FORT was 47.5 ± 1.4% (range 9.1–91.7%).

As expected, at baseline we observed a positive relationship between serum AMH levels and the number of small antral ($r = 0.59$; $P < 0.0001$) and pre-ovulatory follicles ($r = 0.17$; $P < 0.04$). A weak but significant negative relationship was noted between serum AMH and FSH levels on cycle day 3 ($r = -0.16$; $P < 0.04$). Yet, serum AMH levels were not correlated with patient age, BMI, duration of COH or total dose of recombinant FSH. Serum FSH levels also showed a correlation with the number of small antral follicles but weaker than that of AMH ($r = -0.17$; $P < 0.04$) but serum FSH did not correlate with the count of pre-ovulatory follicles. Serum E_2 levels were not correlated either with follicle count at baseline or on dhCG.

Table I Patient characteristics and COH data in the low, average and high serum AMH groups.

	Low AMH <1.69 ng/ml (n = 41)	Average AMH 1.69–3.20 ng/ml (n = 82)	High AMH >3.20 ng/ml (n = 39)	P
Age (years)	35.2 ± 0.6	34.4 ± 0.4	34.4 ± 0.7	0.524
BMI (kg/m^2)	21.9 ± 0.5	21.9 ± 0.3	22.0 ± 0.5	0.966
Indications for IVF–ET				
Male (%)	31.7	39.0	33.3	0.820
Tubal (%)	19.5	11.0	17.9	0.632
Genetic (%)	9.8	14.6	12.8	0.583
Idiopathic (%)	39.0	35.4	36.0	0.895
Small antral follicle (3–8 mm) count	11.3 ± 0.5	15.0 ± 0.4	17.9 ± 0.5	<0.001
Serum AMH levels (ng/ml)[a]	1.30 (0.50–1.68)	2.40 (1.70–3.20)	3.80 (3.21–6.00)	–
Serum Day-3 FSH levels (IU/ml)[a]	7.2 (3.2–13.1)	6.0 (2.7–11.8)	6.4 (4.0–9.5)	0.446
Total recombinant FSH dose (IU)	3011 ± 106	3253 ± 54	3380 ± 64	<0.006
Duration of COH (days)	11.2 ± 0.3	12.1 ± 0.2	12.8 ± 0.1	<0.001
Pre-ovulatory follicle (16–22 mm) count[b]	6.1 ± 0.4	7.1 ± 0.3	7.3 ± 0.4	0.246
FORT (%)[c]	54.2 ± 3.2	47.5 ± 1.9	41.0 ± 1.9	<0.001

Differences among groups were assessed by using analysis of variance or two-sided Pearson χ^2 test when appropriate. IVF–ET, IVF–embryo transfer.
[a]Values are medians (minimum–maximum).
[b]On day of hCG administration.
[c]FORT, follicular output rate defined as pre-ovulatory follicle (16–22 mm) count × 100/small antral follicle (3–10 mm) count.

Population and COH characteristics in AMH groups

Patient characteristics and COH data in the low, average and high-AMH groups are summarized in Table I. Although women in the low-AMH group tended to be older than those in the average and high-AMH groups, differences did not reach statistical significance. Further, BMI and indications for IVF–ET were comparable in all groups. We observed a significant stepwise increase in baseline follicle (3–8 mm) counts from the low to the high-AMH groups, paralleled by a significant increase in total recombinant FSH dose and COH duration. Pre-ovulatory follicle (16–22 mm) counts tended to be lower in the low-AMH group when compared with the other groups. Finally, FORT decreased progressively and significantly from the low to the high-AMH groups.

Factors influencing FORT

Univariate analysis of factors possibly associated with FORT is shown in Table II. As demonstrated, FORT was not correlated with age of patients, basal E_2 and FSH levels or BMI value. Conversely, this novel FORT parameter was negatively correlated with serum AMH level and positively correlated with total recombinant FSH dose and duration of COH. To verify whether the relationship between serum AMH levels and FORT was independent, after adjusting for the remaining correlated variables (total recombinant FSH dose and duration of COH), we conducted a stepwise regression analysis that is summarized in Table III. In this analysis, to avoid multicollinearity with duration of COH, we decided to exclude the total recombinant FSH dose, as both parameters were strongly correlated to each other ($r = 0.88$; $P < 0.0001$). As shown, irrespective of the duration of

Table II Univariate analysis of factors possibly associated with FORT.

	Spearman's correlation coefficient	P
Age	−0.032	0.685
BMI	0.078	0.344
Serum E_2 level	0.030	0.730
Serum Day-3 FSH level	0.033	0.679
Serum AMH level	−0.301	<0.001
Total recombinant FSH dose	0.303	<0.001
Duration of COH	0.381	<0.001

Table III Stepwise regression analysis of variables significantly associated with FORT.

	β-coefficient	P	R^2 of multiple model
Baseline AMH levels	−0.216	<0.002	–
Duration of COH	0.413	<0.001	0.221

Although total recombinant FSH dose was also correlated with FORT values, it had to be excluded from the analysis to avoid multicollinearity with duration of COH.

COH and, *a fortiori*, total recombinant FSH dose, baseline AMH levels were negatively and significantly correlated with FORT.

Discussion

The present investigation proposed a clinical model for studying the possible relationship between serum AMH and antral follicle responsiveness to exogenous FSH in adult women. In this model, we elected to administer similar and sustained recombinant FSH doses to profoundly pituitary-desensitized, BMI-controlled patients who had different pretreatment follicle counts and circulating AMH levels. To quantify follicle responsiveness to FSH, instead of merely reckoning the number of follicles undergoing pre-ovulatory maturation, we used the FORT, an innovative measure that has the advantage of being independent of the pretreatment mass of FSH-sensitive follicles. The present results showed that circulating AMH levels and FORT are correlated, and that this negative relationship is independent of possible confounding covariates, such as the duration of COH or the total recombinant FSH dose.

FORT was calculated by dividing the number of follicles which had reached pre-ovulatory maturation by the number of small antral follicles observed after complete suppression of endogenous gonadotrophins by a time-release GnRH agonist. Yet, the use of such a parameter presents inherent limitations. First, FORT was not, by design, assessable in a fraction of patients having responded poorly to COH and who desired to discontinue treatment (9.5% of cases), since their number of 16–22 mm follicles could not be established. Second, although FORT calculation implies that small antral follicles, ranging from 3 to 8 mm in diameter, respond in a co-ordinated manner to FSH, it is possible that differences exist in the FSH-driven growth of these follicles according to their sizes (Fanchin *et al.*, 2005c). To overcome this limitation, it would have been necessary to track the development of each follicle in response to FSH individually, which is unrealistic when multiple growing follicles co-exist in the same ovary. Third, FORT assumes that only follicles having reached diameters ranging between 16 and 22 mm on dhCG effectively responded to FSH, while it is conceivable that smaller follicles also presented some degree of FSH responsiveness. However, as it is also possible that very small follicles, which could not be counted by ultrasound at baseline, may have also initiated their FSH-driven maturation after the start of COH and reached intermediate sizes on dhCG, the inclusion of average-sized follicles on dhCG into the calculation of FORT should puzzle interpretation of the results. Moreover, further studies focusing on other follicle sizes will be helpful to fine-tune other relevant cutoffs for the calculation of this new FORT parameter. Furthermore, to improve the scientific soundness and validity of FORT, we made the following methodological choices. Primo, to recruit as many follicles as possible despite their pretreatment sizes and homogenize as far as possible the ovarian response to COH, strong initial FSH doses were used in all patients. *Secundo*, strict follicle monitoring policies were respected and, to avoid any unnecessary increase of COH duration, hCG administration was performed as soon as $\geq$4 follicles had reached pre-ovulatory maturation.

A plethora of publications has associated serum AMH levels with the strength of the ovarian response of COH, as reflected by the number of pre-ovulatory follicles and/or oocytes obtained in IVF–ET cycles (Seifer *et al.*, 2002; Hazout *et al.*, 2004; Fanchin *et al.*, 2005a; La Marca *et al.*, 2007; Broer *et al.*, 2009); the negative association we observed between AMH and FORT does not contradict these data, as we also noted a positive relationship between baseline AMH levels and the number of 16–22 mm follicles. Moreover, the differential correlation coefficients between serum AMH levels and small and pre-ovulatory follicles is in keeping with the fact that the percentage of follicles that effectively respond to exogenous FSH is not constant from one patient to another, and that AMH level is related to this percentage. In addition, COH itself may reduce peripheral AMH levels (Fanchin *et al.*, 2003b), presumably through the induced multifollicular maturation and the progressive loss in ability of granulosa cells of pre-ovulatory follicles to produce AMH. Unfortunately, in the present investigation, because serum AMH levels on dhCG were not available, the conceivable relationship between the extent of the decrease in AMH levels during COH and FORT could not be investigated. Additional studies are needed to clarify this issue. It is also noteworthy that the negative relationship between serum AMH and FSH levels observed in the present data, and shown in previous publications (De Vet *et al.*, 2002; Fanchin *et al.*, 2003b), probably is a mere consequence of the putative relationship of both parameters with antral follicle count.

The central finding of the present investigation i.e. the negative relationship between serum AMH levels and FORT is in keeping with the reported inhibitory role of AMH on certain putative FSH actions on ovarian follicles, but by no means it constitutes a conclusive demonstration of this hypothesis. Indeed, previous *in vitro* and *in vivo* studies using both human and animal models have shown that AMH inhibits granulosa cell proliferation (Seifer *et al.*, 1991; Kim *et al.*, 1992; Durlinger *et al.*, 2001) and differentiation (Di Clemente *et al.*, 1994). However, the clarification of the mechanisms underlying these processes still is lacking. Models using *in vitro* growth and maturation of ovarian follicles may be useful to investigate the role of AMH in the responsiveness of antral follicles to FSH under controlled conditions. Another clinical model that could be used to investigate this issue is the polycystic ovary syndrome (PCOS). Indeed, women suffering from PCOS display 2- to 3-fold increased serum AMH levels when compared with non-PCOS women (Pigny *et al.*, 2003; Piltonen *et al.*, 2005), a phenomenon that may be attributed not only to the characteristic excess of growing follicles but also to an increased per-follicle AMH production (Pellatt *et al.*, 2007) in PCOS. It is noteworthy that PCOS patients combine high AMH concentrations, hyperresponse to exogenous FSH (Wang and Gemzell, 1980) and possible hypersensitivity of granulosa cells to FSH (Coffler *et al.*, 2003), which stands in apparent contradiction to the present results. Also, the excess of growing follicles in PCOS does not support the hypothesis that AMH plays an inhibitory role on the initial follicular recruitment (Durlinger *et al.*, 2002), although AMH expression in primordial and transitional follicles has been reported to be insufficient in PCOS (Stubbs *et al.*, 2005). Unfortunately, by design, we chose not to include PCOS patients in the present study, which prevented us from investigating whether serum AMH levels are related to FORT values in this particular group of patients.

Incidentally, the present study did not address the issue of whether FORT constitutes an alternative parameter to assess ovarian and follicular health. Yet, the lack of association between age, basal E_2 and FSH levels and FORT suggests that, with ovarian ageing, antral follicles do not lose significantly their aptitude to respond to FSH. Whether a

compensating mechanism to help maintain ovulatory folliculogenesis exists in older women, and if the decrease in AMH levels is implicated in this process, remain to be elucidated. Also, the present data suggest that the poor ovarian responses to COH observed in older women are essentially linked to the pre-existing depletion of the follicular pool rather than a per-follicle loss of sensitivity to FSH.

The analysis of the fate of oocytes and embryos originating from patients displaying high or low FORT values has not been performed in the present study for three reasons. First, noticeable discrepancies existed in the type of assisted reproductive technology (ART) used (IVF–ET for genetic screening, intrauterine insemination, etc.), which might have influenced the results. Second, the number of ART cycles included in the study lacked the statistical power required to detect possible differences in pregnancy rates according to FORT values. Third, this analysis stood beyond the scope of the physiological investigation (i.e. relationship between circulating AMH levels and responsiveness of antral follicles to exogenous FSH administration). However, this important issue constitutes the objective of studies that are being currently performed by our team and will provide useful information to clarify these questions.

It is also noteworthy that the physiologically plausible, negative relationship between age and serum AMH levels barely reached statistical significance in the present as in previous clinical investigations (Fanchin *et al*., 2003a). Bias in population selection commonly encountered in observational studies may offer a possible explanation for this phenomenon. On the one hand, among patients who effectively enter IVF programmes, older women presenting a very poor ovarian follicle status (and therefore low AMH levels) often are not eligible, which often prevents them from participating in observational trials. On the other, the deliberate non-inclusion of PCOS patients in the present study may have also weakened the expected relationship between age and serum AMH levels, since this group of patients displays high AMH levels and tends to undergo ART at a younger age than other patients (Dewailly *et al*., 2010).

In conclusion, the present findings indicate that antral follicle responsiveness to FSH, as far as it is measurable by FORT, is negatively correlated to the circulating AMH levels in normo-cycling women. This association, which has been controlled for confounding covariates, is in keeping with the theory that AMH inhibits the sensitivity of antral follicles to FSH. However, additional clinical and basic research is needed to challenge the present results and to define the precise role, if any, of AMH in the control of follicle recruitment, growth and sensitivity to FSH.

Authors' roles

V.K.G. and M.G. collecting reported data, interpreting data and writing the paper. J.B.S. and I.R. collecting reported data and reviewing the paper. R.F. reviewing the paper. R.F. designing the reported study, interpreting data, writing and reviewing the paper.

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