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Anti-Müllerian hormone concentrations in the follicular fluid of the preovulatory follicle are predictive of the implantation potential of the ensuing embryo obtained by in vitro fertilization

Abbreviated title: AMH and follicle quality

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Abstract

Context: The strong relationship between serum anti-Müllerian hormone (AMH) levels and the number of antral follicles supports the use of AMH measurements as a quantitative marker of the ovarian follicular status. Yet, it still is unclear whether the aptitude of an individual follicle to produce AMH reflects its reproductive competence.

Objective: This study examined the possible relationship between serum or follicular fluid (FF) AMH concentrations and the fate of the ensuing oocytes and embryos obtained by in vitro fertilization-embryo transfer (IVF-ET) conducted in monodominant follicle cycles.

Design: Prospective study

Setting: University of Paris XI, AP-HP, INSERM U782.

Patients: 118 infertile IVF-ET candidates.

Interventions: Concentrations of AMH, progesterone, and estradiol were measured in the serum on cycle day 3 (d3) and on the day of oocyte pickup (dOPU), and in FF. Cycles were sorted into 3 sets of 3 distinct groups according to whether serum d3, serum dOPU, and FF AMH concentrations were </=30th centile (low AMH), between the 31st and the 70th centile (average AMH) or >70th centile (high AMH) of measurements.

Main outcome measure: Clinical pregnancy and embryo implantation rates.

Results: Clinical pregnancy rates (5.7%, 20.0%, and 39.5%, respectively; P <0.002) and embryo implantation rates (11.8%, 30.8%, and 65.4%, respectively; P <0.001) were markedly different among the low, moderate, and high FF AMH groups, but not among the serum (d3 or dOPU) AMH groups. Fertilization rates and embryo morphology remained similar irrespective of AMH concentrations in the serum or in FF. Incidentally, FF AMH concentrations were negatively correlated with FF progesterone (r=-0.27, P <0.003) and FF estradiol (r=-0.21, P <0.02) concentrations.

Conclusions: Concentrations of AMH in the FF, but not in the serum, constitute a useful follicular marker of embryo implantation and are negatively related to FF progesterone and estradiol concentrations.

Introduction

Growing evidence indicates that anti-Müllerian hormone (AMH), a glycoprotein that is exclusively produced by the granulosa cells of ovarian follicles in the adult female (1), is a unique biomarker of the ovarian follicular status. In contrast with inhibin B and estradiol, AMH is produced, presumably FSH-independently (2,3), in a wide range of follicles that goes from the primary to the small antral stages of folliculogenesis (4-6). In line with this, on cycle day 3, peripheral AMH levels have shown greater sensitivity to ovarian ageing (7), stronger relationship with the number of early antral follicles (8), and improved cycle-to-cycle reproducibility (9), as compared to inhibin B, estradiol, and FSH levels. In addition, serum AMH levels are a useful predictor of the ovarian response to controlled ovarian hyperstimulation (COH) (10-12).

Yet, the possible relationship between AMH production by an individual follicle and its functional quality (i.e. follicle aptitude to release an oocyte able to become a developing embryo) remains to be demonstrated. Indeed, previous studies conducted in different species as the rat (4), the sheep (13), and humans (6) have shown that granulosa cells from atretic follicles fail to express AMH. In addition, in regularly ovulating women, AMH content in individual follicles is related to both the number of early antral follicles on day 3 and their responsiveness to COH (14). These data indicate that women endowed with more antral follicles may also show increased per-follicle AMH levels, which implicitly suggests that peripheral AMH levels reflect not only antral follicle count but also per-follicle AMH production. Since direct information on the relationship between per-follicle AMH production and the outcome of the oocyte/embryo still is lacking, new insights into this sensitive issue could help to clarifying the role of AMH as a qualitative indicator of ovarian follicular status.

The present study was then conducted to investigate the possible relationship between AMH concentrations, measured both in the serum and in the follicular fluid (FF), and the fate of oocytes and embryos generated in IVF-ET conducted in monodominant follicle cycles. Indeed, contrary to COH, in this modality of treatment, a single follicle achieves preovulatory maturation and only one oocyte and embryo are obtained. This may be particularly instrumental in investigating this issue as it allows the adequate traceability between the single follicle and the ensuing oocyte and embryo.

Materials and Methods

Subjects

One hundred eighteen infertile women, 26 to 41 years of age, were studied prospectively. All of them met the following inclusion criteria: 1) both ovaries present, deprived of morphological abnormalities, and adequately visualized in transvaginal ultrasound scans; 2) menstrual cycle length range between 25 and 35 days; 3) no current or past diseases affecting ovaries or gonadotropin or sex steroid secretion, clearance, or excretion; 4) no clinical signs of hyperandrogenism; 5) body mass indexes (BMI) ranging between 18 and 25 kg/m^2. Infertility was due to sperm abnormalities (39%), tubal abnormalities (30%), endometriosis (12%) or was unexplained (19%). In agreement with the inclusion criteria, no patient suffering from polycystic ovary syndrome has been enrolled. An informed consent was obtained from all women and this investigation received the approval of our internal Institutional Review Board.

Cycle monitoring

On cycle day 3 (d3), women underwent blood samplings by venipuncture at approximately 9 AM. Sera were separated and frozen in aliquots at –80 °C for subsequent centralized analysis. Later in the morning, the number and the sizes of early antral follicles were assessed by ultrasound. From cycle day 8 onward, selection of the dominant follicle was monitored by ultrasound. When the mean diameter of the dominant follicle exceeded 12 mm, to prevent the risk of premature LH peak and to control further follicular maturation, women were administered subcutaneously 0.5 mg of a GnRH antagonist (cetrorelix acetate; Cetrotide 0.25 mg, Serono Pharmaceuticals, Boulogne, France) and 150 IU of hMG (Menopur, Ferring Pharmaceuticals, Gentilly, France) daily until the day of hCG (Gonadotrophine Chorionique "Endo", Organon Pharmaceuticals, Saint-Denis, France) administration. The choice of starting hMG treatment once follicle dominance had been achieved aimed at preventing the rescue of additional subordinated follicles. Eventually, women received a 5,000-IU hCG injection intramuscularly when the dominant follicle diameter exceeded 16 mm. The single oocyte was picked up approximately 34 hours after hCG administration (dOPU) and ET was performed 2 days after oocyte pickup. Top quality embryo was defined on day 2 as those having no multinucleated blastomeres, four or five blastomeres, and less than 20% anucleated fragments (15). According to the present study's design, each patient had only 1 oocyte retrieved and only 1 embryo obtained and transferred. Luteal phase was supported with micronized progesterone (Estima Gé, Effik Pharmaceuticals, Bièvres, France, 600 mg/day) administered daily by vaginal route starting on the evening of ET.

Serum and follicular fluid collection

On dOPU, women underwent a blood sampling by venipuncture at approximately 9 AM. Sera were separated and frozen in aliquots at –80 °C for subsequent centralized analysis. Under transvaginal ultrasound guidance, the follicular fluid (FF) from the dominant follicle was gently and thoroughly aspirated using a 10-mL syringe, then maintained at steady temperature conditions (37 °C)

until the oocyte was found and isolated. Meanwhile, the aspiration needle was kept steady inside the follicle and, in case of negative oocyte recovery, sequential follicular flushings were performed using 10-mL syringes filled with 3 mL of a balanced salt solution (Tyrode's salt solution, Eurobio Pharmaceuticals, Courtaboeuf, France). All flushing volumes were discarded. Oocyte pickup failure was defined by a negative oocyte recovery after 3 consecutive follicular flushings. The FF was then centrifuged at 3,000 g for 15 minutes at 4 °C to eliminate cellular elements and subsequently frozen at –80 °C for centralized hormonal analysis. Time elapsed between follicular aspiration and FF cryopreservation did not exceed 30 minutes.

Hormonal measurements in serum and FFs

Blood samples obtained on d3 and dOPU were assayed for AMH, progesterone and estradiol. Serum AMH levels were determined using a "second generation" enzyme-linked immunosorbent assay (reference A16507; Immunotech Beckman Coulter Laboratories, Villepinte, France). Intra- and interassay coefficients of variation were <6% and <10%, respectively, lower detection limit at 0.13 ng/mL, and linearity up to 21 ng/mL for AMH. Serum progesterone and estradiol 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 0.1 ng/mL, linearity up to 60 ng/mL, and intra- and interassay coefficients of variation were 8% and 9%, respectively. For estradiol, lower detection limit was 15 pg/mL, linearity up to 1,000 pg/mL, and intra- and interassay coefficients of variation were 8% and 9%, respectively. Conversion factor to SI units are 7.14 for AMH, 3.18 for progesterone, and 3.67 for estradiol.

For AMH, progesterone, and estradiol assays in the FF, we used similar methodology as described above. To avoid possible bias due to FF volume variability, hormone concentrations in the FF were adjusted to its protein content, as reported elsewhere (14,16). Proteins were measured according to the conventional Biuret reaction (17) using an automated multi-analysis system (AU640, Olympus, Rungis, France). FF hormone levels were expressed as ng/g of protein for both AMH and progesterone and mg/g of protein for estradiol.

Ultrasonographic measurements

Ultrasonographic measurements were performed using a 5.0-9.0 MHz multi-frequency transvaginal probe (Voluson 730 Expert, General Electric Medical Systems, Paris, France) according to a methodology previously described (8, 9). In brief, on d3, all antral follicles that measured 3-10 mm in mean diameter were carefully counted in both ovaries. On the day of hCG administration, the size of the dominant follicle was the mean of two orthogonal diameters.

Definition of AMH groups

Cycles were sorted arbitrarily into 3 sets of 3 different groups according to serum d3 and dOPU and FF AMH concentrations. Cutoffs for defining low, average, and high AMH concentrations corresponded arbitrarily to the round values of the 30th and 70th centiles of each measurement. Hence, according to these criteria, serum d3 AMH levels determined 3 different groups of patients: low-d3 (AMH </=1.0 ng/mL, n=31), average-d3 (AMH 1.1- 2.0 ng/mL, n=45), and high-d3 (AMH >2.0 ng/mL, n=42); serum dOPU AMH levels determined 3 other distinct groups: low-dOPU (AMH </=1.0 ng/mL, n=39), average-dOPU (AMH 1.1- 2.0 ng/mL, n=33), and high-dOPU (AMH >2.0 ng/mL, n=46); and FF AMH levels determined 3 additional groups: low-dOPU (AMH </=50.0 ng/g of protein, n=35), average-dOPU (AMH 50.1- 100.0 ng/g of protein, n=40), and high-dOPU (AMH >100.0 ng/g of protein, n=43).

Statistics

The measure of central tendency used was the mean and the measure of variability was the standard error. Medians and ranges were used when normality of data distribution could not be ascertained. Comparisons between continuous variables from the low, average, and high AMH groups were performed using ANOVA when data distribution was normal or the Kruskal-Wallis test when normality could not be confirmed. To determine the respective influence of different independent variables such as ages, number of antral follicle count, hormonal values on pregnancy and implantation rates we used binomial logistic regression and results were expressed as P and 95% confidence intervals (CI). Paired comparisons were made with the paired Student's *t*-test or the Wilcoxon signed rank test when appropriate. Relationship between two continuous variables was assessed by correlation when they were independent from 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 present study was powered to detect anticipated differences of 25% in embryo implantation rates at >80% power at 0.05 significance level. A *P* value <0.05 was considered statistically significant.

Results

Patient characteristics, cycle monitoring and embryology data

Population characteristics in the low, average, and high serum and FF AMH groups are summarized in Table 1. As mentioned, women's ages, BMIs, menstrual cycle lengths, and indications for IVF-ET were comparable in the 3 sets of AMH groups (d3, dOPU, FF). In line with this, neither serum nor FF AMH levels were significantly correlated with women's ages, BMIs, menstrual cycle lengths. Overall, on cycle day 3, median antral follicle (3-10 mm) count was 9 (range: 1-24). As expected, this measure was positively correlated with AMH levels on d3 (r=0.74; *P* <0.0001), dOPU (r=0.71; *P* <0.0001), and in the FF (r=0.29; *P* <0.002).

Cycle monitoring and embryology data in the low, average, and high serum and FF AMH groups are presented in Table 2. The total dose of hMG used for maintaining dominant follicle growth was similar irrespective of AMH levels in the serum and FF. It is noteworthy that the time necessary to achieve follicle maturation (>16 mm in diameter) was longer in patients showing higher serum d3 AMH. Indeed, the day of hCG administration was positively correlated with serum AMH levels on d3 (r=0.31; P <0.001) and on dOPU (r=0.29; P <0.001), but not with FF AMH levels. As serum AMH levels on d3 (r=0.29; P <0.003) and on dOPU (r=0.26; P <0.008) were correlated with the day in which GnRH antagonist-hMG treatment was started but not with the remaining time required to reach follicle maturation, it is possible that the slower follicular growth in patients having higher serum AMH levels occurred exclusively before GnRH antagonist-hMG treatment. Moreover, on the day of hCG administration, the mean size of the dominant follicle and serum estradiol levels were comparable in the 3 sets of AMH groups and were not correlated with serum or FF AMH levels.

We observed a decrease in the prevalence of oocyte retrieval failure from the low to the high FF AMH groups, irrespective of the number of follicular flushings performed. This phenomenon was not observed in the d3 and dOPU AMH groups. Fertilization rates and the prevalence of top quality embryos available remained comparable in the 3 sets of groups.

Hormonal data

Overall fluctuations of serum AMH, progesterone, and estradiol levels from d3 to dOPU and their absolute FF concentrations are illustrated in Figure 1. Whereas progesterone and estradiol levels increased significantly (P <0.0001), median AMH levels remained steady between d3 at 1.56 ng/mL (range: 0.13-7.26) and dOPU at 1.50 ng/mL (range: 0.13-6.96). Further, we observed, on dOPU, a positive correlation between serum and FF levels of AMH (r=0.47; P <0.0001), progesterone (r=0.53; P <0.0001), and estradiol (r=0.37; P <0.0001). In addition, FF AMH levels were negatively correlated with FF progesterone (r=-0.27; P <0.0004) and estradiol (r=-0.21; P <0.03) levels. In contrast, serum AMH, progesterone, and estradiol levels were correlated neither on d3 nor on dOPU. Further, serum AMH levels (d3 and dOPU) and FF AMH levels were correlated with early antral follicle counts on d3 (r=0.75; P <0.0001; r=0.71; P <0.0001, and r=0.29; P <0.002, respectively). Incidentally, as expected, before adjustment of values to protein content, AMH was roughly 2.5 fold as concentrated in the FF of preovulatory follicles as in the serum on dOPU (3.82 ng/mL versus 1.50 ng/mL, respectively).

Clinical pregnancy, ongoing pregnancy, and implantation rates

Clinical pregnancy, ongoing pregnancy, and embryo implantation rates are presented in Figure 2. As shown, clinical pregnancy (gestational sac observed at ultrasound scans at around 7 weeks of amenorrhea), ongoing pregnancy (>12 weeks of amenorrhea) rates per oocyte retrieval as well as embryo implantation rates (total number of gestational sacs x 100/total number of embryos

transferred) increased dramatically from the low to the high FF AMH groups (5.7%, 20.0%, and 39.5%, P <0.002; 5.7%, 17.5%, and 32.6%, P <0.01; and 11.8%, 30.8%, and 65.4%, P <0.001, respectively). In contrast, the slight differences among these IVF-ET outcome parameters did not reach statistical significance in the 2 sets of serum AMH groups.

Accordingly, binomial logistic regression analysis indicated that only FF AMH levels were significantly associated with clinical pregnancy (P <0.001; CIs, 1.00-1.01) and embryo implantation rates (P <0.003; CIs, 1.00-1.01), in contrast with other parameters such as patient's ages (P <0.86; 0.87-1.17 and P <0.40; 0.89-1.30, respectively), antral follicle count on d3 (P <0.15; 0.96-1.24 and P <0.06; 0.98-1.34), d3 AMH (P <0.80; 0.55-2.13 and P <0.35; 0.27-1.59), and dOPU AMH (P <0.23; 0.31-1.32 and P <0.73; 0.30-2.31). Further, the present study was not adequately powered to address the possible relationship between ovarian AMH production and pregnancy loss. Overall, 4 first-trimester pregnancy losses out of 27 clinical pregnancies (14.8%) were observed in our series and we could not detect any statistically significant variation in the incidence of this event among women who diplayed low, intermediate, or high d3, dOPU, and FF AMH levels.

Discussion

This study used the clinical model of monodominant follicle IVF-ET to determine whether AMH production by a single preovulatory follicle, assessed by FF AMH measurements, is positively related to the fate of the ensuing oocyte and embryo. Although peripheral AMH levels have hitherto been shown to reflect quantitatively the available antral follicle pool (8,7,10,11), a qualitative relationship between AMH production and follicle competence is also conceivable.

The present results support the hypothesis that a direct link exists between aptitude of granulosa cells to produce AMH and functional quality of the oocyte, as reflected by its competence to become an embryo endowed with adequate implantation potential. This striking relationship implies a number of cellular mechanisms. First, it is possible that granulosa cell metabolism and embryogenic competence of the oocyte are interrelated. Indeed, some previous studies have shown that the degree of apoptosis of both mural and cumulus granulosa cells negatively affects the developmental competence of the oocyte (18,19). In agreement with this, atretic human (6) and animal (4,13) follicles do not express AMH. In addition, a plethora of studies have identified a relationship between granulosa cell by-products measured in pooled FF, such as steroids, glycoproteins, proteolytic enzymes, etc., and oocyte/embryo outcome (16,20-24). Furthermore, growing evidence indicates that, in preovulatory follicles, the oocyte directly activates several physiological processes that occur in its surrounding granulosa cells, including plasminogen activator production (25), and LH receptor (27), kit ligand (27), and AMH (28) gene expression. Incidentally, the observation that AMH mRNA expression is lower in mural than in cumulus granulosa cells (29) is in keeping with this hypothesis.

Moreover, we observed a remarkable lack of variation of serum AMH levels between two distinct points in the menstrual cycle (d3 and dOPU). This finding corroborates the hypothesis that circulating AMH levels remain steady throughout the cycle, probably due to multi-staged follicular production (4-6) and presumable FSH-independence (2,3) of AMH. In addition, in contrast to FF AMH concentrations, we failed to relate peripheral AMH levels on d3 or dOPU with embryo implantation. Accordingly, the incidence of oocyte retrieval failure -a phenomenon that has been attributed to follicle quality defects (30)- was related with FF but not serum AMH levels. The differential predictability of FF versus serum AMH levels may be explained by the fact that circulating AMH levels reflect quantitatively the whole pool of AMH-producing follicles but are less effective to discriminate per-follicle AMH production. In keeping with this hypothesis is our observation that antral follicle counts are more strongly related to serum AMH levels than FF AMH levels as well as the conflicting literature on the possible link between peripheral AMH levels and embryo implantation (12,31). Finally, our observation of a positive correlation between serum AMH levels (d3 and dOPU) and the time necessary to achieve follicle maturation (>16 mm in diameter), mainly due to a shorter time to dominance in low AMH patients, is in conformity with the putative reduction in the follicular phase length in ovarian-aged women (32).

It is also noteworthy that the negative impact of low per-follicle AMH production on pregnancy and implantation outcome could not be anticipated by the analysis of oocyte fertilization and top-quality embryo rates. Indeed, oocyte fertilization aptitude and embryo morphology remained statistically similar among FF or serum AMH groups, though a trend for better quality embryos was noted in high AMH groups. Given that serum AMH levels have been recently associated with embryo morphology (33), this issue deserve further confirmation in adequately powered studies.

The present data indicated a negative relationship between AMH and steroid levels in the FF. Although the exact reasons for this phenomenon remain unknown, the reported inhibiting effect of AMH on aromatase activity and estrogen production (34) constitutes a plausible explanation for the negative correlation between FF AMH and estradiol levels. Further, the remarkable negative correlation between FF AMH and progesterone concentrations confirm our previous results (14) and may be explained by at least two mechanisms. On the one hand, AMH may be implicated in the regulation of progesterone production by the preovulatory follicle. Accordingly, Kim *et al.* (35) have previously demonstrated that the administration of recombinant human AMH to cultured luteinized granulosa cells inhibits their basal and epidermal growth factor (EGF)-stimulated progesterone production. On the other hand, luteinization itself may lead to an additional decrease of AMH production by the granulosa cells. This hypothesis is strengthened by the reduced expression of AMH and its type II receptor mRNA in corpora lutea as compared to small or large antral follicles from rats (4), and the decline in serum AMH levels observed after hCG administration in controlled ovarian hyperstimulated cycles (36). The mechanisms underlying the decrease of AMH production by luteinized follicles and its physiological role remain unclear. Yet, the present results are in line with

previous reports indicating that, despite preovulatory maturation and luteinization, follicles still contain sizeable amounts of AMH (1,14). The issue on whether this phenomenon represents an active AMH production by preovulatory luteinized follicles and/or it is merely the resultant of an early-stage follicle production remain to be studied.

In conclusion, the results of the present study indicate that FF AMH concentrations are strongly and positively associated with embryo implantation. They suggest that FF AMH levels reflect granulosa cell functioning and oocyte health better than do serum AMH levels, and may constitute an alternative marker of ovarian ageing. In addition, by extrapolation, FF AMH measurements should help to distinguish the embryos that are the most likely to achieve implantation in IVF-ET conducted in stimulated cycles. Indeed, embryo selection is a precondition to improve outcome of IVF-ET in stimulated cycles without increasing multiple pregnancy rates. This issue is of special interest as the present investigation failed to find any significant difference in the morphological scoring of embryos originated from high or low AMH-producing follicles. Yet, additional prospective studies are necessary to challenge the hypothesis that FF AMH concentrations are useful to assist the selection of the best embryos to transfer.

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Legends

Figure 1 White box-and-whiskers depict the dynamics of serum AMH, progesterone, and estradiol levels from cycle day 3 (d3) to the day of oocyte pickup (OPU) in monodominant follicle IVF-ET cycles. Horizontal lines inside the boxes represent median levels. Upper and lower limits of the boxes and whiskers represent the 75^{th} and 25^{th} centiles and 90^{th} and 10^{th} centiles. Values outside the 90^{th} and 10^{th} centile limits are represented as dots. Note that, contrary to serum progesterone and estradiol levels, which showed an expected significant increase (P <0.0001), serum AMH levels remained steady from d3 to dOPU. Gray box-and-whiskers illustrate FF hormone levels, in particular, the residual yet sizeable AMH production by follicles having undergone preovulatory maturation and luteinization. Note: conversion factor to SI units are 7.14 for AMH, 3.18 for progesterone, and 3.67 for estradiol.

Figure 2 Clinical pregnancy (gestational sac observed at ultrasound scans at around 7 weeks of amenorrhea; gray bars), ongoing pregnancy (>12 weeks of amenorrhea; black bars) rates per oocyte retrieval as well as embryo implantation rates (total number of gestational sacs x 100/total number of embryos transferred; white bars) in the 3 AMH concentration groups. Notice that all 3 IVF-ET outcome parameters increased dramatically from the low to the high FF AMH groups (C panel; P <0.002, P <0.01, P <0.001, respectively) but remained similar in the 2 sets of serum AMH groups (d3 and dOPU; A and B panels, respectively). Note: conversion factor to SI units are 7.14 for AMH, 3.18 for progesterone, and 3.67 for estradiol.

TABLE 1. Patient characteristics in the low, average and high AMH groups

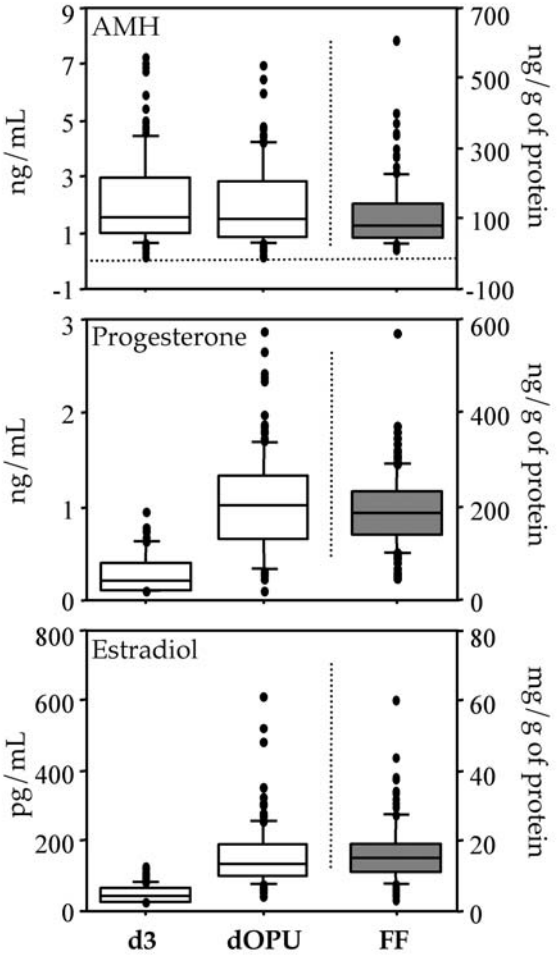
	Serum d3 AMH levels (ng/mL)			Serum dOPU AMH levels (ng/mL)			FF AMH levels (ng/g of protein)		
	Low </=1.0	Average 1.1–2.0	High >2.0	Low </=1.0	Average 1.1–2.0	High >2.0	Low </=50.0	Average 50.1–100.0	High >100.0
Number of cycles	31	45	42	39	33	46	35	40	43
Women's ages (years)[a]	34.1 ± 0.6	33.3 ± 0.5	33.6 ± 0.5	33.9 ± 0.5	33.7 ± 0.6	33.3 ± 0.5	33.5 ± 0.6	33.5 ± 0.6	33.8 ± 0.4
BMI (kg/cm^2)[a]	23.1 ± 0.9	21.2 ± 0.5	22.0 ± 0.4	22.7 ± 0.7	21.8 ± 0.5	21.6 ± 0.4	22.4 ± 0.7	21.2 ± 0.4	22.6 ± 0.6
Menstrual cycle (days)[a]	27.8 ± 0.3	27.5 ± 0.2	27.9 ± 0.3	27.7 ± 0.3	27.6 ± 0.3	27.9 ± 0.3	27.9 ± 0.4	27.6 ± 0.3	27.7 ± 0.3
Indications for IVF-ET (%) :									
Male factor	26	42	48	33	39	45	34	45	40
Tubal factor	32	22	36	23	30	35	37	18	34
Endometriosis	13	20	2	11	21	7	9	15	12
Unexplained	29	16	14	33	10	13	20	22	14

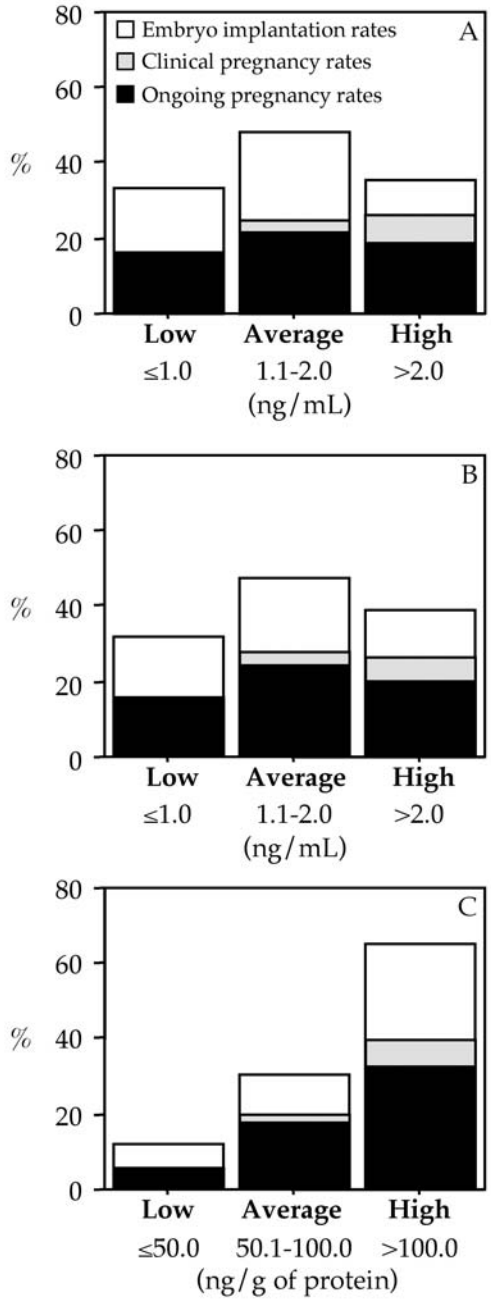
[a]Means ± SE;
Differences among groups are not statistically significant

TABLE 2. Cycle monitoring and embryology data in low, average and high AMH groups

	Serum d3 AMH levels (ng/mL)			Serum dOPU AMH levels (ng/mL)			FF AMH levels (ng/g of protein)		
	Low </=1.0	Average 1.1–2.0	High >2.0	Low </=1.0	Average 1.1–2.0	High >2.0	Low </=50.0	Average 50.1–100.0	High >100.0
Number of cycles	31	45	42	39	33	46	35	40	43
Total hMG dose (IU)	290 ± 25	373 ± 22	353 ± 23	315 ± 22	382 ± 26	342 ± 23	334 ± 19	375 ± 22	324 ± 27
Day of hCG administration	10.7 ± 0.3	12.2 ± 0.3	12.8 ± 0.4[a]	11.5 ± 0.3	11.9 ± 0.4	12.6 ± 0.4	11.7 ± 0.4	12.1 ± 0.3	12.2 ± 0.4
Dominant follicle size (mm)[b]	17.2 ± 0.2	17.5 ± 0.1	17.6 ± 0.2	17.2 ± 0.1	17.7 ± 0.2	17.5 ± 0.1	17.3 ± 0.1	17.5 ± 0.2	17.5 ± 0.2
Serum estradiol (pg/mL)[b]	258 ± 23	272 ± 21	252 ± 15	258 ± 21	283 ± 25	248 ± 15	262 ± 18	285 ± 26	246 ± 19
Number of follicular flushings[c]	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.5 ± 0.1
% of oocyte retrieval failure (numbers)[d]	16 (5/31)	20 (9/45)	10 (4/42)	13 (5/39)	27 (9/33)	9 (4/46)	29[e] (10/35)	8 (3/40)	12 (5/43)
Fertilization rate (%)	60	68	79	59	83	72	71	70	70
Top quality embryos (%)	33	70	55	47	68	52	41	65	53

Continuous variables are means ± SE.
[a] *P*< 0.0004 (serum d3 AMH groups, ANOVA).
[b] On the day of hCG administration.
[c] Required to retrieving the oocyte,if it was not present in the follicular fluid (maximum 3 flushings).
[d] Cases in which no oocyte was retrieved despite 3 follicular flushings.
[e] Statistically different as compared to the average (*P*< 0.02) and high (*P*< 0.04) FF AMH groups.


AMH
ng/mL
ng/g of protein
Progesterone
ng/mL
ng/g of protein
Estradiol
pg/mL
mg/g of protein
d3
dOPU
FF


80
60
40
20
0
%
Embryo implantation rates
Clinical pregnancy rates
Ongoing pregnancy rates
A
Low
≤1.0
Average
1.1-2.0
High
>2.0
(ng/mL)
B
C
≤50.0
50.1-100.0
>100.0
(ng/g of protein)