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Paul Barrière, Géraldine Porcu-Buisson, Catherine Avril, Samir Hamamah. Added value of anti-Müllerian hormone serum concentration in assisted reproduction clinical practice using highly purified human menopausal gonadotropin (HP-hMG). *Journal of Gynecology Obstetrics and Human Reproduction*, 2021, 51 (2), pp.102289. 10.1016/j.jogoh.2021.102289 . inserm-03655083

HAL Id: inserm-03655083

<https://inserm.hal.science/inserm-03655083>

Submitted on 29 Apr 2022

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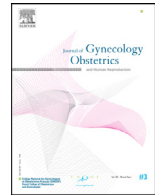
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ELSEVIER

Contents lists available at ScienceDirect

Journal of Gynecology Obstetrics and Human Reproduction

journal homepage: www.elsevier.com

Original Article

Added value of anti-Müllerian hormone serum concentration in assisted reproduction clinical practice using highly purified human menopausal gonadotropin (HP-hMG)



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ARTICLE INFO

Article History:

Received 19 April 2021

Revised 22 November 2021

Accepted 9 December 2021

Available online 11 December 2021

Keywords:

AMH

COS

HP-hMG

In vitro fertilization

ABSTRACT

Introduction: The individual response to controlled ovarian stimulation (COS) depends on several factors, including the initial dose of gonadotropin. In repeated *in vitro* fertilization (IVF) cycles, the initial dose of gonadotropin is mainly established on the basis of the previous attempts' outcomes. Conversely, in naive patients, the ovarian response should be estimated using other criteria, such as the serum concentration of anti-Müllerian hormone (AMH). However, in clinical practice, the initial gonadotropin dose is not systematically adapted to the AMH level, despite the known relationship between AMH and ovarian reserve.

Material and Methods: French non-interventional, longitudinal, prospective, multicentre, cohort study that included infertile women who underwent COS with highly purified human menopausal gonadotropin (HP-hMG 600 IU/mL) during their first IVF/intracytoplasmic sperm injection (ICSI) cycle. Data were collected prospectively during routine follow-up visits from COS initiation to 10–11 weeks after embryo transfer.

Results: Data from 235 of the 297 enrolled women were used for the study. Serum AMH level was negatively correlated with the initial and total HP-hMG doses ($p < 0.001$), and positively correlated with the number of retrieved oocytes ($p < 0.007$). Embryos were obtained for 94.0% of women, and fresh embryo transfer was performed in 72.8% of them. The clinical pregnancy rate was 28.5% after the first embryo transfer.

Conclusion: Selecting the appropriate starting dose of gonadotropin is crucial to optimize the IVF/ICSI procedure. For the first attempt, the serum AMH level is a good biomarker to individualize treatment.

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Introduction

Controlled ovarian stimulation (COS) is a crucial step in *in vitro* fertilization (IVF) procedures with or without intracytoplasmic sperm injection (ICSI). The ovarian response to COS is influenced by several factors, such as woman's age, ethnicity, lifestyle, and ovarian reserve [1–3]. Although the relationship between the ovarian reserve and serum concentration of anti-Müllerian hormone (AMH) has been established [4–7], in clinical practice, the gonadotropin dose at COS initiation is not always adjusted linearly to the AMH level [8]. Therefore, it is important to determine the factors that influence the choice of gonadotropin dose used for COS.

The aim of this study was to determine the relationship between serum AMH level and the starting dose of highly purified human menopausal gonadotropin (HP-hMG; 600 IU/mL) administered to infertile women undergoing their first IVF/ICSI cycle in real practice. This is the first study to evaluate data from French assisted reproductive technology (ART) centres where AMH serum level was measured using a fully automated assay, and where the choice of HP-hMG dose was left to the clinicians (no recommended fixed dose).

Materials and methods

Study design and participants

This non-interventional, longitudinal, prospective, multicentre, cohort study enrolled infertile 18 to 42-year-old women who received HP-hMG (Menopur®; 600 IU/mL) for COS during their first IVF/ICSI cycle and who had at least one recent AMH measurement

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using a fully automated assay, in the last 12 months before inclusion. Twenty-five fertility/ART (private- and public-sector) centres in France participated in the study. Exclusion criteria were stage III/IV endometriosis and/or polycystic ovarian syndrome to avoid possible confounding factors induced by these pathologies with the study aim, untreated major endocrine or metabolic abnormalities, major morphological uterine or ovarian abnormalities, past ovarian surgery, or inclusion in an interventional study to assess infertility treatments.

Women were followed from COS initiation up to 11 weeks after the first embryo transfer. Their demographic and clinical data were collected prospectively by the investigator in a standardized electronic case report form (eCRF), following the routine medical practice. Women were enrolled from October 2016 to December 2017.

Ethical aspects

The study was carried out according to the ethical principles of the Declaration of Helsinki and in accordance with the current Good Epidemiological Practices. The study was approved by the French national committee for processing data related to research in the health field (CCTIRS) and by the French national commission for the protection of private data and rights (CNIL). The study was registered on clinicaltrials.gov (NCT02935335). Each woman included in the study signed a written informed consent.

Study assessments and endpoints

The primary objective of this study was to evaluate the relationship between serum AMH levels, measured with a fully automated assay, and the initial dose of HP-hMG 600 IU/mL used for COS. When analysed as a qualitative variable for the exploratory analysis, four levels of AMH serum (low: <1.1 ng/mL; intermediate: ≥1.1 ng/mL and <2 ng/mL; normal: ≥2 ng/mL and <5 ng/mL; high: ≥5 ng/mL) were considered.

As this was a non-interventional study carried out during the normal management of women undergoing IVF/ICSI in France, it did not entail any additional visit or specific exam.

In all women with a positive β human chorionic gonadotropin test after embryo transfer (i.e. biochemical pregnancy), clinical pregnancy was confirmed by ultrasonography at week 4 or 5 after embryo transfer. Ongoing pregnancy was confirmed by ultrasonography at week 10 or 11 after embryo transfer.

The overall tolerance to the treatment was recorded in all patients who received at least one dose of HP-hMG 600 IU/mL (i.e. Safety population).

Statistical analysis

As this study was observational, statistical analyses were mainly descriptive.

Correlations between quantitative variables were assessed using the Spearman correlation coefficient based on the ranks of the variables to be analysed, with the associated p-value (null hypothesis of zero correlation).

Qualitative variables were compared using the Pearson's Chi2 test or the Fisher's exact test, Gaussian variables with the Student's *t*-test and the Satterthwaite's correction (in case of unequal variance), and semi-quantitative or non-Gaussian variables with the non-parametric Mann-Whitney test (significance level set at 5% for all). The normality of quantitative variables was analysed with the Shapiro-Wilk test.

Analysis of predictive factors was performed, first without selection (5% level), and then using a stepwise multiple linear regression method. The stepwise selection of parameters was done using the forward method and a Wald test p value <5% for entry and allowing the removal of variables with $p > 10\%$.

All statistical analyses were performed with the SAS® statistical package, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

From October 2016 to December 2017, 297 infertile women were enrolled in the study. Among them, 62 were excluded from the analysis, either because they did not receive at least one dose of HP-hMG 600 IU/mL within the study observational period ($n = 39$) or did not achieved triggering ($n = 21$) or did not have at least one result of AMH in the last 12 months before inclusion ($n = 1$) or met an exclusion criterion ($n = 1$). Finally, data from 235 women were used for the study. Their baseline characteristics are summarized in Table 1. Their median age was 33 years and their mean serum AMH level was 2.3 (± 1.7) ng/mL. Serum AMH level was <1.1 ng/mL in 54 women, and ≥ 5 ng/mL in 16 women. Most women (84.3%) were treated with a gonadotropin releasing hormone antagonist protocol, according to the French practice to reduce costs and the risk of ovarian hyperstimulation syndrome compared with agonist protocols [9,10]. Table 2 summarizes the use of HP-hMG 600 IU/mL for COS in the included patients in the analysis. The median initial dose was 225 IU/day (interquartile range [IQR] 150;300). The median treatment duration was 9 days (IQR 8;10) with a median total dose of 2325 IU (IQR 1725;3075). During COS, HP-hMG dose was modified in 42.1% of women: dose increase (21.3%), dose decrease (23.4%), or treatment interruption (i.e. coasting, 2.1%), a patient may have more than one dose modification. The observed dose modification rate was high, although it was previously demonstrated that HP-hMG dose should be appropriately defined at treatment initiation because changes are not associated with a clinical benefit [11–14].

Table 1
Baseline characteristics of the patients analysed.

	N = 235
Age (years, mean \pm SD)	32.5 \pm 4.6
Weight (kg, mean \pm SD)	66.3 \pm 13.4
Body mass index (kg/m ² , mean \pm SD)	24.3 \pm 4.8
Smoking	38 (16.2%)
Infertility	
Primary infertility	140 (59.6%)
Duration (years, mean \pm SD)	3.1 \pm 1.9
Previous ovarian stimulation*	60 (25.5%)
Obstetric history	
Prior pregnancy	100 (42.6%)
≥ 2 pregnancies	41 (17.4%)
≥ 1 live birth	69 (29.4%)
ART	
ICSI	136 (57.9%)
Standard IVF	99 (42.1%)
Antagonist protocol	198 (84.3%)
Pre-treatment with oestradiol	146 (82.5%)
Hormone measurements	
AMH (ng/mL, mean \pm SD)	2.3 \pm 1.7
AMH <1.1 ng/mL	54 (23.0%)
AFC <8	23 (12.3%)
FSH (IU/L, mean \pm SD)§	7.7 \pm 2.8
LH (IU/L, mean \pm SD)§	5.7 \pm 3.0
Oestradiol (pg/mL, mean \pm SD)§	48.9 \pm 51.5
Progesterone (ng/mL, mean \pm SD)§	0.3 \pm 0.3

Categorical data are expressed as frequencies and percentages, quantitative data as means \pm standard deviation.

* For other purpose than IVF; §measured between day 2 and day 4 of the cycle.

AFC, antral follicular count; AMH, anti-Müllerian hormone; ART, assisted reproductive technology; FSH, follicle stimulating hormone; ICSI, intra cytoplasmic sperm injection; IU, international unit; IVF, *in vitro* fertilization; LH, luteinizing hormone, SD, standard deviation.

Table 2
HP-hMG 600 IU/mL use for COS in the analysed patients.

	N = 235
Initial dose (IU/day, mean ± SD)	233.8 ± 73.0
Dose modification*	99 (42.1%)
Dose decrease	55 (23.4%)
Dose increase	50 (21.3%)
Coasting	5 (2.1%)
Total dose (IU, mean ± SD)	2 473.0 ± 971.1
Treatment duration (days, mean ± SD)	9.5 ± 1.9

Categorical data are expressed as frequencies and percentages, quantitative data as means ± standard deviation.

* A patient may have had more than one dose modification. IU, international unit; SD, standard deviation.

Relationship between serum AMH level and HP-hMG dose

The median initial daily doses of HP-hMG 600 IU/mL were 300, 300, 187.5, and 150 IU/day in the low, intermediate, normal, and high AMH subgroups, respectively (Fig. 1). The lower the serum AMH level, the higher the initial dose of HP-hMG. Similar results were obtained for the total HP-hMG dose (median total doses of 3000, 2775, 1912.5, and 1562.5 IU in the low, intermediate, normal and high AMH subgroups, respectively). AMH level was associated with both the initial and the total HP-hMG doses ($p < 0.001$). The age, the smoking status, the AFC and the type of GnRH protocol had also a significant impact on the initial dose of HP-hMG ($p < 0.05$, α risk 5%).

According to the study population which had a normal weight (i.e. mean body mass index: $24.3 \pm 4.8 \text{ kg/m}^2$), the weight was not correlated with the initial dose ($p = 0.2916$) but was significantly associated with the total HP-hMG dose (linear regression analyses, $p < 0.001$). This suggests a dose adaptation according to weight during COS.

Ovarian response and early embryo development

Oocyte retrieval was performed in 228 (97.0%) women (Fig. 2), and the median number of oocytes retrieved was 8 per woman (IQR 5;12) (Table 3). The ovarian response was statistically different in the four AMH groups ($p < 0.007$). Specifically, at least 8 oocytes could be retrieved in 68.0% of women with normal AMH level and in 37.2% of women with low or intermediate AMH level (i.e. $< 2 \text{ ng/mL}$). Fewer than 8 and 4 oocytes were retrieved in 75.5% and 34.7% of patients with low AMH level (i.e. $< 1.1 \text{ ng/mL}$), respectively (Fig. 3). These descriptive results show a clear correlation between AMH serum level and number of retrieved oocytes in the low, intermediate and normal AMH level groups (Fig. 3). This is consistent with the known relationship between ovarian reserve and serum AMH level [4–7]. Results are less clear for the high AMH level subgroup, probably because of the small number of women ($n = 16$) in this subgroup. The median number of fertilized oocytes (IVF or ICSI) was 7 (IQR 5;10) for 8 (IQR 5;12) oocytes retrieved, suggesting that the majority of oocytes retrieved after COS with HP-hMG were mature.

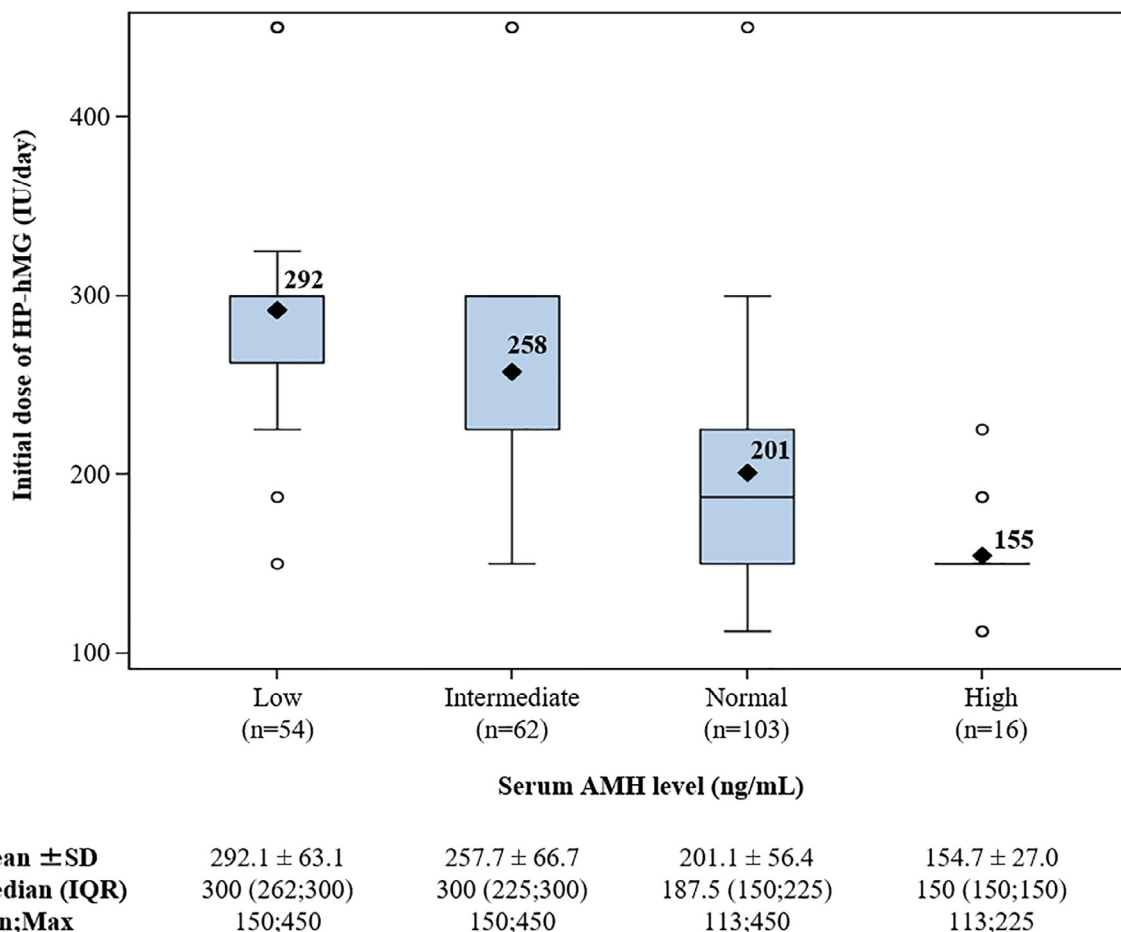


Fig. 1. Boxplot showing the initial HP-hMG dose in infertile women undergoing COS and classified according to their baseline AMH level.

The horizontal line indicates the median, the box the IQR, whiskers extend to the upper adjacent value (75th percentile +1.5IQR) and the lower adjacent value (25th percentile -1.5IQR), the diamond indicates the mean, and dots represent outliers. Serum AMH level was defined as low ($< 1.1 \text{ ng/mL}$), intermediate (≥ 1.1 and $< 2 \text{ ng/mL}$), normal (≥ 1.1 and $< 5 \text{ ng/mL}$), and high if $\geq 5 \text{ ng/mL}$.

IQR, interquartile range; SD, standard deviation.

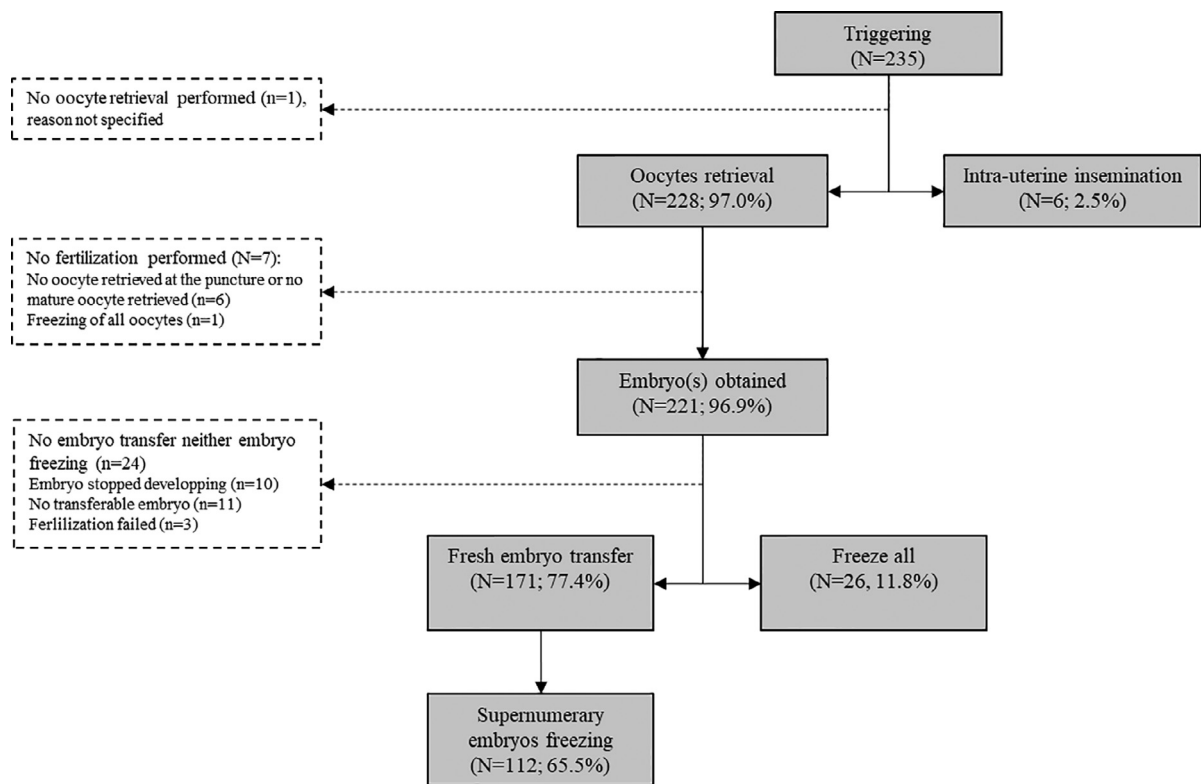


Fig. 2. Study flowchart, ovarian response, and embryo development outcomes.

Embryos were obtained, mainly (62.0%) by ICSI, for 94.0% ($n = 221$) of women. The median number of day 3 embryos was 4 (IQR 3;7), and the median number of day 5/6 blastocysts was 3 (IQR 1;5). This indicated the good quality of the biological material retrieved after COS, considering the high conservative rate of embryos that progressed to the blastocyst stage (i.e. 75%) (Table 3) compared with the usual rate (50%) [15–19].

Fresh embryo transfer was performed in 171 (72.8%) women (Fig. 2), mainly (70.2%) single embryo transfer. Stages D2/D3 embryo (s) and D5/D6 blastocyst(s) were transferred in 103 (60.6%) and 67 (39.4%) women respectively (one patient with missing value). Top quality embryos were transferred in 66.7% of women and no more than two embryos were transferred. Embryo freezing was performed for 138 (58.8%) women, mainly freezing of unused embryos ($n = 112$; 47.7%).

The data obtained from oocyte retrieval up to embryo freezing confirmed the efficacy of HP-hMG 600 IU/mL for COS during IVF/ICSI procedures, and are consistent with the results of a randomized controlled trial showing that the gonadotropin starting dose, chosen according to the ovarian reserve, is associated with an increase of the percentage of women with optimal ovarian response [20].

Table 3
Ovarian response and embryo outcomes.

	N = 235
Number of oocytes retrieved (median (IQR)) [$n = 228$]	8 (5;12)
Number of oocytes inseminated or injected (median (IQR)) [$n = 221$]	7 (5;10)
Number of oocytes with 2 PN (median (IQR)) [$n = 214$]	5 (3;7)
Number of day 2 embryos (median (IQR)) [$n = 183$]	5 (3;7)
Number of day 3 embryos (median (IQR)) [$n = 125$]	4 (3;7)
Number of day 5/6 blastocysts (median (IQR)) [$n = 131$]	3 (1;5)

Data are expressed as medians and interquartile range. 2PN, two distinct pronuclei; IQR, interquartile range; N, number of patients.

Pregnancy rate

The clinical pregnancy rate was 28.5% after the first embryo transfer and was very similar to what usually observed in France [9]. As expected, it was influenced by the woman’s age (30.8% in subjects younger than 36 years vs. 19.2% in women older than 36 years) and by the embryo quality (40.7% after top embryo transfer vs. 14.3% if no top embryo was transferred). Differently from what is reported by some studies [21,22], pregnancy rate was comparable in the four serum AMH level groups, although the number of retrieved oocytes was significantly different among these groups. These results indicate that AMH level is a predictor of the ovarian reserve, but not an indicator of the pregnancy success rate, and suggest that oocyte quality is more important than their quantity for improving the outcome after the first embryo transfer.

The success of the first embryo transfer is a crucial point because any delay in the time-to-pregnancy is perceived as an ordeal; 78.6% of women recognize that waiting 1–2 months for embryo transfer is difficult [23]. Moreover, 25 to 50% of couples abandon after the first or second failed IVF cycle, mainly due to the treatment psychological burden [24]. Therefore, many ART experts consider that bringing home a healthy baby in the shortest period of time with the lowest number of embryo transfers should be the main goal of any IVF treatment [25].

Finally, pregnancy rate tended to be lower (not significant) in women with HP-hMG dose modifications rather than without (24.6% vs 30.9%), particularly in patients with a dose increase (20.9%). This result highlights the subjectivity of dose modification practices and confirms the importance of choosing the best dose at COS initiation by using a dose individualization model based on serum AMH levels.

Limitations of the study

The issues and biases inherent to observational studies are well known [26–28], but these studies provide invaluable insights into

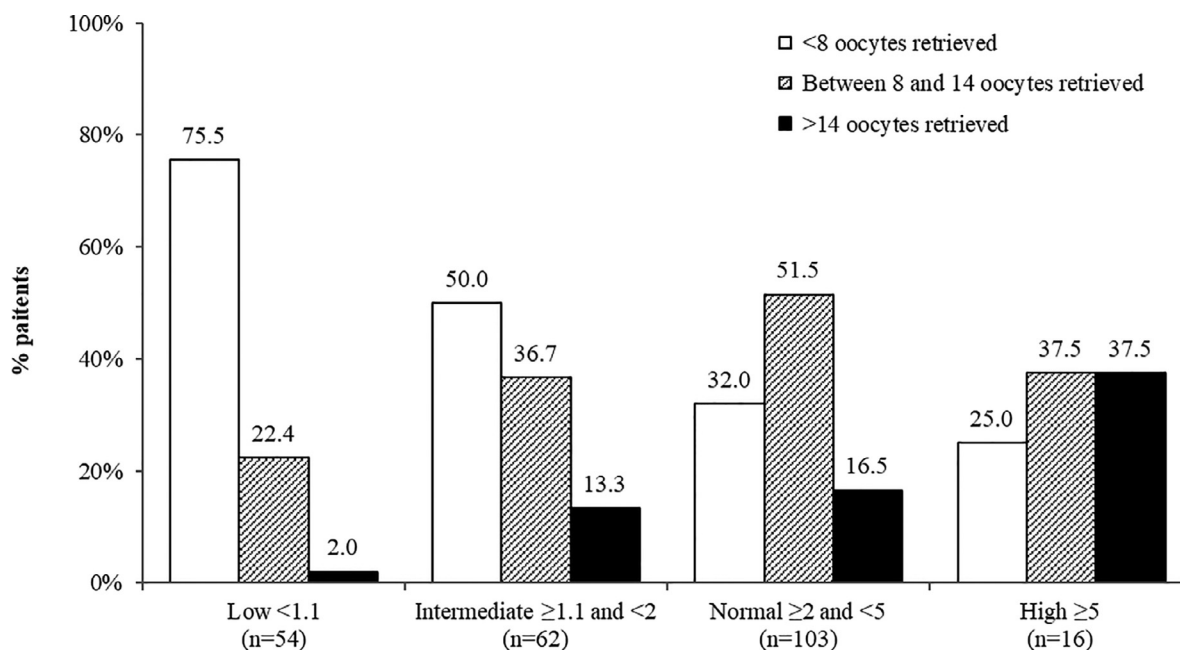


Fig. 3. Number of oocytes retrieved according to the AMH level group.

Serum AMH level was defined as low (<1.1 ng/mL), intermediate (≥1.1 and <2 ng/mL), normal (≥2 and <5 ng/mL), and high (≥5 ng/mL).

the effects of the usual clinical practice. The present study was an observational study and has several limitations, including patient selection bias. To limit this bias, women were enrolled consecutively based only on the selection criteria and on the decision, made before inclusion, to initiate HP-hMG for COS within an IVF/ICSI procedure.

To limit data variability, the study included only women with a serum AMH level measurement performed with a fully automated assay. However, recent data have highlighted significant fluctuations in AMH measurements, and questioned the reliability of a single measurement for clinical decision-making [29]. Despite the use of fully automated assays for AMH quantification, AMH serum levels show significant intra- and inter-cycle variations, not explained by the analytical variability [29]. Additional studies are needed to determine the best time for AMH assessment. Moreover, as the choice of the initial HP-hMG dose is strongly influenced by the ovarian response in previous attempts, only patients at their first IVF/ICSI cycle were included.

Safety

Overall in the safety population ($n = 258$), 25 women (9.7%) reported 31 treatment-related adverse events (AE), mainly non-serious AE (28 AE; 22 subjects). Three patients (1.2%) experienced ovarian hyperstimulation that led to hospitalization. No safety information or information with potential impact on the benefit-risk assessment has arisen.

Conclusion

Considering the influence of AMH level on the ovarian response, the starting dose of HP-hMG is crucial to optimize the IVF procedure. AMH level is a good quantitative predictive biomarker of the ovarian reserve and of the ovarian response to COS in ART. However, more studies are needed to identify biomarkers that strongly correlate with embryo quality and clinical pregnancy outcomes.

Trial registration Number: NCT02935335

Funding

The study was funded by Ferring SAS.

Declaration of Competing Interest

Paul BARRIERE, Géraldine PORCU-BUISSON and Catherine AVRIL report personal fees from Ferring SAS, during the conduct of the study.

Acknowledgements

We thank all physicians who facilitated the recruitment of subjects, and all participants who agreed to take part in the study: ALEX-ANDRE Florence (BEAUMONT, La Chataigneraie Private Hospital), BARRIERE Paul (NANTES, CHU – Biology and Reproductive Medicine), BELAISCH-ALLART Joëlle (SAINT-CLOUD, Des Quatre Villes Hospital), CHAO Choline (SAINT-DENIS, Delafontaine Hospital), CHEVALIER Nicolas (MONTPELLIER St Roch Medical Centre), CHOMIER Martine (ECULLY, Clinic of Val d'Ouest - Rhône Alpin Institute), DUBOURDIEU-FONTAINE Sophie & MENSIER Antoine (SAINT-HERBLAIN, Atlantique Polyclinic), GALLOT Vanessa (CLAMART, Antoine Béclère Hospital), GODEFROY Anne-Sophie (VILLEURBANNE, PMA PROCREO Centre), GRYNBERG Michael (BONDY, Jean Verdier Hospital), GUIVARC'H-LEVEQUE Anne (RENNES), HOCKÉ Claude (BORDEAUX, Pellegrin Hospital), LESTRADE Florence & SCHWEITZER Thérèse (METZ, Hospital Women, Mother, Children), MAIGNIEN Chloé (PARIS, Port Royal Hospital), MICHELSON-LECHAT Xénia (BRUGES, Jean Villar Polyclinic), NAOURI VISCHÉL Michèle (PARIS), PASQUIER Maud (CRÉTEIL, Intercommunal Hospital Centre), PIRRELLO Olivier & RONGIERES Catherine (SCHILTIGHEIM, Medical, Surgical and Obstetrical Centre [CMCO]), PORCU-BUISSON Géraldine (MARSEILLE, Institute of Reproductive Medicine), SCHNEIDER Marc (SAINT MARTIN D'HERES, Belle-donne Clinic), WAINER Robert (POISSY, Intercommunal Hospital Centre), YAZBECK Chadi (PARIS).

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