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Chapter 7

USING AMH FOR DETERMINING A STRATIFIED GONADOTROPIN DOSING REGIMEN FOR IVF/ICSI AND OPTIMIZING OUTCOMES

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ABSTRACT

The use of biomarkers and companion diagnostic approaches should provide valuable tools to aid in the optimization of gonadotropin regimens for better treatment outcomes, improved benefit/risk ratio as well as increased cost-effectiveness. Controlled ovarian stimulation with gonadotropins for IVF/ICSI aims to obtain an adequate number of competent oocytes with the minimum risks for the woman. A large variability in ovarian response across patients given the same dose of gonadotropin is a well-recognized phenomenon. This chapter reviews a strategy for gonadotropin dosing by matching specific patient category characteristics to provide an optimal ovarian response while leading to a reduction of safety risks, fewer cycle transfer cancellations and thereby maximizing the chances for successful treatment outcome. This approach presents a way forward where full information from a diagnostic test (AMH) can be used to begin the journey of truly individualizing and personalizing ovarian stimulation for women undergoing infertility treatment.

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1. INTRODUCTION

Individual patient information can be used for improving diagnosis as well as tailoring better treatment specific for the individual patient [1, 2]. Stratification of patients by using one or more clinical biomarkers could be applied in order to provide therapeutic solutions matching specific patient populations' characteristics [3]. When a biomarker is linked with a specific treatment response, patients can be categorized based on that biomarker allowing the population in each category to receive the most adequate medical treatment.

The concept of incorporating individualized decisions applies to many disease areas, and its application in the management of infertility is not excluded. Choice of infertility treatment modality, controlled ovarian stimulation (COS) protocol (e.g., long GnRH agonist or GnRH antagonist, etc.), gonadotropin regimen and dose, insemination approach, type of luteal phase support, number of embryos/blastocysts transferred, timing of transfer or cryopreservation policy, among others aspects, are examples of personalization of treatment. Yet, objective and more systematic considerations of personalized medicine principles could be applied to infertility treatments, especially in the area of stratification of patients planned to undergo COS [4, 5]. The use of biomarkers and companion diagnostic approaches should provide valuable tools to aid in the optimization of gonadotropin regimens for better treatment outcomes, improved benefit/risk ratio as well as increased cost-effectiveness.

The desired outcome of IVF/ICSI treatment is a healthy baby, and the likelihood of a successful treatment depends on many factors. However, the predictive biomarkers of implantation [6] and pregnancy/live birth [7-11] for women attending a fertility consultation are only moderately characterized. Nevertheless, IVF/ICSI treatment can be divided into different segments for which biomarkers could, in an acceptable degree, predict specific treatment responses. In particular, COS is an area for clinical application of stratification of patients to gonadotropin treatment.

COS with gonadotropins for IVF/ICSI aims to obtain an adequate number of competent oocytes with the minimum risks for the woman. A large variability in ovarian response across patients given the same dose of gonadotropin is a well-recognized phenomenon [12-19]. The concept of "one starting dose of gonadotropins fits all" has limitations, especially in patients at risk of poor or excessive ovarian response. For patients with a low ovarian reserve, a stimulation cycle using a standard starting dose of gonadotropin during the first 5-6 days would be expected to be associated with an elevated risk of cycle cancellation due to insufficient follicular development or no embryos/blastocysts available for transfer, and therefore would compromise efficacy of IVF treatment. The same starting dose of gonadotropin in patients with a high ovarian reserve would have a dramatically increased risk of causing early moderate/severe ovarian hyperstimulation syndrome (OHSS), a complication which can be life-threatening. With the increased attention to safety profile and risk/benefit ratio of available therapies and treatment protocols, and with the availability of tools to identify patients at risk of an excessive response, not only severe OHSS but also less severe signs and symptoms of hyperstimulation are more and more being considered unacceptable from a clinical perspective. The clinical community therefore has identified that an area of improvement for COS lies in the clinical management of patients with an increased likelihood for poor or excessive response to gonadotropin therapy, with the latter also constituting a major opportunity for safer use [4, 20-23]. Such a stratification of risk patients can be done

based on clinical patient characteristics, or preferably by diagnostic biomarkers. The development of more patient-tailored regimens based on initial patient characteristics has not only been a demand from the treating physicians. As reflected in the latest guidance from the National Institute for Health and Clinical Excellence in the United Kingdom [24], there is also a recommendation from health policy makers for considering individualized starting doses of gonadotropins by using predictive factors such as those related to patient characteristics and diagnostic markers of ovarian reserve with the objective of improving COS regimens. A strategy for gonadotropin dosing matching specific patient category characteristics could provide an optimal ovarian response, eventually leading to a reduction of safety risks and fewer cycle transfer cancellations, and thereby maximize the chances for successful treatment outcome.

2. BIOMARKER EVALUATION FOR OPTIMIZING GONADOTROPIN DOSING

A properly constructed stratified dosing regimen should be the result of planned and prospective application of a biomarker(s) predicting ovarian response and adverse reactions in adequately designed gonadotropin dose-response studies, rather than being developed from retrospective evaluations of clinical data. The implementation of a stratified dosing approach to COS has until recently been compromised by the absence of a robust biomarker of ovarian reserve that will accurately predict ovarian response to gonadotropins, and which can be generalized across IVF clinics.

Early follicular phase FSH and antral follicle count (AFC) have been, and still are, two parameters widely used to predict ovarian reserve and response to gonadotropins. Since the study by Seifer and coworkers [25], in which the basal serum level of anti-Müllerian hormone (AMH) was found to be associated with ovarian response to gonadotropins in women undergoing IVF treatment, many advantages of AMH over FSH (as well as over patient age, inhibin B and estradiol) have been demonstrated [10, 22, 23, 26, 27]. Moreover, AMH offered at least similar level of accuracy and clinical value for the prediction of ovarian response to gonadotropins as AFC in several single-center observational cohort studies [28-32], and in two meta-analyses [33, 34]. However, in marked contrast to these reports, three recent large, prospective, multicenter trials in IVF/ICSI patients consistently concluded that AMH was a better predictor of the number of oocytes retrieved as well as categorization of low and high responders than AFC [26, 35, 36]. Due to the limitations of AFC in terms of sonographer-dependent variability and technical aspects of ultrasound equipment [37] and the increasing advantages of AMH testing in terms of patient convenience and assay robustness, AMH is more and more being recognized as the preferred biomarker of ovarian response to COS.

2.1. Predictive Value of AMH - Data from Randomized Controlled Trials

The value of AMH as biomarker in IVF/ICSI patients undergoing COS has mainly been evaluated in single-center observational cohort studies [22]. It is well-known that observational cohort studies may be subject to confounding and selection bias between the

treatment groups [38], whereas randomized controlled trials (RCTs) can be designed to avoid these issues and are therefore suitable to adequately investigate the relationship between a biomarker and relevant outcomes, in this case the relationship between AMH and ovarian response.

In our group, the predictive value AMH in IVF/ICSI patients undergoing COS has been evaluated using data obtained from three RCTs (N = 1,745) performed as part of clinical development programs for urinary derived and recombinant gonadotropins [19, 26, 36, 39]. This included retrospective evaluation of the prediction performance of AMH (N = 1,480), as well as prospective stratification of patients according to AMH levels prior to stimulation (N = 265). These investigations reiterate the finding that AMH is better correlated with oocytes retrieved than basal FSH, inhibin B, and AFC, as illustrated in Figure 1 displaying the higher potential of AMH for predicting ovarian response compared with the other biomarkers.

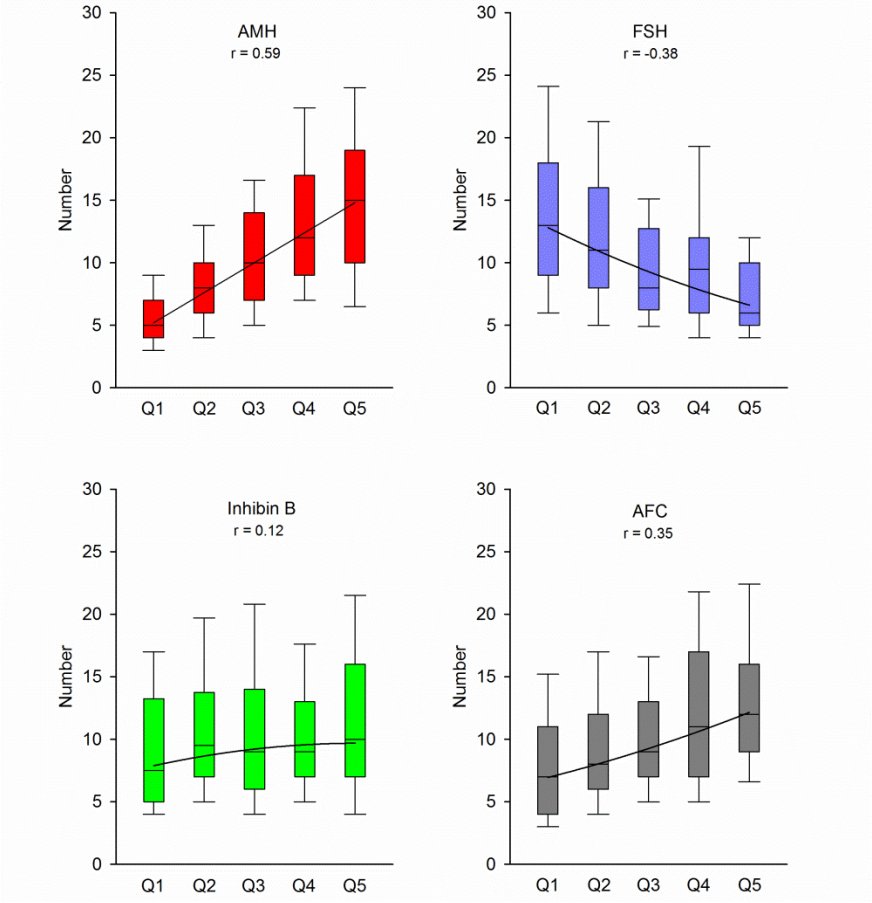


Figure 1. Box-and-whisker plots for numbers of oocytes retrieved grouped by baseline AMH, FSH, inhibin B and AFC quintiles in patients (n=363) treated with rFSH at a starting dose of 150 IU in a GnRH antagonist protocol [26]. Values are median (lines), 25th - 75th percentile (boxes), and 10th - 90th percentile (whiskers) [AMH (pmol/L): Q1 ≤ 10.0, Q2 10.1-18.6, Q3 18.7-28.9, Q4 29.0-42.8, Q5 ≥42.9; FSH (IU/L): Q1 ≤ 5.7, Q2 5.8-6.5, Q3 6.6-7.2, Q4 7.3-8.5, Q5 ≥8.6; Inhibin B (ng/L): ≤ 56.0, Q2 56.1-74.9, Q3 75.0-92.0, Q4 92.1-109.7, Q5 ≥109.8; AFC (n): Q1 ≤ 11, Q2 12-13, Q3 14-15, Q4 16-19, Q5 ≥20]. r = Spearman's rank correlation coefficient.

Furthermore, there is a good association between AMH levels and the number of dose adjustments performed by the clinicians, the frequency of cycle cancellations due to either poor or excessive response, and of early moderate/severe OHSS [26, 36].

AMH is consistently found to be indicative of dose adjustments on stimulation day 6, with low and high AMH associated with increases and decreases, respectively, in the daily gonadotropin dose [36]. AMH shows a high accuracy for the prediction of poor (AUC = 0.897) or excessive response (AUC = 0.813), and is a significantly ($p < 0.05$) better predictor than FSH, inhibin B and AFC (Figures 2A and B) [26]. The optimal cut-offs for poor and excessive response seem to be around AMH values of 12 and 31 pmol/L, respectively, for patients with a gonadotropin starting dose of 150 IU/day in a GnRH antagonist protocol [26]. It is noteworthy that in these multicenter studies AMH was shown to not only be a more robust biomarker of the ovarian response to gonadotropins overall, but also at individual study center level, as the correlation coefficient for AMH and number of oocytes retrieved was numerically higher than that for AFC in most centers (83-89%) [39].

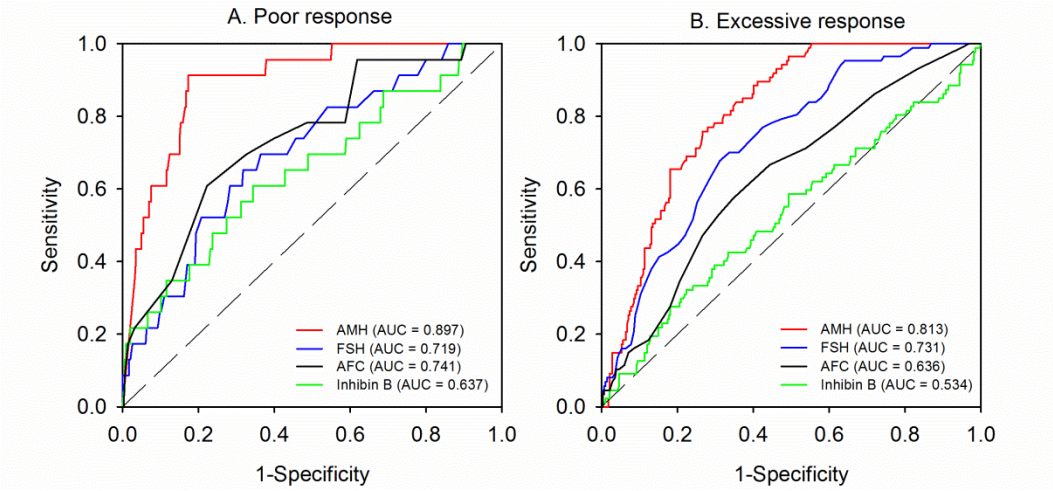


Figure 2. ROC curves for predicting poor response (<4 oocytes retrieved or cycle cancellation due to poor response) (A) and excessive response (≥15 oocytes retrieved or cycle cancellation due to excessive response) (B) after stimulation with rFSH at a starting dose of 150 IU in a GnRH antagonist protocol [26].

In summary, these large RCTs further support the validity of AMH as the most informative predictor of ovarian response to gonadotropins, as repeatedly suggested in the literature (reviewed by Nelson, 2013 [23], Fleming et al., 2013 [40], La Marca and Sunkara, 2013 [41], Toner and Seifer, 2013 [42], Broer et al., 2014 [43], Dewailly et al., 2014 [44]). The clinical value of AMH may also improve further with the introduction of robust assays in an automated platform with high reproducibility [45].

3. CLINICAL APPLICATION OF STRATIFIED MEDICINE – THE FE 999049 (FOLLITROPIN DELTA) CASE

3.1. AMH Incorporated in Phase 2 Development

Based on the existing evidence, it seemed justified to apply a suitable biomarker of ovarian response when developing a new gonadotropin preparation together with a new stratified dosing regimen. Consequently, AMH has been prospectively incorporated in the clinical development program for FE 999049, a novel recombinant FSH [19, 46]. FE 999049 (follitropin delta) is a recombinant human FSH expressed from a cell line of human fetal retinal origin (PER.C6) with an amino acid sequence identical to the native human FSH sequence and existing recombinant FSH preparations derived from Chinese hamster ovary (CHO) cell lines (i.e., follitropin alfa and follitropin beta). The human cell line was chosen to resemble the glycosylation profile of native human FSH. In fact, the sialic acid content of FE 999049 is different and more complex, with both α2,3 and α2,6 sialylation compared with the CHO derived FSH products, which only contain α2,3 sialylation (WO 2012/168680). In healthy women, administration of identical bioactive doses (international units [IU] based on the Steelman-Pohley *in vivo* rat bioassay) of FE 999049 and follitropin alfa resulted in slower clearance for FE 999049 and significantly higher follicular and endocrine responses with FE 999049 [46]. This difference in clinical response despite administration of similar dose in terms of IU of biological activity indicates that the *in vivo* rat bioassay is not predictive of the bioactivity of FE 999049 in humans. A difference in the clearance of FE 999049 between rats and humans is considered the most likely explanation for the limited prediction of the *in vivo* rat bioassay for the FE 999049 potency in humans. Therefore, FE 999049 is dosed in microgram (μg) of protein content rather than in IU of biological activity.

The clinical development program for FE 999049 provided a unique opportunity to establish an individualized treatment strategy in IVF/ICSI patients undergoing COS. The first step in the validation phase for AMH in conjunction with FE 999049 was to use a stratified randomization by AMH in a phase 2 dose-response trial [19]. The main objectives of this study were to evaluate the dose-response relationship of the novel recombinant human FSH with respect to ovarian response in patients undergoing COS for IVF/ICSI, and to assess the influence of the initial serum AMH concentrations on the dose-response curve. Five fixed doses ranging from 5.2 to 12.1 μg of FE 999049 were administered daily throughout the stimulation, without any dose adjustments (see Arce et al. 2014 [19] for detailed description of methods). The randomization was stratified by AMH levels at screening [lower AMH stratum (5.0-14.9 pmol/L) and higher AMH stratum (15.0-44.9 pmol/L)] determined at a central laboratory using the Beckman Coulter Gen II assay (unmodified method, but samples being transported/stored at ambient temperature between 1 and 5 days to avoid possible complement interference). The stratification by AMH removed a potential confounding factor by making the different treatment groups more comparable according to their potential to respond to gonadotropins.

In this study, a significant ($p < 0.001$) linear dose-response relationship between FE 999049 and number of oocytes retrieved was established, overall and for each of the two AMH strata (Figure 3). As expected, the slopes of FE 999049 dose-response curves differed significantly between the two AMH strata. A 10% increase in FE 999049 dose resulted in 0.5

(95% confidence interval 0.2–0.7) and 1.0 (95% confidence interval 0.7–1.3) more oocytes in the low and high AMH stratum, respectively. Interestingly, 31-97% more oocytes were retrieved in high AMH stratum compared with low AMH stratum when administered the same FE 999049 dose, and the magnitude of the differences in ovarian response between the two AMH strata was considered to be of clinical relevance across all dose levels. Thus, the importance of the initial AMH level in the dose-response relationship and the critical implications of the information from this biomarker for recommendation of appropriate FE 999049 doses should be recognized.

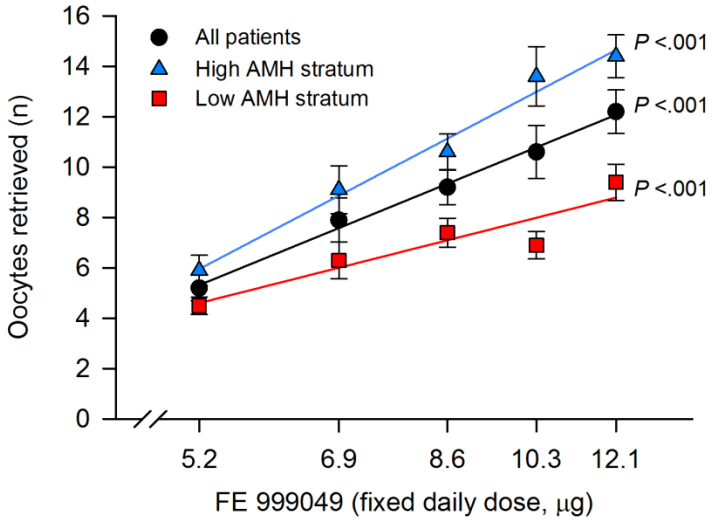


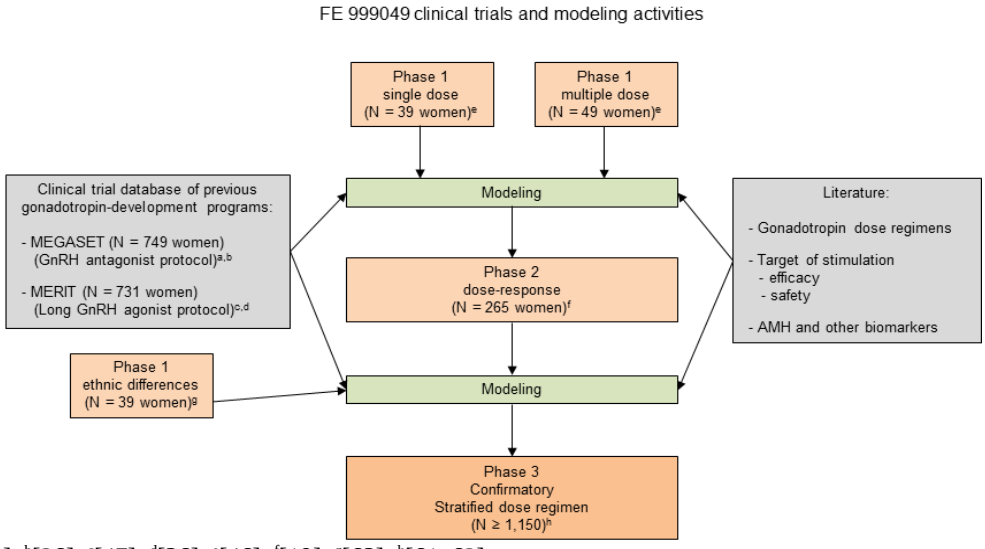
Figure 3. Oocytes retrieved by FE 999049 dose group; overall and by AMH stratum [19]. Values are mean ± S.E. P-values reflect the dose-response relationship.

3.2. Modeling and Simulation

The establishment of the dosing regimen for FE 999049 was initiated based on the findings of the phase 2 trial and the information available from our previous gonadotropin development programs [26, 36, 47, 48]. Literature data available on prediction of ovarian response to gonadotropins with biomarkers and on optimal targets of ovarian response were also considered (Figure 4).

After having documented in phase 2 the relation between ovarian response and dose of FE 999049 as well as the influence of AMH on the dose-response relationship, the next step was to determine the most important aspects predicting the ovarian response to stimulation with FE 999049. The goal was to establish a stratified dosing regimen which can be applied across clinics. In principle, the objectives of this new dosing regimen would be to obtain a more predictable and adequate ovarian response in terms of reducing the risk of poor and excessive response, reducing the risk of OHSS, and eventually improving cost-effectiveness of the gonadotropin treatment. The number of oocytes retrieved was the initial obvious parameter for assessment of ovarian response, as it reflects FSH action and is related (or surrogate) to relevant clinical parameters for consideration when establishing the optimal dosing regimen for FE 999049: risk of cycle cancellation due to poor or excessive response,

risk of early moderate/severe OHSS and excessive response and interventions to prevent these scenarios, risk of no blastocysts available for transfer, and proportion of patients with blastocysts available for fresh transfer and/or freezing.



[a][48], [b][26], [c][47], [d][36], [e][46], [f][19], [g][63], [h][61, 62].

Figure 4. Process for establishing FE 999049 dosing regimen in a phase 3 program.

The process for identifying the individualized starting dose of FE 999049 consisted of the following steps:

1) Development of a pharmacokinetic (PK) model to identify factors that affect serum FSH levels after dosing with FE 999049.
2) Development of a pharmacodynamic (PD) model to identify factors that affect number of oocytes retrieved after dosing with FE 999049.
3) Establishment of the target for ovarian stimulation, i.e., the ideal range of number of oocytes retrieved, and also to consider the risk of cycle cancellation due to poor or excessive response, risk of early moderate/severe OHSS and preventive interventions, and availability of blastocysts for transfer and freezing.
4) Identification of an individualized FE 999049 dosing regimen in accordance with the established target for ovarian stimulation.

After this stepwise process for development of a dosing regimen, the proposed stratified approach to FE 999049 dosing required full prospective clinical validation in IVF patients including clinical documentation of the value and utility compared with a non-stratified approach.

3.2.1. Pharmacokinetic (PK) Model Step

A PK model was established to define the parameters that affected the circulating FSH concentration after dosing with FE 999049. The total serum FSH concentration during stimulation is constituted of both endogenous secretion from the pituitary gland and

exogenous administration, and is influenced by a number of factors, such as patients' characteristics, the dose of FE 999049, and the stimulation protocol, among others. The PK model mimicked the two sources contributing to the FSH concentration by using a one-compartment model with first-order absorption and absorption lag-time and a time-dependent endogenous FSH level (as suppression of FSH production increases over time due to feedback mechanisms). The PK model showed that the serum FSH concentration during stimulation with FE 999049 was directly related to the dose of FE 999049, and inversely related to the body weight of the woman. This is attributed to the fact that exogenous FSH is distributed within the extracellular fluid space, and the apparent volume of distribution and clearance are both proportional to body weight. Thus, in line with the PK model, secondary analysis of data collected in the phase 2 trial showed that the body weight was inversely associated with the follicular development and the serum levels of estradiol, inhibin B and inhibin A during treatment with FE 999049, with lower weight patients having greater responses than higher weight patients [49]. Consequently, the woman's body weight needs to be taken into account in the gonadotropin dosing regimen to optimize the ovarian response.

3.2.2. Pharmacodynamic (PD) Model Step

A PD model was developed to estimate the impact of different FE 999049 doses on the number of oocytes retrieved and other parameters, including age and biomarkers of ovarian reserve/response to gonadotropins, i.e., basal serum levels of FSH, AMH and inhibin B, and AFC. The PD model established the serum AMH concentration before start of stimulation as the best single predictor of number of oocytes retrieved. None of the other baseline parameters contributed with substantial additional explanation of the variation of the data. The FE 999049 dose by body weight and AMH concentration yielded the highest explanation of the variation in number of oocytes retrieved, whereas the FE 999049 dose by body weight together with either basal FSH, inhibin B, AFC or age yielded lower explanations (Table 1).

Table 1. Impact of baseline parameters on number of oocytes retrieved estimated in a PD model

Covariate	Explained variation
Dose by body weight +	
AMH	35%
FSH	23%
Inhibin B	17%
AFC	26%
Age	15%
Dose by body weight + AMH +	
FSH	38%
Inhibin B	35%
AFC	38%
Age	35%

The PD model was a sigmoid Emax model (Emax = expected maximal number of oocytes retrieved), evaluating the contribution of covariates, individually and combined, in explaining the variation in the data (i.e., the number of oocytes retrieved). Covariates were included one by one by starting with the covariate explaining the largest fraction of the total variation and keeping only covariates that were statistically significant and adding at least 5% points to the total variation. Data are based on Arce et al. 2014 [19].

Furthermore, adding any of these parameters to the combination of FE 999049 dose and AMH concentration had either no or only limited value, with age providing no additional explanation to the variation.

These estimates are in line with previous reports from the multicenter RCTs which consistently concluded that AMH is a better predictor of ovarian response to COS than AFC and that inclusion of AFC in models of ovarian response did not provide any additional predictive value beyond that provided by AMH [26, 35, 36, 39].

3.2.3. Target for Ovarian Response

After having established the relationship between the dose of FE 999049 and the number of oocytes retrieved according to different AMH levels, the next step was to define the overall target for ovarian response. It has been suggested that there is an optimal number (or range) of retrieved oocytes in relation to the chance of achieving a pregnancy, although the literature is not fully in agreement regarding the target range or threshold. Thus, 6 metaphase II oocytes [50], 10 oocytes [51], 13 oocytes [52], 5-14 oocytes [53], 5-15 oocytes [54] and 7-15 oocytes [55] have been suggested as appropriate targets of ovarian stimulation. However, cycles with no embryos/blastocysts available for transfer and cycle cancellations due to OHSS were not included in these characterizations of an optimal ovarian response [50, 51, 52]. The risk of moderate/severe OHSS is markedly increased in women with more than 15 retrieved oocytes compared to lower oocyte yields [55]. Furthermore, an excessive ovarian response is not only a concern of safety but may also be a concern of efficacy, because supraphysiological levels of estradiol and progesterone from multiple corpora lutea may have detrimental effects on endometrial receptivity [56, 57]. It has been reported that pregnancy rates decrease with retrievals above 13 oocytes [52], 15 oocytes [54], 18 oocytes [51] or 20 oocytes [58], while others have not reported an effect on efficacy with increasing oocyte yield [59, 60].

The availability of more oocytes would be expected to eventually lead to more blastocysts for transfer or cryopreservation, as long as the quality and developmental competence of the additional oocytes remain intact with increasing response. In the FE 999049 dose-response trial, the fertilization rate and the blastocyst/oocyte ratio decreased significantly with increasing FSH doses in both AMH strata [19]. No linear relationship was observed between FSH dose and number of blastocysts, indicating further that the increased oocyte yield at higher doses does not result in a similar increase in the numbers of blastocysts. In fact, the expected relation between number of oocytes retrieved and number of blastocysts was only observed up to a certain number of retrieved oocytes, after which more oocytes was not associated with an increasing rate of blastocysts. A significant ($p < 0.001$) relation was observed between number of oocytes retrieved and number of blastocysts, both total and of good quality, but this was driven only by the initial phase of the relationship (Figures 5A and 5B). Interestingly, once 11 oocytes were retrieved, additional oocytes did not lead to an increase in number of blastocysts. This indicates that increasing the FE 999049 dose, and thereby increasing the number of oocytes retrieved, may not lead to notable increases in the total number of blastocyst or high-grade blastocysts once a certain threshold (i.e., around 11 oocytes) is reached. Receiver-operating-characteristics (ROC) curve analysis indicated that patients obtaining around 8 oocytes would have a good probability of obtaining at least 2 blastocysts of a good quality.

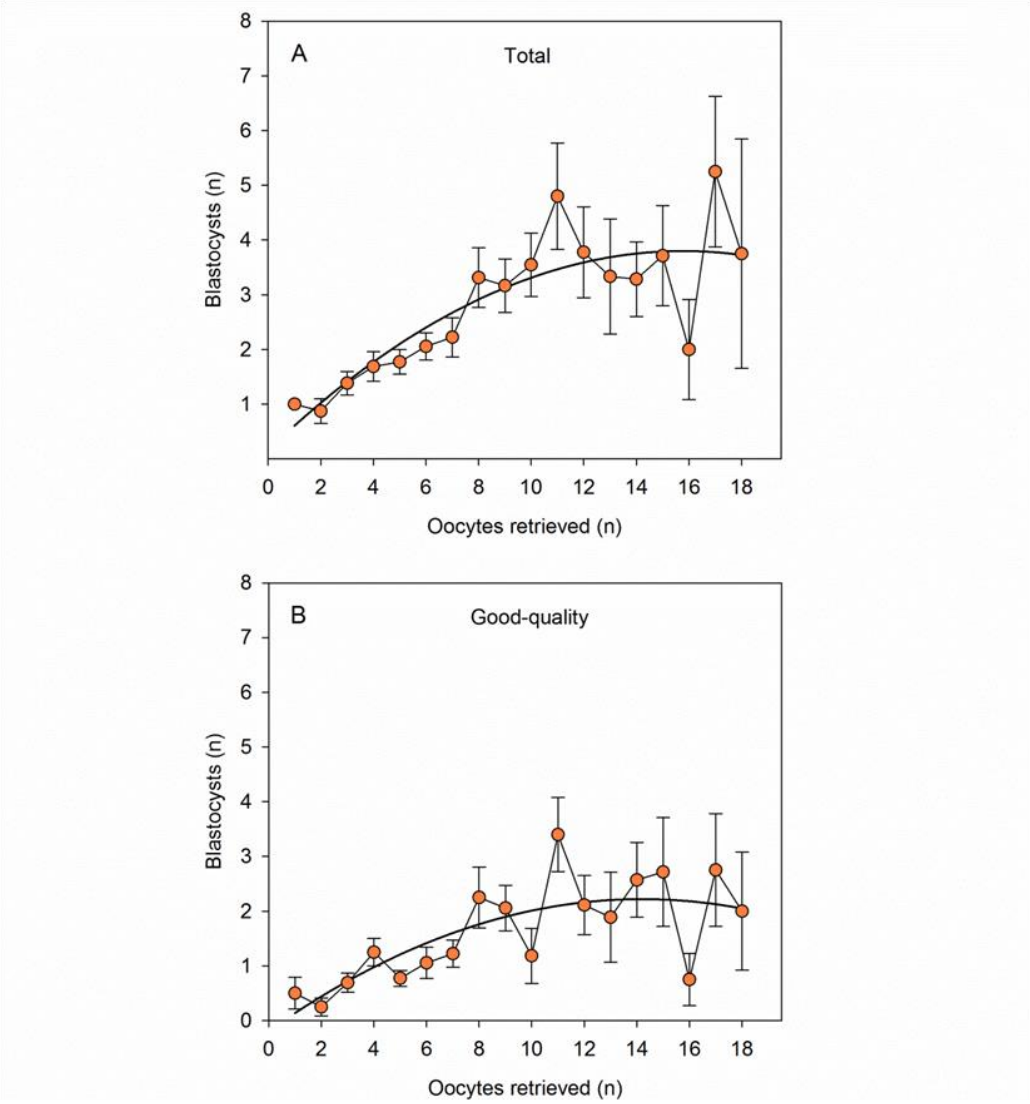


Figure 5. Relation between number of oocytes retrieved and number of blastocysts; total (A) and of good-quality (B). Values are mean ± S.E.

Based on these arguments, the appropriate ovarian response used for the development of the FE 999049 dosing regimen was outlined as 8-14 oocytes retrieved (if possible, with a target of 11 oocytes) to have a high probability of obtaining at least 2 good blastocysts available for transfer(s). Under these assumptions, the proportion of patients with less than 4 oocytes should be minimized due to the risk of unavailability of blastocysts for transfer in fresh and potentially subsequent frozen embryo replacement cycles, and the proportion of patients with 20 oocytes or more, and preferably 15 or more, should be also minimized due to the risk of OHSS.

3.2.4. AMH-Stratified FE 999049 Dosing Regimen

The expected number of oocytes retrieved for each FE 999049 dose (expressed in µg for patients with AMH < 15 pmol/L and in µg/kg body weight for patients with AMH ≥15 pmol/L) and for each AMH value were estimated using the PD model (Figures 6A and 6B).

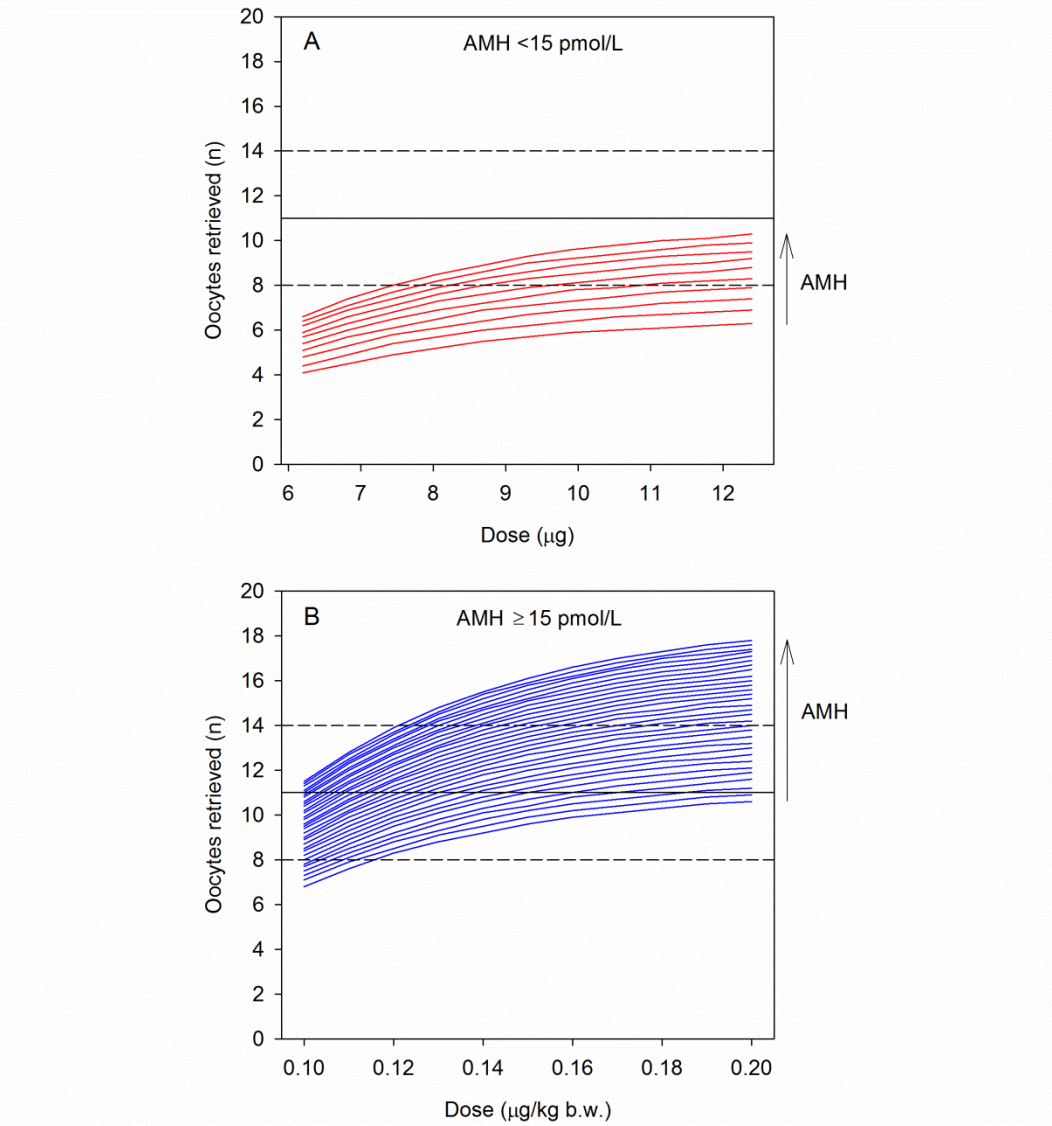


Figure 6. Estimated number of oocytes retrieved by FE 999049 dose for increasing levels of AMH between 5 and 14 pmol/L (A), and between 15 and 45 pmol/L (B). The horizontal dotted lines and the horizontal solid line illustrate the range of 8-14 oocytes retrieved and the target of 11 oocytes retrieved, respectively.

Patients with an AMH value < 15 pmol/L are predicted to have a flatter dose-response and a lower maximum potential than those with higher AMH. Thus, even great increases in dose will have limited impact on the number of oocytes retrieved, especially in patients with diminished ovarian reserve, in whom the ideal target of oocytes retrieved cannot be obtained

even with the highest starting gonadotropin dose. For patients with AMH ≥15 pmol/L (Figure 6B), a relatively large incline of the estimated slope is seen, indicating that even small increases in FE 999049 dose can be associated with a major increase in the number of oocytes retrieved. In this group of patients, the slopes are generally steeper with increasing AMH values and furthermore within each AMH value the slopes are steeper at lower FE 999049 dose levels than at higher dose levels.

Using the above-mentioned target for ovarian stimulation, a FE 999049 dosing regimen based on AMH can been proposed. Since an AMH-based dosing regimen for the first treatment cycle of FE 999049 would be expected to provide the targeted stimulation goal for most doses, FE 999049 is intended to be used in a fixed-dose regimen throughout the stimulation, without a need for dose adjustments during the cycle. The doses achieving or being closest to the target ovarian response were calculated for each AMH value leading to an individualised dosing regimen for FE 999049, which is illustrated in Figure 7. For a patient with AMH < 15 pmol/L, the daily FE 999049 dose proposed would be the highest starting dose recommended irrespective of her body weight, as this parameter has only negligible impact on ovarian response at this dose level. For a patient with AMH ≥15 pmol/L, the daily FE 999049 dose would be adjusted based on the actual AMH value and the body weight, with a ceiling dose to avoid too high doses in heavy patients. This ceiling dose level should lie within the ones tested in the dose-response trial which were associated with documented efficacy (i.e., pregnancies/live births) and safety. A continuous regimen (i.e., doses based on a continuous scale of AMH values, as illustrated in Figure 7) rather than a categorical (i.e., by dose divided into a few AMH categories) is obviously preferable.

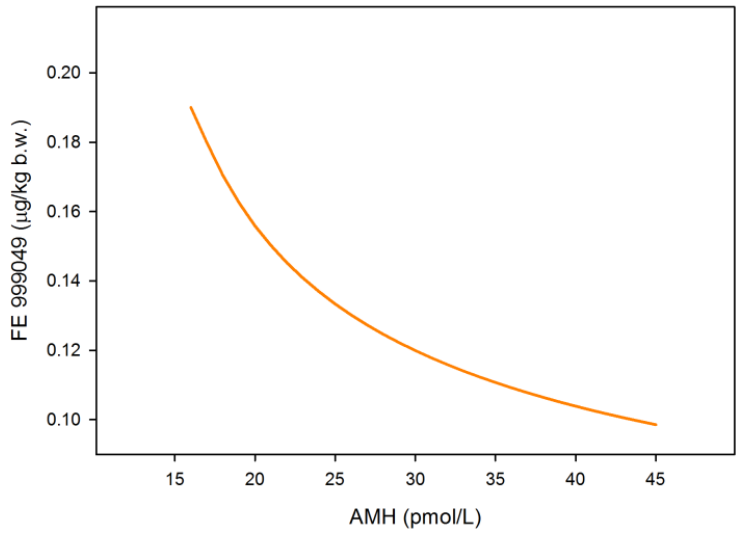


Figure 7. FE 999049 individualised dosing regimen adjusted for body weight for patients with AMH ≥15 pmol/L. All patients with AMH <15 pmol/L are given the same daily dose of 12 µg. For patients with AMH ≥15 pmol/L, the maximum dose is 12 µg/day.

Figure 8A displays the expected distribution of patients according to the estimated number of oocytes retrieved by different AMH values when all patients are given the same dose of 9 µg. It is predicted by the PD model that administration of the same FSH dose to all patients results in a relatively high proportion of low AMH patients having only few retrieved

oocytes. Furthermore, increasing AMH levels are predicted to be associated with an increased proportion of patients with 20 or more oocytes and thereby being at risk of developing OHSS. Figure 8B illustrates the distribution of patients according to the estimated number of oocytes retrieved by different AMH values using the individualised dosing regimen for FE 999049.

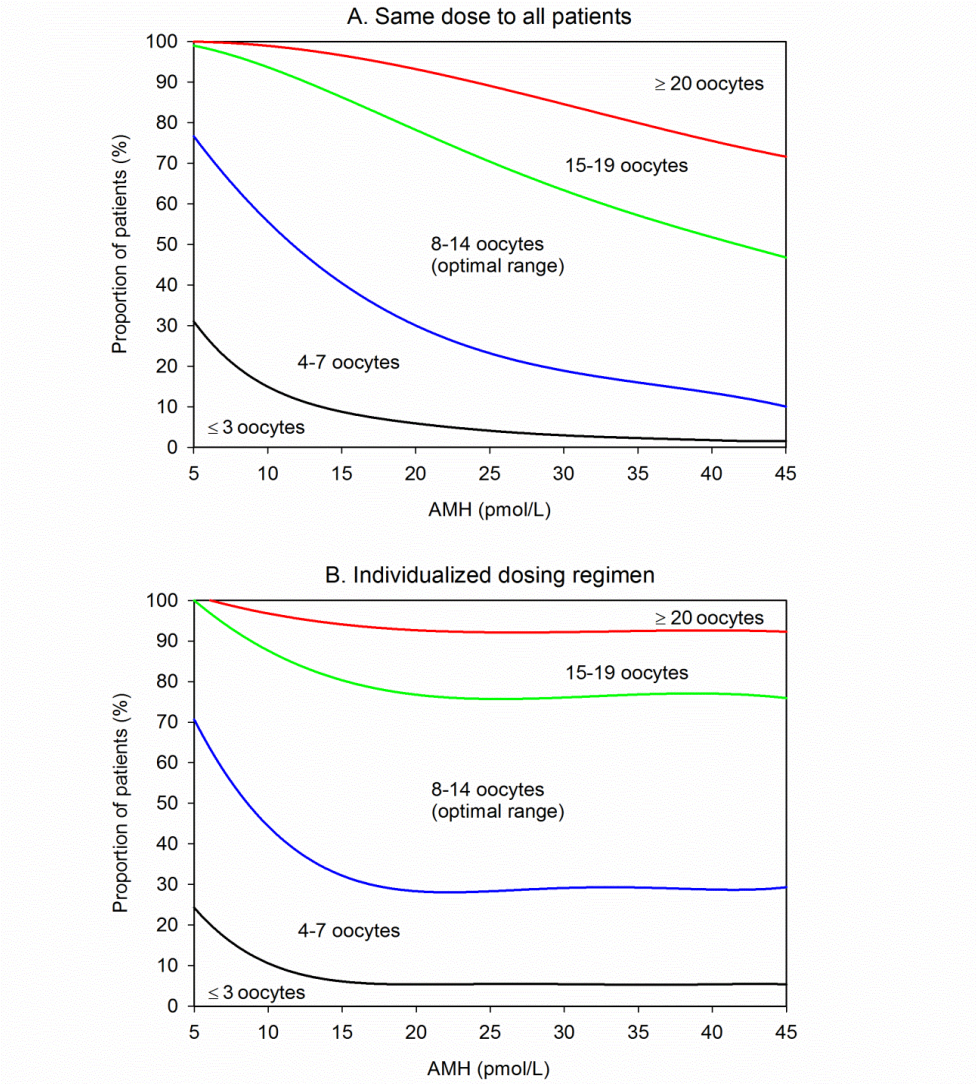


Figure 8. Modeled distribution of patients' ovarian response by AMH level when all patients are either administered the same dose of FE 999049 of 9 µg (A), or the dose proposed in the individualized dosing regimen (B).

The main difference between the two different treatment regimens is the more uniform ovarian response estimated to be achieved with an individualized FE 999049 dosing regimen across all patients (i.e., full range of AMH values). For patients with low AMH, the proportion of patients with less than 4 oocytes retrieved is estimated to be reduced with the proposed individualized dosing regimen, which is anticipated to result in more treatment cycles with blastocysts available for transfer. It should be noted that an increase in dose

beyond a certain level in patients with a very low AMH is not expected to result in more oocytes, as a very low AMH value is an indication of very few follicles recruitable by exogenous gonadotropin stimulation. For patients with high AMH, the frequency of patients with 20 or more oocytes retrieved is estimated to be reduced with the individualized FE 999049 dosing regimen; this should eventually lead to a lower risk of early moderate/severe OHSS and/or to fewer interventions needed to prevent OHSS. The frequency of cycle cancellations due to excessive response or alternatively triggering with GnRH agonist are also expected to be reduced.

3.3. Clinical Validation

Success for this stratified approach requires both adequate analytical performance of AMH and adequate clinical performance of FE 999049, as well as accuracy of the PD model linking the AMH level with the dose of FE 999049. Thus, both the efficacy and the safety of the recommended doses are dependent on the analytical performance of the biomarker assay. The impact of maximum variation of the AMH value, i.e., the combined intra-individual, lot-to-lot and across-lab variation in determining AMH, on the prescribed FE 999049 dose has to be established to ensure that patients are not inappropriately exposed to lower or higher doses than required. In that respect, maximal variations of AMH from the true AMH level for each subject are to be considered for the model and should lead to only minor and non-clinically relevant differences in oocytes retrieved. A dosing regimen based on continuous AMH values is expected to have advantages over any dosing regimen based on AMH category levels, as potential variations in AMH measurements from true AMH is associated with the smallest shifts in the FE 999049 doses along the AMH scale.

Finally, the clinical validation of the proposed stratified approach to FE 999049 based on AMH values requires large randomized clinical trials evaluating clinical endpoints related to the efficacy and safety of the gonadotropin, as well as the clinical benefits associated with the stratified strategy [61, 62]. The value of a stratified approach needs to be first established by documenting its ability to at least maintain overall success rates (i.e., pregnancy and live births), and eventually also by documenting clinical benefits such as providing a more predictable and adequate ovarian response, thus decreasing the proportion of patients with excessive and poor ovarian response, reducing the risk for OHSS and/or preventive interventions for OHSS, and improving the cost-effectiveness of gonadotropin treatment.

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