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Middle East Fertility Society Journal (2013) xxx, xxx–xxx

Middle East Fertility Society

Middle East Fertility Society Journal

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ORIGINAL ARTICLE

Choosing the optimal dose of human menopausal gonadotropins for ovarian stimulation in ICSI cycles

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Received 4 June 2013; accepted 12 June 2013

KEYWORDS

Ovarian response;
Gonadotropin dose;
Age;
BMI;
FSH;
Estradiol

Abstract *Objective:* To identify the most important predictive variables for ovarian response and establishing a model that could predict the most suitable starting gonadotropin (Gn) dose to optimize ovarian stimulation thus avoiding the undesirable side effects of ovarian hyperstimulation and minimizing cancelation rates.

Study design: Retrospective observational multicenter study.

Materials and methods: Data of 233 normo ovulatory females below the age of 39 undergoing their first intracytoplasmic sperm injection (ICSI) trial were collected. All patients were on long protocol and human menopausal gonadotropin (HMG) was used for ovulation induction. Patients with at least 5 oocytes retrieved and good quality embryos transferred were included in the analysis.

Results: Multivariate analysis revealed that predictive variables of statistical significance on Gn dose were age, body mass index (BMI), follicle stimulating hormone (FSH) and estradiol after downregulation (E_2-DR). Fitting these factors in a model to calculate the starting Gn dose revealed this equation:

Dose = 1.035 Age + 2.355 FSH + 0.340 BMI + 0.241 E_2–DR – 15.266.

The concordance probability index for this model is 60%.

Conclusion: Age, basal FSH, BMI and E_2 after downregulation are important predictors of ovarian response when considering a long protocol of ovarian stimulation and could help in selecting the appropriate starting dose of GN.

1. Introduction

Safe and effective ovarian stimulation is a pivotal step in the success of assisted reproduction techniques (ART). It is important to categorize patients planning for intracytoplasmic sperm injection (ICSI) as normal, poor or high responders, thus choosing the appropriate dosage of Gn for every patient that could yield a suitable number of oocytes (1). This is a real challenge and usually depends on the clinician's experience rather than an objective method for planning the proper starting dose.

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Peer review under responsibility of Middle East Fertility Society.

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http://dx.doi.org/10.1016/j.mefs.2013.06.004

The adequate yield of oocytes is ~~even~~ controversial. An appropriate response has been arbitrarily defined as retrieval of 8–10 oocytes as this should result in sufficient high quality embryos being available for transfer. In centers where cryopreservation is available the retrieval of 10–15 oocytes is considered a success as this allows multiple embryo transfer attempts per ovarian stimulation cycle and contributes to higher cumulative pregnancy rates (2). While in centers where cryopreservation is not available, choosing an appropriate dosage of Gn that could yield a modest number of oocytes is considered crucial as it avoids oocyte and drug wastage. Therefore every center should tailor its own dosage of Gn according to the ovarian response required and to its definition of successful induction protocol.

At the same time, retrieval of less than 5 or more 20 oocytes is regarded as an unfavorable response as the former would increase the incidence of cycle cancelation and the latter would increase the risk of ovarian hyperstimulation syndrome (OHSS) (3). Therefore being able to predict poor and high responders would enable clinicians to manage the induction cycle in the best way possible to meet the preset expectations.

Choosing the appropriate Gn dosage to retrieve an optimum number of oocytes is however complicated as many individual patient variables affect that response (4). We could categorize these variables as physical including age and BMI (5), hormonal including concentrations of FSH, E2, inhibin B and anti-mularian hormone (AMH) (6,7) and ultrasound markers of ovarian responsiveness which have emerged as important predictors of treatment success as the antral follicle count (AFC), ovarian volume and ovarian stromal blood flow (8).

Identifying the most effective variables affecting Gn requirement during COH for IVF/ICSI is the primary goal of the current study. We tried to include these variables in an equation that could help choosing the appropriate starting Gn dose to achieve an adequate ovarian response in order to avoid unnecessary side-effects of ovarian hyperstimulation and minimize cancelation rates.

The starting Gn dose chosen could be used in the first 5 days of ovulation induction until follow-up folliculometry at day 6 is done. Modification of the Gn dose could be achieved at day 6 to obtain the needed follicular output.

2. Materials and methods

233 ICSI cycles done at kasr el aini infertility center and a private IVF center during the period from January 2011 to June 2012 met our inclusion criteria. 225 had ovum pick-up and only 219 did embryo transfer and the latter were analyzed.

2.1. Inclusion criteria

1. Age between 19 and 39 years.
2. Regular menstrual cycle.
3. Day 3 FSH <13 IU/l.
4. Both ovaries present.
5. Long protocol of GN RH agonist used for downregulation.
6. Human menopausal gonadotropins used for ovulation induction.
7. Oocyte yield between 5 and 20.

2.2. Exclusion criteria

1. Polycystic ovarian syndrome.
2. Short or antagonist protocols.
3. FSH used for ovulation induction.
4. Inadequate ovarian response ≤ 5 oocytes retrieved.
5. Excessive ovarian response ≥ 20 oocyte retrieved.
6. Development of OHSS.
7. Presence of ovarian cysts.
8. Intake of medications as steroids, non-steroidal anti-inflammatory and anti-psychotics during ovulation induction.

The study was approved by the research committee of the Obstetrics and Gynecology department, kasr El-Aini hospital.

2.3. Data records included

1. Patient age.
2. Type, cause and duration of infertility.
3. BMI.
4. Ultrasound criteria as AFC in the early follicular phase.
5. Hormonal criteria: basal FSH, E_2 and AMH if present as well as E_2 level after downregulation and before starting induction.
6. Total dose of HMG used for ovulation induction.
7. Estradiol level at the day of HCG trigger.
8. Number of follicles ≥ 14 mm and ≥ 17 mm in diameter on the day of HCG trigger.
9. Number of oocytes retrieved.
10. Number of metaphase II oocytes.
11. Number of fertilized oocytes.
12. Number of good embryos transferred.
13. Clinical pregnancy.

The primary outcome was the correlation between the different individual variables affecting ovarian response and the HMG dose and the quantification of the significance of each variable as a potential predictor of the Gn dose.

The secondary outcome was to investigate the possibility of deriving an equation including the most significant predictors of the Gn dose in order to calculate the optimal starting dose of HMG required to achieve a satisfactory response to ovarian stimulation.

Validation of the equation was done by using the starting Gn dose calculated in ovulation induction in 100 patients with the same inclusion criteria. Follow-up of their initial ovarian response on day 6 of ovulation induction was done.

3. Statistical method

Univariate and multivariate analysis models were used to test for the preferential effect of the independent variables on the total gonadotropin dose and ovarian response in the form of the number of oocytes. *P* values less than 0.05 was considered statistically significant. Multivariate regression models were used to create the best fit equation to predict the gonadotropin dose. All predictors that achieved a *P* value <0.05 in univariate analysis were included in the equation trials. The final equation included the most significant combination of predictors. Statistical Package for Social Sciences (SPSS) version 15 for MS Windows was used (SPSS Inc., Chicago, Il, USA).

Please cite this article in press as: Hashish NM, Shaeer EK Choosing the optimal dose of human menopausal gonadotropins for ovarian stimulation in ICSI cycles, Middle East Fertil Soc J (2013), http://dx.doi.org/10.1016/j.mefs.2013.06.004

4. Results

The study included 233 participants, 225 completed the procedure till ovum pick-up and 219 underwent embryo transfer (ET). The average age was 29.1 ± 5.1 years, BMI 29.8 ± 5.9 kg/m^2, duration of infertility 5.7 ± 4.3 years, 84.9% suffered from primary infertility and 15.1% secondary infertility. The average levels of basal hormones were as follows, FSH 6.4 ± 2.2 IU/L, LH 5.7 ± 3.2 IU/L, E_2 47.5 ± 31.7 pmol/L, prolactin 7.5 ± 8.6 ng/ml. Average AFC was 10.3 ± 4, E_2 after downregulation 10.9 ± 13.2 pmol/L (Table 1).

The average number of gonadotropin ampules was 42.3 ± 17.4, duration of stimulation12 ± 1.9 days, E_2 on day of HCG 2364 ± 1051 pmol/L, number of follicles ≥ 17 mm 8.1 ± 3.9, number of follicles ≥ 14 mm 5 ± 2.9, number of oocytes retrieved 10.9 ± 4.7, number of mature oocytes 8.6 ± 4.9, number of fertilized oocytes 5.9 ± 3.4, mean fertilization rate 68%, total number of embryos 5.1 ± 2.7, number of embryos transferred 2.94 ± 1.25 and the pregnancy rate was 34.7% (Table 2).

Predictive variables that had statistically significant influence on Gn dose were age, FSH, BMI, E_2 at downregulation. AFC did not significantly affect the Gn dose although it significantly affected the ovarian response, therefore it was not included in our equation which is directly concerned with constructing a predictive model for determining the Gn dose (Table 3).

The significant predictive factors were then used to formulate the best fitting equation. The equation aims at calculating the starting Gn dose which the patient can use in the first 5 days before coming for her first folliculometry on day 6 of ovulation induction and assessing the ovarian response thereby modifying the dose if needed.

$$\textbf{Dose} = 1.035\ \textbf{Age} + 2.355\ \textbf{FSH} + 0.340\ \textbf{BMI} + 0.241\ \mathbf{E_2}\ \textbf{afterdownregulation} - 15.266$$

The starting daily dose of Gn was calculated by dividing the total Gn dose (predicted from the equation) by 12 which is the average duration (days) of ovulation induction in our study.

The predicted starting Gn dose was tried on 100 patients with the same criteria mentioned before in our inclusion criteria and was found to have satisfactory ovarian response in 60% of cases, while 40% had their dose modulated (25% of cases had their dose reduced, 15% had their dose increased).

Table 1 Basic demographic and clinical characteristics of participants.

Demographic characteristics	Mean ± SD
Age (years)	29.1 ± 5.1
BMI (kg/m^2)	29.8 ± 5.9
Type of infertility: primary (%)	(198/233) 84.9%
Secondary (%)	(35/233) 15.1%
Duration of infertility (years)	5.7 ± 4.3
FSH (IU/L)	6.4 ± 2.2
LH (IU/L)	5.7 ± 3.2
E2 (pmol/L)	47.5 ± 31.7
PRL (ng/ml)	7.5 ± 8.6
E_2 after downregulation (pmol/L)	10.9 ± 13.2
AFC	10.3 ± 4.0

Data are given in mean ± SD or percentage (%).

Table 2 Cycle characteristics.

Cycle characteristics	Mean ± SD
Number of Gn ampules	42.3 ± 17.4
Duration of stimulation (days)	12 ± 1.9
Peak E_2 (pmol/L)	2364 ± 1051
Number of follicles > 17 mm	8.1 ± 3.9
Number of follicles > 14 mm	5 ± 2.9
Number of oocytes retrieved	10.9 ± 4.7
Number of mature oocytes	8.6 ± 4.9
Number of fertilized oocytes	5.9 ± 3.4
Mean fertilization rate	68%
Total number of embryos	5.1 ± 2.7
Number of embryos transferred	2.94 ± 1.25
Pregnancy rate	34.7%

Data are given in mean ± SD or percentage (%).

Table 3 Predictive value of different patient and cycle characteristics.

	Response variable 1 Gn Dose	Response variable 2 Number of oocytes
	(*P*-value)	(*P*-value)
Age	0.000	0.002
FSH	0.003	0.002
BMI	0.036	0.129
AFC	0.282	0.000
LH	0.765	0.640
E_2	0.077	0.027
E_2 after downregulation	0.002	0.550
PRL	0.015	0.206
Infertility duration	0.137	0.539

The concordance probability index (C index) predicts that approximately 60% of cases will be given the proper dose to achieve a satisfactory preliminary ovarian response according to our constructed predictive model.

The concordance probability index shows the degree of association between the predicted Gn dose and the required dose observed after follow-up folliculometry to achieve an appropriate ovarian response. This index was used to validate the predictive ability of the equation.

5. Discussion

Although defining the optimal starting dose of Gn for each patient is one of the most important issues in the management of ART cycles, there is as yet no clear consensus at the most relevant and practical parameters that will predict ovarian response especially during the first treatment cycle where there is no previous history to refer to in order to optimize the result of each cycle for every individual patient (9). Therefore, this study revealed important correlations between predictive variables like age, FSH, BMI and serum E_2 after downregulation and the dose of Gn used to achieve an adequate ovarian response.

A number of studies have evaluated the predictive value of relevant parameters for ovarian response and Gn dose. In accordance with the current study, chronologic age was a common parameter in their findings as one of the most predictive

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variables (1,8,10). However, a contradicting study mentioned that age was not the only predictive variable as women of the same age can be at different stages in the process of follicular depletion due to the wide range of age at the onset of menopause (11).

Basal FSH is the assay most often used as a screening test for ovarian reserve however its predictive accuracy is limited by patient intercycle variability. A study by Abdallah and colleagues in 2004 suggested that elevated basal FSH reflects a quantitative rather than a qualitative decline of the ovarian reserve and is not necessarily a contraindication to IVF treatment (12), therefore its value could positively correlate with the Gn dose.

In the current study, BMI significantly affected the Gn dose. It has been proved that the adipose tissue is considered an inbuilt source of estrogen, therefore; in agreement with our study; BMI strongly correlates with the amount of Gn consumed in COH (13).

There is a clear correlation between the number of antral follicles seen at the beginning of the follicular phase during a natural cycle and the ovarian response. These are the potentials of the ovary that we try to exploit during controlled ovarian hyperstimulation (14). However, our analysis of predictive variables revealed that AFC reflected follicular output rather than the Gn dose and thus was not included in our model.

Serum estradiol concentration after downregulation is a reflection of the magnitude of the patient's response to pituitary downregulation and should be taken into consideration while choosing the starting Gn dose. To our knowledge, its relation to the Gn dose has not been investigated before.

Predictive variables like ovarian volume and ovarian stromal blood flow were not included because of their limited application in clinical practice all over the world. AMH was not included because of its high cost, thus cannot be routinely done. Other variables such as smoking were excluded from the analysis due to their low prevalence in our community.

The current study tried to formulate an equation to determine the optimal starting dose of Gn for ovulation induction based upon the significance of the most important predictive factors. A new combination of predictors based on multivariate regression analysis was used. Age, basal FSH, BMI and E_2 after downregulation were fitted in a simple equation easy to apply to choose the appropriate starting HMG dose during the first 5 days of ovulation induction and before doing follow-up folliculometry on day 6 when dose adjustment could be done according to ovarian response. Our study showed that an equation could be a guide to a starting dose in addition to ultrasound and hormonal follow-up which could never be ignored in tailoring the dose for every patient.

Many trials to develop a scoring system for calculating the appropriate Gn dose have been attempted. Popovic and Todorovic in 2003 have developed a model based on four predictors, the total number of antral follicles, total doppler score, serum testosterone concentrations and smoking habit (15) but his scoring system was not widely adopted in clinical practice because of the inclusion of two predictive factors that are not measured routinely in clinical practice; Doppler score and testosterone concentrations. In addition smoking is not widely spread in all communities to be included as a significant predictor of ovarian response.

In 2006, Howles and colleagues attempted another trial using a combination of 4 other variables that were identified as most important predictors of ovarian response. These included baseline serum FSH concentrations in the early follicular phase, BMI, age and AFC (16). These factors were modeled into a dosing algorithm to calculate the dose of rFSH which was applied in a clinical trial in 2009 (17). Cancelation rate was high due to the application of a low starting dose of 75 IU for the stimulation of multiple follicular development in some cases which is an infrequent dose in routine clinical practice. In addition, the scoring system was very complicated. Olivennes and colleagues; the directors of the study advised modification of the algorithm and introduction of new variables that could yield a more practical model.

6. Conclusion

We could reach a conclusion that taking into consideration the most important predictive biomarkers for Gn dose while deciding on the starting dose of ovulation induction could enrich our clinical experience and justify our choices in tailoring the most effective and safe dose for each patient in order to achieve an adequate ovarian response.

We recommend further studies on a wider sample of patients exploring the importance of different markers that could help in choosing the most effective Gn dose that would achieve an appropriate ovarian response.

Conflict of interest

None.

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