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Authors:

Joan-Carles Arce, Bjarke M Klein, and Antonio La Marca

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The rate of high ovarian response in women identified at risk by a high serum AMH level is influenced by the type of gonadotropin

Joan-Carles Arce¹, Bjarke M. Klein², and Antonio La Marca³

¹Reproductive Health, Ferring Pharmaceuticals A/S, Copenhagen, Denmark, ²Global Biometrics, Ferring Pharmaceuticals A/S, Copenhagen, Denmark, and ³Mother–Infant Department, University of Modena and Reggio Emilia, Modena, Italy

Abstract

The aim was to compare ovarian response and clinical outcome of potential high-responders after stimulation with highly purified menotropin (HP-hMG) or recombinant follicle-stimulating hormone (rFSH) for *in vitro* fertilisation/intracytoplasmic sperm injection. Retrospective analysis was performed on data collected in two randomized controlled trials, one conducted following a long GnRH agonist protocol and the other with an antagonist protocol. Potential high-responders ($n = 155$ and $n = 188$ in the agonist and antagonist protocol, respectively) were defined as having an initial anti-Müllerian hormone (AMH) value >75th percentile (5.2 ng/ml). In both protocols, HP-hMG stimulation in women in the high AMH category was associated with a significantly lower occurrence of high response (≥ 15 oocytes retrieved) than rFSH stimulation; 33% versus 51% ($p = 0.025$) and 31% versus 49% ($p = 0.015$) in the long agonist and antagonist protocol, respectively. In the potential high-responder women, trends for improved live birth rate were observed with HP-hMG compared with rFSH (long agonist protocol: 33% versus 20%, $p = 0.074$; antagonist protocol: 34% versus 23%, $p = 0.075$; overall population: 34% versus 22%, $p = 0.012$). In conclusion, the type of gonadotropin used for ovarian stimulation influences high-response rates and potentially clinical outcome in women identified as potential high-responders.

Keywords

GnRH agonist, GnRH antagonist, gonadotropins, live birth, ovarian response

History

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Introduction

Individualization of the treatment strategy is currently one of the most relevant topics in reproductive medicine. The basis for individualization of treatment in patients undergoing their first *in vitro* fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) cycle is prediction of the ovarian response to gonadotropin stimulation, forecasting poor, normal or high response [1]. Clinicians may then choose between various treatment strategies to maximize efficacy and safety in the different response categories. Albeit it has been suggested that a specific type of gonadotropin-releasing hormone (GnRH) protocol may be more suitable for either potential hyper-responders or potential poor-responders [2–5], no studies have explored whether a specific type of gonadotropin preparation may offer additional advantages in certain groups of patients, such as patients at risk of hyper-response.

Hyper-responders are usually defined as women with high numbers of oocytes retrieved following a standard protocol of controlled ovarian stimulation (COS). Although these patients are generally considered a good-prognosis group regarding reproductive success, it is currently debated whether a high ovarian response is associated with decreased chance of successful

outcome as compared with a normal response. Two large retrospective studies suggest that pregnancy and live birth rates in fresh embryo transfer cycles are directly related to oocyte yield with an almost linear relationship between live birth and increasing number of oocytes retrieved, with a decline in live birth rates at high oocyte yields [6,7]. In contrast, other retrospective analyses have described that a high ovarian response does not compromise pregnancy rates [8,9]. Differences in patient populations or treatment protocols may explain the inconsistent results in the literature concerning outcome in high-responders.

It has been demonstrated that the relatively good chance of success in women with potential for being high-responders could be further increased by using a GnRH antagonist protocol with a starting gonadotropin dose of 150 IU daily [10,11], but it is not established if the type of gonadotropin preparation should be taken into consideration to further modulate the ovarian response. Indeed, highly purified menotropin (HP-hMG) and recombinant follicle-stimulating hormone (rFSH) are associated with differential follicular growth [12,13], which may be attributed to differences in FSH isoforms and overall profile of isoforms, as well as the luteinizing hormone (LH)-activity component in HP-hMG [14]. On this basis, it can be hypothesised that the effective number of high-responders may be different when women with high numbers of recruitable follicles are treated with either HP-hMG or rFSH.

The aim of the present study was to evaluate the impact of the type of gonadotropin preparation (HP-hMG versus rFSH) used for COS on ovarian response and clinical outcome in potential high-responders undergoing IVF/ICSI treatment. The women

Address for correspondence: Joan-Carles Arce, Ferring Pharmaceuticals A/S, Reproductive Health, Global Clinical & Non-Clinical R&D, Kay Fiskers Plads 11, DK-2300 Copenhagen S, Denmark. Tel: +45 28787606. Fax: +45 28176606. E-mail: jca@fering.com

were classified as being at risk of a high response based on a high serum level of anti-Müllerian hormone (AMH) at start of stimulation. AMH has been demonstrated to be a reliable surrogate marker for the functional ovarian follicle reserve [15]. Further, a high basal concentration of AMH has been shown to be associated with excessive response to gonadotropin stimulation [16–22].

Materials and methods

This study was a retrospective analysis of data prospectively collected in two randomized controlled trials comparing treatment outcome in patients undergoing stimulation with HP-hMG (Menopur; Ferring Pharmaceuticals or rFSH (follitropin alfa, Gonal-F; Merck Serono and follitropin beta, Puregon; MSD) following a long GnRH agonist protocol or a GnRH antagonist protocol, as described elsewhere [12,13].

Study populations

The main inclusion criteria for the long agonist trial were women aged 21–37 years; primary infertility diagnosis being tubal factor, unexplained infertility, or mild male factor; FSH 1–12 IU/l. The main inclusion criteria for the antagonist trial were women aged 21–34 years; primary infertility diagnosis being unexplained infertility or mild male factor; FSH 1–12 IU/l. In both trials, women with polycystic ovaries were excluded.

Study protocols

In the long agonist protocol, down-regulation was performed with triptorelin (0.1 mg/d) (Decapeptyl; Ferring Pharmaceuticals). The gonadotropin dose was fixed at 225 IU/d for the first 5 d, followed by dose-adjustments according to ovarian response. In the antagonist protocol, the gonadotropin dose was fixed at 150 IU for the first 5 d and adjusted according to ovarian response from day 6 when GnRH antagonist (ganirelix, Orgalutran; MSD) was initiated (0.25 mg/d) and continued throughout gonadotropin-treatment. In both protocols, hCG (250 µg) (choriogonadotropin alpha, Ovitrelle; Merck Serono) was administered when three follicles of ≥ 17 mm were observed. Oocyte retrieval took place 36 ± 2 h later. Luteal support was provided by vaginal administration of progesterone (Crinone 90 mg/d, Merck Serono; Utrogestan 600 mg/d, Seid) starting the day after oocyte retrieval and for at least 13–15 d after embryo transfer. In the long agonist protocol, 1–2 embryos was transferred on day 3 and in the antagonist protocol 1 blastocyst was transferred on day 5. Delivery of (at least) one live-born neonate defined live birth.

Serum assays

AMH was analysed by enzyme-linked immunosorbent assay (long agonist trial: Immunotech Beckman Coulter AMH ELISA [A11893], Marseilles, France; antagonist trial: Beckman Coulter Gen 2 ELISA [A79765] Webster, TX, US; 1 ng/ml = 7.14 pmol/l). The AMH assays had a sensitivity of 0.35 and 0.08 ng/ml and total imprecision (% coefficient of variation) of <9.5 and <7.7 in Immunotech Beckman Coulter and Beckman Coulter Gen 2, respectively. FSH, estradiol and progesterone were analysed by electrochemiluminescence immunoassay (Roche-Diagnostics ECLIA).

Statistical analysis

In total, the two trials comprised 1372 women with an AMH value on stimulation day 1. Women were classified as potential high-responders if initial AMH was in the uppermost quartile of the observed AMH distribution. In both protocols, the 75th percentile

was identical (5.2 ng/ml = 37.4 pmol/l). One hundred fifty-five women treated in the long GnRH agonist protocol (76 and 79 in the HP-hMG and rFSH groups, respectively) and 188 women in the GnRH antagonist protocol (87 and 101 in the HP-hMG and rFSH groups, respectively) were classified as potential high-responders.

In each protocol, baseline characteristics, end-of-stimulation data, ovarian response and embryo data were compared between the women grouped according to their AMH value on stimulation day 1 (>75 th versus ≤ 75 th percentile). Similar analyses were performed for the potential high-responders comparing gonadotropin treatments (HP-hMG versus rFSH) within each protocol. Continuous and categorical data were compared using the Wilcoxon test and the Chi-Square or Fisher's exact test, respectively. For the potential high-responders, risk of high response (≥ 15 oocytes retrieved) and chance of live birth were compared between treatments using the Chi-square test. The observed differences in live birth rates between gonadotropin-treatment groups were further analysed in the pooled population of potential high-responders from both protocols to determine if they could be attributed to baseline characteristics or end-of-stimulation variables. For each variable, a logistic regression model was fitted including treatment group and the variable in question in the linear predictor. Only fresh treatment cycles were included in the present dataset.

Results

High AMH category versus non-high AMH category

In both the long agonist protocol and the antagonist protocol, the women in the high AMH category were characterized by younger age, longer menstrual cycle length, higher AFC, lower FSH and larger ovarian volume at start of stimulation than women in the non-high AMH category ($p \leq 0.003$ for each variable) (Table 1).

Independent of the protocol used, women with high AMH exhibited significantly ($p < 0.001$ for each variable) higher serum levels of estradiol and progesterone as well as increased number of growing follicles ≥ 12 mm at end of stimulation than women with no-high AMH. Further, the women with high AMH had significantly ($p \leq 0.003$ for each variable) more oocytes retrieved, increased occurrence of high response, higher frequency of early OHSS and interventions for hyper-response. In the long agonist protocol, cycle cancellation due to ovarian hyper-response occurred more frequently among women in the high AMH category ($p = 0.001$). At end of stimulation, no clinically relevant differences were noted in endometrial thickness or echogenicity patterns between the two AMH categories (Table 1).

Significantly more embryos on day 3 (long agonist protocol: $p = 0.029$) or blastocysts on day 5 (antagonist protocol: $p < 0.001$) were available in women with high AMH, but the proportion of women with top-quality embryo(s) or good-quality blastocyst(s) were similar in the two AMH categories (Table 1).

HP-hMG versus rFSH stimulation in high AMH category

Within each protocol, there were no clinically relevant differences between the two gonadotropin-treatment groups in the high AMH category regarding demographics, fertility history and markers of ovarian reserve (Table 2). BMI was significantly lower in rFSH-treated women, but was not of clinical relevance. At end of stimulation, higher estradiol levels ($p = 0.012$) and lower progesterone levels ($p < 0.001$) were observed with HP-hMG in the antagonist and long agonist protocol, respectively.

HP-hMG was associated with lower median number of oocytes retrieved in women with high AMH compared with rFSH (long agonist protocol: -3 oocytes, $p = 0.007$; antagonist protocol: -2

Table 1. Demographics and baseline, end-of-stimulation, oocyte and embryo data of the women grouped by the AMH concentration at start of stimulation (quartiles 1–3 versus quartile 4).

| Variable | Long GnRH agonist protocol | | | GnRH antagonist protocol | | |
|--|--|---|----------|--|---|----------|
| | AMH Q1-Q3 ≤75th (≤5.2 ng/ml) (n = 468) | AMH Q4 >75th (>5.2 ng/ml) (n = 155) | p Value* | AMH Q1-Q3 ≤75th (≤5.2 ng/ml) (n = 561) | AMH Q4 >75th (>5.2 ng/ml) (n = 188) | p Value* |
| Clinical characteristics | | | | | | |
| Age (years) | 31 (29, 34) | 30 (28, 32) | <0.001 | 31 (29, 33) | 30 (28, 32) | <0.001 |
| BMI (kg/m ²) | 21.9 (20.3, 24.0) | 21.3 (20.1, 23.5) | 0.113 | 21.9 (20.3, 23.8) | 21.8 (20.5, 23.5) | 0.868 |
| Cycle length (days) | 28 (28, 29) | 29 (28, 30) | <0.001 | 28 (28, 29) | 29 (28, 30) | <0.001 |
| First treatment cycle, n (%) | 327 (70%) | 104 (67%) | 0.517 | 427 (76%) | 134 (71%) | 0.186 |
| Day 1 (before start of stimulation) | | | | | | |
| Ovarian volume (ml) | 8.5 (6.0, 11.8) | 10.4 (7.7, 14.3) | <0.001 | 10.6 (8.0, 14.2) | 13.2 (9.6, 16.8) | <0.001 |
| AFC (n) | 10 (7, 14) | 11 (8, 18) | <0.001 | 14 (11, 17) | 18 (15, 22) | <0.001 |
| AMH (ng/ml) | 3.0 (2.1, 4.0) | 7.0 (5.8, 8.5) | <0.001 | 2.4 (1.4, 3.6) | 6.9 (6.0, 8.7) | <0.001 |
| FSH (IU/l) | 3.8 (3.0, 4.9) | 3.4 (2.7, 4.4) | 0.003 | 7.2 (6.2, 8.5) | 6.5 (5.7, 7.6) | <0.001 |
| End-of-stimulation | | | | | | |
| Estradiol (nmol/l) | 5.5 (4.0, 7.3) | 8.5 (6.2, 13.0) | <0.001 | 5.7 (4.1, 8.2) | 8.7 (6.3, 13.3) | <0.001 |
| Progesterone (nmol/l) | 2.6 (2.0, 3.4) | 3.2 (2.4, 3.9) | <0.001 | 2.5 (1.9, 3.2) | 2.9 (2.1, 3.8) | <0.001 |
| Progesterone/estradiol ratio | 0.46 (0.35, 0.63) | 0.36 (0.24, 0.49) | <0.001 | 0.42 (0.30, 0.60) | 0.32 (0.21, 0.44) | <0.001 |
| Follicles ≥12 mm (n) | 10 (8, 13) | 15 (12, 19) | <0.001 | 10 (7, 13) | 15 (11, 18) | <0.001 |
| Endometrial thickness (mm) | 11 (9, 12) | 11 (10, 12) | 0.079 | 10 (9, 12) | 11 (10, 12) | 0.039 |
| Endometrial echogenicity pattern (hypo, iso, hyper) (%) | 39, 49, 13 | 34, 51, 15 | 0.544 | 40, 51, 9 | 36, 54, 10 | 0.668 |
| Cycle cancellation for ovarian hyper-response, n (%) | 2 (<1%) | 7 (5%) | 0.001 | 1 (<1%) | 1 (<1%) | 0.439 |
| Early OHSS (moderate/severe), n (%) | 1 (<1%) | 7 (5%) | <0.001 | 4 (<1%) | 8 (4%) | 0.003 |
| Intervention for ovarian hyper-response, n (%) | 2 (<1%) | 11 (7%) | <0.001 | 16 (3%) | 19 (10%) | <0.001 |
| Oocyte retrieval | | | | | | |
| Women with oocyte retrieval, n (%) | 446 (95%) | 145 (94%) | 0.392 | 537 (96%) | 185 (98%) | 0.088 |
| Oocytes retrieved (n) | 9 (6, 12) | 14 (10, 18) | <0.001 | 8 (5, 11) | 12 (9, 17) | <0.001 |
| Women with ≥15 oocytes retrieved, n (%) | 76 (16%) | 65 (42%) | <0.001 | 63 (11%) | 76 (40%) | <0.001 |
| Fertilisation and embryo data | | | | | | |
| Fertilisation rate (%) | 60 (33, 75) | 52 (29, 70) | 0.091 | 60 (43, 75) | 58 (42, 71) | 0.193 |
| Embryos on day 3 (n) | 2 (1, 5) | 3 (2, 6) | 0.029 | | | |
| Women with top-quality embryo(s) on day 3, n (%)† | 199 (45%) | 73 (50%) | 0.229 | | | |
| Blastocysts on day 5 (n) | | | | 2 (1, 4) | 3 (1, 6) | <0.001 |
| Women with good-quality blastocyst(s) on day 5, n (%)‡ | | | | 266 (50%) | 106 (57%) | 0.068 |
| Women with transfer, n (%)¶ | 397 (89%) | 122 (84%) | 0.119 | 462 (86%) | 159 (86%) | 0.976 |

Values are median (IQR) unless otherwise indicated.

*Wilcoxon test (continuous data); Chi-Square test or Fisher's exact test (categorical data).

†Top-quality embryos were defined as 4–5 cells on day 2, ≥7 cells on day 3, equally-sized blastomeres and ≤20% fragmentation on day 3 and no multinucleation.

‡Good-quality blastocysts were defined as blastocysts with expansion and hatching score ≥4 and with inner cell mass and trophectoderm grades of A or B, using the definitions described by Gardner & Schoolcraft [23].

¶Among women with oocytes retrieved.

oocytes, $p = 0.033$) (Table 2). In both protocols, the percentage of women with a high ovarian response was significantly lower for HP-hMG compared with rFSH (long agonist protocol: 33% versus 51%, $p = 0.025$; antagonist protocol: 31% versus 49%, $p = 0.015$) (Figure 1A). Therefore, the risk of high response was consistently reduced with HP-hMG by 35 and 37%, respectively. There were no apparent differences between the two gonadotropin groups concerning cycle cancellations due to excessive response, early moderate/severe OHSS or interventions for excessive response in either protocol.

Within each protocol, fertilisation rate, number of embryos/blastocysts available for transfer, women with top-quality embryo(s)/good-quality blastocyst(s) and percentages of women with transfer were similar between the HP-hMG and rFSH groups in the high AMH category (Table 2). However, in both protocols a statistical trend ($p < 0.10$) for improved live birth rate per started cycle was observed for HP-hMG compared with rFSH

(Figure 1B). When restricted to women with embryo transfer, the difference in live birth rate between HP-hMG and rFSH was statistically significant ($p = 0.043$) in the antagonist protocol.

When the data of women with high AMH from both protocols were integrated, HP-hMG treatment was associated with significantly lower incidence of high response [32% (52/163) versus 49% (89/180), $p < 0.001$] and increased live birth rate per started cycle [34% (55/163) versus 22% (39/180), $p = 0.012$] as well as per embryo transfer cycle [41% (55/133) versus 26% (39/148), $p = 0.008$] compared with rFSH treatment. The logistic regression analysis (Supplementary Table 1) indicated that the type of GnRH protocol did not explain the difference in live birth rates between HP-hMG and rFSH, as it remained significant ($p = 0.012$) in the adjusted analysis. The probability of a live birth significantly increased with the availability of a top-quality embryo/good-quality blastocyst for transfer ($p < 0.001$), while an increased progesterone level ($p = 0.042$) and increased

Table 2. Comparison of baseline, end-of-stimulation, oocyte and embryo characteristics between HP-hMG- and rFSH-treated women with potential for being high-responders by a high AMH at start of stimulation.

| Variable | Long GnRH agonist protocol | | | GnRH antagonist protocol | | |
|--|----------------------------|-------------------|----------|----------------------------|-------------------|----------|
| | AMH Q4: >75th (>5.2 ng/ml) | | | AMH Q4: >75th (>5.2 ng/ml) | | |
| | HP-hMG (n = 76) | rFSH (n = 79) | p Value* | HP-hMG (n = 87) | rFSH (n = 101) | p Value* |
| Clinical characteristics | | | | | | |
| Age (years) | 30 (28, 32) | 30 (28, 32) | 0.743 | 30 (28, 33) | 30 (28, 31) | 0.039 |
| BMI (kg/m ²) | 22.5 (20.7, 23.8) | 20.8 (19.8, 22.8) | 0.002 | 22.1 (21.0, 23.9) | 21.6 (20.1, 23.0) | 0.022 |
| Cycle length (days) | 29 (28, 30) | 29 (28, 30) | 0.682 | 29 (28, 30) | 29 (28, 31) | 0.382 |
| First treatment cycle, n (%) | 52 (68%) | 52 (66%) | 0.731 | 57(66%) | 77 (76%) | 0.105 |
| Day 1 (before start of stimulation) | | | | | | |
| Ovarian volume (ml) | 10.3 (7.9, 13.9) | 10.5 (7.7, 14.8) | 0.807 | 13.4 (9.1, 17.0) | 13.0 (9.9, 16.7) | 0.885 |
| AFC (n) | 12 (8, 20) | 11 (8, 16) | 0.486 | 18 (15, 22) | 18 (15, 22) | 0.934 |
| AMH (ng/ml) | 7.0 (5.9, 8.5) | 7.0 (5.7, 8.4) | 0.912 | 7.1 (6.2, 8.7) | 6.8 (6.0, 8.3) | 0.347 |
| FSH (IU/l) | 3.2 (2.6, 4.4) | 3.6 (2.8, 4.4) | 0.257 | 6.7 (5.6, 7.7) | 6.4 (5.7, 7.5) | 0.251 |
| End-of-stimulation | | | | | | |
| Estradiol (nmol/l) | 8.7 (6.4, 13.0) | 8.4 (6.1, 12.8) | 0.736 | 9.7 (6.8, 14.8) | 7.8 (5.5, 12.4) | 0.012 |
| Progesterone (nmol/l) | 2.7 (1.9, 3.6) | 3.6 (2.8, 4.5) | <0.001 | 2.8 (2.1, 3.9) | 3.0 (2.1, 3.8) | 0.857 |
| Progesterone/estradiol ratio | 0.31 (0.21, 0.42) | 0.43 (0.30, 0.52) | <0.001 | 0.26 (0.20, 0.41) | 0.34 (0.24, 0.47) | 0.011 |
| Follicles ≥12 mm (n) | 15 (12, 18) | 16 (13, 19) | 0.274 | 14 (11, 18) | 16 (12, 19) | 0.064 |
| Endometrial thickness (mm) | 11 (10, 12) | 11 (10, 12) | 0.522 | 11 (10, 12) | 11 (10, 12) | 0.478 |
| Endometrial echogenicity pattern (hypo, iso, hyper) (%) | 44, 43, 13 | 24, 60, 17 | 0.033 | 31, 58, 11 | 41, 50, 9 | 0.386 |
| Cycle cancellation for ovarian hyper-response, n (%) | 3 (4%) | 4 (5%) | 1.000 | 0 (0%) | 1 (<1%) | – |
| Early OHSS (moderate/severe), n (%) | 3 (4%) | 4 (5%) | 1.000 | 3 (3%) | 5 (5%) | 0.727 |
| Intervention for ovarian hyper-response, n (%) | 5 (7%) | 6 (8%) | 1.000 | 7 (8%) | 12 (12%) | 0.470 |
| Oocyte retrieval | | | | | | |
| Women with oocyte retrieval, n (%) | 72 (95%) | 73 (92%) | 0.555 | 85 (98%) | 100 (99%) | 0.475 |
| Oocytes retrieved (n) | 12 (9, 16) | 15 (11, 20) | 0.007 | 12 (8, 15) | 14 (10, 19) | 0.033 |
| Women with ≥15 oocytes retrieved, n (%) | 25 (33%) | 40 (51%) | 0.025 | 27 (31%) | 49 (49%) | 0.015 |
| Fertilisation and embryo data | | | | | | |
| Fertilisation rate (%) | 50 (27, 73) | 56 (35, 69) | 0.826 | 57 (43, 69) | 60 (41, 73) | 0.663 |
| Embryos on day 3 (n) | 3 (2, 6) | 4 (2, 6) | 0.806 | | | |
| Women with top-quality embryo(s) on day 3, n (%)† | 38 (53%) | 35 (48%) | 0.561 | | | |
| Blastocysts on day 5 (n) | | | | 3 (1, 6) | 3 (1, 6) | 0.969 |
| Women with good-quality blastocyst(s) on day 5, n (%)‡ | | | | 55 (65%) | 51 (51%) | 0.060 |
| Women with transfer, n (%)¶ | 61 (85%) | 61 (84%) | 0.848 | 72 (85%) | 87 (87%) | 0.655 |

Values are median (IQR) unless otherwise indicated.

*Wilcoxon test (continuous data); Chi-Square test or Fisher's exact test (categorical data).

†Top-quality embryos were defined as 4–5 cells on day 2, ≥7 cells on day 3, equally-sized blastomeres and ≤20% fragmentation on day 3 and no multinucleation.

‡Good-quality blastocysts were defined as blastocysts with expansion and hatching score ≥4 and with inner cell mass and trophectoderm grades of A or B, using the definitions described by Gardner & Schoolcraft [23].

¶Among women with oocytes retrieved.

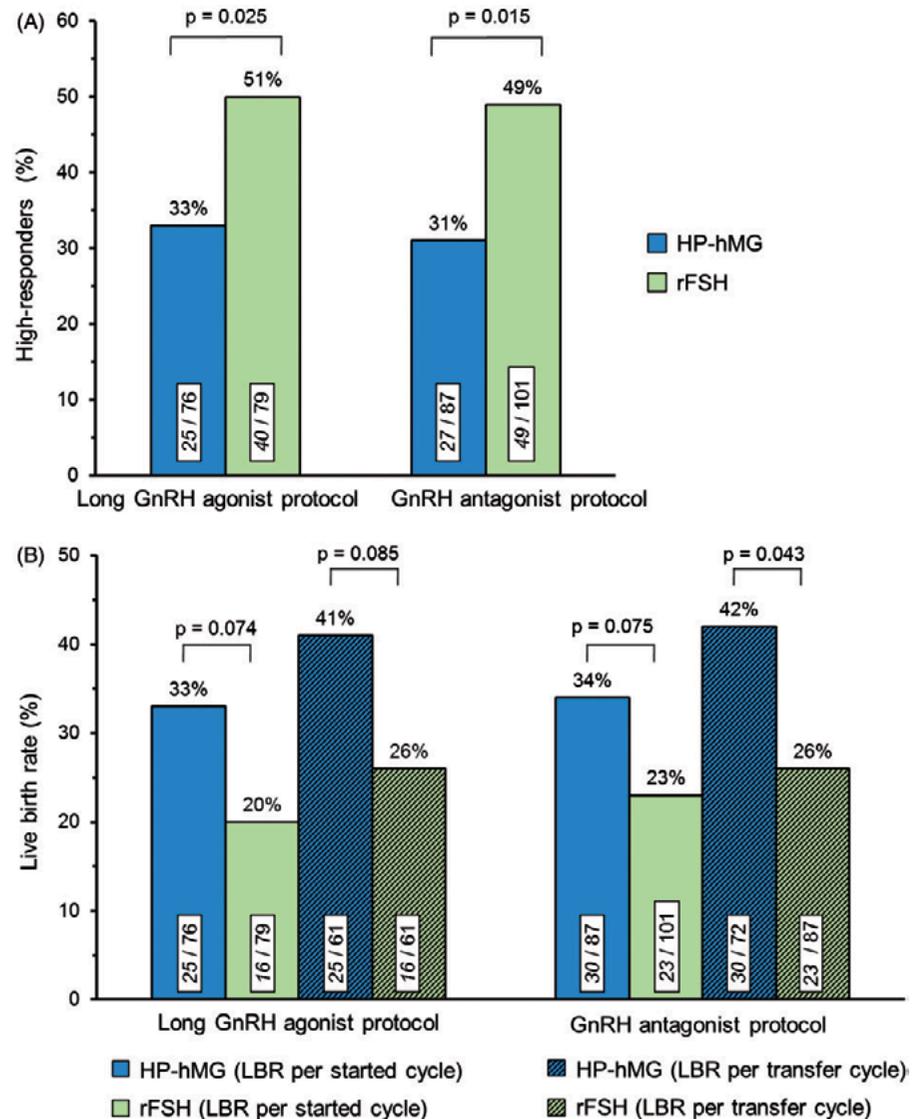
progesterone/estradiol ratio ($p=0.042$) at end of stimulation significantly decreased the probability of live birth (Supplementary Table 1). However, in all adjusted analyses the difference between the two gonadotropin preparations remained significant ($p<0.05$) indicating that the higher live birth rate in women with high AMH and stimulated with HP-hMG could not be attributed to differences in the baseline and end-of-stimulation variables examined.

Discussion

Several previous studies have shown that AMH can accurately identify women who are at risk of having an excessive ovarian response to COS [16–22]. In the present study, the prevalence of patients with a high ovarian response (i.e. ≥15 oocytes retrieved) was approximately three times higher in women with high AMH (>5.2 ng/ml) than in women in the non-high AMH category in

both the long GnRH agonist and GnRH antagonist protocol. Recent meta-analyses comparing outcome of GnRH agonist versus antagonist indicate that the incidence of severe OHSS is significantly lower in antagonist protocols [24,25]. The use of GnRH antagonist has therefore been advocated in predicted high-responders, such as patients with high basal AMH [11,26,27] and PCOS patients [4,5]. In the present study in women with high AMH, similar incidences of high response, early moderate/severe OHSS as well as need of intervention because of ovarian hyper-response were observed in the antagonist and long agonist protocols. Additional adjustments of the treatment regimen, beyond the type of protocol, may therefore be required in patients with high AMH to reduce high-response rate and maximize safe use of gonadotropins. Furthermore, the present study suggests that consideration should be made to the actual gonadotropin preparation to choose the optimal stimulation strategy for each patient, as HP-hMG was associated with a substantially lower

Figure 1. (A) Occurrence of high ovarian response (≥ 15 oocytes retrieved) and (B) live birth rates (LBR) among the women classified as potential high responders by a high initial AMH level. Values within bars are n /total. p Values are based on the Chi-Square test.



high-response rate than rFSH. Hence, the risk of developing a high response where an excessive response is predicted may be reduced by approximately one third with the elective use of HP-hMG, even in the GnRH antagonist protocol.

The rationale for a more moderate ovarian response with HP-hMG, and thereby a reduced risk of hyper-response with HP-hMG compared with rFSH, may be attributed to the FSH and/or LH components in the preparations. Like other glycoproteins, FSH displays a high degree of structural heterogeneity due to differences in the amount and/or composition of the carbohydrate structures, in particular sialic acid residues. Human-derived FSH shows more complex isoform heterogeneity than rFSH expressed by Chinese hamster ovary (CHO) cells. This is most likely because the CHO cells lack the enzymatic functions to construct the more complex oligosaccharide structures found in humans [28]. The composition of the carbohydrate moieties has a significant impact on the *in vivo* bioactivity of the various isoforms of FSH by affecting the metabolic clearance rate, the binding properties to the FSH receptors on the granulosa cells of the ovary and its ability to activate the receptors [29–33]. Hence, despite administration of similar amounts of bioactive FSH as measured by the Steelman-Pohley *in vivo* rat assay [34], the different FSH isoform profiles of HP-hMG and rFSH may influence the *in vivo* biopotency in humans and thereby the rate of high ovarian response among the potential high-responders.

Another hypothesis to be considered is that exposure to the LH activity in HP-hMG early in the stimulation induces an initial higher level of androgens compared with stimulation with rFSH [35]. A higher androgen level has been suggested to increase the sensitivity of the follicle to FSH via up-regulation of the FSH receptors at an early stage of the follicle development leading to decrease in granulosa cell proliferation and, therefore, affecting the androgen-estrogen tonus [35,36]. The shift in favour of androgens early in the stimulation with HP-hMG may induce a more selective follicle recruitment process, thereby influencing the number of follicles/oocytes that will develop during the COS [35].

Interestingly, when using either type of GnRH analogue in women with high AMH there were consistent trends of increased success rates with HP-hMG compared with rFSH. The logistic regression analyses in the overall population did not identify any specific variable that explained the different live birth rates between HP-hMG and rFSH, but indicated that progesterone levels and progesterone/estradiol ratios at the end of stimulation as well as availability of top-quality embryos/good-quality blastocysts influenced live birth rates. Several studies have reported that elevated progesterone levels in the late follicular phase decrease pregnancy/live birth rates [37–40], which is considered to be due to advancement of the endometrium [41,42], without affecting oocyte/embryo quality [43–45].

Also, an elevated progesterone/estradiol ratio on the day of hCG has been suggested to better reflect “premature luteinisation” [46] and to be associated with lower pregnancy rates in both agonist [47] and antagonist protocols [48]. It should be noted that the progesterone/estradiol ratio was significantly lower after HP-hMG treatment in both protocols in the present study due to the more estrogenic microenvironment induced by the HP-hMG preparation [35]. Finally, it has been suggested that the type of stimulation protocol and the magnitude of the ovarian response may have direct effects on oocyte quality and aneuploidy rate [49–52], and incidences of embryo chromosome abnormalities has been reported to be higher for women with high response to stimulation [52,53]. The present study reinforces that progesterone, progesterone/estradiol ratio and embryo quality plays a role in treatment outcome in patients at risk of hyper-response based on high serum AMH levels. The presence of LH-activity in the menotropin preparation may explain the potential treatment outcome differences between the HP-hMG and rFSH groups by influencing some of the endocrine [12,13,35] or embryo-quality parameters [35,54,55].

In conclusion, the present study suggests that women prospectively identified as potential high-responders by a high initial AMH have a lower rate of high ovarian response with HP-hMG than with rFSH during COS for IVF/ICSI treatment. The potential impact on clinical outcome should instigate additional investigations for confirming prospectively this finding and elucidate further the mechanisms implicated.

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