Clinical pregnancy in a woman with idiopathic hypogonadotropic hypogonadism and low AMH: utility of ovarian reserve markers in IHH

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Received: 5 May 2014 / Accepted: 28 July 2014 / Published online: 12 August 2014
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Abstract

Objective Serum anti-mullerian hormone (AMH) has been proposed as a useful marker of ovarian reserve that is cycle-independent and predictive of outcome in assisted reproduction cycles. However, there is evidence that AMH production is gonadotropin-dependent, and that under the influence of FSH, growing follicles contribute to circulating AMH levels. Therefore, AMH testing may not be universally reflective of the primordial follicle pool in certain conditions. We demonstrate that in patients with idiopathic hypogonadotropic hypogonadism (IHH) and deficient gonadotropin production, AMH and antral follicle count (AFC) may not be reliable markers of ovarian reserve.

Design Case report.

Setting Fertility clinic at a tertiary academic hospital.

Patient A 30-year-old nulligravid patient with IHH who presented for fertility treatment with low FSH (0.3 IU/L), LH (0.1 IU/L), estradiol (77 pmol/L) and AMH levels (0.65 pmol/L), and an unmeasurable AFC.

Intervention A three-month course of priming with oral micronized 17 β -estradiol, followed by daily injections of human menopausal gonadotropins (hMG).

Main outcome measure AMH level and follicular development.

Results After 60 days of stimulation with hMG, the patient's AMH level increased to a peak of 1.27 pmol/L. After 102 days of stimulation, her estradiol level rose to 480 pmol/L and a 19 mm dominant follicle was detected. The patient successfully conceived with intrauterine insemination.

Conclusion Ovarian reserve testing in patients with IHH can be challenging due to the contracted appearance of the ovaries and deficient FSH production. In these patients, AMH levels may underestimate ovarian reserve due to the lack of FSH-dependent growing follicles. When treated with a long course of hMG, these patients may exhibit increased AMH levels and demonstrate adequate follicular development.

Introduction

As age alone is a poor predictor of ovarian response, various markers of ovarian reserve have been proposed to more accurately reflect the primordial follicle pool, and predict outcome in assisted reproduction cycles. Markers of ovarian reserve used clinically include day 3 FSH, inhibin, the clomiphene challenge test, ovarian volume, antral follicle count (AFC), and serum anti-mullerian hormone (AMH) levels. Of these, AFC and AMH have been shown to be more closely correlated with ovarian primordial follicle count, even after adjustment for age [1].

AMH in particular has been lauded as having superior performance over dynamic ovarian reserve testing. AMH is a member of the TGF- β superfamily initially identified as the signal produced by the fetal testis that induces Mullerian duct regression during male sexual differentiation [2]. More recently, AMH has been shown to be expressed in granulosa cells of primary ovarian follicles once recruited from the primordial pool, with expression persisting until the early antral follicle stage. In humans, expression is highest in follicles less than 4 mm in diameter, and declines to negligible levels beyond 8 mm [3–5]. As a surrogate measure of the primordial follicle

Capsule We report a case of a woman with idiopathic hypogonadotropic hypogonadism and low AMH who responded to a long course of hMG stimulation with increased AMH levels and conceived successfully. This report suggests that AMH may not be an accurate marker of ovarian reserve in patients with endogenous gonadotropin deficiency.

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pool, AMH has demonstrated clinical utility in differentiating the causes of ovarian dysfunction [6], predicting outcome in ART [7, 8], and predicting ovarian hyperstimulation syndrome [9]. AMH may also have a role in predicting time to menopause [10], and as a possible diagnostic criterion for polycystic ovary syndrome [6]. However, as with any new technology, there have been challenges with the widespread application of AMH testing. Two notable limitations are the lack of standardization of the various AMH assays, and the paucity of knowledge about the physiologic role and regulation of AMH [6, 11].

Controversy exists regarding the regulation of AMH production. The traditional view is that AMH production is gonadotropin-independent. This was in part derived from studies that demonstrated minimal variation of AMH levels throughout the menstrual cycle. As AMH production has declined when follicles are selected for dominance, cyclic variation is thought to be minimal enough that measurement can be performed at any timepoint in the cycle [12]. However, more recent evidence indicates that AMH levels may fluctuate more than previously thought, with higher levels in the follicular phase compared to the luteal phase [13–16]. Further evidence for gonadotropin regulation of AMH includes observational data that prolonged suppression with GnRH analogues results in a decline in AMH levels [17]. An additional clinical scenario where prolonged gonadotropin deficiency has been associated with decreased AMH levels is pregnancy [18]. Finally, consistent with the theory that AMH is not fully gonadotropin-independent is the observation that some women with idiopathic hypogonadotropic hypogonadism (IHH) and endogenous gonadotropin deficiency can have low AMH levels. There has been a case report of a patient with IHH and low AMH levels where baseline AMH was not predictive of ovarian response. Following a long stimulation with human menopausal gonadotropins (hMG), this patient demonstrated increased AMH production, good follicular development, and conceived [19]. These observations call into question the universal ability of AMH in reflecting the primordial follicle pool and prognosticating ovarian response, especially in gonadotropin-deficient states.

We present a case of a 30-year-old nulligravid patient with IHH who was referred to Mount Sinai Hospital's Centre for Fertility and Reproductive Health for fertility treatment. In terms of her developmental history, she was born at term but small for gestational age, weighing 2.5 lbs. Her prenatal course had been uncomplicated. She achieved normal developmental milestones. In adolescence, she recalled having late thelarche compared to her peers. Family history was non-contributory, and her mother and sister both underwent normal menarche and had regular cycles. She presented to an endocrinologist at age 21 with primary amenorrhea and delayed secondary sexual characteristics. On examination, her weight was 160 lbs and no dysmorphism was noted. Breast examination was Tanner Stage III and evidence of adrenarche was noted with normal

hair distribution. Investigations revealed FSH and LH levels <1 IU/L, and an estradiol level of 93 pmol/L (25 pg/mL). MRI of the head was normal. Karyotyping revealed a normal 46XX chromosomal complement. She was diagnosed with IHH and placed on a low dose oral contraceptive pill (OCP), on which she had regular menses.

She had discontinued OCP use 6 months before her initial fertility consultation, hoping to conceive. Since then, she had not had any menses. Her diet and activity level were normal, and she had maintained a stable body weight. She had no significant past medical history and was not on any medications. Her partner was a 35-year-old healthy gentleman with a normal semen analysis. On transvaginal ultrasound, her ovaries could not be definitively identified, and there was a thin endometrium measuring 1 mm. To locate the ovaries, a pelvic MRI was done which revealed a small amount of ovarian tissue in both adnexal regions, measuring 1 cm in maximum dimension in the right adnexa. An infantile uterus measuring 2×1.6 cm was noted. Her FSH, LH and estradiol levels were 0.3 IU/L, 0.1 IU/L and 77 pmol/L (21 pg/mL) respectively. Her AMH level was 0.65 pmol/L.

Given the diminutive appearance of the ovaries and the low AMH level, the possibility of coexistent primary ovarian insufficiency was considered. A literature search was performed, which revealed a report of a similar patient who demonstrated increased AMH levels and follicular development after 3 months of priming with escalating doses of hMG [19]. Our patient was counseled and wished to proceed with a trial of stimulation.

Materials and methods

The patient was started on oral micronized 17β -estradiol (Estrace; Shire Pharmaceuticals, Saint-Laurent, QC) 2 mg daily for 3 months, with monthly medroxyprogesterone acetate-induced withdrawal bleeds to "prime" the uterus. Following this, hMG (Repronex; Ferring, Toronto, ON) was started at 75 IU sc daily. The dose of hMG would be titrated based on response. The first day of stimulation was designated as Cycle 1 day 1, and the course of her stimulation is summarized in Table 1.

The main outcome measures were AMH level and follicular development. AMH measurements were performed using the Generation II Assay (Beckman Coulter, Mississauga, ON) and reported in pmol/L. Interassay variability was 12.5 % and the detection limit was 0.6 pmol/L. Follicular development was assessed by transvaginal ultrasonography.

Results

In the patient's first cycle, her AMH level started at 0.99 pmol/L. After 60 days of stimulation with 75 IU of hMG

Table 1 Summary of patient's stimulation

Day of stimulation	hMG dose (IU)	AMH (pmol/L)	FSH (IU/L)	Estradiol (pmol/L)	Developing follicles (mm)
Cycle 1					
1	75	0.99	0.2	29	
14	75			52	
25	75			59	
32	75			31	
39	75			36	
46	75	1.12		29	
53	75	0.86		39	
60	75	1.27		38	
67	75	1.26		33	
74	75	<0.57		24	
81	75	<0.57	7.8	44	
88	75	0.63		47	
95	150 ^a			66	
102	150	<0.57		480	19
103 ^b				682	20
Cycle 2					
1	150		0.3	48	
7	150			69	
10	150			157	12
12	150			349	13
15	150			1020	15
16	150			1510	17
17 ^b				2030	20

^a Dose of hMG increased to 150 IU on Cycle 1 day 91

^b Day of ovulation trigger with 250 µg of recombinant human choriogonadotropin. IUI was performed 36 h after trigger

daily, her AMH peaked at 1.27 pmol/L. Her estradiol levels did not change appreciably and no dominant follicles were detected by the third month of stimulation. At that point, the patient was counselled and given the options of continuing stimulation with a higher dose of hMG or stopping treatment. She chose to continue and her dose of hMG was increased to 150 IU daily on day 91. On day 102 of stimulation, her estradiol level rose to 480 pmol/L (131 pg/mL) and a 19 mm dominant follicle was detected on ultrasound. She was triggered the next day with 250 µg of recombinant human choriogonadotropin alfa (Ovidrel; EMD Serono, Mississauga, ON), with an estradiol of 682 pmol/L (186 pg/mL) and a 20 mm follicle. Her endometrial lining measured 0.9 cm. An intrauterine insemination (IUI) was performed 36 h after trigger. She received progesterone (Prometrium; Merck, Kirkland, QC) 200 mg per vagina daily for luteal support, starting the day after IUI. Two weeks later, she had a negative βhCG test, progesterone was discontinued, and she had a withdrawal bleed.

Forty days after her withdrawal bleed, she was still amenorrheic. On ultrasound, her ovaries were quiet and her estradiol level was 48 pmol/L (13 pg/mL). This was assigned as Cycle 2 day 1, and based on her response in the first cycle, she

was started on hMG 150 IU daily. Her AMH was not measured during this treatment cycle. On day 10 of stimulation, a follicle measuring 12 mm emerged. She was triggered on day 17 with an estradiol of 2030 pmol/L (553 pg/mL), a 20 mm dominant follicle, and a 0.9 cm endometrial lining. An IUI was performed 36 h after trigger, and the post-trigger protocol was the same as the first cycle. Her first βhCG level measured 2 weeks after the IUI was 235 IU/L. Two days later, her βhCG had risen to 496 IU/L. An early pregnancy ultrasound at 7+3 wks gestation showed a single viable gestation with a fetal heart, and she was referred on for obstetrical care. Her pregnancy was on-going at the time of this report.

Discussion

This case highlights the challenges of assessing ovarian reserve and predicting response to stimulation in patients with IHH. The contracted appearance of gonadotropin-naïve ovaries can make follicle measurement difficult, precluding the use of AFC as a marker of ovarian reserve. Day 3 FSH also has limited utility, as endogenous levels in IHH are low by definition. AMH is a reliable ovarian reserve marker in

patients with normal endogenous gonadotropins, but has not been validated in patients with IHH due to the low prevalence of this condition (1/8000 to 1/50000) [20]. To our knowledge, there has been one case report showing suppressed AMH levels in a patient with IHH [19].

There may be a threshold of gonadotropin exposure necessary to induce normal AMH production by the granulosa cells. IHH is characterized by delayed puberty and amenorrhea, due to deficient hypothalamic GnRH secretion which results in a partial or complete lack of endogenous gonadotropins. Our patient had extremely suppressed FSH and LH levels (<1 IU/L), and low baseline AMH levels. In contrast, patients with functional hypogonadotropic hypogonadism (FHH) have not been shown to have suppressed AMH levels [21]. FHH is a reversible condition characterized by hypogonadotropic amenorrhea, often related to excessive weight loss, exercise, or stress [20]. Patients with FHH have disordered hypothalamic GnRH secretion, leading to reduced LH pulsatility and decreased but not absent FSH production. Patients with FHH may have sufficient circulating gonadotropin concentrations to recruit follicles from the non-AMH producing primordial pool to a later stage of development with higher AMH production.

In the previous reported case of IHH, the patient's AMH levels markedly increased after 3 months of priming with exogenous gonadotropins. She developed multiple follicles and conceived. This is consistent with the theory that sufficient gonadotropin exposure is required to recruit the growing follicles that make a significant contribution to circulating AMH levels. Our patient also exhibited an initial increase in AMH levels with hMG stimulation, although less marked than previously described [19]. Her AMH increased from a baseline of 0.99 pmol/L to a peak of 1.27 pmol/L after 60 days of stimulation, then began to decline again to <0.57 pmol/L. This decline in AMH during controlled ovarian stimulation has been demonstrated in patients with normal endogenous gonadotropins and may reflect a reduction in AMH-producing small antral follicles as stimulation progresses [22, 23], although in our case recruitment of a dominant follicle was not seen until approximately 30 days after the decline in AMH. However, the previous case report on IHH showed the opposite finding, with AMH levels increasing throughout the stimulation as multiple dominant follicles emerged. This discrepancy in AMH dynamics during stimulation may be related to the difference in follicular development in our patient compared to the previous case (mono- vs. multi-follicular). It is also possible that our patient had some element of premature ovarian insufficiency, which limited the recruitment of AMH-producing follicles. In any case, this discrepancy underscores the complexity of AMH dynamics in folliculogenesis. Our current understanding of the relative contribution of small gonadotropin-independent and larger gonadotropin-dependent follicles to circulating AMH concentrations is lacking and needs further study [3].

Based on this report and others, we conclude that AMH may not be an accurate marker of ovarian reserve in patients with deficient endogenous gonadotropin production. Our data supports the hypothesis that a proportion of circulating AMH comes from gonadotropin-dependent follicles. We have demonstrated that long-term priming with 3–4 months of exogenous gonadotropins in a patient with IHH and low AMH levels can result in increased AMH levels and normal follicular development. These findings could potentially be extrapolated to patients with other congenital gonadotropin-deficient conditions such as Kallman syndrome, but more studies need to be done on these and other patients. A better physiologic understanding of AMH regulation is also necessary. From a clinical point of view, our report reinforces the difficulty of ovarian reserve testing in patients with IHH. We caution clinicians against diagnosing such patients with poor ovarian reserve based on baseline AMH levels, without priming with gonadotropins. Baseline AMH may not reliably predict ovarian response, and these patients may be prone to inadvertent hyperstimulation. Therefore, we recommend a “low and slow” approach to gonadotropin stimulation. Our case also highlights the academic value of following AMH levels in these patients during gonadotropin stimulation, to better understand the dynamics of AMH production.

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