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Anti-Müllerian hormone dynamics during ovulation induction treatment with recombinant follicle-stimulating hormone in women with polycystic ovary syndrome

Hormon anty-Müllerowski (AMH) jako marker folikulogenezy w jajnikach kobiet z zespołem policystycznych jajników

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Abstract

Introduction: Anti-Müllerian hormone (AMH) has been suggested as a predictor of ovarian response to ovulation induction and controlled ovarian hyperstimulation.

Patients and methods: Twenty-six women, wishing to become pregnant and who showed resistance to clomiphene citrate, were included in the study. All women received recombinant follicle-stimulating hormone (recFSH).

Results: In the group of good responders, luteinising hormone (LH) and oestradiol levels were lower than in the group of non-responders. Free testosterone levels, free androgen index, and insulin resistance were higher in the group of non-responders. In the group of good responders, AMH levels decreased on successive days of ovarian stimulation and a greater slope of AMH levels was observed in patients with a higher number of increasing follicles.

PCOS patients have low FSH and high AMH levels. It could be suggested that the serum AMH decrease preceded growth of many follicles, which is a consequence of the FSH stimulation. In anovulatory PCOS women, gently increasing the serum FSH level reduces the AMH excess, thus relieving the inhibition from the latter on aromatase expression by selectable follicles and allowing the emergence of growing follicles. Patients with severe hyperandrogenism, insulin resistance and high level of LH do not respond to stimulation.

Conclusions: The decrease of AMH levels in PCOS women after one week of ovarian stimulation is a practical, valuable indicator which could predict the patients with a high risk of ovarian hyperstimulation. Anovulating PCOS patients with severe hyperandrogenism, insulin resistance and hyperinsulinaemia should not be qualified for recFSH ovarian stimulation. **(Endokrynol Pol 2013; 64 (3): 203–207)**

Key words: *anti-Müllerian hormone, ovulation induction, polycystic ovary syndrome*

Streszczenie

Wstęp: Hormon anty-Müllerowski (AMH) jest uznawany za marker odpowiedzi jajników na stymulację owulacji.

Materiał i metody: Do badania włączono 26 kobiet pragnących zajść w ciążę i wykazujących oporność na leczenie cytrynianem klomifenu. Wszystkie pacjentki były stymulowane rekombinowaną folitropiną.

Wyniki: W grupie dobrze odpowiadającej na stymulację stężenia lutropiny i estradiolu były niższe niż w grupie nieodpowiadającej. Stężenie wolnego testosteronu, indeks wolnych androgenów i insulinooporność były większe w grupie nieodpowiadającej na stymulację. W grupie odpowiadającej stężenie AMH obniżyło się w kolejnych dniach stymulacji i spadek ten był wyraźniejszy u pacjentek z większą liczbą wzrastających pęcherzyków.

Pacjentki z PCOS wykazują niskie stężenia FSH i wysokie AMH. Uważa się, że obniżenie stężenia AMH poprzedza wzrost pęcherzyków w trakcie stymulacji rekombinowanym FSH. U bezowulacyjnych pacjentek z PCOS łagodny wzrost w surowicy FSH hamuje AMH, odblokowując ekspresję aromatazy przez wybrane pęcherzyki, co pozwala na wyłonienie rosnących pęcherzyków. Kobiety z nasilonym hiperandrogenizmem, insulinoopornością i wysokim poziomem LH nie odpowiadają na stymulację.

Wnioski: Obniżenie stężenia AMH u pacjentek z PCOS po tygodniu stymulacji rekombinowanym FSH jest praktycznym, cennym markerem pozwalającym wyłonić pacjentki z wysokim ryzykiem zespołu hiperstymulacji. Bezowulacyjne pacjentki z PCOS z ciężkim hiperandrogenizmem, insulinoopornością i hiperinsulinemią nie powinny być kwalifikowane do stymulacji owulacji rekombinowanym FSH. **(Endokrynol Pol 2013; 64 (3): 203–207)**

Słowa kluczowe: *hormon anty-Mullerowski, stymulacja owulacji, zespół policystycznych jajników*

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Introduction

Follicle-stimulating hormone (FSH) is an appropriate first-line treatment for women with polycystic ovary syndrome and anovulatory infertility [1]. Anti-Müllerian hormone (AMH) has been suggested as a predictor of ovarian response to ovulation induction and controlled ovarian hyperstimulation. In human

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ovaries, AMH is produced by granulosa cells, with the highest expression being in small antral follicles, and continues to be expressed in the growing follicles until they have reached the size and differentiation state at which they are to be selected for dominance [2].

Many recently published studies have confirmed elevated concentrations of AMH in the blood of women with polycystic ovary syndrome (PCOS) [3–5]. As is well known, PCOS is characterised by an increase in follicle number of small antral follicles. AMH controls folliculogenesis by reducing follicle sensitivity to FSH, and leads to anovulation when secreted in excess amounts in polycystic ovary syndrome. It has been proved, however, that follicle number only added 5.3% to variance in the concentration of AMH, and raised production of hormone is an intrinsic property of granulosa cells in PCOS [4,6,7]. Although the cause of increased AMH production in PCOS remain unclear, this fact is important in explaining the mechanism of folliculogenesis disorders in this syndrome, and also for the effective stimulation of ovulation.

Serum AMH levels in young normo-ovulatory women correlate not only with age and the number of antral follicles, but also with FSH levels. Durlinger et al. proved in an *in vitro* study that AMH hormone inhibits antral follicle responsiveness to FSH [8]. Exogenous FSH administration is followed by a significant reduction in AMH levels. Several studies have demonstrated AMH secretion changes during controlled ovarian hyperstimulation (COH). The changes consist of a progressive reduction of serum hormone levels, which correlate with the dose of gonadotrophins used [5, 9, 10]. Therefore, the serum AMH levels during COH are good markers to predict ovarian response. Moreover, AMH may permit identification of the extremes of ovarian stimulation. A possible role for its measurement may be in individualisation of treatment strategies in PCOS anovulatory women.

The question then arises: what is the practical value of AMH determinations in patients with PCOS, treated with the recFSH stimulation? The answer to this question is the main objective of this study.

Material and methods

Twenty-six women attending the Gynaecological Endocrinology Department between January 2011 and March 2012 were included in the study. All patients gave signed informed consent. Procedures followed were in accordance with ethical standards and the study received approval from the Silesian Medical University Ethics Committee.

The diagnosis of PCOS was made on the basis of chronic oligomenorrhea, and either clinical hyperandrogenism (hirsutism — Ferriman-Gallwey score > 8) or hyperandrogenemia (high free testosterone or high free androgen index > 8%). All of the women studied showed typical features of polycystic ovaries detected by ultrasound, considered to be one of the criteria for diagnosis of this syndrome. Other entities that could cause excess androgen activity were excluded. Thus, patients included in the study met both the Rotterdam diagnostic criteria of PCOS, and also the Androgen Excess PCOS Society diagnostic criteria of this syndrome.

Women included in the study were aged 24–35 years, wishing to become pregnant, and showed resistance to clomiphene citrate. Previously performed examinations have established that these women are characterised by a chronic anovulation (ultrasound evaluation of ovarian follicular maturation and serum levels of oestradiol and progesterone determinations). All patients before treatment underwent measurement of plasma levels of oestradiol, total and free testosterone, sex hormone binding globulin (SHBG), luteinising hormone (LH), FSH, thyroid stimulating hormone (TSH), prolactin, 17-hydroxyprogesterone (17OHP), and AMH, as well as vaginal ultrasound.

All women were investigated during only one cycle and received recombinant FSH (recFSH) (Puregon; Organon Nl) using the low-dose protocol. Patients received 50 IU recFSH daily for the first seven days. If ovarian stimulation had been ineffective and follicles had not reached the diameter of 10 mm, the dose of recFSH was increased to 100IU for the next seven days. Treatment was started on day 3 after onset of either spontaneous or progesterone-(Luteine; Adamed Pl) induced menstruation. During treatment, patients were hospitalised. Treatment was monitored by daily transvaginal ultrasound examination. The ultrasound examination (Voluson 730 Expert) was performed with special regard to number and diameter of ovarian follicles.

The end-point of the study was achieved by one or more follicles being more than 17 mm in diameter. When the follicle reached a sufficient diameter before 14 days of treatment, it was interrupted. If the follicles did not grow within 14 days of treatment, the study was stopped. After evaluation, women were divided into two groups:

Patients who responded to stimulation with recFSH — the dominant follicle 17 mm or more (20 patients — we called this group the good responders)

Patients who did not respond to stimulation with recFSH (six patients — non-responders).

Blood samples for the determination of AMH were taken three times: before treatment, on the last day of stimulation, and afterwards.

The collected blood was centrifuged and sera were stored at –70°C until analysed (no longer than 30 days). Blood serum concentrations of AMH were determined using ELISA (DRG Instruments GmbH, Germany). The

Table I. *Difference in hormonal evaluation between group of good responders and group of non-responders before stimulation*
Tabela I. *Różnice stężeń hormonów między pacjentkami dobrze odpowiadającymi i nieodpowiadającymi na stymulację przed rozpoczęciem stymulacji*

Result	Medium level in group of good responders	Medium level in group of non-responders	Difference between groups of good responders and non-responders	p
AMH [ng/mL]	7.72	6.62	1.1	0.78974
FSH [mIU/mL]	5.56	6.67	–1.11	0.17559
LH [mIU/mL]	8.50	11.68	–3.18	0.01314
Oestradiol [pg/mL]	44.78	57.18	–12.4	0.03907
Prolactin [ng/mL]	22.04	22.88	–0.84	0.69994
Free testosterone [ng/mL]	2.91	5.02	–2.11	0.01939
Insulin [mIU/mL]	9.01	11.05	–2.04	0.01314
HOMA	2.13	2.64	–0.51	0.04586

Table II. *AMH levels in the group of good responders (group 1) and the group of non-responders*
Tabela II. *Stężenie AMH w grupie dobrze odpowiadającej i nieodpowiadającej na stymulację*

	AMH day 1	AMH day 7	Difference between day 1 and 7 (p)	AMH day 14	Difference between day 1 and 14 (p)	Difference between day 7 and 14 (p)
Group 1	7.725	6.025	0.00197	4.314	0.00056	0.00197
Group 2	6.617	6.833	$p > 0.5$	6.377	$p > 0.5$	$p > 0.5$

assays were performed according to the manufacturer's instruction.

Results

In the group of good responders, LH and oestradiol levels were lower than in the group of non-responders (LH: 8.505 ± 4.971 *v.* 11.683 ± 2.491, $p < 0.05$; oestradiol: 44.780 ± 14.168 *v.* 57.18 ± 6.4, $p < 0.05$). There was no difference in the levels of FSH, prolactin and AMH between the group of good responders and the group of non-responders (Table I).

Free testosterone levels and FAI were higher in patients who did not respond to stimulation of ovulation (free testosterone: 5.02 ± 1.6 *v.* 2.912 ± 1.7; FAI: 9.2 ± ± 5.4 *v.* 4.8 ± 3.9, $p < 0.05$) (Table I).

Insulin fasting levels and the Homeostasis Model Assessment (HOMA) were higher in the group of non-responders than patients who responded to recFSH (insulin: 11.05 ± 1.8 *v.* 9.01 ± 4.1, $p < 0.05$; HOMA: 2.64 ± ± 0.49 *v.* 2.1 ± 1.07, $p < 0.05$) (Table I).

In all patients who responded to recFSH, AMH levels decreased on successive days of ovarian stimulation ($p < 0.05$). (Fig. 1). In patients with a higher number of dominant follicles, the decrease of AMH followed earlier and the stimulation was interrupted after one week. In the non-responders, AMH results were similar on all days of evaluation.

Moreover, we observed a greater slope of AMH levels in patients with higher number of increasing follicles (diameter > 17 mm) in the group of good responders ($p < 0.05$) (Table II).The slope of AMH levels was greater in patients with higher levels of LH in the group of good responders (Table II).

Discussion

AMH is generally considered as a regulator of the early stages of follicular development [10]. AMH induces reduction in the response of the growing follicles to FSH. Thus, FSH-stimulated preantral follicular growth *in vitro* is suppressed in the presence of AMH ([10]. La Marca et al. [10] proved that in normal menstruating infertile women, recFSH treatment decreases AMH levels. Moreover, the authors found a correlation between decrease of AMH and increase of oestradiol, and a positive correlation between basic level of AMH and the level of oestradiol after recFSH treatment in normal women. In PCOS patients, the observations are different. PCOS patients have low FSH and high AMH levels. In these patients, there is a relationship between AMH levels and menstrual disorders and also between AMH levels and the antral follicle count (AFC) [11–13]. The treatment of anovulating PCOS patients is difficult especially in those who are resistant to clomiphene stimulation.

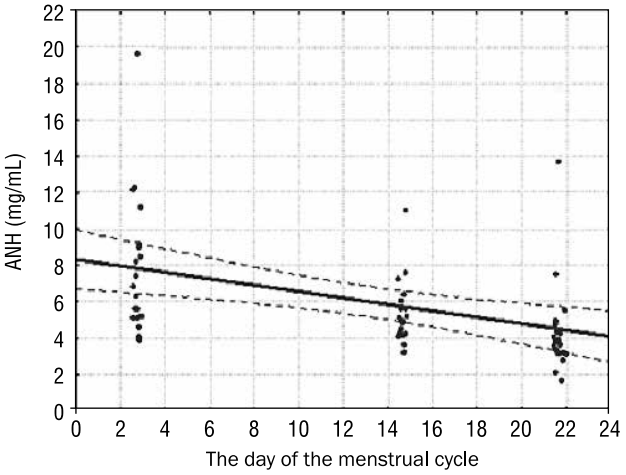

Figure 1. *The changes of AMH levels on successive days of ovarian stimulation in the group of good responders*

Rycina 1. *Zmiany stężenia AMH w kolejnych dniach stymulacji w grupie dobrze odpowiadającej na stymulację*

PCOS patients are in the group of high risk of hyperstimulation. It has been shown that *in vitro* FSH significantly reduced AMH expression in cultured GCs from PCOS patients [14]. From these results, it could be suggested that the serum AMH decrease preceded the follicle dominance, which is a consequence of the FSH stimulation [5]. La Marca et al. [10] examined AMH levels during ovarian stimulation in healthy anovulatory patients. During FSH administration, FSH and oestradiol levels increased and the levels were significantly higher than in the spontaneous cycle [10]. AMH levels decreased progressively from day 2 to day 6 in FSH-treated cycles but did not change in spontaneous cycles [10]. A significant slope of AMH levels was observed in our patients with PCOS who responded to recFSH stimulation. In patients with several growing follicles, the decrease of AMH followed earlier and was more significant. Catteau-Jonard et al. [5] obtained similar results in PCOS patients. They suggested that in anovulatory PCOS women, gently increasing the serum FSH level reduces the AMH excess, thus relieving the inhibition from the latter on aromatase expression by selectable follicles and allowing the emergence of a dominant follicle [5].

Our results confirmed that PCOS patients treated with recFSH could be divided into two groups. The patients with lower levels of LH, FAI and HOMA respond to recFSH stimulation. Patients with severe hyperandrogenism, insulin resistance and high level of LH on the third day of the cycle do not respond to stimulation. Moreover, the slope of AMH levels was higher in patients who develop a dominant follicle after stimulation.

There is a significant difference in the levels of LH, oestradiol, free testosterone, insulin and HOMA between the two groups of patients. However, there are no differences in the levels of AMH, FSH and prolactin on the second day of the cycles. We suggest that basic levels of the above parameters are not good markers to predict the success of ovarian stimulation. Similar results were obtained by Lie Fong et al. [15]. But these authors also suggested that there is a lack of change in AMH concentrations during low-dose ovarian stimulation contrary to controlled ovarian hyperstimulation. In our results, there was a decrease of AMH level in patients with many growing follicles after seven days of ovarian stimulation, contrary to stable levels of AMH in patients who did not respond to recFSH stimulation. Biasoni et al. [16] analysed AMH and ovarian sensitivity index (OSI), which was calculated dividing the total administered FSH dose by the number of retrieved oocytes. They found a significant negative correlation between AMH and OSI that is stronger than the one between AMH and the total number of retrieved oocytes. In our investigation, it was interesting that non-responders had significantly higher levels of LH, free testosterone, FAI and HOMA. This indicates that in patients with severe PCOS there is decreased response to recFSH. We suggest that these patients may require higher doses. We may conclude that severity of PCOS, which is connected with a greater number of small follicles and a high level of AMH, requires higher doses of recFSH.

Conclusions

A decrease in AMH levels in PCOS women after one week of ovarian stimulation is a practical, valuable indicator which could predict the patients with a high risk of ovarian hyperstimulation. Anovulating PCOS patients with severe hyperandrogenism, insulin resistance and hyperinsulinemia should not be qualified for recFSH ovarian stimulation.

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