



Research Article

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Anti-Müllerian Hormone (AMH) is A Good Predictor for Ongoing Pregnancy in Women Undergoing IVF/ICSI in Antagonist Cycles



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Abstract

Observation cohort study, assessed pregnancy rate based only on the AMH algorithm in 888 patients/ 2148 cycles in antagonist protocol for In vitro-fertilising/intracytoplasmatic sperm injection (IVF/ICSI). Patients are divided into 3 groups based on the value of AMH. The first group had AMH <12pmol/L group two between 12-32pmol/L and group three > 32pmol/L. Patients with low AMH <12pmol/L (n=291 patients/764 cycles) had maximal stimulation with corifollitropin-alfa (n=473/62%) or 300 IU/day of rFSH (n=289/38%) as well 300 IU/day of HP-HMG(n=2/0,3%). Patients with AMH 12-32 pmol/L (n=401 patients/941 cycles) had standard stimulation with 150 IU/day of rFSH (n=789/84%), or HP-HMG 150 IU/day (n=103/11%) as well corifollitropin-alfa (n=49/5%). Patients with AMH>32pmol/L(n=196 patients/ 443 cycles) had minimal stimulation with 112 IU/day of HP-hMG (n=244/55%) or rFSH 112 IU/day (n=199/45%). The ongoing pregnancy rates per started cycle in the three AMH groups first cycle were 15%, 25% and 38% respectively. The chance of achieving an ongoing pregnancy was reduced in women with low AMH whereas high AMH was associated with an increased chance of achieving an ongoing pregnancy, regardless of the patient's age. The proportion with pregnancy in the low AMH group was significantly ($p < 0.001$) lower than in the high AMH group. The conclusion is that among high AMH patients, has statistically significantly high ongoing pregnancy rate. However, a randomised study with enough power with our protocol should be performed.

Keywords: Anti-Müllerian hormone; Antral Follicle Count; IVF/ICSI; Pregnancy rate; Ovarian reserve

Abbreviations: AMH: Anti-Müllerian hormone; AFC: Antral Follicle Count

Introduction

Anti-Müllerian hormone (AMH) is a member of the TGF-beta family and is expressed by the small preantral and early antral follicles. The AMH level reflects the size of the primordial follicle pool and the best biochemical marker of ovarian function across an array of clinical situations [1]. In adult women, AMH levels gradually decline as the primordial follicle pool declines with age [2]. The AMH level appears to be an early, reliable, direct indicator of declining ovarian function. In patients planning IVF, an AMH level correlate with the number of oocytes retrieved after stimulation, and is the best biomarker for predicting poor and excessive ovarian response [3,4].

Unlike the day 3FSH, AMH can be measured anytime during the menstrual cycle and typically demonstrates minimal intracycle variability since the growth of small preantral follicles that express it is continuous, not cyclical. Reliable AMH kits [5] are readily available from multiple vendors and AMH measurements can be obtained from large clinical reference laboratories [6,7].

Anti-Müllerian hormone (AMH) is a very important parameter for adequate ovarian stimulation for women undergoing IVF/ICSI treatment [8-10]. A multicentre observational Japanese study has shown that AMH had a significantly higher predictive value for normal and high responders than FSH and E2 [11]. A prospective cohort study suggested that Anti-Müllerian hormone (AMH) is strongly associated with live birth rates after IVF/ICSI [12]. The number of oocytes in a linear increase depends on an AMH level [13].

We implemented the use of Anti-Müllerian hormone (AMH) as a biomarker for ovarian stimulation [14-16] since 2014. Our target was to achieve around 10 oocytes, with a range of 8-14. This was done by segmenting the patients into 3 groups given the maximum, standard or low dose of FSH stimulation according to their AMH levels.

Materials and Methods

Observation cohort study, where data were retrospectively collected, by 888 patients undergoing their 2148 cycle antagonist

protocol, at the Fertility Clinic Rigshospitalet, Copenhagen University Hospital from January 2014 to June 2017. All included patients were stimulated according to an AMH-based FSH dosing-algorithm.

Patients with low AMH <12pmol/L (n=291 patients/764 cycles) were stimulated with a weight-adjusted dose of Elonva® (n=473/62%) according to labelling: women >60kg received 150µg and women <60kg received 100µg, or 300 IU/day of rFSH (n=289/38%) as well 300 IU/ of HP-HMG (n=2/0,3%). Patients with AMH 12-32pmol/L (n=401 patients/941 cycles) had standard stimulation with 150IU/day of rFSH (follitropin-alfa or -beta) (n=789/84%), or HP-HMG 150 IU/day (n=103/11%) as well corifollitropin-alfa (n=49/5%). Patients with AMH >32pmol/L (n=196 patients/ 443 cycles) were stimulated with Menopur® (HP-hMG) 112 IU/day (n=244/55%) or rFSH (follitropin-alfa or -beta)112 IU/day (n=199/45%). The FSH dose was fixed the first seven days of stimulation. After seven days of stimulation, the FSH dose could be adjusted based on

sonographic assessment. The standard criteria for triggering of ovulation were at least 3 follicles ≥17mm.

Oocyte retrieval was done 36 hours after Ovitrelle (hCG)/Suprefact®GnRH triggering, and a transfer was done at day 2 or day 5. Luteal support was given using vaginal progesterone Lutinus® 100mg 3 times daily for 2 weeks. Lutinus® All data were prospectively collected and recorded in an SSPS version 22 and STATA 13.1. database. The study was approved by the Danish Patient Safety Authority (Number 3-3013-2242/1).

Result

Baseline characteristics of the 888 patients treated in antagonist protocol in 2148 cycle were stimulated according to an AMH-based FSH dosing algorithm Table 1. As seen, 291 (32.8%) were in the low AMH group, 401 (45.1%) in the intermediate and 196 (22.1%) in the high AMH group. Age, AMH and AFC were significantly different between AMH-groups (p<0.001), whereas weight and BMI are not significant.

Table 1: Baseline characteristics of 888 AMH based hormone stimulation in their first IVF/ICSI cycle.

Serum AMH (Pmol/L) Groups	<12	12-32	>32	All	P- Value
Patients, n(%)	291(32.8)	401(45.1)	196(22.1)	888(100)	
Cycles, n(%)	764(35.6)	941(43.8)	443(20.6)	2148(100)	
Age (Years) mean±SD	35.0±3.7	32.4±4.3	31.7±4.1	33.1±4.2	0.001
AMH (Anti-Müllerian horm.) mean±SD	6.5±2.9	20.2±5.5	57.7 ±27,2	24.1±23.2	0
AFC (Antral follicle count) mean±SD	8.4±4.7	19.4±6.8	40.3±13.6	20.4±14.2	0
Weight (kg) mean±SD	66.2±12.1	63.0±11.6	63.8±10.4	65.4±11.6	0.457
BMI (kg/m ²) mean±SD	23.5±3.8	23.4±3.7	22.6±3.2	23.1±3.7	0.474
Causes of infertility					
Anovulation, n(%)	9(3.1)	22(5.5)	65(33.2)	96(10.8)	
Tubal factor, n(%)	16(5.5)	27(6.7)	6(3.0)	49(5.5)	
Uterine factor, n(%)	8(2.7)	8(2.0)	1(0.5)	17(1.9)	
Endometriosis, n(%)	20(6.9)	21(5.2)	6(3.0)	47(5.3)	
Single women, n(%)	45(15.5)	47(11.7)	16(8.2)	108(12.2)	
Male factor, n(%)	105(36.1)	181(45.2)	75(38.3)	361(40.6)	
Unexplained, n(%)	88(30.2)	95(23.7)	27(13.8)	210(23.7)	

Data are described with means and standard deviation, Kruskal-Wallis test and median test.

P-value < 0.05 was considered statistically significant. Statistics, comment in text.

Table 2: Cycle characteristics and outcome in relation to the AMH-based algorithm first cycle IVF/ICSI, in antagonist protocol.

Serum-AMH, Groups	<12	12-32	>32	All	P-Value
Patients, n(%)	291(32.8)	401(45.1)	196(22.1)	888(10)	
Elonva(Corifollitropin-alfa)	203(69.8)	13(3.2)	0(0)	216(24.3)	
Gonal F or Puregon(rFSH)	86(29.5)	327(81.6)	26(13.3)	439(49.5)	
Menopur(hMG)	2(0.7)	61(15.2)	170(86.7)	233(26.2)	
Stimulation duration (days) mean±SD	8.5±1,7	8.5±1.4	9.5±2.1	8.7±1.7	0.004
Cycles converted to IUI, n (%)	3(1.0)	5(1.2)	2(1.0)	10(1.1)	0.953
Oocyte retrievals, patients, n, (%)	288 (99.0)	396(98.8)	194(99.0)	878(98.9)	0.953
Number of follicles aspirated, mean±SD	5.9±3.3	9.2±4.2	9.8±5.3	8.2±4.5	0
Number of retrieved oocytes, mean± SD	4.9±3.2	7.8±3.8	8.0 (4.5)	6.9±4.1	0
Embryo transfer, n(% of oocyte retrievals)	214 (73.5)	338(84.3)	157(80.1)	709(79.8)	0.002

Dual transfers, n(%)	32(11)	33(8.2)	10(5.1)	75(8.4)	0.704
Embryos cryopreserved, mean±SD (range)	0.3±0.4	0.4±0.5	0.4±0.5	0.3±0.5	
Positive hCG, n (% of started cycles)	70(24.1)	148(36.9)	88(44.9)	306(34.5)	0.001
Positive hCG, n (% of transfer)	70(32.7)	148(43.8)	88(56.1)	306(43.2)	0.001
Ongoing pregnancies, n(% of started cycles)	44(15.1)	100(24.9)	75(38.3)	219(24.7)	0.001
Ongoing pregnancies, n(% of transfer)	44(20.6)	100(29.6)	75(47.8)	219(30.9)	0.001
Twins, n(% ongoing pregnancies)	4(1.4)	0(0.0)	0(0.0)	4(0.5)	
Ovarian hyperstimulation syndrome (OHSS), n (%)	0(0)	2(0.5)	5(2.5)	7(0.8)	0.028

1. Data are described with means and standard deviation, Kruskal-Wallis test and Chi-square test.

2. Statistics, comment in text.

Table 2 shows first cycle response parameters and clinical outcome in the three AMH groups. The ongoing pregnancy rates per started cycle in the three AMH groups were 15%, 25% and 38%, respectively (p<0.001) and per transfer 21%, 30% and

48% respectively (p<0.001). The chance of achieving an ongoing pregnancy was reduced in women with low AMH, whereas high AMH was associated with an increased chance of achieving an ongoing pregnancy.

Table 3: Cycle characteristics and outcome in relation to the AMH-based algorithm first cycle IVF/ICSI, in antagonist protocol.

Serum-AMH, Groups	<12	12-32	>32	All	P-Value
Cycles, n (%)	764(35.6)	941(43.8)	443(20.6)	2148(100)	
Stimulation duration (days) mean±SD	8.5±1.7	8.6±1.5	9.2±1.9	8.7±1.7	0
Elonva	473(61.9)	49(5.2)	0(0)	522(24.3)	
Gonal F or Puregon	289(37.8)	789(83.9)	199(44.9)	1277(59.4)	
Menopur	2(0.3)	103(10.9)	244(55.1)	349(16.3)	
Cycles converted to IUI, n (%)	12 (1.6)	7(0.7)	7(1.6)	26(1.2)	0.217
Number of follicles aspirated, mean±SD	6.0±3.4	9.5±3.9	10.3±4.9	8.4±4.4	0
Number of retrieved oocytes, mean± SD	5.1±3.3	8.1±3.8	8.6 (4.4)	7.2±4.1	0
Oocyte retrievals, patients, n, (%)	746 (97.6)	933(99.1)	436(98.4)	2119(98.6)	0.042
Embryo transfer, n (% of oocyte retrievals)	558 (73.0)	796(84.6)	368(84.4)	1722(80.2)	0.001
Dual transfers, n (%)	156 (20.4)	179(19.1)	74(16.9)	409(19.0)	0.013
Embryos cryopreserved, mean ± SD (range)	0.1±0.1	0.3±0.5	0.4±0.5	0.3±0.5	0.003
Positive hCG, n (% of started cycles)	177(23.2)	327(34.8)	182(41.1)	686(31.9)	0.001
Positive hCG, n (% of transfer)	177(31.7)	327(41.1)	182(49.5)	686(39.8)	0.001
Ongoing pregnancies, n (% of started cycles)	110(14.4)	208(22.1)	140(31.6)	458(21.3)	0.001
Ongoing pregnancies, n (% of transfer)	110(19.7)	208(26.1)	140(38.1)	458(26.6)	0.008
Twins, n (% ongoing pregnancies)	9(1.2)	5(0.5)	9(2.0)	23(1.1)	0.041
Ovarian hyperstimulation syndrome (OHSS), n (%)	0(0)	3(0.3)	11(2.5)	14(0.7)	0.001

1. Data are described with means and standard deviation, Chi-square test, Kruskal-Wallis test one-way ANOVA

2. Statistics, comment in text.

Table 3 shows response parameters and clinical outcome for all cycles 2148. The ongoing pregnancy per started cycle in the AMH groups were 14%, 22% and 32% respectively (p<0.001) and per transfer 20%, 26% and 38 respectively (<0.001). In the first cycle, the pregnancy rate is high compared to all cycles. The

pregnancy rate is low after the first cycle, but if we are based on the AMH group, there is still significant pregnancy rate between AMH groups. The pregnancy rate is high in the group with high AMH and low in the group with low AMH.

Table 4: Ongoing pregnancies, number and percentages of started cycles, and of transfer in relation to cycle number and age.

Serum -Amh, Groups	<12Pmol/L	12-32 Pmol/L	>32 Pmol/L	All	P-Value
Cycle, n=1	291(32.8)	401(45.1)	196(22.1)	888(100)	
Age (Years) mean±SD	35.0±3.7	32.4±4.3	31.7±4.1	33.1±4.2	0.001
Ongoing pregnancies, n (% of started cycles)	44(15.1)	100(24.9)	75(38.3)	219(24.7)	0.001
Ongoing pregnancies, n (% of transfer)	44(20.6)	100(29.6)	75(47.8)	219(30.9)	0.001
Age (Years)<35 mean±SD	30.9±2.6	29.9±2.9	29.6±2.6	30.0±2.8	0.17
Age <35 Ongoing pregnancies, n (% of started cycles)	21(19.5)	73(27.4)	53(38.1)	147(28.7)	0.005

Age <35 Ongoing pregnancies, n (% of transfer)	21(25.0)	73(33.2)	53(48.6)	147(35.6)	0.001
Age (Years)>35 mean±SD	37.4±1.5	37.2±1.4	36.9±1.5	37.3±1.4	0.105
Age >35 Ongoing pregnancies, n (% of started cycles)	23(12.5)	27(20.0)	22(38.6)	72(19.1)	0.006
Age >35 Ongoing pregnancies, n (% of transfer)	23(17.7)	27(22.9)	22(45.8)	72(24.3)	0.004
Cycles, n ≥2	473(37.5)	540(42.9)	247(19.6)	1260(100)	
Age (Years) mean±SD	35.4±3.6	33.6±4.2	32.5±4.1	34.1±4.1	0.001
Ongoing pregnancies, n (% of started cycles)	66(14.0)	108(20.0)	65(26.3)	239(19.0)	0.002
Ongoing pregnancies, n (% of transfer)	66(19.2)	108(23.6)	65(30.8)	239(23.6)	0.007
Age (Years)<35 mean±SD	31.7±2.7	31.0±3.1	30.3±2.7	31.0±3.0	0.149
Age<35 Ongoing pregnancies, n (% of started cycles)	35(17.8)	71(21.3)	53(30.6)	159(22.6)	0.009
Age<35 Ongoing pregnancies, n (% of transfer)	35(23.0)	71(25.1)	53(37.6)	159(27.6)	0.008
Age (Years)>35 mean±SD	38.0±1.2	37.8±1.2	37.6±1.2	37.9±1.2	0.213
Age>35 Ongoing pregnancies, n (% of started cycles)	31(11.2)	37(18.0)	12(16.2)	80(14.4)	0.101
Age>35 Ongoing pregnancies, n (% of transfer)	31(16.1)	37(21.1)	12(17.1)	80(18.3)	0.448

1. P-value < 0.05 was considered statistically significant.

2. Statistics, comment in text.

Table 4 shows the pregnancy rate the relationship to the first cycle and the second or more cycle based on AMH and age. In the first cycle, age did not affect pregnancy relative to AMH groups. The pregnancy rate was significantly high in groups with high AMH, while in patients>35-years-old pregnancy rate was generally low, but still significantly high in groups with high AMH. In the second or more cycle of patients ≤35-years-old, age had no influence, and patients with high AMH had a high pregnancy rate. Patients >35-years-old in second or more cycle had no significant difference in pregnancy rate between AMH groups.

Discussion

The present study provides results from 2148 ART cycles where a stimulation based on the AMH level FSH dosing algorithm. The main findings of the present study were patients with AMH levels above 32pmol/L and thus a predicted high rate of pregnancy. Several studies based on the number of oocytes that predict high pregnancy rates [16-19]. We know that patients with high AMH can develop more follicles, and as a result, many oocytes come with aspiration. It has always been difficult to keep the balance between having a high pregnancy and minimizing OHSS. Patients with anovulation have high AMH and tend to have many follicles on stimulation, but they may also have a low response if stimulated with an inadequate dose of FSH [20-22]. In our study we could show that patients with high AMH, even some of them, do not have many oocytes nevertheless, had better pregnancy rate in the first cycle regardless of age. In patients >35-year-old, the rate of pregnancy decreases but is still present, for patients with high AMH has a high pregnancy rate compared to patients with low AMH.

Patients who had completed number two or more cycles pregnancy is still high in the group of patients with high AMH aged <35 years. In patients aged>35 years, with two or more cycles we could not find different inter-group pregnancy rates,

regardless of AMH level. AMH is a very good predictor of pregnancy prognosis. Patients with high AMH are at risk group for OHSS therefore we choose AMH algorithm to minimize OHSS and maintain good pregnancy rate. We have mildly stimulated patients with AMH> 32 where some of the patients have responded with very few follicles but the pregnancy rate was satisfactory and none of them had OHSS [23-25]. Several studies show that a number of oocytes 8-14 will provide better pregnancy rate [16-18]. We agree that patients with high AMH and young age can reach in most cases the number of oocytes we want. Our study confirms that AMH is a really good prediction for oocytes retrieval and pregnancy rate, especially in patients <35 years-old , whatever there is a first, second or more cycle.

Low AMH patients do not have a good pregnancy prognosis, the number of oocytes retrieval is not high and the pregnancy rate is low [26-30]. Our study shows that even though age was <35 years, the patients with low AMH had significant low pregnancy compared to patients with high AMH. AMH level reflects pregnancy chances in all groups, we can see differences in pregnancy rate in all AMH groups. Group of patients with AMH <12pmol/L had poor pregnancy and group of patients with AMH> 32pmol/L had the best pregnancy rate. Patients with AMH 12-32pmol/L normal responder stimulated with standard dose FSH, the pregnancy rate was better than the group of patients with low AMH, but low compared to the group of patients with high AMH> 32pmol/L.

Conclusion

The main finding suggest that an AMH is a good predictor for pregnancy prognosis in patients at the least <35 years old. Patients with high AMH have a significantly better pregnancy rate compared to patients with low AMH. The AMH algorithm can be used to select the starting dose of FSH to minimize OHSS and retains good pregnancy rate. However, a randomised study with enough power with our protocol should be performed

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