The LH/AMH ratio as a predictive value for the outcome of assisted reproductive techniques

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ABSTRACT

Purpose: This study was conducted to test the luteinizing hormone/anti-Müllerian hormone (LH/AMH) ratio as a possible new predictive factor for outcomes after controlled ovarian hyperstimulation (COH) in women undergoing in vitro fertilization (IVF) / intracytoplasmic sperm injection (ICSI) treatment.

Methods: This retrospective cohort study included 164 women submitted to their first IVF/ICSI treatment.

Results: Pregnancy and live birth rates were 21.5% and 18.6%, respectively. In a generalized linear model for the prediction of oocyte quantity after COH, age (odds ratio, OR, 0.98, 95% confidence interval, CI, 0.97;0.99), smoking (OR 0.83, 95%CI 0.68;0.99), AMH serum levels (OR 1.06, 95%CI 1.03;1.09) and a higher LH/AMH ratio (OR 0.97, 95%CI 0.96;0.98) were associated with oocyte quantity. For both ongoing pregnancy and live birth rates, only age was found to be predictive (OR 0.92, 95%CI 0.86;0.97, and 0.87, 95%CI 0.78;0.96; respectively).

Conclusion: The LH/AMH ratio is a new predictive parameter for oocyte quantity after COH. However, for the clinically more relevant outcome parameters of ongoing pregnancy and live birth, only patient age was significantly predictive.

KEYWORDS

Ovarian reserve test, anti-Müllerian hormone, luteinizing hormone, follicle-stimulating hormone, IVF, ICSI, outcome, pregnancy rate, live birth.

Introduction

Since there is a wide variation in the ovarian aging process, and even some young women respond poorly to controlled ovarian hyperstimulation (COH) [1], a test or a predictive model able to provide reliable information about the individual woman's chances of achieving pregnancy after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment would certainly be desirable. The identification of potential low and high responders to COH could be of high clinical value.

The term "ovarian reserve" is widely used to refer to the number of oocytes in the ovaries and their quality. Several ovarian reserve tests have been evaluated with the goal of reliably predicting outcome after IVF/ICSI treatment. However, as reviewed in detail, no single ovarian reserve test offering more than modest predictive properties has been identified to date [2]. Several tests have been known for some time and include measurement of early-follicular-phase blood values of follicle-stimulating hormone (FSH), estradiol, inhibin B, and anti-Müllerian hormone (AMH), the antral follicle count (AFC), measurement of ovarian volume and ovarian blood flow, the clomiphene citrate challenge test, the exogenous FSH ovarian reserve test, and the gonadotrophin agonist stimulation test [2]. In addition, early follicular levels of luteinizing hormone (LH) and their relationship to FSH levels have been evaluated in the past few years [3-5]. An elevated day 3 FSH/LH ratio has been demonstrated to be a sign of diminished ovarian reserve and poor response to hyperstimulation [3,4]. Unfortunately, in an analysis based on age, day 3 FSH/LH ratio was accurate only in younger women [5]. In women of reproductive age, AMH is secreted by small antral follicles [6,7]. It is structurally related to inhibin and activin, and, as a protein hormone, it belongs to the transforming growth factor ß- family [8]. The gene responsible for AMH is located on chromosome 19p13.3, and the genetic locus for the AMH receptor II is located on chromosome 12, which has a role in controlling the formation of primary follicles [9]. A large meta-analysis that included 5705 women undergoing IVF demonstrated that AFC and AMH levels, in addition to age, might predict a poor response. However, a combination of both parameters did not improve prediction, and other ovarian reserve tests did not add any information. Moreover, when testing predictive parameters for ongoing pregnancy, only age was of moderate value [10]. Thus, for the main outcome parameters of IVF, namely pregnancy and live birth rates, there are, as yet, no laboratory parameters that can be used for prediction.

Weghofer and Gleicher have suggested that the human reproduction system aims to maintain a "normal equilibrium" in

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the "presence of opposing forces". This refers, for example, to the activities of androgens and estrogens, as well as FSH and LH [11]. The rationale behind testing the predictive value of the interaction between LH and AMH was based on previous findings and assumptions. First of all, whereas LH alone does not seem to be predictive of IVF outcome, the FSH/LH ratio does [4,12]; second, we realize that LH and AMH are produced and secreted at totally different sites, and that LH has no effect on AMH expression by lutein granulosa cells in normo-ovulatory women. However, LH does lead to an up-regulation of AMH messenger RNA production in oligo-/anovulatory women with polycystic ovary syndrome. In addition, LH reduces AMH receptor II expression in normo-ovulatory women [13]. These data seem to indicate that there is a pathophysiologically relevant link between LH and AMH, at least in women with an ovulatory imbalance. On the basis of these considerations, we set out to evaluate the clinical value of the LH/AMH ratio for the prediction of oocyte quantity after COH, pregnancy, and live birth rates in our patient population. We also considered the predictive value of several "canonical" ovarian reserve tests.

Materials and Methods

Study population and study design

This retrospective study included 177 women who underwent their first IVF/ICSI treatment cycle at the Department of Gynecologic Endocrinology and Reproductive Medicine of the Medical University of Vienna, between January 2008 and January 2012. Inclusion criteria were the use of a standard antagonist protocol, age <42 years, regular menstrual cycles (every 25–35 days), total antral follicle count of 8–18, and basal day 3 FSH levels <12 mIU/mL in unstimulated cycles.

Data were retrieved by retrospective chart review. The main outcome parameters were oocyte quantity after COH and ongoing pregnancy rate, indicated by a positive heartbeat during week five after embryo transfer (ET), as well as live birth rate. The study was approved by the ethics committee of the Medical University of Vienna, Austria (IRB number 044/2010).

Laboratory determinations

All patients were tested for serum AMH, FSH, LH, estradiol, and thyroid-stimulating hormone (TSH) levels on the second day of the menstrual cycle before COH. All examined serum parameters were determined in the central laboratory of the General Hospital of Vienna, Vienna, Austria, using commercially available assays. Enhanced chemiluminescence immunoassay systems were used to determine serum levels of LH, FSH, and estradiol. An enzyme-linked immunosorbent assay was used to determine AMH levels. Details of these tests applied are provided online at http://www.kimcl.at.

IVF treatment

All women were treated using an antagonist protocol, as published previously [14]. On day 2 of the menstrual cycle, transvaginal sonography was performed and a blood sample was obtained for hormone analyses (AMH, FSH, LH, estradiol, TSH, prolactin), which were performed, using standard protocols, at the Central Laboratory of the General Hospital of Vienna, Department of Laboratory Diagnostics, Medical University of Vienna. The stimulation was started on day 3 with a basal dosage of 200 IU of recombinant FSH (Puregon; AESCA Pharma). Monitoring was performed by transvaginal sonography. When necessary, the FSH dosage was adjusted according to the follicle number and diameter. When adequate stimulation was achieved (≥3 follicles of R18 mm in diameter), a 10,000 IU dose of human chorionic gonadotropine (hCG) (Pregnyl; AESCA Pharma) was administered. Oocyte retrieval was performed 35 hours after hCG injection. Conventional IVF following standard techniques was used for fertilization. A maximum of two embryos were transferred through a Wallace catheter between days 3 and 5 after oocyte retrieval. All patients received 10 mg of dydrogesterone (Duphaston; Solvay Pharma) orally twice daily and 200 mg of progesterone (Utrogestan; Meda Pharma) vaginally three times daily for luteal support. Biochemical pregnancy was defined as a positive urinary hCG test on day 14 after transfer. A clinical pregnancy was defined as an intrauterine pregnancy with an embryo with positive heartbeat, verified by transvaginal sonography five weeks after ET.

Parameters analyzed

We included the following parameters in the multivariate models to predict the number of retrieved oocytes, as well as the rates of pregnancy and live birth: age; body mass index (BMI); female smoking; FSH; LH; estradiol; AMH; and the LH/AMH ratio. For the latter, AMH values <0.01 were analyzed as 0.01 to allow calculation of the LH/AMH ratio.

Statistical analysis

Nominal variables are reported as numbers and frequencies, and continuous variables as medians and interquartile ranges (IQR). Statistical analysis was performed using a logistic regression model to test the statistical significance of all coefficients for the prediction of pregnancy and live birth. A generalized linear model with a Poisson link function was used for the prediction of oocyte quantity. Odds ratios (OR) are given, including the 95 per cent confidence interval (95% CI). P-values <0.05 were considered statistically significant. Statistical analyses were performed with the SPSS software package, version 19 (SPSS, Chicago).

Results

Table 1 provides details of patient characteristics. The median number of retrieved oocytes was 5 (IQR 3–8). Pregnancy and live birth rates were 21.5% and 18.6%, respectively. In a generalized linear model for the prediction of oocyte quantity after COH, increasing age, smoking, a lower AMH value, and a higher LH/AMH ratio were associated with a lower oocyte quantity (Table 2). We then calculated two logistic regression models for the prediction of (i) ongoing pregnancy (Table 3), and (ii) live birth after IVF (Table 4). In both analyses, only age was significantly associated with IVF outcome (OR 0.92 and 0.87, respectively), with increasing age leading to lower pregnancy and live birth rates.

Table 1 Basic patient characteristics.

Number of patients	177
Age (years) [a]	34 (30-38)
Body mass index (kg/m^2) [a]	23.0 (20.4-26.3)
Tubal factor [b]	32 (18.1)
Male factor infertility [b]	123 (69.5)
Endometriosis [b]	30 (16.9)
Polycystic ovary syndrome [b]	19 (10.7)
Idiopathic infertility [b]	4 (2.3)
Number of retrieved oocytes [a]	5 (3-8)
Length of stimulation (days) [a]	11 (10-12)
ICSI [b]	71 (47.0)
Pregnancy rate [b]	38 (21.5)
Live birth rate [b]	33 (18.6)

Data are provided as *a* median (interquartile range) or *b* n (%)
Multiple citations possible

Table 2 Generalized linear model using a Poisson link function for prediction of oocyte quantity after controlled ovarian hyperstimulation.

	Odds ratio	95% confidence interval	Wald's chi square	LR test*
Smoking	0.83	(0.68;0.99)	3.812	0.050
Age	0.98	(0.97;0.99)	5.612	0.018
Body mass index	1.00	(0.98;1.01)	0.138	0.711
FSH	0.99	(0.95;1.02)	0.659	0.071
LH	1.02	(0.99;1.05)	2.258	0.133
Estradiol	1.00	(1.00;1.01)	1.295	0.255
AMH	1.06	(1.03;1.09)	19.100	<0.001
LH/AMH ratio	0.97	(0.96;0.98)	4.464	0.035

* LR test = likelihood ratio test

Table 3 Multivariate logistic regression model for the prediction of ongoing pregnancy after IVF/ICSI.

	Pregnancy (n=38)	No pregnancy (n=139)	Odds ratio	95% confidence interval	Wald's chi square	LR test [a]
Age (years) [b]	30 (26;34)	33 (29;38)	0.92	(0.86;0.97)	3.528	0.030
BMI (mg/m^2) [b]	23.2 (20.2;28.9)	23.5 (20.9;26.3)	1.01	(0.92;1.11)	0.023	0.879
Smoking [c]	9 (23.7)	31 (22.3)	0.88	(0.30;2.58)	0.050	0.823
Endometriosis [c]	8 (21.1)	22 (15.8)	1.18	(0.28;5.07)	0.051	0.821
FSH (U/l) [b]	6.5 (5.5;8.6)	6.7 (5.4;8.3)	1.01	(0.83;1.23)	0.015	0.903
LH (mIE/ml) [b]	5.2 (3.5;8.8)	5.2 (4.0;7.1)	1.10	(0.93;1.30)	1.206	0.272
E2 (pg/ml) [b]	37.0 (26.0;43.0)	37.0 (27.8;52.0)	0.99	(0.97;1.02)	0.617	0.432
AMH (mg/dl) [b]	1.9 (1.2;3.0)	1.9 (0.8;3.5)	0.93	(0.73;1.18)	0.359	0.549
LH/AMH ratio [b]	2.1 (1.1;4.9)	2.7 (1.6;5.3)	0.95	(0.83;1.10)	0.425	0.515

a LR test = likelihood ratio test; data are provided as *b* median (interquartile range) or *c* n (%)

Table 4 Multivariate logistic regression model for the prediction of live birth after IVF/ICSI.

	Live birth (n=33)	No live birth (n=144)	Odds ratio	95% confidence interval	Wald's chi square	LR test [a]
Age (years) [b]	29 (25;33)	33 (29;38)	0.87	(0.78;0.96)	7.124	0.008
BMI (mg/m^2) [b]	23.4 (21.5;29.2)	23.4 (20.6; 26.3)	1.04	(0.93;1.15)	0.442	0.506
Smoking [c]	7 (23.3)	33 (22.9)	0.61	(0.18;2.12)	0.603	0.437
Endometriosis [c]	7 (23.3)	23 (16.0)	2.07	(0.45;9.63)	0.863	0.353
FSH (U/l) [b]	6.1 (5.4;8.6)	6.7 (5.4;8.4)	0.97	(0.79;1.20)	0.080	0.777
LH (mIE/ml) [b]	6.1 (3.0;10.9)	5.2 (4.0;7.0)	1.15	(1.97;1.36)	2.729	0.099
E2 (pg/ml) [b]	37.0 (26.0;43.0)	37.0 (28.0;51.8)	0.98	(0.95;1.01)	1.314	0.252
AMH (mg/dl) [b]	1.9 (1.2;3.8)	1.9 (0.9;3.5)	0.95	(0.77;1.18)	0.218	0.641
LH/AMH ratio [b]	2.2 (1.0;5.9)	2.7 (1.6;5.1)	0.93	(0.93;1.05)	0.059	0.808

a LR test = likelihood ratio test; data are provided as *b* median (interquartile range) or *c* n (%)

Discussion

This retrospective study demonstrated that a lower LH/AMH ratio was associated with higher oocyte quantity during IVF/ICSI treatment, making this a new predictive parameter for oocyte quantity, in addition to classical predictive parameters, such as age, smoking, and AMH. To the best of our knowledge, this is the first study to evaluate the LH/AMH ratio from this perspective.

The measurement of AMH in reproductive medicine is the subject of some debate [15]. It is now evident that AMH concentrations correlate with the number of retrieved oocytes. However, many studies were unable to find an association between AMH serum levels and the outcome of IVF and ICSI, in terms of pregnancy rate and live birth rate [14-17]. Only a few trials suggested the opposite: Merhi and colleagues, for example, found that an AMH cut-off level of 0.2 ng/ml seemed to be a meaningful threshold for the prediction of the clinical pregnancy rate in women with severely diminished ovarian reserve [18]. Moreover, the combination of abnormally high FSH and AMH levels was found to reflect highly beneficial outcomes of IVF [19]. In women undergoing ICSI, AMH was also highly predictive for pregnancy and live birth [20].

However, the observation of many previous studies, namely that AMH was not reliable in the prediction of pregnancy and life birth rates, is in accordance with our results. Our study also confirmed the correlation between AMH serum levels and the number of retrieved oocytes (OR 1.06, $p< 0.001$). In our study, we were unable to find a correlation between AMH serum levels on day two of the cycle and pregnancy rate or live birth rate. Many factors can play a role here. One is the fact that AMH levels in serum do not predict the chromosomal abnormalities of oocytes or embryos that may occur with ovarian aging and diminishing ovarian reserve, as Tremellen also reported [21, 22]. These findings suggest that the most determinant factor for oocyte quality is not the size of the ovarian pool, which is measured by AMH, but the age of the oocyte. Moreover, the fact that AMH levels decline with age is independent of oocyte aging and is likely due to the reduction of the follicle pool [23, 24].

We combined LH and AMH serum levels by calculating the LH/AMH ratio. The connection between LH and oocyte maturation is not fully understood, but it is known that the LH peak in the middle of the cycle causes a resumption of meiosis, the rupture of the follicular wall, cumulus-oocyte expansion, and transformation of granulosa cells into luteal cells through a signaling pathway dependent on epidermal growth factor and amphiregulin. Moreover, LH promotes the growth and differentiation of ovarian granulosa cells, and thereby, the formation of the corpus luteum [23, 25-27]. Although, hypothetically speaking, higher LH levels at the start of the cycle may result in better maturation of ovarian granulosa cells, and thus, in better function of the corpus luteum after IVF treatment (which is already the case for AMH) [28, 29], this presumably better function of the corpus luteum did not lead to a higher rate of implantation after IVF (Table 3). Notably, LH was not an independent predictive parameter, which is in accordance with a previous study in women undergoing long-protocol COH that failed to demonstrate LH as a predictive parameter for ovarian response, conception, or pregnancy outcome [30].

However, the new parameter, LH/AMH ratio, adds additional information to the multivariate model for the prediction of oocyte quantity (OR 0.97, $p=0.035$). As demonstrated in Table 2, the predictive value of AMH is obviously higher than that of the LH/AMH ratio. However, the latter is still an independent predictive factor in the multivariate model. Notably, the lower the LH/AMH ratio was (in other words, the higher the AMH and the lower the LH in the ratio), the better. One might argue that although a certain baseline LH level is needed for high-quality maturation of ovarian granulosa cells, higher AMH levels, indicating a sufficient ovarian pool, are beneficial to the patient. Hypothetically, the LH/AMH ratio could be representative of egg quality, since oocyte number and egg quality have been suggested to be directly related to each other [29]. Of note, the LH/AMH ratio did not predict pregnancy and live birth rates, and, thus, we do not consider it to be associated with the chance of implantation.

Despite the fact that we found various factors influencing the number of retrieved oocytes, our data provide a further demonstration that there is no serum parameter able to predict clinical pregnancy and live birth rates after IVF treatment. Unfortunately, the LH/AMH ratio did not meet our expectations as only age was associated with these outcome parameters, which stands in accordance with another report [10].

This study must be interpreted within the context of its retrospective design. In conclusion, we provided the first demonstration of LH/AMH ratio as a new predictive parameter for oocyte quantity after COH, in addition to age, smoking, and AMH. However, for the clinically more relevant outcome parameter of ongoing pregnancy rate, only patient age was significantly predictive. Future studies are warranted to confirm our results and, should they be confirmed, elucidate the reason for the association between the LH/AMH ratio and oocyte number, which could hypothetically be linked to egg quality.

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