Eur J Med Res (2007) 12: 604-608

Correlations between Anti-Müllerian Hormone, Inhibin B, and Activin A in Follicular Fluid in IVF/ICSI Patients for Assessing the Maturation and Developmental Potential of Oocytes

S. Cupisti, R. Dittrich, A. Mueller, R. Strick, E. Stiegler, H. Binder, M. W. Beckmann, P. Strissel

University Clinics, Dept. of Obstetrics and Gynecology, Laboratory for Molecular Medicine and Laboratory for Endocrinology, Erlangen, Germany

Abstract

Objective: The objective of the present study was to evaluate the correlation between anti-müllerian hormone (AMH), inhibin B, and activin A in follicular fluid from patients receiving treatment with in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), to identify a parameter to assess the maturation and developmental potential of oocytes.

Materials and methods: AMH, inhibin B, and activin A were measured in follicular fluid from 27 patients undergoing IVF/ICSI treatment for male-factor infertility, tubal occlusion, endometriosis, or anovulation. The values were correlated with the serum estradiol level, the numbers and maturation of the oocytes, and the outcome of IVF/ICSI.

Results: A positive correlation was found between AMH in follicular fluid and the number of oocytes retrieved. High inhibin B levels in follicular fluid and high serum E_2 levels indicated a normal ovarian response to stimulation, corresponding to the oocyte numbers, while low inhibin B and 17-β-estradiol (E_2) levels indicated poor responders to stimulation. An activin A/inhibin B ratio of less than 1 and very high inhibin B levels correlated with large numbers of oocytes, while a ratio of 1–2 and high inhibin levels correlated with regular numbers of oocytes. An activin/inhibin ratio of more than 3 and low inhibin levels were found in poor responders. Pregnancies occurred predominantly in the group with a normal or high response. Patients with elevated ratios for 17-β-estradiol/AMH, oocyte numbers/AMH, and metaphase II oocyte numbers/AMH had the best chances of becoming pregnant, indicating an inverse correlation between AMH and the maturation and developmental potential of the oocytes.

Conclusions: In IVF/ICSI patients, a positive correlation was found between AMH, inhibin B, and the activin A/inhibin B ratio in follicular fluid, on the one hand; and between serum 17-β-estradiol levels and the numbers of oocytes retrieved, on the other. The activin A/inhibin B ratio correlated with the number of oocytes retrieved. The ratio for 17-β-estradiol, oocyte numbers, and metaphase II oocytes relative to AMH indicated the best developmental potential, and it can therefore be assumed that there is a negative correlation between AMH levels and the maturation and quality of oocytes.

Introduction

Serum anti-müllerian hormone (AMH), which induces suppression of the müllerian ducts in males, is thought to play a role in ovarian function and has been reported to be an important indicator of normal menstrual cycles [1]. The main source of AMH, inhibin B, activin A, and 17-β-estradiol is the granulosa-cell lining of the ovarian follicles. There have been several reports that serum inhibin B levels, as a further indicator of ovarian function, correlate with age and – in combination with 17-β-estradiol – with the number of oocytes and the pregnancy rate in patients receiving treatment with in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [2, 3, 4, 5, 6]. Both parameters have been investigated in serum at different time points throughout the cycle. However, no parameters are currently available for assessing the maturation and quality of oocytes to indicate their developmental potential.

The aim of the present study was to investigate AMH, inhibin B, and activin A in the follicular fluid of IVF/ICSI patients, along with serum 17-β-estradiol levels, and to correlate these parameters with oocyte numbers, numbers of metaphase II oocytes, and pregnancies by calculating the various corresponding ratios.

Materials and Methods

Twenty-seven patients undergoing IVF/ICSI treatment were included in the study. From each patient, measurements of AMH (pmol), inhibin B (pg/ml), and activin A (ng/ml) were made, and the activin A/inhibin B ratio was determined. An enzyme-linked immunosorbent assay (ELISA) was used (activin A: Oxford Bio-Innovation-LTD, inhibin B: DSL, AMH: Asbach medical Products). Each patient's hormonal status was determined by measuring prolactin (ng/ml), luteinizing hormone (LH, mU/ml), follicle-stimulating hormone (FSH, mU/ml), 17-β-estradiol (pg/ml), and progesterone (pg/ml). All of the endocrine and hormonal parameters were measured in pooled follicular fluid on the day of oocyte retrieval. 17-β-estradiol, progesterone, and LH were measured in serum on the same day. The numbers of oocytes retrieved and the numbers of mature metaphase II oocytes were recorded, and the ratios for 17-β-estradiol/AMH, oocyte numbers/AMH, and metaphase II oocyte numbers/AMH were determined. We used descriptive statistics only.

Results

Patients' Characteristics

The IVF/ICSI group consisted of 27 patients, aged 25–43. There were 22 cases of primary sterility and five cases of secondary sterility. The patients with secondary infertility included two following delivery and three following abortion. The cause of sterility was pure male subfertility in 13 patients; in two cases, testicular sperm extraction (TESE) procedures had been carried out. Nine patients had male-factor infertility combined with additional problems – five with anovulation and four with tubal occlusion. Only three patients had tubal occlusion alone, and only two patients had severe endometriosis (American Fertility Society class IV). Eighteen patients had normal baseline hormonal levels for LH, FSH, 17-β-estradiol, prolactin, testosterone, and dehydroepiandrosterone sulfate (DHEAS). Three patients had elevated DHEAS levels. Two patients had elevated FSH values, and two patients had elevated prolactin levels. One patient had an elevated testosterone value. Fourteen patients had at least one previous treatment cycle, while 13 patients were receiving their first treatment cycle. Twenty patients underwent ICSI treatment, two patients were treated after TESE procedures, and five patients received IVF alone.

Anti-Müllerian Hormone

In the IVF/ICSI patients, AMH levels on the day of oocyte retrieval ranged from 12.47 to 200 pmol, with a median of 33.65 pmol. The highest value (200 pmol) was found in a 30-year-old patient, from whom 16 oocytes were collected. Two other patients had AMH levels over 100 pmol, and 15 and 20 oocytes were collected in these cases, respectively. Two patients with low AMH levels under 20 pmol had one and three oocytes collected, respectively. There was no obvious correlation with the age of the patient or activin A levels (correlation coefficient $r = 0.39$).

Inhibin B

Inhibin B ranged from 3022 to 54,982 pg/ml and correlated positively with 17-β-estradiol, oocyte numbers, and the outcome of the IVF/ICSI treatment. Eight patients with inhibin >40,000 pg/ml had 17-β-estradiol values between 1004 and 3538 pg/ml. Four patients with high 17-β-estradiol values > 3000 pg/ml had high oocyte numbers, with more than 14 oocytes. Seven pregnancies occurred. Low inhibin B levels of less than 6000 pg/ml were found in five patients, four of whom had fewer than five oocytes collected and had estradiol values < 1000 pg/ml.

Activin A

Activin A values ranged from 14.92 to 24.51 pg/ml, with no correlation with any of the other parameters, and in particular no correlation with the numbers of oocytes collected ($r = 0.05$).

Activin A/Inhibin B Ratio

The activin A/inhibin B ratio correlated negatively with 17-β-estradiol and oocyte numbers. A ratio of less than 1 corresponded to high 17-β-estradiol levels and oocyte numbers. A ratio of 1–2 in four patients corresponded to a normal 17-β-estradiol level after ovarian stimulation for IVF (1429–2151 pg/ml) and normal oocyte numbers (< 10), while a ratio greater than 3 in four patients indicated a low-response situation, with low 17-β-estradiol levels (269–834 pg/ml) and few oocytes (< 5).

Estradiol

17-β-estradiol in serum ranged from 269 to 4442 pg/ml and showed a strong positive correlation with inhibin B levels and oocyte numbers. Four patients with low 17-β-estradiol values of less than 1000 pg/ml had inhibin B values of less than 6000 pg/ml, and less than five oocytes were retrieved.

Ratios for Estradiol/AMH, Oocyte Numbers/AMH, Metaphase II/AMH, and IVF Outcome (Table 2)

The calculated ratio of serum 17-β-estradiol to AMH ranged from 9.18 to 313.39. Four of five patients with

Table 1. Patients with an activin/inhibin ratio >3.

Patient no.	Age (pmol)	AMH (pg/ml)	Inhibin B (ng/ml)	Activin A	Ratio (pg/ml)	E_2	Oocytes	Outcome
8	42	33.65	3022	12.02	3.97	309	2	No pregnancy
20	36	14.1	3051	16.7	5.47	269	1	No pregnancy
26	41	42.42	5138	18.2	3.54	817	3	No pregnancy
27	35	24.53	5196	18.07	3.47	834	1	No pregnancy

AMH, anti-müllerian hormone; E_2, estradiol; ratio: activin/inhibin ratio.

Table 2. Ratios for estradiol/anti-müllerian hormone (AMH), oocyte number/AMH, and metaphase II/AMH relative to the outcome of in-vitro fertilization treatment.

Activin A/ inhibin B ratio	Estradiol/ AMH ratio	Oocyte/ AMH ratio	Metaphase II/ AMH ratio *	Oocytes	Metaphase II oocytes	Outcome
0.38	54.79	0.307	0.307	11	11	Pregnancy
1.17	65.39	0.2463	0.2463	6	6	0
0.39	45.08	0.1796	0.1796	4	4	Pregnancy
1.35	14.09	0.0229	0.0229	2	2	0
0.47	101.2	0.2406	0.0802	3	1	0
0.59	313.39	0.4502	0.4221	16	15	Pregnancy
0.43	37.43	0.198	0.198	11	11	0
3.97	9.18	0.0594	0	2	0	0
0.7	24.62	0.4056	0.2821	23	16	0
2.11	35.22	0.1184	0.1016	7	6	0
0.43	22.12	0.1244	0.0913	15	11	0
0.28	38.89	0.4444	0.4074	12	11	Twin pregnancy
0.6	111.14	0.5679	0.3932	13	9	Pregnancy
0.23	22.8	0.228	0.19	12	10	0
0.89	44.52	0.2005	0.1102	20	11	0
0.3	14.03	0.08	0.06	16	12	0
0.38	113.11	0.4476	0.2877	14	9	Pregnancy
0.39	55.97	0	0	0	0	0
0.53	68.66	0.3399	0.0755	9	2	0
5.47	19.08	0.0709	0	1	0	0
0.82	66.92	0.3541	0.1517	7	3	0
1.38	39.15	0.2192	0.1644	8	6	0
1.37	83.66	0.3509	0.3509	9	9	0
0.843	109.27	0.5453	0.4772	16	14	Pregnancy
0.483	86.92	0.509	0.3915	13	10	0
3.54	19.26	0.0707	0	3	–	0
3.477	34	0.0408	0	1	0	0

* $r = 0.728571$.

values > 100 became pregnant. The ratio of oocyte numbers to AMH ranged from 0 to 0.56. Six of seven patients with values > 0.44 became pregnant. The ratio of metaphase II to AMH ranged from 0 to 0.47. Five of seven patients with values > 0.30 became pregnant. Interestingly, all patients with values > 0.40 became pregnant. One patient with a ratio of 0.39 conceived but had an abortion.

The patients with normal to high responses to ovarian hyperstimulation were aged 25–39, had high inhibin B levels (< 10,000 pg/ml) and high 17-β-estradiol levels (> 1000 pg/ml) and had up to 23 oocytes retrieved. Seven pregnancies occurred in patients aged 25–39, with 4–16 oocytes collected. The activin/inhibin ratio was < 1 in all cases.

The four patients with a poor response to ovarian hyperstimulation were over the age of 35 (range 35–43). They had low inhibin B and estradiol levels, and fewer than five oocytes were retrieved. No pregnancies occurred. The activin A/inhibin B ratio was > 3 in all cases.

Discussion

Previous reports in the literature on AMH, serum inhibin B, and 17-β-estradiol following IVF [4, 5, 6] have shown that poor responders to IVF treatment had significantly lower luteal AMH levels, while high responders had significantly higher AMH and stimulated luteal inhibin B and 17-β-estradiol levels than normal responders. The authors found that a combination of factors, including follicular phase, AMH, and stimulated inhibin B levels, could be used to predict the oocyte numbers. Only AMH showed a significant difference between pregnant and nonpregnant women in both phases of the cycle. AMH did not change 24 h after FSH stimulation, either in the early follicular phase or in the luteal phase. The most significant correlations were found with follicular-phase inhibin B and AMH. AMH is thought to play a role in ovarian function and is essential for inducing suppression of the müllerian ducts in males [7]. AMH is an important indicator of ovarian function and is reported to be the single best predictor of ovarian reserve in controlled hyperstimulation in IVF patients [2]. In the present study, AMH alone correlated positively with oocyte numbers.

In healthy adult women, serum inhibin B levels are usually high during the follicular phase of the menstrual cycle and low during the luteal phase [8]. Inhibin B correlates with both LH and FSH, but more strongly with FSH. It increases during the follicular phase, indicating that the greatest production by follicles takes place in the early developmental stages [8]. Inhibin B is strongly associated with the numbers of oocytes after IVF and with the fertilization rate [2, 3].

The present study provides support for the finding of a positive correlation between follicular-fluid inhibin B and the response to stimulation, estradiol, and oocytes collected. A correlation between estradiol values and oocyte numbers and the pregnancy rate in IVF patients has been reported in several studies [4, 5, 6]. Even in the small numbers of patients included in the present study, a positive correlation was found between follicular-fluid inhibin B and serum estradiol levels and oocyte numbers.

Activin A is known to promote follicular development, inhibit androgen production, and increase FSH and insulin secretion, and to be negatively affected by polycystic ovaries and FSH and positively affected by weight and androstenedione levels. Activin A levels do not change with stimulation and do not correlate with oocyte quality or fertilization [4, 5, 6, 9]. This is in agreement with the findings for follicular-fluid activin A in the IVF/ICSI patients in the present study.

A study by Akande et al. [10] reported on inhibin A, inhibin B, and activin A in follicular fluid in natural cycles in women with tubal damage, unexplained infertility, and endometriosis. Interestingly, no correlation was found between the levels of inhibin A, inhibin B, activin A, and oocyte quality or fertilizing capacity in the three groups of women. The patients included in the present study differed from those in the study by Akande et al. [10], as male-factor infertility was the predominant cause of IVF/ICSI.

Another study reported on inhibin A, inhibin B, and activin A in individual follicles after IVF [11]. With increasing follicle size, the concentration of inhibin A in the fluid in single follicles increased, while that of inhibin B decreased. The three parameters did not significantly differ in follicles with different oocyte qualities. Activin A appeared to be a marker for follicular maturation. In the present study, no correlation was found between activin A and any other parameter investigated.

Even in the small patient population studied here, it was found that the activin A/inhibin B ratio indicated an ovarian response to hormonal stimulation in IVF/ICSI patients. A ratio < 1 indicated high responders, 1–2 indicated normal responders, and > 3 indicated low responders to hormonal stimulation. It will be important to confirm this finding by further research in larger numbers of patients.

However, oocyte numbers alone do not necessarily imply the presence of mature oocytes with developmental potential. Large numbers of small follicles could also produce high values for serum estradiol and follicular-fluid inhibin B – comparable to the values produced by smaller numbers of large follicles. Measurements of estradiol and inhibin B alone are therefore not capable of indicating the maturation of follicles and oocytes and therefore their developmental potential. Fanchin et al. [12] have shown that serum AMH shows values comparable to those of follicular-fluid AMH and that AMH decreases in proportion to the maturation process of follicles.

The aim of the present study was therefore to evaluate AMH, inhibin B, and activin A in follicular fluid from IVF/ICSI patients in order to assess not only the response to stimulation treatment but also the maturation of oocytes, in relation to the numbers of metaphase II oocytes found and pregnancies occurring. It was found that oocyte quality and pregnancy can best be predicted by using the ratio of the metaphase II oocyte numbers to AMH – indicating that with increasing follicular maturation, AMH production decreases, thus these oocytes have the best developmental potential for pregnancy. Further clinical studies will be important to validate the present findings.

Conclusions

Most studies investigating the significance of endocrine parameters in predicting ovarian reserve in response to stimulation for IVF have focused on combinations of various parameters such as serum AMH, inhibin B, and 17-β-estradiol. These parameters are usually used to assess oocyte numbers, but criteria for assessing oocyte maturation have not previously been available. This study confirms previous findings for parameters in the follicular fluid of patients undergoing IVF/ICSI. It was found that the activin A/inhibin B ratio has some significance for indicating ovarian function, but also that the metaphase II/AMH ratio appears to be predictive for the quality of the oocytes.

References

1. Gruijters MJ, Visser JA, Durlinger AL, Themmen AP. Anti-Müllerian hormone and its role in ovarian function. Mol Cell Endocrinol. 2003 Dec 15; 211(1–2): 85–90.
2. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranierl DM, Serhal P. Antral follicle count, anti-müllerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? BJOG. 2005 Oct; 112(10): 1384–90.
3. Muttukrishna S, Suharjono H, McGarrigle H, Sathanandan M. Inhibin B and anti-Müllerian hormone: markers of ovarian response in IVF/ICSI patients? BJOG. 2004 Nov; 111(11): 1248–53.
4. Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, Gal M, Zylber-Haran E, Margalioth EJ. Dynamic assays of inhibin B, anti-Müllerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod. 2005 Nov; 20(11): 3178–83.
5. Eldar-Geva T, Margalioth EJ, Gal M, Ben-Chetrit A, Algur N, Zylber-Haran E, Brooks B, Huerta M, Spitz IM. Serum anti-Müllerian hormone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogenism. Hum Reprod. 2005 Jul; 20(7): 1814–9.

6. Eldar-Geva T, Spitz IM, Groome NP, Margalioth EJ, Homburg R. Follistatin and activin A serum concentrations in obese and non-obese patients with polycystic ovary syndrome. Hum Reprod. 2001 Dec; 16(12): 2552–6.
7. Oppelt P, Strissel PL, Kellermann A, Seeber S, Humeny A, Beckmann MW , Strick R. DNA sequence variations of the entire anti-Müllerian hormone (AMH) gene promotor and AMH protein expression in patients with the Mayer–Rokitansky–Küster–Hauser syndrome. Hum Reprod. 2005 Jan; 20(1): 149–57.
8. Sehested A, Juul AA, Andersson AM, Petersen JH, Jensen TK, Müller J, Skakkebaek NE. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. J Clin Endocrinol Metab. 2000 Apr; 85(4): 1634–40.
9. Casper FW, Seufert RJ, Schaffrath M, Pollow K. Concentrations of inhibins and activin in women undergoing stimulation with recombinant follicle-stimulating hormone for in vitro fertilization treatment. Fertil Steril. 2001 Jan; 75(1): 32–7.
10. Akande AV, Asselin J, Keay SD, Cahill DJ, Muttukrishna S, Groome NP, Wardle PG. Inhibin A, inhibin B and activin A in follicular fluid of infertile women with tubal damage, unexplained infertility and endometriosis. Am J Reprod Immunol. 2000 Feb; 43(2): 61–9.
11. Lau CP, Ledger WL, Groome NP, Barlow DH, Muttukrishna S. Dimeric inhibins and activin A in human follicular fluid and oocyte-cumulus culture medium. Hum Reprod. 1999 Oct; 14(10): 2525–30.
12. Fanchin R, Louafi N, Lozano DHM, Frydman N, Frydman R, Taieb J. Per-follicle measurements indicate that anti-müllerian hormone secretion is modulated by the extent of follicular development and luteinisation and may reflect qualitatively the ovarian follicular status. Fertil Steril. 2005 Jul; 84(1): 167–73.
13. Al-Qahtani A, Muttukrishna S, Appasamy M, Johns J, Cranfield M, Visser JA, Themmen AP, Groome NP. Development of a sensitive enzyme immunoassay for anti-Müllerian hormone and the evaluation of potential clinical applications in males and females. Clin Endocrinol (Oxf). 2005 Sep; 63(3): 267–73.
14. Ethier JF, Findlay JK. Roles of activin and its signal transduction mechanisms in reproductive tissues. Reproduction. 2001 May;121(5): 667–75.
15. Gurbuz B, Yalti S, Ficicioglu C, Tasdemir S. The relation of serum and follicular fluid leptin and ovarian steroid levels in response to induction of ovulation in in vitro fertilization cycles. Eur J Obstet Gynecol Reprod Biol. 2005 Feb 1; 118(2): 214–8.
16. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. Serum antimüllerian hormone/müllerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril. 2004 Nov; 82(5): 1323–9.
17. Karande VC. Managing and predicting low response to standard in vitro fertilization therapy: a review of the options. Treat Endocrinol. 2003; 2(4): 257–72.
18. Penarrubia J, Fabregues F, Manau D, Creus M, Casals G, Casamitjana R, Carmona F, Vanrell J A, Balasch J. Basal and stimulation day 5 anti-Müllerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment. Hum Reprod. 2005 Apr; 20(4): 915–22.
19. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-müllerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol. 2005 Feb; 45(1): 20–4.
20. van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod. 2002 Dec; 17(12): 3065–71.

Received: October 4, 2006 / Accepted: August 20, 2007

Address for correspondence:
Dr. Ralf Dittrich
Dept. of Obstetrics and Gynecology
Erlangen University Hospital
Universitätsstrasse 21–23
91054 Erlangen
Germany
Tel.: + 49 9131 8533553
Fax: + 49 9131 8533552
Email: Ralf.Dittrich@gyn.imed.uni-erlangen.de