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REPRODUCTIVE MEDICINE

Different serum anti-Müllerian hormone concentrations are associated with oocyte quality, embryo development parameters and IVF-ICSI outcomes

Tulay Irez · Pelin Ocal · Onur Guralp · Meral Cetin · Begum Aydogan · Sezai Sahmay

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Abstract
Objective To evaluate the association between different basal serum levels of anti-Müllerian hormone (AMH) and oocyte-embryo quality and IVF outcomes.
Materials and methods Two hundred and nine infertile women who underwent in vitro fertilization treatment with intracytoplasmic sperm injection (ICSI) between January 2009 and February 2011 were included in the study. Mean age, BMI, FSH, E_2, inhibin B, duration of infertility, total gonadotropin dose, antral follicle count, morphology of all oocytes, percentage of MII, early cleavage rate, the number of good quality embryos in transfer and ongoing pregnancy (>12 weeks) rates were evaluated.
Results Six groups were formed according to the percentiles as <10% (≤0.89 ng/ml; $n = 21$), 10–25% (0.89–1.40 ng/ml; $n = 31$), 25–50% (1.40–2.89 ng/ml; $n = 53$), 50–75% (2.89–4.83 ng/ml; $n = 28$), 75–90% (4.83–8.06 ng/ml; $n = 55$), >90% (>8.06 ng/ml; $n = 21$). Central granulation, cytoplasmic granulation, oocyte postmaturity, percentage of embryos, early cleavage and percentage of transferred good quality embryos were significantly different in five groups (ANOVA test). Ongoing pregnancy rate (PR) was the lowest in <10% (9.5%), and the highest in 50–75% group (39.3%). ($P = 0.040$)
Conclusion Different AMH levels may predict the quality of oocytes, presence of postmaturity and nucleoli Z score, early cleavage and ICSI outcomes.

T. Irez · P. Ocal (✉) · O. Guralp · B. Aydogan · S. Sahmay
Department of Obstetrics and Gynecology, Cerrahpasa School of Medicine, Istanbul University, IVF Unit, Istanbul, Turkey
e-mail: drpelinocal@hotmail.com

M. Cetin
Department of Obstetrics and Gynecology,
School of Medicine, Cumhuriyet University, Sivas, Turkey

Keywords AMH · Oocyte quality · Embryo quality · ICSI outcomes

Introduction

Traditionally, embryo selection is performed by using embryo morphology as the guideline. Other additional selection methods include oocyte and zygote morphology, blastomere symmetry and blastocyst culture [1]. Recently, observation of embryonic first mitosis has been emphasized [2]. Several studies have shown that embryonic early cleavage (EC), which occurs at 25–27 h post insemination for *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI), can be an additional indicator of viable embryos [3]. In general, "good" quality cleaving embryos display stage-specific cell division, have blastomeres of fairly equal size with few to no cytoplasmic fragments [1, 3]. Recent studies continue to add new information to what has been reported regarding the appearance of a good quality embryo and the processes it undergoes before implantation [4, 5].

In assisted reproductive technology (ART), serum levels of several key hormones are used to evaluate the ovarian reserve and to monitor the growth of gonadotropin-stimulated follicle. Traditional methods used to predict the response to ovarian stimulation have mainly included the measurement of basal serum concentrations of hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and inhibin, on the third day of the cycle or ultrasonographic indices such as pretreatment ovarian volume and number of early antral follicles. [6] Recently, anti-Müllerian hormone (AMH) also referred to as Müllerian-inhibiting substance (MIS), has been proposed as a novel marker for predicting ovarian response to

gonadotropin stimulation. [7] Serum levels of AMH has shown poor response in <1 ng/ml, normal response in 1–4 ng/ml and high response in >4 ng/ml [6–9].

In the present study, we prospectively assessed the significance of basal AMH levels as a marker of oocyte/embryo quality in intracytoplasmic sperm injection (ICSI) cycles. We evaluated whether or not different serum concentrations of AMH in early follicular phase are associated with ovarian response, oocyte and embryo quality, embryo development parameters and pregnancy outcome in patients which undergo ovulation induction in ICSI cycle.

Materials and methods

From January 2009 to February 2011, 209 women undergoing IVF with ICSI were included in this study in our IVF Center. An informed consent from all women, and approval of Human Ethics Committee of Istanbul University were obtained.

Our inclusion criteria were (1) age under 38 years, (2) presence of both ovaries, (3) patients without TESE or frozen TESE.

All patients received GnRH agonist, leuprolide acetate 1 mg/day s.c. (Lucrin®, Cedex, France) beginning on the 21th day of previous cycle. Leuprolide acetate was reduced to 50 μg/day and gonadotropin 150–225 IU (Gonal F®, Serono, Swiss or Puregon®, Schering Plough, Istanbul) was started i.m. daily. Transvaginal ultrasound scan was performed on days 7 and 9 of cycle during ovarian stimulation and every 1 or 2 days thereafter, as required. Whenever follicle size larger than 12 mm was seen, serum estradiol was measured. The dose of the gonadotropin was adjusted according to the follicular growth. When more than two follicles of ≥17 mm were seen, HCG (Pregnyl®, Schering Plough, Istanbul) 10,000 IU or (Ovitrelle® 250 mcg Serono, Swiss) were injected to induce final oocyte maturation and 36 h later, oocyte retrieval was carried out transvaginally under ultrasound guidance. After 2 h of incubation, oocytes were removed from their cumulus complex using enzyme hyaluronidase (80 IU/ml; Medicult®, Copenhagen, Denmark) and hand-drawn glass pipettes. Following this denudation, ICSI was performed as published previously [10]. Maturation and morphological features of the oocytes were investigated before ICSI. Analyzed anomalies included dark granulation of the cytoplasm, refractory bodies and postmaturity. Fertilization was characterized by the presence of two pronuclei 18–20 h after injection. Normal fertilization was confirmed by the presence of two pronuclei and two polar bodies 16–20 h (day 1) after insemination.

The zygotes were scored according to the Z-scoring system [11]. The system took account of nuclear size and alignment, and nucleoli (nucleolar precursor bodies, NPB) number and distribution. Briefly, Z-1 zygotes had equal numbers of NPB aligned at the pronuclear junction. The absolute number was not counted but it was between three and six. Z-2 zygotes had equal number and sizes of nucleoli (between three and six) which were equally scattered in the two nuclei. Z-3 zygotes had equal numbers of NBP of equal sizes in the same nuclei but with one nucleus having alignment at the pronuclear junction and the other with scattered nucleoli. Zygotes with unequal numbers (a difference of more than two nucleus) and/or sizes of nucleoli were also considered as Z-3. Z-4 zygotes were those with pronuclei that were separated, of very different sizes or peripheral location (Fig. 1). On the same day, examination for EC was performed, 25–27 h after insemination. Embryos displaying two cells at inspection were designated as 'EC'. The embryos that had not yet cleaved to the two-cell stage were designated as 'late cleavage'. Embryos were further examined for their quality at

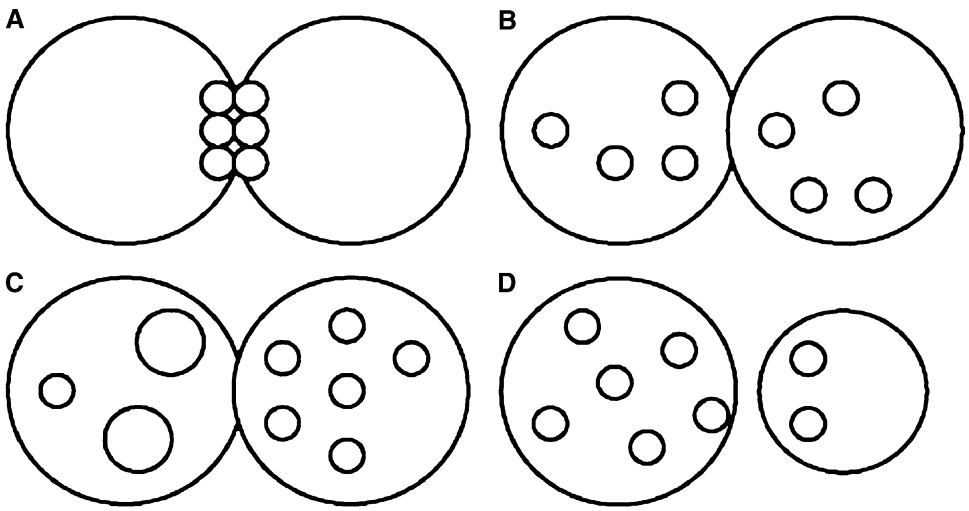

Fig. 1 Shown nucleoli Z scores. **a** Z1, **b** Z2, **c** Z3, **d** Z4

44–46 h (day 2) and at 66–68 h (day 3). The day 2 and day 3 embryo scoring system was as follows: For day 2 embryos, namely: score 1: embryo with blastomeres of equal size and no cytoplasmic fragmentation; score 2: embryo with blastomeres of equal or unequal size and cytoplasmic fragmentation which covers ≤10% of the embryo surface; score 3: embryo with blastomeres of equal or unequal size and 11–49% overall cytoplasmic fragmentation; score 4: embryo with blastomeres of equal or unequal size and cytoplasmic fragmentation which covers ≥50% of the embryo surface [12]. For the day 3 embryo scoring system, this was the same as described above except score 2 denotes ≤20% cytoplasmic fragmentation and score 3 denotes 21–49% cytoplasmic fragmentation.

Fertilization rate and the number of eight blastomered embryos at the third day were recorded. Day 3 morphology was checked and selected embryos were transferred.

Luteal phase was supported with progesterone 200 mg administered by vaginal route three times daily (Progynex® jel, Koçak, Istanbul, or Crinone gel® 8%, Merck Serono, Istanbul) or by 100 mg progesterone injection IM daily (Progynex® ampoule, Koçak, Istanbul) until the day of the pregnancy test 12 days after the embryo transfer.

We recorded age, duration of infertility, body mass index (kg/m^2), total gonadotropin doses, antral follicle count (AFC), and levels of AMH, FSH, inhibin B and E_2 on hCG day. On day 3 of a spontaneous menstrual cycle within 3 months of onset of ovarian stimulation, blood samples for assay of FSH and AMH were obtained by venopuncture at approximately 08.30 a.m.

Measurements of AMH were determined in duplicate using the AMH/MIS enzyme-linked immunosorbent assay kit (Diagnostic Systems Lab, Webster, TX, USA).The sensitivity of the assay was 0.017 ng/mL. The intra- and inter- assay variations were <5 and <8%, respectively.

The FSH, inhibin B and E_2 concentrations were estimated using the immulite semi-automated assay system. In the third day of menstrual cycles, a transvaginal ultrasound scan was performed to assess the total number of antral follicles measuring 2–5 mm in diameter and to confirm normal anatomy of the pelvic organs. E2 levels were evaluated on the day of HCG administration.

Secondary outcome measures were clinical or ongoing pregnancy that were determined by detection of fetal heart beat through abdominal ultrasonography at eight gestational weeks (GW) after the initiation of ART cycles and healthy pregnancies after 12 GW, respectively.

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) for Windows package (SPSS, version 11, Chicago, USA) Values were presented as mean ± standard deviation. Comparison of age, BMI, duration of infertility, total gonadotropin doses, AMH, inhibin B and FSH and hCG-day E2 levels, and AFC with oocyte and embryological parameters were performed with ANOVA and Tukey post hoc analysis. Comparison of two independent groups was done using Student's *t* test, Mann–Whitney *U* test and X^2 test. Statistical significance was considered to be reached at *P* values of <0.05.

Results

Six groups were formed according to the percentiles of AMH concentrations as <10% (≤0.89 ng/ml; $n = 21$), 10–25% (0.89–1.40 ng/ml; $n = 31$), 25–50% (1.40–2.89 ng/ml; $n = 53$), 50–75% (2.89–4.83 ng/ml; $n = 28$), 75–90% (4.83–8.06 ng/ml; $n = 55$), >90% (>8.06 ng/ml; $n = 21$).

There were statistical differences among the groups in terms of number of granular oocytes ($P = 0.019$), post-mature oocyte ($P = 0.026$), percentage of embryos ($P = 0.012$) and number of grade I eight-cell embryos ($P = 0.045$).

Percentage of EC embryos was significantly lower in groups 5 and 6 compared to group 3 and 4. Moreover there was no EC in group 6 (Table 1).

Number of total oocytes and antral follicles, inhibin B levels, number of central granular and post mature oocytes, nucleoli Z score 1, FSH levels, duration of infertility, total gonadotropin dose and mean age were found to be associated with AMH levels (Tables 2, 3).

There was no statistical difference in sperm concentration, total sperm motility, percentage of grade A motile sperms and sperm morphology (P =0.640; $P = 0.840$; $P = 0.172$; $P = 0.386$ respectively).

There was no statistical difference among the groups in terms of number of oocytes used in ICSI ($P = 0.210$), M2 ratio ($P = 0.810$), woman age ($P = 0.216$), normal polar body ratio ($P = 0.363$), oocyte size ($P = 0.502$), EC rate ($P = 0.399$), number of third day eight-cell embryos ($P = 0.190$), total number of embryos ($P = 0.217$) and fertilization rate ($P = 0.250$).

There were 2 (9.5%) pregnant women in ≤10% group and 11 (39.3%) pregnant women in ≥90% group (P = 0.040) (Table 2).

The number of pregnant women with polycystic ovary syndrome (PCOS) in the study groups was 61. PCOS rate was significantly higher in group 5 and 6 compared to other groups. (P = 0.000) Among 61 women with PCOS, AMH showed negative correlation with EC rate, and positive correlation with central granulation (P = 0.004, R: −0.293 and P = 0.001, R: 0.310).

Table 1 Embryo development parameters in the five groups of IVF-ICSI patients

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AMH percentile	≤10	10–25	25–50	50–75	75–90	≥90
AMH range (ng/ml)	≤0.89	0.89–1.40	1.40–2.89	2.89–4.83	4.83–8.06	≥8.06
AMH concentration (Mean ± SD)	0.54 ± 0.30	1.15 ± 0.14	2.11 ± 0.39	3.31 ± 0.27	5.46 ± 1.27	10.4 ± 2.08
Number of women	21	31	53	28	55	21
Number of oocytes	3.42 ± 1.68	5.25 ± 3.49	7.71 ± 4.62	7.10 ± 3.90[a]	11.30 ± 4.68[b]	11.52 ± 5.61[c]
ICSI attempted	2.4 ± 1.68[d]	5.48 ± 3.33[e]	8.90 ± 3.17	7.63 ± 5.21	9.88 ± 2.90	9.86 ± 3.02
Number of embryos	3.06 ± 1.35[f]	3.24 ± 2.18[g]	4.47 ± 3.24	4.11 ± 3.08	5.61 ± 2.85	5.33 ± 3.30
Early cleavage rate	6.55 ± 4.66[h]	8.55 ± 2.13[i]	11.30 ± 5.20	9.66 ± 1.67	2.1 ± 1.0[j]	0.0 ± 0.0[k]
Number of trans.embryos	1.76 ± 0.92	1.91 ± 1.13	2.47 ± 0.96	2.10 ± 0.99	2.71 ± 0.84[l]	2.42 ± 0.92
Transferred eight-cell grade I (*n*)	1.71 ± 0.95[m]	1.80 ± 0.94[n]	2.11 ± 1.07	1.80 ± 0.86	2.29 ± 0.98	2.21 ± 1.05
Post mature oocyte (*n*)	0.73 ± 0.61[o]	1.73 ± 1.05[p]	2.30 ± 1.81	1.74 ± 1.17	3.25 ± 2.00	2.90 ± 0.84
Cytoplasmic granulation (*n*)	2.4 ± 1.50[q]	3.38 ± 2.35	4.12 ± 3.52	3.29 ± 2.23	5.94 ± 3.50[r]	4.04 ± 3.42
PN score 1 (*n*)	2.32 ± 0.96[s]	3.82 ± 2.04[t]	5.20 ± 2.11	4.31 ± 3.20	6.10 ± 4.11	5.16 ± 4.16
Nucleoli Z score 1 (*n*)	2.00 ± 0.1[u]	2.5 ± 1.86	2.33 ± 1.33	2.19 ± 1.10	4.20 ± 3.40[v]	4.12 ± 1.68[w]
Central granulated oocytes	0.00 ± 0.00[x]	0.11 ± 0.10[y]	0.16 ± 0.08[z]	2.89 ± 1.60[†]	3.60 ± 2.98	4.80 ± 2.98

Tukey test: [a]Compared to group 5 ($P = 0.001$); [b]Compared to group 1–4 ($P = 0.001$); [c]compared to group 1 ($P = 0.001$), group 2 ($P = 0.001$), group 3 ($P = 0.013$), group 4 ($P = 0.001$); [d]compared to group 5 ($P = 0.001$) and 6 ($P = 0.001$); [e]compared to group 5 ($P = 0.001$) and 6 ($P = 0.001$); [f]compared to group 5 ($P < 0.048$); [g]compared to group 5 ($P < 0.017$); [h]Compared to group 2 ($P = 0.041$), group 3 ($P = 0.001$), group 4 ($P = 0.002$), group 5 ($P = 0.001$); [i]Compared to group 3 ($P = 0.006$), group 4 ($P = 0.03$), group 5 ($P < 0.001$); [j]compared to group 1–4 ($P = 0.001$); [k]Cannot be tested; [l]compared to group 1 ($P = 0.021$) and group 2 ($P = 0.012$); [m]compared to group 5 ($P < 0.022$); [n]compared to group 5 ($P < 0.026$); [o]Compared to group 2–6 ($P < 0.001$); [p]Compared to group 5 and 6 ($P < 0.001$); [q]compared to group 5 ($P = 0.002$); [r]compared to group 1 ($P = 0.002$), group 2 ($P = 0.009$), group 3 ($P = 0.03$), group 4 ($P = 0.005$); [s]compared to group 2–6 ($P < 0.01$); [t]compared to group 3 ($P = 0.001$) and group 5 ($P < 0.001$); [u]compared to group 5 and 6 ($P = 0.001$); [v]compared to group 2–6 ($P < 0.01$); [w]compared to group 2–6 ($P < 0.01$); [x]cannot be tested; [y]compared to group 4–6 ($P < 0.001$); [z]compared to group 4–6 ($P = 0.001$); [†]compared to group 5–6 ($P < 0.01$)

Table 2 Clinical characteristics of the five groups of IVF-ICSI patients

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AMH percentile	≤10	10–25	25–50	50–75	75–90	≥90
Woman age	32.0 ± 3.0	32.3 ± 3.7	30.9 ± 3.8	30.0 ± 3.7	29.3 ± 3.7[a]	28.4 ± 3.1[b]
Total unit of gonadotropin (IU)	3066 ± 1229	3012 ± 1116	2424 ± 1045	2264 ± 717	2062 ± 1508[c]	1711 ± 531[d]
Duration of infertility (years)	7.5 ± 4.1	7.4 ± 4.2	7.0 ± 4.0	7.6 ± 3.3	6.5 ± 3.3	5.6 ± 3.5
BMI (kg/m^2)	25.8 ± 2.8	25.9 ± 4.2	24.3 ± 3.7	25.9 ± 4.3	24.5 ± 4.1	24.1 ± 4.0
Inhibin B	54.6 ± 51.1	79.6 ± 74.4	94.3 ± 43.9	77.2 ± 39.6	93.9 ± 60.4	96.9 ± 42.4
FSH (mIU/ml)	10.0 ± 5.9[e]	6.7 ± 3.5	6.7 ± 2.8	6.8 ± 4.6	5.4 ± 1.5	5.5 ± 1.6
AFC	4.1 ± 2.5	6.2 ± 3.0	7.1 ± 3.2	6.2 ± 4.6	13.3 ± 6.3[f]	13.0 ± 6.6[g]
Presence of PCOS	7.1%	3.8%	13.3%	15.8%	68%[§]	88.9%[§]
Ongoing Pregnancy rate (%)	9.5[†]	19.4	34.0	39.3	30.9	19.0

Values are given as mean ± SD

Student's *t* test, χ^2, Mann–Whitney *U* test and Tukey analysis were used for statistical analysis

AFC Antral Follicle Count, *AMH* Anti-Müllerian hormone, *BMI* Body mass index, *FSH* Follicle stimulating hormone, *HCG* Human chorionic gonadotropin, *IVF-ICSI* In vitro fertilization-intracytoplasmic sperm injection, *PCOS* Polycystic ovary syndrome

Tukey test: [a] Compared to group 2 ($P = 0.004$); [b] Compared to group 1 ($P = 0.020$) and 2 ($P = 0.003$); [c] compared to group 1 ($P = 0.010$) and 2 ($P = 0.004$); [d] compared to group 1 ($P = 0.002$) and 2 ($P = 0.001$); [e] compared to all other groups ($P < 0.001$); [f] compared to group 1 to 4 ($P < 0.001$); [g] compared to group 1 to 4 ($P < 0.001$)

[†] Chi-square test, Compared to group 4, $P = 0.040$

[§] Chi square test, compared to group 1 to 4, $P < 0.001$

Table 3 Correlation of AMH and AFC with IVF-ICSI outcomes (Anova test)

	AMH	AFC
Women age	0.018*	0.012*
Total unit of gonadotropin	0.007*	0.036*
Inhibin B	0.003*	0.112
FSH	0.006*	0.942
AMH	–	0.000*
AFC	0.000*	–
Presence of PCOS	0.000†	0.000†
Central granulation	0.001*	0.003*
Number of oocytes retrieved	0.004*	0.014*
PN score 1	0.388	0.005*
Early cleavaged embryo rate	0.005*	0.444
Nucleoli Z score1	0.016*	0.399
ICSI attempted oocytes	0.233	0.008*
Postmature oocyte	0.026*	0.213
Small size polar body	0.000*	0.143

AFC antral follicle count, *AMH* anti-Müllerian hormone, *FSH* follicle stimulating hormone, *HCG* human chorionic gonadotropin, *IVF-ICSI* in vitro fertilization-intracytoplasmic sperm injection, *PCOS* polycystic ovary syndrome

* Significant

† Chi-square test

Discussion

The assessment of oocyte and embryo quality in human in vitro fertilization is getting increasing attention from embryologists. An ideal oocyte is thought to have a clear, moderately granular cytoplasm, a small perivitelline space, an intact first polar body and a round, colorless zona pellucida. Several intracytoplasmic and extracytoplasmic abnormalities have been described, but whether these abnormalities might be predictive of oocyte competence is controversial and the selection methods proposed are still poorly effective.

Compared to other ovarian tests, AMH seems to be the best marker reflecting the decline of ovarian reserve in reproductive age [6, 7]. Concomitantly, oocyte quantity and embryo quality decrease with advancing age. Hence, it was postulated that AMH in serum constitutes a marker for embryo quality [13]. In addition, basal serum AMH concentrations predict ovarian response during IVF cycles [14, 15].

Present data strongly support previously published manuscripts dealing with the prognostic value of AMH on oocyte and embryo quality, the number of oocytes, FSH levels, antral follicle count and ICSI outcomes [13, 16]. In this study, we have also found that AMH level was important for ongoing PR and AMH had a negative correlation with woman age ($P = 0.018$), FSH ($P = 0.006$) and a positive correlation with total unit of gonadotropin ($P = 0.007$), AFC ($P = 0.000$), inhibin B ($P = 0.003$), the number of oocytes ($P = 0.004$).

Seifer et al. compared AMH levels in day 3 serum from women with $\leq$6 versus $\geq$11 oocytes retrieved in preparation for IVF and they detected that mean serum AMH concentrations were significantly higher in $\geq$11 compared to $\leq$6 oocyte group (1.0 ± 0.4 vs. 2.5 ± 0.3 ng/mL, $P < 0.0001$) [17]. In our study, the number of oocytes also were higher in high level AMH groups ($P = 0.004$).

Majumder et al. (2010) suggested that AMH levels correlated with the number of top quality embryos available for transfer and the number of embryos frozen, but not with failed fertilization and failed cleavage [18].

Takahashi observed that oocytes were more likely to be fertilized when their follicle was able to produce high levels of AMH, as follicular fluid AMH levels from follicles with fertilized oocytes were more than three times higher than from follicles with non-fertilized eggs [19].

Morphology of the first PB can be a reliable indicator of oocyte age and the presence of a well-shaped, non-fragmented PB was associated with increased pregnancy rates [15]. In our study PB morphology (fragmented small PB) was also important for poor pregnancy rates and extremely higher AMH levels (Table 3, $P = 0.000$).

EC embryos were shown to be associated with high implantation rates [2, 5]. In our study, compared to other groups EC rate was significantly higher in the third and fourth group of AMH. This finding supports the hypothesis that AMH is associated with implantation rates. [17, 20]

Some authors used the median of all AMH values measured (at the time of hCG administration, 2.7 ng/ml) to predict implantation success [17, 20]. This may in part be explained by the fact that these authors performed AMH measurement on the day of ovulation induction (not on cycle day 3), a time when AMH values usually decline because of the presence of growing follicles and may thus fail to reflect the actual competence of the oocyte or embryo [21].

Eldar-Geva et al suggested a threshold basal AMH level of 2.52 ng/ml for significant prediction of ongoing pregnancy [21]. Peñarrubia et al. [7] suggested a threshold of 0.69 ng/ml discriminated between cancelled and ongoing cycles. Tremellen et al. [22] used a threshold value of 1.13 ng/ml, and suggested that plasma AMH assessment could predict poor ovarian reserve on a subsequent IVF cycle, with a sensitivity of 80% and a specificity of 85%. Wunder et al. [23] performed a study in 276 women and have shown that the concentrations of AMH and inhibin B in both serum and FF were significantly higher in the group of women who became pregnant in the corresponding treatment cycle than in those who did not conceive. In our study, ongoing PR was the lowest in <10% (9.5%), and the

highest in 50–75% group (39.3%); however, the difference was weakly significant ($P = 0.040$). Therefore, more patients are needed to draw definitive decisions about ongoing PR. The number of patients in each group was not equal in our study, since it is very difficult to find patients with AMH levels of $\geq$10 and $\leq$1 ng/mL.

Ebner et al. [11] evaluated 141 ICSI patients, measured serum AMH levels on cycle day 3 and subdivided all women into three groups using the 25th and 75th percentiles. Cycle cancellation rate was correlated with AMH levels ($P < 0.05$). The 25th percentile (<1.66 ng/ml) and 75th percentile (>4.52 ng/ml) groups showed oocytes of lower quality [dark central granulation, aggregation of smooth endoplasmic reticulum (sER)] compared to the median group (50th percentile) (1.66–4.52 ng/ml). Basal serum FSH did not allow for adequate prognosis in terms of gamete appearance. Fertilization and further cleavage up to blastocyst stage was not affected by AMH levels [11]. In our study, we also detected that the number of oocytes with dark central granulation was higher in high AMH levels and AMH levels did not affect the fertilization.

In our study, different levels of AMH has a statistically significant relationship with antral follicle count, oocyte morphology, cytoplasmic central granulation, polar body size, percentage of EC embryos and the number of Z1 scored embryos.

Hazout et al. [24] demonstrated that day 3 serum AMH level and IVF outcome were strongly associated; and higher AMH concentrations were associated with a greater number of mature oocytes, a greater number of embryos, and ultimately a higher clinical pregnancy rate.

Although AMH is a useful marker for the assessment of ovarian reserve and responsiveness, most of the studies showed that it was not able to predict pregnancy accurately [20–22, 24]. Cupisti found that AMH levels in individual follicles were inversely correlated with the maturation and developmental potential of oocytes [26]. To achieve a successful pregnancy following IVF, a number of additional parameters, such as male factor, sperm parameters, embryo development and quality, endometrial receptivity are needed to be considered [27]. In our study, sperm parameters were similar in all AMH groups and mean sperm concentration was also in normal range.

In our study in the group 3 had highest PR, EC embryos, the nucleoli Z1 scored embryos and group 6 (includes lower pregnancy rate) did not include EC embryos. Oocyte central granulation which was highest in the sixth group, is a predictor of oocyte dismorphism and in turn a bad prognostic factor for pregnancy [28, 29].

AMH has significant positive correlation with PCOS. In our study group, worse embryologic parameters were seen in groups 5 and 6, and this may be attributed to PCOS. However, not all women with PCOS had the same oocyte quality results. Considering only 61 women with PCOS, AMH showed negative correlation with EC rate, and positive correlation with central granulation ($P = 0.004$, R: -0.293 and $P = 0.001$, R: 0.310). Although 61 women with PCOS is not adequate to draw definitive conclusions we may speculate that AMH may show women with better oocyte quality in the PCOS group. This may be further investigated in bigger trials.

In conclusion, our results demonstrated that serum AMH levels were highly correlated with the number of antral follicles, and the oocyte quality and embryo development. It appears that AMH serum levels are associated with ovarian response in ART cycles and can be served as a novel marker for ovarian reserve. Furthermore, with respect to significant difference in clinical pregnancy outcome, serum levels of AMH may be used as a marker for predicting the clinical pregnancy rate. However, further studies are needed to determine whether AMH can accurately predict the ART outcomes.

Conflict of interest None.

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