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Original Research Article

Relation between anti-mullerian hormone and blastocyst rate in patients less than 35 years old

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ABSTRACT

Background: Anti-mullerian hormone is the most commonly used predictor for in vitro fertilization cycles outcome from the quantity point of view but not the quality.
Methods: The study included retrospective analysis of 247 cycles out come up to blastocyst rate. Patients included in the study were divided in to three groups according to Anti-Mullerian hormone value.
Results: The primary outcome included cycles outcome rates up to blastocyst rate, cycles without blastocyst, cycles with 100% blastocyst. While secondary outcome included oocyte number, number of mature oocyte, number of fertilized oocytes, number of cleaved oocytes, number of blastocyst. We found that the mean number of blastocyst is significantly affected by AMH in favor of group C, but the blastocyst rate was not significantly affected between the three groups group A (58.16%), group B (52.56%), group C (55.49%) with p value 0.621 which is not significant. The rate of cycles with 100% blastocyst rate was not significantly different between the groups, however the rate of cycles without blastocyst was higher in group B (13.58%) but it did not differ between the groups A (7.4%) and group C (7.9%). The difference was not significant between the three groups.
Conclusions: AMH can be a good predictor of IVF cycles outcome based on the ovarian yield and numerical outcome but it is not a good predictor of the blastocyst rate which could indicate the quality of the outcome of the cycles in patients 35 years of age or less.

Keywords: Anti-Mullerian hormone, Blastocyst, IVF

INTRODUCTION

Since the early days of in vitro fertilization embryo transfer was done in the cleavage stage of embryos as the extended culture to day 5 was impossible. Recently, fertility centers started extending the culture of the embryos till day 5.[1] This was helped by Improvement of culture media, as the one stage culture media improved the results of embryos incubated till day 5.[2] Blastocyst transfer helped in better selection of embryos as the best survives, it also helped to decrease embryos available for transfer so it lowers the incidence of multiple pregnancy.[3] Recently there is increased demand for preimplantation genetic screening and diagnosis due to increased maternal age, recurrent implantation failure and or recurrent miscarriages, which was used to be done through cleavage stage biopsy but after improved embryo culture till day 5 the tropho-ectoderm biopsy became more available and more reliable and acceptable as the day 5 biopsy will give better results and decreases the incidence of mosaicism.[4,5] it also decreases the injury to the embryos, gives more cells available for testing, and provides more DNA with less possible fragmentation that helps in the diagnosis.[6-8] This is beside the improvement of freezing outcome due to vitrification which leads to better survival of the blastocyst, for that most of IVF

centers prefer to proceed with blastocyst freezing for another cycle.[9] All these factors had pushed most of the IVF centers to go for blastocyst culture and transfer to increase the pregnancy rate.[10] When the IVF laboratory decided to proceed to day 5 culture of the embryos so they know that the number of embryos available for transfer and or biopsy will be less, and the possibilities of having no embryos available for transfer could happen so the predictor factors helps to decide to proceed with day 5 culture or no after patient counselling.[11]

Before patients start the cycle and during the counselling, they need to review the predicting factors that helps to explain the outcome of the IVF treatment cycle for the couples as it also helps in individualized treatment and what to expect.[12] In the early days of IVF the predicting factors used were: maternal age, antral follicle count together with basal follicle stimulating hormone, serum inhibin, ovarian volume but now the Anti-Mullerian hormone (AMH) is the dominant predicting factor for ovarian reserve, protocol of treatment and the cycle outcome.[13,14] Anti-Mullerian hormone (AMH) is one of the transforming growth factor-β superfamily.[15] It is produced in the ovary mainly by granulosa cells and its levels are not affected by day of menstrual cycle or pregnancy.[16] It has been shown that the link between AMH and cycle prognosis and reproductive outcome in many ways as serum AMH correlates strongly with number of oocytes retrieved, and in some earlier studies they found an association of AMH with implantation, pregnancy, and/or live birth after assisted conception, but others have found no relation.[17-19] However, the association between AMH and IVF outcomes has been shown to be affected by age, with too much variability in extremes of reproductive age and the same also for infertility subgroup.[20,21] In spite of all this still the implementation of AMH value as a sole predictive factor for IVF outcome can't be ignored. Different factors could affect the cycle outcome such as the maternal age, controlled ovarian stimulation protocol, the sperm parameters so in this study we unified most of these parameters such as the age as all patients included were 35 years old or less, in cycles with normal sperm parameters, antagonist protocols so the predictive ability of the AMH will not be affected by other factors.

METHODS

This is a retrospective cohort study in large IVF center in Abu Dhabi in period between January 2020 and December 2020, the study included analysis of cycles outcome of patients less than 35 years old going through ICSI cycles with embryos cultured till day 5 for blastocyst stage.

Inclusion criteria

Patients with primary or secondary infertility aged 35 years old or less, with normal male factors going through antagonist protocol cycles were included in the study.

Exclusion criteria

Patients more than 35 years old, sever male factors, patients for day 3 transfer or day 3 biopsy were excluded from the study.

The patients had their AMH done before starting the cycle. Patients had gone through antagonist protocol cycles after counselling and consenting, controlled ovarian stimulation will be through recombinant follicle stimulation hormone and\or highly purified combined follicle stimulation hormone and luteinizing hormone by about 75-450 unit daily according to each patient situation started on day 2 or day 3 of the menstrual cycle with follow up of follicles size using the transvaginal scan till the follicles reached the desired size then to trigger the ovulation with either human chorionic gonadotropins (HCG) or gonadotropin releasing hormone (GnRh) agonist according to each case. Oocyte retrieval to be done under conscious sedation and under ultrasound guidance, the collected oocytes to be fertilized using the intracytoplasmic sperm injection with Olympus IX73 Narishige Takanome micromanipulator, then all fertilized oocytes to be kept in the Esco Medical MIRI bench top incubator and incubated in 6202 Stage 1-Step media for blastocyst culture.

Statistical analysis

Patients were divided to three group according to AMH level, first group with AMH less than 1, 2 ng/ml, second group included patients with AMH more than 1, 2 ng/ml till less than 3 ng/ml, then the third group included patients with AMH 3 ng/ml or more. The primary outcome included the cycles outcome rate starting by maturation rate up to blastocyst rate, blastocyst formation rate per cleaved embryos and per fertilized oocytes, cycles with 100% blastocyst rate and cycles ended with no blastocyst, secondary outcome included number of oocytes, number of mature oocytes, number of fertilized oocytes, number of cleaved embryos. The outcome will be expressed in mean with standard deviation, the difference in outcome between the three groups will be calculated using the one-way ANOVA including Tukey HSD and the significance will be when $p<0.05$, and the Chi-square test to compare and fined the significance between the rate of cycle without blastocyst and cycles with 100% blastocyst. significance will be when $p<0.05$.

RESULTS

The study included 247 patients; patients divided according to their AMH in to the three groups. Group A with AMH less than 1.2 ng/ml, group B with AMH 1.2 to less than 3 ng/ml, group C with AMH 3 ng/ml or more. The mean age for all the groups was 30.57with SD±3.8, ranging from 21 years old to 35 years old, with AMH mean 5.53 ng/ml ranging from 0.5 to 20.33 ng/ml, the mean of the collected oocytes was 15.56 with SD ±8.47 ranging from 2 oocytes to 45 oocytes, the mean of the

mature oocytes were 11.86 with SD ±7.037 ranging from 2-42 oocytes, fertilized oocytes mean was 10.10 with SD ±6.21 ranging from 2 to 36 fertilized oocytes, fertilization rate mean 86.40%, the mean of cleaved embryos was 9.89 with SD ±6.15 range from 1 to 28 embryo, mean cleavage rate 98%, mean number of embryos cultured for day 5 were 9.26 with SD ±5.87 and an average of 1 to 28 embryo, mean number of blastocyst was 5 with SD ±4.14 and average from 0 to 17 blastocyst, with mean blastocyst rate per cleaved embryo 54.82% and mean blastocyst rate per fertilized oocyte 52.39%.

Table 1: demographic criteria of 3 groups.

Variables	A	B
Number of patients	27	81
Mean age	31.22	31.11
Mean AMH	0.85	2.02

Group A included 27 case with mean age 31.22 with SD ±2.75 and range from 25 to 33 years old, mean AMH 0.85 ng/ml, mean number of oocytes collected 9.89 with SD ±4.24 , mean of mature oocytes 7.74 with SD ±4.86, mean fertilized oocytes 6.67 with SD ±3.88, mean fertilization rate 87.98%, mean of cleaved embryos 6.67 with SD ±3.88, mean cleavage rate 100%, mean number of embryos cultured for day 5 was 6.33 with SD ±3.83 , mean blastocyst was 4 with SD ±2.81 , mean blastocyst rate per cleaved embryo was 58.16% , blastocyst rate per fertilized oocyte was 56.75%. Group B included 81 case with mean age 31.11 with SD ±3.27, and rang from 21 to 35 years old, mean AMH value was 2.02 ng/ml, mean number of collected oocytes 12.22 oocyte with SD ±5.03, mean number of mature oocytes 9.11oocytes with SD ±4.46, mean number of fertilized oocytes 7.82 with SD ±37,mean number of fertilization rate 85.81%, mean number of cleaved embryos 7.80 with SD ±4.39, mean of cleavage rate 99%, mean number of embryos cultured for day 5 was 7.56 with SD ±4.40, mean number of blastocyst was 4 with SD ±3.66.

Table 2: Rate of outcome (primary outcome).

Variables	A	B	C	P value
Maturation rate	78.3	74,7	76,6	0.445
Fertilization rate	87.98	85.81	86.44	0.383
Cleavage rate	100.00	99.00	97.00	0.0137
Blastocyst rate per cleavage	58.16	52.56	55.49	0.621
Blastocyst rate per fertilized oocytes	56.75	50.88	52.42	0.669

Mean blastocyst rate per cleaved embryo was 52.56%, mean blastocyst rate per fertilized oocyte was 50.88%. Group C included 139 patient with mean age 30.12 with SD ±3.87and ranging from 21 to 35 years old, mean AMH value was 8.49 ,mean number of oocytes was 18.60 with SD ±8.94, mean number of mature oocytes was 14.25 with SD ±7.78 , with mean number of fertilized oocytes 12.10 with SD ±6.93, mean fertilization rate 86.44%, mean number of cleaved embryos was 11.76 with SD ±6.89, mean cleavage rate was 97%, mean number of embryos cultured till day 5 was 10.82 with SD ±6.41, mean number of blastocyst was 6 with SD ±4.18, mean blastocyst rate per cleaved embryo was 55.49, and the mean blastocyst rate per fertilized oocyte was 52.42.

Table 3: Number of cycles that ended with no blastocyst (primary outcome).

Variables	A	B	C	Total
Number	2	11	11	24
Rate	7.4	13.6	7.9	9.72

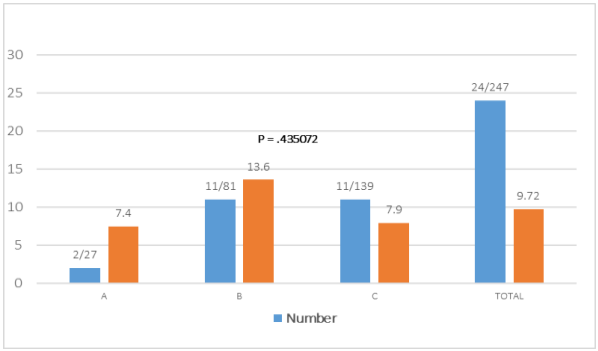

Figure 1: Number of cycles that ended with no blastocyst.

Using the one-way ANOVA test to find the p value and the significance between the outcomes of the three groups we found that in view of oocytes collected the p value between the three groups was significant as the p value was <0.0001 and in between each of the two groups A:B p value was 0.254, A:C p value was 0, B:C p value was 0.0006. Regarding the mature oocyte we can see that the p value between the groups <0.0001, and in between each of the two groups A:B p value was 0.532, A:C p value was 0, B:C p value was 0.00022. For the fertilized oocytes the p value was <0.0001, and in between each of the two groups A: B p value was 0.578, A:C p value was 0.0001, B:C p value was 0.00077. For the fertilization rate the p value was 0.838 which is not significant, and in between each of the two groups A: B p value was 0.805, A:C p value was 0.807, B:C p value was 0.99. Regarding the cleaved embryos in the three groups the p value was <0.0001, and in between each of the two groups A:B p value was 0.589, A:C p value was 0.0004, B:C p value was 0.0018. With the cleavage rate we see the p value 0.0137, and in between each of the two groups A:B p value was 0.986, A:C p value was 0.098, B:C p value was 0.138. Regarding the embryos that to be cultured till day

5 the p value between the three groups was <0.0001, and in between each of the two groups A:B p value was 0.498, A:C p value was 0.0014, B:C p value was 0.0081, with the number of blastocyst available the p value was 0.00049, and in between each of the two groups A:B p value was 0.723, A:C p value was 0.0049, B:C p value was 0.045.

Table 4: rate of cycles with 100% blastocyst (primary outcome).

Variables	A	B	C	Total
Number	3	8	8	19
Rate	11.1	9.9	5.6	7.7

The blastocyst rate per cleaved embryos we can see the p value 0.621, and in between each of the two groups A:B p value was 0.571, A:C p value was 0.879, B:C p value was 0.858, and the blastocyst rate per fertilized oocyte was 0.669, and in between each of the two groups A:B p value was 0.560, A:C p value was 0.728, B:C p value was 0.961.

Table 5: outcome of cycles (secondary outcome).

Variables	A	B	C	P value
Mean no. of oocytes	9.89	12.22	18.6	<0.0001
Mean no. of mature oocyte	7.74	9.11	14.25	<0.0001
Mean no. of fertilized oocytes	6.67	7.82	12.1	<0.0001
Mean no. of cleavage oocyte	6.67	7.8	11.76	<0.0001
Mean no. of embryos cultured till D5	6.33	7.56	10.82	<0.0001
Mean no. of blastocyst	4	4	6	<0.00049
Mean no. of oocytes	9.89	12.22	18.6	<0.0001

The number of cycles which ended with no available blastocyst was 24 out of the 247 cycles 9.72% of the cycles, in group A it was 2 out of the 27 cycles (7.4%), in group B it was 11 out of 81 cycles (13.58%), and in group C it was 11 out of 139 cycles (7.91%), the Chi-square statistics is 106645, the p value is 0.435072, the results is not significant at p<0.05. The number of cycles that had 100% blastocyst rate in the study was 19 out of the 247 (7.7%), in group A 3 out of 27 cycles (11.11%), in group B 8 out of 81 cycles (9.9%), and in group C 8 out of 139 cycles (5.6%). The chi-square statistics is 1.4675. The p value is 0.480112, the result is not significant.

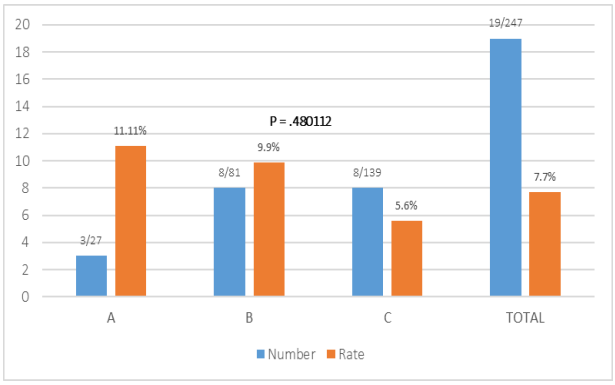

Figure 2: rate of cycles with 100% blastocyst.

DISCUSSION

As observed in current study that there was significant positive correlation between the AMH value and qualitative cycles out come as noted in the number of oocytes and the available mature ones that's ready for insemination and that the number of fertilized oocytes accordingly was significantly higher in group C which represents the high responders, this is also reflected on the number of cleaved embryos and embryos available for blastocyst culture and mean blastocyst that can be for embryo transfer, preimplantation genetic testing and or freezing as by logic when you have more oocyte you will have more mature and more will be fertilized then cleaved and you will obtain more blastocyst in case of normo-spermic cycles as in our study. On the other hand, when we go inside the study, we found that the difference was less in case of comparison of group A which represent the low reserve patients and group B which represent the normal responding patients. This leads us to conclude that the significant difference was great due to inclusion of high responder patients in our study. From other point of view we could see that the difference in the rate of cycle outcome was not significant between the three groups which till us about the quality of oocyte obtained in the three group and that the AMH value did not predict the quality in this age, together with the rate of cycles ended with no blastocyst as we can see that the rate of no blastocyst was almost the same in low reserve, normal responders and high reserve group which is also a second indicator of the quality of oocytes in this age. This is in addition to cycles ended by 100% blastocyst rate in the three group are almost the same without any significant difference in the rate. These results are with agreement of studies done by a lot of researchers to empathize the role of AMH in predicting the cycle out come as done by Nelson SM., et al., in 2015, who found that AMH is a better predictor of ovarian response during the IVF cycles than antral follicle count, also in study done by Arce et al which concluded that there is positive correlation between AMH value and oocyte yield and AMH is better predictor of high and low ovarian response.[17,22] And in study by Sun et al in found that the AMH and had positive correlation with number of oocytes and both affect the IVF cycle outcome. On the

other hand study done by Reichman et al and concluded that AMH is a good predictor of the number of oocytes and cycle cancellation but poor predictor in regard to pregnancy outcome which might indicate oocyte quality.[23,24] Also we can see in study done by Kavoussi et al that the AMH results can be good predictor of oocyte quality in patents 35 years old or less which is against our finding but in the study done by Kavoussi did not exclude male factor, and did not unify the protocol of treatment but his conclusion was based on the available number of blastocyst which is also observed by our study and its logical that when the number of oocytes is higher so the obtained blastocyst will be also higher but if we compare the rate we will find no significant difference.[25] That if you get any number of oocyte the number of blastocyst will be around 50% and the rate that you will not obtain blastocyst around 7 to 13% and the incidence you will get 100% blastocyst 9-11% no matter the AMH value in patients 35 years old or less. On the other hand, this may be different in older aged patient. As had been concluded by study done by da Silva et al and found that AMH predicts the quality of ovarian response to stimulation, regardless of patient age, but in his study he depended mainly on the number of mature oocyte as an indicator of oocyte quality, this is in comparison to a study done by Mutlu et al who found that antral follicle count is better predictor than AMH in regard to clinical pregnancy rate in poor responders which could support our work in that the AMH has less predictability on quality of cycle outcome.[26,27] In other study by Shan- Zhou et al on different factors that predict the ovarian reserve and cycle outcome in patients more than 40 years old found that the antral follicle count/patient age is the best predictor of cycle out come more than other factors including the AMH.[28]

Limitations

Limitations of our study included the number of cycles and retrospective aspect of the study, prospective studies that includes more cycles are required for more information.

CONCLUSION

AMH is very important hormone in individualizing the protocol of ovarian stimulation in IVF cycles and in prediction of cycle outcome but in our study, we found that it is a good predictor of oocyte number and its consequences as regard the number of fertilized, cleaved, blastocyst available in means of quantity of cycle outcome. But we are uncertain about its predictability of quality of cycles outcome in patients 35 years old or less.it is important to explain and council the patients before starting the cycle about the probability of having blastocyst and possibility of cycles that might ends with no blastocyst based on her parameters and tailor the treatment cycle accordingly in order to achieve best results.

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