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Mature Oocyte Morphological Score and Its Utility in Assisted Reproduction Cycles

Priyanka Chevuturi[1], Avani Pillai[2], Prasanth CP[3]

ABSTRACT

Background: Oocyte quality is an important factor in determining the success rates of assisted conception cycles. We wanted to know the correlation of various oocyte morphological and physical parameters with quality of the embryos.

Methods: This is a single institutional prospective observational study conducted after institutional ethical committee approval over 18 months in 90 consenting women undergoing in-vitro fertilization. Ovarian stimulation protocols, gonadotropin doses and ovulation trigger were decided according to the patient's clinical profile. After retrieval, oocytes were incubated for 2 hours and denudation was done followed by morphological assessment of each oocyte under inverted microscope and ICSI was done with morphologically normal sperm. All injected oocytes were cultured singly and the grade of embryo according to the Istanbul consensus for cleavage stage embryos was noted for each oocyte.

Statistical Analysis/Results: 720 mature oocytes upon ICSI with morphological sperm yielded 408 (56.6%) Grade A, 125 (17.4%) Grade B embryos which were considered transferrable and 187 (26%) Grade C embryos and Unfertilized oocytes. Each oocyte morphological parameter was studied against the grade of embryo it formed.

Conclusion: We developed a mature oocyte morphological score with each variant of the oocyte parameter for both significant and non-significant oocyte parameters.

Key words: Oocyte quality, Oocyte scoring, IVF outcomes

1. MBBS MS (Obgyn), (M. Ch Reproductive Medicine and Surgery), M.Ch Resident, Reproductive Medicine and Surgery, Amrita Institute of Medical Sciences, Kochi.
2. DGO DNB MRCOG FRM EFRM, Additional Professor, Reproductive Medicine and Surgery, Amrita Institute of Medical Sciences.
3. M.Sc. M.Phil. Senior Embryologist and IVF Lab-Incharge

Corresponding Author: Priyanka Chevuturi

Introduction:

Infertility is considered as disease of the reproductive system and is defined as failure to achieve a clinical pregnancy after one year or more of regular unprotected sexual intercourse. The chance of couple conceiving depends on many factors and oocyte quality is one of the important factors determining the success of assisted conception cycles. Major determining factor determining the in-vitro fertilization (IVF) outcomes remains to be female age. Apart from maternal age, factors affecting the quality of oocyte are certain oocyte secreted factors, environmental factors, smoking, certain nutritional habits and intracellular temperatures. The prognostication of the ART cycle outcome can be done at or before the beginning of the cycle or during the cycle. The first approach would help in proper patient counselling and is the usual preferred approach. These mainly involves counselling based on the demographic factors including age, BMI, basal hormonal status, ovarian reserve tests including Follicle stimulating Hormone (FSH), Anti mullerian Hormone (AMH), Antral follicle count (AFC) and Estradiol, response to previous stimulation etc. The prognostic variables during the treatment cycle include folliculogenesis, endometrial response, oocytes retrieved, quality of oocytes, number and grade of embryos. Since these factors represent the unexpected changes in ovarian response, quality of oocytes, fertilization and cleavage of embryos, these can be utilized in a more appropriate counselling during the treatment cycle. The former group fails to communicate properly regarding certain unexpected or unpredictable events that can occur during treatment cycle. The counselling regarding the prognosis is very important in IVF cycles because this is one of the fields in Medicine which have maximum treatment failures and hence the clinician often need to face the grief and displeasure of the patients. This will help the patients too to face the situation. The currently available tests for prognosticating the IVF outcome are not uniformly applicable to all patient populations. The predictive values of many of these tests are quite debatable. Many models were developed for the prediction of clinical pregnancy over the last few years but many of these failed to achieve proper validation in further studies and hence are not currently very popular. Oocyte quality has been described in many studies as having positive correlation with embryo morphology[1], embryo quality[2], growth and development[3], cleavage rates[1] and pregnancy rates.[4]

Objective:

To formulate a total oocyte score in predicting clinical pregnancy

Materials And Methods:

Institutional ethical committee approval was taken before beginning the study and informed consent has been taken from all the study participants. This is a prospective observational study conducted over a period of 18 months in 90 women (720 mature oocytes) at Amrita Fertility Centre, AIMS, Kochi. All patients undergoing autologous IVF cycles irrespective of the indication for IVF and ICSI performed with morphologically normal sperm were included in the study. However, presence of endometrioma, uncontrolled endocrinological conditions, thin endometrium, history of recurrent miscarriage or implantation failure, uterine anomalies were excluded as these factors may affect the clinical pregnancy rates independently. Median age of the patients was 31.6± 3.6 years.

Patients were stimulated with gonadotropins according to institutional protocol and posted for oocyte retrieval after 35-36 hours after ovulation trigger. All the injected oocytes were assessed subjectively for the cytoplasm appearance, 1st polar body morphology, Oolemma breakage, Perivitelline space, shape of oocyte and zona, presence or absence of Refractile bodies, Vacuoles and Smooth Endoplasmic Reticulum. Once the oocytes were injected, they were cultured individually and a fertilization assessment was made on Day 2 or 3 post ICSI and embryos were graded according to Istanbul consensus.[5] A maximum of 3 (lesser in most cases) A or B grade embryos both of which were considered as good quality embryos were transferred either in a fresh or a frozen embryo transfer. Biochemical pregnancy (positive serum β hCG with value > 50 IU two weeks after embryo transfer) and clinical pregnancy (presence of gestational sac during the ultrasound scan performed two weeks after positive β hCG) were recorded. An objective oocyte score was calculated from the quality of embryos the oocyte formed and clinical pregnancy status of the patient.

Statistical analysis software used was IBM SPSS version 20.0 and categorical variables were expressed by frequency and percentage, continuous variables were expressed as median. Chi-square test was used to test association of categorical variables with grade of embryos. Score of oocytes was formulated by Multiple ordinal logistic regression (Odd's ratio with 95% CI). Cut-off mean oocyte score to predict clinical pregnancy was done by ROC curve analysis and Diagnostic measures.

Results:

Table 1: Distribution of patient characteristics (n = 90)

Variable	Median(Q1-Q3)
Age in years	31.6± 3.6
Total number of Embryos	6(3-10)
Gonadotropin Dose (IU)	300(225-450)
Embryos transferred	3(2-3)

Table 2: Univariate analysis of association of oocytes characteristics with grade of the embryos (n=720)

Parameters	Category	Grade			P value
		Grade C and Unfertilized oocytes	Grade B embryos	Grade A embryos	
Cytoplasm	Central granulation/ Coarse granulation) (n=167)	80(48.1)	36(21.3)	51(30.6)	<0.001
	Fine granulation (n=353)	89(25.1)	57(16.1)	208(58.8)	
	Normal (n=200)	18(9.1)	33(16.4)	149(74.4)	
Polar body	Fragmented / Big (n=101)	43(42.7)	13(12.7)	45(44.5)	<0.001
	Round (n=107)	42(39.3)	27(24.8)	38(35.9)	
	Normal (n=513)	103(20)	86(16.8)	325(63.3)	
Zona pellucid shape	Oval (n=18	10(55)	2(10)	6(35)	0.011
	Round (n=702)	178(25.3)	124(17.6)	402(57.2)	
Zona Pellucida Thickness	Thick (n=78)	41(52.3)	9(11.6)	28(36)	<0.001
	Normal (n=642)	146(22.8)	116(18.1)	379(59.1)	
Oocyte breakage	Sudden/Difficult breakage (246)	102(41.6)	29(11.9)	114(46.5)	<0.001
	Normal (474)	85(17.9)	96(20.2)	293(61.8)	
Perivitelline space	Large (n=200)	80(40)	34(16.9)	86(42.9)	<0.001
	Normal (n=520)	107(20.6)	92(17.6)	321(61.8)	
Perivitelline space granulate	Granulated (n=230)	89(38.5)	39(17.1)	102(44.4)	<0.001
	Normal (n=490)	98(20.1)	86(17.5)	305(62.3)	
Size of the oocyte	Normal (n=720)	187(26)	125(17.4)	408(56.6)	-
	Giant oocyte (0)	0	0	0	
Vacuole	Present (n=170)	66(38.7)	36(21)	69(40.3)	<0.001
	Absent (n=550)	122(22.1)	90(16.3)	339(61.6)	
Smooth Endoplasmic Reticulum	Present (n=51)	34(66.1)	5(10.7)	12(23.2)	<0.001
	Absent (n=669)	154(23)	120(17.9)	395(59.1)	
Refractile body	Present (n=102)	48(47.3)	19(18.8)	35(33.9)	<0.001
	Absent (n=618)	139(22.5)	106(17.1)	373(60.3)	
Oocyte shape	Oval (n=17)	9(55.6)	3(16.7)	5(27.8)	0.012
	Normal (n=703)	178(25.3)	122(17.4)	403(57.3)	

Table 3: Multivariate analysis of association of oocytes characteristics with grade of the embryos

Variables	Category	P value	Odds Ratio (95% CI)
Cytoplasm	Central granulation or coarse granulation	-	<0.001
	Fine granulation	<0.001	3.0 (2.0 ,4.3)
	Normal	<0.001	3.8(2.4,6.0)

Variables	Category	P value	Odds Ratio (95% CI)
Polar body	Fragmented or Big	-	1
	Round	0.398	0.79(0.46,1.4)
	Normal	0.662	1.1(0.71,1.7)
Zona pellucid shape	Oval	0.013	1
	Normal		3.4 (1.3,8.9)
Zona Pellucida Thickness	Thick	0.004	1
	Normal		2.0(1.3,3.3)
Oocyte breakage	Sudden/Difficult breakage	0.018	0.018
	Normal		1.5(1.1,2.1)
Perivitelline space	Large	0.089	1
	Normal		1.3(0.96,1.9)
Perivitelline space granulate	Granulated	0.004	1.0
	Normal		1.7(1.2,2.4)
Smooth Endoplasmic Reticulum	Present	0.0006	1
	Absent		2.9 (1.6,5.4)
Oocyte shape	Oval	0.229	1
	Normal		1.8(0.68,4.9)
Vacuoles	Present	0.009	1
	Absent		1.6(1.1,2.3)
Refractile body	Present	0.024	1
	Absent		1.6(1.1,2.5)

Table 4: Mature oocyte morphological scoring system

Oocyte Parameter	Category	Score
Cytoplasm	Central granulation or Coarse granulation	1
	Fine granulation	3.0
	Normal	3.8
Zona pellucida shape	Oval	1
	Normal	3.4
Zona Pellucida Thickness	Thick	1
	Normal	2.0
Oocyte breakage	Sudden/Difficult breakage	1
	Normal	1.5
Perivitelline space granulations	Granulated	1.0
	Normal	1.7
Smooth Endoplasmic Reticulum	Present	1
	Absent	2.9
Vacuoles	Present	1
	Absent	1.6
Refractile body	Present	1
	Absent	1.6
Polar Body	Fragmented or Big	1
	Round	0.79
	Normal	1.1
Peri-vitelline space	Large	1
	Normal	1.3
Oocyte shape	Oval	1
	Normal	1.8

Table:5 Distribution of Clinical and Biochemical pregnancy

Clinical Pregnancy	Frequency	Percent	Biochemical Pregnancy	Frequency	Percent
Yes	41	45.6	Yes	47	52.2
No	49	54.4	No	43	47.8

Among the total 90 patients 41(45.6%) had clinical pregnancy and 47(52.2%) had biochemical pregnancy.

Table 6: ROC curves for mean oocytes score for the prediction of clinical pregnancy

Area	Std. Error	p value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.756	0.050	<0.001	0.657	0.854

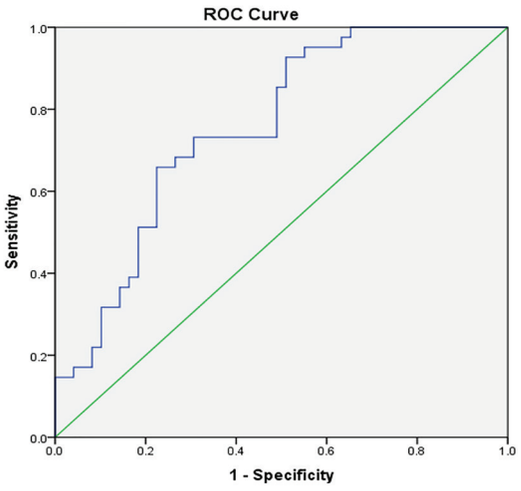

Fig:1. : ROC curves for mean oocytes score for the prediction of clinical pregnancy

Table 7: Diagnostic measures

Sensitivity	Specificity	Predictive Value positive	Negative predictive value	Accuracy
73.2%	69.4%	66.7%	75.6%	71%

Table 8: Association of mean oocytes score cutoff value with clinical pregnancy

Mean Oocytes Score	Clinical Pregnancy		p value
	Yes N (%)	No N (%)	
≥22.4	30 (66.7%)	15(33.3%)	<0.001
<22.4	11 (24.4%)	34 (75.6%)	

The results showed that the cut-off score of > 22.4 could predict a clinical pregnancy with sensitivity 73.2%, specificity 69.4% and accuracy 71%. (p value<0.001)

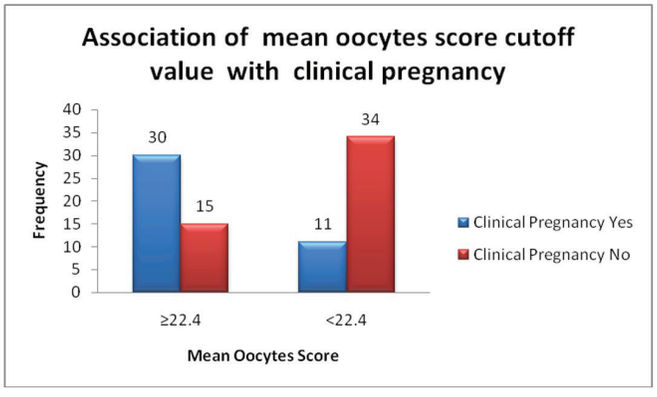

Fig:2.: Association of mean oocytes score cut-off value with clinical pregnancy

Discussion:

Table 1 describes the baseline patient characteristics, total number of embryos formed, average gonadotropin dose used in each patient and average number of embryos transferred in each patient.

Table 2 describes the univariate analysis where each categorical variant of a particular oocyte parameter was studied against the grade of embryo it formed after Intra-cytoplasmic sperm injection. Table 3 describes multivariate ordinal regression analysis association of oocytes characteristics with grade of the embryos. In this model, eight variables such as Cytoplasm (p value<0.001), Zona pellucida shape (p value=0.013), Zona Pellucida Thickness (p value=0.004), Oocyte breakage (p value=0.018), Perivitelline space granulations (p value=0.004), smooth endoplasmic reticulum (p value=0.0006), presence of Vacuole (p value=0.009) and Refractile body (p value=0.0244) showed statistical significance. Among the significant predictors, most significant predictor was cytoplasm with an Odd's risk of 3.8 vs 1 for normal and coarsely granulated cytoplasm respectively.

Mature oocyte morphologic score was generated by considering odds ratios obtained for each oocyte morphologic characteristic. Eight parameters mentioned above i.e. Cytoplasm characteristics, Zona pellucida shape, Zona Pellucida thickness, Peri vitelline space granularity, Smooth Endoplasmic reticulum, Vacuoles, Refractile bodies, Oocyte breakage were

independently statistically significant and the other three i.e. Oocyte shape, Size of Peri vitelline space and Polar body were not independently statistically significant in predicting the quality of embryos. Both significant and non-significant parameters were included in the score so as not to rule out the possibility of occurrence of simultaneous presence of multiple such parameters in a single oocyte with poorer outcomes (embryo quality and subsequent results). We have considered these parameters due to the results published in various studies regarding their importance in determining the embryo quality and IVF outcomes.[6,7,8,9,10]

Therefore, a relative weightage was given to each analysed oocyte if one or more of the following morphologic characteristics were present: oval Zona pellucida, thick Zona Pellucida, Sudden/Difficult oocyte breakage, large refractile body, granulated perivitelline space, centrally located granular cytoplasm and vacuoles, large or fragmented or round polar bodies, large peri vitelline space and shape of oocyte. The oocytes that received the lowest score were expected to have the least implantation potential (due to the poorer embryo quality). Similar scoring system was proposed with only a few significant oocyte parameters by Rienzi et al.[11]

The total oocyte score was calculated by summing all the individual parameter scores of a given oocyte and mean oocyte score by dividing the sum of all total oocyte scores with number of mature oocytes.

Table 5 depicts the clinical and biochemical pregnancy distribution among the population upon fresh/frozen embryo transfer of at least 2 embryos and a maximum of 3 embryos at cleavage stage. The mean oocyte score was studied against the Clinical Pregnancy rate to establish a cut-off of the oocyte score for predicting pregnancy. ROC curves were constructed to find the predictability of our oocyte scoring system in predicting clinical pregnancy outcome and showed statistical significance (p value <0.001). The area under the curve of mean oocyte score for the prediction of clinical pregnancy was 0.756±0. 050. The cutoff value of mean oocyte score was 22.4 with sensitivity 73.2% and specificity 69.4%.

Based on the above details, >22.4 is considered as cut-off value in predicting clinical pregnancy and in our study. The results showed that a cut-off total oocyte score >22.4 could predict clinical pregnancy with an accuracy of 71%. This may be used in future to select embryos developed from oocytes with higher score and transfer them earlier to possibly reduce the time for pregnancy.

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Conflicts of Interest: None

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