Original Article

Prognostic Role of Pronuclear Morphology as a Marker of Embryo Quality

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Abstract

Background: Selection of transferred embryos for assisted reproduction required special care. Scoring of the morphological features for 18, 44, and 68 h after intracytoplasmic sperm injection. **Objectives:** The study aims to evaluate the correlation between pronuclear morphology and embryo quality. **Materials and Methods:** This prospective study included 85 intracytoplasmic sperm injection (ICSI) cycles. The following pronuclear morphological features were evaluated: pronuclear centering, proximity, number of pronuclear precursor bodies their polarization, orientation of pronuclei, and presence of cytoplasmic hallo. All are related to day 3 embryo development and morphology. **Results:** A total of 207 embryos were enrolled in the study. Of these, 67 embryos were considered as poor quality, and 140 embryos were considered as good quality. There was an insignificant difference between the good embryo quality group and bad embryo quality group in orientation, proximity centering, number of nucleolar precursor bodies, polarization, and c-halo ($P > 0.05$). A total score of 14 or more gives a sensitivity of 60% and spasticity of 56% to get a good embryo quality. **Conclusion:** Pronuclear morphology cannot be used as a prognostic marker for cleavage stage embryo quality.

Keywords: Embryo, ICSI pronuclear score, quality, zygote

Introduction

The ovarian environment, which is composed of a diverse range of physiologically active chemicals, serves as the oocyte's microenvironment during its development and maturity, which affect ultimately embryo quality.[1] Careful embryo selection affects the result of the assisted technique.[2] A lot of embryo scoring strategies were used. However, the most frequently used systems are the cleavage rate, size, and shape of the blastomeres together with the percentage of anucleated fragments.[3]

Previous research found that the optimal cleavage rate would be the most critical criterion when choosing day 2 and 3 embryos for intrauterine transfer.[4,5] Symmetry of embryos and multinucleation of blastomeres have also been shown to influence the implantation potential.[6] Accordingly, the human pronuclear stage is scored and evaluated depending based on pronuclear alignment, size, number, evenness, and heterogeneity of cytoplasm.[6-9] Pronuclear scoring system positively associated with the potential of implantation and live birth in zygote transfers.[10]

As it is for other systems of evaluating embryo morphology,[11] two principle systems for the assessment of pronuclear morphology were suggested by Scott and Smith[12] and Tesarik *et al*.[13]

Materials and Methods

From 85 ICSI cycles, 207 zygotes were involved in our study, which was done in an infertility treatment center in Alsadr Medical City. between September 2013 and December 2014. Couples enrolled in this study were treated by intracytoplasmic sperm injection. Ovarian

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stimulation was done, and 10,000 IU of human chorionic gonadotrophin (hCG) was given as a trigger for the final maturation of the oocyte.

The timing of the trigger was decided when the three follicles were ≥18 mm in diameter. Ovum pickup was done with transvaginal ultrasound guidance under light general anesthesia 35–36 h posthCG. Oocytes were gathered immediately into (37°C) *in vitro* fertilization culture medium (Fertipro) and CO_2 incubator. The semen samples of the male partner were analyzed and prepared by sperm wash and swim-up method. Oocyte injected with husband sperm using an inverted microscope (Olympus, Hachioji, Tokyo, Japan) equipped with a micromanibulator (RI).

Around 16–18 h after injection, oocytes were examined for signs of fertilization.

Normal fertilization was reflected by the presence of two distinct pronuclei.

"According to the appearance of a halo, a halo-positive zygote or a halo-negative zygote were classified" [Figure 1].

The Zygote Scoring System was used in this study.[10] The parameters that were considered in this system were: "orientation, proximity, and centering of pronuclei. Number and polarization of nucleoli (nucleolar precursor bodies, NPB) and finally the appearance of cytoplasmic hallo." Each parameter was scored from 1 to 3 (from the least quality to the best) cleaved (day 2 or 3) embryo scoring.

The morphology of the embryo was assessed 48–68 h after injection. The embryos graded from I to IV were as follows:

Grade I: have even regular blastomer with no or very few fragmentation rate;
Grade II: have uneven or irregularly shaped blastomer with not more than 10% fragmentation rate;
Grade III: fragmentation up to 50%; and
Grade IV: more than 50% of the blastomers were fragmented. It represents the worst quality embryos.

Results

The total number of embryos involved in this study were 207. Of these, 67 embryos were considered as poor embryo quality, and 140 embryos were considered as good embryo quality.

The relationship between pronuclei (PN) score and embryo quality is shown in Table 1. There were insignificant differences between the good embryo quality group and bad embryo quality group in orientation, proximity centering, no.NPB (number of NPB), c-polarization, and c-halo ($P > 0.05$).

About orientation in the good embryo quality group, 35.71% had a score of 3, proximity centering 51.43% had a score of 3, no NPB 68.57% had a score of 3, c-polarization 32.86% had a score of 3, and c-halo 20.71% had a score of 3.

The median of all scores was calculated, there was an insignificant difference between the good embryo quality group and the bad embryo quality group regarding total score, the median of both groups was equal to 13 [Table 1].

Factors associated with embryo quality at binary logistic regression were reported in Table 2. There was an insignificant association between studied factors and embryo quality ($P > 0.05$).

The reference group was bad quality embryo group. Receiver operating characteristic (ROC) curve of the total score for the good quality embryo group compared with the bad quality group is shown in Figure 2. A total score of 14 or more gives a sensitivity of 60% and spasticity of 56% to get a good embryo quality.

Discussion

Evaluation of the developmental potential of embryos is the single most important factor for their selection for transfer, and to achieve high pregnancy rates they suggested a score to identify embryos suitable for transfer.[14,15]

Till recently, literature data about the association between zygote morphology and the biological outcome of embryos was inconclusive, which may be due to differences in criteria used for PN evaluation and time of PN assessment.[16]

This study evaluated the prognostic effect of the PN morphology, we did not find any relationship between PN score and embryo quality as another study.[13,17-22]

The dynamic nature of morphological sequential changes occurring in the pronuclear zygote has a critical role in future embryo development and can be used as markers of embryo progress[23] Orientation of the PN decides the cleavage plane and will be vital for the polarity of embryo.[24] Inside the pronuclei, NPB behaves in a dynamic pattern at the time of PN movement; initially appear scattered, and this is followed by their polarization with alignment along the line of pronuclear contact.[25] In the cytoplasm, a clear halo appears in the peripheral area during fertilization due to the movement of organelles such as mitochondria from the cortex towards the center of the zygote around the PN.[26]

In our study, all these morphological changes were examined for pronuclear scoring to identify their correlation with embryo quality.

Asynchrony in the formation and polarization of pronuclei may be a sequential, linked event associated

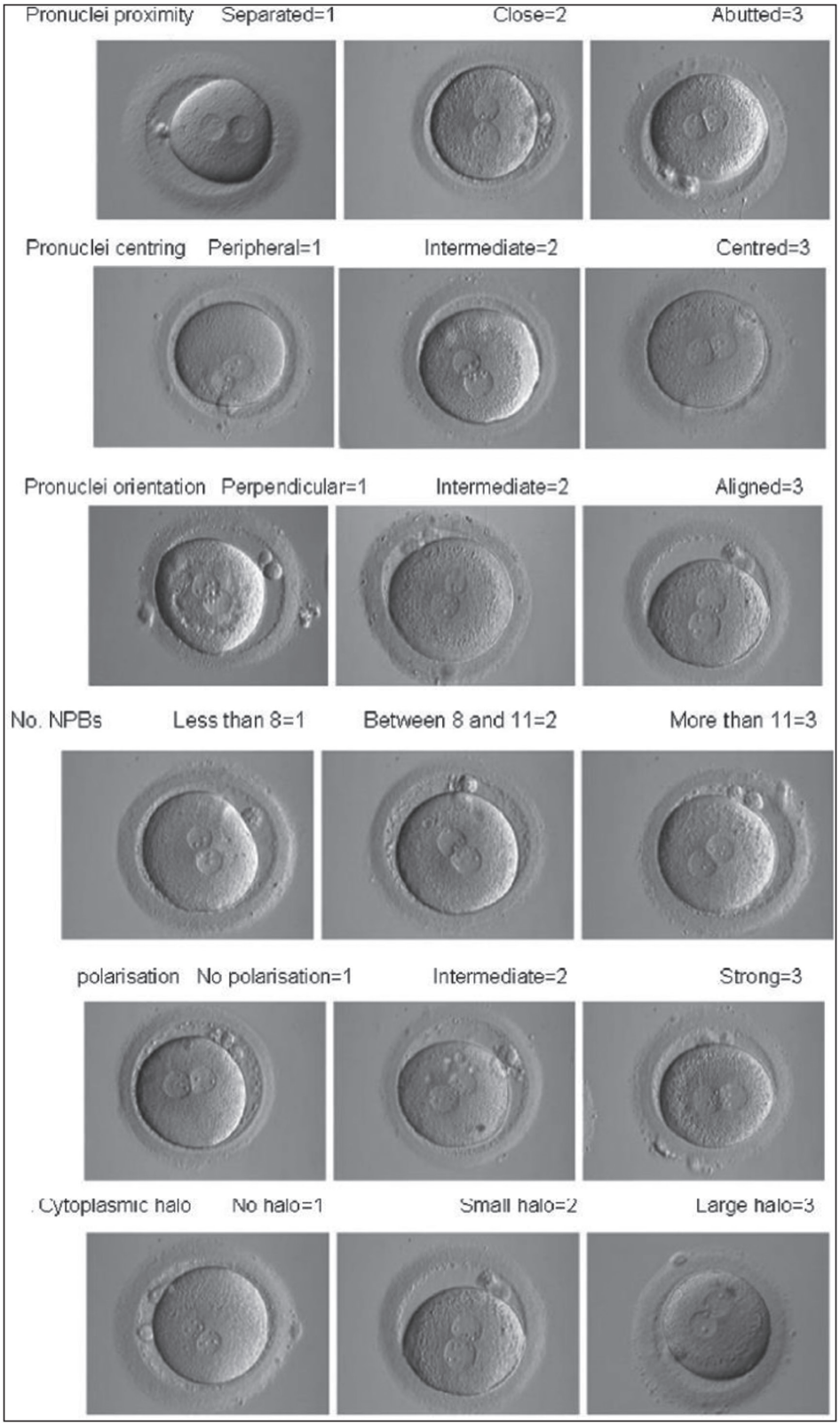


Figure 1: The Zygote Scoring System

Table 1: The relationship between PN score and embryo quality

Character	Score	Poor embryo quality	Percentage (%)	Good embryo quality	Percentage (%)	*P* value
Orientation	1	20	29.85	47	33.57	0.78
	2	24	35.82	43	30.71	
	3	23	34.33	50	35.71	
Proximity centering	1	21	31.34	45	32.14	0.82
	2	9	13.43	23	16.43	
	3	37	55.22	72	51.43	
	1	2	2.99	9	6.43	0.55
	2	19	28.36	34	24.29	
	3	46	68.66	97	69.29	
No.NPB	1	2	2.99	9	6.43	0.52
	2	19	28.36	34	24.29	
	3	46	68.66	96	68.57	
c-Polarization	1	6	8.96	21	15.00	0.45
	2	39	58.21	73	52.14	
	3	22	32.84	46	32.86	
c-Halo	1	18	26.87	48	34.29	0.55
	2	34	50.75	63	45.00	
	3	15	22.39	29	20.71	
Total		67		140		207
Total score median		13		13		0.63

P value < 0.05 is considered significant

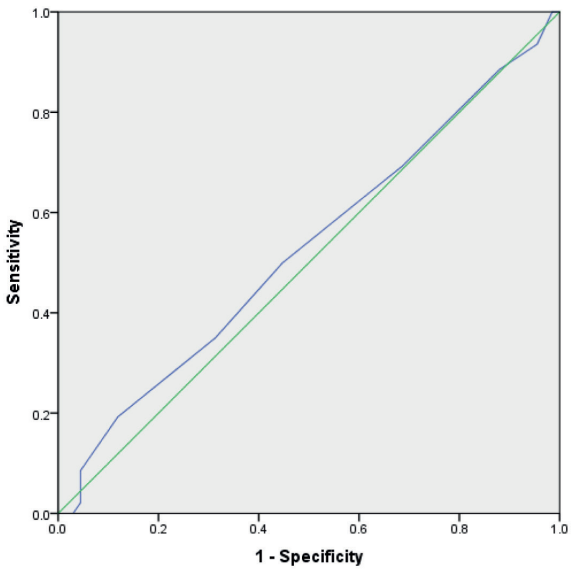
Table 2: "Binary logistic regression" of predictors factors for embryo quality

Character	Score	*P* value	Odds ratio	CI	
				Upper	Lower
Orientation	1	0.589	0.824	0.408	1.664
	2	0.832	1.081	0.526	2.220
	3				
Proximity centering	1	0.772	1.101	0.574	2.114
	2	0.538	1.313	0.552	3.124
	3				
	1	0.338	2.156	0.448	10.385
	2	0.649	0.857	0.442	1.663
	3				
no.NPB	1	0.338	2.156	0.448	10.385
	2	0.649	0.857	0.442	1.663
	3				
c-Polarization	1	0.332	1.674	0.592	4.735
	2	0.734	0.895	0.472	1.697
	3				
c-HALO	1	0.445	1.379	0.604	3.150
	2	0.912	0.958	0.453	2.029
	3				

with chromosomal abnormalities, with their consequences appearing after activation of the embryonic genome.[27-29]

Another studied factor is the appearance of cytoplasmic halo. Payne *et al*.[23] were the first to report a suboolemmal district of clear cytoplasm soon before the composition of the male and female pronuclei. This concern a focused clear field of cortical cytoplasm repeatedly progressing to involve all cytocortex (character). This wonder is a proverb showing the switch of mitochondria and various cytoplasmic organelles to the perinuclear domain.[30] This feature may be a part of the cycle regulation and activation by mobilization of calcium and adenosine triphosphate release.[31-34] Again, our study documented that there is no significant correlation between this phenomenon and embryo quality.

Nuclear precursor bodies are another parameter evaluated in the zygote score. A lot of studies indicated



Area	Cutoff value	Sensitivity	Specificity	95% CI	
				Lower Bound	Upper Bound
0.58	14	60%	56%	0.445	0.610

Figure 2: ROC curve of total score for good quality embryo group compared to bad quality group

that the morphology of the pronuclear oocyte has a direct influence on continuous *in vitro* progress.[12,14,35]

The absence of polarization of the NPB may be a morphological expression of a lack of chromatin polarization, which indicates a slower and much poorer development.[36]

As noticed, high-quality zygotes can develop into low-quality embryos and vice versa. Zygotes cannot predict preimplantation embryo morphology and developmental potential.

Embryonic development demonstrates that the morphology of zygote variates within a short period.[37] Therefore, a single microscopic evaluation may be confusing and such alterations may explain the inconsistent study and reports.[16,38]

Conclusion

Pronuclear morphology cannot be used as a prognostic marker for cleavage stage embryo quality.

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Conflicts of interest

There are no conflicts of interest.

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