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ASSISTED REPRODUCTION TECHNOLOGIES

Embryo quality but not pronuclear score is associated with clinical pregnancy following IVF

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Abstract
Purpose The association between pronuclear (PN) scoring of embryos from assisted reproductive technology (ART) and clinical pregnancy remains controversial. We hypothesized that embryos with PNs scored on the day of fertilization check offer better embryo selection on day 3 and higher CPR compared to non-PN scored embryos.
Methods Patients (19–46 years) undergoing IVF/ICSI cycles at Montefiore's Institute for Reproductive Medicine and Health between January 2006 and December 2009 were included in our study. We analyzed fresh day 3 cycles only with autologous oocytes and partner's fresh sperm (n=344). A total of 1,899 embryos were included. We compared CPR from non-PN scored embryos (Group 1, n=835) with PN scored embryos (Group 2, n=1,064). Composite scores by patient were developed based on embryo disposition. We also assessed traditional embryo grading derived from cell number, fragmentation and cell symmetry. Data analysis included chi square and t test to determine if PN scoring was associated with improved CPR, and to compare the additional variables.
Results CPR between Group 1 and Group 2 were not different (p=0.91). CPR was significantly associated with female age, number of mature oocytes retrieved, number of day 3 embryos and grade of embryos transferred on day 3 (p<0.05).
Conclusion PN scoring was not associated with improved CPR in day 3 embryo transfers. Mean grade of transferred embryos continues to be a well-established, independent predictor of CPR. We conclude that further refinement of embryo grading by PN scoring is not beneficial.

Keywords PN scoring · Embryo grading · IVF · ICSI · Clinical pregnancy rates

Capsule Pronuclear scoring is not associated with improved clinical pregnancy rates in day 3 embryo transfers.

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Background

Following assisted reproductive technologies (ART), embryos are selected for uterine transfer based on the timing and rate of cell division, and gross morphology. Grading of day 3 embryos comprises cell number, degree of fragmentation, cell symmetry and presence or absence of cytoplasmic pitting, and has been shown to predict ART outcomes [1–3]. Better morphology grade embryos have been associated with significantly improved implantation rates and live birth rates [4, 5].

On day 1 after fertilization, pronuclear (PN) morphology and arrangement of nucleolar precursor bodies (NPB) provide the earliest information on sperm and egg interaction. NPB numbers, size and distribution within the PN membrane have been correlated to embryo quality, development and in vitro fertilization (IVF) outcome [6–11]. Specific PN morphology patterns, i.e. Pattern 0 [6] and Z1 or Z2 [10] have been associated with an increase in implantation and pregnancy rates [7, 8, 11–15]. Recently however, a number of

investigators have not been able to demonstrate an association between PN scoring and improved ART outcomes [16–18].

Aside from using chromosome analysis to select euploid embryos for transfer, embryo morphology criteria most predictive of clinical pregnancy following ART remains equivocal. Our objective was to examine the association of PN scoring and clinical pregnancy rate (CPR) in a large sample size. We hypothesized that embryos with PN score on the day of fertilization check offered better embryo selection on day 3 and resulted in higher CPR compared to embryos that with no PN score.

Materials and methods

Patients (ages 19–46 years) undergoing ART at Montefiore's Institute for Reproductive Medicine and Health between January 2006 and December 2009 were included in our study. Approval for the study was obtained from the institutional review board of Montefiore Medical Center. We analyzed fresh day 3 IVF-ET only with autologous oocytes and partner's fresh sperm (n=344). A total of 1,899 embryos were included. Integrated in our data analysis were female age, maximum FSH, number of mature oocytes retrieved, number of embryos transferred, number of embryos on day 3 and the grades of embryos transferred. We then compared cycle parameters and outcomes from non-PN scored embryos (Group 1, n=835) to PN scored embryos (Group 2, n=1,064). Group allocation was decided based on the year of the retrieval; Group 1 cycles were retrieved January 2006–December 2007 and Group 2 cycles were retrieved January 2008–December 2009.

IVF cycle characteristics evaluated included serum level of estradiol (pg/mL) and progesterone (ng/mL) on the day of and the day after triggering of ovulation with human chorionic gonadotropin (hCG), type of oocyte insemination, number of eggs retrieved, number of mature eggs, total number of embryos available on day 3, number of embryos cryopreserved and the outcome of the fresh cycle (i.e. clinical pregnancy defined as intrauterine gestational sac on transvaginal sonogram). Hormone levels were measured to monitor proper ovarian stimulation; patients who were cancelled due to hypo- or hyper-response were not included in this study. Oocytes were retrieved on day 0 and inseminated via insemination (IVF) or intracytoplasmic sperm injection (ICSI). On day 1, 16–18 h after insemination, all embryos were checked for fertilization. Group 1 embryos were further evaluated for PN grading [10]. PN grades given included Z1, Z2, Z3-1, Z3-2, Z3-3, Z3-4, Z4-1 and Z4-2 based on Scott's grading system [11]. These grades were then converted to generate a PN score of 1.0, 2.0, 3.0, 4.0; PN Grade of Z1=PN Score of 1.0, PN Grade Z2=PN Score 2.0, PN Grades Z3-1, Z3-2, Z3-3, Z3-4= PN Score 3.0, and PN Grade Z4-1 and Z4-2=PN Score 4.0. The composite scores per cycle were then calculated for all embryo dispositions by averaging PN scores of all embryos with identical disposition (embryos transferred, frozen or discarded).

All embryos in Group 1 and Group 2 were checked on day 2 for cell number and fragmentation. On day 3 all embryos were checked for cell number, fragmentation and cell symmetry, then embryos were scored according to SART scoring system [1]. In order to compile day 3 embryo data for comparison between patients, we developed a composite grade for all embryo dispositions by patient (embryos transferred, frozen or discarded). Based on cell number, percent fragmentation and cell symmetry, all day 3 embryos were assigned a grade 1.0–3.0, as per criteria described in Table 1. The composite scores were then calculated for all embryo dispositions by averaging day 3 grades of all embryos with identical disposition. Uniformity of grading in the laboratory was routinely monitored (every 6 months) for inter-technician variation using standardized proficiency testing services.

Data analysis included chi square and Student's t-test to determine if PN scoring improved CPR. P values<0.05 were considered to be statistically significant.

Results

We compared a number of cycle variables in pregnant versus non-pregnant patients, a summary of which is presented in Table 2. A total of 1,899 embryos from 344 cycles were studied. The overall pregnancy rate of this cohort was 37.2 %. The use of conventional IVF, ICSI or IVF/ICSI split was not significantly different between Groups 1 and 2. (P= 0.07). For patients in Group 2, we developed composite PN scores, by patient, based on embryo disposition. Therefore each patient had a composite PN score for embryos transferred, frozen and discarded. Composite PN scores were not different between patients who became pregnant and those who did not (Table 3). Further, pregnancy rates for patients with or without PN scores were not significantly different (37.5 % and 37.9 % respectively, p=0.91).

Embryo quality on day 3 prior to transfer is routinely assessed. While we did not observe a difference in CPRs between PN scored and non-PN scored embryos, a significantly lower CPR was associated with higher female age (p<0.01), fewer mature oocytes retrieved (p=0.04), number of embryos on day 3 (p<0.01), and a lower mean grade of embryo(s) transferred on day 3 (p<0.01) (Table 2).

Secular trend is a drift in outcomes associated with a change that is not cyclical or seasonal and exists over a long period of time, and can occur in an IVF program [19]. To confirm that there were no doctor, embryology or media-related shifts in grading during the study period, we compared grades of transferred embryos between Group 1 and Group 2.

Table 1 Embryo grading system

Grade	Number of cells	% Fragmentation	Cell symmetry
1.0	6–8 cells	<5 % fragmentation	Equal blastomeres
1.5	6–8 cells	≤10 % fragmentation	Equal blastomeres
2.0	4–6 cells	≤25 % fragmentation	Almost equal blastomeres
2.5	Cell number did not double in 24 h	≤30 % fragmentation	Unequal blastomeres
3.0	Cells did not divide	>50 % fragmentation	Unequal blastomeres

Embryo grades were determined based on numeric conversion of cell number per embryo, percent fragmentation and cell symmetry, which were evaluated on day 3 of embryo development

Composite day 3 mean grades of embryos transferred were not significantly different (p=0.8) between Group 1 (n=184, mean ±SEM=1.7±0.4) and Group 2 (n=160, mean±SEM=1.7± 0.4) (p=0.8). There was no impact of grader (embryologist) or media on PN or embryo day 3 grade (p=0.8).

Discussion

In this study, we found that PN scores on day 1 were not associated with improved CPR following day 3 embryo transfer, indicating NPB patterns are not associated with improved embryo selection, leading to improved pregnancy rates. We did not find any correlation between PN scores and IVF cycle parameters or outcomes. We did observe that the composite grade of embryos transferred on day 3 was a predictor of clinical pregnancy. As expected, the embryos with better morphology on day 3 resulted in clinical pregnancy. Younger female age, number of mature oocytes retrieved and number of embryos viable on day 3 were significant predictors of clinical pregnancy.

Pronuclei develop in zygotes where sperm has successfully penetrated an oocyte, whether by conventional IVF or ICSI.

Table 2 Demographics and clinical characteristics of all the participants by pregnancy outcome

	Clinical Pregnancy n=(128)	Not Pregnant n=(216)	p-value
Female age, years	34.5 (4.3)	36.1 (4.5)	<0.01
Max FSH (mIU/mL)	7.6 (2.7)	7.7 (2.3)	0.71
Number of mature oocytes retrieved	10.0 (4.8)	8.6 (5.0)	0.04
Number of embryos transferred	2.8 (0.8)	2.7 (1.0)	0.26
Number of embryos on Day 3	6.2 (3.4)	5.1 (3.3)	<0.01
Mean grade embryos transferred	1.6 (0.4)	1.7 (0.4)	<0.01
Mean PN score transferred embryos	2.1 (0.7)	2.0 (0.6)	0.63

Data presented as mean (SD) or prevalence. Mean PN score includes only embryos transferred; 951 embryos of the original 1,899 were either cultured out to day 5 or discarded

The assembly of NPB requires pronuclear chromatin and is indicative of the end of cell cycle phase G1 and the start of synthesis and processing of ribosomal RNA [20–22]. PN scoring in human IVF was originally reported as a predictor of embryo development [6, 7, 10, 11]. The two most well known methods of PN analysis have been described by Tesarik as *Patterns 0–5* [6] and by Scott as *Z scores 1–4* [10]. Early literature supported the predictive power of PN scoring following IVF and ICSI. This includes a study of 787 embryos that reported PN scores predicted the rate of embryo development on day 3 [23], a 2007 study that observed PN patterns from 569 embryos predicted both good and poor quality embryos depending upon the pattern [20], and a study of 2,836 zygotes that showed PN scores were predictive of developmental ability on day 3 as well as implantation potential [24].

More recently however, there have been studies reporting a lack of correlation between PN score and ART outcome [16–18, 25, 26]. Cell number and embryo grade are more predictive of implantation rate than PN morphology in a study of 852 embryos when all or none of the embryos implanted [18]. Nicoli et al., 2007 found that 1,078 PN-scored embryos showed no relationship between PN patterns and embryo quality or clinical pregnancy [27]. A smaller study of 331 embryos which were also biopsied, reported no association between PN pattern, embryo quality or chromosome status [28]. Some of the discrepancy seen in the literature could be the result of inexact timing of PN observation; if a laboratory is not consistent, PN patterns may be visible but change over time and depending on the PN check relative to fertilization,

Table 3 Composite PN scores and pregnancy outcome of participants in Group 2

	Clinical pregnancy	Not pregnant	p-value
Transferred embryos, n=160	2.1 (0.7)	2.0 (0.6)	0.63
Frozen embryos, n=18	1.8 (0.8)	2.3 (0.6)	0.15
Discarded embryos, n=107	2.2 (0.6)	2.2 (0.7)	0.82
Mean PN score transferred embryos, n=948	2.1 (0.7)	2.0 (0.6)	0.63

Data presented as mean (SD)

could result in discrepant PN scoring. This could be especially variable in conventional IVF embryos in which cases the sperm could penetrate the zona pellucida at any time after being added to the culture dish.

The length of time an embryo is out of the incubator may play a role in embryo stress leading to clinical pregnancy, and different embryologists may have slightly different techniques, which could influence the amount of time an embryo is out of the incubator during PN scoring [29]. Our study tested whether PN scoring with day 3 morphology was beneficial; our theory was based on the assumption that the more information acquired from the embryo before transfer the better. However, we found that day 3 morphology alone was associated with pregnancy outcome and refining embryo selection by adding PN scoring did not improve pregnancy outcomes. Fortunately, we did not see any negative impact on embryos from PN scoring, indicating time out of the incubator was not detrimental to embryo grade nor to CPR in our lab.

Selecting the best embryos which result in a pregnancy is a challenge across all IVF laboratories. While we did not see a correlation between PN scoring and pregnancy, we did observe a positive correlation between embryo quality, a high composite embryo score and clinical pregnancy, indicating embryo morphology on day 3 is predictive. Our conclusion is that early embryo analysis, more specific than 2PN fertilization, is not informative. Based on this study, we have resumed a group (up to 3 embryos) micro drop culture model and are no longer culturing zygotes and embryos individually to track associations between PN scoring and embryo quality prior to embryo selection for transfer. The advantages of group culture are two-fold: the embryos can benefit from endogenous growth factors when in the same drop [30, 31], and the time embryos are out of the incubator for observation is reduced when multiple embryos are cultured together.

The advantages of our study included a larger sample size than many of the previously published studies assessing the relationship between PN patterns and pregnancy rate, and that we included secular trend data analysis. This study was retrospective in design, a limitation we recognize. In addition, while we observed PN scores consistently within the same time frame relative to fertilization (16–18 h after insemination) for all patients in Group 2, it is possible that variation within this 2 h window may have confounded our findings.

Given the lack of importance of PN scoring, perhaps investigating other embryo characteristics on day 1 and/or 2 of development would be more beneficial for embryo selection. Very recently, time-lapse monitoring systems have been developed and early data suggests the precise time of cell division and cleavage pattern are associated with improved implantation and pregnancy rates [32–34]. Therefore even if PN score is not predictive, zygote and early embryo division prior to day 3 may still be helpful in predicting embryo potential. If and until time-lapse technology becomes standard-of-care in the IVF laboratory, standard morphology including cell number, symmetry and fragmentation on day 3 remains the gold standard for embryo selection for transfer of cleavage stage embryos.

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