PRONUCLEAR TRANSFER IN ICSI PATIENTS: OUR RESULTS AT "SAPIENZA" UNIVERSITY OF ROME, ITALY

Linari A,MS, Bezerra Espinola MS,PhD, Micara G,MS,Tranquilli D,MS, Boninsegna C,MS and Aragona C,MD

Infertility and Assisted Reproduction Unit, Obstetrics and Gynecology Department, "Sapienza" University, Rome, Italy

Abstract

Objective: ssessment of pronuclear embryo transfer efficacy (Day 1) compared to Day 3 embryo transfer at the Infertility and Assisted Reproduction Unit, Ob/Gyn Dept, "Sapienza" University, Rome, Italy.

Material and method:A retrospective study comprised of 461 ICSI patients were retrospectively evaluated between May 2010 and December 2011. In 183 cycles embryo transfer was performed 26-28 hours after ICSI (Group A) and in 278 cycles embryo transfer was performed on Day 3 (Group B). Zygotes at pronuclear stage were classified according to a new simplified criteria. Only "good quality" embryos were transferred in both groups.

Results:o statistical significant differences were found between group 1 and group 2.

Conclusion: In conclusion, pronuclear "good" embryo transfer remains a viable and easy option for IVF, resulting in clinical pregnancy and delivery rates comparable to those reported from Day 3 transfer.

Introduction

Many different non-invasive methods based on various morphological criteria have been used to analyze potential development of zygotes and embryos. Embryo quality is generally determined considering several parameters, i.e. cleavage rate, blastomeres regularity, fragmentation degree, multinucleation presence, cytoplasmic irregularities and development ability until blastocyst stage. In the last few years it has been highlighted the role of prolonged embryo culture from three to five/six days in order to improve IVF outcomes.

Zygote morphological characteristics (two pronuclei presence and regularity after fertilization) have also been used to predict embryo quality (1-9). Scott (10) improved her previous zygote pronuclear scoring system using five categories (Grade 1-5) based on nucleoli number and distribution, referred as Z-score system. Furthermore, polar bodies orientation respect to pronuclei position has been used as an additional parameter to categorize zygotes. All together these morphological parameters determine a very complicated system for zygote quality assessment, which has been formalized by Gianaroli (11). However, given the big number of possible combinations for all parameters, all pronuclear grading systems are quite difficult to be used for clinical purposes, especially in large IVF centers.

Aim of the present study is to report our experience regarding pronuclear zygote transfer performed the day following oocyte retrieval at "Sapienza" University of Rome using a simplified classification developed in our laboratory and to compare results with Day 3 embryo transfer.

Correspondence
Cesare Aragona MD, Infertility and Assisted Reproduction Unit, Obstetrics and Gynecology Department, "Sapienza" University,
Rome, Italy.
Fax: +39 0649972548
E-mail: cesare.aragona@uniroma1.it

Material and methods

The study was reviewed and approved by Institutional Review Board and all patients gave informed written consent.

Patients

461 ICSI Cycles were retrospectively evaluated at IVF Unit, "Sapienza" University of Rome – Italy from May 2010 to December 2011. Female patients undergoing ICSI cycles aged from 26 to 42 (mean age 35.92 ± 3.6), while male patients' age ranged from 30 to 51 (mean age 39.68 ± 4.8).

All patients included in this study had a normal karyotype, normal hormonal assessments, negative vaginal or urethral cultures and had no malignancy or systemic diseases.

All patients who underwent a standard infertility evaluation were nulliparous with previous failed IVF cycles ranging from 0 to 4 and none of them showed basal FSH >10 mIU/ml or E2 > 40 pg/ml on cycle Day 3. No difference was found between the two groups.

Ovarian stimulation and oocyte retrieval

ICSI cycle management consisted of down regulation with a long protocol starting from day 21 of the pretreatment cycle with a GnRH agonist (Decapeptyl 0.1 ml, daily subcutaneous (s.c.), IPSEN/BIOTECH, Paris, France). Once ovarian suppression was assessed by E2 profiles and ovarian ultrasound scan (US), daily subcutaneous administration of 150 IU urinary or recombinant FSH was commenced.

From the seventh day of stimulation, daily monitoring of follicles size by US was performed and plasma levels of E2 and progesterone were measured. From this stage, the dose of FSH was adjusted depending on the individual response of each patient. Criteria used for triggering ovulation with 10.000 IU hCG (Gonasi HP 5000® IBSA) s.c. were plasma E2 between 1000 and 3000 pg/ml and at least four follicles >18 mm mean diameter (two perpendicular measurements) with plasma Progesterone < 1.5 ng/ml. Oocyte retrieval was performed 36 hours after hCG administration, by transvaginal US-guided follicular aspiration under i.v. sedation.

Semen preparation and insemination

Preparation of semen samples collected by masturbation was performed following the World Health Organization (WHO) standard protocol (12). ICSI was performed according to procedure reported by Palermo (13) and exclusively on metaphase II oocytes.

Fertilization assessment

Oocyte fertilization was assessed 16-18 hours (Day 1) after ICSI to confirm two pronuclei presence. Morphological parameters used to evaluate pronuclear grading were: 1) pronuclei position and size, 2) nucleoli number, size and distribution in the pronuclei, 3) pronuclei orientation respect to polar bodies according to Gianaroli (11). We routinely adopted a simplified score, classifying pronuclei, according to our experience, in four categories, from PN1 (best) to PN4 (worst).

PN1 is assigned when pronuclei are centralized, juxtaposed, with large size nucleoli aligned in a row at pronuclear junction or scattered in both pronuclei and polar bodies aligned or perpendicular to pronuclear axis. When pronuclei are as PN1 but peripheral, with nucleoli as PN1 but with polar bodies neither aligned nor perpendicular, or with asymmetric nucleoli pattern or with small size nucleoli, zygotes are defined PN2.

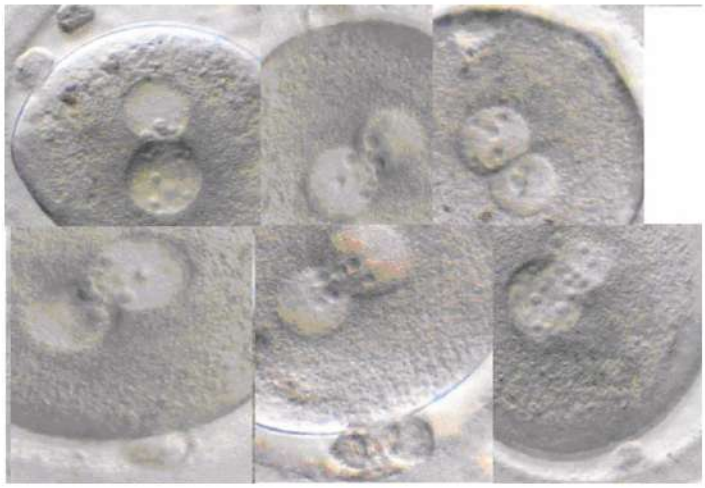
PN3 is consisted with juxtaposed pronuclei, equal in size and peripheral, with big nucleoli in a row or scattered, but with polar bodies neither aligned nor perpendicular to pronuclei axis, or when nucleoli were asymmetrically disposed or small and scattered in peripheral pronuclei. PN4 is when pronuclei are not juxtaposed or disequal in size or fragmented with all type of nucleoli pattern and all polar bodies position respect to pronuclei axis. The Simplified Pronuclear Score (as modified from Gianaroli) routinely used in our laboratory is presented in Table I e Figure 1.

Table (I):
New Simplified Pronuclear Score

Pronuclear Position	Nucleolar Characteristics	Pronuclei / Polar Bodies	Pronuclear Cathegories (Pn)
A	1 , 2	α , β	1
A	1 , 2	γ	2
A	3 , 4	α , β , γ	2
B	1 , 2	α , β	2
B	1 , 2	γ	3
B	3 , 4	α , β , γ	3
C	1 , 2 , 3 , 4	α , β , γ	4
D	1 , 2 , 3 , 4	α , β , γ	4
E	1 , 2 , 3 , 4	α , β , γ	4

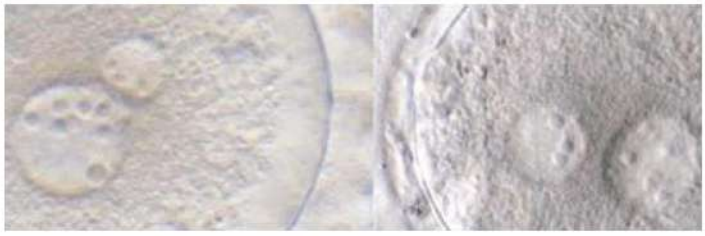
Fig.1: New Simplified Pronuclear Score images

PN1



PN2

PN3



PN4

Embryo Quality Assessment

In our laboratory embryos are routinely scored at 40-42 hours (Day 2) and 68-72 hours (Day 3) after ICSI according to Veeck classification. A morphologic grade (I, II, III, IV, V) is assigned according to blastomeres number and fragmentation percentage (14, 15).

Embryo Transfer

Embryo transfer was performed on Day 1 (Group A) or Day 3 (Group B) after oocytes retrieval and ICSI, under US guidance, using a Wallace embryo transfer catheter. All transfer procedures were performed by the same physician.
Only "good quality" embryos were randomly transferred on Day 1 or Day 3.
-Embryo transfer on Day 1:

- 183 transfers were performed 26-28 hours after ICSI (pronuclear stage).
- 398 pronuclear stage zygotes (PN1, PN2) were transferred.

- Embryo transfer on Day 3:

- 278 transfers were performed in Day 3 after ICSI (cleavage stage).
- 634 embryos (EG I, EG II) were transferred.

All patients undergoing embryo transfer received supplemental intramuscular progesterone (50 mg/day; Prontogest; AMSA, Italy) from the day of embryo transfer until β-hCG assay.

Establishment of Pregnancy

Biochemical pregnancy is initially determined 14 days after embryo transfer by a positive qualitative serum β-hCG assay (>50 mIU), followed by repeated quantitative β-hCG evaluations. A clinical pregnancy is defined as at least one positive heartbeat embryo revealed by transvaginal sonography at 4 to 5 weeks after embryo transfer. Implantation rate is defined as gestational sacs number, detected by ultrasound scan, divided by the number of transferred embryos.

Statistical Analysis

Data were retrospectively collected and analyzed by t Student and Chi-square tests when frequencies were compared, while t tests only was utilized when different groups mean values were compared. P values <0.001 and <0.05 are considered to indicate a statistically significant difference, while being N.S. not significant.

Results

Results are shown in Table II.

Table (II) :

pronuclear embryo transfer versus Day 3 embryo transfer:

	Group A (Day 1)	Group B (Day 3)	Significativity
N. ICSI Cycles	183	278	
Mean Age	35,3 (± 3,7)	35,1 (± 3,5)	N.S.
N. Pronuclear Embryos (PN1, PN2) or Embryos (EG I, EG II) Transferred	398	634	
N. Pronuclear Embryos (PN1, PN2) or Embryos (EG I, EG II) Transferred for patient	2,17 (± 0,6)	2,28 (± 0,5)	N.S.
Pregnancy Rate (%)	32.7%	33.9%	N.S.
Implantation Rate (%)	19,8%	20,3%	N.S.
Abortion Rate (%)	18,8 %	18,6 %	N.S.
Multiple Prangnancies (%)	16,2 %	17,1%	N.S.

Discussion

Although many factors influence results of Assisted Reproductive Technologies (ART) procedures (e.g. stimulation response, endometrial receptivity, oocyte maturity, paternal contribution and culture conditions) embryo morphology is certainly regarded as one of the most important. Current systems of embryo scoring are based on their morphology (pronuclear morphology, cleavage rate, blastomere number and morphology) (16) which has been shown to be closely linked to embryo viability (17). There has been reported a relationship between zygote morphology at 16-18 hours post insemination and its ability to continue development (10, 18). Various pronuclear scores have been proposed to identify zygotes in relation to particular characteristics (pronuclei size and position, nucleoli number, size and alignment and pronuclei orientation respect to polar bodies) (2, 3, 4, 10). Beneficial reports on scoring criterion are followed by others which do not see any improvement using it (19) and it has been claimed as not justifiable to spend additional time for scoring zygotes if the outcome is questionable or of no benefit at all.

In our study, we have evaluated pronuclei morphology utilizing a simplified pronuclear scoring system alternative to that proposed by Gianaroli (11). Zygotes have been catalogued in four classes ("PN "1", PN "2", PN "3" and PN "4"). Pronuclear transfer was already experienced by our centre and successfully reported in several studies (1, 2, 10, 20). Due to IVF restrictive Italian law from February 2004 to May 2009, we firstly elected to perform pronuclear stage embryo transfer at 1 Day after oocytes retrieval rather than Day 3 transfer. Pronuclear transfer was performed in part because no more than 3 oocytes could be inseminated but also when less than 3 oocytes were retrieved.

On May 2009, even though IVF Italian law was reviewed, removing some limitations, pronuclear transfer was continued. Retrospective study reported was undertaken comparing outcome of cycles with transfer at pronuclear stage (Day 1) versus cycles with transfer at Day 3. In the light of present experience, we confirm that it is successfully possible to select and transfer high quality pronuclear stage embryos. The success with pronuclear embryo transfer could be related to the less anoxic environment of human uterus. In fact this allows early embryo to continue to perform oxidative phosphorylation at a stage when glucose utilization through glycolysis is not possible due to lack of phosphofructokinase (2), in presence of an early secretory endometrial pattern.

Conclusions

Our results indicate that "the new proposed simplified pronuclear score" is a practical method to classify zygotes, in order to identify and transfer "good quality" embryos. Besides this, pronuclear transfer clinical success, already well documented in literature, has been confirmed useful when longer in vitro culture is unfit. In fact when small numbers of oocytes/embryos are present, a shorter in vitro culture could be suggested considering uterus environment suitable for embryo development and future implantation. In conclusion, transfer of "good" pronuclear embryos remains a viable and easy option for IVF, resulting in clinical pregnancy and delivery rates comparable to those reported for traditional Day 3 transfer.

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