The validity of the 0PN fertilization pattern

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Background

The evaluation of zygotes with non-standard ProNuclear (PN) patterns, such as 0PN, 1PN, and 3PN, along with other rare variations, has gained increasing interest as a strategy to optimize embryo availability and maximize cumulative clinical success per cycle [(1)]. However, this approach raises both clinical and ethical concerns, particularly regarding the controversial hypothesis that 0PN zygotes may develop through pre- and post-implantation stages without pronuclear formation. Time-Lapse Technology (TLT) serves as a valuable tool in addressing these uncertainties by enabling continuous and precise monitoring of embryo morphokinetics [(2)].

Aims

This study aims to determine whether the 0PN fertilization pattern, assessed through static observation within the 16–18 Hours Post-Insemination (HPI) window, permits embryo development in the absence of conventional pronuclear formation.

Results

This retrospective study included patients who underwent ovarian stimulation (GnRH antagonist protocol with hCG/agonist trigger) between 2013 and 2020, yielding a total of 6052 oocytes. All oocytes were inseminated via ICSI and cultured in a TLT incubator. The ICSI cycles resulted in 5059 zygotes displaying at least one PN, 249 degenerated oocytes, and 744 zygotes classified as true 0PN. Embryo development was monitored until the blastocyst stage.

Morphokinetic analysis revealed critical associations between pronuclear behavior and blastocyst development. Among 4478 2PN zygotes, those in which PN fading occurred between 18–20 hpi exhibited the highest blastocyst formation rate (<16 hpi: N=10, 40.0%; 16–18 hpi: N=44, 47.7%; 18–20 hpi: N=309, 65.4%; >20 hpi: N=4115, 53.4%; p<0.001). Conversely, in 87 cases where PN formation was delayed beyond 16 hpi, blastocyst formation was significantly reduced (54.5% vs. 31%; p<0.001).

Among the 744 true 0PN zygotes, 94 (13%) underwent at least one cleavage event; however, none progressed beyond the compaction stage (cell count: mean±SD 3.1±1.6, median 3, range 1–10). The majority exhibited abnormal cleavage patterns, including direct, reverse, and chaotic divisions (N=71, 76%), while others displayed significant vacuolization (N=15, 16%) and/or extensive fragmentation (N=36, 38%). Statistical regression analysis, adjusted for confounders such as maternal age, male factor, and PN type, confirmed these associations through Generalized Estimating Equations (GEE).

Conclusions

A small proportion of zygotes capable of reaching the blastocyst stage exhibit pronuclear formation beyond the conventional 16–18 hpi window. In the rare cases where cleavage occurs without pronuclear formation, development invariably ceases at an early stage. The application of TLT highlights the limitations of traditional static observation, which fails to detect a significant number of zygotes, with implications for fertilization assessment, blastocyst prediction, and the utilization of zygotes with atypical pronuclear patterns. Furthermore, TLT provides strong evidence against the hypothesis that blastocyst formation can occur in the absence of pronuclei. Independent validation of these findings is warranted, along with further investigations into fertilization morphokinetics in conventional IVF zygotes.

Bibliography

1 *Capalbo A, Cimadomo D, Coticchio G, Ottolini CS. An expert opinion on rescuing atypically pronucleated human zygotes by molecular genetic fertilization checks in IVF. Hum Reprod. 2024;39(9):1869-78.*

2 *Coticchio G, Mignini Renzini M, Novara PV, et al. Focused time-lapse analysis reveals novel aspects of human fertilization and suggests new parameters of embryo viability. Hum Reprod. 2018;33(1):23-31.*