TITLE: UNEVEN PRONUCLEATES (PN) ARE ASSOCIATED WITH SLOWER EMBRYO DEVELOPMENT

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BACKGROUND: Assessing biomarkers that may affect embryo viability is difficult and time consuming. Artificial Intelligence tools may allow for timely and in-depth analysis of biomarkers that could be associated with embryo viability, bringing biological insights to embryo assessment that are traditionally missed.

OBJECTIVE: To assess the impact of uneven PNs on embryo development and viability.

MATERIALS AND METHODS: This is a retrospective comparative study performed in a single academic affiliated fertility center with 460 2PN embryos cultured in a time-lapse incubator between March 2020 and April 2022. CHLOE, an artificial intelligence (AI) support tool for embryologists, automatically annotated: PN size (um^2), morphokinetic annotations (tPNf-tEB), CHLOE Blast Score, CHLOE EQ score, embryo quality (Good vs poor). PN size difference at 18 hours post insemination (hpi) was categorized into two groups: even (less than 20% difference in size, n=374) and uneven (>20% difference in size, n=43). The groups were compared in terms of overall blastulation. For statistical analysis, chi-square and binary logistic regression were used for comparison of blastulation, regression analysis was used for chloe EQ score, and t-test was used for morphokinetics. Data presented as even vs uneven.

RESULTS: Uneven PNs lead to slower development of the embryo (table 1). PN unevenness did not affect embryo quality as assessed by CHLOE EQ Score (0.44 ± 0.41 vs 0.53 ± 0.44, p=0.214), Blast Score (0.43 ± 0.37 vs 0.42 ± 0.34), proportion of morphologically good quality embryos (45% vs 53%) or blastocyst conversion rate [67% (273/407) vs 70% (32/46), p=NS]. PN unevenness, was not affected by embryo area (0.13 ± 0.01 vs 0.12 ± 0.008um2, p=0.319), zona pellucida thickness (0.07 ± 0.01 vs 0.07 ± 0.009um, p=0.430), perivitelline space (0.04 ± 0.01 vs 0.04 ± 0.01um, p=0.847)

CONCLUSION: Unevenness of PNs is associated with slower development, nonetheless it does not affect blastulation and embryo quality.

Support: Fairtility

Reference

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Table 1. Time to developmental milestones (hours post insemination) of the embryo with uneven and even pronucleates

Event	Uneven PN (hours)	Even PN (hours)	P-value
tPNf	9.0 ± 6.4	11.8 ± 10.3	0.09
T2	10.1 ± 4.9	13.0 ± 5.4	0.002
T3	12.8 ± 5.4	17.9 ± 8.7	<0.001
T4	16.8 ± 8.6	vs 18.2 ± 3	0.02
T5	23.1 ± 10.3	vs 27.45 ± 4.15	<0.001
T6	21.9 ± 10.1	vs 25.8 ± 8.4	0.009
T7	24.8 ± 10.6	28.9 ± 9.3	0.01
T8	26.0 ± 10.4	31.5 ± 10.6	0.004
T9	28.6 ± 9.35	34.9 ± 9.6	<0.001
tM	31.9 ± 8.4	39.6 ± 8.5	<0.001
tSB	36.3 ± 7.2	48.7 ± 7.8	<0.001
tB	38.9 ± 6.8	53.7 ± 9.4	<0.001
tEB	41.2 ± 7.2	56.3 ± 9.4	<0.001

Abbreviations; PN: pronuelceates, tPNF:pronuclei fading , T2: two-cell, T3three-cell, T4: four-cell, T5: five-cell, T6:six-cell, T7: seven-cell, T8: eight-cell, T9: nine-cell, tM: morula, tsB:strat of blastulation, tB:full blastocyst, tEB:expanded blastocyst