P-191 Time-lapse videography reveals morphometric and morphokinetic differences in the pronuclei of male and female human zygotes

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Study question: Do morphometric and morphokinetic profiles of pronuclei (PN) following intracytoplasmic sperm injection (ICSI) vary between male and female human zygotes?

Summary answer: Male and female zygotes displayed different PN morphometrics and morphokinetics. Additionally, variations were identified between sperm-originated (SPN) and oocyte-originated (OPN) pronuclei.

What is known already: Previous studies have investigated the use of PN-associated parameters via static observations as indicators of zygote viability, including size equality or juxtaposition. However, recent clinical application of time-lapse videography (TLV) provides a novel opportunity to assess these pronuclear events with greater accuracy and precision of morphometric and morphokinetic measurement. A number of recent TLV studies have also investigated potential live birth prediction by such PN associated measures, however whether or not there are gender associated differences in such measures which could in turn confound live birth prediction is unknown. Study design, size, duration: This retrospective cohort study included 94 consecutive autologous single day 5 transfer cycles (either fresh or frozen) performed between January 2019 and March 2020. Only ICSI cycles (maternal age <40 years) leading to a singleton live birth (43 males and 51 females) were included for analysis. All oocytes were placed in the EmbryoScope incubator for culture immediately post sperm injection with all annotation performed retrospectively by one embryologist (L-SO).

Participants/materials, setting, methods: Timings included 2nd polar body extrusion (tPb2), SPN(tSPNa)/OPN(tOPNa) appearance (differentiated by proximity to Pb2) and PN fading (tPNF). Morphometrics were evaluated at 8 (stage 1), 4 (stage 2) and 0 hour before PNF (stage 3), measuring PN area (um2), PN juxtaposition, and nucleolus precursor body (NPB) arrangement. Means ± standard deviation were compared using student t test or logistic regression as odds ratio (OR) and 95% confidence interval (CI), and proportional data by chi-squared analysis.

Main results and the role of chance: Logistic regression indicated that male zygotes had longer time intervals of tPb2_tSPNa than female zygotes (4.8±1.5 vs 4.2±1.0 h, OR=1.442, 95% CI 1.009-2.061, p=0.044), but not tPb2_tOPNa (4.7±1.8 vs 4.5±1.3 h, OR=1.224, 95% CI 0.868-1.728, p=0.250) and tPb2_tPNF (19.9±2.8 vs 19.1±2.3 h, OR=1.136, 95% CI 0.957-1.347, p=0.144). SPN increased in size from stage 1 through 2 to 3 (435.3±70.2, 506.7±77.3, and 556.3±86.4 um2, p=0.000) and OPN did similarly (399.0±59.4, 464.3±65.2, and 513.8±63.5 um2, p=0.000), with SPN being significantly larger than OPN at each stage (p<0.05 respectively). However, relative size difference between SPN and OPN was similar between male and female zygotes at 3 stages (33.6±61.7 vs 38.6±50.8 um2, p=0.664; 38.5±53.1 vs 45.7±71.9 um2, p=0.585; 38.4±77.4 vs 45.8±63.9 um2, p=0.615; respectively). More male than female zygotes reached central PN juxtaposition at stage 1 (77% vs 51%, p=0.010), stage 2 (98% vs 86%, p=0.048) and stage 3 (98% vs 86%, p=0.048). Furthermore, more OPN showed aligned NPBs than in SPN at stage 1 (45% vs 29%, p=0.023), but similar proportions at stage 2 (64% vs 50%, p=0.056) and stage 3 (76% vs 72%, p=0.618). There were no gender associated differences detected in NPB alignment in either SPN or OPN (p>0.05 respectively).

Limitations, reasons for caution: The retrospective design does not allow for control of unknown confounders. Sample size is considered relatively small. PN area measurement may not truly represent volume as PN may not be perfectly spherical. Findings were based on women <40 years old so may not apply to older population.

Wider implications of the findings: These findings augment and extend previous studies investigating PN parameters via static observations. The reported variations between male and female embryos may confound live birth prediction when using pronuclei morphometrics and morphokinetics. Larger scaled studies are warranted to verify these findings.

Trial registration number: Not applicable