**Understanding the implications of follicular output rate (FORT) and follicle to oocyte index (FOI) on human embryo morphokinetics**

**Objective**: The follicular output rate (FORT, calculated as the number of preovulatory follicle measuring between 16 and 22 mm in diameter per antral follicle count [AFC] × 100) and follicle-to-oocyte index (FOI, calculated as the number of retrieved oocytes per AFC) are quantitative markers of the ovarian response to gonadotrophins, which accurately reflect the follicular development dynamics. The objective of this study was to evaluate the effects of follicular output rate (FORT) and follicle to oocyte index (FOI) on embryos morphokinetics.

**Methods**: This historical cohort study was performed in a private university-affiliated IVF centre between February 2019 and December 2021. Kinetic data were analysed in 8,376 embryos, individually cultured in a time-lapse imaging (TLI) incubator, derived from 2,470 patients undergoing ICSI cycles. The timing of specific events from the point of insemination was determined using TLI. Recorded kinetic markers were timing to pronuclei appearance (tPNa) and fading (tPNf), timing to two (t2), three (t3), four (t4), five (t5), six (t6), seven (t7), and eight cells (t8), and timing morulation (tM), timing to start blastulation (tSB) and to blastulation (tB). The durations of the second (cc2, t3-t2) and third cell cycles (cc3, t5-t3) and the timing to complete synchronous divisions t2-tPNf (s1), t4-t3 (s2) and t8-t5 (s3) were also calculated. The effects of FORT and FOI the levels on morphokinetic events and ICSI clinical outcomes were investigated. For that, embryos were split into groups according to FOI value: FOI< 50 (Low-FOI, n=247 cycles and 894 embryos) and FOI≥ 50 (high-FOI, n=2,223 cycles and 7,482 embryos) and according to the FORT value: FORT values below the 33rd percentile, FORT< 27.3 (low-FORT, n= 753 cycle and 2,556 embryos), FORT values between the 33rd and the 67th percentile, FORT: 27.3–47.6 (medium-FORT, n=874 cycles and 2,970 embryos), and FORT values above the 67th percentile, FORT> 47.6 (high-FORT, n=843 cycles and 2,850 embryos). Embryo morphokinetics and ICSI outcomes were compared among the FOI and FORT groups.

**Results**: A significant difference was noted in almost all morphokinetic parameters, where embryos derived from cycles with an FOI <50 presented slower development than embryos derived from cycles with an FOI ≥50, considering tPNf, t2, t4, t6, t7, t8, tM, tB, s1, s2, s3, and cc2. A significantly higher KID score D5 was observed among embryos derived from cycles with an FOI ≥ 50 when compared with those with an FOI < 50. Additionally, increased blastocyst formation and implantation rates were noted among cycles with higher FOIs (Table 1). An increased time to complete morphokinetic events was observed among embryos derived from cycles with low FORT, followed by those with medium FORT, while embryos derived from cycles with high FORT presented a faster development competence: tPNa, t2, t4, t5, t6, t7, t8, tsB, s2, s3. Embryos derived from cycles with high FORT presented a higher Kid Score D5, followed by those derived from cycles with medium FORT, and embryos from cycles with low FORT presented the lowest KID score. Significantly higher rates of blastocyst formation and implantation were observed in embryos derived from cycles with high FORT, followed by those with medium FORT, while embryos from cycles with low FORT presented the lowest blastocyst formation and implantation rates (Table 1).

**Conclusion:** This study highlights the association between FORT and FOI and embryo morphokinetic development, suggesting that FORT and FOI are both quantitative and qualitative markers of oocyte retrieval related to ovarian activity and ovarian stimulation. It could be suggested that higher FOI and FORT are associated with the retrieval of better-quality oocytes, which in turn results in better embryonic development. Significant positive relationships were observed between embryo development and these parameters, which would not be detected in embryos cultured in conventional incubators. It is possible that the deselection of these embryos may have prevented an effect on clinical pregnancy, but additional research is needed to validate this assumption.

**Keywords:** Time-lapse microscopy; morphokinetic assessment; Follicular Output Rate (FORT), Follicle-to-Oocyte index (FOI)

**Table 1: Comparison of known implantation diagnosis (KID) score D5 and intracytoplasmic sperm injection (ICSI) outcomes between the low and high** **Follicle-To-Oocyte (FOI) index groups and between the low, medium and high follicular output rate (FORT) groups.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Low FOI**  | **High FOI**  | **p value** | **Low FORT**  | **Medium FORT**  | **High FORT**  | **p value** |
| **n** | 894 | 7532 |  | 2,556 | 2,970 | 2,850 |  |
| **Kid score** | 5.1 ± 0.09 | 5.60 ± 0.03 | < 0.001 | 5.4 ± 0.5a | 5.5 ± 0.5 a,b | 5.6 ± 0.6 b | 0.021 |
| **Blastocyst rate (%)** | 53.6 ± 0.92 | 44.85 ± 1.87 | < 0.001 | 49.2 ± 1.4a | 50.8 ± 1.4a | 55.5 ± 1.4b | < 0.001 |
| **Implantation rate (%)** | 24.8 ± 0.32 | 26.08 ± 0.53 | 0.037 | 23.6 ± 0.4a | 24.5 ± 0.4a | 27.1 ± 0.5b | < 0.001 |

Values are percentage ± standard error, unless otherwise noted. a ≠ b ≠ c.