# Is there an association between morula timing and duration on the pregnancy outcome from single blastocyst transfer cycles?

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## INTRODUCTION

- Embryo developmental timings are being increasingly used by embryologists to select the best embryo to transfer in assisted reproductive treatment.
- Time to formation of the morula has been suggested to relate to pregnancy outcome (1).
- Duration of the morula stage has not been assessed previously.
- The Edinburgh Fertility Centre (EFC) currently do not assess the morula stage when selecting a blastocyst for transfer on day 5.
- This study will help to determine if the assessment of morula timing or duration can be used as tool to help select blastocysts for transfer in the EFC patient population.

## AIM

To determine if the time to formation of the morula or duration of the morula stage is different in blastocysts that resulted in clinical pregnancy compared to those that resulted in non-pregnancy.

## METHODS

- Retrospective case-control study including 411 single blastocyst transfer cycles.


Morula — Start of blastulation — Blastocyst

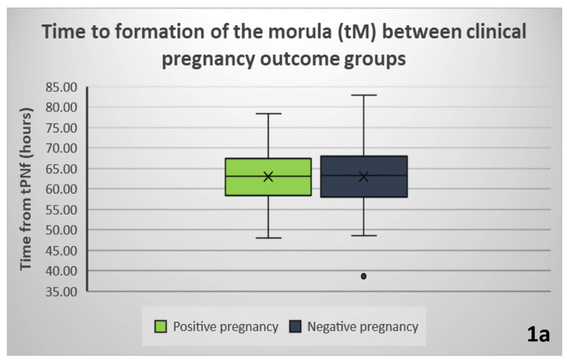
- Developmental timings (including time to morula, tM) of transferred blastocysts were exported from the EmbryoScope™ time-lapse incubator software and the duration of the morula stage was calculated (tSB-tM and tB-tM).
- Pregnancy outcomes were obtained from lab database records.
- Mean embryo timings were compared by students t-test or non-parametric equivalent. tM was measured from the time of pronuclear fading (tPNf). tM timings were split into quartiles to select an optimal range and pregnancy rates between groups assessed by Chi-square test.

## RESULTS

There was no difference in the mean time to morula or duration of the morula stage between blastocysts that resulted in pregnancy and those that did not result in pregnancy (table 1).

Table 1. Mean timing of morula formation and duration in blastocysts that resulted in positive and negative clinical pregnancies

| Clinical pregnancy | Developmental time in hours from tPNf Mean ±SD (n) | | |
|---|---|---|---|
| | tM | tSB-tM | tB-tM |
| **Positive** | 62.99 ± 6.06 (223) | 7.99 ± 4.22 (223) | 17.26 ± 4.94 (221) |
| **Negative** | 62.97 ± 6.84 (188) | 8.08 ± 4.96 (188) | 17.90 ± 5.07 (185) |
| **P value** | 0.97 | 0.70 | 0.20 |




The duration of the morula stage showed a similar distribution between pregnancy outcome groups (fig 1b and c) but the time to morula formation appeared to extend to later times in the group that did not result in pregnancy (fig 1a).

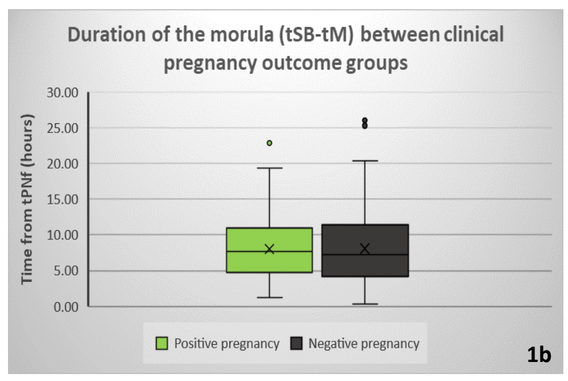



Figure 1. Distribution of timings for morula formation (1a) and duration (1b and 1c) in blastocysts that resulted in positive (green plot) or negative (grey plot) clinical pregnancy outcomes

Table 2. Pregnancy rate when timings of morula formation in blastocysts are split into quartiles

| | Time to tM from tPNf (hours) | Clinical pregnancy rate (%) | n |
|---|---|---|---|
| **Q1** | <58.2 | 50 | 102 |
| **Q2** | 58.2-63.2 | 61 | 108 |
| **Q3** | 63.2-67.7 | 52.5 | 99 |
| **Q4** | >67.7 | 52.9 | 102 |

Blastocysts with a tM of 58.2-63.2 hours (Q2) had the highest clinical pregnancy rate (table 2) but this was not significantly different compared to blastocysts with a tM outside of this range (51.8%, p=0.10).

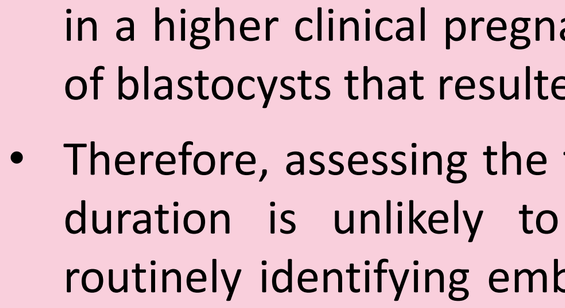



Figure 2. Pregnancy rates when timings of morula formation in blastocysts are within or out-with the cut-off limits

When the range of tM was adjusted to 59-64 hours, this gave a significant difference in pregnancy rates (fig 2). This difference was not observed when applied to an independent cohort of 86 blastocysts transferred in 2020.

## CONCLUSION

- There is no difference in the mean timing of morula formation or duration between blastocyst transfers that resulted in pregnancy or non-pregnancy.
- Although a 5-hour window of time to morula formation was identified in blastocysts that resulted in a higher clinical pregnancy, this captured only 35% of blastocysts that resulted in a positive pregnancy.
- Therefore, assessing the time to morula formation or duration is unlikely to be an effective tool for routinely identifying embryos that are more likely to result in a clinical pregnancy.

References
1. Harada Y, Maeda T, Fukunaga E, Shiba RO, S, Kinutani M, Horiuchi T. Selection of high-quality and viable blastocysts based on timing of morula compaction and blastocyst formation. Reproductive Medicine and Biology. 2020;19(1):58-64