THE MORPHOKINETICS OF EMBRYOS SHOWING REVERSE CLEAVAGE DURING THE 1ST, 2ND OR 3RD CLEAVAGE CYCLE AS DETERMINED WITH TIME-LAPSE VIDEOGRAPHY.

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INTRODUCTION

Cleavage abnormalities observed via time-lapse videography are increasingly used as biomarkers for embryo deselection with little understanding of their biology, despite such embryos having poor implantation potential (1). This study aimed to investigate morphokinetic characteristics of embryos displaying reverse cleavage (RC) during the 1st, 2nd or 3rd cleavage cycle.

MATERIALS AND METHODS

167 IVF/ICSI autologous treatment cycles using fresh oocytes (female age 35.0 ± 4.6 yr) were included. All women attended Fertility North between January 2017 and December 2018. Embryo annotation using Embryosviewer® (Vitrolife, Sweden) software allowed morphokinetic profiles of RC embryos (n=241) to be compared with their unaffected siblings in the 1st, 2nd and 3rd cleavage cycle up to Day 3. The sibling-embryo design eliminated patient and laboratory related confounding factors(4, 5, 6).

RC was defined as cell fusion following division (type I) or failed cytokinesis after karyokinesis (type II [2]). Embryonic milestones relative to pronuclear fading (PNF) removed timing variations due to the insemination method (3). Timed milestones included 2-cell (T2), 3-cell (T3), 4-cell (T4), 5-cell (T5), 6-cell (T6), 7-cell (T7) and 8-cell (T8). Relative timing parameters included CC2 (duration of the 2-cell stage or T3-T2), CC3 (duration of the 4-cell stage or T5-T4) and synchrony of cell division at the 2-cell (S2=T4-T3) and 4-cell stages (S3=T8-T5).

Statistical analysis was performed using the Microsoft® Excel Student t-test, where a p value of <0.05 was considered statistically significant and all timing parameters were expressed in the form of mean and standard deviation.

RC IN THE 1ST CLEAVAGE CYCLE

Comparisons between RC affected and unaffected (normal cleavage pattern) sibling embryos showed significantly delayed subsequent development (T2, T3, T4 and CC2; all p<0.01) when RC occurred in the 1st cleavage cycle (figure 1).

Currently the cause of RC remains unknown, although previous reports have shown potential association with sperm motility, ovarian stimulation regime, defects in the cell membrane, however not female age (2,7). Results in the present study suggest that embryos displaying RC may have an underlying intrinsic defect, and perhaps studies at the ultrastructural and molecular level may offer further insights.

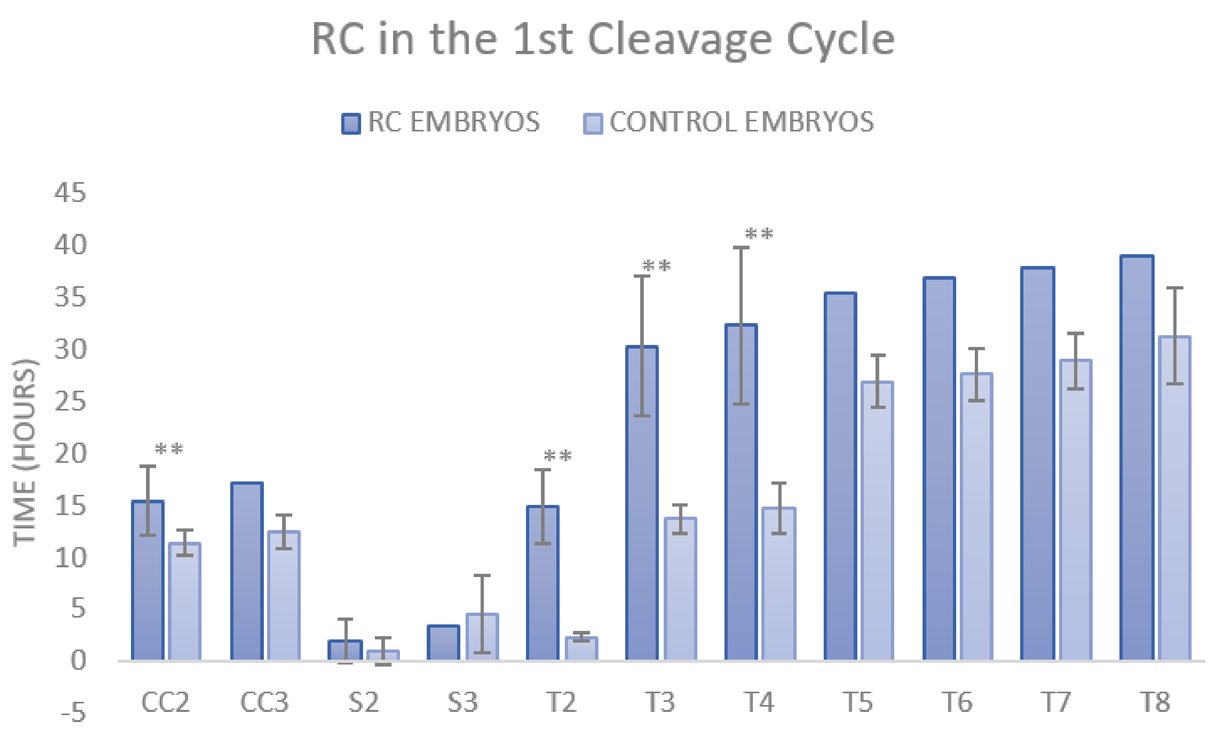

*Figure 1. The morphokinetic's and cleavage cycle parameters of embryos showing reverse cleavage were compared to those embryos showing no cleavage abnormality (control embryos). *p<0.05, **p<0.01.*

RC IN THE 2ND CLEAVAGE CYCLE

Figure 2 shows similar relative timing parameters between RC embryos and control embryos prior to the RC event in the 2nd cleavage cycle. A developmental delay was detected in embryos post RC in the 2nd cleavage cycle (T4, T7, T8, CC3, S2, and S3; all p<0.01) (T5 and T6; both p<0.05) compared to control embryos (figure 2).

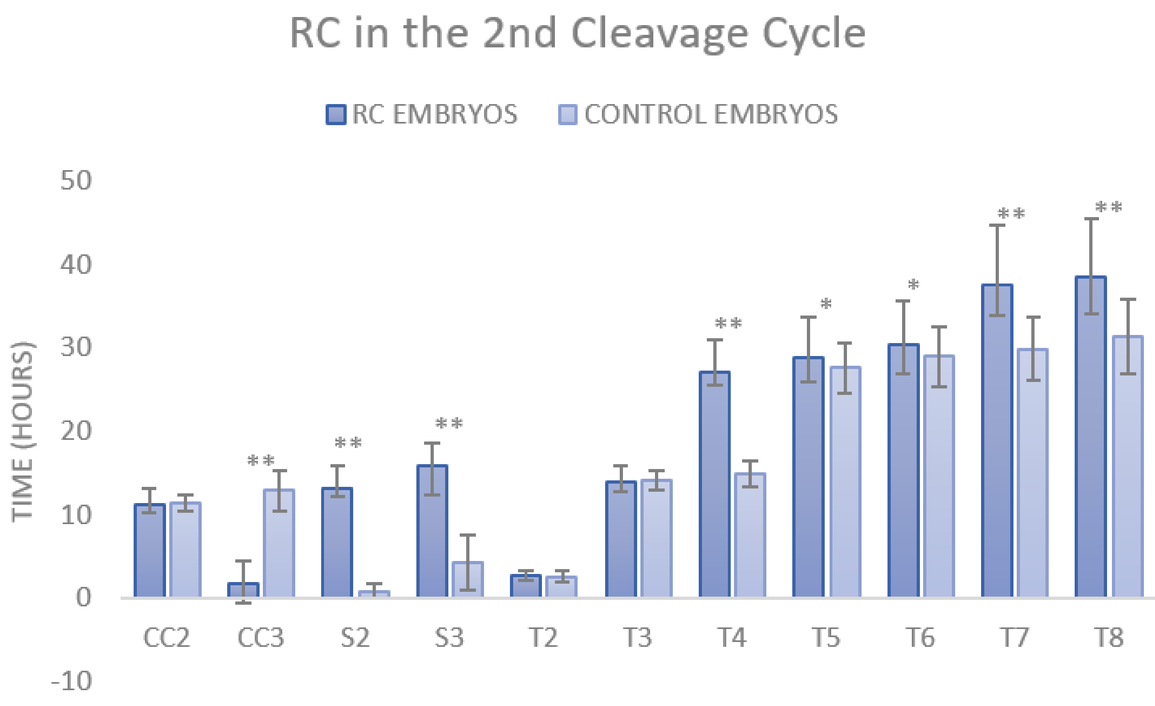

*Figure 2. The morphokinetic's and cleavage cycle parameters of embryos showing reverse cleavage were compared to those embryos showing no cleavage abnormality (control embryos). *p<0.05, **p<0.01.*

RC IN THE 3RD CLEAVAGE CYCLE

RC embryos demonstrated significantly slower development when RC occured in the 3rd cleavage cycle (T7, T8 and S3, all p<0.05) after the cleavage abnormality occured, however no significant difference in the measured timing parameters was detected before the onset of abnormal cleavage.

CONCLUSION

In summary, altered morphokinetic profiles are displayed by RC embryos after the occurrence in all three cleavage cycles, which could potentially confound morphokinetic comparisons if not separated from their unaffected sibling embryos. Further study is warranted in order to fully understand the biological mechanisms of such events and the impact upon the genetics of the embryo.

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