THE USE OF TIME-LAPSE VIDEOGRAPHY TO DESCRIBE THE MORPHOKINETIC PROFILES OF EMBRYOS DISPLAYING DIRECT CLEAVAGE AT DIFFERENT STAGES OF DEVELOPMENT

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INTRODUCTION

Cleavage abnormalities observed directly via time-lapse videography (TLV) are invariably associated with low pregnancy rates. Direct cleavage (DC) cell division is an abnormality that results in 3 or more daughter cells, which is differentiated from large anuclear fragments by tracking via TLV (1). The aim of this study was to investigate the morphokinetic characteristics of embryos displaying DC during the 1st, 2nd or 3rd cleavage cycle.

MATERIALS AND METHODS

A total of 167 IVF/ICSI autologous treatment cycles using fresh oocytes (female age 33.8 ± 4.3yr) were included. All women attended Fertility North between January 2017 and December 2018. Embryo annotation using Embryosviewer® (Vitrolife, Sweden) software allowed morphokinetic profiles of DC embryos (n=244) to be compared with their unaffected sibling embryos up to Day 3.

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.

DC IN THE 1ST CLEAVAGE CYCLE

It is a novel finding that DC embryos exhibited slower developmental progression prior to the onset of the cleavage abnormality as demonstrated by significantly delayed T2 in the 1st cleavage cycle ($p<0.01$). Figure 1 clearly shows DC embryos reached developmental milestones earlier than their unaffected siblings in the 1st cleavage cycle (T3, T4, T5, T6, T7, T8; all $p<0.05$) after the cleavage abnormality due to the additional daughter cell(s) generated during the division. CC2 and CC3 were significantly shortened at the 1st cleavage cycle (figure 1; both $p<0.01$) whilst S2 and S3 were prolonged during this cleavage cycle (figure 1; both $p<0.01$).

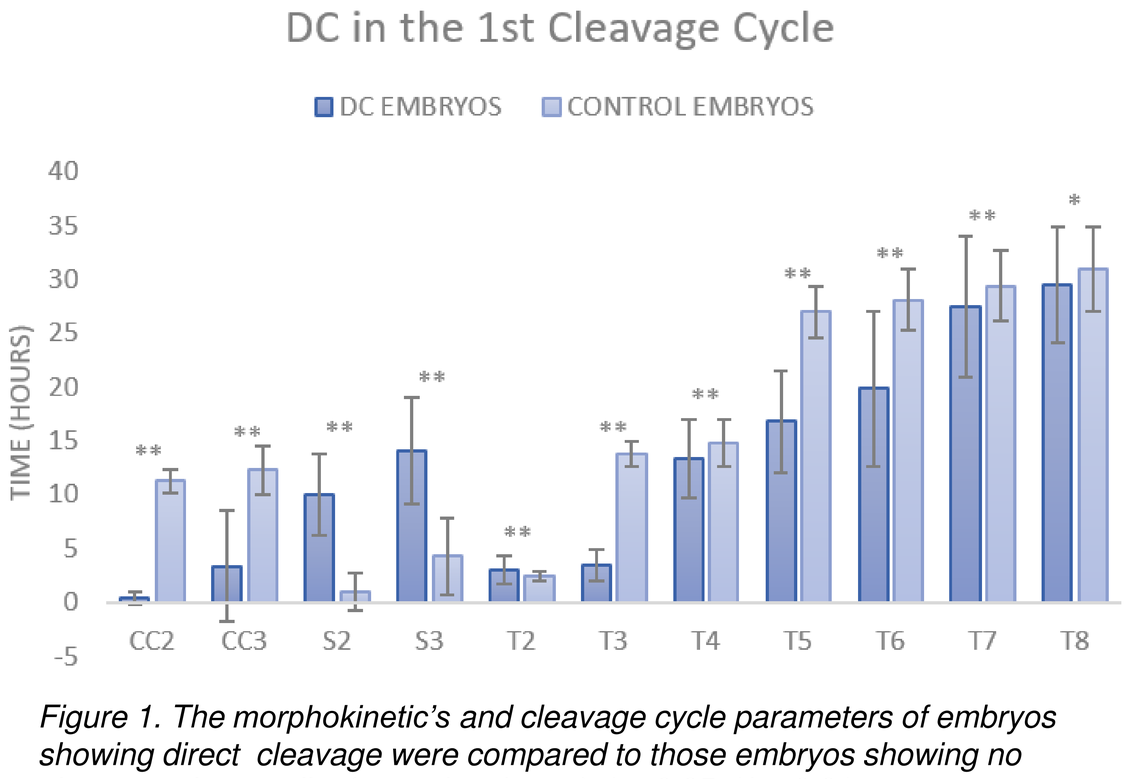

*Figure 1. The morphokinetic's and cleavage cycle parameters of embryos showing direct cleavage were compared to those embryos showing no cleavage abnormality (control embryos). *p<0.05, **p<0.01.*

DC IN THE 2ND CLEAVAGE CYCLE

DC also slowed down before the cleavage abnormality occurred in the 2nd cleavage cycle showing a lag in T2, T3 and T4 ($p<0.01$). DC embryos in the 2nd cleavage cycle significantly outpaced their no cleavage abnormality counterparts (T5, T6, T7; all $p<0.05$) as seen in figure 2. CC3 was shortened when DC occurred in the 2nd cleavage cycle, whereas S3 was longer comparatively (figure 2; $p<0.01$).

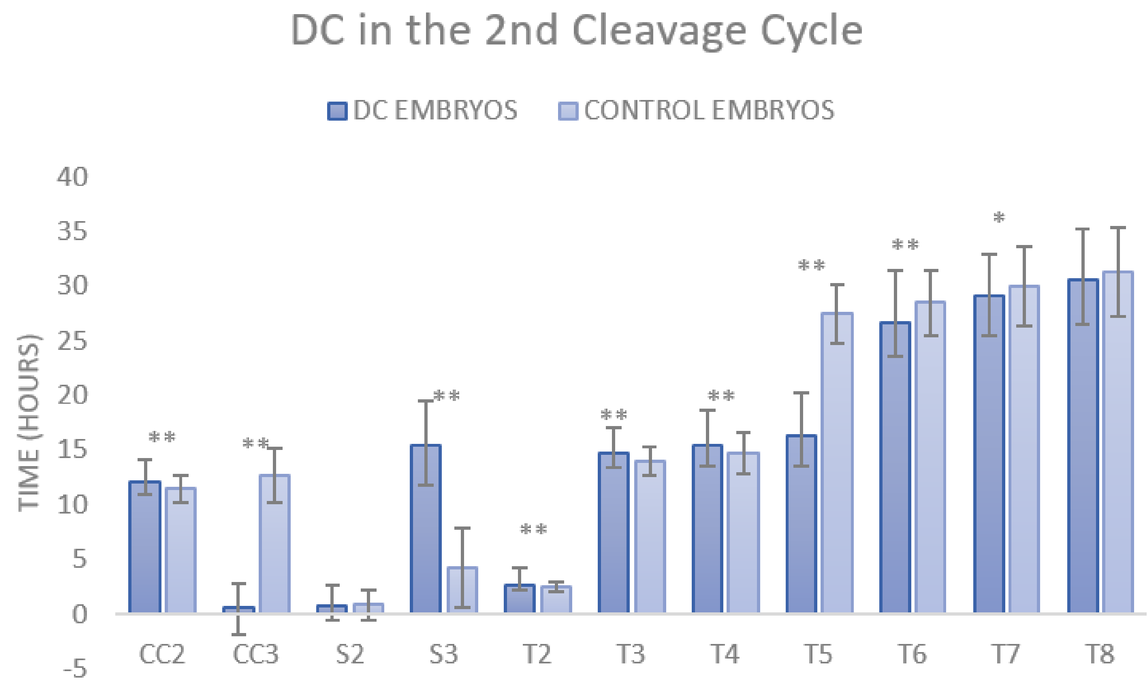

*Figure 2. The morphokinetic's and cleavage cycle parameters of embryos showing direct cleavage were compared to those embryos showing no cleavage abnormality (control embryos). *p<0.05, **p<0.01.*

DC IN THE 3RD CLEAVAGE CYCLE

Prior to the onset of DC, the postponement of T3 ($p<0.05$) was once again observed when DC occurred in the 3rd cleavage cycle. Kalatova et al, illustrated that the immediate cellular mechanism of tripolar mitosis may originate from excessive duplication of the centrosome before mitotic spindle formation (3), which may explain the observed delay prior to the onset of DC in the present study, where embryos with impaired centrosomal function may require extra time regulating associated replication activities before the occurrence of DC.

DC embryos in the 3rd cleavage cycle did not differ significantly from control embryos in the rate at which they reached developmental milestones after the onset of abnormal cleavage, which was not expected since DC in one of the 4-cell stage blastomeres would have led to shorter T6 and onwards. A 3 day cultured observation period post oocyte collection, forgoes the opportunity to detect later stage (e.g., 9-cell or beyond) acceleration of embryo growth. Additionally "self-correction" is believed to play a role in later stage embryos lessening the impact of abnormal cells (4).

CONCLUSION

In summary, results indicate significantly changed morphokinetic profiles of DC embryos both before and after their occurrence at each of the three cleavage cycles. It is therefore important that such embryos are identified and removed from morphokinetic data collections.

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