THE EFFECT OF LIPID SEGREGATION WITH OR WITHOUT ZONA PELLUCIDA LASER DRILLING ON POST THAW EMBRYO DEVELOPMENT OF IN VITRO PRODUCED BOVINE EMBRYOS

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INTRODUCTION

- Studies have shown that increased levels of lipids within bovine embryos during freezing can increase levels of intra-cellular damage thus lowering development rates.
- The objective of this study was to determine if lipid segregation with or without zona pellucida cutting on post thawed in vitro produced bovine embryos would effect survivability and development.

MATERIALS and METHODS

- Good quality (n=357) d6 (Tuli influenced) bovine IVF embryos were randomly allocated by stage (32-cell to blastocyst) and grade (1 & 2) into the following 5 treatment groups prior to freezing in 1.5M Ethylene Glycol (EG):

➢ Treatment 1: Control - No treatment.
➢ Treatment 2: (CB) - 7.5 µg/ml Cytochalasin B for 20 min.
➢ Treatment 3: (CBCF) - CB with 20 min Centrifugation (CF) at 16,000g, for lipid segregation.
➢ Treatment 4: (CBCFLAH) - CB with 20 min CF – Freeze then Laser Assisted Hatching (LAH) upon thaw.
➢ Treatment 5: LAH only post-thaw.

- FREEZING: 5 min in 1.5M EG, seeding at -6ºC with a ramp of 0.5ºC/min from -6ºC to -32ºC before plunging in LN_2. Treatments 2-4 contained CB in 1.5M EG.
- THAWING: Air thaw for 7 s and then in 35ºC H_2O for 10 seconds.
- LAH: Immediately post-thaw, the zona pellucida of embryos from treatment groups 4&5 were laser drilled (power setting = 90%, Pulse length = 600 µsec) using the XY Clone® laser. (*Photo 1).*
- CULTURE: All embryos post thaw were assessed for survivability (>60% intact/viable appearing cells) and development (cultured for 24 hours and determined developed if progressed to the next stage). (*Table 1).*
- STAINING: Post 24 hr culture, embryos were stained in warmed holding medium containing 2.5 µg/ml Bisbenzimide Hoechst 33342 & 5 µg/ml Propidium Iodide for 15 minutes. Embryos were submitted to ultra-violet (UV) light for cell counts.
- EMBRYO CELL COUNTS: Under UV light, red fluorescent cells (dead) were counted first before blue fluorescent cells (total), (*Photo 2).*

Photo 1: Treatment 4 (CBCFLAH) compacted morula post thaw before and after zona pellucida laser drilling.

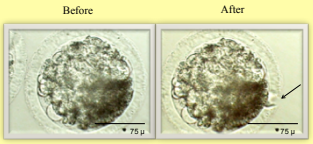
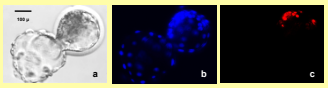


Photo 2: Treatment 4 (CBCFLAH) of a bovine IVF compacted morula frozen/thawed and cultured for 24 h.



The hatching blastocyst was submitted to: a) Bright field, b) UV - blue fluorescence for total cell counts and c) UV -red fluorescence for dead cell counts.

RESULTS

- There were no mean Treatment differences observed between survivability and development (P<0.05), (*Table* 1).
- Due primarily to low numbers in embryos in stages other than compacted morula (CM) no differences among treatments were detected.
- Within the CM stage, CBCFLAH was not different than LAH, CBCF and Control but exhibited a significantly greater percentage of live cells than CB (77.0, 71.9, 68.8, 68.3, 65.5% respectively; p<0.05), (*Table 3).*
- CBCFLAH and LAH exhibited a significantly greater number of both total and live cells than Control (P<0.05), (*Table 2).*

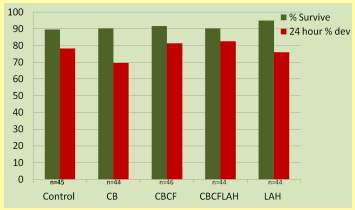
ACKNOWLEDGEMENTS

- Financial support for this project was supported by a grant from Texas A&M University (USA)- CONACYT (Mexico) and Ovagenix. The authors also acknowledge the contributions of Dr. John Dobrinski of Minitube America, Verona, WI.

CONCLUSIONS

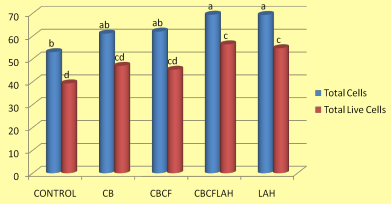
- These data indicate that LAH post-thaw alone or in combination with CBCF improves both total cell number and embryo viability following cryopreservation.
- Further research is needed to determine if these findings, when applied to fresh, frozen/thawed or vitrified/warmed transferrable bovine IVF embryos, can improve pregnancy results.

Table 1: Post thaw survivability and development rates between Treatment groups for bovine IVF compacted morula (CM).



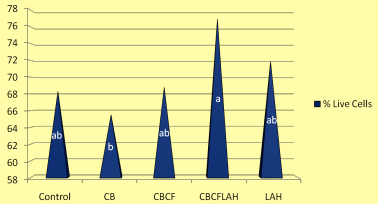
No significant difference P<0.05, ANOVA

Table 2: Mean number of Total and Live Cells of bovine IVF CM frozen/thawed and cultured for 24 h.



a-d Values with different superscripts within same color columns are significantly different (P<0.05, Student's t).

Table 3: Percent live cells by treatment of bovine IVF CM frozen/ thawed and cultured for 24 h.



ab Values with different subscripts are significantly different (P <0.05, Student's t).