Improved Bovine Embryo Production Using Novel *In Vitro* Culture Systems

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INTRODUCTION

- Testing new media conditions for embryo production is an essential component for improving *in vitro* development
- The objective of this study was to compare media used in two bovine embryo production systems (Control and Embryo Trans Biotech: ETB).

MATERIALS & METHODS

Experiment 1: Abattoir-procured oocytes were matured, fertilized and cultured in the following media under a 5% CO_2, 5% O_2, 90% N_2 in a 38.5°C humidified atmosphere:

Control:

- Maturation (IVM) - Medium 199 with Earles salts supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin, 0.2 mM sodium pyruvate, 2 mM L-Glutamine, and 5.0 µg mL^{-1} of Folltropin®-V.
- Fertilization (IVF) - 500 µl pre-equilibrated modified Tyrode-lactate medium (Pryor et al. 2011, Therio. 75, 24-33).
- In vitro Culture (IVC) - Seventeen hours post-insemination, presumptive zygotes were cleaned of cumulus cells and cultured in Bovine Evolve supplemented with 4 mg mL^{-1} of Probumin BSA under oil for 7 days (8 days post-IVF) where cleavage and viability rates were assessed (Table 1, Fig. 1 & 2).

ETB:

- IVM – ETB BO-IVM
- IVF – ETB BO-IVF
- IVC – ETB BO-IVC

Experiment 2: Same protocol as above except a modified ETB BO-IVC was used (ETB mod.).

Cell Counts: Embryos were fixed in cold methanol, washed in PBS/0.1% Tween 20 and mounted in 10 µg mL^{-1} Hoechst/glycerol to stain the nuclei. Cell counts were performed manually at 200X using UV light microscopy (Fig. 3 & 4).

Statistical Analysis: Percentage data were transformed using arcsine square root function prior to analysis and means compared using a Student's *t*-test; alpha = 0.05.

- Additionally, we also evaluated the media effects on C quality oocytes (1 layer of cumulus cells; n=205), which were evenly divided between Control vs. ETB over 5 replicates. Due to the lower numbers, Chi Square analysis was performed to compare outcomes (Fig. 2).

Experiment	Treatment	(n)	% Cleaved	% Viable	% HBL	% HBL/Expanded BL
1	Control	193	81.0[a]	42.9[a]	9.3[a]	39.3[a]
1	ETB	206	80.5[a]	48.4[a]	11.6[a]	36.8[a]
2	Control	211	80.5[b]	29.2[b]	5.8[b]	20.5[b]
2	ETBmod	216	82.8[b]	51.9[c]	23.9[c]	45.8[c]

Table 1. In vitro development of bovine embryos at d8 post-IVF for Control vs. ETB systems.
[a,b,c] Values in a column and within experiment with different superscripts were different, P<0.05.

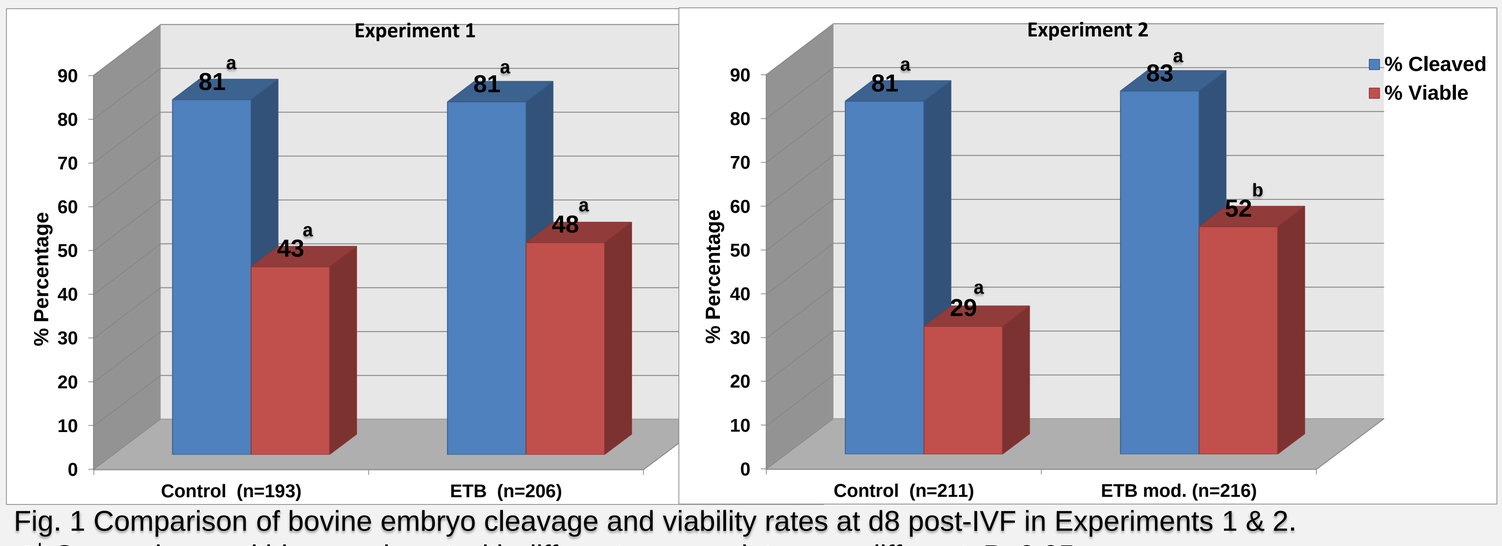

Fig. 1 Comparison of bovine embryo cleavage and viability rates at d8 post-IVF in Experiments 1 & 2.
[a,b] Comparisons within experiment with different superscripts were different, P<0.05.

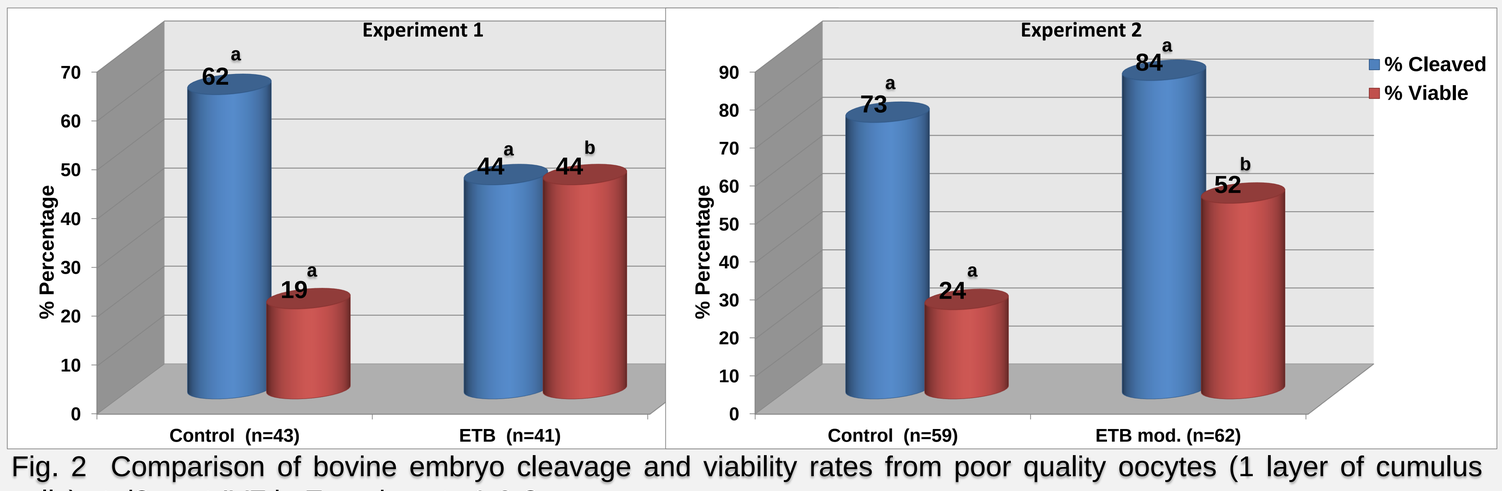

Fig. 2 Comparison of bovine embryo cleavage and viability rates from poor quality oocytes (1 layer of cumulus cells) at d8 post-IVF in Experiments 1 & 2.
[a,b] Comparisons within experiment with different superscripts were different, P<0.05.

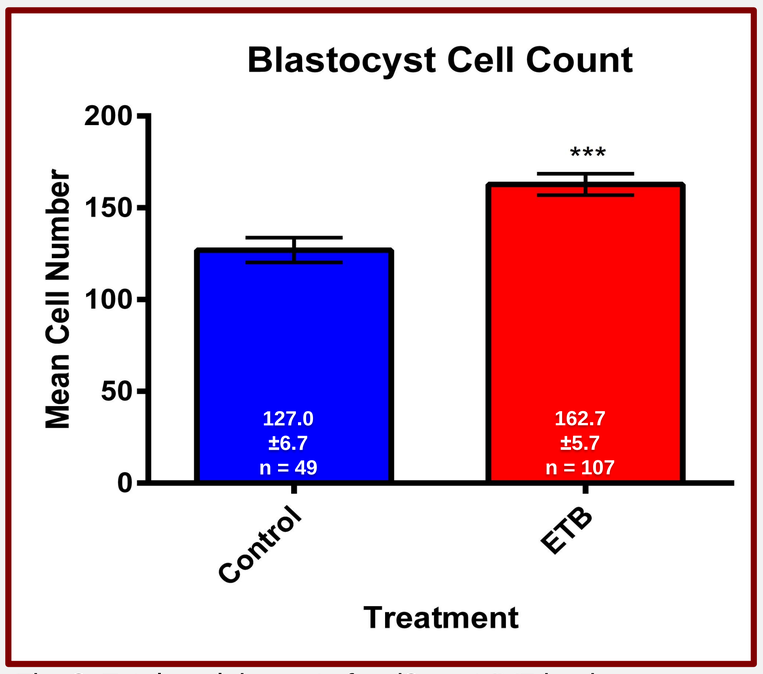

Fig. 3 Total nuclei counts for d8 post-IVF bovine embryos with ±SEM. *** Indicates significant difference, P<0.05

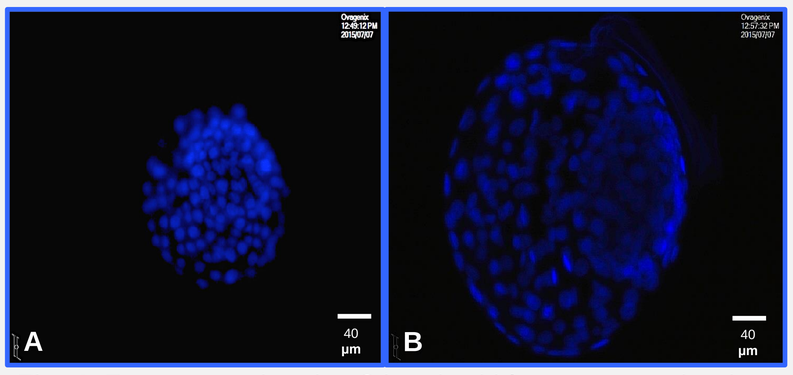

Fig. 4 Representative day 8 hatched blastocysts stained for nuclei counts (200X magnification). A). Control, 150 nuclei. B). ETB, 250 nuclei.

RESULTS

- Experiment 1: No differences in rates of cleaved or viable embryos were observed between treatment groups (Fig. 1, Table 1).
- Experiment 2:
  - The modified version of ETB produced an increase in viable embryos compared to Control (Fig. 1, Table 1).
  - Mean cell counts for viable embryos were significantly different following culture in modified ETB (Fig. 3).
  - Embryo viability decreased in the Control media but were maintained using the modified ETB between experiments with seasonal temperatures ranging from 23.8°C (Exp1) to 33.8°C (Exp2).
  - Viability rates for poor quality oocytes were significantly higher for ETB than Control across both experiments (Fig. 2).

CONCLUSION

- ETB's modified BO-IVC media produced more higher quality embryos under varying conditions, produced higher cell counts for BL and enhanced the rate of development compared to Control.
- Continued research is underway to ascertain pregnancy rates following fresh or frozen/thaw embryo transfer.

ACKNOWLEDGMENTS

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