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Comparative analysis of fertilization success between intracytoplasmatic sperm injection and conventional *in vitro* fertilization in equine reproduction

M. G. Souza[A], S. R. Teague[A], M. L. Martin[A], R. L. Beck[A] and R. E. Martinez[B]

[A]In Foal Inc., Millsap, TX, USA
[B]Tarleton State University, Stephenville, TX, USA

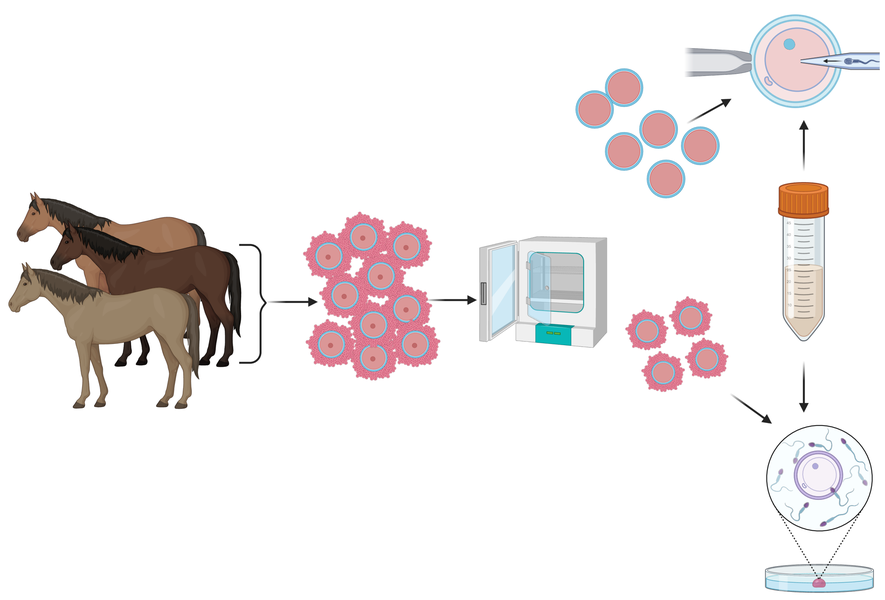
INTRODUCTION

- ICSI has been the standard for *in vitro* embryo production in horses due to limited success with conventional IVF
- Recent advancements, including the study by Felix et al. (2022), demonstrated successful outcomes with equine IVF
- These findings suggest that IVF could become a viable alternative to ICSI for commercial application
- Comparative studies are needed to evaluate fertilization and developmental success between ICSI and IVF protocols

OBJECTIVE

- To compare fertilization success between intracytoplasmic sperm injection (ICSI) and conventional *in vitro* fertilization (IVF) using equine oocytes
- To evaluate differences in cleavage rates, blastocyst development from cleaved embryos, and overall blastocyst development per cumulus–oocyte complex (COC) between ICSI and IVF treatments

METHODS



- **Oocyte Collection & Allocation:** Oocytes were collected via ovum pickup (OPU) from 12 mares. Cumulus–oocyte complexes (COCs) were placed into maturation medium and allocated in a 60:40 ratio to ICSI and IVF treatments
- **Fresh Semen:** Fresh semen from the same ejaculate was used for both fertilization techniques
- **Fertilization Techniques:**
  - **ICSI:** Only MII-stage oocytes (n=101) were injected
  - **IVF:** The number of MII oocytes was estimated to be (n=62) based on the maturation rate observed in oocytes denuded for ICSI
- **Embryo Assessment:**
  - Cleavage-stage embryos: Identified by cellular division.
  - Blastocysts: Identified by the presence of blastomeres
- **Outcomes Evaluated:** Cleavage rates, blastocyst development from cleaved embryos, and overall blastocyst development per initial COC
- **Statistical Analysis:** Fisher's exact test ($P < 0.05$; trends at $P < 0.1$)

RESULTS

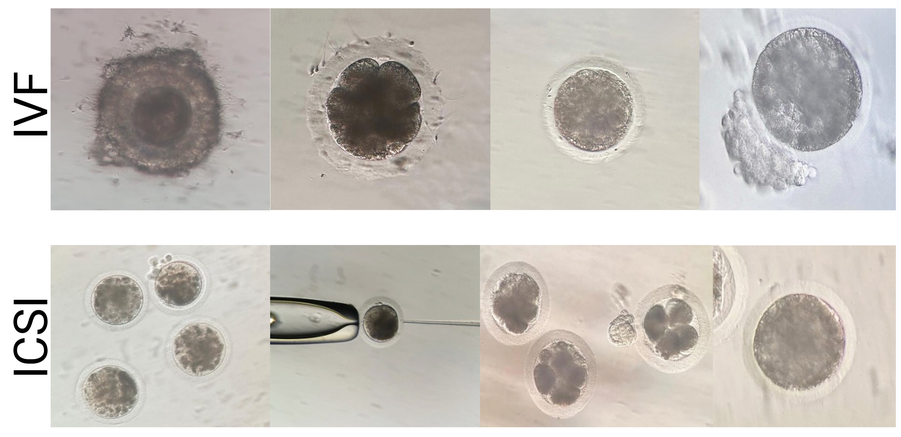


Figure 1. Representative images of embryos derived from equine oocytes fertilized using intracytoplasmic sperm injection (ICSI) and conventional *in vitro* fertilization (IVF).

- **Cleavage Rates:** IVF achieved a significantly higher cleavage rate (55/62; 89%) compared to ICSI (63/101; 63%; $P = 0.0003$).
  - This demonstrates that a larger proportion of fertilized oocytes progressed to the cleavage stage under IVF treatment
- **Blastocyst Development (from Cleaved Embryos):** A trend was observed for higher blastocyst development from cleaved embryos in IVF (26/55; 47%) compared to ICSI (20/63; 32%; $P = 0.093$)
  - This suggests potential differences in developmental competence
- **Overall Blastocyst Development (per Initial COC):** IVF yielded significantly greater overall blastocyst production (26/111; 23%) compared to ICSI (20/181; 11%; $P = 0.023$)
  - This highlights the higher efficiency of IVF in producing blastocysts from the total oocyte pool

CONCLUSION

- **IVF** demonstrated higher **cleavage rates** and **overall blastocyst development** compared to **ICSI**, suggesting superior efficiency
- A **trend toward increased blastocyst development from cleaved embryos** with IVF was observed, though not statistically significant
- Overall, **IVF** appears to be a more effective reproductive technology for equine oocyte fertilization, offering potential for improved success in commercial equine breeding programs

FUTURE STUDY PLANS

By using the experimental model in this study:

- Evaluate pregnancy outcomes, including a comparison of pregnancy loss rates between fertilization methods using these embryos.
- Explore gene expression and epigenetic modifications in embryos produced via IVF and ICSI to understand the molecular basis for differences in fertilization success and early development.
- Examine the influence of stallion and mare factors, such as age, breed, or reproductive health status, on fertilization success

REFERENCES

Felix MR, Turner RM, Dobbie T, Hinrichs K. Successful in vitro fertilization in the horse: production of blastocysts and birth of foals after prolonged sperm incubation for capacitation. May 10;108(5):849. Biol Reprod. 2022;107(6):1551-1564.

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