Advances in the equine artificial reproductive toolbox

Jennifer Clulow

Charles Sturt University, Wagga Wagga jeclulow@csu.edu.au

Whilst advances in equine reproduction have been well documented in the literature, adaption of these tools and technologies into clinical practice is often slow. The reason for this varies but is largely due to the restrictions of traditional breeding systems or economic sensitivities. Despite this, equine reproduction has undergone significant transformations over the past few decades primarily driven by advancements in artificial reproductive technologies. This has also opened new avenues for genetic conservation, the management of reproductive health, improved biosecurity by reducing horse movements and ultimately, improved breeding efficiency.

1. Semen Preservation – enhancing genetic potential

The storage of stallion spermatozoa has improved the logistics of mare breeding by improving the flexibility of management practices in addition to reducing the reliance on horse movement. Cryopreservation of stallion spermatozoa remains the only viable means of long-term storage of sperm and advances in this area has not been the focus of many researchers recently [1]. Whilst cryopreservation media and methos have been well established, there is still no quality assurance or international standard for a what constitutes a frozen sperm insemination dose in the horse. Furthermore, the higher costs and reduced fertility rates associated with breeding management of mares with frozen sperm means that many breeders continue to rely on the more traditional cooled liquid storage of stallion sperm. Recent advances in sperm storage includes the formulation of a completely synthetic, ambient temperature medium [2] which prolongs the storage time for stallion spermatozoa for up to 14 days post collection. The development of this alternative, 'non-chilled' medium required that the deleterious effects of reactive oxygen species produced by oxidative phosphorylation (OXPHOS) are curtailed via other means, such as the addition of effective antioxidants and by supporting efficient OXPHOS. Therefore, when compared to our traditional cooled systems where sperm metabolism is reduced but not eliminated resulting in a maximum storage time of 72 hours, this ambient temperature medium effectively supports mitochondrial energy production (OXPHOS) allowing longer liquid storage durations [3]. Other stallion spermatozoa research has been directed in the diagnostic field including sperm function assays and evaluation of DNA integrity which may not contribute to overall improved breeding outcomes, are useful in identifying and investigating stallion fertility issues [4-10].

2. Artificial Insemination and embryo transfer - enhancing reproductive efficiency

Artificial insemination (AI) has become the cornerstone of equine breeding management allowing the widespread use of stallion genetics without the need for natural mating. Improvements in the semen processing and timing of insemination have increased both the success rates and widespread use of these techniques. When coupled with embryo transfer (ET), breeding management for superior mares can be pushed to the physiological limits, allowing multiple foals to be produced each year. In addition to the exploitation of genetically superior mares, ET also allows mares with reproductive challenges to continue to contribute to the population. Whilst AI and ET cannot overcome inherent infertility such as reproductive

senescence or oocyte degeneration, these mares can continue to contribute genetically, provided the uterine environment can support the developing blastocyst until embryo retrieval. Success rates have been improved following a better understanding of recipient selection by ensuring that the mare displays a long preceding oestrus prior to recruiting that mare in the recipient pool [11]. Historically, the transfer of equine embryos was highly technician dependant with experienced operators achieving >70% pregnancy rates following transfer. The technique as described by Wilsher and Allen [12] has reduced the variation noted between experienced and novice practitioners, allowing the adaptation of this technology into clinical practice by lessor experienced operators with reasonable success [13].

3. In Vitro Fertilisation – pushing the boundaries of reproductive technologies

Undoubtedly, the greatest advances in artificial reproductive technologies in the horse have occurred in the laboratory with a large uptake of *in vitro* technologies becoming widespread across Europe, North and South America. Whilst the first *in vitro* fertilisation (IVF) derived foals were reported in the early 1990s [14], subsequent success has been limited in this field due to a lack of understanding of stallion sperm capacitation. Despite several attempts by researchers utilising sperm capacitation methods used in other species, attempts to establish commercial equine IVF protocols have been unsuccessful [15]. As such, most success in *in vitro* embryo production (IVEP) in the horse has been with the use of Intracytoplasmic sperm injection (ICSI).

Equine IVEP requires specialised and expensive equipment in addition to trained personnel, which has contributed to the slow adoption of the technology into horse breeding systems. Furthermore, transvaginal aspiration of oocytes/ovum pick up (TVA or OPU respectively) also required specialised equipment and trained veterinarians to achieve adequate oocyte retrieval and good results in the laboratory [16]. Despite this, there has been growth in the number of TVA/ICSI derived foals worldwide and to a lesser extent, here in Australia. IVEP has opened new possibilities in equine reproduction, overcoming issues such as low sperm numbers, low oocyte numbers in aged mares, hostile uterine environments and in some cases, infertility. The ability to freeze or vitrify ICSI derived embryos is a further advantage for the technology, facilitating embryo transfer logistics by eliminating the need for recipient synchronisation. Unfortunately due to the delay in equine IVEP progress compared to other domestic species, there has been a lag in the development of adjunct *in vitro* technologies such as oocyte cryopreservation [17]. This is certainly an area for future research.

The most recent developments in this space have been the successful capacitation of sperm *in vitro*, allowing equine IVF story to be revisited [15,18-20]. Further research is required to determine whether IVF has advantages over ICSI as a means of IVEP quality and quantity and if there is a difference in pregnancy rate. However, it has been shown that blastocyst production via IVF with frozen-thawed sperm is at worst comparable or exceeds the number of blastocysts produced via ICSI. Given that IVF requires less expensive laboratory equipment investment, IVF may provide a more cost-effective means of IVEP for commercial breeding operations. Further research is needed to determine if there are advantages for embryo quality and pregnancy rates however, it is hypothesized that allowing normal sperm function, physiological fusion and activation of the oocyte can only have positive outcomes [15].

4. Challenges of future directions

Despite significant progress, several challenges persist in equine reproduction. The high cost of ART procedures and the need for specialised expertise limits accessibility for some breeders.

Additionally, the regulatory landscape varies between breeds and regions which can affect the implementation and standardisation of reproductive technologies. Despite this, there are many layers to the artificial reproductive toolbox may help to troubleshoot fertility problems in all broodmares to assist in pinpointing where in the reproductive tract the problem arises[21] e.g. failure of ovulation, failure of fertilisation, failure of embryo development or failure to maintain a pregnancy. Furthermore, developments *in vitro* have made investigation of sperm function and early embryo development possible. Even the discovery of the elusive factor responsible for maternal recognition in this species could be within reach!

As awareness of animal rights grows, the social licence to utilise horses in equestrian sports is becoming increasingly fragile. The advent of ARTs has raised important ethical questions regarding the manipulation of equine reproduction. While these technologies offer significant benefits, they also present challenges related to animal welfare, genetic diversity and limit the opportunity for horses to engage in natural mating behaviours. In March 2024 the Swedish warmblood association banned the registration of ICSI offspring emphasising that "horse welfare always outweighs purely commercial interests for SWB" [22]. TVA or OPU for ICSI is an invasive surgical procedure which is not without risk to the mare. It is therefore imperative that the procedure is performed by trained veterinarians and restrictions are put in place to ensure adequate pain relief and monitoring of the animal following the procedure. Improving analgesia provided both during and after OPU is certainly an important area where the procedure can be improved to ensure positive mare welfare outcomes. This area was a focus of discussion at the most recent ESER Symposium in 2024 resulting in research collaborations to further investigate pain scores and efficacy of different analgesia approaches.

The widespread use of ARTs is a double-edged sword. It has the potential to reduce genetic diversity within equine populations and potentially propagate deleterious genetic disorders. Conversely, it also provides the opportunity for early genetic testing of disorders in addition to sex selection, which may limit overproduction and presence of know genetic disease. Whilst we develop and implement these technologies, it is important that we continue to promote programs and research that maintain diverse breeding practices, prioritise welfare of the individuals and genetic robustness of the population to continue to improve the integrity and health of equine populations.

References

1. Gibb Z, Aitken RJ. Recent Developments in Stallion Semen Preservation. *Journal of equine veterinary science* 2016;43:S29-S36.
2. Gibb Z, Clulow JR, Aitken RJ, et al. First Publication to Describe a Protocol for the Liquid Storage of Stallion Spermatozoa for 7 Days. *Journal of equine veterinary science* 2018;66:37-40.
3. Clulow J, Gibb Z. Liquid storage of stallion spermatozoa – Past, present and future. *Animal reproduction science* 2022;247:107088.
4. Peña FJ, Martín-Cano FE, Becerro-Rey L, et al. Proteomics is advancing the understanding of stallion sperm biology. *PROTEOMICS* 2024;24:2300522.
5. Orsolini MF, Meyers SA, Dini P. An Update on Semen Physiology, Technologies, and Selection Techniques for the Advancement of In Vitro Equine Embryo Production: Section I. *Animals* 2021;11:3248.
6. Castaneda C, Juras R, Kjollerstrom J, et al. Thoroughbred stallion fertility is significantly associated with FKBP6 genotype but not with inbreeding or the contribution of a leading sire. *Animal genetics* 2021.
7. Hernández-Avilés C, Ramírez-Agámez L, Varner DD, et al. The stallion sperm acrosome: Considerations from a research and clinical perspective. *Theriogenology* 2023;196:121-149.

8. Egyptien S, Deleuze S, Ledeck J, et al. Sperm Quality Assessment in Stallions: How to Choose Relevant Assays to Answer Clinical Questions. *Animals* 2023;13:3123.
9. Hernández-Avilés C, Ramírez-Agámez L, Varner DD, et al. Spermatozoal acrosome dysfunction and its role in stallion subfertility. *Journal of Equine Veterinary Science* 2024;143:105213.
10. Yaseen M, Swegen A, Gibb Z. Comparative analysis of equine semen preservation techniques and DNA damage detection using Comet and SCSA assays. *Journal of Equine Veterinary Science* 2025;145:105335.
11. Cuervo-Arango J, Claes AN, Ruijter-Villani M, et al. Likelihood of pregnancy after embryo transfer is reduced in recipient mares with a short preceding oestrus. *Equine Veterinary Journal* 2018;50:386-390.
12. Wilsher S, Allen WR. An improved method for nonsurgical embryo transfer in the mare. *Equine Veterinary Education* 2004;16:39-44.
13. Cuervo-Arango J, Claes AN, Stout TA. Effect of embryo transfer technique on the likelihood of pregnancy in the mare: a comparison of conventional and Wilsher's forceps-assisted transfer. *Veterinary record* 2018;183:323-323.
14. Palmer E, Bézard J, Magistrini M, et al. In vitro fertilization in the horse. A retrospective study. *J Reprod Fertil Suppl* 1991;44:375-384.
15. Felix MR, Turner RM, Dobbie T, et al. Successful in vitro fertilization in the horse: production of blastocysts and birth of foals after prolonged sperm incubation for capacitation†. *Biology of Reproduction* 2022;107:1551-1564.
16. Cuervo-Arango J, Claes AN, Stout TAE, et al. Mare and stallion effects on blastocyst production in a commercial equine ovum pick-up-intracytoplasmic sperm injection program. *Reproduction fertility and development* 2019;31:1894-1903.
17. Angel-Velez D, De Coster T, Azari-Dolatabad N, et al. New Alternative Mixtures of Cryoprotectants for Equine Immature Oocyte Vitrification. *Animals* 2021;11:3077.
18. Martin-Pelaez S, Fuente Adl, Takahashi K, et al. IVF with frozen-thawed sperm after prolonged capacitation yields comparable results to ICSI in horses: A morphokinetics study. *Theriogenology* 2025;232:39-45.
19. Broothaers K, Angel-Velez D, De Coster T, et al. Capacitation of frozen-thawed semen improves ICSI-outcomes in horses. *Journal of Equine Veterinary Science* 2025;145:105269.
20. Broothaers K, Angel-Velez D, Smits K. Higher blastocyst rates after IVF with frozen-thawed semen compared to ICSI in horses. *Journal of Equine Veterinary Science* 2025;145:105268.
21. Clulow J, Nugent S, Adkins A. Successful pregnancies in a mare after laparoscopic unilateral ovariohysterectomy. *Clinical Theriogenology* 2021;13:52-57.
22. SWB. SWB tillåter inte registrering av OPU/ICSI-avkommor från 2025-03-01. 2024; https://swb.org/nyheter/swb-tillater-inte-registrering-av-opu-icsi-avkommor-fran-2025-03-01/#. Accessed 10/06/25, 2025.