Academic Insights and Industry Application of OPU and ICSI in Horses

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Keywords: ART, OPU ICSI, embryo production, equine reproduction, micromanipulation.

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1. Abstract

Assisted reproductive technologies (ART) in horses have evolved significantly over the past two decades, with Ovum Pick-Up (OPU) and Intracytoplasmic Sperm Injection (ICSI) now among the most promising tools for enhancing fertility management and genetic advancement in elite equine breeding programs. At The University of Queensland (UQ), we have developed a research-integrated OPU-ICSI platform that supports scientific inquiry while directly addressing the needs of the Australian equine industry.

Built on extensive international experience in micromanipulation, ICSI, and cloning technologies, including the first equine clones in South America and Australia, our group has produced over 250 equine embryos within five months. A portion of these embryos have been successfully transferred, resulting in confirmed pregnancies, with the first ICSI foals expected in August 2025. These outcomes demonstrate that high-quality *in vitro* embryo production in horses is feasible and scalable in Australia, even under regional and logistical constraints.

Despite this progress, a major barrier to national expansion remains the limited availability of training in OPU and the lack of laboratories with consistent, high-quality outcomes. Based on current and projected demand, we estimate that four to six specialized laboratories with trained staff will be needed across Australia to ensure broader access to this technology.

Here we outline our insights of the current status of this technology and and impact of UQ's OPU-ICSI platform, emphasizing its scientific foundation, industry relevance, and capacity to reshape equine breeding in Australia.

2. Introduction

Australia's equine industry is geographically dispersed and includes high-performance sport horses, thoroughbreds, and recreational horses. However, Australia's vast size means many mares are situated far from breeding centres, limiting access to reproductive services during the breeding season, often requiring extended periods of agistment and routine reproductive manipulation. Conventional artificial insemination and embryo transfer (ET) programs are further challenged by the prevalence of aging mares, whose declining fertility reduces the likelihood of successful pregnancies. Furthermore, the inability to achieve success with poor fertility frozen semen in standard AI and ET programs limits the consistency and cost-effectiveness of producing viable embryos and live foals. These limitations affect the ability of breeders to make rapid genetic gains and efficiently manage valuable individuals and stored semen.

The combination of OPU with ICSI offers a unique opportunity to overcome many of these challenges. This technique enables embryo production from mares regardless of age, fertility status, or competition schedule, and from stallions with poor semen quality or limited availability. As an example, between 2017 and 2024, over 2,000 foals were registered from four deceased stallions: Chacco-Blue, Cumano, Clinton, and Cassini I. Notably, Chacco-Blue alone (passed away more than 10 years ago) accounted for 1600 new foals in this period, underscoring the technique's impact on preserving and propagating elite genetics (**Hippomundo 2025**). This advancement allows breeders to continue utilizing valuable bloodlines from animals that cannot reproduce naturally or by conventional ART.

More importantly, OPU-ICSI in horses it is having a transformative impact by eliminating the traditional dependence on the breeding season, allowing oocytes to be collected and embryos to be produced throughout the entire year. This year-round capability not only increases the efficiency of breeding programs but also provides unprecedented flexibility for managing the reproductive timelines of high-performance mares and stallions, ultimately expanding access to elite genetics and optimizing the use of veterinary and laboratory resources across seasons.

In contrast to its widespread use in Europe and North America, OPU-ICSI remains significantly underutilized in Australia, despite the growing interest from breeders and veterinarians. Several key factors contribute to this limited uptake. One of the primary barriers is the lack of veterinarians efficiently trained in OPU. This is a technically demanding procedure that requires not only theoretical knowledge but also extensive hands-on experience under expert supervision. OPU is performed via transvaginal ultrasound-guided aspiration of follicles, which involves inserting a needle through the vaginal wall into the ovary. This must be done with extreme precision to avoid damaging surrounding tissues or causing unnecessary discomfort or stress to the mare. Inadequate technique can compromise mare health or translate into low oocyte recovery rates. These risks underscore the importance of proper training and consistent practice, which currently are not widely available in Australia.

Another major constraint in the broader adoption of OPU-ICSI in Australia is the limited number of laboratories with the infrastructure, expertise, and consistency required to process equine oocytes and support embryo development to the blastocyst stage. Producing high-quality embryos *in vitro* is a complex process that demands specialized equipment, tightly controlled culture conditions, and skilled embryologists with experience in handling equine gametes. At present, only a small number of laboratories in Australia have demonstrated reliable and repeatable success rates, and even fewer are set up to receive oocytes from external veterinarians or breeding operations. This creates a significant logistical bottleneck, especially for clinics or farms located in rural or regional areas. While some veterinarians are interested in offering OPU services locally, they often lack access to a laboratory willing and able to process the oocytes they collect.

At UQ, our goal has been to build a research platform that serves both academic and industry purposes. We aim to improve the efficiency of the technology, train future practitioners, and reduce the number of OPU sessions required to obtain embryos from each mare. If we achieve the desired annual embryo yield from just two or three OPU sessions per mare, we will reduce procedural stress, low costs, and ultimately support better animal welfare.

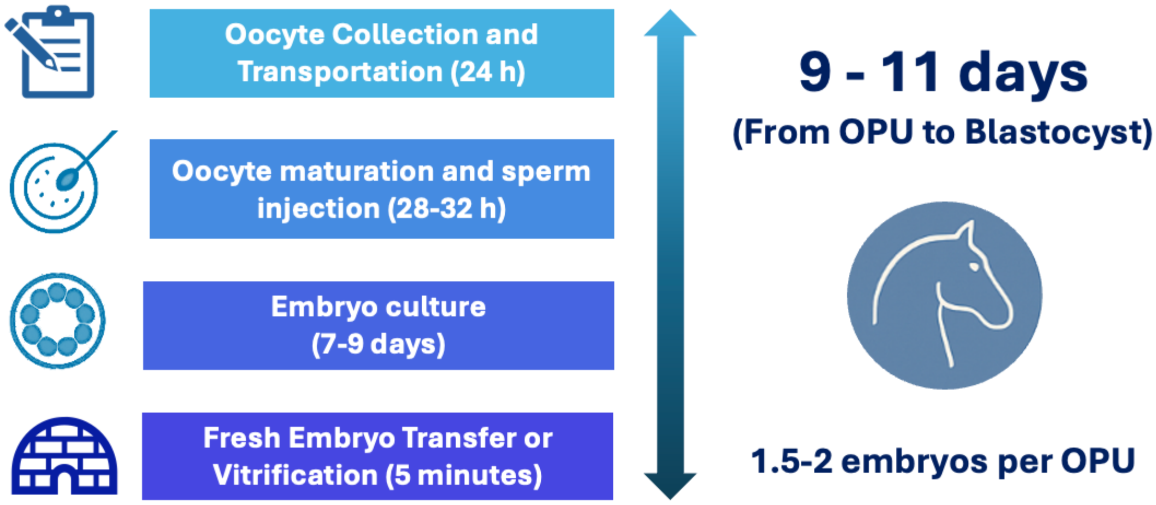

Figure 1: Equine *in vitro* embryo production timeline. The process begins with the collection and transportation of immature oocytes from the mare to the laboratory (24 hours), followed by intracytoplasmic sperm injection (ICSI), typically performed after 28 to 32 hours of *in vitro* oocyte maturation. The injected oocytes are then cultured *in vitro* for 7 to 9 days until blastocyst development. Embryos are either transferred fresh into synchronized recipient mares or cryopreserved via vitrification. The entire process from OPU to blastocyst formation takes approximately 9 to 11 days.

3. Building on Expertise

Our equine OPU-ICSI program is built on a foundation of technical expertise developed almost two decades. Our group has experience in micromanipulation, cloning, gene editing, and embryo production across species, including cattle, pigs, cats, dogs, and many other species besides horses. This experience includes the successful generation of the first cloned horses in both South America and Australia, the first cloned zebra embryos, as well as the first ICSI-derived embryos in donkeys and kangaroos.

This background enabled us to quickly adapt ICSI protocols for equine oocytes, which are known to be more sensitive than those of other species. Some key challenges we addressed include performing ICSI with and without piezo-assisted injection using available microinjectors, developing media solutions locally due to limited availability in Australia of commercial products used in other countries and optimizing sperm selection protocols to match stallion-specific semen profiles.

Our experience is further strengthened by close collaboration and knowledge exchange with human IVF clinics in different countries. This connection has enabled the transfer of high-standard laboratory procedures into the equine ART setting. Because many technical aspects of OPU-ICSI in horses parallel those used in human medicine, we have been able to adapt some of the most recent advances in clinical embryology to the equine model, resulting in greater reproducibility and improved embryo development outcomes. In fact, the horse is emerging as a valuable translational platform for studying the effects of ART, including ICSI, *in vitro* culture and early pregnancy development, which are otherwise difficult to investigate in human embryos due to ethical constraints.

More importantly, our lab benefits from a dynamic academic environment, with multiple PhD and master's students contributing to method development, testing, and validation across various species. This structure helps maintain constant calibration of equipment and quality assurance of reagents, ensuring that all systems operate under optimal conditions.

4. Collection and *In Vitro* Maturation of Equine Oocytes

Through a Collaborative Research Agreement with UQ, our industry partners perform transvaginal ultrasound-guided OPU using adapted aspiration equipment on unstimulated mares. This approach reduces hormonal use while still achieving consistent oocyte recovery. However, hormonal treatments to increase the number of recruited follicles are under development and they are likely to offer promising applications, especially in mares with persistently low antral follicle counts.

We closely support veterinarians in training, helping them improve collection efficiency and minimize oocyte handling risks. We also offer the opportunity for those in early stages of OPU training to send surplus oocytes to our lab for research rather than discarding them. This collaboration benefits both scientific research and technical development in the field.

Oocytes are typically transported to our laboratory within 24 hours. As long as temperature is maintained between 21 and 23 degrees Celsius, there is no significant loss in developmental potential. This step is particularly important in Australia due to the extreme environmental conditions in some regions. Commercial holding or flushing media are used to keep oocytes in an immature state during transit.

We have developed a reliable *in vitro* maturation system using our in-house media. Maturation is evaluated by first polar body extrusion, with current success rates of around 70% after 28 to 30 hours. While polar body extrusion reflects nuclear maturation, it is well known that cytoplasmic and epigenetic maturation are equally critical and not visible under the microscope. These factors vary among oocytes, making each oocyte unique with differences in developmental competence. Thus, oocyte maturation is indeed one of the most crucial steps towards successful production of embryos in the lab.

5. Sperm Injection and Embryo Culture

ICSI is carried out using standard inverted microscopes and microinjectors. Sperm are selected based on motility and morphology, with recent integration of laser-assisted selection techniques that allow identification of viable sperm within immotile populations. This is particularly useful in cases involving poor-quality semen.

Sperm are prepared using methods such as swim-up and microfluidic separation with devices like the VetCount Harvester (GYTECH). The choice of method depends on the specific characteristics of each ejaculate. Capacitation agents are included to support fertilization competence.

After ICSI, embryos are cultured in modified DMEM/F12-based media adapted to the metabolic needs of the equine embryo. Cleavage is assessed on Day 4 with most blastocysts forming by Days 7 to 8. Although some embryos develop by Day 9 or 10, these are generally associated with lower developmental potential and higher pregnancy loss. Nonetheless, a small proportion of these delayed embryos can still result in healthy foals, and decisions about transfer should be made based on embryo quality and recipient availability.

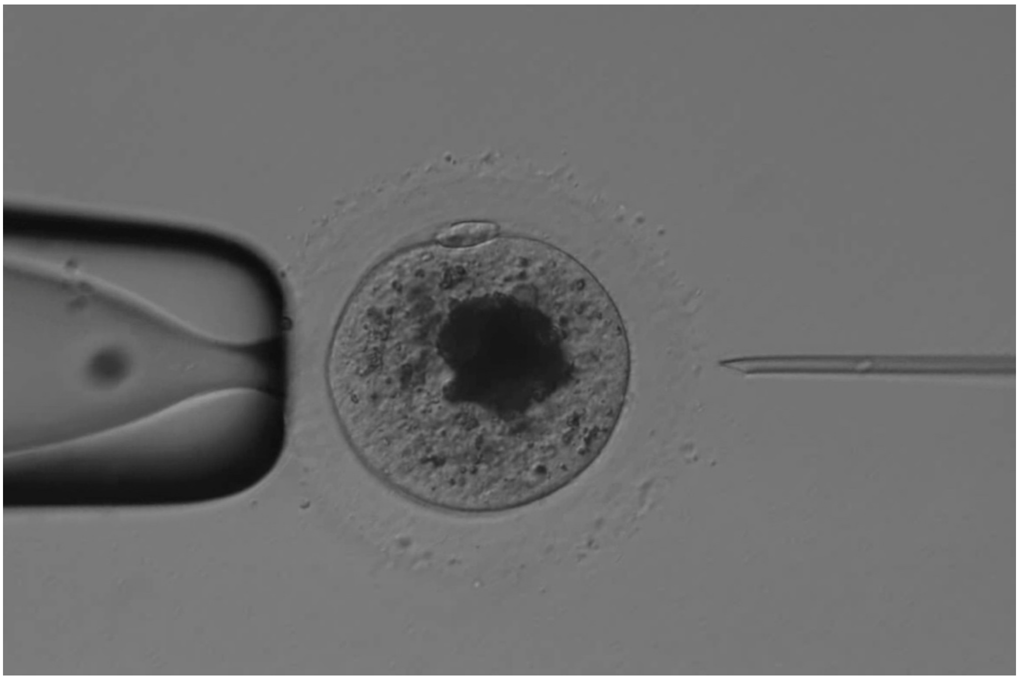

Figure 2: Matured equine oocyte at the metaphase II (MII) stage positioned sperm injection (ICSI). A single spermatozoon is visible within the injection needle, aligned for penetration through the zona pellucida and oolemma.

6. Embryo Vitrification

One of the key advantages of ICSI-based embryo production is the ability to cryopreserve embryos at the blastocyst stage using vitrification. This technique offers high post-thaw survival rates, preserves embryo quality, and provides greater flexibility in scheduling embryo transfers by decoupling embryo production from recipient availability. Vitrification also enables long-term embryo banking, creating new business and export opportunities, and supporting genetic management strategies by allowing embryos to be stored until optimal transfer conditions are met. This is particularly valuable given the relevance of recipient selection for *in vitro* produced embryos and the limited access to suitable recipient mares in some regions.

At UQ, we use commercially available vitrification kits developed for human embryos. These systems are user-friendly, well-validated, and deliver consistently high survival rates after warming. Our protocol enables routine vitrification of blastocysts without compromising developmental competence or pregnancy outcomes. This not only improves the efficiency of embryo use but also enhances the practicality of scheduling transfers based on mare cycles or client needs.

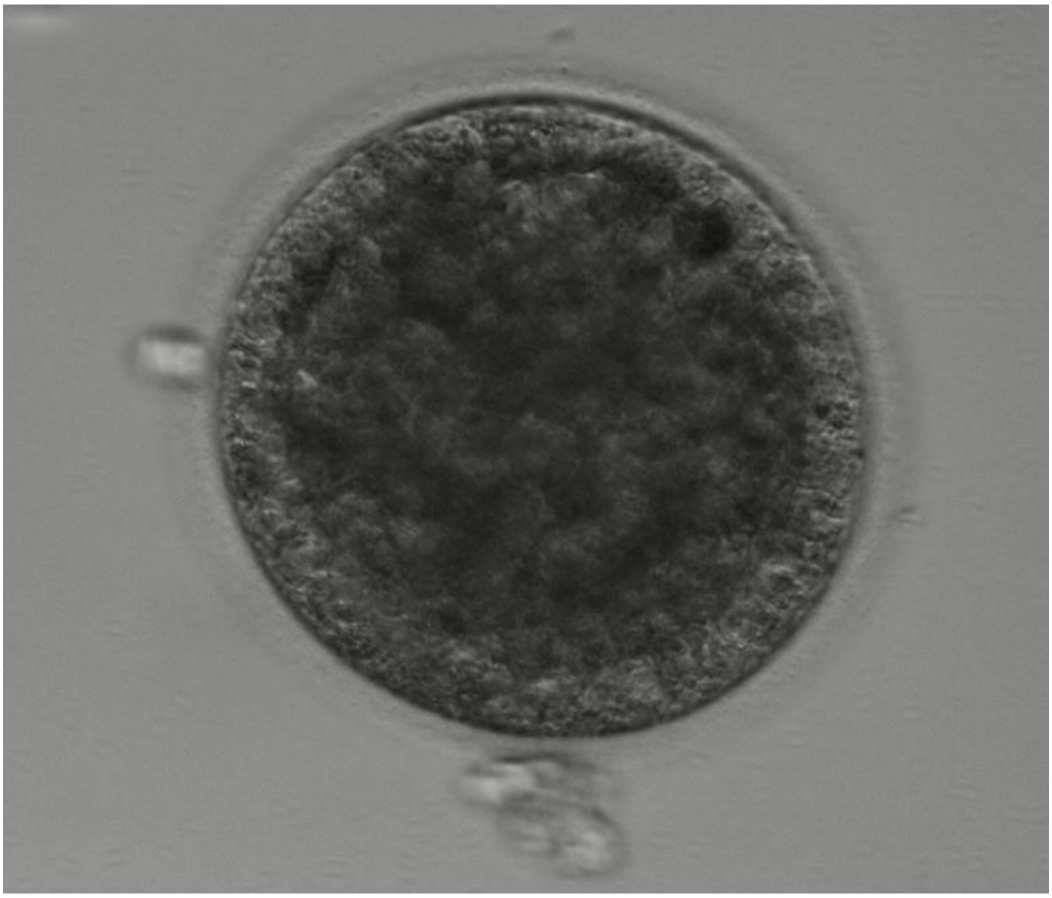

Figure 3: Day 7 equine blastocyst produced via intracytoplasmic sperm injection (ICSI). The embryo is at the appropriate developmental stage for vitrification, and stored in LN2, or preparation for immediate fresh transfer into a synchronized recipient mare.

7. Collaborating with Academia

While collaborations with academic institutions bring access to cutting-edge research and innovation, they also involve certain structural differences compared to commercial services. At UQ, our ICSI platform operates within a research and teaching framework. This means that before beginning a project, formal agreements may be required to ensure compliance with institutional guidelines and research ethics. Also, because this platform is embedded within an academic schedule, we currently can perform ICSI twice per week, with plans to increase this to three sessions in the upcoming season. This structure allows us to maintain quality control but also limits the number of oocytes we can process in a given week.

It is also important to recognise that academic laboratories are spaces for learning, experimentation, and discovery. While we aim to provide consistent and high-quality outcomes, research inherently involves variability. Some approaches may perform well in controlled studies but not translate as expected later on. At times, methods we believe will improve results may not work as predicted. These are not failures, but valuable data points that help refine protocols and expand our understanding. Our commitment is to continual improvement and transparency. Every embryo produced, and every experiment that falls short of expectations contributes to the body of knowledge that advances equine reproductive technologies in Australia and beyond.

Our overall results to date include a total of 141 OPU procedures that yielded 1,196 oocytes processed in 41 ICSI sessions. Mare breeds include Quarter Horse, Warmblood, Standardbred, Stock Horse, Shire and Thoroughbred. Mare's age ranged from 2 to 25 years old. Of these recovered oocytes, 819 (68.5%) matured and ICSI was performed. Cleavage occurred in 539/819 (65.8%), with 22.6% (185/819) forming Day 7–8 blastocysts and 26.4% (216/819) by Days 9–10. The average was 1.53 blastocysts per mare. Outcomes improved from 2024 to 2025: cleavage rates rose from 61.8% to 72.5%, Day 7–8 blastocyst rates from 20.0% to 26.9%, and blastocysts per mare from 1.32 to 1.98. The overall 45-day pregnancy rate following transfer of fresh and vitrified embryos was 70%. Notably, we have been able to produce embryos from deceased stallions and mares. Also, ICSI blastocysts were obtained from mares undergoing treatment for PPID, chronic laminitis, EMS, and chronic uveitis.

8. Conclusion

The establishment of a high-throughput, research-integrated OPU-ICSI platform at The University of Queensland represents a major step forward for equine ART in Australia. By combining scientific rigor, years of experience, and strong industry partnerships, we have demonstrated that medium-scale, reliable *in vitro* embryo production is achievable.

Moving forward, broader adoption of OPU-ICSI will require additional laboratory capacity, targeted training of veterinarians and embryologists, and further investment in infrastructure. With continued industry support, this platform has the potential to become a lead training centre for future equine embryologist with the subsequent impact on equine reproductive efficiency, genetic advancement, and animal welfare**.**

9. References

Hippomundo. (2025, May 13). *Old blood, new champions: The rise of ICSI-bred sport horses from late stallions*. Hippomundo. https://www.hippomundo.com/de/news/old-blood-new-champions-the-rise-of-icsi-bred-sport-horses-from-late-stallions