REPRODUCTION CENTRE

Information for clients

Goulburn Valley Equine Hospital

905B Goulburn Valley Hwy, Congupna
(03) 58 299 566 | info@gvequine.com.au
www.gvequine.com.au

Stallion service

Semen Collection

GVEH boasts an experienced team of veterinarians, technicians and handlers trained in semen collection and evaluation. Stallions that are trained to mount a phantom can be collected using an artificial vagina. Ejaculated semen can then be evaluated and used for direct insemination (fresh) into a mare, chilled for shipment across Australia and New Zealand for insemination elsewhere, or cryopreserved (frozen) for long term storage and use.

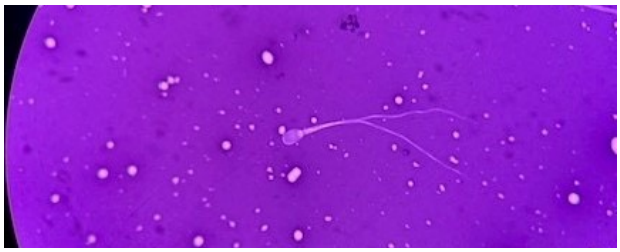
Semen Collection

Breeding management

GVEH stands a number of stallions every breeding season. Our qualified team of staff will manage their routine collections for chilled semen shipments or insemination of fresh semen directly into the mare. At GVEH we aim to optimize your stallion's fertility and reproductive potential by ensuring each insemination dose is collected, evaluated, calculated and shipped in the best possible condition. Availability is limited, so please talk to our reproduction team to arrange to potentially stand your stallion at GVEH for the breeding season.

Breeding Soundness Evaluation

The breeding soundness evaluation includes an assessment of the stallion's general health, external genitalia, libido, behaviour, semen evaluation, venereal disease testing and semen longevity evaluation. Additional diagnostic tests which can be discussed on a case-by- case basis may include testicular ultrasound, ultrasound of internal reproductive organs and specialized sperm viability and function tests.



Semen Morphology – duplicate principal piece (tail)

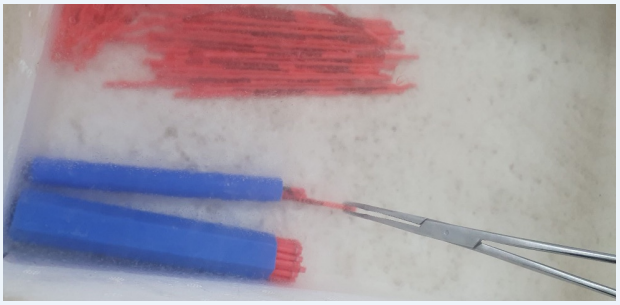
Semen Freezing

GVEH offers semen cryopreservation (freezing) during the months of February-September. Stallions will typically board at GVEH for several weeks. During their stay semen collection, evaluation and freezing will be performed, as well as any biosecurity requirements for semen destined for international use. Because cryopreserved (frozen) semen can be stored in liquid nitrogen indefinitely, it offers several advantages to equine breeding industries. Valuable genetics can be preserved for years and shipped interstate or internationally.

It is important to remember that not all stallions will 'freeze well'. Meaning, not every stallion will produce commercial quality frozen semen following the cryopreservation process. Generally, it is believed that a small percentage of stallions will tolerate freezing poorly, a small percentage of stallions will freeze well, while the majority of stallions will tolerate freezing 'adequately'. It is also important to note that frozen semen is generally regarded as having reduced fertility compared to fresh and chilled semen. For this reason, it is recommended to freeze semen that shows adequate motility and morphology prior to the freezing process.

Semen Freezing cont.

Once your stallion has been collected and evaluated, his suitability for semen freezing can be discussed at this time. Stallions that have not been bred or collected regularly will typically show improvements in motility and morphology with repeated, daily 'clean out' collections, until they reach their daily sperm output. We generally advise that for most stallions, this may involve performing 3-6 collections prior to beginning to freeze the stallion's ejaculate. The number of frozen semen doses that can be prepared and stored for each collection will vary from stallion to stallion. As a rough guide, approximately 5-10 doses per ejaculate may be expected.



Frozen semen straws immersed in liquid nitrogen for processing

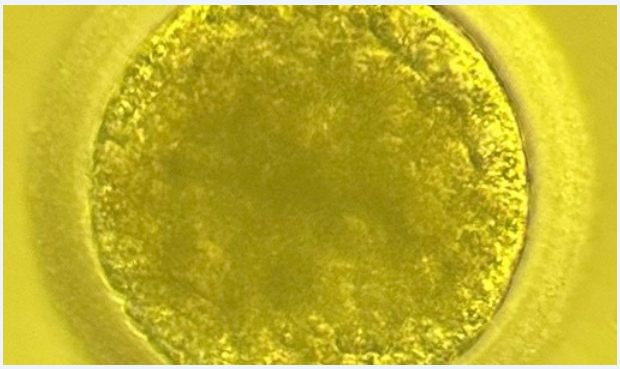
GVEH is an approved quarantine facility. This means that we can produce frozen semen doses suitable for export. Please be advised that quarantine requirements for frozen semen will vary from country to country. Availability is limited, if you have a stallion that you are interested in preparing frozen semen from, whether it is for personal or commercial use, local or internationally, please contact our reproduction center for more details.

Frozen semen and ICSI

Frozen semen is frequently used for creating intra-cytoplasmic sperm injected (ICSI) embryos. As only one sperm is selected for ICSI into each oocyte, a relatively small amount of semen is required for each ICSI session. This can be advantageous, as one single frozen semen straw can potentially be used for multiple ICSI sessions.

Frozen semen and ICSI cont.

Semen frozen specifically for the purpose of ICSI can also be prepared at more dilute concentrations than standard semen doses for artificial insemination (AI). In this regard, ICSI has useful applications for frozen semen that may be in limited supply.



ICSI Embryo

Epididymal Harvest

Epididymal flush or harvest is a procedure that is carried out post castration or post-mortem to flush sperm out of the epididymis, in order to salvage semen. The semen will be analysed and processed for cryopreservation to be used for artificial insemination (AI) or ICSI.

Depending on the stallion, 5-25 breeding doses for AI can typically be obtained. It is important to note that epididymal sperm are regarded as having less fertilizing potential as ejaculated sperm, and the quality of the semen recovered (and likely breeding potential) will vary from stallion to stallion. Other important considerations (particularly for stallions that have been euthanized or died) is the potential impact of medical conditions or medications the stallion may have been receiving prior to epididymal harvest.

This being said, acceptable pregnancy rates can be achieved using frozen epididymal sperm harvested immediately fresh from testes, or from testes cooled and shipped (within 24 hours) to GVEH for processing. Epididymal harvest can be particularly advantageous in some circumstances, such as when a stallion may suffer catastrophic injury or die suddenly, or it can be requested at the time of an elective castration.

Epididymal Harvest

Mare Services

Breeding Management

The GVEH reproduction centre offers a variety of breeding contracts for the complete reproductive management of broodmares. Our breeding management contracts aim to offer an affordable, high-quality service. We pride ourselves on attention to detail and offering tailored breeding plans. Mares can be agisted at GVEH for breeding management, walk into GVEH for their appointments or be managed on farm for mares within our clinic radius. Our contracts include live cover/Thoroughbred breeding, fresh or chilled artificial insemination (AI) or frozen semen AI.

Embryo Transfer

There are many reasons you may want to consider using embryo transfer for your mare. It can be beneficial to allow young mares more time in competition, it offers the potential to produce more than one pregnancy per breeding season, it will negate risks associated with late gestation and parturition for mares with conditions that may be exacerbated by pregnancy or parturition such as laminitis, abdominal wall rupture or pelvic fracture,

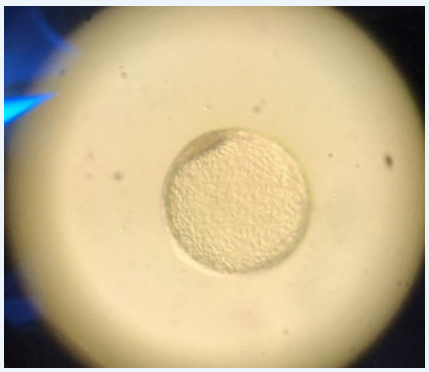
Embryo Transfer cont.

or to allow problem breeders to produce pregnancies that have difficulty maintaining the early embryo or later stages of gestation.

Embryo transfer can refer to the transfer of both ‘in vivo’ and ‘in vitro’ produced embryos. We will discuss the production of ‘in vitro’ produced embryos via intra-cytoplasmic sperm injection (ICSI) under oocyte pick up below. For now, we will be discussing the production of ‘in vivo’ produced embryos. Mares are bred using fresh, chilled or frozen semen. Following breeding, embryo flush is typically performed 7-8 days post ovulation to recover an embryo that can be transferred immediately into a recipient on site.

At GVEH we are fortunate to be home to a large herd of recipient mares that are specially selected for their reproductive health and suitability for raising a foal. This allows us to have access to a selection of healthy, cycling mares throughout the breeding season to transfer embryos into. Alternatively, you may choose to use your own recipient mare. Mares can have their complete reproductive management for breeding, embryo flush and transfer performed at GVEH. Alternatively, some mares will be bred at another location and walk into GVEH for an embryo flush appointment and transfer of any recovered embryos.

Following embryo recovery, embryos do not necessarily have to be transferred fresh. It is also possible for embryos to be chilled for shipment to another location. This means that GVEH can also accept chilled embryos that have been flushed and recovered by outside clinics via same-day or overnight shipment (up to 24 hours), for transfer into one of our recipient mares. A final option is for a recovered embryo to be cryopreserved by vitrification or slow freezing for storage and transfer at a later date.

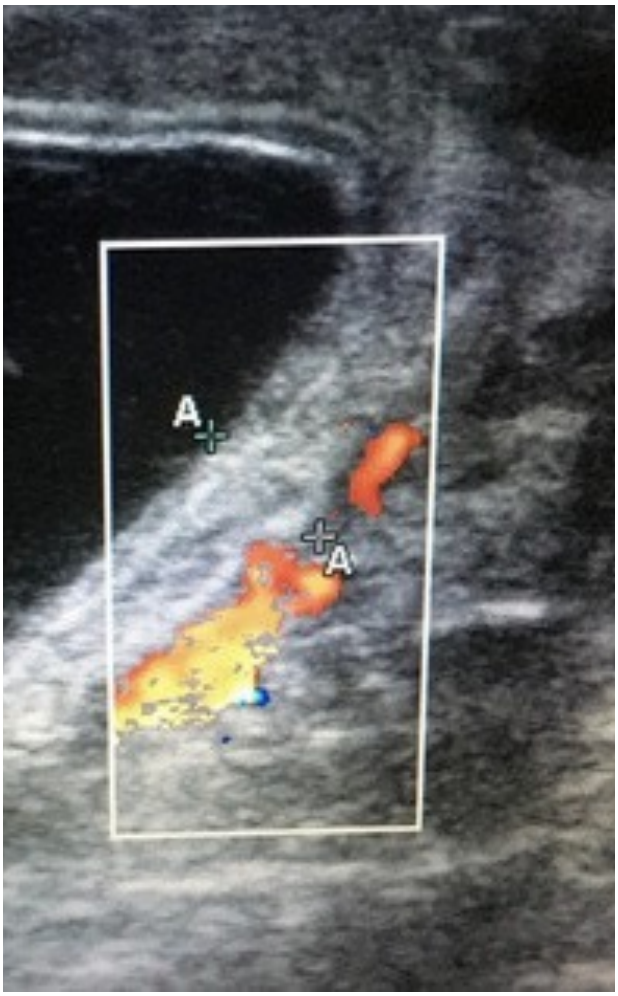


Embryo Transfer Day 8 Blastocyst

Pregnancy Monitoring and Placental Scanning

There's nothing quite as exciting as witnessing the wonder of the fetoplacental unit in real time through ultrasonography. Transrectal and transabdominal ultrasound of the pregnant mare can be performed both on farm and at the GVEH. Parameters such as fetal heart rate, blood flow, fetal mobility, fetal fluid quality, depth, placental thickness, separation and more can be carefully observed to gain insight into pregnancy health and assist in the diagnosis and management of conditions such as placentitis.

GVEH offers affordable placental scans and pregnancy monitoring plans for the high-risk mare. If you have a mare that has a history of pregnancy loss, you may wish to contact our reproduction department for more information.



Placenta Scan

Fetal Sexing

Performing fetal sexing provides advantages to breeders for making early plans for rebreeding, sale or purchase of stock. There are two windows of gestation described for fetal sexing to be performed. GVEH advises clients that we prefer to perform fetal sexing between 60-70 or 110-130 days gestation. The early window identifies the fetal sex based on the location of the genital tubercle (fetal precursor to the clitoris and penis) while the late window is based on identifying the presence of male or female gonads and genitalia (penis, prepuce, clitoris) or mammary glands.

Fetal Sexing - Mammary Glands

Fetal Abortion Post-Mortem

There are many causes of fetal loss and abortion in Australian broodmares. While clinical indicators such as history, presentation and examination of the mare can provide clues as to the cause of abortion for some cases, this will usually be speculative without a post-mortem examination of the fetus and fetal membranes.

Identifying the cause or likely causes of abortion in mares allows breeders to implement strategies to prevent further fetal loss on the farm for current and future foaling seasons. The fetal post-mortem examination can help to answer questions such as (not limited to):

- Does the mare need to be quarantined following abortion to prevent the spread of infectious causes of abortion or an abortion storm?
- Should the mare be monitored during future pregnancies for placentitis or placental insufficiency?
- Should the farm implement strategies to prevent Equine Amniotis and Fetal Loss (caterpillar abortions)

GVEH offers complete abortion post-mortem reports and consultation including; thorough examination of the placenta, fetus and fetal organs, comprehensive histology, bacterial culture and sensitivity and Chlamydia psitacci and EHV-1 (Equine Herpes Virus) PCR testing. This will be completed by board certified reproductive veterinarians. Alternatively, examination can be tailored to fit your budget and risk factors, based on the history of your mare and breeding farm. Please contact our reproduction department for more details on fetal-post mortem testing and prices.

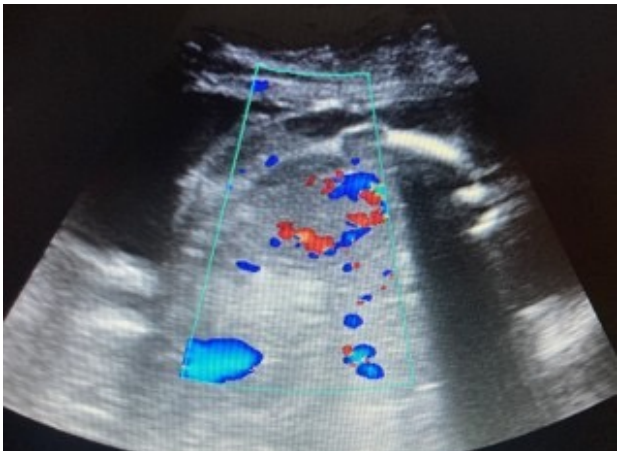
Breeding Soundness Evaluation and Problem Mare Management

At GVEH, we love a good problem mare puzzle! The breeding soundness evaluation aims to identify causes or contributing factors to subfertility in broodmares, and which treatment strategies are likely to enhance your breeding outcomes. The breeding soundness evaluation of the mare typically involves reproductive examination with palpation and ultrasound, vaginal and cervical examination, uterine culture and cytology via low volume lavage and endometrial biopsy.

Breeding Soundness Evaluation and Problem Mare Management cont.

Additional diagnostic tests that can be discussed on a case-by-case basis also include hysteroscopy (visualizing the inside of the uterus with an endoscope), endocrine testing and cytogenetic analysis.

If you have a problem breeder, having a breeding soundness evaluation performed prior to the beginning of the busy breeding season is ideal, in order to have a breeding strategy in place ahead of time.



Fetal Sexing - Filly Gonad

Reproductive Surgery

The normal mare has a number of anatomic defenses against contamination of the uterus which may become compromised with advancing age/repeated pregnancies, or through foaling trauma.

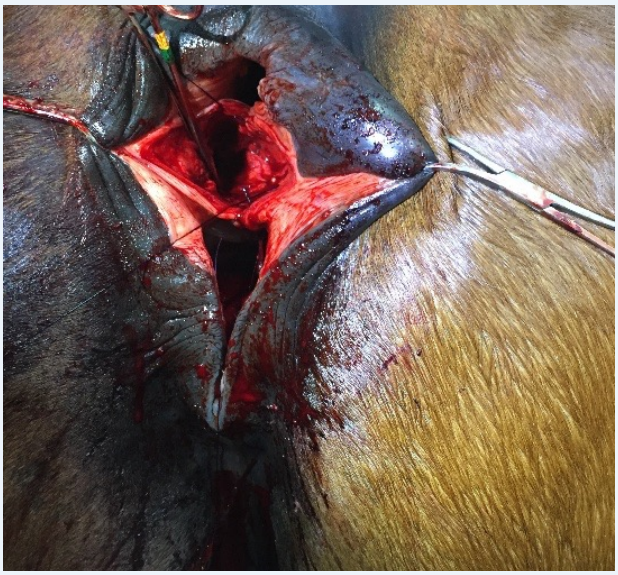
Conditions such as vestibulo-vaginal sphincter or cervical incompetence, rectal fistulas, perineal lacerations or urine pooling can benefit from reproductive surgery.

These conditions may be obvious in some cases (e.g. perineal laceration) immediately post- partum from foaling trauma, or be identified during evaluation of the mare for problem breeding.

GVEH can offer reproductive surgeries including but not limited to; episioplasty, urethral extension, perineal laceration repair, ovariectomy.

Reproductive Surgery cont.

One of several important considerations prior to performing reproductive surgery is the reproductive potential of the mare after successful surgical correction i.e. is your mare likely to still be a useful/ viable breeding prospect even if the defect is corrected? GVEH is happy to receive referrals from your regular veterinarian or evaluate your mare for potential surgical correction.



Third degree perineal laceration repair

Twin Reduction

Due to the high risk of poor outcomes related to twin pregnancies in the mare (placental insufficiency, abortion, dystocia, still born, premature or weak foals, to name a few) twin management is exceptionally important for broodmares. Twins are preferred to be reduced prior to pregnancy fixation (day 16 gestation) when mobility of the embryonic vesicle facilitates manual reduction and a successful outcome is maximized.

There are many techniques described for the management of twins post fixation and their selection is based on the stage of gestation and to some degree, clinician preference. These include but are not limited to; manual manipulation, fetal oscillation, aspiration of fetal fluids and fetal intra-cardiac injection.

Twin Reduction cont.

Management of twins post fixation can be technically difficult and it is preferable to have twin pregnancies examined as early as possible, so that a treatment plan with the most likely successful outcome can be made. If you would like to refer a twin pregnancy to GVEH for examination and management, please contact our reproduction department as soon as possible.

Twin pregnancy- day 14

High Risk Pregnant Mare Monitoring/Foaling Down

A high-risk pregnant mare may be a mare that has a medical condition that can be exacerbated by foaling or pregnancy e.g. laminitis, or conditions of pregnancy that put her at a high risk of dystocia or a weak foal e.g. prepubic tendon rupture, placentitis, placental separation. A high-risk pregnant mare and her foal are more likely need urgent and intensive medical intervention.

Boarding a high-risk pregnant mare at a facility such as GVEH allows her access to veterinary care as needed. Mares with pregnancy conditions such as placentitis can have regular pregnancy monitoring implemented to adjust their treatment accordingly. If foals are born sick and compromised, intensive medical care can be implemented without delay. In the unfortunate event that your mare requires emergency Cesearian section, this can also happen without delay.

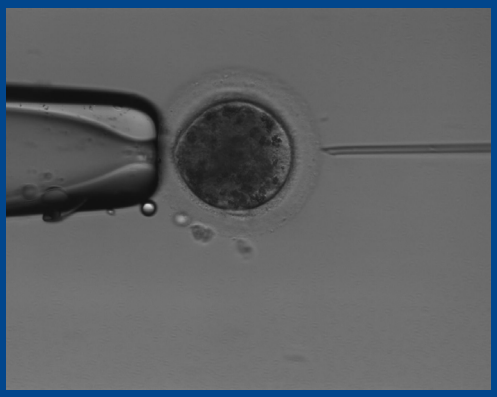
Oocyte Pick Up (OPU) for Intra-cytoplasmic Sperm Injection (ICSI)

GVEH is excited to be able to offer oocyte pick up (OPU) for the purpose of intra- cytoplasmic sperm injection (ICSI).

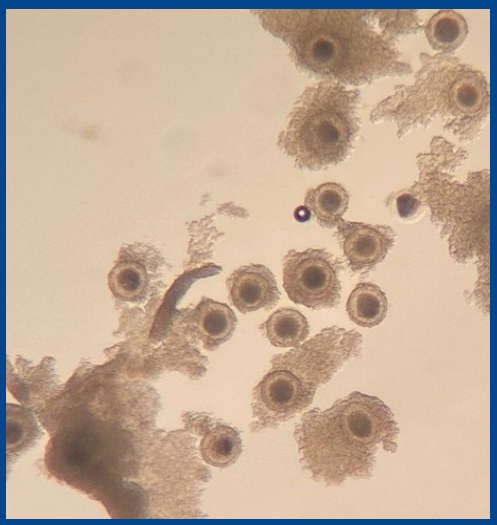
What is ICSI?

ICSI is a technique of in vitro breeding where a single sperm cell is injected into the ooplasm of a mature oocyte for fertilization and embryo production. There are multiple steps involved in the production of ICSI embryos. It requires highly skilled personnel to perform these techniques and manage these laboratories, in order to provide optimum conditions for maturing oocytes and growing ICSI embryos.

GVEH has two ICSI laboratories available to ship oocytes to; the University of Queensland and the University of Newcastle. For more information, please see our OPU/ ICSI forms or talk to a member of the reproduction team. The following information will aim to present an overview of the procedures conducted after the arrival of immature oocytes to an ICSI laboratory.



ICSI



Equine oocytes harvested from OPU

Oocyte maturation

GVEH aims to harvest a number of immature oocytes from a mare during an OPU session (as many as possible). Because immature oocytes are not ready for fertilization, when these oocytes arrive at the laboratory they are first incubated in conditions which promote oocyte maturation. During oocyte maturation the oocyte nucleus advances in meiotic division and arrests at a stage when fertilization can occur. Oocyte maturation also involves important changes to the cytoplasm of the cell which will support fertilization and early embryonic development.

Sperm Selection

ICSI can be performed using fresh, chilled or frozen semen. Frozen semen is frequently used, as it is able to be stored at the laboratory and thawed as needed for ICSI procedures. Sperm selection techniques aim to select a population of viable sperm from the raw sample based on sperm properties such as motility, density or DNA content.

ICSI Procedures

The ICSI procedure requires the use of a high powered, inverted microscope with micromanipulation tools that allow 3-dimensional visualization and control of a holding pipette (to stabilize the oocyte by gentle suction) and microinjector pipette (to perform ICSI injection).

A single sperm is selected by the embryologist that appears to be motile and morphologically normal. The sperm must be caught using the microinjector pipette and rendered immobile by breaking the sperm at the midpiece. The sperm is aspirated into the microinjector pipette and is ready for ICSI injection. ICSI injection is performed, and the sperm is injected into the ooplasm of the oocyte.

Oocyte pick up (OPU)

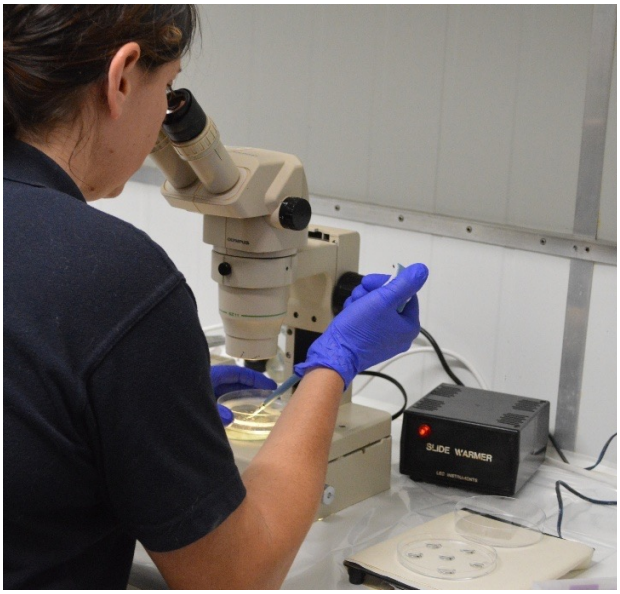
Embryo Culture

The injected oocytes are then placed into embryo culture medium in tri-gas incubators to promote embryonic development. Developing embryos are highly sensitive to environmental factors such as media properties, air quality, light, temperature, pH, and a variety of potential toxins such as volatile organic compounds. Meticulous attention to detail in maintenance and quality control is important for the success of ICSI laboratories.

Injected oocytes will typically be monitored for the presence of early embryonic cleavage days 4-5, and for development to blastocyst stage from day 7-10.

Embryo Culture cont.

When the embryologist is satisfied that a blastocyst has formed (typically day 7-8), the embryo can be transferred fresh or cryopreserved. Embryos are frequently cryopreserved by vitrification or slow freezing so that they can be stored and shipped to a recipient mare facility such as GVEH, to be warmed and transferred at a later date.



Oocyte Processing

Oocyte Pick up (OPU)

What is OPU?

Oocyte pick up (OPU), also known as transvaginal aspiration (TVA) is a technique for harvesting oocytes from a mare's ovaries. An ultrasound probe is placed in the vagina of the mare, and the veterinarian manipulates the ovary per rectum to secure one ovary in front of the vaginal ultrasound probe, allowing visualization of the follicles. A 12-gauge double lumen needle is manipulated through a needle guide built into the vaginal probe. The needle is used to puncture, scrape and flush the follicles with media and the flushed media is aspirated into a collection bottle. The collected media can then be filtered and searched for oocytes.

What is OPU? cont.

Finally, oocytes are washed and packaged at room temperature for same-day or overnight shipment to the ICSI laboratory. Our reproduction team places emphasis on the care of your oocytes from retrieval to arrival at the ICSI laboratory. Management of factors such as temperature, contamination or exposure to potential toxins during harvest, processing and shipping can all impact the success of ICSI procedures.

How are mares restrained for the procedure?

Mares are positioned in modified stocks that allow them to stand comfortably under heavy sedation. An intravenous catheter is placed prior to the procedure to facilitate sedatives, pain relief and smooth muscle relaxants (to induce relaxation of the rectum) to be administered as needed for your mare. A member of the GVEH team will be appointed to monitor the level comfort and sedation of your mare throughout the procedure.

Mild discomfort for 1-3 days may be seen in some mares. This is more likely to be seen in young maiden mares. Pain relief can be administered, but should be discussed with a GVEH veterinarian first. Prophylactic antibiotic is also administered for 3 days following the procedure.

Mare restrained for OPU

Are there risks associated with OPU?

Like any veterinary procedure, complications are possible and it is important that you understand these risks before having the procedure performed via a discussion with one of our reproductive veterinarians. OPU is performed by a board-certified reproductive veterinarian with advanced training in these techniques. It is advised for mares to stay at GVEH the evening following OPU so that they can be monitored overnight.

OPU probe and needle

Why do OPU/ICSI?

There are a number for advantages to performing OPU/ICSI embryo production:

- The donor mares do not need to be cyclic- this allows OPU/ICSI procedures to be performed outside the breeding season.
- **Problem breeders:**
  - OPU/ICSI provides an avenue for potential embryo production for mares with cervical, uterine or oviductal pathology the impedes their capability to create embryos from in-vivo breeding
  - Donor mares with irregular estrous cycles are candidates for OPU/ICSI as a normal pre-ovulatory follicle and ovulation is not required
- Convenience in maintaining competition schedules and training- the number of reproductive examinations is minimal compared to in-vivo breeding, which can be advantageous for mares in competition
- Sub-fertility in the stallion- some subfertility issues in a stallion may be circumvented through ICSI procedures
- Semen in limited supply- comparative to other breeding strategies, only a small amount of semen is required for ICSI procedures. See Stallion services- frozen semen and ICSI

When should I perform OPU/ICSI?

The reproductive efficiency of OPU for the collection of immature oocytes is heavily dependent on the number of follicles available for aspiration, and GVEH recommends your mare ideally have a minimum of 10 follicles (>5mm) total present.

OPU/ICSI can be performed when your mare is cycling, or in spring/autumn transition. This will require your mare to be examined within a few days of having the procedure performed to determine that her follicular growth is sufficient, and depending on the mare, may require repeat examination for optimal timing. GVEH is currently offering OPU procedures February-May and August- September. This will be subject to change dependent on laboratory availability.

What is the likely success OPU/ICSI?

Like in-vivo breeding techniques, the success of OPU/ICSI is going to vary between individual mares and stallions. The following information is presented as a general guide. It is advised to expect 1-2 OPU/ICSI sessions for the production of at least one embryo from the average mare. Recovery rates from OPU (oocytes per follicle aspirated) vary, but are typically between 50-80%.

Approximately 60-80% of these will reach maturation in the ICSI laboratory and be available for ICSI injection. The rates of embryo loss for ICSI embryos is often higher than that typically achieved by in-vivo breeding, such that the live foal rate per transferred embryo is typically reported as 50-60%.

Goulburn Valley
Equine Hospital

905B Goulburn Valley Hwy, Congupna
(03) 58 299 566 | info@gvequine.com.au

www.gvequine.com.au

Version 1, May 2025