# OVUM PICK UP (OPU) AND INTRACYTOPLASMIC SPERM INJECTION (ICSI)

## OPU

OPU or TVA (transvaginal follicular aspiration) is the procedure involving puncturing, emptying and re-filling of ovarian follicles (flushing) via repeated suction and scraping in order to collected oocytes.

The procedure is performed after extensive preparation of the mare under sedation while the mare is standing in the crush. Anti-inflammatories, antibiotics and intestinal relaxant are administered. A urinary catheter is placed in the bladder and the mare receives an epidural in the majority of cases to assure the procedure is as safe and comfortable as possible. The comfort and well-being of the mare is of the highest priority to us and if mares are not settled, we do not continue.

During the procedure, the veterinarian will rectally locate and reposition the ovary. A specialised ultrasound probe with needle guide is inserted in the vagina of the mare. The ovary is manually fixed through the rectum and visualised through the vaginal wall with the ultrasound probe. The follicles visualised by the ultrasound probe are then individually punctured and flushed multiple times using a long double lumen needle inserted through the needle guide. The procedure is repeated on the other ovary. The procedure takes between 20-50 minutes depending on the number of follicles present on the ovaries.

## ART LABORATORY

The retrieved fluid is taken to our laboratory and examined and assessed for the presence of oocytes under a microscope. Once the follicular fluid has been examined and oocytes identified, the oocytes are washed, cleaned, graded and moved into a maturation medium.

The time OPU-retrieved oocytes require to mature to a stage where they can be fertilised depends on a number of factors. Cumulus morphology, mare age, stage of the mare's cycle and suitable maturation culture are thought to be the most influential on the length of time the oocyte needs to reach metaphase II, characterised by the first polar body extrusion. Successfully cultured oocytes spend between 15-36 hours in the maturation medium. Currently around 60% of oocytes retrieved after OPU reach the maturation stage ready to be fertilised.

## ICSI (INTRACYTOPLASMIC SPERM INJECTION)

The metaphase II oocytes are prepared for ICSI which is performed on an inverted microscope with heated stage and micromanipulators. The oocyte is fixed by the holding pipette at the end of one of the manipulators. Another manipulator guides the injection pipette which holds the individual sperm. The sperm is then injected into the oocyte.

Once the oocytes are fertilised, they are positioned in culture medium and then placed in an incubator. The injected oocytes (now zygotes) are incubated until an embryo (blastocyst) has developed which can take 7 to 10 days.

The successfully produced embryos can then be transferred in a suitable recipient mare during the breeding season using conventional insemination techniques or frozen and thawed at a suitable time. This is particularly useful when OPU/ICSI is performed outside the breeding season or when no suitable recipient mares are available.

## SEMEN

Semen used for ICSI can be fresh, chilled or frozen but frozen semen is commonly used. Because very few sperm are needed, only a very small piece is cut off one straw, while preserving the remainder in the liquid nitrogen for future use. The small amount of sperm can be processed in various ways, all to ultimately help select quality motile sperm. Individual sperm are subsequently selected under the microscope and immobilised with the ICSI-microscope injection pipette. Immobilisation is attained by damaging the tail of the spermatozoon. Once injected in the oocyte, the damaged membrane of the tail releases specific proteins (sperm protein factor) which are responsible for initiating the cascade of oocyte activation and cell division.

Mare owners are fully responsible for the conditions relating to the stallion/semen in relation to its use for ICSI. Stallion semen cannot be used for ICSI unless Willinga Vet Services Pty Limited (WVS) has received confirmation from the stallion's owner or its agent that the mare's owner has met its contractual obligations with respect to the use of that semen.

It is the responsibility of the mare's owner to keep up to date and be aware of the requirements for the semen used for ICSI. It is the responsibility of the mare's owner to organise the registration of any ICSI foal in the desired breeding organisation.

# RISK AND RESPONSIBILITY

There is risk associated with both rectal examination and transvaginal aspiration of follicles of the ovaries of the mare. The WVS team makes the mare's well-being and safety the highest priority. Strict hygiene, prophylactic antibiotics, anti-inflammatories, epidural anaesthesia and sedation are routinely administered. The procedure is performed in a quiet and controlled environment ensuring the mare is relaxed. If at any time during the procedure the conditions change, the risks and the mare's situation will be assessed and, if required, the procedure will be stopped and possibly rescheduled for a later date.

In general, the risks associated with OPU/ICSI are low but the mare's owners must be aware that complications may occur with OPU. Although uncommon, possible complications include rectal tears, rectal, vaginal and ovarian bleeding, rectal and ovarian abscesses and peritonitis, which in rare cases can all lead to death of the mare. A small number of mares can experience slight discomfort, fever or loss of appetite for a few days after an OPU/ICSI procedure.

OPU/ICSI is performed at the owner's risk and WVS accepts no liability for any damage caused or loss suffered, including the death of the mare. Any insurance of the mare against these risks is the responsibility of the owner.

## STORAGE OF EMBRYOS AND SEMEN

Storage of semen and embryos at Willinga Park Vet Hospital is at the owner's risk. Accidental loss of semen and or embryos by fire, services failure, weather event is a possibility and WVS is not liable for these events. Notwithstanding that semen storage tanks are regularly checked, liquid nitrogen levels are monitored every 2 weeks and storage tanks are refilled every 6 weeks, WVS will not be liable for semen or embryos that are lost through tank failure. Any insurance against this risk is the responsibility of the Mare owners

## PREGNANCY LOSS

There is a 60% change of producing a viable embryo from one OPU session. After transfer of the embryo to a suitable recipient, 70% of transferred embryos result in a pregnancy.

The owner accepts that there is approximately a 15% risk of pregnancy loss in the first 45 days of gestation which is slightly higher than in non-ICSI pregnancies.

The incidence of monozygotic twins or triplets of a single in vitro produced embryo is low (1.6%). Detection prior to development of the endometrial cups is crucial because of the poor outcome irrespective of an attempt to reduce the twin or triplet to a single embryo. WVS cannot be held liable for any pregnancy loss due to twin/triplet reduction.

## AGISTMENT

All costs and risk associated with agistment of the mare at Willinga Park Vet Hospital are the responsibility of the owner. WVS cannot be held liable for injury, disease or death of the mare while resident at Willinga Park Vet Hospital. WVS is authorised to secure emergency care whenever necessary, and this may incur additional fees.

# OWNER AND INVOICE INFORMATION

MARE: MOBILE NUMBER:

MARE OWNER: EMAIL ADDRESS:

I hereby declare that I have read and understood the information in the sheet.

I consent to the procedure being carried out on my mare.

I acknowledge and accept the WVS Terms and Conditions at **willingapark.com.au/vet-client-downloads**

By signing this contract, I warrant that I am the true and lawful owner of the mare or if not the owner, then I have the actual authority to act as the owner's agent for purposing of entering into this contract and to make any and all decisions regarding the animal and its veterinary care.

I agree that I will be fully responsible for all charges incurred under this contract and that I will indemnify, defend and hold harmless Willinga Park Vet Hospital from any and all claims, of every kind and nature, arising as a result of or in connection with this contract (including any claim that such person lacked the right or authority to execute the contract on behalf of the true owner).

SIGNATURE: DATE: