

Salvaging Epididymal Sperm 'It's not too late'

The loss of a breeding stallion can not only be a distressing and emotional time for a stallion owner, it can furthermore have devastating financial implications. Customarily the breeding potential of a stallion is lost with death, nevertheless advances in cryopreservation techniques have meant that stallions can now have genetic material collected and stored indefinitely. In ordinary circumstances, valuable breeding stallions have semen collected and cryopreserved routinely during periods of competing rest, or when fresh breeding programmes have concluded. However, if this is not always possible, and when stallion death is sudden and unanticipated, semen may now be salvaged and cryopreserved retrospectively.

Collection and cryopreservation of epididymal spermatozoa can be used as a terminal procedure in stallions with irreparable conditions, such as when fractures or colic have occurred or where the testes have suffered a severe trauma or torsion. In addition, epididymal semen may also be collected and cryopreserved following elective castration. This procedure enables us to salvage valuable genetics which would have otherwise been lost at a time when there is no other option.

The production of spermatozoa (spermatogenesis) takes approximately 57 days in the stallion. Sperm are produced in the testes and mature during the 7-10 day period it takes to transit the length of the convolutedly coiled epididymis. The intricate process of developmental progression that occurs at this time gives the spermatozoa the capability of fertilisation and motility. The epididymides are the principal storage sites of the testes and contain billions of spermatozoa. Epididymal spermatozoa have restricted motility which is significantly increased once the sperm are ejaculated, and mixed with the secretory fluids of the accessory glands such as seminal plasma.

For the procedure the stallion has one or both testes removed using standard castration techniques, leaving the tail of the epididymis and as much of the ductus deferens as possible intact. The deferent duct is then ligated with a suture material to prevent sperm from leaking in transit. Each testis is washed with saline and placed in a sterile plastic bag. The

testes must be kept chilled and are packaged into an insulated shipping container with ice packs, a buffer is used to separate the testes from the ice packs to prevent freezing. The samples are then couriered to the centre in Shropshire for processing.

The success of this procedure is very much dependent on the speed at which the testes are transported to the centre, from the time the animal was castrated or the time of death. In most instances, spermatozoa will survive and maintain capacity for fertilization for up to 24 hours after the death of a stallion. It is recommended that sperm be harvested and cryopreserved as soon as possible following death, euthanasia, or elective castration of a stallion. At Stallion AI Services samples are requested to arrive within 12 hours of castration to allow for ample processing time of the testes.

Once at the semen laboratory, the testes are dissected and the sperm cells are harvested from the epididymis using a combination of techniques including aspiration, flushing and flotation. The semen sample is centrifuged and a density calculation is carried out before final dilution. The sample is then packaged and cryopreserved using standard laboratory techniques.

Although this procedure is normally carried out at short notice and often under difficult circumstances for the stallion owner, it is essential that bio-security is not overlooked in order to prevent future disease outbreak. At Stallion AI Services the donor stallion is required to undergo a full pre-entry health testing programme, this includes a blood sample tested for Equine Infectious Anaemia (EIA), Equine Viral Arteritis (EVA) and a swab for Contagious Equine Metritis (CEM). These tests can be done at the point of castration.

The number of breeding doses collected differs largely from stallion to stallion depending on testicle size and the density of the semen sample collected. On average the final number of doses varies from between 10-40 doses, based on collecting and processing epididymal spermatozoa from both testes.

There is not an abundance of data on the fertility results of epididymal semen salvaged and cryopreserved in this manner. Although, it is known that a mare inseminated with

cryopreserved epididymal semen will have lower pregnancy rates when compared with a mare inseminated with ejaculated frozen semen from the same stallion. However, with specialised processing techniques being used at Stallion AI Services, post thaw results of up to 50% Progressive Linear Motility (PLM) and positive pregnancy results are being achieved.

For the future, Intracytoplasmic Sperm Injection (ICSI) may be the preferred method of use for epididymal semen. This procedure requires only one sperm cell to be injected into an oocyte and therefore would help to conserve spermatozoa banks. When using ICSI to obtain a viable pregnancy, epididymal semen produces the same pregnancy rates as that of ejaculated frozen spermatozoa.

Stallion AI Services always recommend that this procedure should be used as a last resort and not as a replacement for traditional freezing methods. Although pregnancy results are being achieved, fertility is likely to be lower than that of semen collected and frozen in the traditional manner. However, care taken to collect the testes and epididymides properly, combined with correct packaging and shipping, could have the greatest influence on salvaging viable sperm. As the developments in assisted reproductive techniques continue, it is doubtless that there will be a higher instance of viable pregnancies obtained from cryopreserved epididymal sperm from valuable stallions.