The effects of different Cryoprotectants on Stallion Epididymal Spermatozoa

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ABSTRACT

Sudden death, catastrophic injury, castration or any other event that makes semen collection or mating impossible may prematurely conclude a stallion's reproductive life. From the point of any one of these events onwards, the genetic potential of that horse is lost. Fortunately frozen semen has proven to be an effective way to preserve the genetics of an individual stallion. Indeed, the first foal produced from frozen semen was in Canada in the 1950s and was the result of frozen "epididymal" sperm. Since then, despite numerous studies, no more pregnancies were reported from artificial insemination with frozen epididymal sperm until studies investigating stallion fertility were performed at the TBA Equine Fertility Unit, UK by Prof. Twink Allen and Dr Lee Morris in the late 1990s.

What is epididymal sperm? In the reproductive system of all male mammals the sperm leave the testes via a long coiled tube called the epididymis. They are stored there until ejaculation. Stallion sperm capable of fertilization can be harvested from the epididymis and frozen for future use. However, the fertility of thawed-frozen epididymal spermatozoa has previously been shown to be lower than that of ejaculated sperm. Understanding the factors that affect the fertility of this stored epididymal sperm may also shed some light on factors affecting the overall fertility of stallions. Recently, the beneficial effects of using formamides as cryoprotectants in combination with a variety of amino acids and complex sugars for the freezing epididymal spermatozoa has been shown to be useful for both epididymal sperm and subfertile stallions.

The aim of this research project was to determine the origin of these beneficial effects, whether they be due to different cryoprotectants or different amino acid and sugar combinations. It has also been proposed that epididymal sperm are less fertile due to their lower motility. Based on this observation stimulation of motility in epididymal sperm would theoretically enhance its ability to reach the oviduct where the oocyte awaiting fertilization resides. This theory was also tested in this project by exposing thawed-frozen epididymal spermatozoa to the post thaw media SW3 (Jel Media, Auckland NZ).

The epididymal sperm were recovered from 5 colts at the time of castration. Each semen sample was split into different treatment groups using the same base media and frozen with different combination of cryoprotectant, Glycerol, Formamide or a combination of both. The semen was frozen and stored in liquid Nitrogen until required for insemination. After thawing the motility, plasma membrane integrity, acrosome status and DNA fragmentation was analysed to acquire information on sperm viability and longevity. The reproductive tracts of mares were examined by ultrasound during oestrus and inseminated at 4h prior to ovulation with semen frozen with either Glycerol or a Formamide combination. At 8 days after ovulation the uterus of each mare was either flushed for an embryo or the mare was scanned for pregnancy at 14 days after ovulation to determine conception rates.

After thawing, there were no significant differences in the motility parameters amongst the treatment groups. However, the addition of SW3 media to the post thaw samples frozen with Glycerol resulted in significantly higher motility than when added to epididymal spermatozoa frozen in the other cryoprotectants. However, the addition of SW3 media to the post thaw samples frozen with Glycerol resulted in significantly higher motility than when added to epididymal sperm frozen in the other cryoprotectants. There were no significant differences in plasma membrane integrity amongst treatment groups, nor were there any significant differences in DNA fragmentation or chromatin integrity.

To date the conception rates of the epididymal sperm frozen with the formamide combination was higher (62%) than the sperm frozen in glycerol alone (13%). Furthermore, with our new semen freezing method we have found that hysteroscopic insemination is no longer required for epididymal sperm and that deep uterine horn insemination can produce good pregnancy rates under good veterinary management.

If the semen was frozen with Glycerol as the cryoprotectant, then there was a beneficial effect of adding SW3 media (JEL Media, NZ) to the frozen thawed epididymal sperm. However, the increased fertility of epididymal spermatozoa frozen with the glycerol:formamide combination rather than glycerol alone, suggests that it is the effect of the cryoprotectant and not the complex amino acids that contributes to the improved fertility of the frozen epididymal spermatozoa. These results may also provide some insight into the fertility of frozen semen from subfertile stallions.

The key finding of this study is the development of a semen freezing method for epididymal sperm that will produce good pregnancy rates using deep uterine insemination that is available to many vets with an interest in frozen semen inseminations.

Over the years, various techniques for semen collection and preservation have been developed, but few researchers have focused on maximising the use of epididymal sperm cells from stallions. It is of great importance that experiments involving epididymal sperm are undertaken using techniques which allow higher pregnancy rates from low doses of sperm to maximize its use when in limited supply. This research will not only have great benefits to the sport-horse industry but it will also have important implications for the conservation of endangered species and understanding factors that affect the fertility of semen from all stallions.