REPRO TRACKS

by Cliff Lamb, Texas A&M University

Reproductive Frontier

Use of cryopreservation to store and ship our cattle genetics.

The advent of cryopreservation (process of cooling and storing cells, tissues or organs at very low or freezing temperatures to save them for future use) allows cattle producers to store semen, embryos and other genetic material for later use or shipment worldwide.

A minority of livestock producers realize the effects this technology has had on global production of livestock. Not only has the technology simplified artificial insemination (AI) and embryo transfer (ET) management, granting more flexibility, but it has also allowed easy transport of cells globally with limited harm to the genetic material.

Consider the effects on humans, where gametes or embryo cryopreservation have brought fertility to a new level by providing patients with terminal diseases like cancer the possibility of having children after aggressive treatment.

Still, like other reproductive technologies, cryopreservation far from mimics physiological conditions, and therefore can result in lower pregnancy rates or greater pregnancy losses. In cattle, pregnancy rates from ET tend to be 10-15% lower when frozen rather than when fresh embryos are utilized.

There is considerable variability between gametes (the male and female cells that unite to create an embryo) and embryos of different species in terms of membrane characteristics and lipid content, which has resulted in varying success among species and often within species. This may partially explain why semen from some bulls does not handle freezing as well as other bulls'.

The process

Cryopreservation can be performed in two ways: slow freezing and vitrification. In both strategies, cells are treated with cryoprotective agents, such as glycerol, ethylene glycol or dimethyl sulfoxide (DMSO).

When slow freezing is utilized, the cooling rate is about one degree Celsius (C) per minute, it is achieved with rate-controlled freezers. While commonly used and yielding acceptable pregnancy rates, this process takes about three hours to be completed, demands a higher sample volume, may be accompanied by ice crystal formation and can cause mechanical damage to the cells.

The vitrification procedure was developed later as an alternative to the standard slow freezing. Vitrification is an ultrarapid cooling technique that requires a high concentration of cryoprotectants and utilizes liquid nitrogen to reach a cooling rate between 15,000 and 30,000 degrees C per minute. With this technique, an ice-free solidification of water-based solutions will occur at subzero temperatures due to the elevation in viscosity (contribution of cryoprotectants). Therefore, there is no formation of ice crystals, since water is transformed into a glassy, vitrified stage.

The process takes less than 10 minutes and generates less mechanical damage to the cells when compared to the slow-freezing process. However, the high amount of cryoprotectants may still be

toxic to the cell, and efforts must be directed to develop a vitrification protocol that increases the speed of temperature change while keeping a low concentration of cryoprotectants.

For embryo cryopreservation in cattle, ethylene glycol currently appears superior to the other cryoprotectants. Research has shown enhanced post-warming survival rates when lipogenesis (formation of lipids) was inhibited by adding conjugated linoleic acids or lipolysis agents to the cryopreservation media. Embryo survival after cryopreservation is a multifactorial process, mainly influenced by lipid content and composition. Recent advances in vitrification might have partially mitigated some of these issues, but not all of them and not in all species.

Cryopreservation of oocytes is more challenging compared to embryos for a few reasons: 1) oocytes have a greater cellular volume, making them more susceptible to forming ice crystals; 2) oocytes have an inferior membrane elasticity, so they can be easily injured during freezing; and, 3) cryoprotectants can increase cytosolic calcium concentrations during either vitrification or warming procedures.

The increased calcium in the cell cytoplasm induces the hardening of the zona pellucida, a similar process to avoid polyspermy and prevent the penetration of sperm and further fertilization.

Cytosolic lipids are also limiting factors to the cryopreservation, even though they are important for oocyte maturation and development. Cattle oocytes have better freezability among all species and are more likely to develop into blastocysts after *in-vitro* fertilization (IVF).

Advancements

Improvements in bovine cryopreservation have been made over the years, providing even better results, such as using polymers as cryoprotectants to lessen toxicity, using Cryotop® (a method of vitrification) or chemicals, such as conjugated linoleic acid, to decrease injury to the cytoskeleton. Cleavage rates are usually lower than immediate/fresh oocyte survival rates, but they range between 50-60%, which is highly acceptable.

Sperm cryopreservation is what we largely use for freezing and distributing semen. It is a procedure that is successful because of the large quantities of sperm per unit (15 to 20 million cells for conventional semen and 2 to 6 million cells for sex-sorted units). Cattle sperm are less sensitive to lower temperatures compared to porcine, ovine and horse sperm; allowing cattle producers the opportunity to collect, store and distribute semen with more success than the other species.

While research continues to advance and improve success of cryopreservation techniques for gametes, embryos and sperm, studies are still required to overcome particular issues within multiple species. These issues tend to be associated with lipid content of cells, the selection of less toxic cryoprotectants and genetic differences between semen from bulls that may have excellent freezing outcomes compared to bulls with poor freezing outcomes.

Cryopreservation plays a significant role in our ability to enhance reproductive technologies and distribute genetics around the world, and methods continue to improve daily. AJ

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