Cryopreservation of small ruminant semen made simple

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Abstract

Successful cryopreservation of small ruminant semen is critical to prosperous artificial insemination programs. Semen collection with the intent to cryopreserve is achieved by either via the use of an artificial vagina or an electroejaculator. Semen collected during the natural breeding season most often has the best prefreezing and postthawing viability. Ejaculates from rams and bucks can be preserved using commercially available OptiXcell II extender and freezing via 'floatation over liquid nitrogen technique' for a short interval.

Keywords: Semen, cryopreservation, small ruminant, artificial insemination

Introduction

Semen collection and cryopreservation provide an excellent opportunity for small ruminant producers via artificial insemination with frozen semen. Laparoscopic artificial insemination (LAI) with fresh semen yields the best results with a high-quality frozen semen. There are multiple variables surrounding the collection that can affect the quantity and quality of semen doses. Furthermore, extending and freezing semen can affect the postthaw quality of semen. Not all frozen semen is created equally. Using high quality semen for LAI is critical for obtaining acceptable or better conception rates.

Semen collection

Semen is collected from bucks and rams either via an artificial vagina (AV) during their mount on a female in behavioral estrus or via electroejaculation (EE). Although semen collected using an AV is preferred, semen collected via EE provides acceptable postthaw semen quality. Generally, EE collection provides a higher volume of semen; however, it has lower concentration of sperm because of additional seminal plasma in the ejaculate. Additional seminal plasma reduces resistance to cold shock and decreases postthaw sperm survival. Furthermore, EE collection has a risk of urine contamination. Additionally, EE also increases the chance of having cellular debris and dirt from the prepuce in the final extended ejaculate.

Impact of season

Generally, semen collection during breeding season produces the highest volume of quality semen for either fresh LAI or cryopreservation for later use. On average, depending on season, a buck or ram produces an ejaculate ranging from 0.5 to 1.5 ml, Semen that is collected during the breeding season will provide a higher volume of slightly less concentrated semen. It is not uncommon, in breeding season, to obtain 0.8 - 2.0 ml of semen from a mature male small ruminant with a concentration of 2 - 6 x 10^9 sperm/ml. Semen collected outside of breeding season typically yields 0.4 - 0.6 ml with a concentration of 5 - 9 x 10^9/ml. Furthermore, collection during summer months has heat stress effect on male fertility. Collection during winter months adds a substantial degree of difficulty to ensure that cold shock is not encountered. Semen contact with temperatures lower than 37°C can substantially lower fertility.

Evaluation of semen quality

There is no single measure that can effectively evaluate buck or ram semen quality. Considering a combination of measures provides the best assessment of semen fertility. Semen volume, concentration, progressive motility, and morphology are all values that can help determine a buck or ram's fertility after a fresh collection. Furthermore, these affect the quantity of straws that can be frozen and have a direct effect on postthaw quality. It is imperative to thaw a straw of semen after the freezing process to evaluate at least the survivability (+/- motility) and concentration of the semen in that straw. Teaching clients to request this information when purchasing frozen semen can be helpful.

Immediately after collection, the ejaculate is placed into a digital dry block heater at 34°C. A 50 µl aliquot of semen is diluted with 450 µl of OptiXcell II (IMV Technologies USA, Maple Grove, MN) extender at 34°C to a 1:10 dilution factor. A 7.5 µl drop of diluted semen is placed onto the disposable base sampling chip of the iSperm Semen Analyzer (Aidmics Biotechnology Co., Ltd, Taipei City 10647, Taiwan) with a pipette. A cover chip is placed over the base chip before the sample

collector is attached to the optical lens module on the iPad. Initial sperm total motility percentage and concentration (10^9 sperm per ml) of the sample is obtained with a 4-view analysis on the iSperm device.

Extension and freezing

A liposome-based extender OPTIXcell II™ (IMV Technologies, Maple Grove, Minnesota, USA) is preferred. Extenders are freshly prepared on each collection day. The stock solution of extender is stored in a 4°C refrigerator. OptiXcell II is prepared by diluting 15 ml of OptiXcell II extender with 30 ml of ART distilled water for reproductive techniques. The prepared extenders are left to equilibrate in a 34°C digital dry block warmer for 20 minutes before use.

The amount of extender required is calculated to reach a final dilution of 200 x 10^6 sperm/ml, ~ 100 x 10^6 sperm per 0.5 ml straw. OptiXcell II, held at 34°C in a digital dry block warmer, is slowly incorporated into the ejaculate in a dropwise manner in order to avoid osmotic shocks. The conical tube with extended semen is gently rocked until thoroughly mixed. Conical tubes of extended semen are taken out of the 34°C digital dry block warmer and kept for 20 minutes at ambient room temperature. During this equilibration, the semen is loaded into 0.5 ml French straws at ambient temperature. Straws are sealed using a commercial ultrasonic sealer or with a PVC powder and water combination. The straws are then placed onto a metal semen straw rack in a 4°C refrigerator to equilibrate.

After 4 hours has elapsed, the straws are frozen. The freezing process involves floating the samples on a metal and Styrofoam rack 7 cm above the surface of liquid nitrogen in the vapors for 10 minutes before plunging into the liquid nitrogen for storage. Straws are then placed into 10 or 20 straw canes under liquid nitrogen before transferred to a cannister in a liquid nitrogen tank for storage.

Postthaw evaluation

Semen straws are removed from liquid nitrogen storage tank and placed in a 37°C water bath for 1 minute and then dried with a paper towel. Straws are cut with scissors and samples are emptied in glass vials kept on 37°C digital dry block warmer and swirled for 1 minute. A 7.5 µl sample is loaded with a pipette to the iSperm base chip and analyzed for total motility and concentration.

Conflict of interest

None to declare.

Meeting the demand for laparoscopic artificial insemination in sheep and goats

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Abstract

Demand for laparoscopic artificial insemination in small ruminants is growing exponentially. Use of a well-designed estrus synchronization protocol allows does and ewes to be inseminated in a timely manner with good conception rates. Females are sedated with an intramuscular cocktail to facilitate inversion and laparoscopic access to their uterus for deposition of fresh or frozen semen directly into the lumen of both uterine horns.

Keywords: Laparoscopy, artificial insemination, small ruminants

Introduction

Use of assisted reproductive technologies (ART) is becoming more popular within small ruminant industries. Requests for laparoscopic artificial insemination (LAI) services increase each year as producers look for ways to advance genetics in a quick and cost-effective manner. Availability of global importation of semen from bucks and rams to the United States has further driven the demand for this surgical procedure. Laparoscopic artificial insemination allows semen to be deposited intrauterine in these 2 species with difficult to pass or impassable cervixes. The thought of performing laparoscopic surgery in small ruminants can be daunting to the veterinarian. With a basic understanding of synchronizing estrus and a review of laparoscopic equipment and procedure, a general practitioner should be able to competently add laparoscopic artificial insemination to their list of service offerings.

Estrus synchronization

A standard small ruminant controlled internal drug release (CIDR) synchronization protocol is utilized. Does and ewes have a CIDR placed on day 0. A dose of prostaglandin is given on day 11. The CIDR is removed on day 12 and a dose of PG600® (Intervet/Merck Animal Health, Madison, NJ) is given intramuscularly. Food and water are removed mid-day on day 13 and a teaser ram or buck is turned in with females. Laparoscopic artificial insemination is performed on day 14 at 48 - 54 hours after CIDR removal.

Sedation

Does and ewes are sedated prior to LAI using a combination of ketamine, xylazine, and butorphanol. Ketamine (1 mg/kg) is combined with 20 mg/ml xylazine (0.05 mg/kg) and butorphanol (0.025 mg/kg) to make a stock solution for the LAI procedure. Ewes are given intramuscularly 0.02 ml/kg whereas does respond variably to an intramuscular dose of 0.01 - 0.02 ml/kg. After 10 minutes, the sedated females are ready to be stood alongside an artificial insemination table and rolled into dorsal recumbency.

Patient preparation

Once in dorsal recumbency on the artificial insemination table, the front limbs of the female are hooked under the corresponding leg brackets at pastern joint level. Hind limbs are secured to their corresponding leg brackets using the attached rope and ratcheted down. Female's eyes are covered using a towel and halter combination or a custom-designed blindfold. A 30 x 30 cm area cranial to the udder is clipped using commercial sheep shears followed by a surgical blade. The area subsequently undergoes a surgical scrub using alternating chlorhexidine scrub-soaked gauze and sterile saline-soaked gauze.

Artificial insemination procedure

Artificial insemination table is inverted. Two stab incisions (3 - 5 cm) are made through the skin ~ 12 cm cranial to the udder on either side of midline. A teat cannula or Veress needle is inserted bluntly through 1 stab incision, puncturing into the abdominal cavity. Tubing leading from a carbon dioxide insufflator is connected and the abdomen is insufflated at 5 - 7 liters per minute. Teat cannula or Veress needle is then removed and immediately replaced with a trocar. A laparoscope is introduced via the trocar and the uterus is visualized. Semen is requested to be prepared while the second trocar is placed through the second skin stab incision. The prepared artificial insemination gun is inserted through the second trocar by an assistant and handed off to the technician performing the insemination. Needle of the artificial insemination sheath is

aligned perpendicular to the base of 1 uterine horn. A quick stab is made, presumably penetrating the uterine lumen. Assistant plunges half of the semen dose. Gun is then removed from that uterine horn and moved to the opposite horn. Similar stab technique is used to insert the needle into the second uterine horn, and the remaining semen in the straw is deposited. Insemination gun is removed, trocar stopcocks are opened, and excess carbon dioxide is drained from the abdomen. Then trocars are removed and 1 staple is placed in the skin over each incision. Blindfold is removed, and the female is rolled off the table. Ewes readily walk away whereas goats may lie sedated for up to 45 minutes.

Conflict of interest

None to declare.