Embryo transfer and pregnancy diagnosis

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Abstract

Embryo transfer (ET) is a very effective technique for quickly improving the genetics. While the application of this technology is extensive in the cattle industry, it is a growing technology for small ruminants with considerable progress being made with regard to estrus synchronization and superovulation. Early pregnancy diagnosis and determination of fetal number are of significant value in goat reproductive herd health management. The value of pregnancy determination is in the identification of non-productive females and does bearing multiple fetuses.

Keywords: Embryo transfer, pregnancy diagnosis, gender determination, doe

Introduction

Embryo transfer is a very effective technique for quickly improving the genetics. While the application of this technology is extensive in the cattle industry, it is a growing technology for small ruminants. Rapid genetic improvement is accomplished by shortening the generational interval and can potentially double the genetic gain in a herd. The main limitations for wider use in goats are the naturally occurring anestrus period, the inconsistency of response to superovulatory protocols, fertilization failure, and the need for surgical collection and transfer of embryos. However, considerable progress has been made with regard to estrus synchronization and superovulation.

Early pregnancy diagnosis and determination of fetal number are of significant value in goat reproductive herd health management. Frequently, clinical signs such as failure to return to estrus after breeding, enlarging abdomen, and developing mammary glands to make a presumptive diagnosis of pregnancy. However, there are many limitations to using these clinical signs to diagnose pregnancy. The value of pregnancy determination is in the identification of non-productive females and does bearing multiple fetuses.

Embryo transfer

Breeding programs based on multiple ovulations and embryo transfer use genetically superior females to increase the genetic diversity and progress in the herd. A successful ET program requires advanced planning and attention to detail in donor and recipient selection, synchronization of donor and recipient, and successful recovery and transfer of high quality embryos.[1] Embryo transfer can be performed in or out of the breeding season. However, the best response is obtained during the breeding season when donors and recipients are cycling normally.

Donor and recipient management

Donor and recipient selection and management are critical for the success of an ET program. Recipient and donor does must be synchronized to cycle together. Donors respond most successfully to estrus synchronization and superovulation when they are young, healthy, and cycling normally with does between two and five years of age responding best to synchronization and superovulation protocols.[2] Donors should be in good body condition but not over-conditioned. They should also be current on vaccinations for any infectious diseases that are locally prevalent. Donors should be acclimated to new pastures or pens, feeding, and handling two to four months prior to the beginning of the ET program to help minimize stress.[3] Stress appears to be cause premature luteal regression in some breeds (e.g., Boer).

Recipients should also be healthy animals with proven reproductive ability that are in good body condition (BCS 3 to 3.5) and cycling normally.[2] Does that are two to four years of age with a history of good maternal characteristics and adequate potential for milk production are preferred. In addition, recipients should also be current on vaccinations.

Synchronization

Most ET programs rely on the use of exogenous hormones to induce and synchronize estrus in donors and recipients. Synchronization protocols commonly use progestin sponges or controlled internal drug release devices (CIDRs). The accurate detection of estrus is critical to the success of ET programs. Teaser bucks are often used to provide more accurate detection of estrus. The same method of estrus detection should be used for both donor and recipients; however, superior results have been obtained if progestin sources are removed from recipients 12 hours before they are removed from donors.[1]

Superovulation

Superovulation of donor animals is achieved by injecting equine chorionic gonadotropin (eCG) or pituitary extracts of follicle stimulating hormone (FSH). The eCG is administered in a single dose 48 hours prior to removal of the progesterone source. Equine CG has a longer half-life and is associated with overstimulation of the ovaries leading to the release of large numbers of oocytes, an increased proportion of unfertilized embryos and poorer quality embryos. In addition, antibody formation is a common side effect after treatment with eCG, even at small doses, which could have a negative impact on future fertility of the donor.[4] The donor may also be superovulated with an FSH product either alone or in combination with eCG two days before the end of the artificially created luteal phase.[5] The FSH preparations, usually of porcine or ovine origin, have a half-life of six hours and are administered twice a day beginning 48 hours before removal of the progestin source. The FSH preparations are superior to eCG in ovulation rates, fertilization rates and percentage of good-quality embryos.

Does typically exhibit estrus between 24 and 36 hours after progestin removal. Gonadotropin releasing hormone (GnRH) has been administered at 30 to 36 hours after removal of the progestin to induce a strong pre-ovulatory luteiniing hormone (LH) surge which is required to maximize the probability of ovulation. Careful and frequent observation of does for estrus with the aid of a teaser animal will ensure accurate detection and recording of the time of estrus. Donors may be hand-mated 12 to 24 hours after estrus detection. Laparoscopic deposition of frozen-thawed semen into the uterine horns 24 hours after the animal is first seen in estrus produces optimum results. If donor animals are to be bred naturally, one buck should be housed with one or two super-ovulated does.[6]

Embryo recovery and handling

Embryos are usually harvested from the donor's uterus on day 5 to 7 after breeding. In most cases, surgical collection of embryos is performed. However, laparoscopic and non-surgical embryo collections have also been developed. There are numerous methods to recover embryos including surgical techniques, laparoscopic techniques and non-surgical techniques.

Surgical embryo collection involves fasting the animals prior to the procedure in preparation for anesthesia. Once under general anesthesia, the animal is prepared for surgery. During the surgery, the uterus and ovaries are exteriorized and examined. The uterus is then flushed with harvest medium to harvest embryos by catheterizing each uterine horn. The abdominal incision is then closed in routine fashion. Prostaglandin F2α (PGFα) should then be administered to the donor to ensure lysis of luteal tissue. If embryos are to be collected prior to the fourth day following breeding, oviductal flushing is necessary.

Laparoscopic collection of embryos can also be performed be exteriorizing the tip of the uterine horn and flushing in the same manner described for surgical embryo collection. Laparoscopic collection reduces the severity of adhesions that result from the handling required in the surgical approach. Laparoscopic collection of embryos requires considerable skill and is not practical as a routine field procedure. This method allows the operator to visualize the ovary and corpus luteum (CL) and more easily exteriorize the uterine horn. Advantages of laparoscopic collection of embryos include reduced surgical time and a small abdominal incision.

Non-surgical or transcervical embryo collection methods avoid the risk of postsurgical adhesions and maintains the value of genetically superior donors after embryo collections. Several reports of successful non-surgical embryo collections in goats have been published.[7-9] Embryo recovery rates

appear to be similar to those attained with surgical embryo collections. However, surgical recovery is still the most common method used for commercial recovery of embryos.

The flush medium that is recovered during flushing is examined with a dissecting microscope. The embryos are retrieved and placed into a holding dish after washing. Before transfer into recipients or freezing, the embryos are carefully evaluated for stage of development and quality. Embryos in the morula or blastocyst stages are expected when embryos are collected at day 5 or 6. The International Embryo Transfer Society (IETS) has defined handling procedures to reduce the risk of disease transmission during ET.[1] The embryos are pulled into a small-bore intravenous catheter attached to a 1 mL syringe for immediate transfer into recipients. Embryos may also be frozen if immediate transfer is not available or desired.

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Transfer of embryos

Recipients are chosen for transfer of embryos based on the greatest synchrony of estrus to the donor doe which is one of the most important factors affecting success of ET. Most transfers are performed surgically. The recipient is anesthetized and prepared for surgery. During surgery, the ovaries are examined for the presence of a CL. Once a CL is identified, the uterine horn on the ipsilateral side is exteriorized. Embryos harvested prior to day 4 are then transferred into the oviducts through the fimbriae using a catheter or pipette. Older embryos (embryos collected after day 4) are transferred into the uterine horns. Before closing the abdominal incision, one should examine the catheter or pipette to ensure that no embryos have been retained. Two embryos are most commonly transferred per recipient.[1]

Laparoscopic transfer of embryos is performed by placing the animal under general anesthesia and preparing the recipient for surgery. The embryos are loaded into a 0.5 mL straw which is placed into a Cassou insemination gun with an injection tip. The Cassou gun is inserted into the abdomen through a cannula, the needle inserted into an avascular area of the tip of the uterine horn and the plunger of the gun depressed to express the embryos into the uterine horn.[1]

An average of eight to ten0 transferable embryos can be expected per flush with acceptable pregnancy rates of 60% to 80% for the transfer of two fresh embryos per recipient.[10] Pregnancy rates from the transfer of frozen embryos are much lower. There are many factors that can affect the success of an ET program.

Pregnancy diagnosis

Early pregnancy diagnosis and determination of fetal number are very important for goat reproductive herd health management. Often, signs such as failure to return to estrus after breeding, enlarging abdomen, and developing mammary glands are used to make a presumptive diagnosis of pregnancy. However, there are many limitations to using these clinical signs to diagnose pregnancy. Pathologic conditions of the uterus and ovaries, physiologic anestrus late in the breeding season, and out-of-season breeding also may cause post-breeding anestrus in non-pregnant does. In addition, some does exhibit estrus behavior during pregnancy.

Ultrasonography and hormonal assays are the most useful methods for pregnancy diagnosis. Abdominal palpation, ballotment and radiography have limited usefulness. The value of pregnancy determination is in the identification of non-productive females and does bearing multiple fetuses. Early identification of multiple fetuses allows for the implementation appropriate nutritional and management programs. This includes dividing the herd into groups based on pregnancy status to improve the health care of these animals as well as decreasing production costs.

Imaging

Ultrasonography. Ultrasonography for pregnancy determination has included amplitude modulation (A-mode or amplitude modality), Doppler, and real-time (B-mode or brightness modality) imaging. A-mode imaging has been used to detect pregnancy between 70 and 100 days of gestation where fluid density is interpreted as pregnancy. A-mode is considered accurate (90-95%) but the

accuracy falls significantly after 145 days of gestation. However, A-mode is considered unreliable because it may yield false positive results in cases of hydrometra or a large bladder.

Doppler ultrasonography can be used to detect movement (fetal movements, fetal heart beat, and blood flow through the middle uterine artery) which may indicate pregnancy. The external Doppler technique has an accuracy of 100% during the second half of gestation but is not as effective at 50 to 75 days or earlier. Transrectal Doppler ultrasonography may be attempted at 25 to 30 days after breeding; however, waiting until 35 to 40 days produces better results. False negative and false positive results are common and determining fetal number is quite difficult.[1]

Pregnancy diagnosis via ultrasonography in the doe is now performed almost entirely with real-time (B-mode) ultrasonography. Linear array real-time ultrasound transducers can be used transrectally to diagnose pregnancy between 18 and 60 days of gestation. A plastic extension can be made from polyvinyl chloide pipe to allow easy introduction of the transducer into the rectum. A 5- or a 7.5 MHz transducer is recommended for rectal scans. After 60 days of gestation, the gravid or pregnant uterus is pulled over the brim of the pelvis and down into the abdomen which can make transrectal ultrasonography difficult.[1] Transabdominal ultrasonography with a 3.5- or 5-MHz linear or sector scanner is used 28 to 30 days of gestation. The transducer is placed on the hairless or clipped area of the abdomen high in the inguinal area, preferably in the right flank. A fluid such as ultrasound gel, vegetable oil, or methylcellulose should be used to couple the ultrasound transducer to the skin. The transducer beam is aimed toward the pelvis, and the abdomen is scanned by slowing sweeping the transducer cranially. Identification of the triangular-shaped bladder provides an excellent landmark. The uterus is typically located dorsal or cranial to the bladder. Pregnancy may be diagnosed by finding a fetus and numerous fluid-filled uterine luminal sections (less reliable). The fetal bones form shadows, and the fetal ribs produce a characteristic striated appearance. The fetal heartbeat can be first detected by 26 days and easily recognized as early as 30 days to 35 days after breeding. Twin pregnancies are best determined between 45 and 90 days of gestation.[1]

Radiography. The use of radiography is used more commonly in small ruminants than in domesticated livestock species but is still rarely used even in these species. Radiographs are only useful after ossification of the fetal bones. Radiography is highly accurate and may also be used to accurately assess fetal number. Although, the use of radiography is limited due to the cost and late stage of fetal bone ossification.

Abdominal palpation and ballottement

During the last half of gestation, the gravid uterus or fetus may be palpated through the abdominal wall.[1] Ballottement may also be used to diagnose pregnancy in late gestation with a large fetus. Ballottement is performed by gently pushing a fisted hand into the caudoventral abdomen of the female. In a pregnant female in late gestation, the fetus should be pushed away from the fist and then return in a fluid wave to "bump" the fist. Ballottement is unable to assess the viability of the fetus and may only be accurately performed later in gestation.

Hormone assays

The measurement of hormone assays in blood, milk, or urine provides an alternative method of pregnancy when ultrasonography may not be available.

Estrone sulfate. Estrone sulfate is the major estrogen produced by the fetoplacental unit during pregnancy. Measurement of estrone sulfate has been used to diagnose pregnancy in cattle, goats, and sheep. Estrone sulfate can be detected in the urine, serum or milk after day 50 of gestation and are nearly 100% accurate. A positive test indicates a viable fetus. However, false positive results are possible with hemolyzed serum samples. In addition, false negatives may occur if samples are collected before 50 days of gestation.[1] Due to the limited number of laboratories performing this assay and the expense of the test, very few people use this assay for pregnancy diagnosis.

Pregnancy-specific protein B. Pregnancy-specific protein B (PSPB) is produced by the binucleate giant cells of the placenta throughout gestation. This hormone assay can be used to detect

pregnancy any time after 30 days of gestation in goats. Both false positive and false negative results are possible and few laboratories offer this test. Multiple fetuses result in higher levels of PSPB.[11]

Progesterone. The progesterone assay is not a test for pregnancy but more accurately detects non-pregnant animals. Goats require progesterone from the CL throughout gestation to maintain pregnancy. Plasma or serum progesterone concentrations below 1 to 2 ng/mL at 21 days in the doe indicate that the doe is not pregnant due to the absence of a CL.[1] An elevated progesterone concentration may indicate pregnancy, hydrometra, pyometra, early embryonic death, fetal mummification, or irregular estrous cycle. The accuracy of blood progesterone levels is 80% to 100% for non-pregnancy and 67% to 100% for pregnancy.[1]

Fetal age determination

The size of the fetus as determined by ultrasonography can be used to estimate gestational age. The crown-to-rump length of the fetus in relation to gestational age has been estimated for Saanen and Alpine goat breeds. A crown-to-rump length of 40 mm is equivalent to 45 days of gestation, 100 mm to 60 days, and 250 mm to 90 days. Fetal age has also been estimated for dairy and pygmy goats by using biparietal diameter with ultrasonography.[12,13]

Fetal sex determination

Fetal sex determination can be made using real-time ultrasonography by visualization of the genital tubercle of the fetus between 55 and 75 days of gestation.[1] The genital tubercle begins its development between the hind limbs and moves back towards the tail in females and forwards towards the umbilicus in males. In addition, females should have two teats visible between the hind limbs with the absence of the triangular-shapes scortum.[1] The penis and prepuce are located just caudal to the umbilical cord.[14] Fetal sexing is more difficult with twin or triplet pregnancies.

Conclusion

Advanced reproductive technologies have helped advance the goat industry. Embryo transfer has become a very effective technique for quickly improving genetics within a herd. While the application of this technology has been used extensive in the cattle industry, it is a growing technology for small ruminants. Rapid genetic improvement is accomplished by shortening the generational interval and can potentially double the genetic gain in a herd. Embryo transfer is becoming more common as progress has been made with regard to estrus synchronization and superovulation. In addition, early pregnancy diagnosis and determination of fetal number have become important aspects in goat reproductive herd health management. Imaging, palpation or ballotment, and hormone assays are the most common methods of pregnancy diagnosis. Early pregnancy determination allows the producer to identify non-productive females and does bearing multiple fetuses which might need additional nurtition.

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