# Artificial Insemination and Embryo Transfer in Goats

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**Abstract:** Artificial insemination (AI) is an important technology for improving animal production. Through consistent use of AI, herd genetics can be advanced at a rapid rate. While AI is a technology that enables the dissemination of selected male genetics, embryo transfer (ET) is a technology that enables the dissemination of selected female genetics and, by using ET, frozen embryos can be moved around the world at significantly reduced costs compared to movement of adult animals. Utilization of either AI or ET requires careful doe management and is most effective when these technologies are used in conjunction with estrus or ovulation synchronization. This paper will address the topics of estrus and ovulation synchronization, artificial insemination and embryo transfer in goats with a focus on the practical application of synchronization and AI for herd improvement.

**Key Words:** estrus synchronization, ovulation synchronization, timed AI, ET, goats

## I. Introduction

Artificial insemination (AI) is a key technology that can be used for improving animal production. Through consistent use of AI, herd genetics can be advanced at a rapid rate using semen from sires selected based on expected progeny differences (EPDs) or selection indexes targeted to improve various traits including weaning weights, growth rates, slaughter weights, milk production, physical type as well as maternal longevity and production efficiency. Introduction of AI to the US dairy cattle industry, coupled with essentially universal acceptance of computerized dairy records management systems, revolutionized the domestic dairy industry such that today the US dairy herd is at its lowest number of cows yet greatest production per animal in history (USDA-ERS, 2013; Wolf, 2003), effectively supplying milk for the largest population in US history. More recently, swine producers have also recognized the significant advantages of implementing AI to advance animal production programs within their industry (Broekhuijse, et al, 2012; Roca, et al, 2006).

While AI is a technology that enables the dissemination of selected male genetics, embryo transfer (ET) is a technology that enables the dissemination of selected female genetics. In this way, productive genes carried by dams can be more rapidly spread within a population by having less valuable dams carrying offspring of elite females instead. In addition, through the use of ET, frozen embryos can be moved around the world more easily and safely compared to movement of postnatal animals (Givens, et al, 2007; Stringfellow and Givens, 2000).

Utilization of either AI or ET requires careful doe management and is most effective when these technologies are used in conjunction with estrus or ovulation synchronization. Synchronization allows groups of does to be managed together, focusing labor needs and reducing time input. Therefore, this paper will address the topics of estrus and ovulation synchronization, artificial insemination and embryo transfer in goats focusing on practical application of these technologies for herd improvement with particular emphasis on synchronization and AI. Discussion will be limited to application of these technologies during the normal breeding season rather than for breeding management during the anestrous season.

## II. Synchronization of Estrus and Ovulation

### A. Estrus Synchronization

The goal of any synchronization program is to group animals so they enter into the same physiological state as simultaneously as possible. In the case of estrus synchronization, the goal is to have does in heat as close to the same time as possible, allowing animals to be heat checked as a group with individual does bred after they show estrus. Labor efforts are highly focused allowing one to take advantage of AI for breeding. In practice, most methods for estrus synchronization result in a group of does coming into estrus over about a 48 h period, requiring both morning and evening heat checks before, during and after this targeted time interval. When using AI, does found in estrus are bred 12 h after they are first detected in heat by the AM-PM rule; that is, if a doe is found in heat in the morning, she would be bred that evening and if in heat in the evening, she would be bred the following morning.

A number of useful methods are available to producers for estrus synchronization in goats during the breeding season. These include progesterone-based or prostaglandin-based protocols as well as

combined methods utilizing both progesterone- and prostaglandin-based treatments.

***II.A.1. Progesterone-based synchronization***

Progesterone-based synchronization consists of treatments with either the natural hormone, progesterone, or with synthetic derivatives of progesterone known as progestogens. As an example, natural progesterone can be found in a controlled intravaginal drug releasing device (CIDR, Pfizer Inc.) (Rathbone, et al, 1998). Examples of progestogens include melengestrol acetate (MGA), altrenogest or norgestomet. These agents mimic the action of the corpus luteum (CL), a progesterone-secreting structure located on the ovary. If a doe has a CL that is secreting progesterone, she will not show estrus. The CL also functions to maintain pregnancy, if the doe has been bred successfully. Whether using the natural hormone or a progestogen, similar physiological responses occur within the animal. However, differences exist between the various progestogens with regard to routes of administration, dosages required, effectiveness and cost (Abecia, et al, 2012; Whitley and Jackson, 2004).

Because CIDRs are commercially available and simple to use for most producers, the remainder of this discussion will focus on natural progesterone-based synchronization using CIDRs. Treatment consists of a single CIDR being gently inserted into a doe's vaginal vault with the device remaining in place for varying periods until removed. Elevated levels of progesterone released by the CIDR prevent the doe from coming into estrus until the device is removed. After CIDR removal, the doe will come into heat about 36-72 h later and can be bred by AI. CIDRs can be administered for varying periods, typically ranging from 9 to 21 days. Any does not settled after AI at the first estrus following CIDR removal will remain synchronized for the second estrus 3 weeks later.

Retention rates for CIDRs in goats are about 90% (Omotese, et al, 2010). In general, these devices work well with females of moderate to large frame size. Doelings or adult does of smaller frame breeds may have difficulty accommodating the size of the CIDR in their reproductive tracts. CIDRs can be purchased from commercial animal health and livestock suppliers and do not require a prescription. However, because CIDRs are only approved for use in sheep in the United States, their use in goats would be considered extra-label.

***II.A.2. Prostaglandin-based synchronization***

Prostaglandin F2alpha (PGF) is a naturally occurring hormone that can be used to bring females into estrus. It is effective only if females are normally cycling and have a corpus luteum (CL) present on their ovaries. The CL is the ovarian structure that produces progesterone in the female, keeping her out of estrus. In a normal estrous cycle, secretion of PGF from the uterus destroys the CL and the doe comes into heat.

PGF is marketed as the product dinoprost (Lutalyse, Pfizer, Inc.). To obtain PGF, or any of its synthetic analogs, a prescription is required from your veterinarian. Synchronization with PGF typically involves administration of 2 treatments, with the first given on Day 1 and the second on Day 10. Lutalyse dosages can vary from 7.5mg to 15mg per injection. In our experience, the 15 mg dosage provides consistent and reliable results (Bowdridge, et al, 2013). However, lower doses can also be effective (Al Yacoub, et al, 2011). Does will come into heat after either the first or second injection and can be bred by AI at either time. To better conserve labor, animals are usually not heat checked after the first injection but only after the second PGF injection. Eighty to 90% of the treated does will show estrus 36-96 h after the second PGF injection (Bowdridge, et al, 2013). Animals can be bred by AI using the AM-PM rule. There is no decrease in fertility at the synchronized estrus when PGF is used.

***II.A.3. Combined CIDR and PGF synchronization***

Effective protocols for estrus synchronization in goats can also involve the use to both CIDRs and PGF. Use of a combined protocol can alleviate the fertility depression associated with longer term CIDR use, resulting in good synchronization and fertility at the estrus immediately post-treatment. A relatively simple combined CIDR-PGF protocol involves a short, 6-day CIDR treatment. A CIDR is inserted on Day 1 in the morning and six days later is removed (am or pm on Day 6). An injection of PGF must be given either at the time of CIDR insertion or CIDR removal. If PGF is given at the time of CIDR removal, does will come into estrus 36-72 h later and can be bred by AI based on estrus detection and the AM/PM rule.

***II.A.4. Use of Bucks to Breed Synchronized Females***

Although producers will make the greatest genetic gains coupling estrus synchronization protocols with AI, it is important to note that estrus synchronization can also be combined with natural breeding. Use of estrus synchronization in this context allows

producers to take advantage of grouping females for their breeding and subsequent kidding periods, making overall reproductive management of the doe herd more predictable. Although few studies exist that have evaluated buck to doe ratios when females have been estrus synchronized, based on studies in cattle (Farin, et al, 1989; Pexton, et al, 1989), it is recommended that only mature males be used for this purpose. A mature buck can be expected to handle ratios of 1:15-20 synchronized females with good pregnancy rates to the synchronized estrus.

**II.B. Ovulation Synchronization**

Ovulation synchronization, as its name implies, is a method that allows producers to synchronize the release of the egg from the ovary, rather than simply synchronizing the female's estrus behavior. The major advantage of ovulation synchronization is that a producer does not need to perform any heat checking of their does prior to breeding. Rather than relying on detection of estrus behavior that is frequently challenging to reliably determine in does, ovulation is synchronized and the females are bred by AI at a fixed time during the treatment protocol. This is referred to as "timed AI" or TAI. Ovulation synchronization further focuses labor needs to the preselected day of breeding only because heat checking is not used and breeding dates can be precisely scheduled in advance of the breeding season. Pregnancy rates following ovulation synchronization with TAI using a single dose of frozen semen (Bowdridge, et al, 2013; Holtz, et al, 2008; Sohnrey and Holtz, 2005) are comparable to those obtained for AI breed-by-estrus protocols.

Successful ovulation synchronization-TAI protocols have been developed for goats (Bowdridge, et al, 2013; Holtz, et al, 2008). These protocols utilize several different hormones in sequence to control CL function, stimulate follicular development and regulate ovulation. Progesterone-free protocols are also available and utilize treatment combinations of PGF and the ovulatory hormone, gonadotropin releasing hormone (GnRH), to induce a synchronized ovulation in preparation for TAI.

Over the past five breeding seasons, we have evaluated pregnancy and kidding rates achieved following three different ovulation synchronization-TAI protocols compared to rates achieved by breed-by-estrus AI controls. Timelines for each of the ovulation synchronization-TAI protocols examined are illustrated in Figure 1. In these studies, does were assigned to receive either the NC.Synch, CIDR.6, CIDR.11 or Control treatments. Does in the NC.Synch, CIDR.6 and CIDR.11 groups were bred

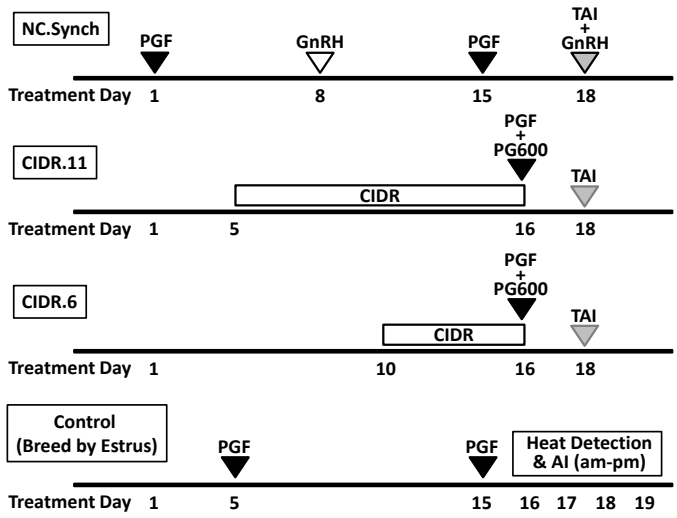


**Figure 1. Ovulation (NC.Synch, CIDR.11, CIDR.6) or estrus (Control) synchronization protocols evaluated in the NCSU goat herd between 2007 and 2012.**

by TAI whereas does in the Control group were bred by AI-based heat checking and the AM-PM rule. All treatments were scheduled to begin in the morning on any treatment day. All inseminations were performed by experienced inseminators using the NCSU simplified catheter-based transcervical method (see below) with a single dose of frozen semen. Pregnancy rates were based on detection by transabdominal ultrasonography at 55 and 85 days post-insemination. Because not all treatments were represented each year, the number of does in each of the treatment groups varied (Figure 2).

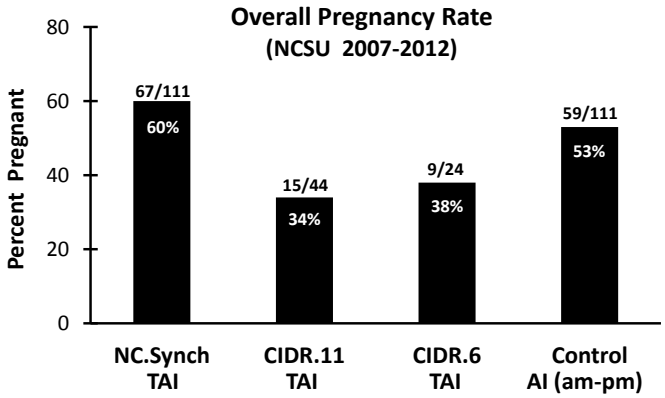


**Figure 2. Pregnancy rates for ovulation or estrus synchronization protocols evaluated in the NCSU goat herd between 2007 and 2012.**

The pregnancy rate following the NC.Synch-TAI treatment was comparable to the rate for the breed-by-estrus AI control ($p$ = 0.28). In contrast, pregnancy rates were significantly ($p < 0.01$) lower in

the CIDR.6-TAI and CIDR.11-TAI groups compared to NC.Synch-TAI. These data demonstrate that satisfactory pregnancy rates can be achieved with a single dose of frozen semen using NC.Synch-TAI protocol. Furthermore, use of an ovulation synchronization protocol eliminates the need for heat checking before AI and allows breeding to occur on a schedule that can be set by the producer while still maintaining pregnancy rates comparable to the traditional breed-by-estrus method.

Use of PG600 in the experiments described above provided a source of the hormone, equine chorionic gonadotropin (eCG), to induce additional ovarian stimulation, potentially increasing the number of kids produced. Repeated use of this hormone in goats, either within a breeding season or across breeding seasons is not recommended. Like cattle, goats will develop antibodies against this hormone, usually after two eCG administrations, making females permanently resistant to its effects (Bodin, et al, 1997; Roy, et al, 1999a; Roy, et al, 1999b). Use of the NC.Synch-TAI method during the breeding season eliminates the need for eCG treatment, does not involve the use of CIDRs and is a very simple ovulation synchronization protocol for producers to follow. It can only be used during the breeding season when does are naturally cycling, however. Veterinary prescriptions are required to obtain the hormones used in this protocol.

## III. Artificial Insemination (AI)

There are two basic techniques used for AI in goats, laparoscopic insemination and transcervical insemination (Cseh, et al, 2012). Pregnancy rates are generally higher for laparoscopic insemination compared to transcervical insemination. However, laparoscopic insemination requires highly skilled inseminators, typically veterinarians or individuals under veterinary supervision, because minor surgical procedures and application of anesthesia is required. In contrast, transcervical insemination techniques can be learned and performed by the producer and do not require surgical entry into the animal or application of anesthesia.

### III.A. Laparscopic Insemination

Laparoscopic insemination involves a limited surgical entry into the abdominal portion of the body cavity of the doe with guided injection of the semen dose directly into each uterine horn. It is typically performed by trained veterinarians and veterinary assistants skilled in the technique. Briefly, for laparoscopic insemination, the doe is anesthetized and placed on a surgical table in dorsal recumbency with her rear quarters elevated above her head. Portions of the abdominal area of her belly are scrubbed and small incisions are made on either side of the midline. Sterile instruments are introduced into each incision and the abdominal cavity inflated with carbon dioxide or sterile air to facilitate visualization of the uterus and ovaries. Insemination is accomplished by visualization of each uterine horn using a laparoscope and deposition of semen into the uterine lumen using a sterile insemination needle. Once the doe is anesthetized, the entire insemination procedure takes about 5-10 minutes. Following insemination, the incisions are sutured and the doe is returned to a recovery pen where she will recover in about 10 minutes.

Pregnancy rates achieved with laparoscopic insemination are approximately 60-80% (Cseh, et al, 2012), making use of this technique an attractive option for producers with large numbers of animals requiring insemination. In contrast, this option may not be cost-effective for producers with fewer animals. Use of ovulation synchronization and TAI protocols, however, can make laparoscopic insemination a more attractive option even for smaller producers because specific insemination dates can be conveniently prearranged.

### III.B. Transcervical Insemination

The cervix of the doe has 4 tightly closed, cartilaginous rings that provide structure to the cervix and, along with cervical mucus, form a protective physical barrier against the entry of foreign particles. To achieve the highest pregnancy rates for AI, semen must be deposited into the uterine body or into each of the uterine horns. Deposition of semen into the uterus requires that all 4 cervical rings must be passed during the insemination procedure. The small size of the doe's reproductive tract, particularly for nulliparous (virgin) or young primiparous (once kidded) does, in addition to the tightness of the cervical rings and their typical lack of alignment can make passing the insemination rod during transcervical AI a challenging task. However, several methods for transcervical insemination have been developed and are available (Cseh, et al, 2012; Sohnrey and Holtz, 2005), some of which are similar to procedures described for nonsurgical embryo transfer in goats (Kraemer, 1989).

#### *II.B.1. Standard AI Method (tube speculum)*

The simplest transcervical AI method involves the use of a tube-like speculum and a standard French-style insemination gun. The speculum, with a detachable light, is inserted into the vaginal vault of the doe and used to visualize the external cervical os which is the entry point into the cervical channel.

Frozen semen is available in 1/4cc or 1/2cc straws and must be appropriately thawed prior to use. Once the semen straw is prepared and placed into the insemination gun, a clean sheath is overlaid to protect the semen and reduce cross-contamination between does. Sheaths can have either standard (rounded) or apex (pointed) ends that can aid in achieving deeper penetration of the cervix. The insemination gun is introduced through the speculum and the inseminator attempts to pass the insemination gun through the cervix and deposit the semen into the uterine body. Following insemination, the gun and speculum are removed and the speculum disinfected between does. The single-use AI gun sheath is disposed of appropriately.

The major advantage of the standard method is that it is a simple and easily mastered technique that is reasonably effective with older, multiparous does. The major disadvantage of this technique is that it is difficult to pass the insemination gun through the small cervix of a young doe or through the cervix if it is highly convoluted. In many cases, use of the standard technique results in deposition of the semen in the cervix if all of the cervical rings cannot be passed. Under controlled conditions, pregnancy rates following the use of the standard technique are low, typically in the range of 20-30% (Sohnrey and Holtz, 2005).

### *III.B.2. Deep Cornual (Uterine) Insemination (Catheter- within-Catheter) Method*

In 2005, Sohnrey and Holtz reported the development of a novel method for transcervical insemination of goats. In their method, semen is deposited deep into the uterine horn (cornua) by means of a catheter-within-catheter technique. This technique relies on use of a soft, small diameter pediatric urinary catheter stiffened with an insemination gun stylet to gain entry into the uterine body and individual uterine horn. To facilitate passage through the cervix, the doe's hindquarters are raised and a Pozzi tenaculum forceps is used to grasp the cervix and align the cervical rings. Once the catheter is positioned into the uterine horn, the stylet is removed and a small diameter insemination tubing is threaded through the urinary catheter and used to deposit fresh or frozen-thawed semen into the upper portion of the uterine horn. The urinary catheter is then repositioned into the opposite uterine horn and the second half of the semen sample is deposited deep into that horn to complete the insemination. With trained technicians, the entire procedure takes about 5-10 min (Sohnrey and Holtz, 2005) and does not involve any surgical entry or anesthesia of the doe. Furthermore, pregnancy rates following deep cornual insemination were greater than those for laparoscopic insemination in their study (Sohnrey and Holtz, 2005). In a subsequent study (Holtz, et al, 2008), pregnancy rates using ovulation synchronization with TAI of a single dose of frozen semen were 58% and kidding rates similar at 53%. These pregnancy rates are comparable to those obtained for beef cattle for first-service insemination after TAI using frozen semen (Lamb, et al, 2010; Larson, et al, 2009).

### *III.B.3. NCSU Simplified Catheter-Based Method*

At North Carolina State University, a modified insemination technique was developed (Farin and Knox, unpublished) based on the deep uterine technique reported by Sohnrey and Holtz (2005) and the nonsurgical embryo transfer technique described by Kraemer (1989). This technique relies on use of the pediatric urinary catheter, insemination gun stylet and Pozzi tenaculum forceps as described by Sohnrey and Holtz (2005) to gain passage through the cervix and entry into the uterine body. In the NCSU method, the tip of the catheter is placed in the uterine body, with catheter position verified by digital palpation through the vaginal vault (Kraemer, 1989) or by predetermined measurement. Once in position, the stylet is removed, a syringe barrel introduced onto the distal end of the pediatric catheter and the semen sample is deposited into the uterine body though the pediatric catheter itself. Once the semen is deposited into the uterine body, the catheter and Pozzi forceps are removed. With skilled technicians, the entire procedure takes about 5-8 min.

Pregnancy rates following estrus synchronization and AI or ovulation synchronization and TAI using the simplified catheter method for transcervical insemination are comparable (Figure 2) to those reported for the deep cornual insemination (catheter-within-catheter) method (Holtz, et al, 2008). Furthermore, pregnancy rates are greater for either of the catheter-based methods compared to the standard AI technique (Bowdridge, et al, 2013; Holtz, et al, 2008; Sohnrey and Holtz, 2005).

## IV. Embryo Transfer in Goats

Embryo transfer (ET) is a reproductive technology that allows producers to take advantage of high-quality genetics in their dams. In addition, ET can be used to rapidly introduce new genetic lines or entirely new breeds into existing herds. For the successful application of ET within a goat herd, intensive reproductive management and excellent record-keeping is required. In addition, careful analysis of cost-benefit ratios must be considered

before an ET program is started. Kids must be highly marketable or of high genetic value to balance the costs associated with developing an ET program.

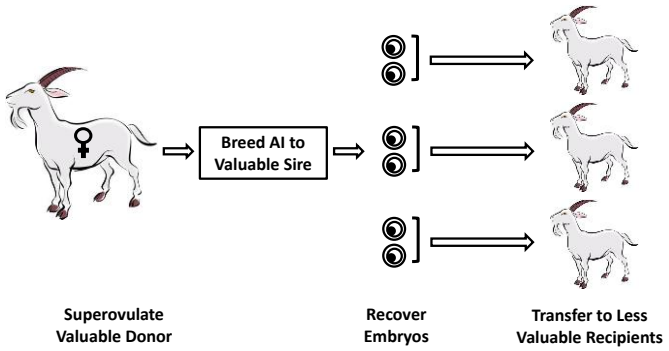


**Figure 3. Overview of the embryo transfer process for goats.**

The basic steps in the ET process are illustrated in Figure 3. Donor females of high genetic quality are superovulated with hormonal treatments that cause them to ovulate more than the normal number of eggs. The donor doe is inseminated with semen from a genetically desirable sire and the fertilized embryos are allowed to grow within the donor's reproductive tract for approximately 1 week. After this period, the embryos are recovered from the uterus of the donor female by uterine lavage (flushing). The recovered embryos are identified, assessed and from 1 to 3 embryos are transferred into the uterus of a recipient female that is of lower genetic quality and whose reproductive cycle has been carefully synchronized to match the cycle of the donor female. In this way, embryos are transferred from the uterus of the original donor into a uterus at the same gestational stage within the recipient female, and thus, can continue development to term unaffected. After an appropriate rest period (2-3 months), the process can be repeated with the original donor female who would be superovulated and bred again to produce more embryos for transfer. Because protocols for freezing goat embryos are available (Al Yacoub, et al, 2010; Youngs, 2011), it is also feasible for recovered embryos to be frozen and held for transfer at a later date.

Although ET in cattle is performed entirely with transcervical (nonsurgical) procedures, ET in goats is performed using either laparotomy (abdominal surgery) or, more frequently, laparoscopy for embryo recovery coupled with laparoscopy for transfer of the recovered embryos into the recipient female (Amiridis and Cseh, 2012). Nonsurgical procedures for embryo recovery in goats have been reported (Melican and Gavin, 2008; Pereira, et al, 1998). However, most donor flushes in goats are still performed using laparoscopic recovery. Producers considering an ET program for their herd must arrange to work with a veterinarian highly experienced in these techniques to have these procedures performed on farm.

Implementation of a successful ET program requires effective donor and recipient management. To successfully produce sufficient numbers of embryos of desired genetics for recovery, donor females are estrus synchronized, superovulated and bred by AI. Successful superovuation protocols for goats have been published (Menchaca, et al, 2010; Rubianes and Menchaca, 2003) and entail a combination of CIDR treatment, PGF or PGF-analog injection, follicle stimulating hormone (FSH) treatment to induce follicular development and GnRH treatment to induce ovulation. An example of a donor treatment schedule is illustrated in Figure 4 (Menchaca, et al, 2010).

Using either laparoscopy or nonsurgical embryo recovery, between 0 and 18 embryos are produced

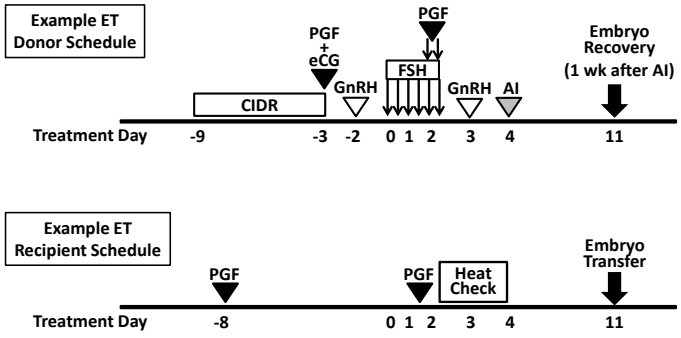


**Figure 4. Example donor superovulation and recipient estrus synchronization treatment schedules for embryo transfer in goats (modified from Menchaca, et al, 2010).**

per donor, with about 4-6 on average (Melican and Gavin, 2008; Menchaca, et al, 2010) . It is important to note that superovulation is not a well-controlled phenomenon and, based on data in cattle, about 25% of donor cow flushes do not produce any embryos whereas about 25% of donor cow flushes produce an excessive number of embryos. Therefore, when using ET, it is often suggested that at least 3-4 donor females be set up for a specific ET session and that the producer should expect, *on average*, about 4-6 embryos to be produced per donor per session.

In conjunction with donor preparation, the recipient females also need to be estrus synchronized so that they are at the same stage of the estrous cycle as the donor females. Recipient does are heat

checked so the first day of their cycle is known, but they are not bred so that they do not have embryos of their own in their reproductive tract. Recipient cycles can be synchronized by a variety of estrus or ovulation synchronization protocols, an example of which is illustrated in Figure 4. Embryo transfers are done by laparoscopy (Shin, et al, 2008) with 1-3 embryos transferred into the recipient female's uterus. A good rule of thumb for estimating the number of recipient females needed for an ET session is to have about 3-4 recipients synchronized for each donor doe scheduled to undergo embryo recovery. If extra embryos are produced and no additional recipients are available, embryos can be frozen and stored for future transfer. Furthermore, if frozen embryos are obtained by the producer, recipient does can be synchronized, heat checked and 1-3 embryos transferred into the recipient's uterus by laparoscopy (Shin, et al, 2008) at an appropriate time convenient for the producer and veterinarian.

## V. Conclusions

Effective estrus synchronization, ovulation synchronization and transcervical insemination methods for AI are now available to goat producers and can be used to increase the efficiency of herd reproductive management. In addition, for producers with high-quality genetic stock or those who want to rapidly change the genetics of their herds, the use of embryo transfer will facilitate attainment of these objectives. Successful implementation of any of these reproductive management technologies, however, requires careful attention to protocol specifics, record-keeping and doe management.

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