UPDATE IN SMALL RUMINANT REPRODUCTION

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The following paper reviews the advanced reproductive technologies currently being used in small ruminant reproduction. Reproductive technologies soon to be commercially available to small ruminant producers are also discussed.

Reproductive Ultrasonography

Reproductive ultrasonography can be used in small ruminants to determine pregnancy status, viable fetal numbers and estimate gestational age. Ultrasonography can also be used to evaluate the male external genitalia and urethra as well as diagnose uterine pathology in ewes and does.

Ultrasonographic diagnosis of pregnancy should be incorporated into the management of all small ruminants. The timely determination of pregnancy status and fetal viability reduces feed expenses associated with nonpregnant females. Determining fetal numbers and stage of gestation also allows producers to plan effective nutritional and lambing strategies. Pregnancy can be diagnosed as early as 25 days of gestation in small ruminants both transrectally and transabdominally, however, pregnancy status is more accurately determined using the transabdominal approach (Gearhart et al., 1988). Routine pregnancy diagnosis is performed between 35 and 50 days of gestation using the transabdominal approach. Transabdominal pregnancy diagnosis is accomplished by placing the ultrasound probe (3.5 or 5.0 mHz) in the fleeceless groin region close to the udder in the standing animal (Buckrell, 1988). The optimum time for accurately assessing fetal numbers using the transabdominal approach is between 51 and 75 days. Pregnancy detection and fetal counts are generally done from the right side of the abdomen because the rumen occupies most of the left flank area (Gearhart et al., 1988). A diagnosis of pregnancy requires the identification of the fetus or fetoplacental unit. A fetal heartbeat can be detected by day 35 and placentomes can be seen as early as day 22. By 40 days, placentomes are obvious “C” or “O” shaped structures. Fetal fluid is anechoic and by 45 days the fetal skeleton is hyperechoic (Sharkey et al., 2001). Owners should be informed that a positive pregnancy diagnosis does not guarantee lambs at the expected time of parturition due to premature mortality of embryos or fetuses.

Ultrasonography can be used in male small ruminants to evaluate the scrotum, testicles, epididymides and urethra using a 5 or 7.5 mHz linear array transducer (Buckrell, 1988). Ultrasonography has been shown to be helpful in the diagnosis of spermatic granuloma and urethral obstruction in the ram (Karaca et al., 1999; Scott, 2000). Mucometra, pyometra, fetal death, mummification and fetal maceration can be diagnosed in the standing female small ruminant using a 3.5 or 5.0 mHz transducer.

Semen Cryopreservation

Small ruminant semen cryopreservation is increasing in popularity in North America. The benefits of semen cryopreservation include the preservation of genetics for future use and a means of disseminating valuable genetics. Males selected for semen cryopreservation should be

genetically superior to their contemporaries (Evans and Maxwell, 1987). All males should undergo and pass a breeding soundness evaluation prior to semen cryopreservation. Semen quality is highest at the peak of the breeding season, therefore, semen cryopreservation should be planned to coincide with the natural breeding season (Evans and Maxwell, 1987). The preferred method of semen collection is using an artificial vagina (AV). Electroejaculation is reserved for those males that cannot be trained to service an AV. Semen obtained in an AV is better quality semen than that obtained by electroejaculation (Gourley and Riese, 1990) , although both can be successfully frozen. Rams and bucks must be trained to service the AV while mounted on a teaser female. A teaser can be a female in natural estrus or an ovariectomized female which is occasionally administered estradiol 17β (0.1 ml intramuscularly). Training should be done during the breeding season when libido is the strongest. It may take several days to train a ram or buck to service the AV (Evans and Maxwell, 1987). Semen collected for freezing should be carefully handled to avoid cold shock. Initially the semen sample is evaluated for volume, motility and sperm concentration. Sample dilution rate is based on the required inseminate volume and the number of spermatozoa. The diluents used for freezing contain a buffer, an energy source, an agent to protect cell membranes during cooling to 5 ºC (usually egg yolk) and a cryoprotective agent (usually glycerol) which protects the sperm against membrane damage during freezing. A suitable medium for dilution and freezing of ram and buck semen is the tris-glucose, egg yolk and glycerol extender (Evans and Maxwell). Once semen is appropriately diluted it is slowly cooled to 4 ºC over two hours. Semen is then loaded into straws (0.25-0.5 ml) and frozen in liquid nitrogen vapor or a programmable freezer. Alternatively the extended semen can be placed in depressions in dry ice and frozen in pellets. The frozen semen is stored in liquid nitrogen. Thawed frozen semen should have at least 40% progressive motility to be considered suitable for long term storage and commercial use (Evans and Maxwell, 1987). There are some rams (5-10%) whose semen will not successfully withstand cryopreservation using the techniques currently recommended (Mylne et al, 1997).

Artificial Insemination

Artificial insemination (AI) programs reduce the risk of disease transmission and can dramatically increase the rate of genetic gain in an individual flock through the use of superior genetics. AI programs also allow producers to breed out-of-season and use genetics from dead or injured animals (Mylne et al., 1997). Females participating in an AI program should have their estrous cycles synchronized to allow efficient use of labor, facilitate out-of-season breeding and to allow for group insemination (Evans and Maxwell, 1987). Most synchronization regimes utilize intravaginal devices containing progestogens. These devices are placed in the female's vagina for 12 to 14 days. Each animal receives an injection of equine chorionic gonadotropin (eCG) before or at the time of progestogen withdrawal in order to increase the number of ovulations and synchronize the timing of the ovulations. The dose of eCG each female receives is season and breed dependent (Gourley et al., 1990; Bretzlaff, 1997). Alternatively two intramuscular injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be used 10-14 days apart to synchronize estrus in a group of females. $PGF_{2\alpha}$ is not used widely for synchronization due to the variability of the response, lowered fertility rates and ineffectiveness during anestrus (Gourley et al., 1990). Sterile or vasectomized teaser males equipped with marking harnesses are introduced following pessary removal to stimulate an earlier onset and synchrony of estrus and ovulation (Evans and Maxwell, 1987). Time of marking by the teaser male will determine

the order in which females are inseminated and will eliminate those females that did not respond to the synchronization regime. AI programs in small ruminants may involve fresh or frozen semen although most programs today involve the use of frozen semen. Fresh semen can be deposited into the female's vagina, cervix and uterus (either transcervically or laparoscopically). Acceptable pregnancy rates using frozen semen are only achieved when thawed semen is deposited into the female's uterus either laparoscopically or transcervically. Pregnancy rates associated with transcervical intrauterine insemination are variable and generally lower than those following laparoscopic insemination probably because semen is deposited in the uterine body rather than in the uterine horns (Mylne et al., 1997).

Laparoscopic AI is gaining in popularity in the United States. Laparoscopic AI should be performed before ovulation for best results. Therefore sheep synchronized with progestogens, eCG and teaser rams should be bred laparoscopically 52-60 hours and 55-60 hours following progestogen withdrawal with fresh and frozen semen, respectively. Approximately 20 million motile sperm are recommended for use in laparoscopic AI to achieve acceptable pregnancy rates. Females to be inseminated should be kept off feed and water for 24-48 hours to reduce the amount of rumen and bladder fill and to reduce the risk of regurgitation (Gourley et al., 1990). Various laparoscopic AI techniques are available but generally the female is placed in dorsal recumbency in a small ruminant cradle with the hindquarters elevated so the animal is at a 45 degree or greater angle. Animals are easier to handle when lightly sedated with xylazine (15 mg IM) five minutes prior to placement in the cradle. Two abdominal punctures are made, one to accommodate the laparoscope and the other to accommodate the insemination guide and needle. Some operators insufflate the abdomen with carbon dioxide to make the abdominal contents visible (Evans and Maxwell, 1987), however, CO2 insufflation is not necessary provided the animal is tilted appropriately. Half of the frozen, thawed semen should be deposited into one uterine horn with the remainder deposited in the other uterine horn. Pregnancy rates following laparoscopic AI using frozen, thawed semen are generally good and range from 50-90% in sheep during the natural breeding season (Gourley et al., 1990).

Embryo Transfer

Embryo transfer (ET) in sheep and goats has been gaining in popularity in North America. The benefits of ET include a reduced risk of disease transmission, the potential for genetic improvement and the ability to store and market genetics. Most authors report an average recovery of 8-12 embryos per embryo flush. Six to eight of these embryos will be fertilized and 5-6 of these embryos will be of acceptable quality to transfer into recipients. Generally, 2-4 live offspring will result from each superovulation and transfer attempt (Buckrell and Pollard, 1997). The estrous cycle on donor and recipient animals are usually synchronized for ET using intravaginal pessaries containing progestogens. These devices are placed in the vagina for a period of approximately 14 days in sheep and 14 to 21 days in goats. Superovulation of donor animals is most frequently accomplished using follicle stimulating hormone (FSH). FSH is administered in decreasing doses (ie. 5, 4, 3, 3, 2, 2 mg) at 12 hour intervals during the last several days of progestogen treatment (Flores-Foxworth, 1997; Buckrell and Pollard, 1997). Alternatively, a single dose of equine chorionic gonadotropin (eCG) can be used. Unfortunately eCG has a long duration of action which, by way of elevated estradiol concentrations, contributes to an increased proportion of unfertilized ova and poor quality embryos. A combination of low

dose eCG given simultaneously with the first and second injections of FSH has been used to successfully superovulate ewes (Buckrell and Pollard, 1997). Donor females are bred laparoscopically approximately 47 and 60 hours following progestogen removal in goats and sheep, respectively (Flores-Foxworth, 1997; Buckrell and Pollard, 1997). The administration of 50 μg of GnRH 36 hours following progestogen withdrawal in FSH stimulated ewes will improve the yield of fertilized ova (Buckrell and Pollard, 1997). Synchronized and super ovulated does are prone to early luteal regression giving rise to poor embryo recovery rates. Supplementing does with a progestogen between breeding and embryo recovery will alleviate this problem (Flores-Foxworth, 1997). Embryos are recovered from donor animals 6 days following laparoscopic artificial insemination. Embryos can be recovered from ewes surgically and laparoscopically and from does surgically, laparoscopically and transcervically. Surgical embryo recovery results in high embryo recovery rates, however, animals often develop post-surgical adhesions which in some individuals may interfere with long term fertility. Laparoscopic embryo collection is less invasive and results in fewer adhesions but requires considerable experience (Flores-Foxworth, 1997; Buckrell and Pollard, 1997). Recently very good embryo recovery rates have been achieved transcervically in Boer goats in a standing position without tranquilization or anesthesia. These does were administered $PGF_{2\alpha}$ 8 or16 hours before embryo recovery to facilitate luteal regression and intrauterine catheter placement (Pereira et al., 1998). Embryos recovered are assessed for stage of development and quality and then are either frozen in ethylene glycol or transferred fresh into synchronized recipients. In order to achieve acceptable pregnancy rates following the transfer of fresh or frozen, thawed embryos, there must be good synchrony (plus or minus 24 hours) between donor and recipient animals (Buckrell and Pollard, 1997). Embryos can be transferred into recipient ewes surgically or laparoscopically and into recipient does surgically, laparoscopically, and transcervically (Flores-Foxworth, 1997; Buckrell and Pollard, 1997).

Sexed Semen

The X-bearing chromosome has 3-7% more DNA than does the Y-bearing chromosome in common mammals (Johnson, 1995). This inherent difference in DNA content allows sperm to be sorted accurately (90%) into populations of X- and Y- chromosome bearing sperm using flow sorting/cell sorting technology that has been modified specifically for sperm (Johnson and Welch, 1999). Initially the sperm are treated with a fluorescent dye which binds to the DNA. The sperm are then exposed to a laser beam which measures the relative fluorescent intensity reflective of DNA content of each sperm and then separates the sperm based on DNA content (Welch and Johnson, 1999). Initially relatively low numbers of sperm could be sorted per unit time using flow cytometer sorting. Now with improved efficiency in orienting the sperm head to the intersecting laser beam sperm sorting rates have increased to a maximum of 11 million sperm per hour (Johnson and Welch, 1999) bringing the technology closer to commercial application. Field trials in heifers using sexed frozen semen have shown pregnancy rates of 70-90% of unsexed controls using only a fraction of the number of sperm (ie. 1-1.5 million sperm in treated animals compared with 20-40 million sperm in control animals) (Seidel et al., 1999). Initially sexed semen will be expensive and will therefore make its use limited to ET and in vitro fertilization (IVF) programs. As the technology improves and the cost decreases sexed semen will be more affordable for routine AI. Sex control will allow small ruminant producers to mate genetically superior females to X-chromosome enriched semen or Y-chromosme enriched semen

to produce genetically superior females and males, respectively. Purebred breeders can combine multiple ovulation and embryo transfer (MOET) and sexed semen technology to lower the cost of producing valuable embryos and offspring of the desired sex (Baker, 1990). Sexed semen will also dramatically benefit in vitro embryo production programs by producing embryos and offspring of the desired sex.

In Vitro Embryo Production

Currently there is great interest in producing embryos from oocytes recovered from the ovaries of live and slaughtered small ruminants. The technology has the potential to generate large numbers of genetically valuable embryos for commercial use. More embryos could be generated with this technology than could ever be generated from traditional MOET programmes. In vitro embryo production also makes it possible to produce offspring from individuals that would not be eligible for MOET programmes (ie. prepubertal animals, gestating animals and aged animals). The production of embryos from prepubertal animals has the potential to significantly shorten the generation interval and accelerate genetic progress (Tervit, 1996). The perfection of in vitro maturation of oocytes and the culture of embryos will also help to advance the commercialization of cloning in small ruminants. In vitro embryo production also provides a conservation tool for rare species of sheep and goats (Wani et al., 1999).

Ovaries from slaughtered animals are the cheapest and most abundant source of primary oocytes for large scale production of embryos through in vitro maturation, fertilization and embryo culture (Wani et al., 1999). Oocytes can also be obtained via laparotomy or through laparoscopic oocyte pick-up (LOPU). LOPU is less invasive than laparotomy and it can be repeated many times with a predictable number of oocytes recovered during each session (Baldassarre et al., 2002). Successful in vitro oocyte maturation, fertilization and embryo culture techniques have been worked out in both sheep and goats (Wang et al., 1998; Keskintepe et al., 1994). Further refinement of these techniques will hopefully make this technology commercially available in the near future.

Cloning

Cloning is a technology that will be commercially available to the small ruminant producer in the near future. Sheep have already been successfully cloned from embryonic cells, fetal cells and adult tissue (Wilmut et al., 1997) and with the advances being made in commercial bovine cloning it will be only a matter of time before small ruminants will be commercially cloned from a skin biopsy. The ability to produce and sell large numbers of cloned embryos from elite animals will allow for the fast dissemination of superior genetics to the commercial population (Baker et al., 1990). The cost of commercial cloning will limit the technology to elite male and female animals' initially but with continued improvements in nuclear transfer technology the cost will decrease making cloning more affordable for other breeding stock.

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