SOP No: **AHT 10**

SUBJECT: **Multiple ovulation and embryo transfer in sheep**

DATE ISSUED: **18.06.2014**

REASON FOR USE: **To synchronise oestrus and stimulate multiple ovulations in sheep for collection and transfer of multiple embryos**

POLICY:

1. **Demonstration and supervision of sponge and CIDR insertion and removal is required with inexperienced staff.**
2. **Some knowledge and/or experience is required for determination of appropriate doses of gonadotrophin.**
3. **Skilled training under supervision**
4. **A sound knowledge of aseptic technique and anaesthesia is required. The person performing the surgery must be either trained or supervised by an experienced and skilled operator.**

PRECAUTIONS:

Sturdy footwear and sun protection.
Sheep should be handled quietly before, during and after the procedure.
Understand the flight zone of the animal.
Animals should not be overcrowded in yards.
Wash hands and exposed body parts thoroughly with soap and water after handling animals.
The animal should be confined to an area to limit movement e.g. a crush.
Surgical cap and mask, surgical gown (freshly laundered), closed shoes
A withholding period may be necessary when using xylazine

EQUIPMENT:

18 gauge needle
21 gauge needle
2 to 10 ml syringes
7 mm trocar and trocar sleeve
Laparoscope
5 mm trocar and trocar sleeve
Intrauterine insemination gun
8 French gauge Foley Catheter
22 gauge teflon IV catheter
Fluorogestone acetate or Medroxyprogesterone acetate or Progesterone (pessary, sponge or CIDR)
Prostaglandin-F2α or synthetic analogue
Follicle stimulating hormone (FSH)
Pregnant mare's serum gonadotrophin (PMSG)
Gonadotrophin releasing hormone (GnRH)
Xylazine (dose according to weight)
Analgesic of choice
2% lignocaine hydrochloride
Antiseptic/fly repellent
Long acting oxytetracycline
Alphaxalone
1-2% isoflurane and oxygen
Betadine
Flushing media

PROCEDURE:

1. Synchronisation

There are currently two main methods of synchronising oestrus in ewes. The first method involves administering progesterone for 12-14 days via an intravaginal pessary, sponge or CIDR (controlled internal drug releasing device). Upon removal of the progesterone device, the ewes will come into oestrus within 2-3 days. The second method involves intramuscular administration of two prostaglandin-F2α injections. A single injection of prostaglandin-F2α at around seven days after initial administration of the progestagen device can also be incorporated into the first method.

a) Progestagen devices

Insertion of sponges. **Each intravaginal sponge is threaded on a string and is inserted into the vagina using an applicator (a plastic tube and a rod) as directed by the product instructions. Before insertion sponges must be treated with an antibiotic cream and the applicator moistened with an antiseptic solution. With young maiden ewes that have tight vaginas, it is better to gently insert a narrow sponge (e.g. Repromap®) using the fingers. In some cases however, exceptionally tight vaginas (due to the presence of the hymen) will make sponge insertion impossible.**

Removal of sponges: **Remove the sponge by pulling the protruding string gently outwards and slightly down. The appearance of a discharge is normal. In the event that the sponge cannot be removed with the string, a speculum allows the sponge to be removed with long forceps.**

Controlled Internal Drug Release dispenser (CIDR): **CIDRs consist of an inert silicone elastomer that releases controlled amounts of progesterone. The CIDR is inserted into the vagina using a special applicator where it remains for 12-14 days. Ewes should come into oestrus approximately 54 hours after CIDR removal.**

b) Prostaglandin-F2α

Two intramuscular injections of prostaglandin-F2α using an 18 gauge needle spaced 9-10 days apart are required for complete synchronisation of the flock. Oestrus occurs, on average, 2-3 days after the second injection.

2. Superovulation of ewes

Superovulation is most commonly achieved by the administration of the exogenous pituitary gonadotrophins FSH. This therapy commences 48 hours before sponge or CIDR removal and can be given intramuscularly using an 18 gauge needle as either a single injection or, as a decreasing FSH injection regime that consists of 8 injections (at 12 hour intervals). Superstimulatory treatment may be accompanied by an intramuscular dose of prostaglandin-F2α, PMSG, or a GnRH analogue. The response of ewes to superovulatory treatment is dependent on many variables and therefore, the dose of FSH and other hormones given will vary for each superovulation program.

3. Intrauterine insemination of ewes

Time of insemination: The time of intrauterine insemination can be between 36-48 hours after the cessation of progesterone treatment with fresh semen and 44-48 hours post sponge/CIDR removal with frozen semen. The volume of semen introduced is usually about 0.1 ml per uterine horn.

Intrauterine insemination by mid-ventral laparoscopy: Laparoscopic AI is performed approximately 39 hours after withdrawal of the vaginal progesterone implant. Ewes are administered an analgesic/sedative (xylazine 2mg per 50 kg ewe intramuscularly using a 21 gauge needle and 2ml syringe - xylazine 20mg/ml is first diluted to 2mg/ml using sterile water). After 15 minutes the ewe is restrained in dorsal recumbency in a laparotomy cradle. Local anaesthetic, 1 ml of 2% lignocaine hydrochloride is administered subcutaneously at the incision sites and a 7 mm laparoscopic trocar in a trocar sleeve is introduced into the abdomen to allow the abdomen to be sufficiently inflated with carbon dioxide. The laparoscope is then inserted through the trocar sleeve. A 5 mm trocar in a trocar sleeve is inserted intrabdominally on the other side of the midline and an insemination gun introduced into the trocar sleeve. After locating the uterus with the laparoscope, each uterine horn is intraluminally inseminated. Care will be taken not to introduce contaminants (e.g. antiseptic solution, blood or water) into the abdomen or the uterus. All equipment will then be removed and incisions will be sprayed with antiseptic/fly repellent. Antibiotic will be administered (long acting oxytetracycline 1ml/10kg).

Care of Animals Following the Procedure: After insemination, ewes should remain quietly in a clean pen and monitored for a least 2-3 hours. A long acting antibiotic should be administered according to the liveweight of the animal.

4. Surgical collection and transfer of sheep embryos

Collection of Embryos from Donors- Pre-operative Preparation: Surgical collection of embryos occurs 6 to 7 days after artificial insemination. Ewes are deprived of food and water 24 hours prior to the operation. After restraining the ewe in a laparoscopy cradle, wool is clipped from an area anterior to the udder and thoroughly cleaned with ivone scrub. Finer clippers are then used to remove short wool. Anaesthesia will be induced with alphaxalone (2-3 mg/kg) intravenously to effect followed by endotracheal intubation and maintenance on 1-2% isoflurane and oxygen. Laparoscopy is used to view ovaries and count the number of corpora lutea. The area of incision then undergoes surgical preparation (Betadine, 70% alcohol and tincture of iodine) and is draped with sterile drapes.

Operative technique: A 7-8 cm skin incision is made paramedially to the right of the ventral midline, followed by a blunt dissection through the muscle layers. Any disrupted arteries are clamped and ligated. The uterine horns are then exteriorised and packed with swabs moistened with sterile saline. An 8 French gauge Foley catheter is introduced via a blunt stab incision in the wall of the uterus at the level of the external bifurcation. The cuff of the catheter is inflated with air and the end held in a sterile petri-dish or egg bowl. A 22 gauge teflon IV catheter is then inserted ~ 3cm proximal to the utero-tubal junction and is used to flush embryo transfer media down the horn and into the Foley catheter. The same procedure is repeated on the other uterine horn. Each site

where the Foley catheter has been introduced to the uterine horn is sutured using 3-0 absorbable suture material in a single interrupted pattern. After both horns have been flushed, the abdominal cavity will be flushed with 250ml of saline solution to minimise the development of post-operative abdominal adhesions. The peritoneum and the muscle layer are then closed with a simple continuous pattern using 2-0 Maxon or PDS (absorbable suture). The skin is then closed with a vertical mattress sutures using 2-0 Maxon. All ewes are given 7 mls of long acting antibiotic and the wound is treated with topical antibiotic spray/fly repellent. The total recovered fluid will be filtered through an Emcon filter then searched under a stereomicroscope. Embryos will be staged and graded according to IETS criteria.

a) Transfer of Embryos into Recipient ewes

After restraining the ewe in a laparoscopy cradle, wool is clipped from an area anterior to the udder and thoroughly cleaned with ivone scrub. Finer clippers are then used to remove short wool. Anaesthesia will be induce with alphaxalone (2-3 mg/kg) intravenously to effect followed by endotracheal intubation and maintenance on 1-2% isoflurane and oxygen. Laparoscopy is used to observe the ovaries and if the presence of a healthy corpus luteum is noted using laparoscopy the procedure continues. The area of incision then undergoes surgical preparation (Betadine, 70% alcohol and tincture of iodine) and is draped with sterile drapes. The site of cannulation to the right of the midline is then enlarged (2-3 cm) so that a pair of sterile Babcock forceps can be introduced to exteriorise the upper part of the uterine horn ipsilateral to the ovary with the corpus luteum. A blunt incision is made into the uterine horn and a unopipette containing the embryo introduced and threaded towards the utero-tubal junction. The embryo is then expelled. The uterus is returned to the abdomen and the muscle and subcutaneous layer closed using 2-0 Maxon in a single interrupted pattern. The skin is then sutured in a similar fashion. The wound is dressed with a topical antibiotic/fly repellent.

Care of Animals after Procedure: **Give all ewes an injection of long acting antibiotic according to live-weight. Leave recipient and donor ewes to recover in a quiet pen and observe for 2-3 hours. Return ewes to pasture and check twice daily for 10 days post-surgery.**

RECOMMENDATIONS:

REVISED:

CHAIR OF AEC

REFERENCES

1. Evans, G. & Maxwell, W. M. C. (1987). Salamon's Artificial Insemination of Sheep and Goats. Butterworths, Australia.
2. Evans, G., Maxwell, W., & Wilson, H. (1994). Superovulation and embryo recovery in Merino ewes. *Theriogenology,* 41: 192
3. Forcada, F., Sanchez-Prieto, L., Casao, A., Palacin, I., Cebrian-Perez, J., Muino-Blanco, T. & Abecia, J. (2012). Use of laparoscopic intrauterine insemination associated with a simplified superovulation treatment for in vivo embryo production in sheep: a preliminary report. *Animal Production Science,* 52, 1111-1116.
4. Menchaca, A., Vilarino, M., Pinczak, A., Kmaid, S. & Saldana, J. (2009). Progesterone treatment, FSH plus eCG, GnRH administration, and Day 0 Protocol for MOET programs in sheep. *Theriogenology*, 72, 477-483.