

<b>SOP No:</b>	<b>AHT 11</b>
<b>SUBJECT:</b>	<b>Multiple ovulation and embryo transfer in cattle</b>
<b>DATE ISSUED:</b>	<b>18.06.2014</b>
<b>REASON FOR USE:</b>	<b>To synchronise oestrus and stimulate multiple ovulations in cattle for collection and transfer of multiple embryos</b>
<b>POLICY:</b>	<b>Some knowledge and/or experience is required for determination of appropriate doses of gonadotrophin, insertion and removal of progestagen devices, artificial insemination and embryo collection and transfer.</b>
<b>PRECAUTIONS:</b>	<b>Wear sturdy footwear and sun protection. Cattle 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.</b>
<b>EQUIPMENT:</b>	<b>18 gauge needle 2 to 10 ml syringes Insemination pipette 8 French gauge Foley Catheter Fluorogestone acetate or Medroxyprogesterone acetate or Progesterone (pessary, sponge or CIDR) Prostaglandin-F2<math>\alpha</math> or synthetic analogue Follicle stimulating hormone (FSH) Pregnant mare's serum gonadotrophin (PMSG) Gonadotrophin releasing hormone (GnRH) 500 ml phosphate-buffered saline (PBS) supplemented with 10% foetal calf serum (FCS) 2% lignocaine</b>
<b>PROCEDURE:</b>	<ol style="list-style-type: none"><li><b><u>Synchronisation</u></b> Synchronisation of the oestrous cycle in cows involves administering progesterone for 7 days via an intra-vaginal CIDR (controlled internal drug releasing device). The CIDR is inserted into the vagina using a special applicator where it remains for 7 days. Upon removal of the progesterone device, the cows will come into oestrus within 2-3 days. An injection of 2.5-5 mg of oestradiol-17<math>\beta</math> or oestradiol benzoate intramuscularly using a 5 ml syringe and an 18 gauge needle on the day of insertion of the progestagen device can also be incorporated into this method.</li><li><b><u>Superovulation</u></b> Superovulation is most commonly achieved by the administration of the exogenous pituitary gonadotrophin FSH. This therapy commences 4 days after initial progestagen CIDR insertion 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-F2<math>\alpha</math>, PMSG, or a GnRH analogue and is commonly accompanied by</li></ol>

intramuscular administration of PMSG or GnRH 12-24 hours after removal of the progestagen device. The response of cows 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. Artificial insemination

*Time of insemination*

Insemination is usually performed 36 hours after withdrawal of the vaginal progestagen implant. This procedure involves securing cows in a crush, inserting an insemination pipette into the vagina of the cow, feeling for the position of the pipette via the rectal wall and depositing semen into the cervix or uterine body of the cow.

4. Non-Surgical Collection and transfer of embryos

a) *Collection of Embryos from Donors*

Around 6-7 days after artificial insemination, embryos are recovered by nonsurgical transcervical flushing with 500 ml phosphate-buffered saline (PBS) supplemented with 10% foetal calf serum (FCS) for each uterine horn by an experienced operator. Cows are first administered a low caudal epidural (4ml 2% lignocaine via 18 gauge needle). The perineum is then washed and cleaned prior to insertion of the Foley catheter with metal stilette into first the distal left uterine horn and then the distal right uterine horn. Fluid is instilled by 60 ml syringe then recovered. This is repeated approximately 8 times before 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. Following the flush, cows will be administered 5ml of a prostaglandin analogue by 18 gauge needle to lyse the corpus luteum.

b) *Transfer of Embryos into Recipient cows*

Recipient cows need to be oestrus synchronised using CIDRs. (controlled internal drug releasing device). The CIDR is inserted into the vagina using a special applicator where it remains for 7 days. Cows are first restrained in a crush and administered a low caudal epidural (4ml 2% lignocaine via 18 gauge needle). The perineum is then washed and cleaned and the cows are rectally palpated to determine which ovary has ovulated. Embryos for transfer are loaded into an insemination pipette. This pipette is then inserted into the vagina of the cow and carefully passed through the cervix. The tip of the pipette is then inserted into the uterine horn ipsilateral to the corpus luteum and the embryo(s) is gently expelled.

RECOMMENDATIONS:

REVISED:



CHAIR OF AEC

REFERENCES

1. Mapletoft, R. J. & Bo, G. A. 2012. The evolution of improved and simplified superovulation protocols in cattle. *Reproduction Fertility and Development*, 24, 278-283.