**Superovulation**

Treatment of a female with gonadotropins (generally FSH) to increase the number of oocytes that are selected to become dominant follicles and ovulate.

A typical treatment response in cattle would be 8 to 10 ovulation

Superovulation Procedures

**Hormones used for superovulation**

**FSH** ( Follicle stimulating hormone)

Short half life ̴ 2 hours

**PMSG (**pregnant mare serum gonadotropi ; eCG

Long half life ̴ 2 – 4 days

**Table (1): FSH dosage for superovulation of cattle**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Bos Taurus** | **Bos Indicus** | **Heifers** |
|  | AM | PM | AM | PM | AM | PM |
| **Day 1** | 5 mg | 5 mg | 4mg | 4mg | 3mg | 3mg |
| **Day2** | 4mg | 4mg | 3mg | 3mg | 3mg | 3mg |
| **Day3** | 3mg | 3mg | 2mg | 2mg | 2mg | 2mg |
| **Day4** | 2mg | 2mg | 2mg | 2mg | 2mg | 2mg |
| **Total** | 28 mg | 22mg | 20 mg |

**Steps of embryo transfer in cows**

Synchronization of recipients with donor

**Goal**: want donor and recipient to be in same stage of estrus cycle

**Reason**: preparation of recipient uterus to support embryogenesis

**How**: treat recipient with synchronization protocol that induces estrus to occur at same time as the donor

**Superovulation of donor female**

**Goal:** hyper stimulate ovaries with gonadotropins

**Reason**: provide higher than normal number of follicles that will ovulate

**How**: inject with FSH

**Inseminate donor with semen from genetically superior bull**

**Goal:** to generate the best fertilization rates and genetic combinations possible

**Reason**: enhance rate of genetic progress

**How**: utilize highly fertile semen and trained inseminators

**Recovery and identification of viable embryos**

**Goal**: nonsurgically collect (flush) embryos from donor for transfer

**Reason**: to recover viable embryos

**How**: block with local anesthetic to relax rectum, at day 6 – 8 a Foleys catheter is inserted into uterus and inflated to prevent retrograde flow of flushing medium, introduce medium, lavage uterus and collect fluid.

**Transfer of viable embryos into synchronized recipients**

**Goal**: to deposit a potentially viable embryo into the uterine horn of each recipient

**Reason**: to achieve pregnancy in each recipient

**How**: a single embryo is placed into the uterine horn ( ipsilateral to the CL) using a transfer pipette

**Embryo grading**

**Criteria for classifying embryos**

* Even number of cells
* Uniform division
* Healthy zona pellucid

**Embryo Quality**

1. Excellent
2. Good
3. Fair
4. Poor
5. Degenerate

**Table (2): Summary of events in super ovulation and embryo transverse?**

|  |  |  |
| --- | --- | --- |
| Estrus day | Donor cow | Recipient cow |
| 9 | 6mg FSH at 7am and 7pm |  |
| 10 | 25 mg FSH at 7am and 7pm | 25 mg PGF2α 7pm |
| 11 | 4 mg FSH at 7am and 7pm25 mg PGF2α at 7pm |  |
| 12 | 3 mg FSH at 7am and 7pm |  |
| 13 if not is estrus | 2 mg FSH at 7am and 7pm |  |
| 13 -15 | estrus |  |
| Onset of estrus | inseminate |  |
| Onset of estrus +12h | inseminate |  |
| Onset of estrus+24h | inseminate | Transverse embryo |
| 7 new cycle | Collect and evaluate embryo with inject PGF2α into donor after flush |  |

**Table (3): superovulation and embryo transverse programs by using PMSG**

|  |  |  |
| --- | --- | --- |
| Estrus days | Donor cow | Recipient cow |
| 10 | PMSG 2500 IU |  |
| 12 | PGF2α 25mg | PGF2α 25mg |
| 14 estrus | inseminate |  |
| 7 day after estrus | Uterine flushing | Embryo transverse |

**Table (4): Embryo transverse programs by FSH**

|  |  |  |
| --- | --- | --- |
| Estrus day | Donor cow | Recipient cow |
| 10 | FSH 5mg am and 5mg pm |  |
| 11 | FSH 5mg am and 5mg pm |  |
| 12 | FSH 5mg am and 5mg pm | PGF2α 25mg |
| 13 | FSH 5mg am and 5mg pm |  |
| 14 estrus | inseminate |  |
| 7 day after estrus | Uterine flushing | Embryo transverse |

 **Table (4): Modified Dulbecco phosphate-buffered medium**

**Part 1**

|  |  |
| --- | --- |
| ingredients | Amount for 10 Liters g |
| NaCl | **80.0** |
| KCl | **2.0** |
| Na2HPO4 | **11.5** |
| KH2PO4 | **2.0** |
| Glucose | **10.0** |
| Streptomycin sulphate | **.5** |
| Na pyruvate | **.36** |
| Na penicillin G | **1,000,000 units** |

**Part 2**

|  |  |
| --- | --- |
| CaCl2 2H2O | 1.32 |
| MgSO4 7H2O | 1.21 |

1. **Dissolve part 1 in 8 liters of deionized, distilled water.**
2. **Dissolve part 2 liters of deionized, distilled.**
3. **Add part 2 to part 1 slowly with constant stirring to prevent precipitation**
4. **Add heat-treated autoserum immediately prior to use: 1% for flushing and 20% for culture.**

**Note: autoserum (( bovine serum albumin used for recovery and holding media)) this serum heat at 56C for 30 minute, the purpose from heating is remove complement embryo toxic.**

1. **Sterilize by passing through Millipore filters with 0.22µ pores.**



**Training manual for embryo transfer in cow**