Embryo transfer technology

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Embryo Transfer in cattle has be come a proven field technique in developed countries. Its advantages are well known. One of the advantages is quick multiplication of superior animals. Although many problems pertaining to Embryo transfer remain to be solved, the technique adopted now are being successfully used in the field by trained and experienced doctors.

Reproductive system of the cow

The cow in addition to contributing microscopic female sex cells (ova) necessary for starting a new calf, provides the environment in which the new individual is conceived and nourished during the early days of its life. These functions are carried out by the primary and secondary organs of reproduction. The primary organs, the ovaries, produce the ova and the female hormones. The secondary organs of production comprises of fallopian tubes (oviducts), the uterus, the cervix, the vagina and the vulva.

The reproductive organs of the heifer are produced long before birth. After birth they develop gradually until the animal becomes sexually active and capable of conceiving and producing a calf. This is called reaching puberty.

At and after puberty, the female reproductive system exihibits a rythmical change called the oestrous cycle. The highlights of this cycle of the period of oestrous, at which time the female is receptive to the male, and soon after the egg is shed. The oestrous cycle may be described in a number of ways. The duration of oestrous cycle is commonly 21 days. But the range may extend from 16-24 days. Based on external signs the oestrous cycle has four parts.

Pro-Oestrus

The period of preparation lasting 2-3 days before oestrus

Oestrus

This is the period of desire, characterised by the pshychic manifestation of heat. It is recognised by the cow's standing still and allowing itself to be mounted by other cattle. The duration of oestrus falls within the range 12 to 26 hours.

Metoestrus

This is the period of 3 or 4 days immediatelly following cessation of heat. Ovulation occurs during this period. Metoestrall bleeding occurs about 35-40 hours after the end of oestrus.

Dioestrus

This is the period lasting for 12 to 18 days from metesrus until the following pro-oestrus. If the fertilisation has occured, the period of diestrus extend

into pregnancy known as the gestation period.

Principal hormones involved in reproduction (described in sequances)

1. Follicle stimulating Hormone (FSH) from the anterior pituitary gland stimulates development of the follicle in the ovary.

2. Cells lining the follicle in turn produce oestrogen hormone which brings signs of heat.

3. The oestrogen triggers the release of Leutinising Hormone (LH) by the anterior pituitary. LH acts on the follicle to cause ovulation.It also stimulates the formation of corpus luteum (CL) at the ruptured follicle.

4. LH also cause the CL to produce progesteron, which prepares the uterus for implantation.

5. If implantation does not take place, the CLdecreases in size about 17 days after estrus (heat) resulting in a decrease of progesteron production. The regression of CL is stimulated by prostaglandin F2 alpha produced in the uterus. Consequently, prostaglanding is very effectively used for oestrus synchronisation and inducement of early abortion.

6. In the absence of high level of progesteron, the block on FSH production by anterior pituitary is removed and increased levels of this hormone begin to circulate causing development of a new follicle iin the ovary. The oestrus cycle repeats itself.

7. If pregnancy occurs, the CL does not regress but is maintained to become the CL of pregnancy and continue to secrete progesteron until just before the birth of the calf.

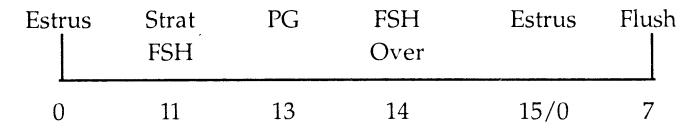
Superovulation of donor cow

Well nourished donor can enter an ET programme within 60 days postpartum. Establishing donors estrous cycle as accurately as possible is an important factor for achieving success. The Oestrus cycle should be normal and of regular length. Before treatment the donors Oestrous cycle must be monitored at least one cycle. Super ovulatory hormones are injected during the midluteal phase i.e. between day 9 to day 13 of the oestrous cycle.

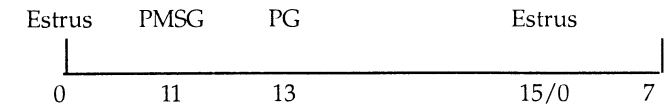
There are several products which induce superovulation in cattle, but currently FSH is used extensively. Before starting FSH treatment, it is necessary to palpate the donor to ascertain the presence of a CL. If no CL is present treatment should be postponed.

Super ovulation Regime

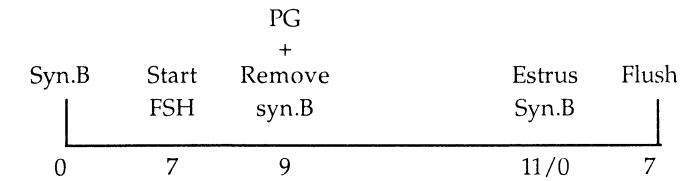
FSH/PG



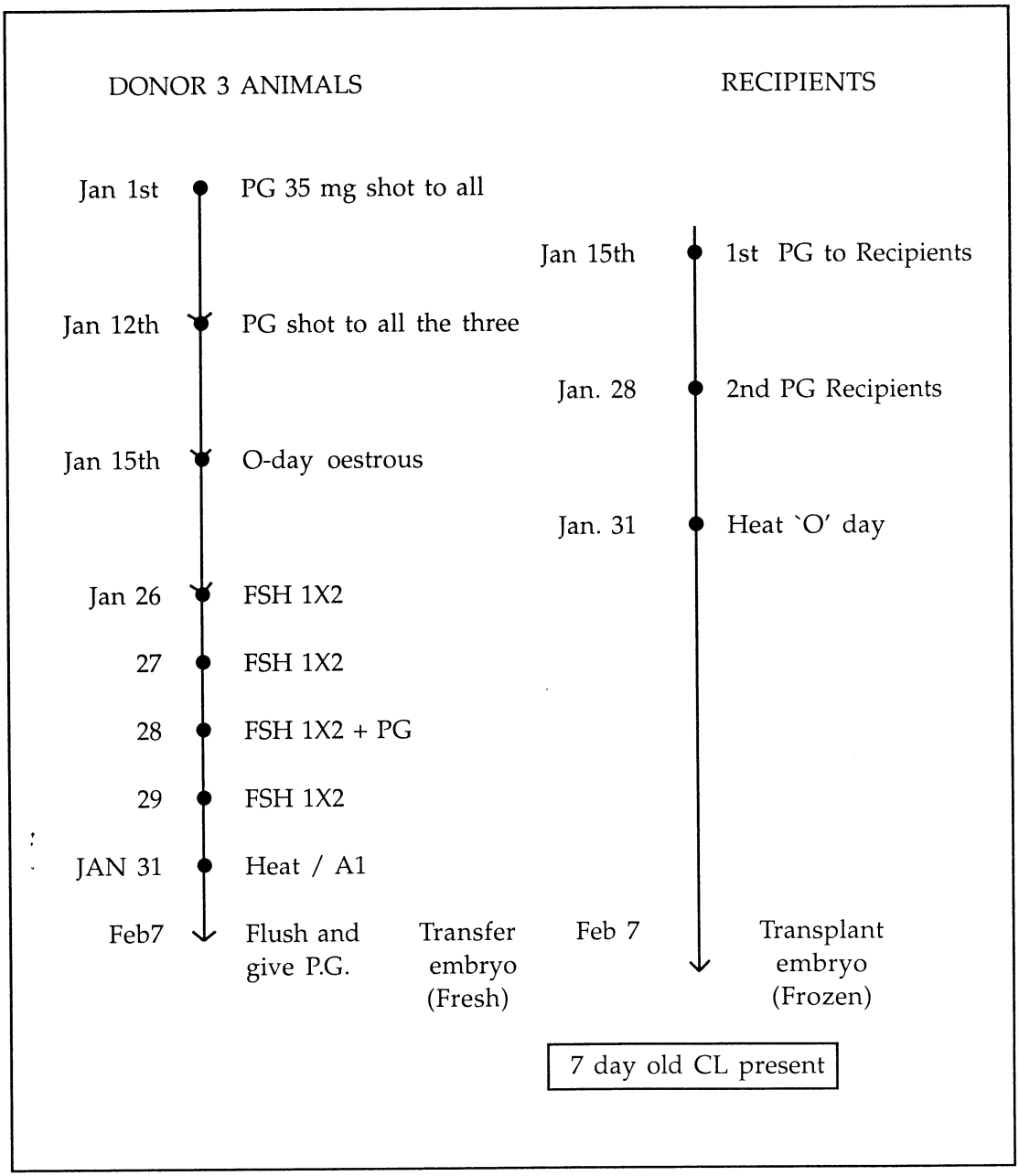
PMSG/PG



FSH/Syn.B



Synchronisation of donors and recipients to heat and transfer



Anterior Pituitary

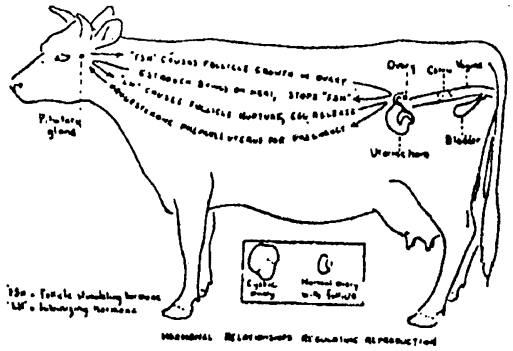
Ovary

FSH causes follicle growth in Ovary

Estrogen produced by follicle brings on heat and stops FSH

LH triggerd by oestrogen from anterior pituitary causes follicle rupture and egg is released and CL formed at ruptured follicle

LH also causes Cl to produce. Progesteron which prepares uterus for pregnancy



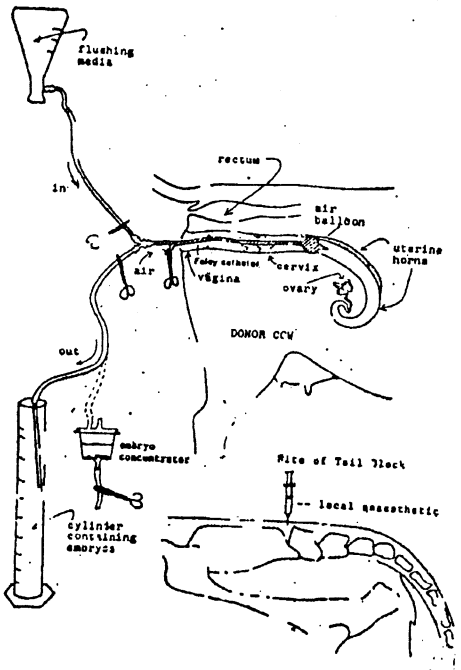
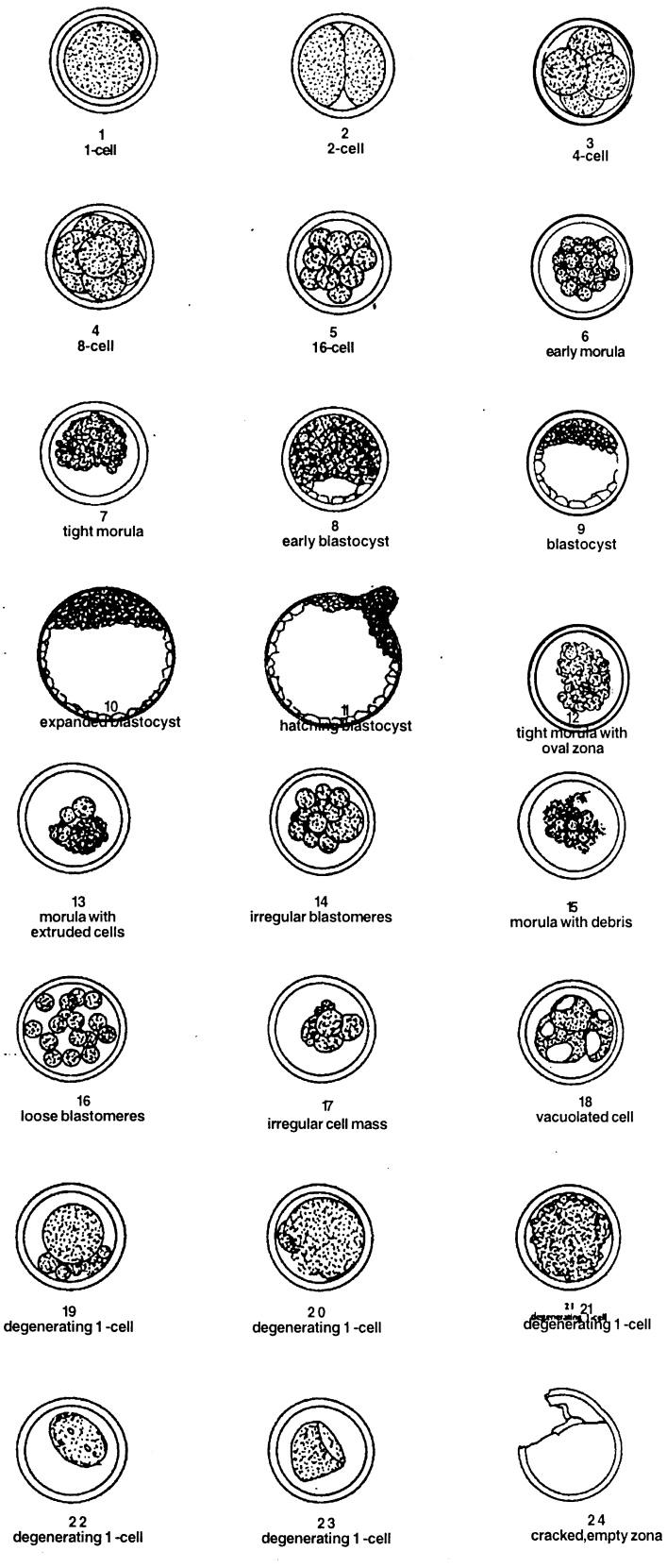


Figure 1. Flushing the Donor Cow



Drawings 1-11 represent normal embryos if they are recovered at appropriate times after estrus.

Drawings 12-24 illustrate increasing abnormalities. Prgnancy rates are very low from 15 to 24.

Number	Description	Days after estrus normally found
1	1-cell	0-2
2	2-cell	1-3
3	4-cell	2-3
4	8-cell	3-5
5	16-cell	4-5
6	early morula	5-6
7	tight morula	5-7
8	early blastocyst	7-8
9	blastocyst	7-9
10	expanded blastocyst	8-10
11	hatching blastocyst	9-11
12	tight morula with oval zona	
13	morula with extruded blastomeres	
14	irregular blastomeres	
15	morula with debris	
16	loose blastomeres	
17	irregular cell mass	
18	vacuolated cells	
19		
20		
21	degenerating 1-cell	
22		
23		
24	cracked, empty zona pellucida	

For superovulating the donor, eight injections are given to donor at 12 hours intervals, starting on either day 10,11 or 12 of the oestrous cycle. Generally tapering dosage of FSH is effective. PG1 F2 alpha is given in combination with the FSH treatment to ensure a predict able interval between termination of gonadotrophin injection and oestrous. When PGF2 alpha is administered to superovulated cows, oestrous follows in 36 to 48 hours after the PGF2 injection.

FSH dosage

1. Upto 450Kg. body wt. 5/5, 4/4, 4/4,4/4-34mg.
2. Upto 600Kg. body wt. 5/5, 5/5, 5/5, 4/4-38mg.

(The injection interval should not be shorter than 8 hours and longer than 12 hours)

Prostaglandin is given in the morning of a day 4 of the FSH treatment. Prostagladin dosage- 35 mg.

Sychronisation

Bringing animals in oestrous on the same day is called synchronisation. This can be achieved by giving prostaglandin injection.

Example for synchronisation of donors

A dairy man has three animals, each weighing more or less same weight. These cows have normal Oestrous cycles, but oestrous dates are far apart. The dairy man wants to flush all the three cows on the same day.

The first step is to synchronise the oestrous cycle of the three donors so that they came into oestrus approximately the same day.

Let us suppose we gave the first prostaglandin shot (35mg) to each cow on January 1st, then the second prostaglandin injection will be given on January 12. The three donors should be in heat on January 15 (3 day after the second PG shot)

Now, then January 15 is day zero and on day 11 (January 26) we start FSH as follows:

Date	Cycle day	AM	PM
January 26	Day 11	FSH 5mg	FSH 5mg
January 27	Day12	FSH 4mg	FSH 4mg
January 28	Day13	FSH 4mg	FSH 4mg
January29	Day14	FSH 4mg +35 mg PGF2	FSH 4mg

On January 31st the donors will be in heat. This January 31st is again day Zero (0). Flush on 7th February (7th day)

Flushing (Collection of Embryos from donor)

Embryos can be collected from the uterine horns of donor animal either by surgical or non-surgical method (7th day) Surgical

1. Flank laprotoms
2. Mid-ventral

After the collection of embryos, it will be screened, graded. Then the embryos are ready for transplantation. If no recipients are there to accept the collected embryos, it can be frozen in flushing media with 10% Glycerol in Liquid Nitrogen.

Flushing Media-Ingrediants (Dulbeccos phosphate Buffered Saline-DPBS)

1.DPBS 2.Sodium pyruvate

3.Streptomycin : 4. Penicillin

5.Glucose or dextrose 6. Bovine Serum Albumin or Foetal Calf Serum

7. Double distilled water pH-7.25 to be adjusted

Recipients:

A good recipient is an open cow whose reproductive organ is capable of receiving an embryo and carrying it to term. The best way to handle large numbers of embryo transfer is to use natural heats. However this is not possible at all times. To overcome this problem, recipients are synchronised with the donor. The most common synchronising agent used is prostaglandin. Prostaglandin can be used during 6 through 16 day of the oestrous cycle. Estrus occurs approximately 60 to 72 hours post injection in non-superovulated animals.

Procedure to Synchronise Recipients

Day Zero-when donor is in estrus, same day or one day later give 1st prostaglandin injection to recipients. Then on 3rd day of donor FSH treatment give a second injection of PG to the same recipients. The donor and the recipients may be in estrus on the same day.

Transplantation

7th day old embryos can be transplanted into the seven day old estrus uterine horns of recipients either by surgical or non-surgical method.

Freezing of Embryos

1. Collect 7 to 8 day old good quality embryos

2. Wash with sterile Dulbeccos Phosphate buffered saline (PBS) + 4% Bovine Serum Albumin (BSA)

3. Place embryos into PBS + 4% BSA + 5% Glycerol for 5 mts.

4. Place them into PBS + 4% BSA + 10% Glycerol for 10-30 mts.

5. Place the embryo into pre-labelled 0.25 ml. french straws. Fill the straw half way with freezing medium (PBS + 4% BSA +10% Glycerol) then on air bubble

of 4 mm, then another column of freezing medium containing the embryo, then 105-2 mm of paraffin oil, then seal the end. Cool straws to -65°C at a rate of 4°c per mt. and hold it for 5 mts. Cool then from-6.5° C to - 30° C at 0.5°C per mt. : When straws reach- 30° C plung them liquid nitrogen immediately.

Thawing

Thaw in 37°C water bath 10-20 seconds. Then deglycerisation is to be done as follows:

Put embryos in

1. Dish containing 6% Glycerol + 0.3 molar sucrose for 10mts.
2. 3% Glycerol +0.3 m Sucrose for 10 mts.
3. 0.3 molar sucrose for 10 mts.
4. Flushing media -5-10 mts.
5. Flushing media- 5-10 mts.

6.FM or holding Media- 5mts.

Then grade it and transfer.

Technical Info :(Latest research and Development)

The traditional thawing procedure of cryostored embryos invoves the removal of cryoprotectant i.e. Glycerol in three steps. Besides being time consuming , the procedure demands preparation and storage of these steps and microscopic handling / examination of the embryos before their transfer under uncontrolled environment,specially under field conditions.

To circumvent this cumbersome procedure, cryopreservation with more permeable solvent like ethylene glycerol has been tried. Few embryos were thawed and directly transferred to the recipients resulting in pregnancy. This method will make the transfer of frozen embryos simple and similar to frozen semen, where the contents of the straw need not be removed for further processing and evaluation.

Reasons for multiple ovulation and embryo transfer failure

1. Successful Embryo Transfer requires good pregnancy rates.
2. Education of producers to accept MOET programme.
3. Poor transportation and inappropriate attitudes of personnel often are overwhelming.
4. MOET is still expensive and not cost effective in commercial stocks.

Justification

Even if Embryo Transfer is expensive it will be justified in practice if it increases genetic response rates since the national economic returns from extra improvement will be large relative to the MOET costs in the breeding herds.

Given the appropriate environment, a motivated staff can fulfill the task of MOET application successfully.