EMBRYO TRANSFER (ET) IN CATTLE

جامعة الإخوة منتوري قسنطينة
UNIVERSITE DES FRERES MENTOURI CONSTANTINE

THERIOGENOLOGY LECTURES – A5

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Embryo Transfer Technology Provides Avenues for Reproductive and Genetic Enhancement

- Embryo transfer requires a set of procedures that allows removal of pre-attachment embryos from the reproductive tract of a donor female and transfers them into the reproductive tract of a recipient female
- **Embryo transfer is a valuable production and research technique**

Embryo Transfer Technology Provides Avenues for Reproductive and Genetic Enhancement

- It is commercially available in some species to increase the productivity of females with desired traits
- The **first successful embryo transfer** procedure was performed in a **rabbit in 1890**

Embryo Transfer Technology Provides Avenues for Reproductive and Genetic Enhancement

- The main advantage of embryo transfer in cattle is **to amplify the number of offspring that donor females with desired genetic traits can produce**
- With embryo transfer, a single donor cow is capable of producing **10 to 20 offspring annually**

Embryo Transfer Technology Provides Avenues for Reproductive and Genetic Enhancement

- Embryo transfer is an important technique used to enhance reproduction in **endangered species**

History of ET

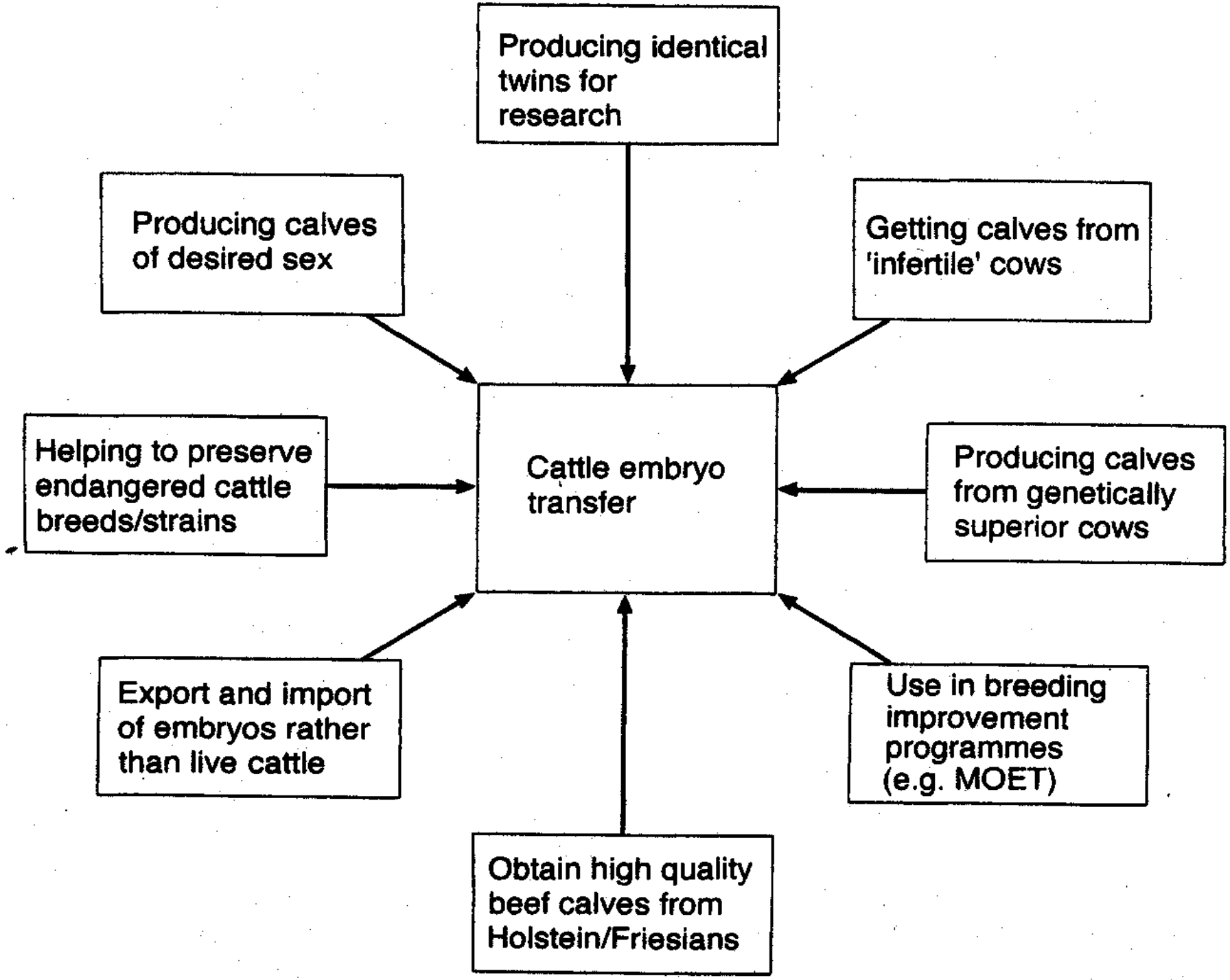
- 1930 : collection of first bovine embryo
- 1951: first successful bovine ET
- 1964: first non-surgical collection
- 1970s: application of ET to cattle industry
- 1983 : in vitro fertilization of a bovine oocyte
- As of 1992 : 162000 registered Holsteins resulted from ET

Definitions of terms

- **Embryo** :
- A fertilized ovum which will eventually develop into the offspring
- Main tissues, organs and systems are formed
- Period between **10 and 45 days**

Definitions of terms

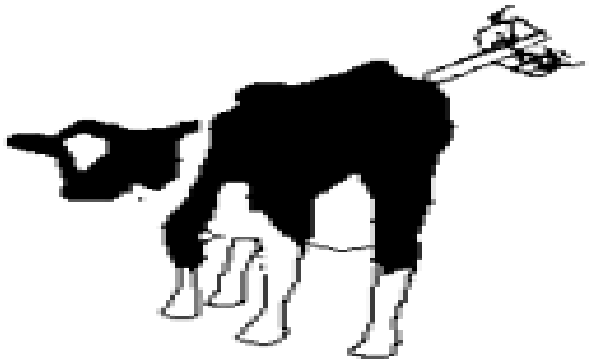
- **Embryo transfer**
- Process by which an embryo is collected (flushed) from one female (donor) and transferred to another female (recipient) to complete the gestation period


Producing identical twins for research
Producing calves of desired sex
Getting calves from 'infertile' cows
Helping to preserve endangered cattle breeds/strains
Cattle embryo transfer
Producing calves from genetically superior cows
Export and import of embryos rather than live cattle
Use in breeding improvement programmes (e.g. MOET)
Obtain high quality beef calves from Holstein/Friesians

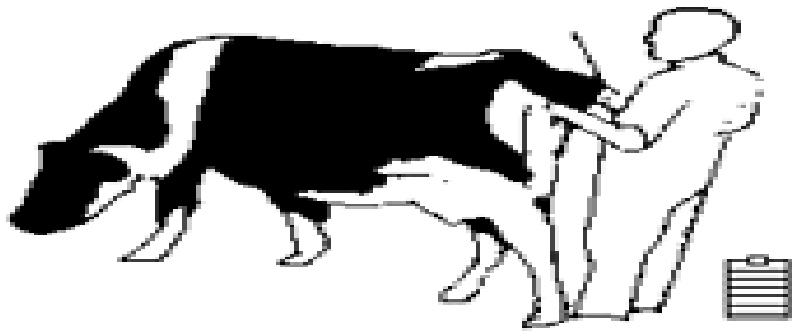
Identical Twins.

Two sets of identical twins produced by embryos splitting at Colorado State University.

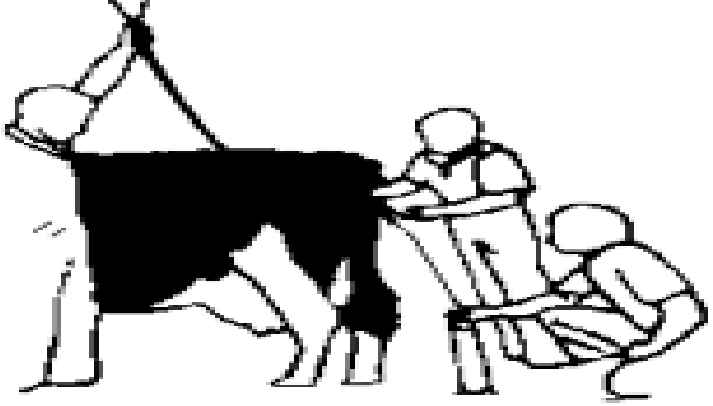
Seidel GE (1984)



Superovulation of donor with gonadotropins



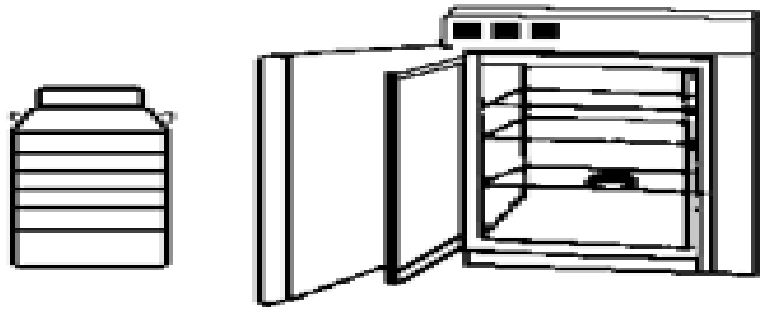
Artificial Insemination (5 days after initiating superovulation)



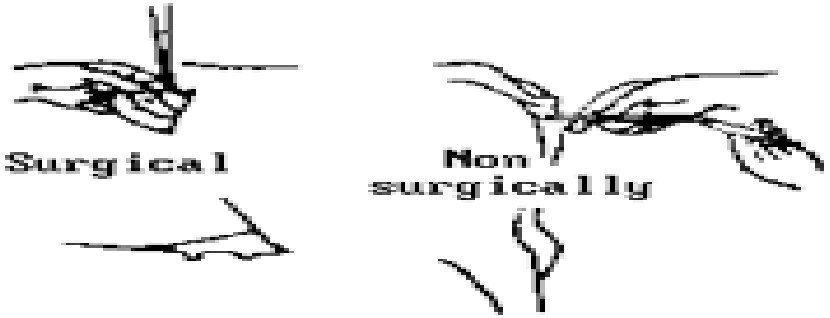
Nonsurgical recovery of embryos (6 to 8 days after insemination)

Foley catheter for recovery of embryos

Isolation and classification of embryos



Storage of embryos indefinitely in liquid nitrogen or at 37 C or room temperature for 1 day



Transfer of embryos to recipients surgically or nonsurgically

Pregnancy diagnosis by palpation through the rectal wall 1 to 3 months after embryo transfer

Birth (9 months after embryo transfer)

The advantages of embryo transfer

- Circumvention of seasonal reproduction
- Enhanced generation of offspring in monotocous species
- Enhanced reproductive potential of endangered species
- Enhanced genetic diversity across a wide geographical region (ship embryos rather than animals)

The advantages of embryo transfer

- Embryo transfer is an essential step in many experimental techniques in the production of clones and transgenic animals

Successful embryo transfer involves :

1. Synchronizing the cycles of donors and recipients
2. Superovulation (hyperstimulation of the ovaries) of the donor
3. Artificial insemination of the donor female
4. Recovery of embryos from the donor
5. Maintenance of viable embryos in vitro
6. Transfer of embryos to recipient females

MANAGING DONOR AND RECIPIENT COWS

Donor selection

1. Genetically superior cow

- Growth rates
- Calving ease
- Milk production and composition
- Disease resistance

Donor Selection

2. Production of usable embryos

- Reproductively sound
  - ⇨ Normal reproductive tract
  - ⇨ Normal postpartum history
    - * **No parturition difficulties**

Donor Selection

- Regular heat cycles
- No more than 2 breedings/conception
- At least 2 months postpartum
- 365 days calving interval
- No conformational or genetic defects

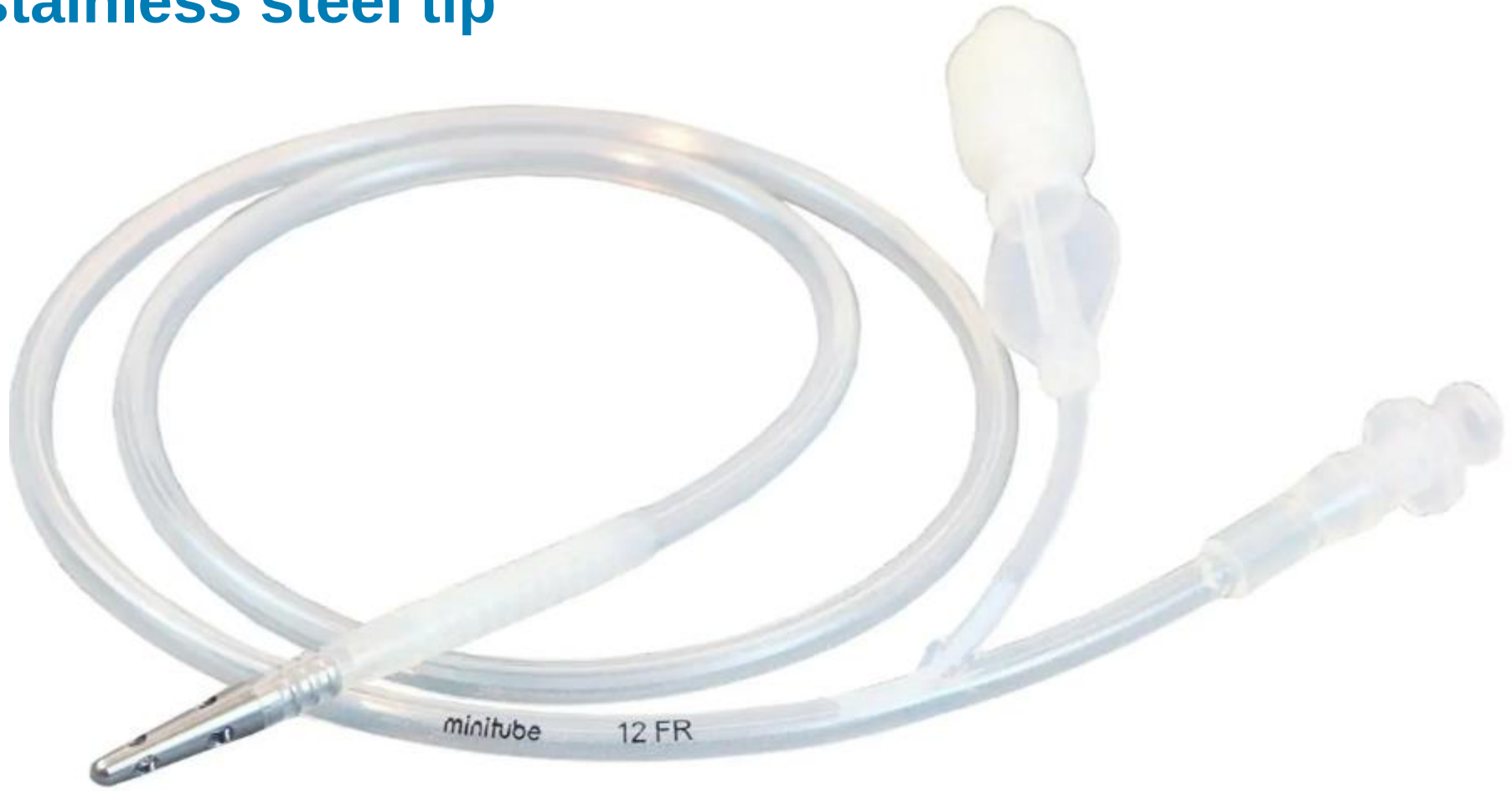
CERVIX DILATOR

Facilitates introduction of embryo collection catheter. With two different shapes to meet individual anatomic requirements

Y-JUNCTION TUBING LUER FOR MINIFLUSH®

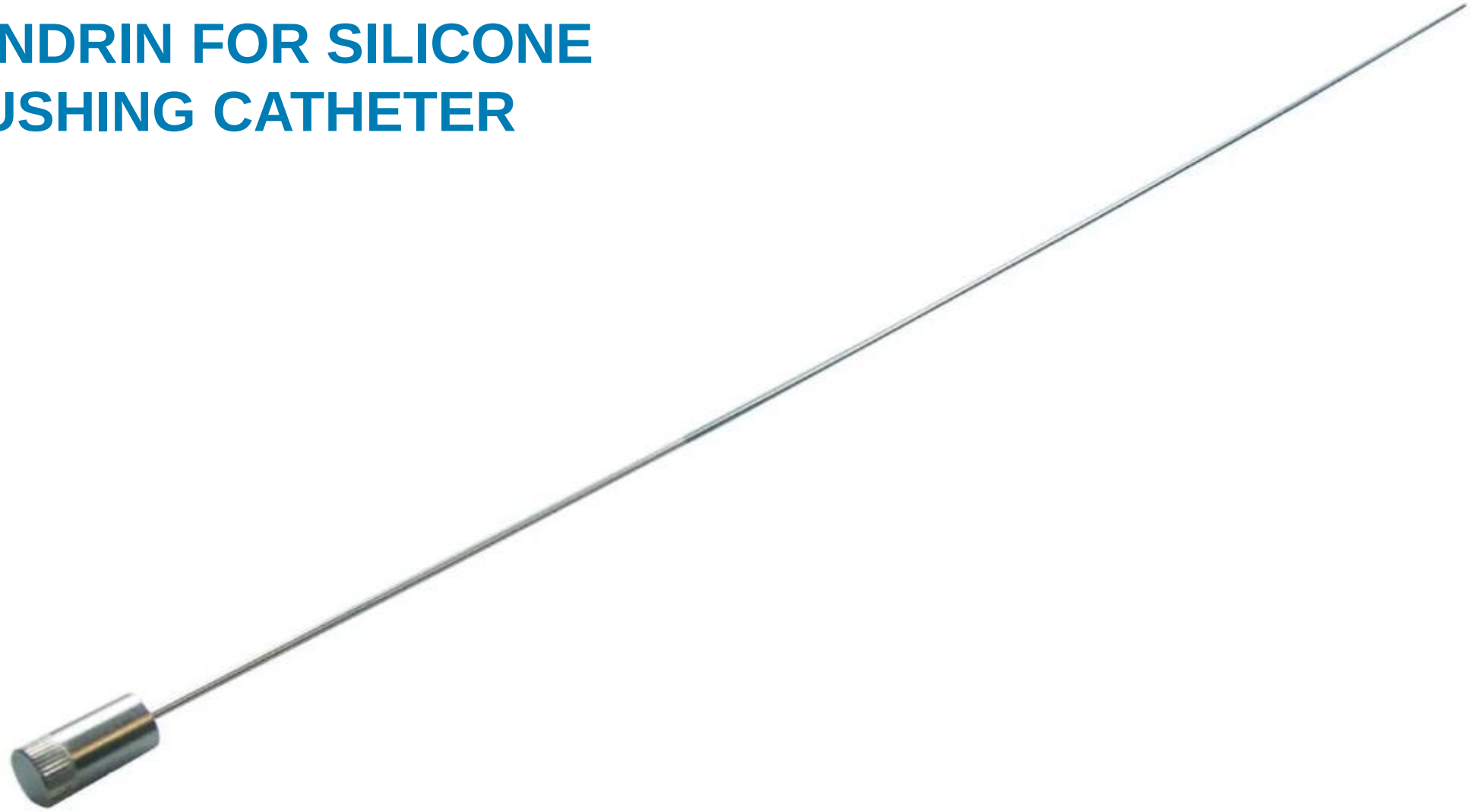
Disposable Y-junction tubing with clamps, to be used in combination with any catheter.
Tubing made of very smooth, flexible material.
For embryo collection with filter, end for filter connection requires no extra adapter

Silicone ET flushing catheter CH 12, with stainless steel tip



Silicone catheter with stainless steel tip and 6 ports. 2-way catheter with Luer lock adapter

MANDRIN FOR SILICONE FLUSHING CATHETER



SILICONE ET CATHETER CH 12, LUER LOCK, 2-WAY, BALLOON 5-15 ML FOR EMBRYO FLUSHING

EMBRYO TRANSPORT KIT AND A PORTABLE INCUBATOR WITH ADJUSTABLE TEMPERATURE

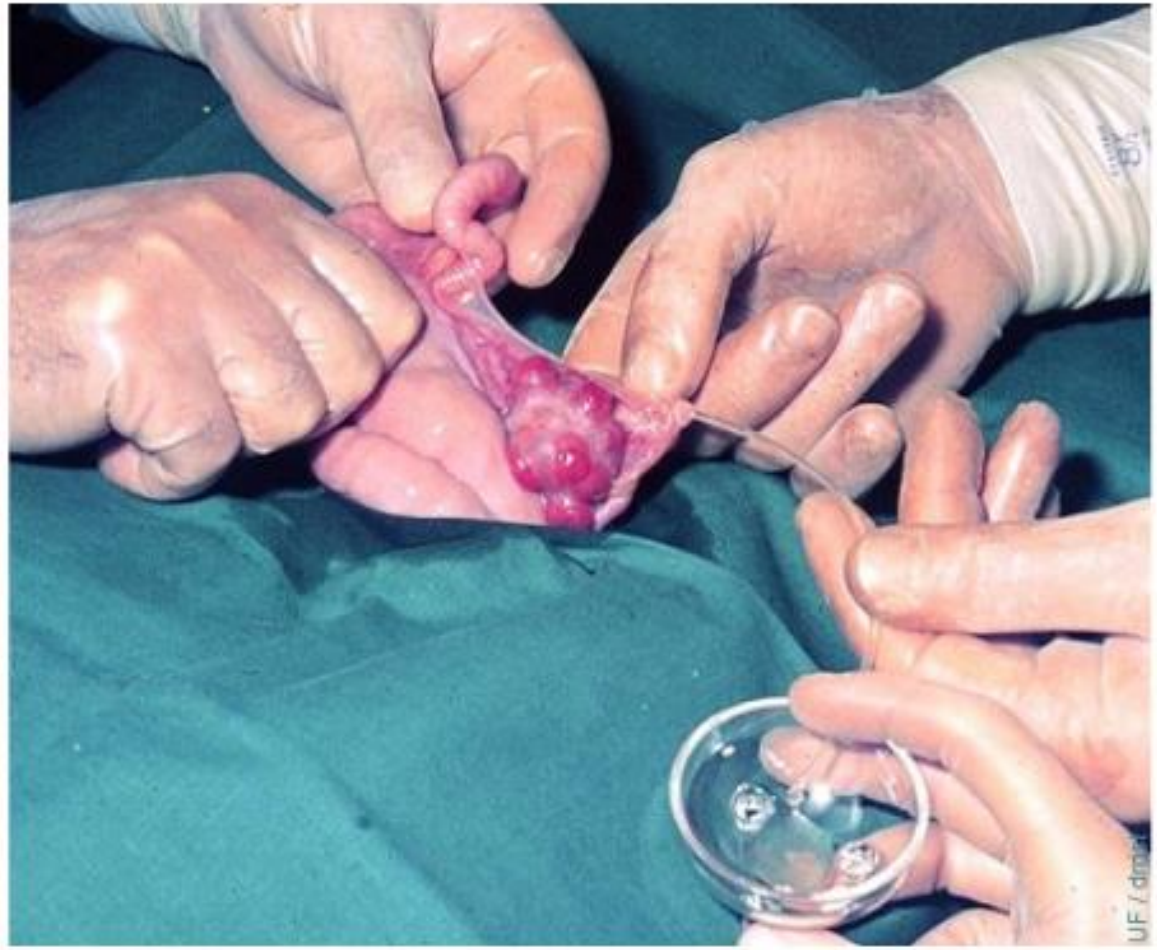
BoviFlush EMBRYO RECOVERY MEDIUM WITH BSA AND ANTIBIOTICS

BoviFreeze, freezing medium for bovine embryos

Donor with Litter of Calves.

Holstein donor cow with six of her calves carried by beef cattle recipients as surrogate dams.

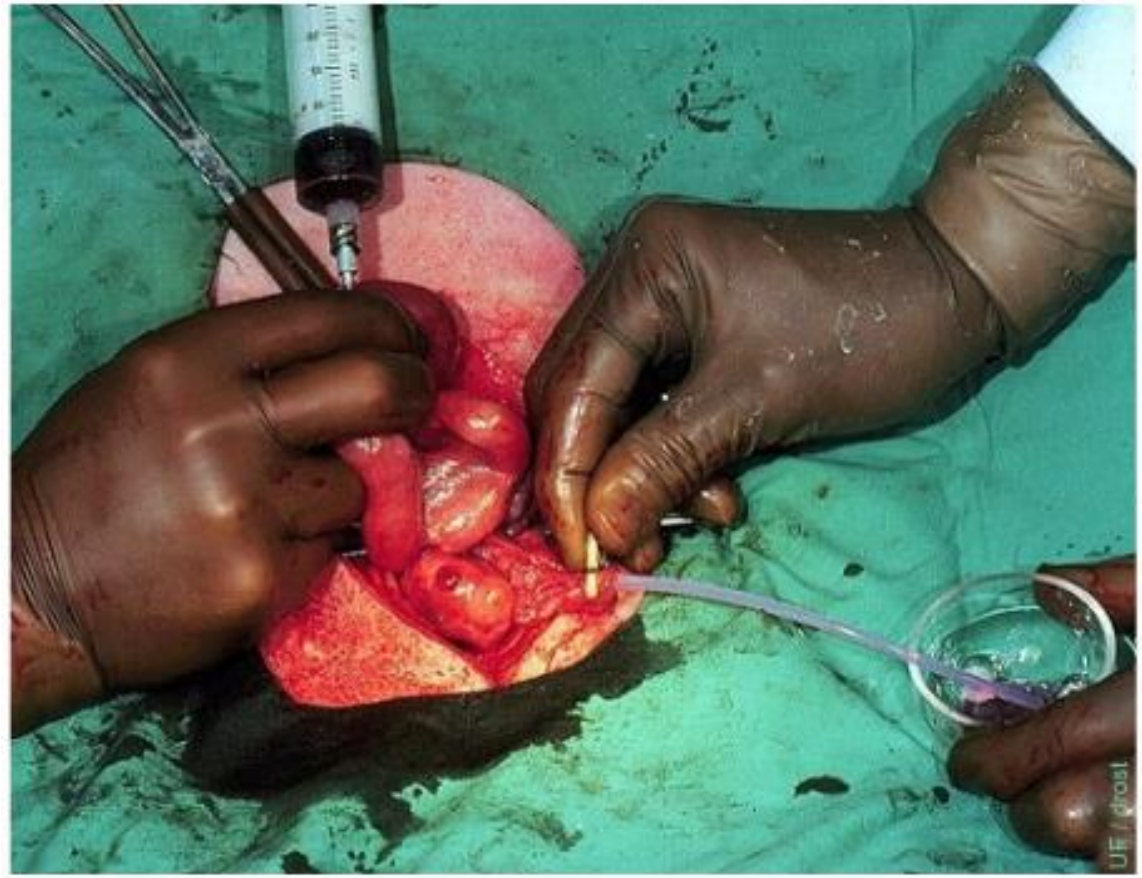
Seidel GE (1984)



Oviductal Flush - Superovulation.

A small diameter Teflon catheter has been inserted into the oviduct and the ipsilateral uterine horn is flushed from its bifurcation, on Day 3 of the cycle of a superovulated donor.

Drost M (1974)



Oviductal Flush - Natural Cycle.

A small diameter Teflon catheter has been inserted into the oviduct and the ipsilateral uterine horn is flushed from its bifurcation on Day 3 of a natural cycle. Note corpus hemorrhagicum.

Drost M (1974)

Loading the Straw.

Loading the embryo from the holding dish into a 0.25 cc straw attached to a tuberculin syringe.

Drost M (1974)

Dairy Cow Recipients.

Commercial dairy cows in the sprinkler pen waiting to be milked. Young dairy cows in good body condition make good recipients and can be kept in the milking herd after delivering the calf.

Drost M (1984)

Nymphomaniac Jersey Cow.

This Jersey cow is a nymphomaniac. She suffers from chronic cystic follicular degeneration and displays a sterility hump, an elevated tailhead, due to relaxation of the pelvic ligaments. Cows with chronic nymphomania do not make good donors, nor recipients.

Nymphomaniac Friesian Cow.

This Friesian cow is a nymphomaniac. She suffers from chronic cystic follicular degeneration and displays a sterility hump, an elevated tailhead, due to relaxation of the pelvic ligaments. Cows with chronic nymphomania do not make good donors, nor recipients.

Utrecht (1976)

Managing Recipients

- Animal identification
- Cycling animals
- Good state of nutrition

Managing Recipients

- Health programs
- Facilities
- Estrus detection program
- Monitoring at parturition

Estrus synchronization

- Synchronization:
- Matching the estrous cycle of a donor and a recipient by the injection of prostaglandin **(PGF2a) to stimulate estrus (heat)**
- **For example, if a 7-day embryo is to be transferred into a recipient, she must be in the seventh day of her estrous cycle**

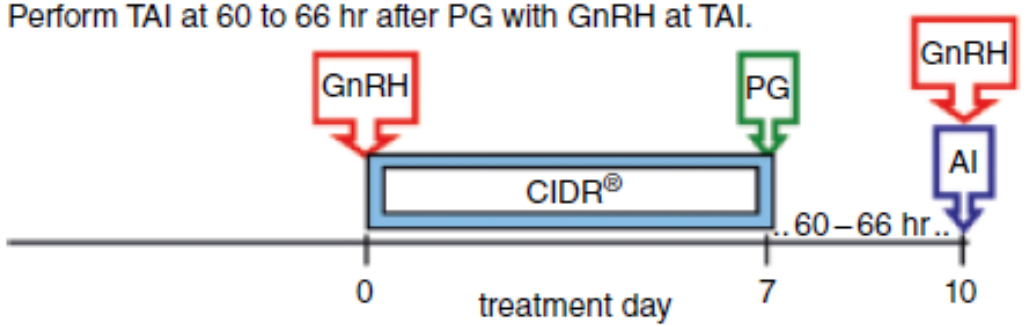
Estrus synchronization

- Recipient must be synchronized with donor or 1 day behind
  - Recipients should ovulate within 12 hours of the donors
- **PGF2a**
  - Two injections 11 days apart
  - Estrus: 3 days after injection

FIXED-TIME AI (TAI)*

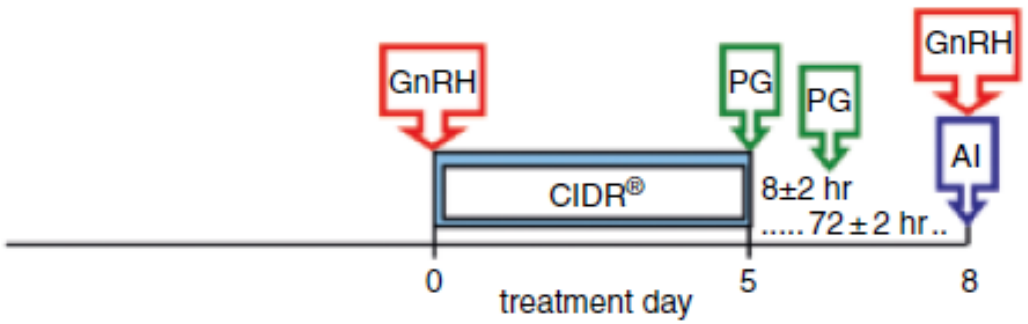
7-day CO-Synch + CIDR®

Perform TAI at 60 to 66 hr after PG with GnRH at TAI.



5-day CO-Synch + CIDR®

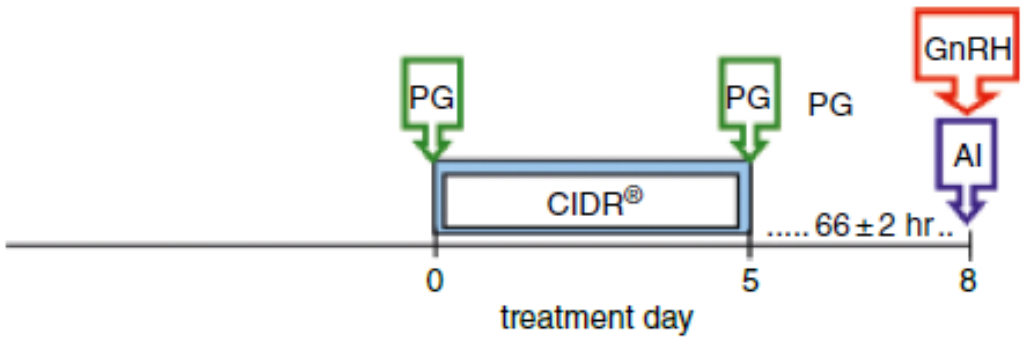
Perform TAI at 72 ± 2 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.



FIXED-TIME AI (TAI)*
for *Bos Indicus* cows only

Bos Indicus PG 5-day + CIDR®

Perform TAI at 66 ± 2 hr after CIDR removal with GnRH at TAI.



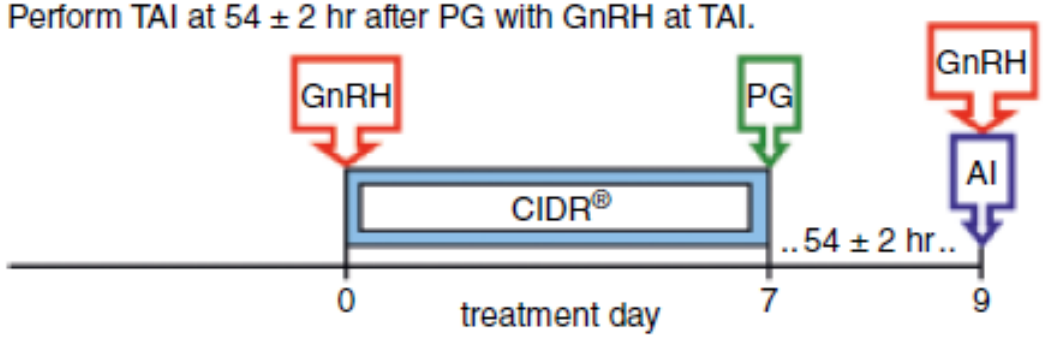
* The time listed for "Fixed-time AI" should be considered as the approximate average time of insemination. This should be based on the number of cows to inseminate, labor, and facilities.

FIXED-TIME AI (TAI)*

Short-term Protocols

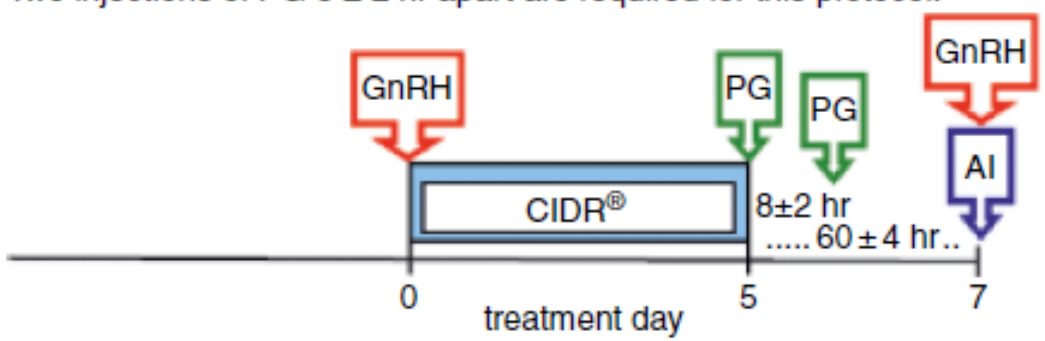
7-day CO-Synch + CIDR®

Perform TAI at 54 ± 2 hr after PG with GnRH at TAI.



5-day CO-Synch + CIDR®

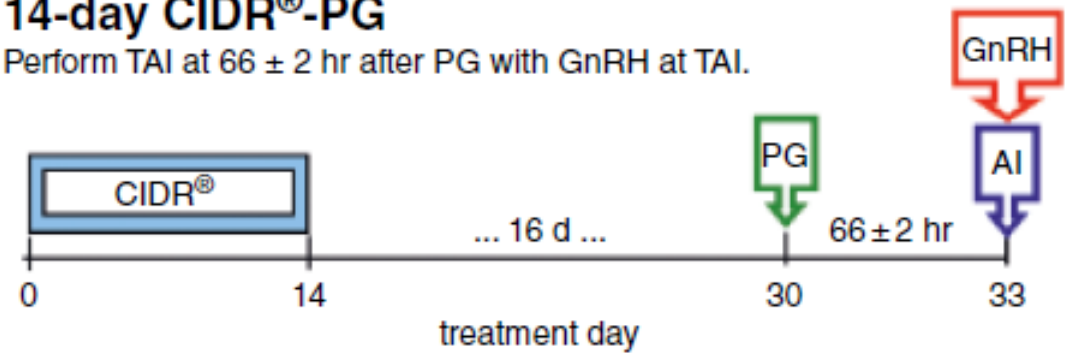
Perform TAI at 60 ± 4 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.



Long-term Protocols

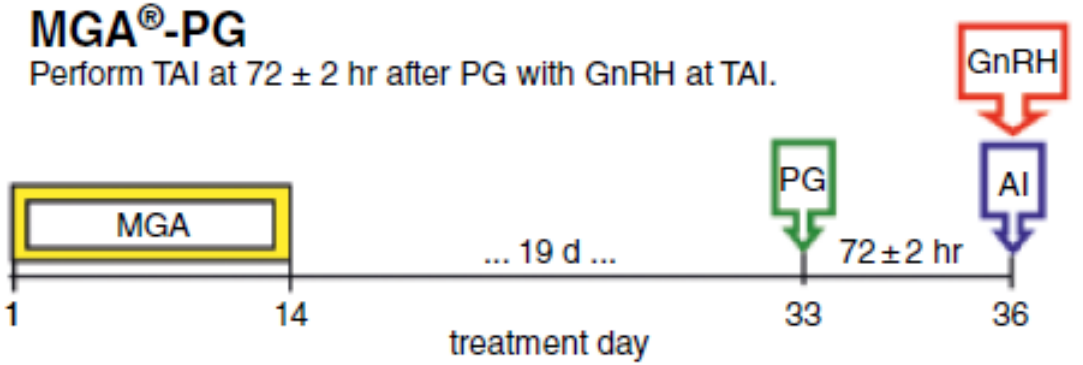
14-day CIDR®-PG

Perform TAI at 66 ± 2 hr after PG with GnRH at TAI.

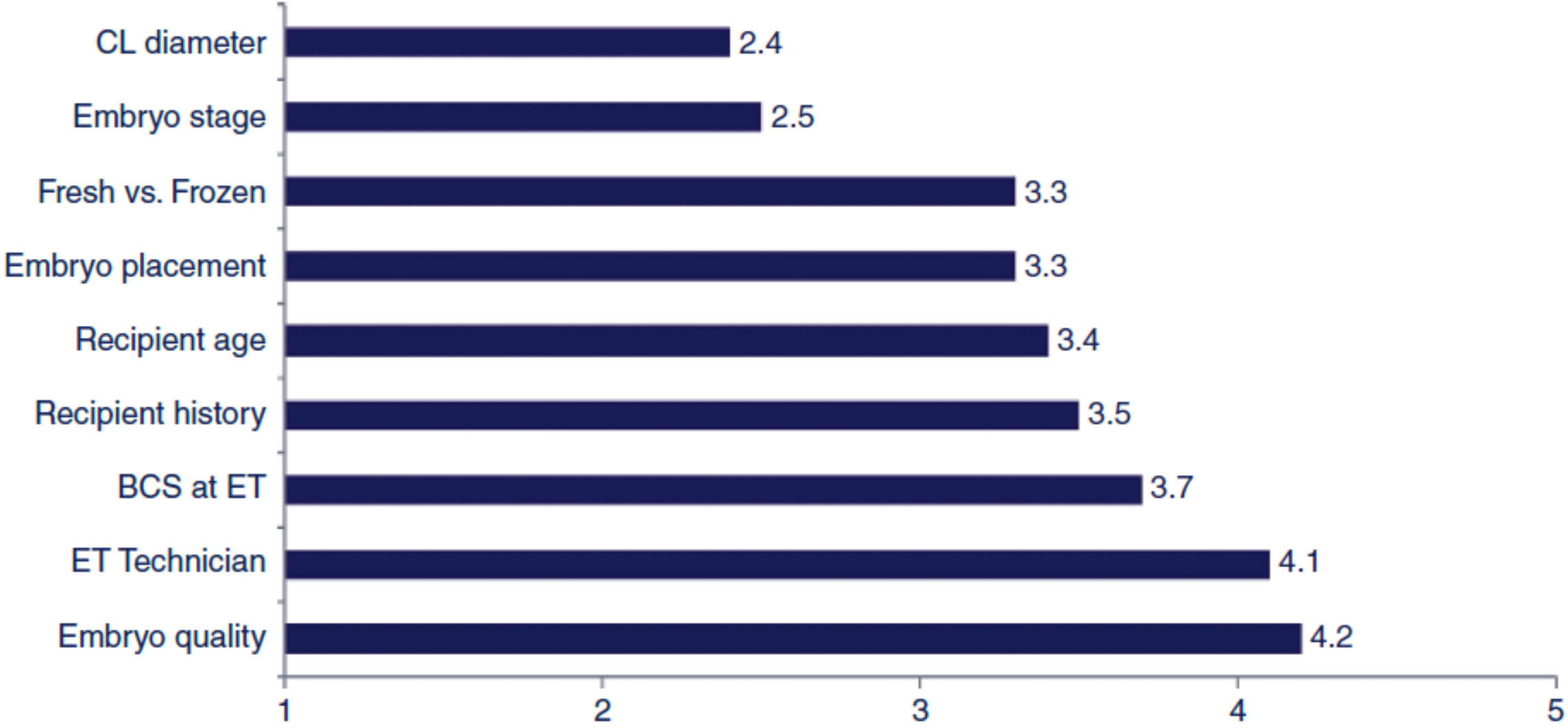


MGA®-PG

Perform TAI at 72 ± 2 hr after PG with GnRH at TAI.



* The time listed for "Fixed-time AI" should be considered as the approximate average time of insemination. This should be based on the number of heifers to inseminate, labor, and facilities.



Perception of the relative impact on fertility of recipients to embryo transfer (1, least impact to 5, greatest impact). BCS, Body condition score; ET, embryo transfer

Superovulation

- Superovulation:
  - The result of treatment of a donor with gonadotropin (especially follicle stimulating hormone, FSH) to produce more than a single ovum

Superovulation

- It is difficult to accurately assess the number of ovulations by palpation of ovarian structures per rectum on the day of embryo recovery when the number of corpora lutea (CL) exceeds 4 to 6 per ovary or when several anovulatory follicles are also present

Superovulation

- An excessive number of anovulatory follicles in the presence of corpora lutea appears to adversely influence the percentage of recovered embryos because of an unfavorable estrogen-to progesterone ratio, which affects gamete and embryo transport through the tubular reproductive tract

Superovulation

- At birth: ovaries contain about 200,000 oocytes
- No new oocytes formed after birth
- Many oocytes degenerate even before puberty (atresia)

Superovulation Treatments with Follicle Stimulating Hormone in the Bovine*

Treatment Day		Treatment 1	Treatment 2
–27		25 mg (5.0 cc) PGF2α[†]	25 mg (5.0 cc) PGF2α
–17		25 mg (5.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
–14		Estrus	Estrus
–4	AM	80 mg (4.0 cc) FSH[‡]	50 mg (2.5 cc) FSH
	PM	80 mg (4.0 cc) FSH	50 mg (2.5 cc) FSH
–3	AM	60 mg (3.0 cc) FSH	50 mg (2.5 cc) FSH
	PM	60 mg (3.0 cc) FSH	50 mg (2.5 cc) FSH
–2	AM	40 mg (2.0 cc) FSH	50 mg (2.5 cc) FSH
		35 mg (7.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
	PM	40 mg (2.0 cc) FSH	50 mg (2.5 cc) FSH
		25 mg (5.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
–1	AM	20 mg (1.0 cc) FSH	50 mg (2.5 cc) FSH
	PM[§]	20 mg (1.0 cc) FSH	50 mg (2.5 cc) FSH
0		Estrus and AI[§]	Estrus and AI[§]
7		Embryo recovery, transfer, and freezing	Embryo recovery, transfer, and freezing

Treatment 1 (decreasing dosage of FSH) as preferred by most ET practitioners,

Treatment 2 (level dosage of FSH) as recommended by the manufacturer

† PGF2α, Prostaglandin F2 α; Lutalyse, Pfizer Animal Health, New York; IM injections.

‡ FSH, Follicle stimulating hormone; Folltropin-V, Bioniche Animal Health USA, Inc., Athens, GA; IM injections.

§ FSH treatment is discontinued when the donor comes into estrus early. If the donor does not come into estrus, FSH treatment may be continued

1 additional day at the dosage level of the last scheduled day.

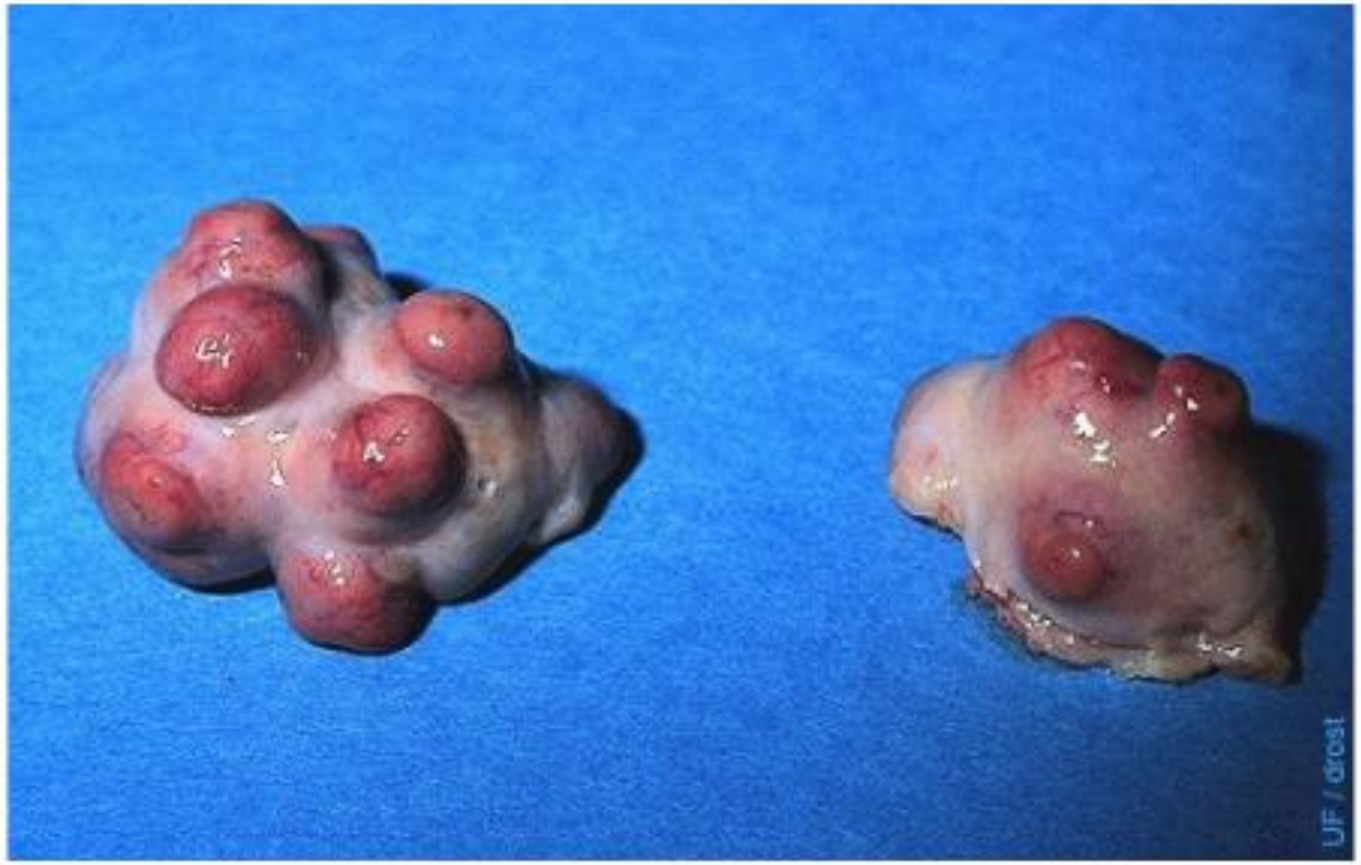
Artificial insemination (AI) of donor at 4 to 6 hours after onset of estrus, repeated

once 10 to 12 hours later.

Superovulation Treatment with Follicle Stimulating Hormone and CIDR without Regard to the Day of the Donor's Estrous Cycle

Treatment Day		Treatment
0		CIDR* inserted vaginally
2	PM	100 µg GnRH†
4	PM	60 mg (3.0 cc) FSH‡
5	AM	60 mg (3.0 cc) FSH
	PM	60 mg (3.0 cc) FSH
6	AM	50 mg (2.5 cc) FSH
	PM	50 mg (2.5 cc) FSH
7	AM	40 mg (2.0 cc) FSH
	PM	40 mg (2.0 cc) FSH, 35 mg (7.0 cc) PGF2α§
8	AM	40 mg (2.0 cc) FSH, 25 mg (5.0 cc) PGF2α CIDR out
9	AM	Estrus and AI
	PM	Estrus and AI
16		Embryo recovery, transfer, and freezing

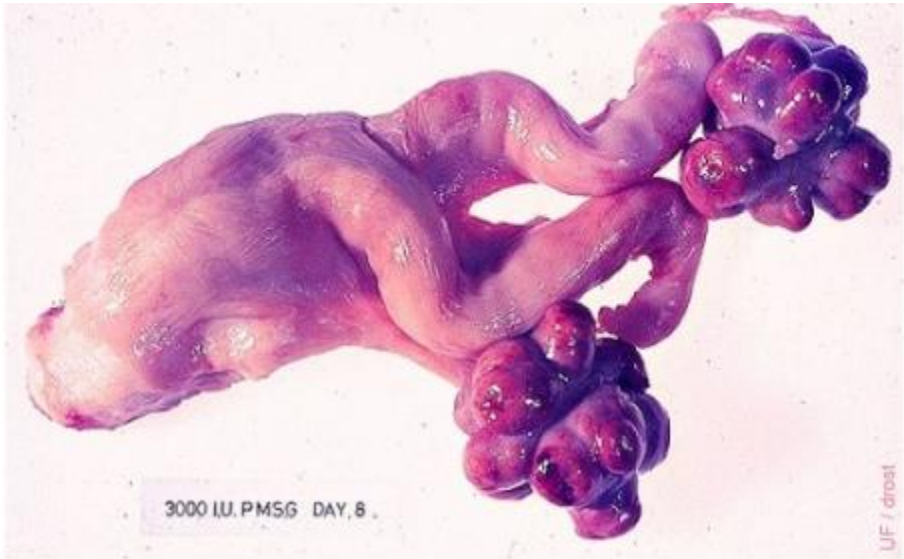
*CIDR, Controlled internal drug release; EAZI-BREED CIDR progesterone insert, InterAg Company, Hamilton, NZ, or Pfizer Animal Health, New York.
†GnRH, Gonadotropin releasing hormone; Cystorelin (Gonadorelin), Merial Limited, Iselin, NJ; IM injection.
‡FSH, Follicle stimulating hormone; Folltropin-V, Bioniche Animal Health USA, Inc., Athens, GA; IM injections.
§PGF2α, Prostaglandin F2α; Lutalyse, Pfizer Animal Health, New York; IM injections.



Superovulated Ovaries (FSH).

Nice superovulatory response: 7 corpora lutea on the left ovary and (only) 1 anovulatory follicle; 4 or 5 corpora lutea on the right ovary. Day 7, routine time for collection.

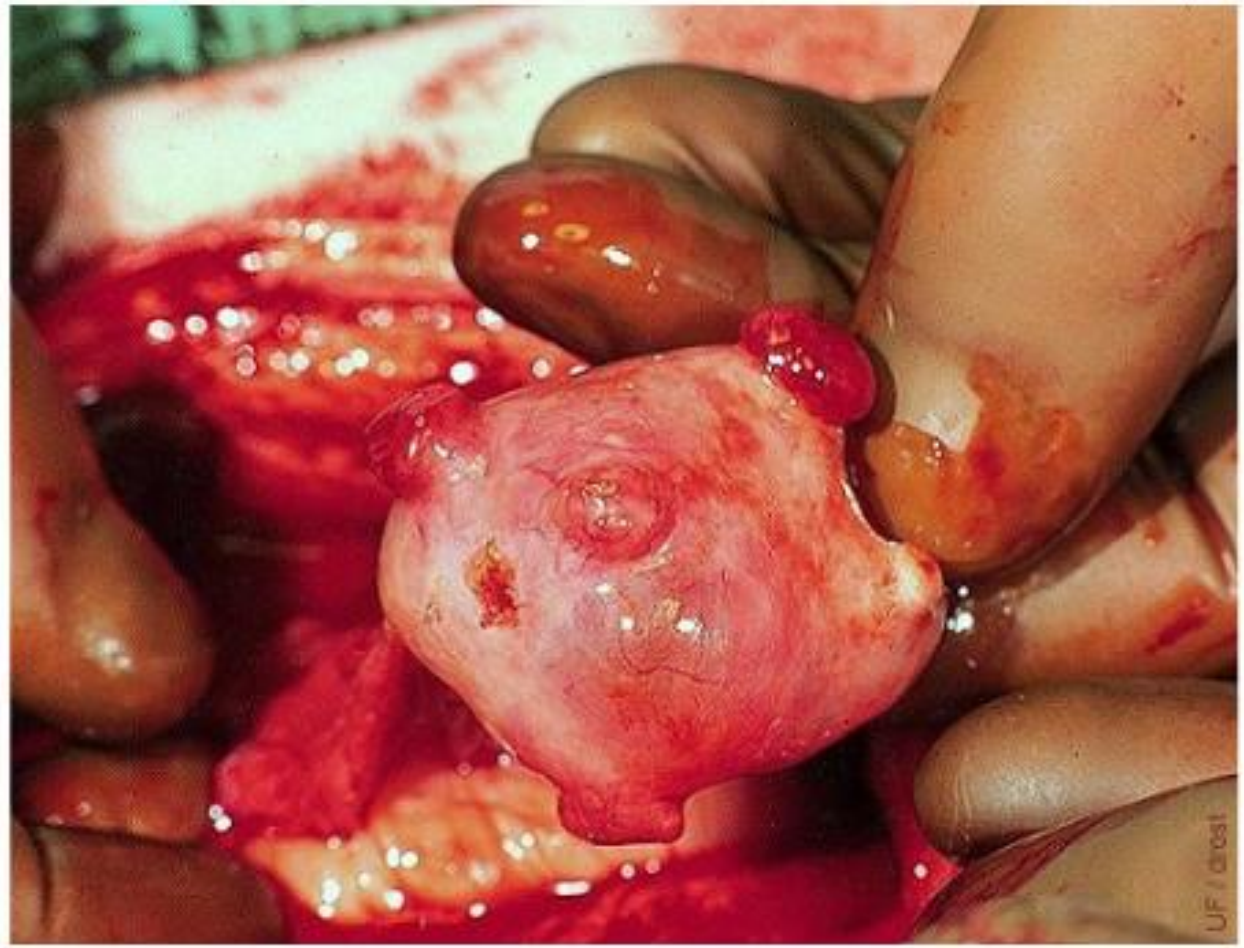
Drost M (1976)



Superovulated Ovaries (eCG).

Excellent response to 3000 IU of eCG (formerly PMSG). The left ovary shows only one small anovulatory follicle. Heifer tract. eCG: equine Chorionic Gonadotropin PMSG: Pregnant Mare Serum Gonadotropin

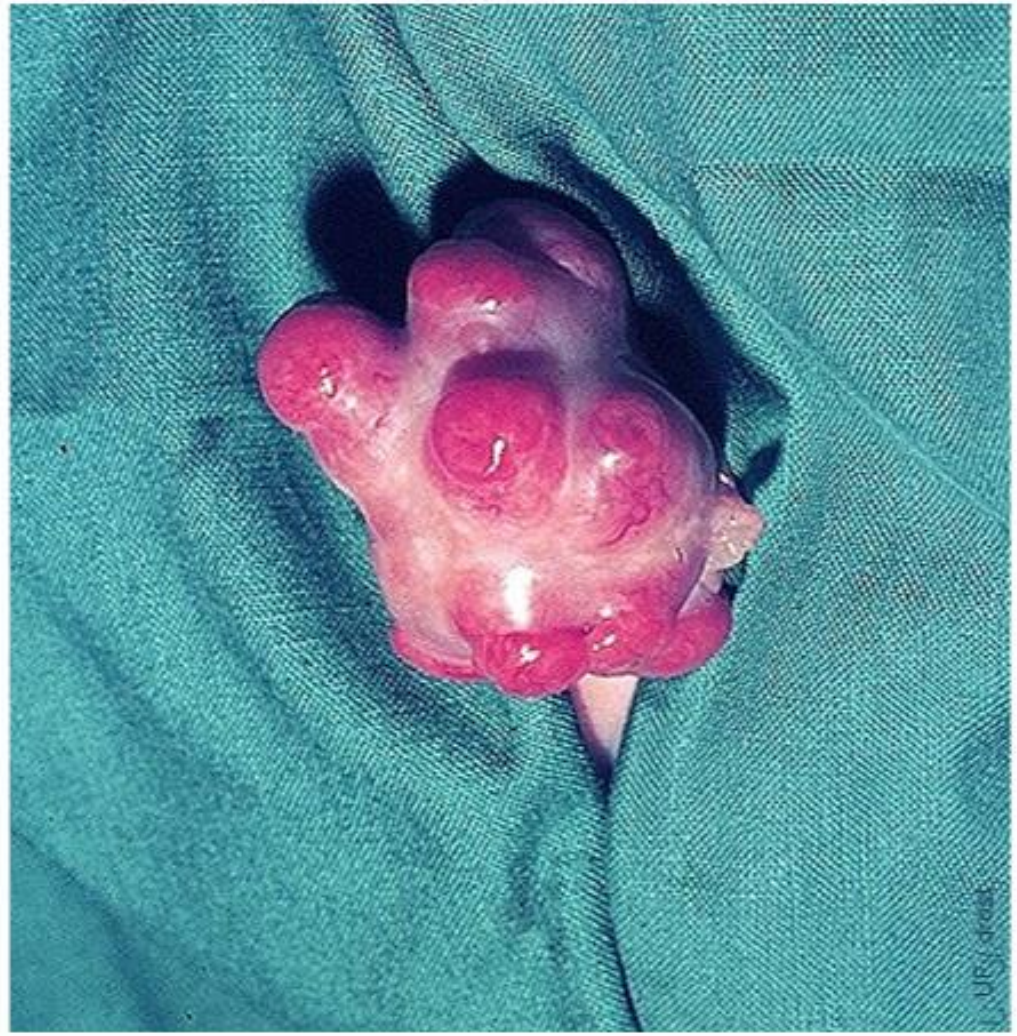
Drost M (1976)



Superovulated Ovary on Day 3.

Three corpora hemorrhagica are readily visible. Also a corpus albicans at the lower tip of the thumb.

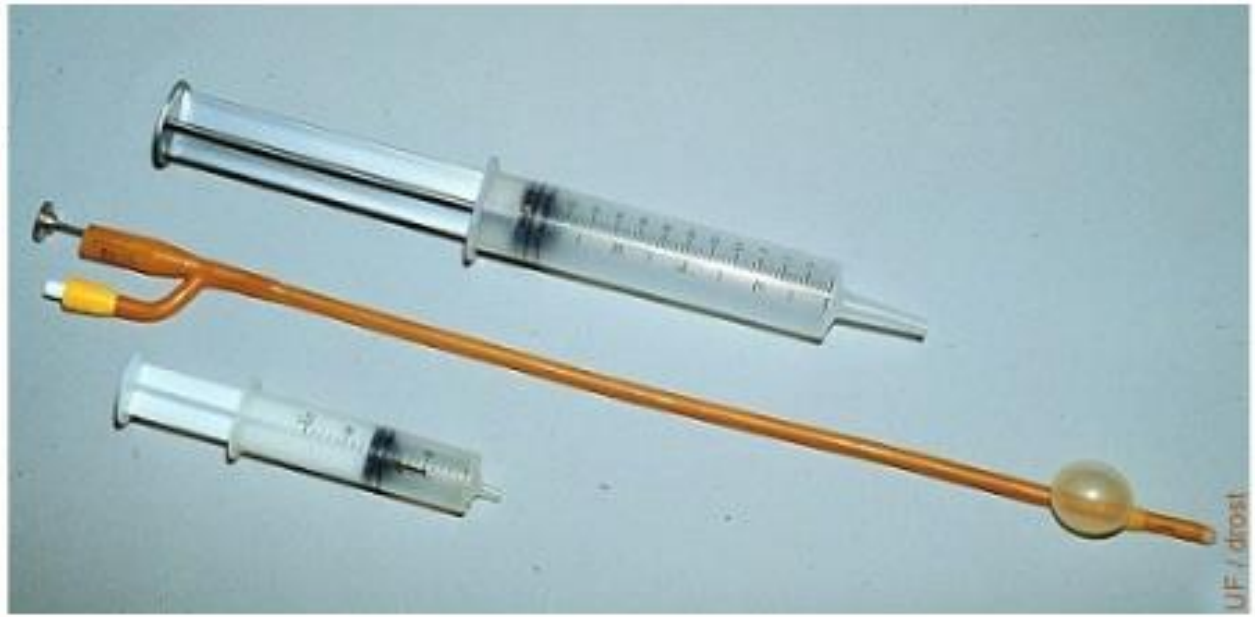
Drost M (1973)



Superovulated Ovary on Day 7.

Day 7 superovulated ovary.

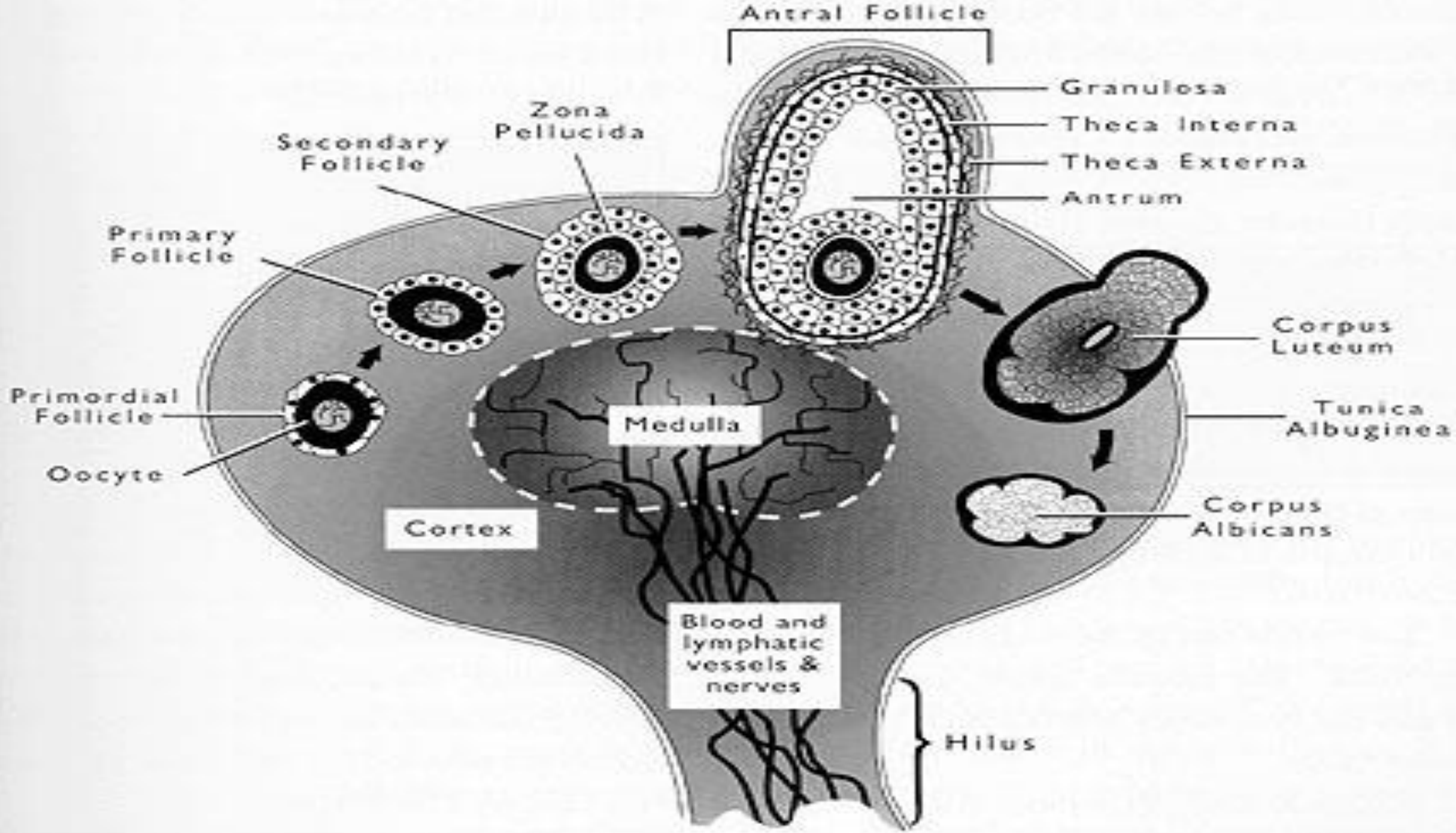
Drost M (1973)



Foley Catheter.

Foley catheters are human urinary balloon catheters. They are 45 cm long and the diameter varies from 12 French gauge (1 French gauge = 0.3 mm) to 24 French gauge. A 20 French gauge is most commonly used in cows, while in heifer a 16 French gauge catheter is is used. The balloon is distended with air or with flushing medium; approximately 15 cc in cows, approximately 12 cc in heifers.

Drost M (1976


Antral Follicle
Granulosa
Theca Interna
Theca Externa
Antrum
Zona Pellucida
Secondary Follicle
Primary Follicle
Primordial Follicle
Oocyte
Medulla
Cortex
Corpus Luteum
Tunica Albuginea
Corpus Albicans
Blood and lymphatic vessels & nerves
Hilus

Superovulation

- Circumvents this natural phenomenon
- Injection of extra FSH
  - Start treatment on any days 9-14 of estrous cycle
  - Inhibin shuts down pituitary FSH
  - Injected FSH is still effective

Superovulation

D1	AM	6 mg	FSH
	PM	6 mg	FSH
D2	AM	4 mg	FSH
	PM	4 mg	FSH
D3	AM	2 mg	FSH
	PM	2 mg	FSH+PGF
D4	AM	2 mg	FSH+PGF
	PM	2 mg	FSH

Superovulation

- 30 % superovulated cows
  - Average 6 usable embryos
- 20 – 30 %
  - No embryos
- 20 – 30 %
  - 3 embryos

Superovulation

- A typical response in cattle would be **8 to I 0 ovulations**, producing **5 to 7 viable embryos**
- But, about **30 % of the cows respond by producing one or fewer viable embryos**
- About **2 % of the cows may produce as many as 30 embryos or more**

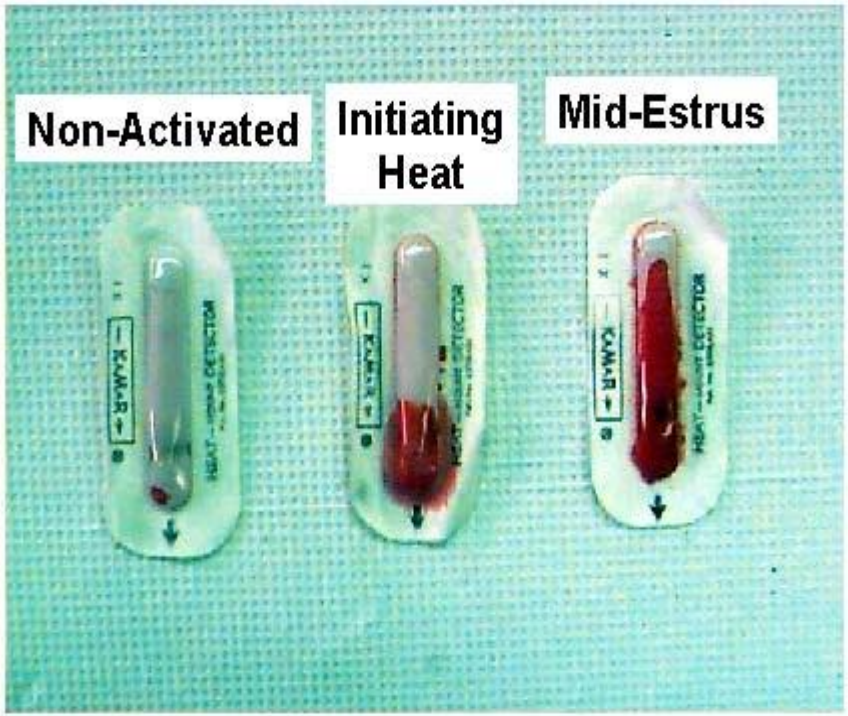
Superovulation

- The physiologic reasons for this wide variation in ovarian response to hyperstimulation are not known

Insemination

- Donor cow should be observed for signs of estrus
  - Estrus detection devices: KAMAR
- 10 % of donors don't show estrus
  - Do not inseminate

KAMAR
Heat - Mount Detector



Insemination

- Accurate estrus detection is of great importance not only for timely insemination of the donor, but also for the determination of the degree of synchronization of estrus and ovulation between the donor and her recipients
- **The age of the embryo is calculated from the time of onset of standing heat**

Insemination

- **Donors should be artificially inseminated twice with a 10- to 12-hour interval, beginning 4 to 6 hours after the onset of estrus, to cover the range of time over which the ovulations may occur.**
- Depending on the quality of the frozen semen, a double inseminating dose may be used at each insemination.

Insemination

- Fresh semen:
  - 10-50 million motile spermatozoa
  - 12 and 24 hrs after onset of estrus

Insemination

- Frozen semen
  - 1-2 doses (straws) 12 and 24 hrs after estrus
- If one insemination is to be done
  - 24 hrs after estrus

Insemination

- Recommendations
  - Semen of highest quality
  - Excellent semen handling techniques
  - Hygienic techniques
  - Minimize reproductive tract manipulation

Recovery of Embryos

- Lidocaine : 3 – 5 ml epidural is administered using an 18-gauge × 3.75-cm needle
- Immediately after the epidural, palpate each ovary and record an estimate as to the number of corpora lutea that are on each
- Unovulated follicles are identified as well as the size of ovaries if they are unusual.
- This information is helpful once the results are in, whether good or bad

Recovery of Embryos

- Avoid using disinfectants around the collection materials
- Simply wipe the perineal region after the tail is tied off to the side
- In the absence of water, wipe the perineal area, clean with a disposable towel
- Once prepared, the cow is ready for collection

Recovery of Embryos

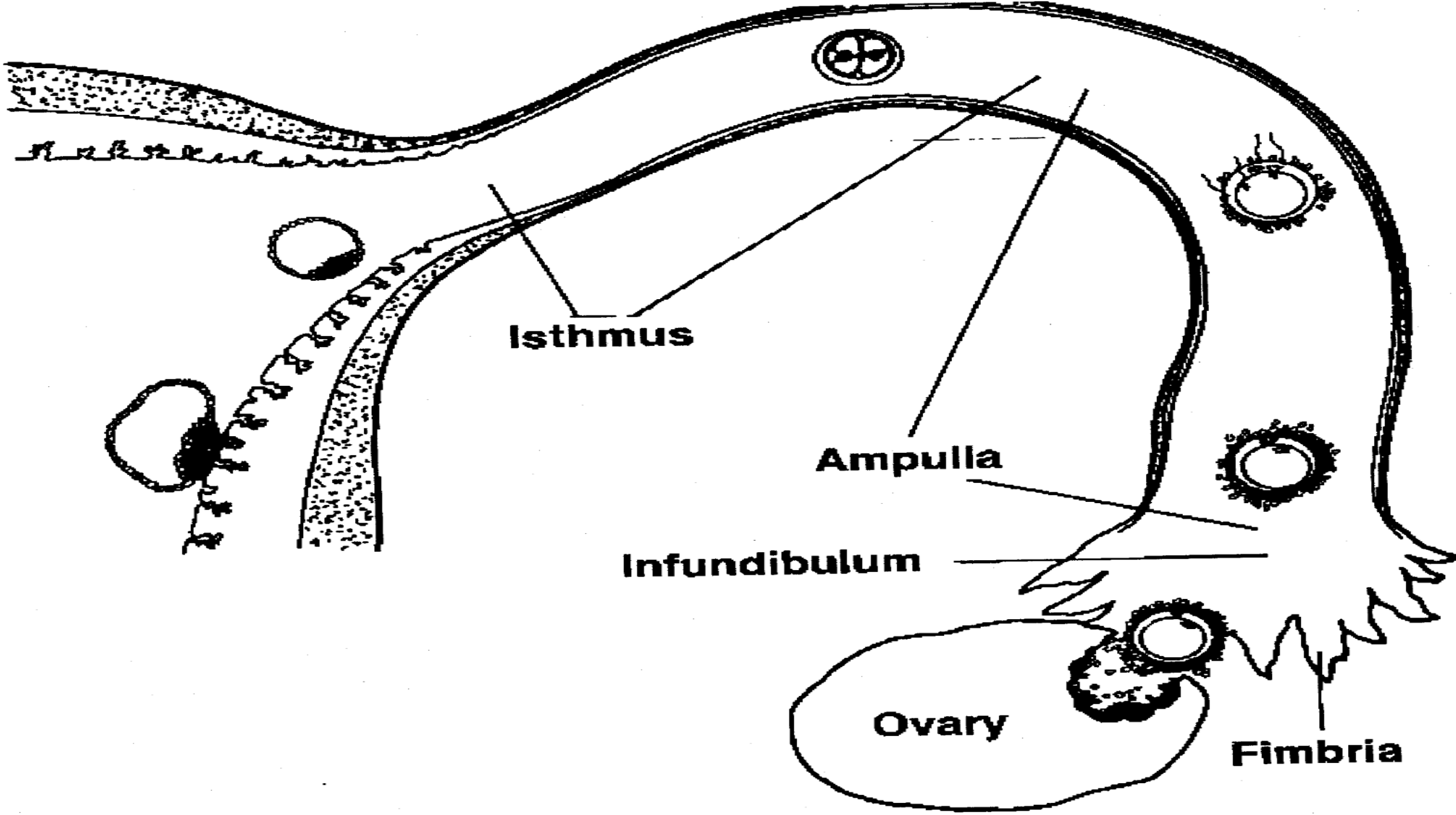
- Non-surgical (transcervical)
  - 6-8 days after estrus (day 0)
  - before day 6
    - Lower recovery rate

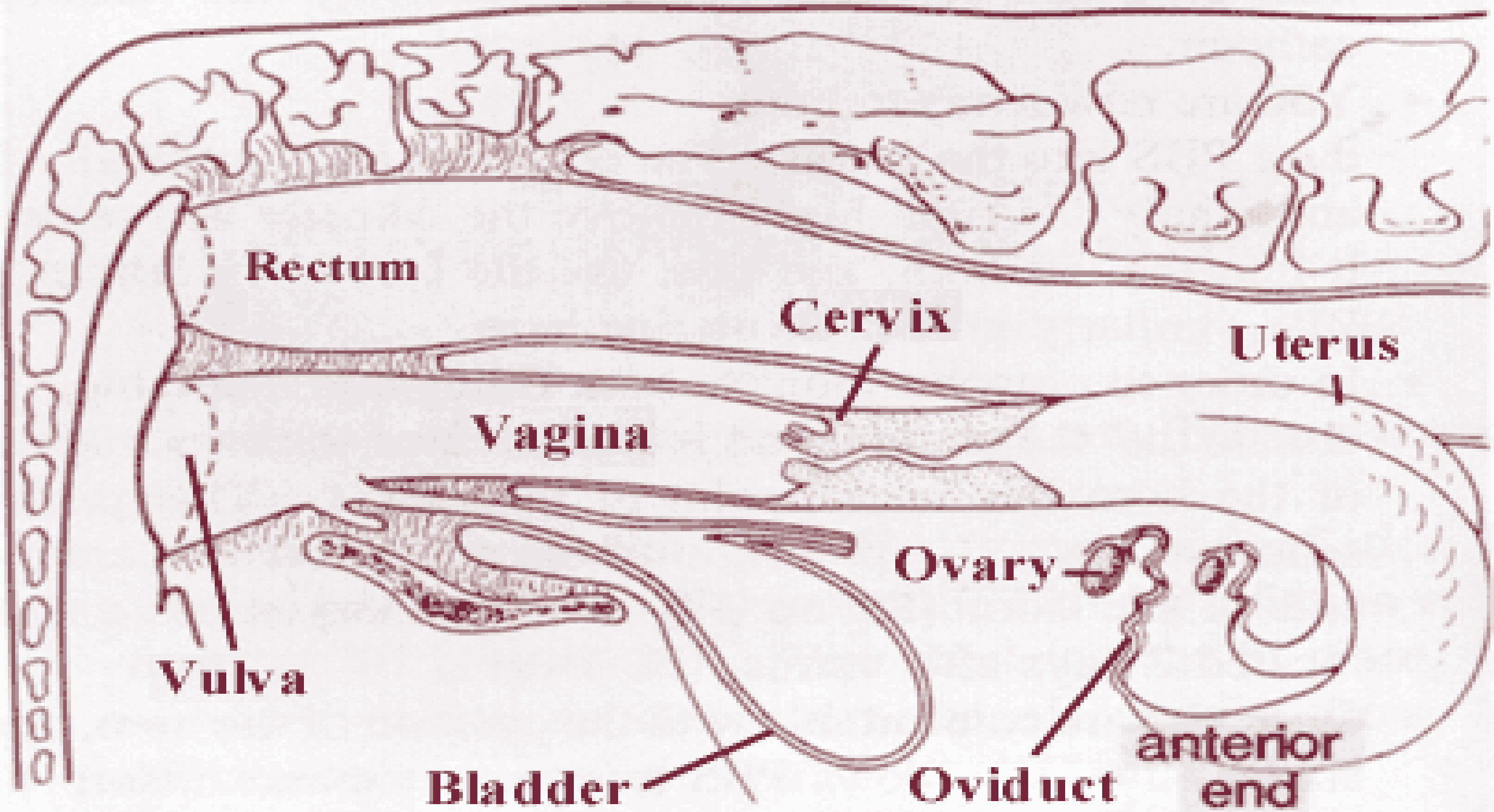
Recovery of Embryos

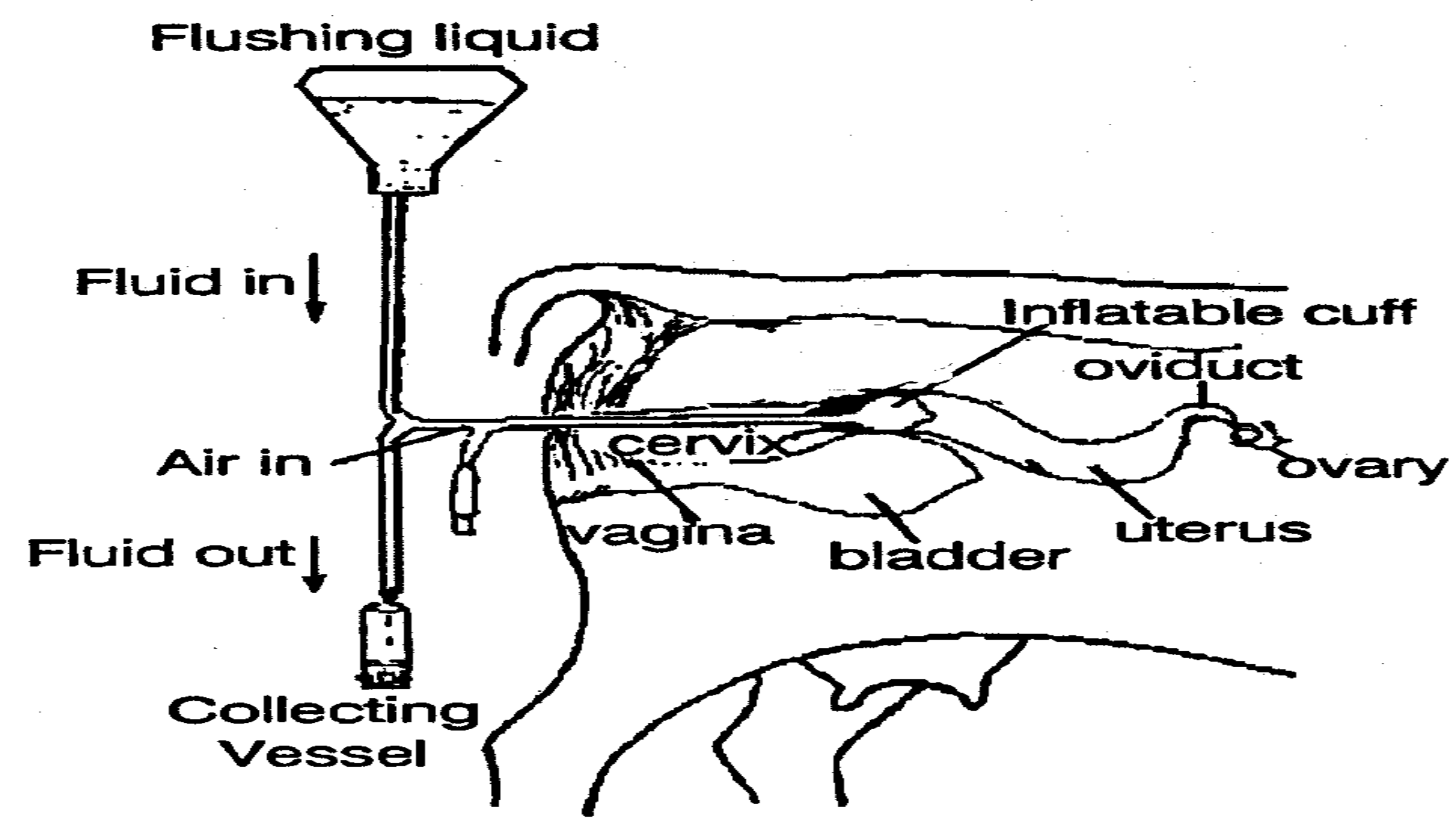
- After day 8
  - Hatched embryos
  - Difficult to recognize
  - Options for freezing and splitting don't work well

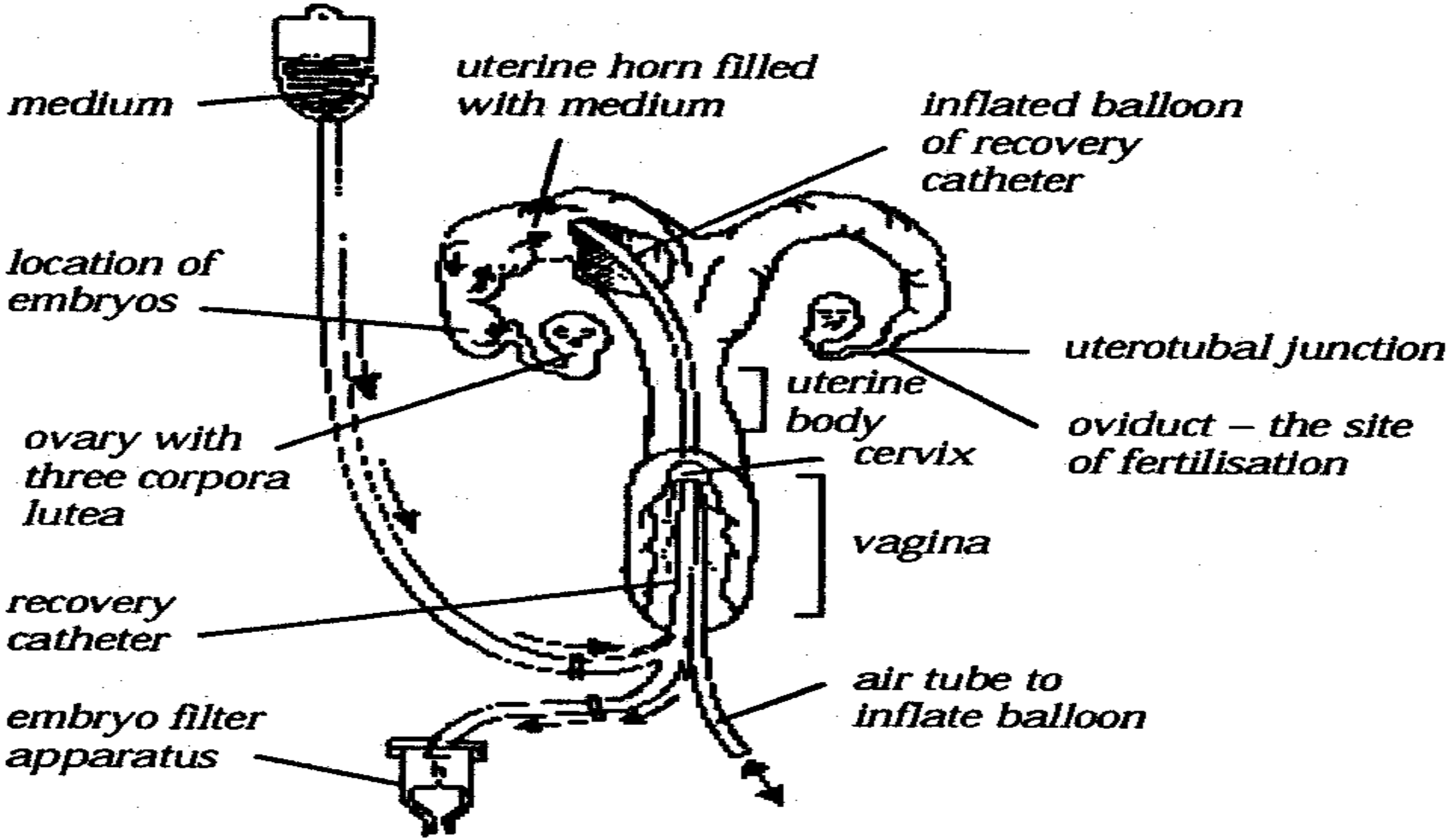
Collection Media

- Ready-to-go medium from Vetoquinol : Vigro Complete Flush™, containing a surfactant (polyvinyl alcohol) and antibiotics (gentamicin and kanamycin)
- Lactated Ringers with 0.1% bovine serum albumin as a surfactant

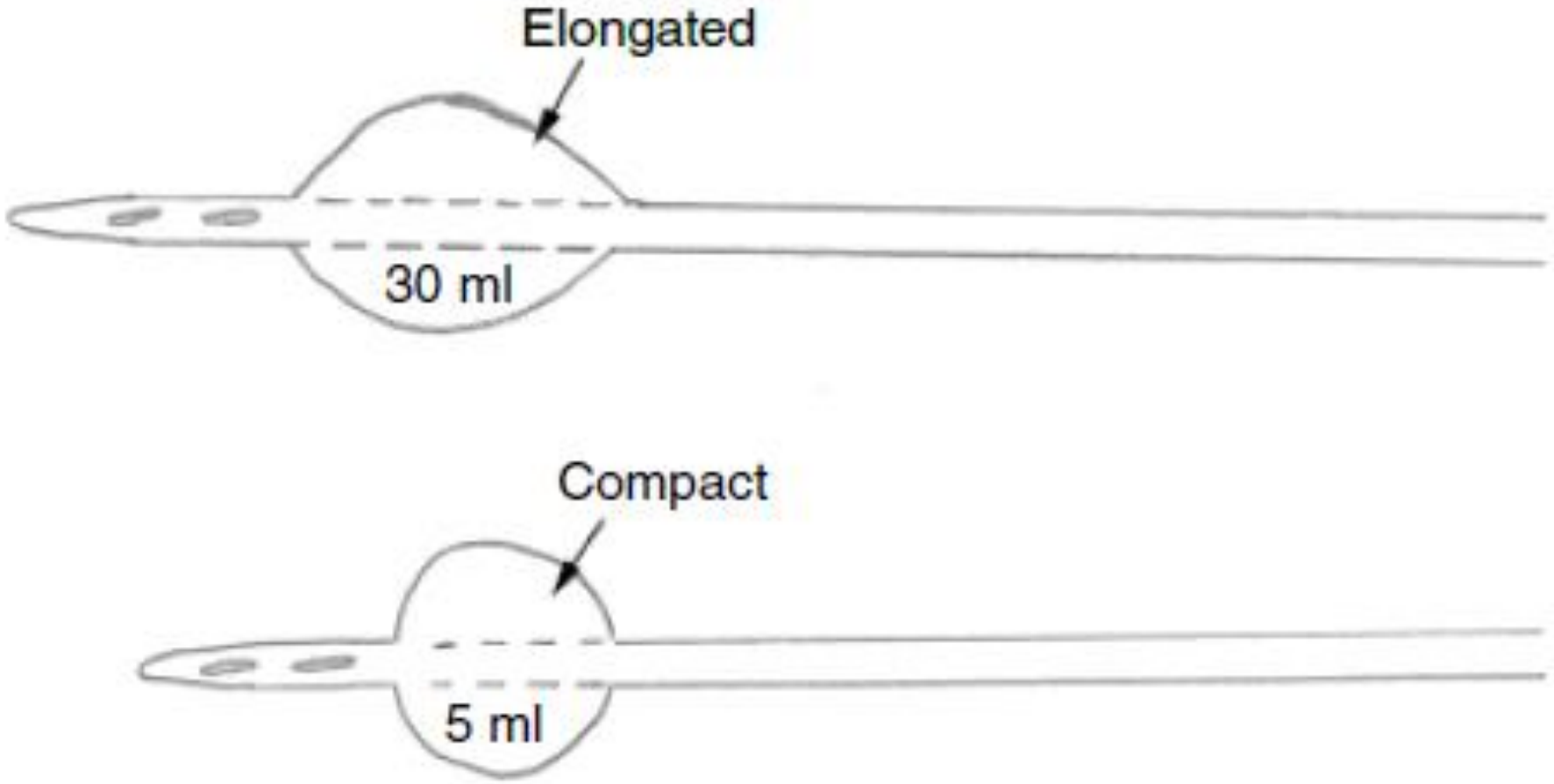

Isthmus
Ampulla
Infundibulum
Ovary
Fimbria


Rectum
Cervix
Uterus
Vagina
Ovary
Vulva
Bladder
Oviduct
anterior
end


Flushing liquid
Fluid in
Air in
Fluid out
Collecting Vessel
Inflatable cuff
oviduct
ovary
cervix
vagina
bladder
uterus

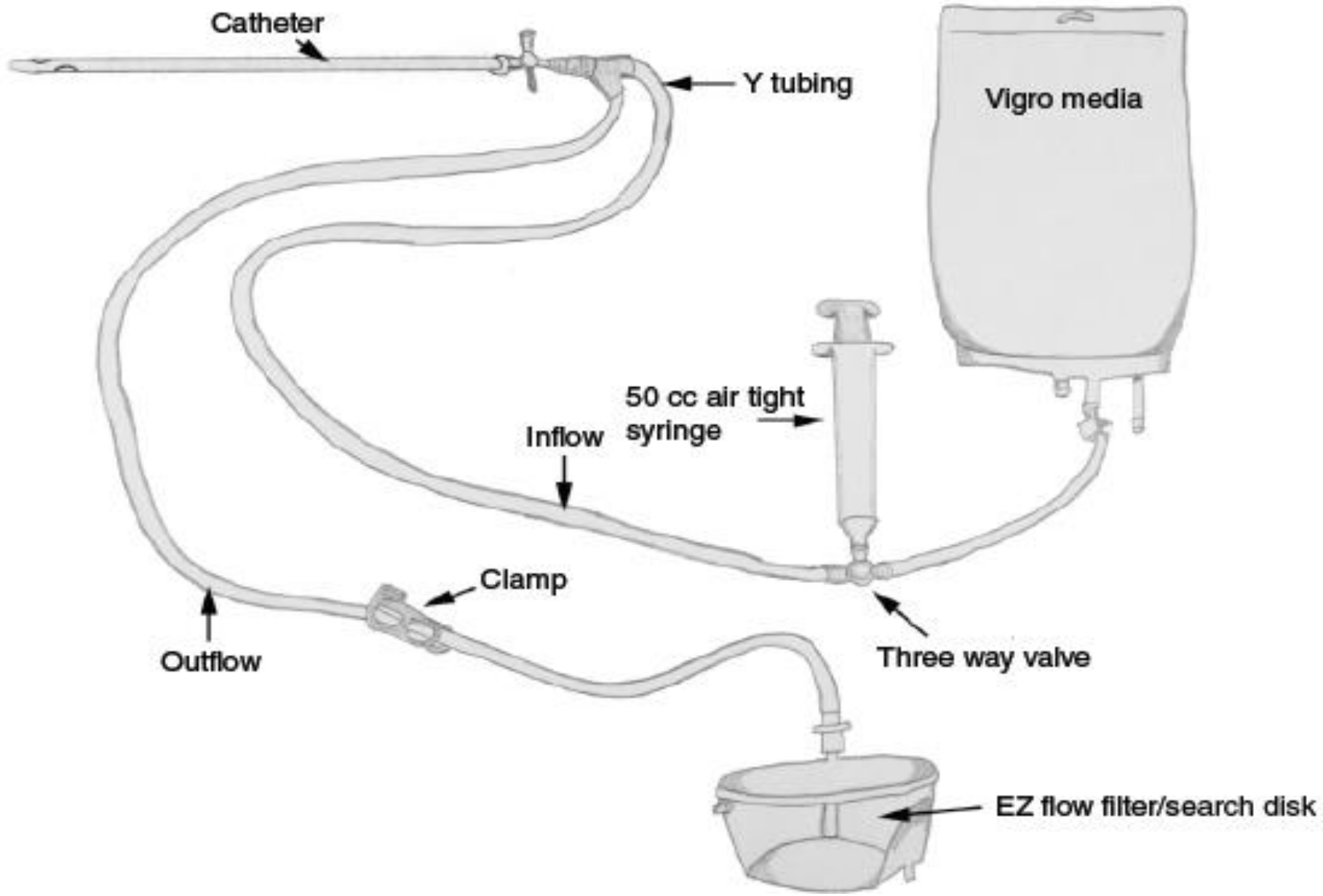


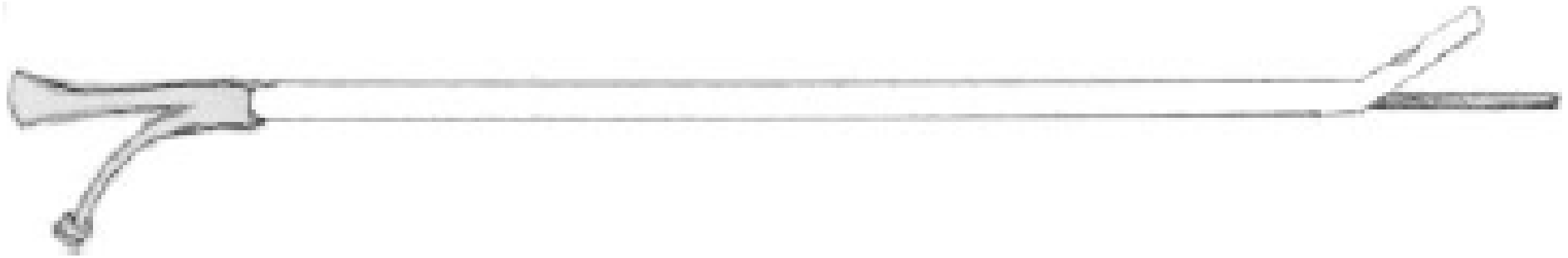
Non-surgical recovery of embryos using a gravity feed system



Shapes of Foley catheter balloon when inflated

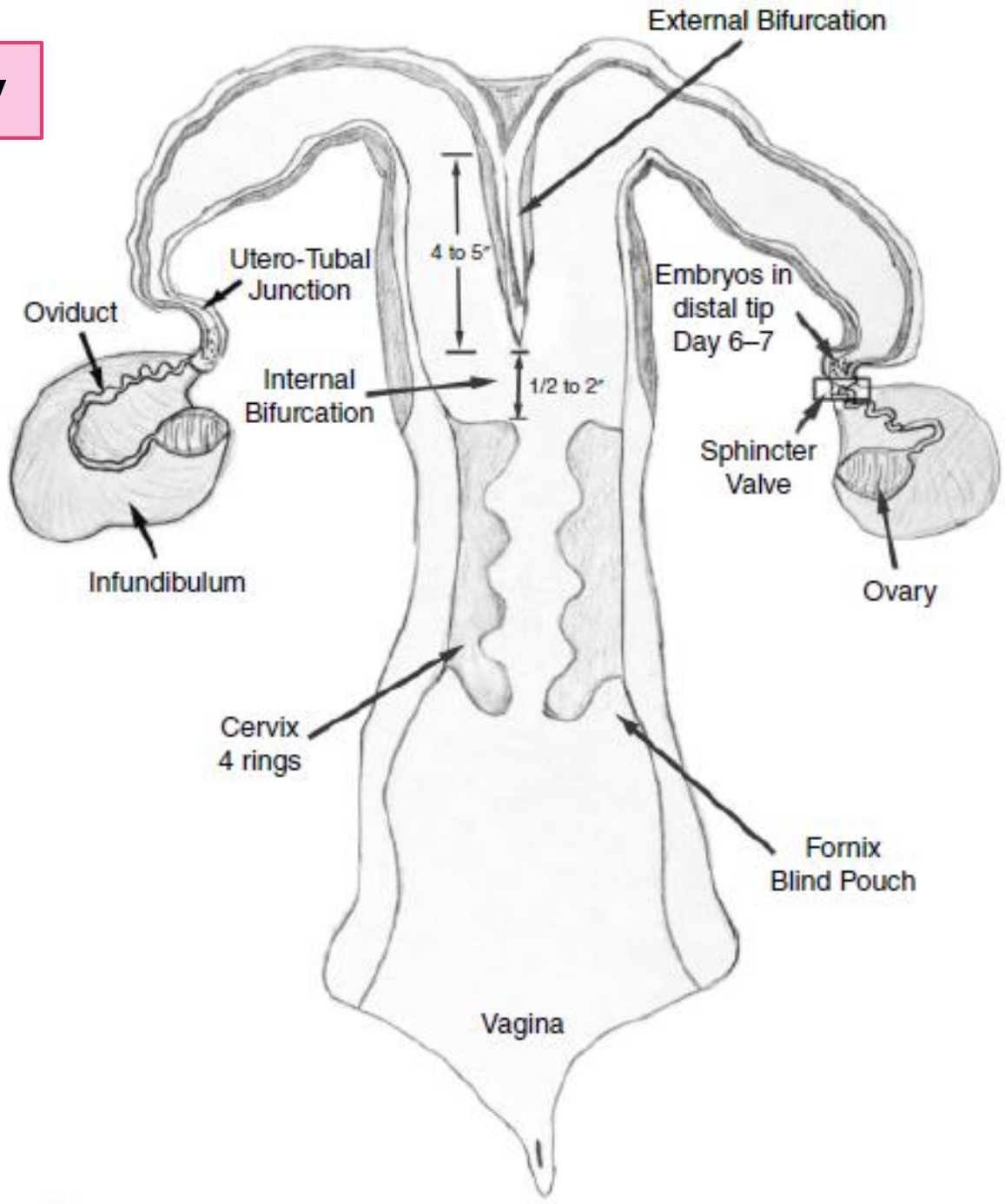
Schematic of flush equipment set-up



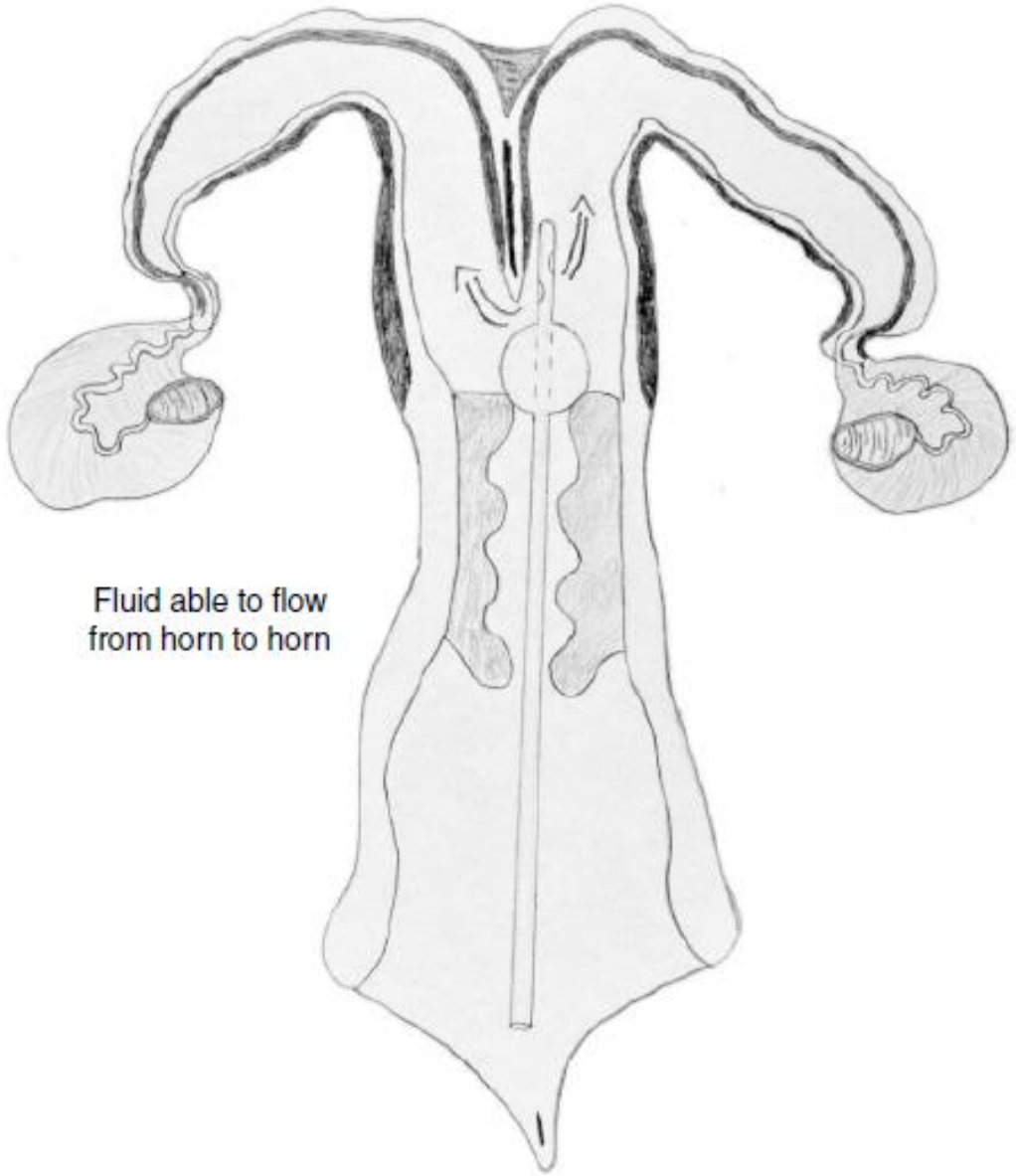


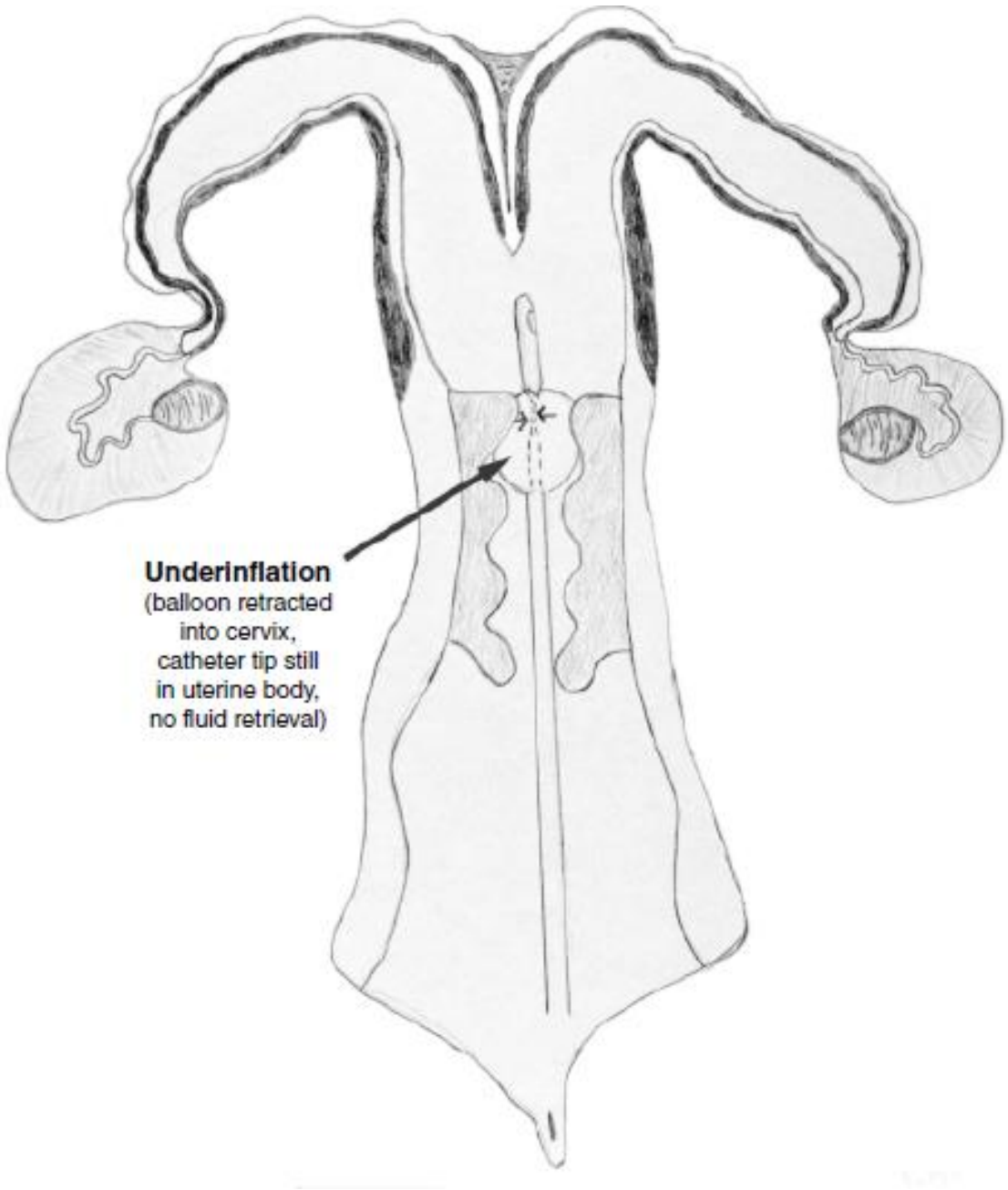
Stylet protruding through catheter

Normal anatomy

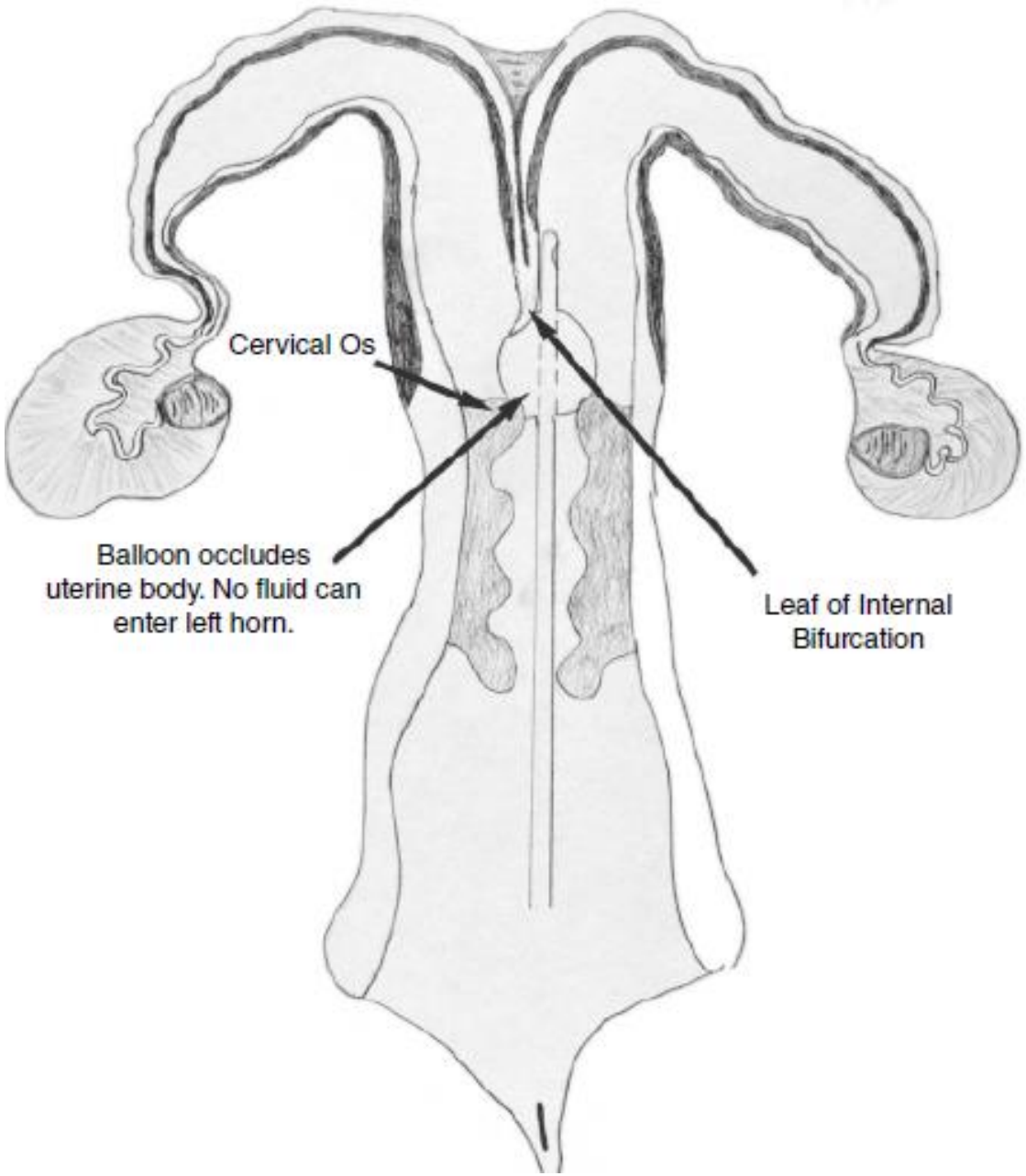


Correct catheter-balloon placement allows flush media to flow freely throughout both horns



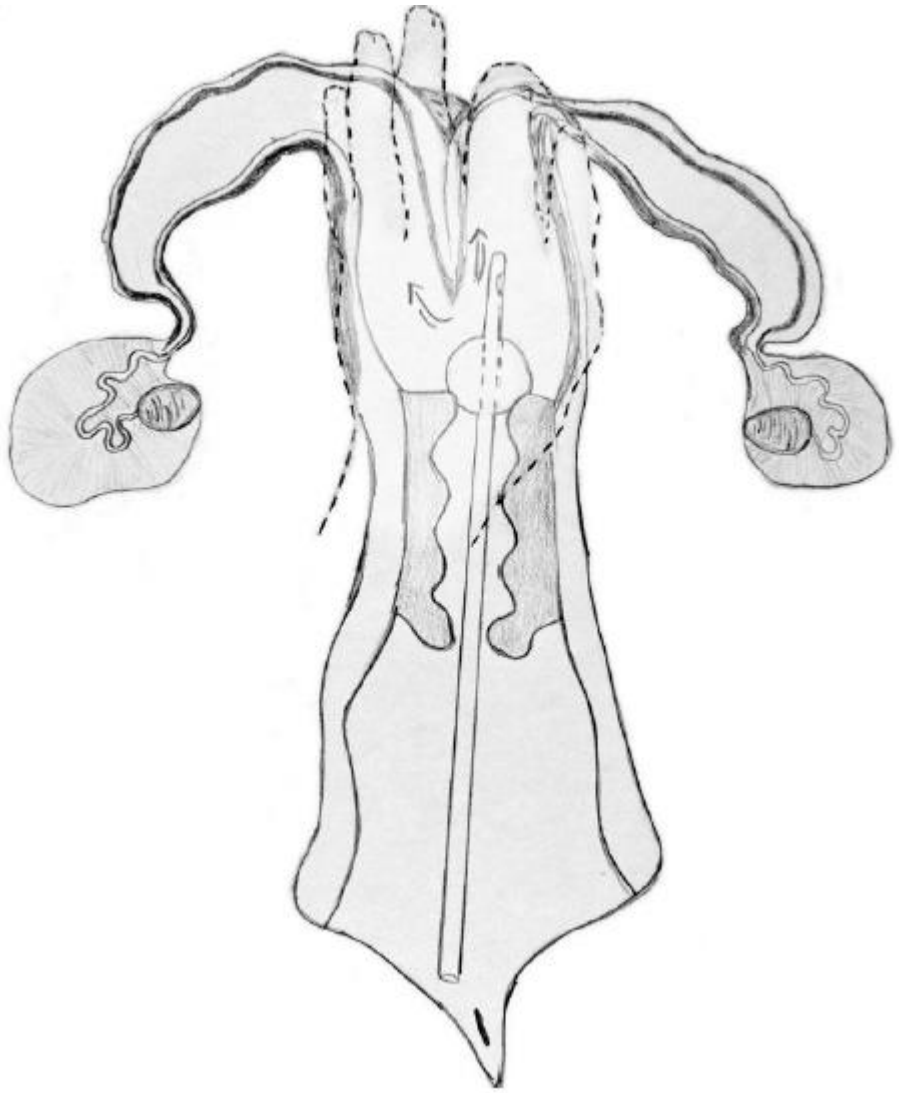


Underinflation.



Overinflation and the occlusion of one horn

One horn can be held off to better
fill the opposite horn



Embryo Flushing Equipment (and Supplier)

- Catheter: 52 cm silicone, 16 French with 5-ml cuff
- Stylet: 60 cm stainless steel
- Y tubing: 150 cm plastic tubing, one end with syringe tip and the other attaches to EZ Way filter

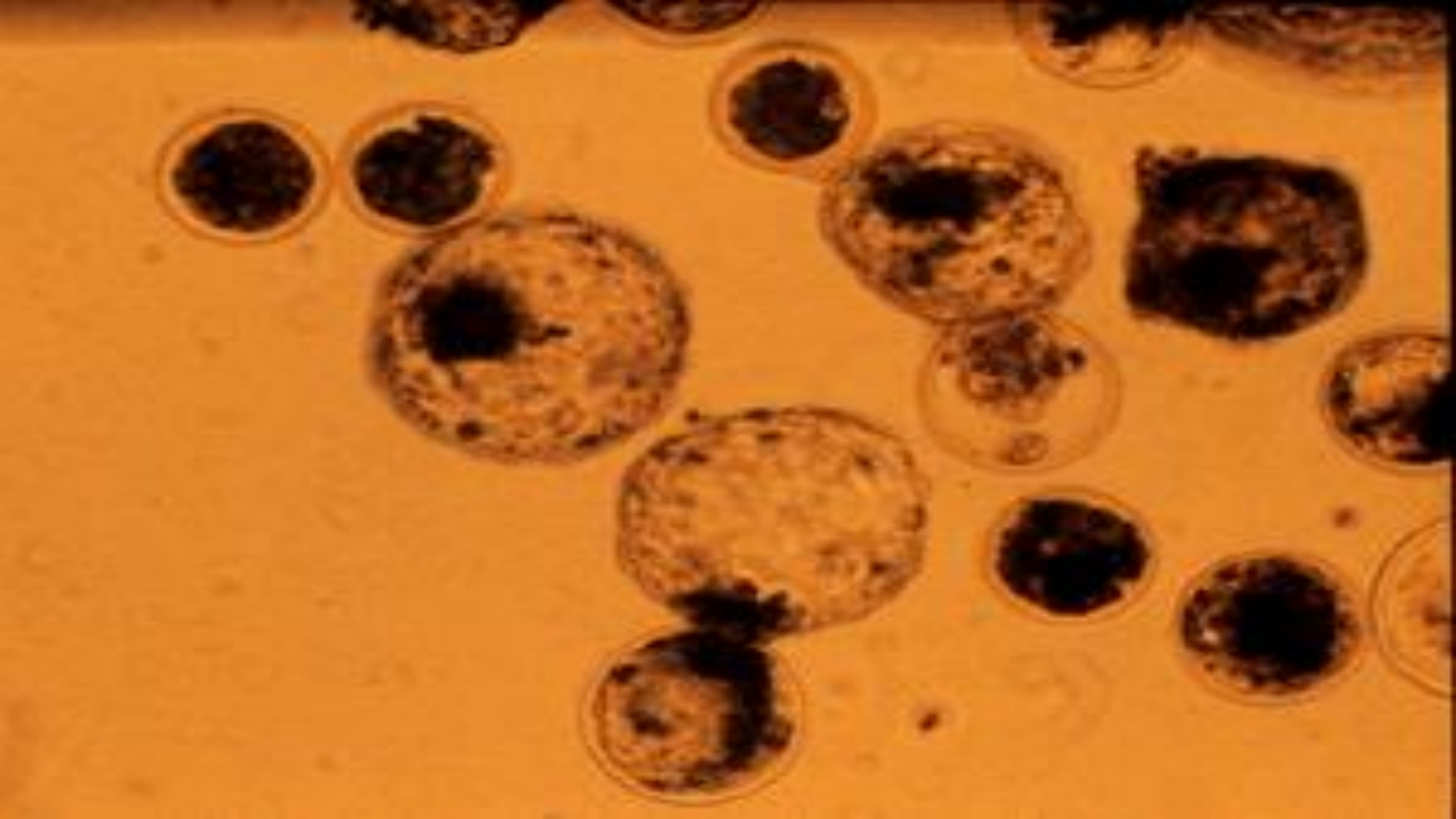
Embryo Flushing Equipment (and Supplier)

- Disposable three-way plastic valve
- 50- or 60-ml airtight syringe
- EZ Way Embryo Filter

Embryo Flushing Equipment (and Supplier)

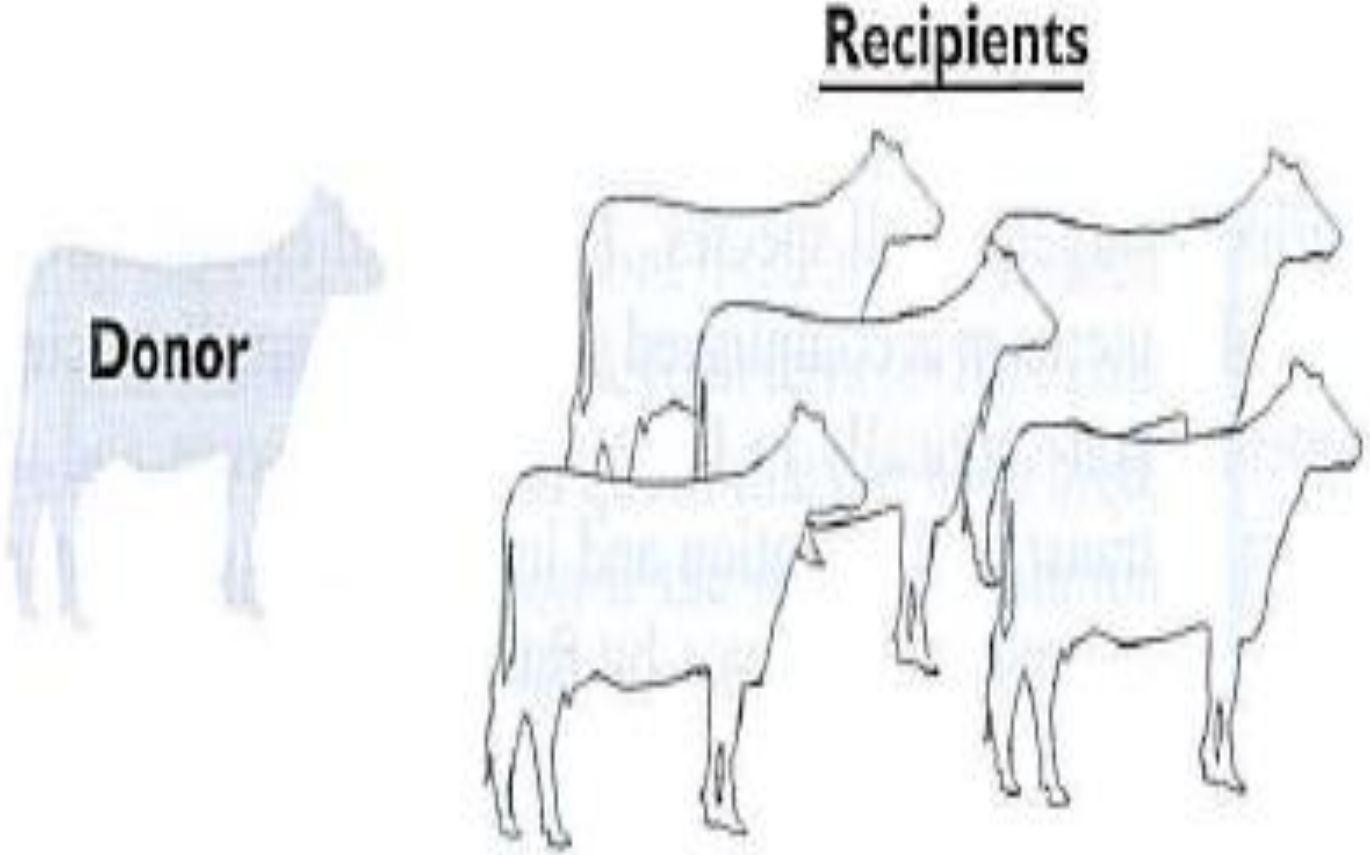
- Obstetric (OB) Lube
- Sterile lube
- Cervical dilator
- Medium: Vigro Complete (Vetoquinol)
- 10-ml syringe to inflate cuff

Foley Catheter



STEP I

Sychronization of recipients with donor



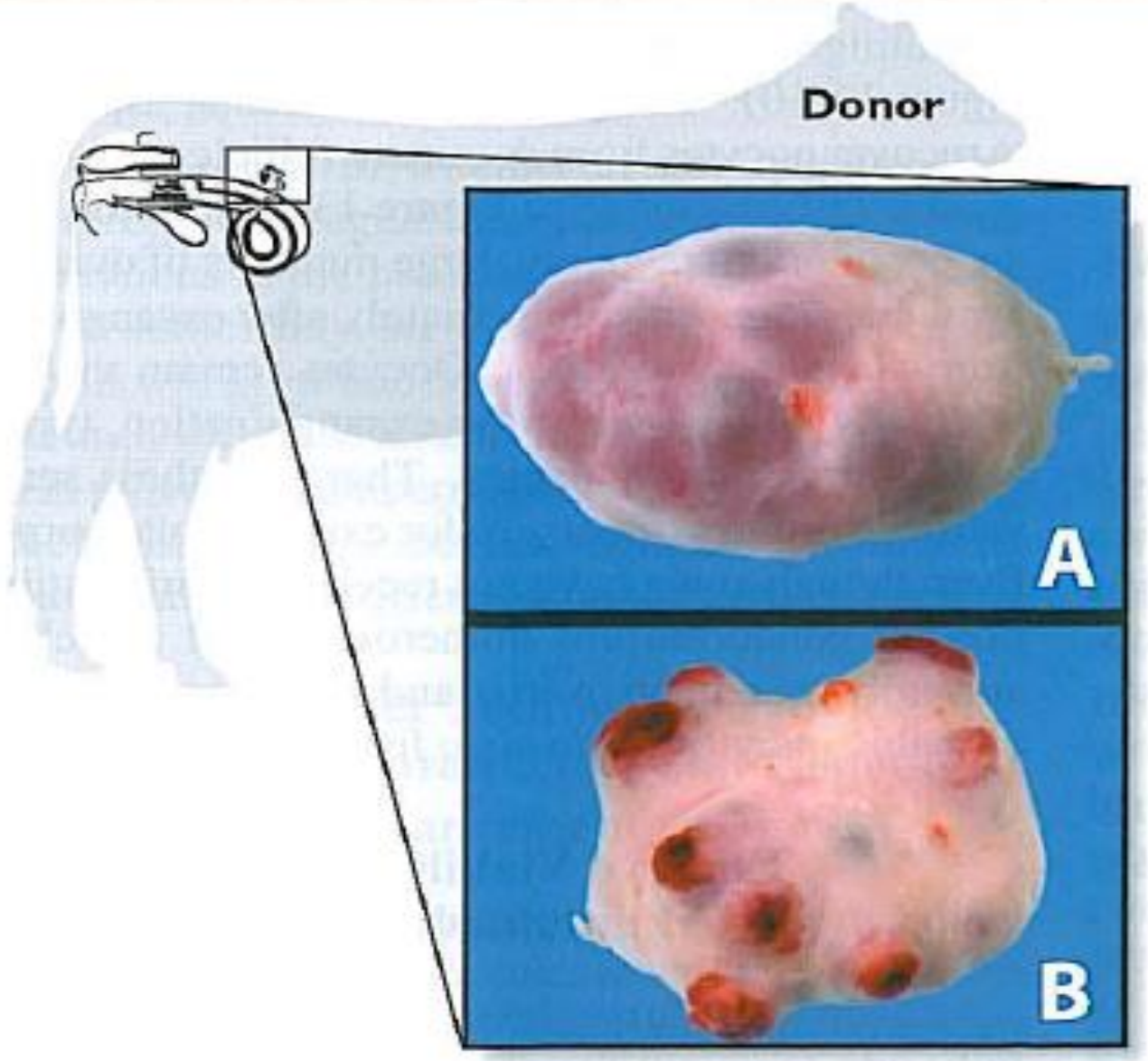
Goal: To synchronize the donor and recipient to be in the same stage of the estrous cycle.

Reason: To prepare the uterus of the recipient to support preattachment embryogenesis.

How: Treat recipient with hormonal regime that induces estrus to occur at the same time as the donor.

STEP 2

Superovulation of donor



Goal: To hyperstimulate ovaries with gonadotropins.

Reason: To provide higher than normal numbers of follicles that reach dominance and ovulate.

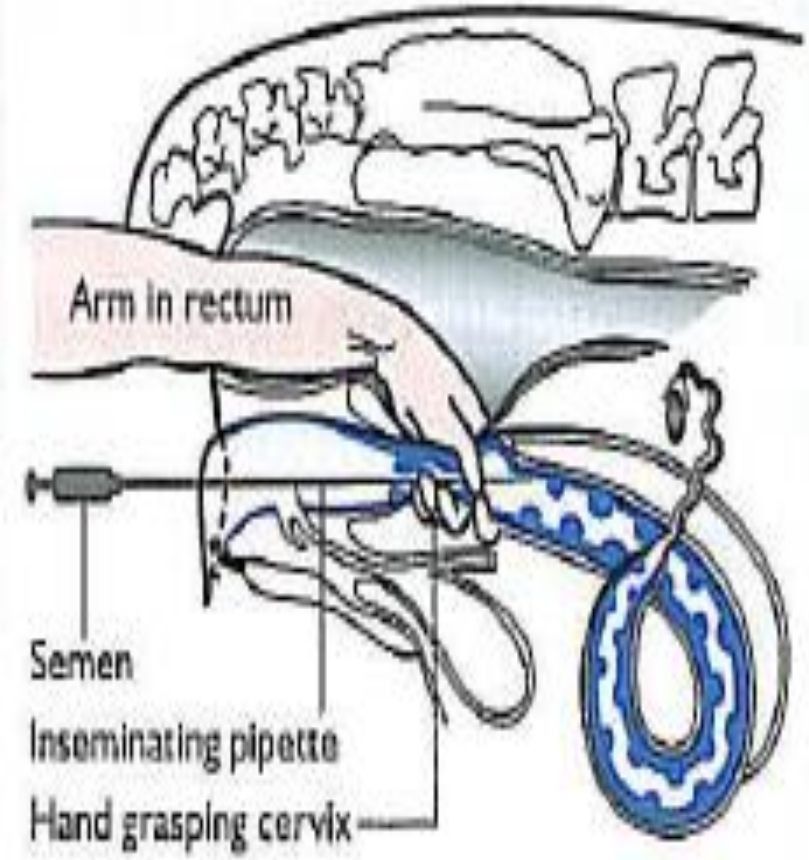
How: Inject donor with gonadotropins to hyperstimulate follicular development. Generally, FSH (or one of its analogs) is used.

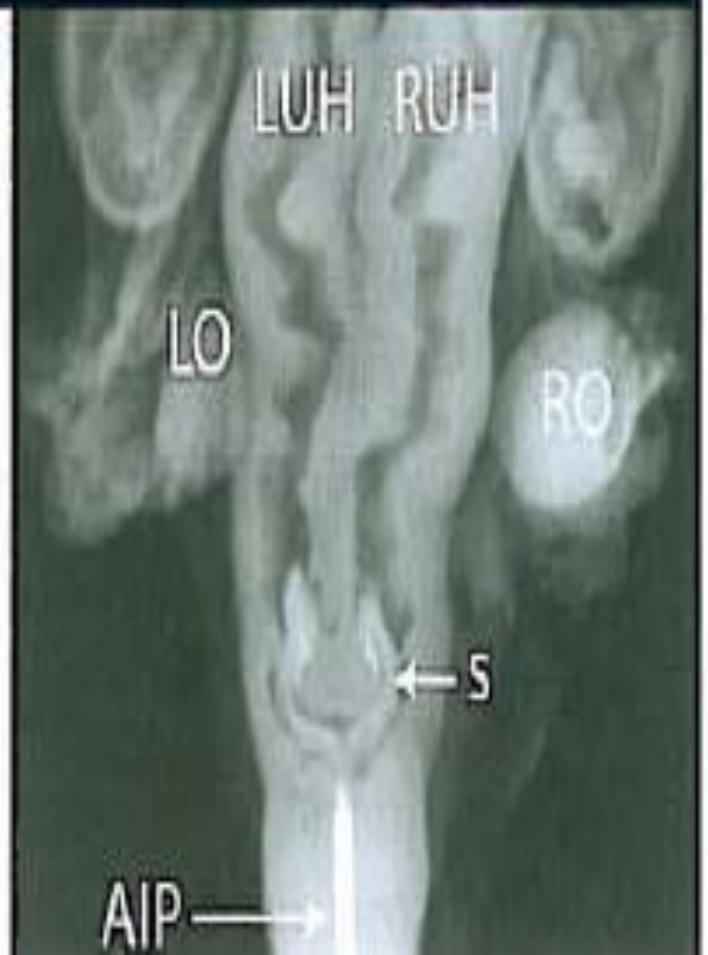
Ovary A- Hyperstimulated ovary. There are 9 follicles visible in this ovary. The donor is in estrus.

Ovary B- 1 day after estrus. There are 9 corpora hemorrhagica visible on this specimen.

STEP 3

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.

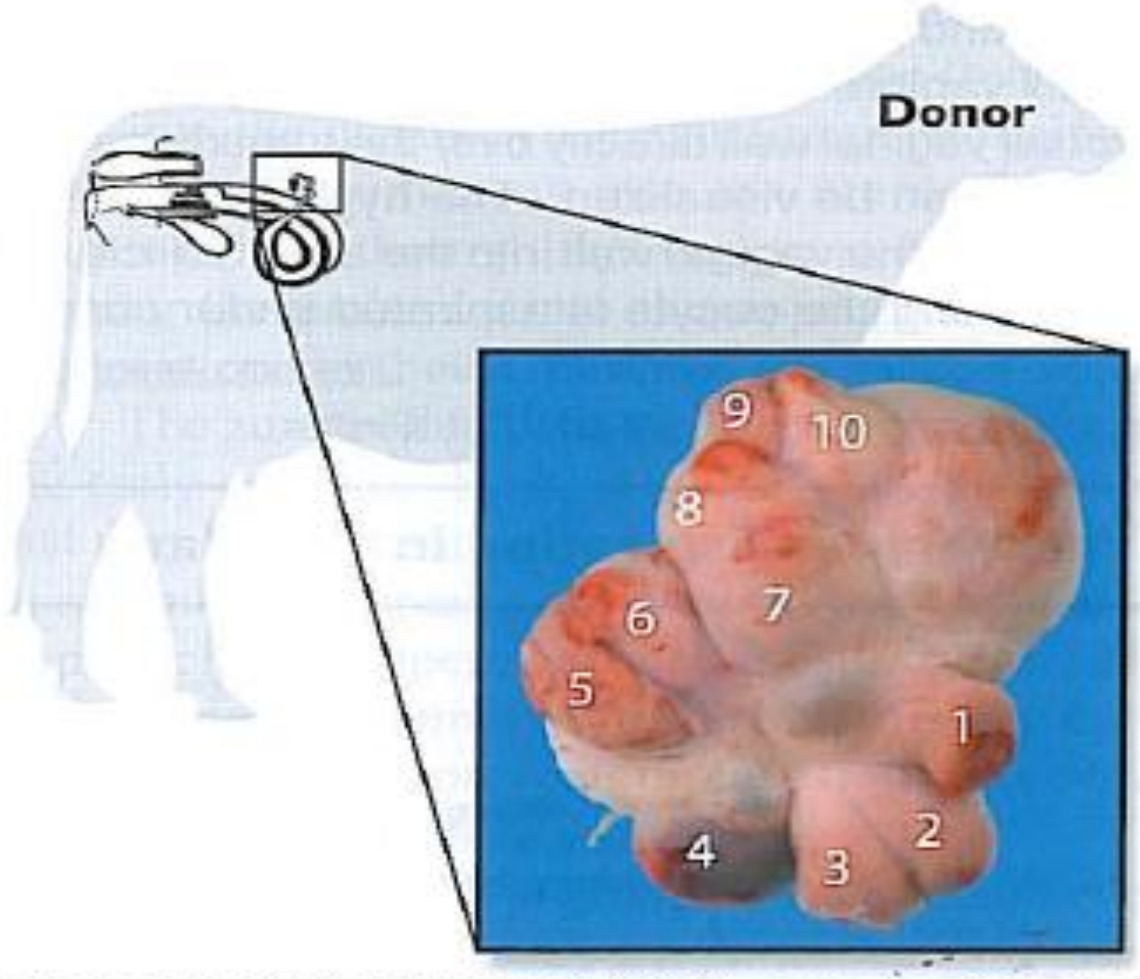
How: Utilize highly fertile semen and well-trained, experienced inseminators.

AIP = AI Pipette, S = Semen, RO = Right Ovary, LO = Left Ovary, RUH = Right Uterine Horn, LUH = Left Uterine Horn

(Ovarian specimens courtesy of Dr. B.R. Lindsey)

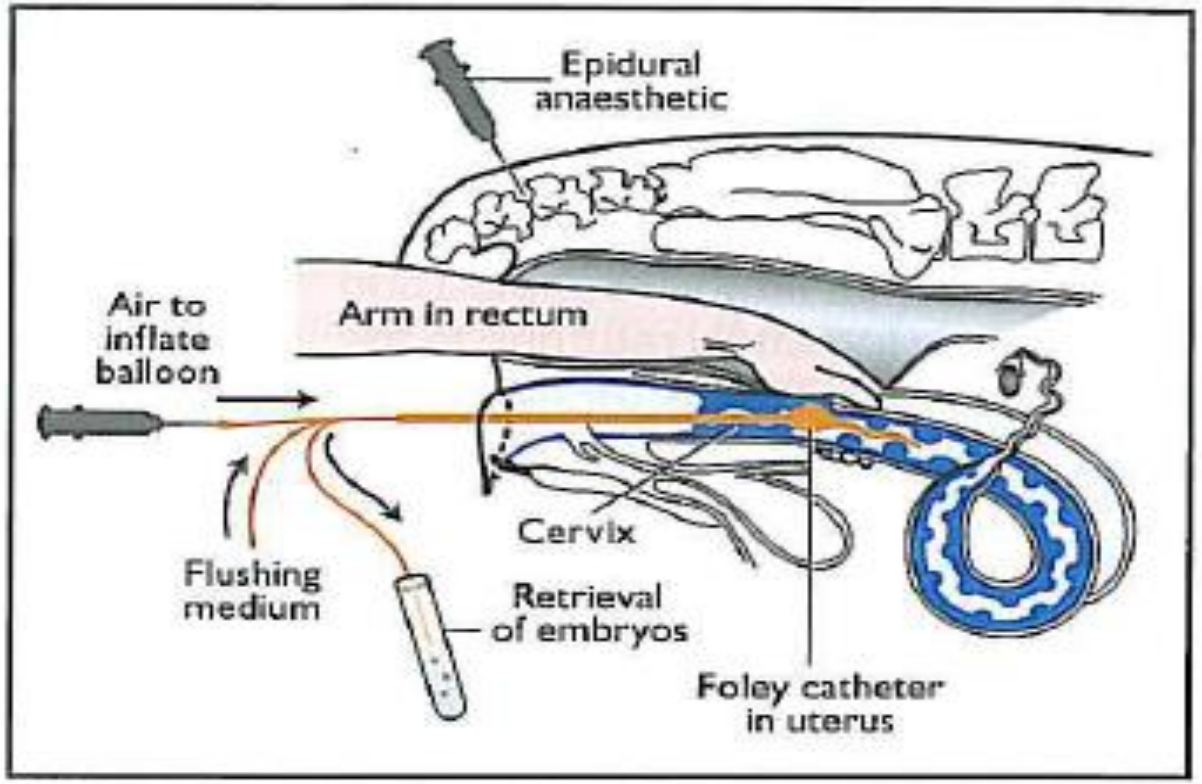
STEP 4

Recovery and indentification of viable embryos



Goal: To nonsurgically collect (flush) embryos from the donor for transfer.

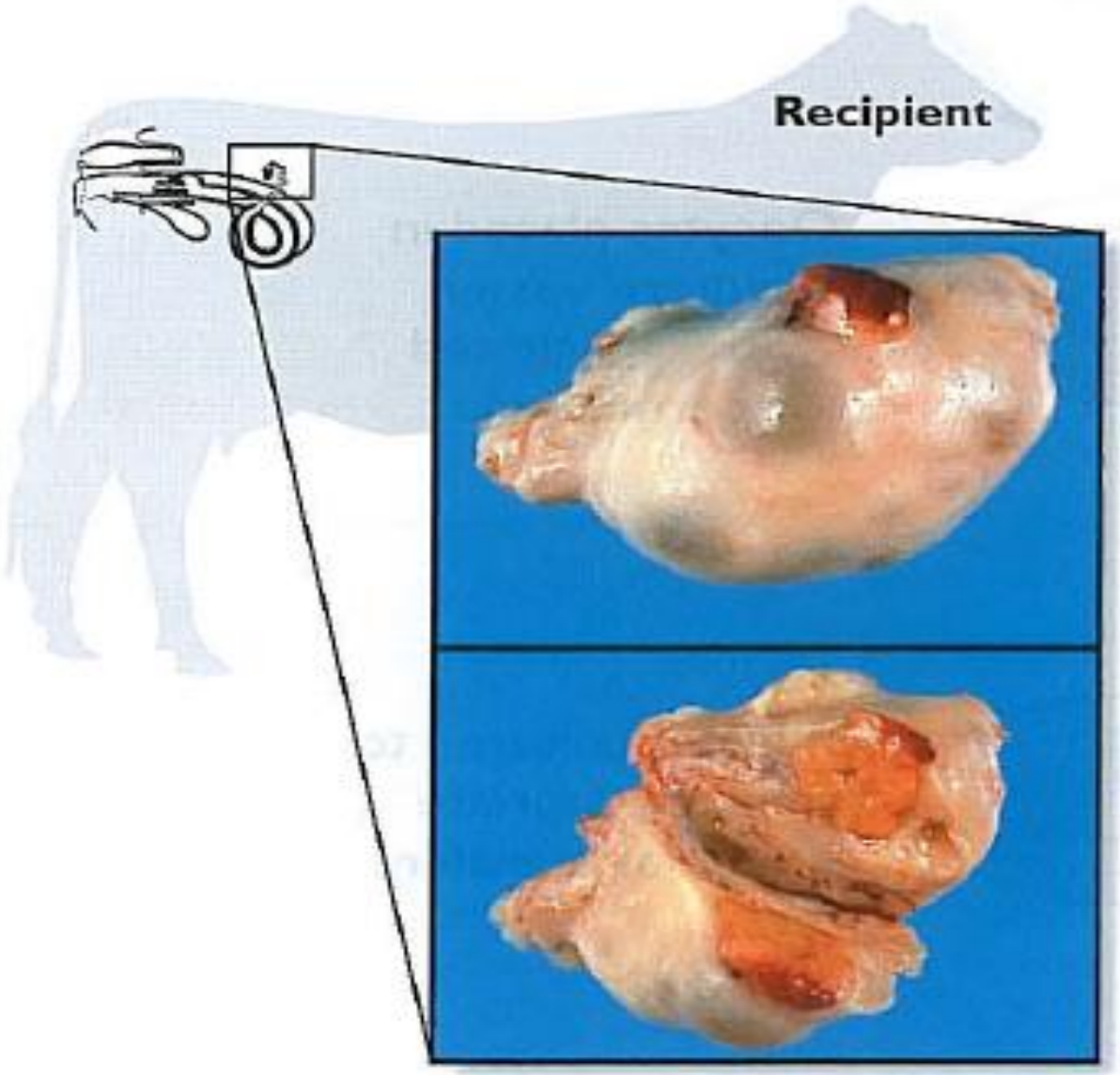
Reason: To recover viable embryos.

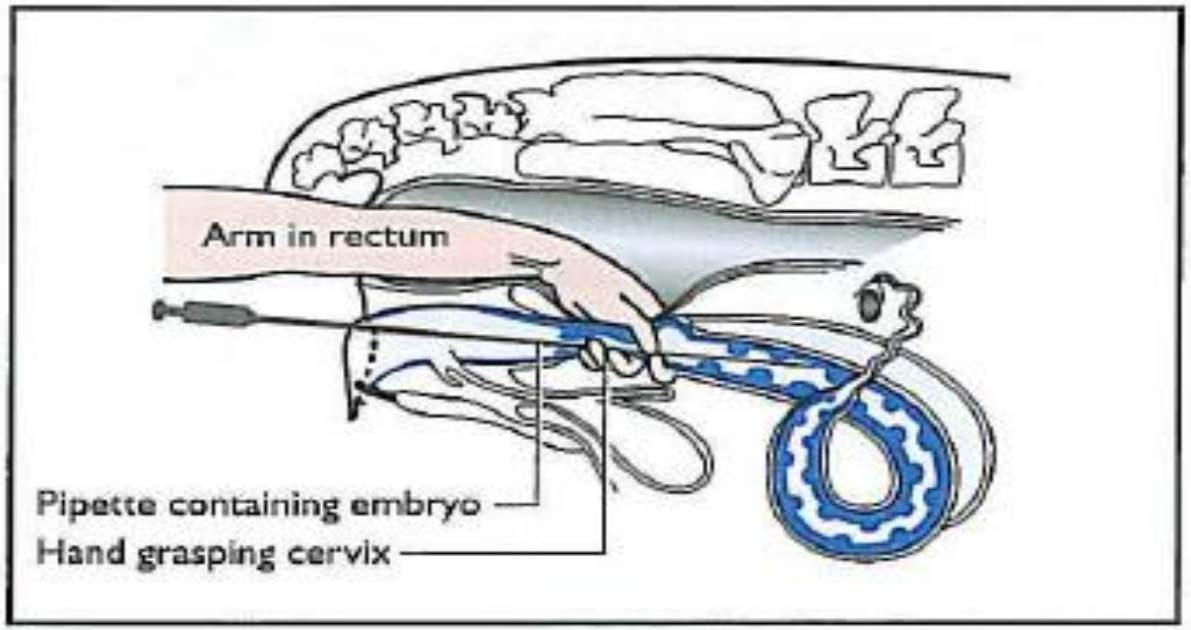


How: Before the procedure is started a local anesthetic is injected to cause relaxation of the rectum. At day 6-8 a specialized catheter is inserted into the uterus. The catheter has a small balloon that can be inflated to prevent retrograde flow of the flushing medium. A flushing medium is then introduced into the uterus, lavaged and then returned through the catheter to a collection vessel. The ovary in the photo has ten-7 day CL.

STEP 5

Transfer of viable embryos into synchronized recipients





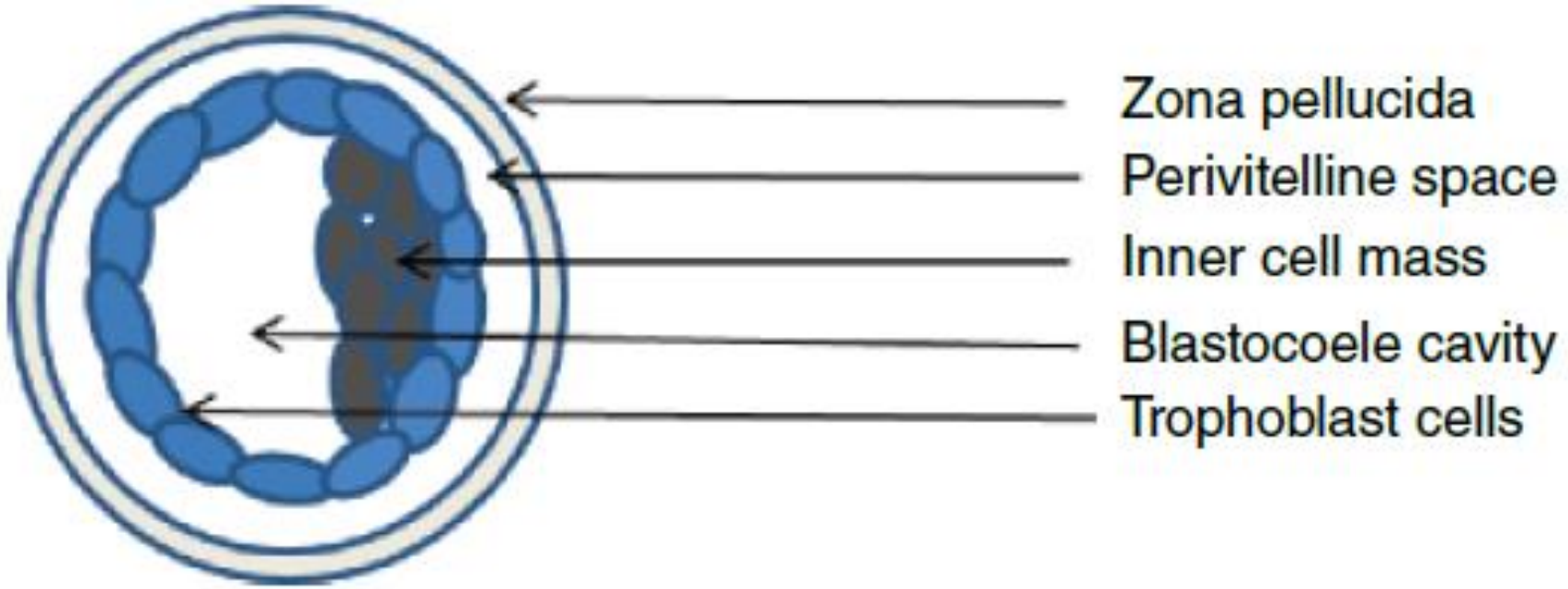
Goal: To deposit a potentially viable embryo into the uterine horn of each recipient.
Reason: To achieve pregnancy in each recipient.
How: A single embryo is placed into the uterine horn using a transfer pipette. Note that both the donor (step 4) and recipient here have CL at similar stages of leutinization. Thus, the uterine environment in the donor and recipient are quite similar.

Prevention of Pregnancy in Donors

- PGF2a
  - Infuse 5 ml Lutalyse into the uterus at the end of the flush
  - Ask the owner to administer 5 ml (25 mg) IM
- Rectal palpation or ultrasound
  - 45-50 days after insemination
  - twice a day on the third day

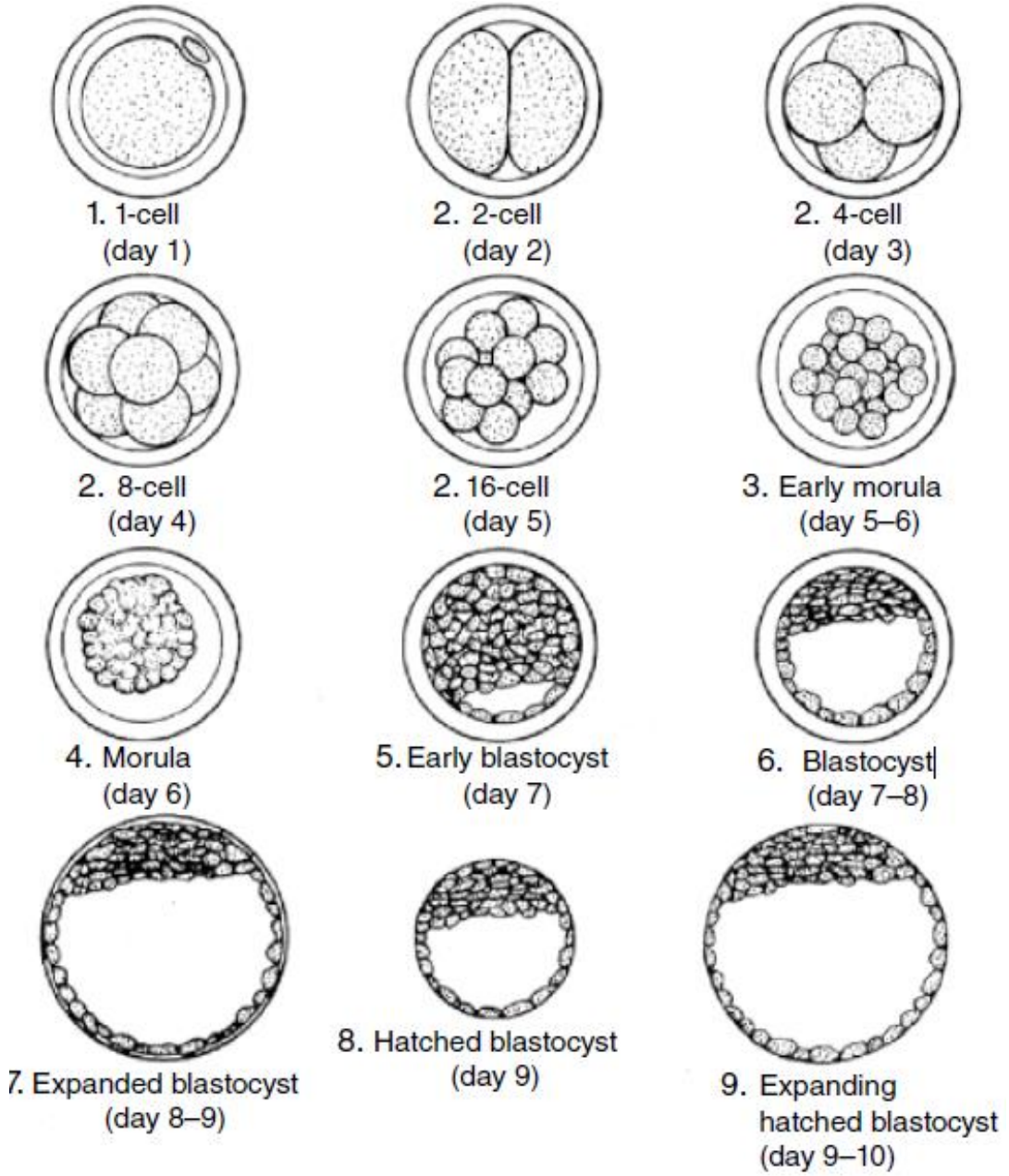
Evaluation of Embryos

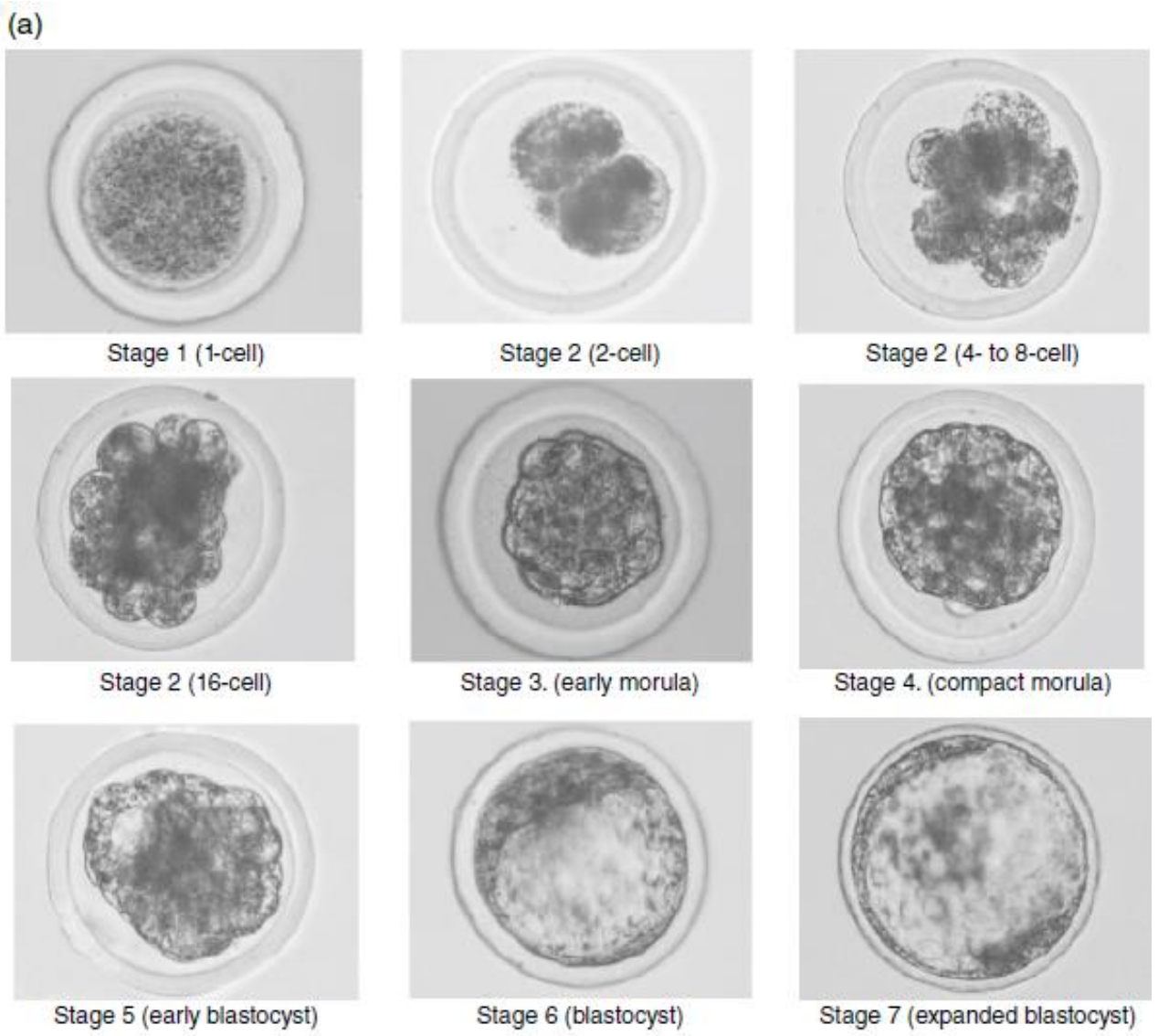
- Embryo quality
- Classified numerically
  - Potential likelihood of success
  - if transferred to a recipient



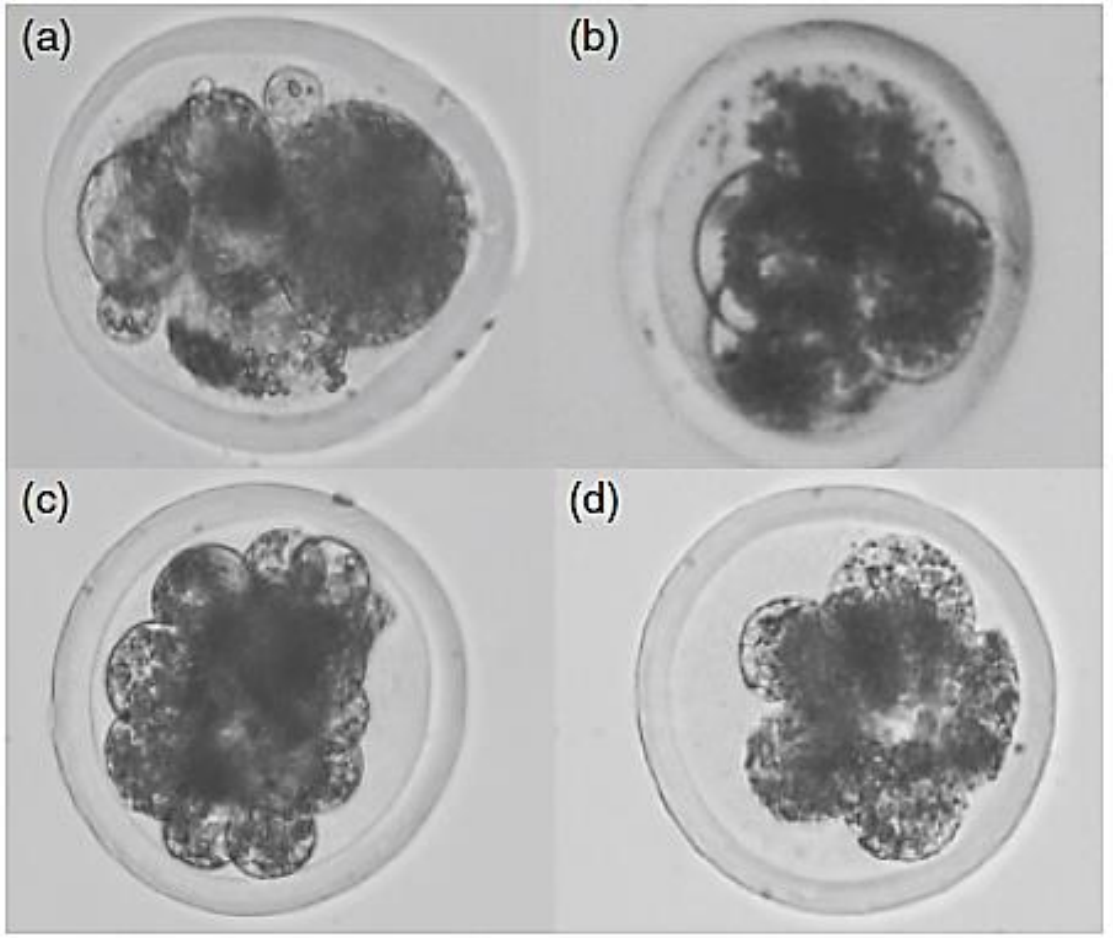
A blastocyst-stage bovine embryo

Normal embryonic development of bovine embryos

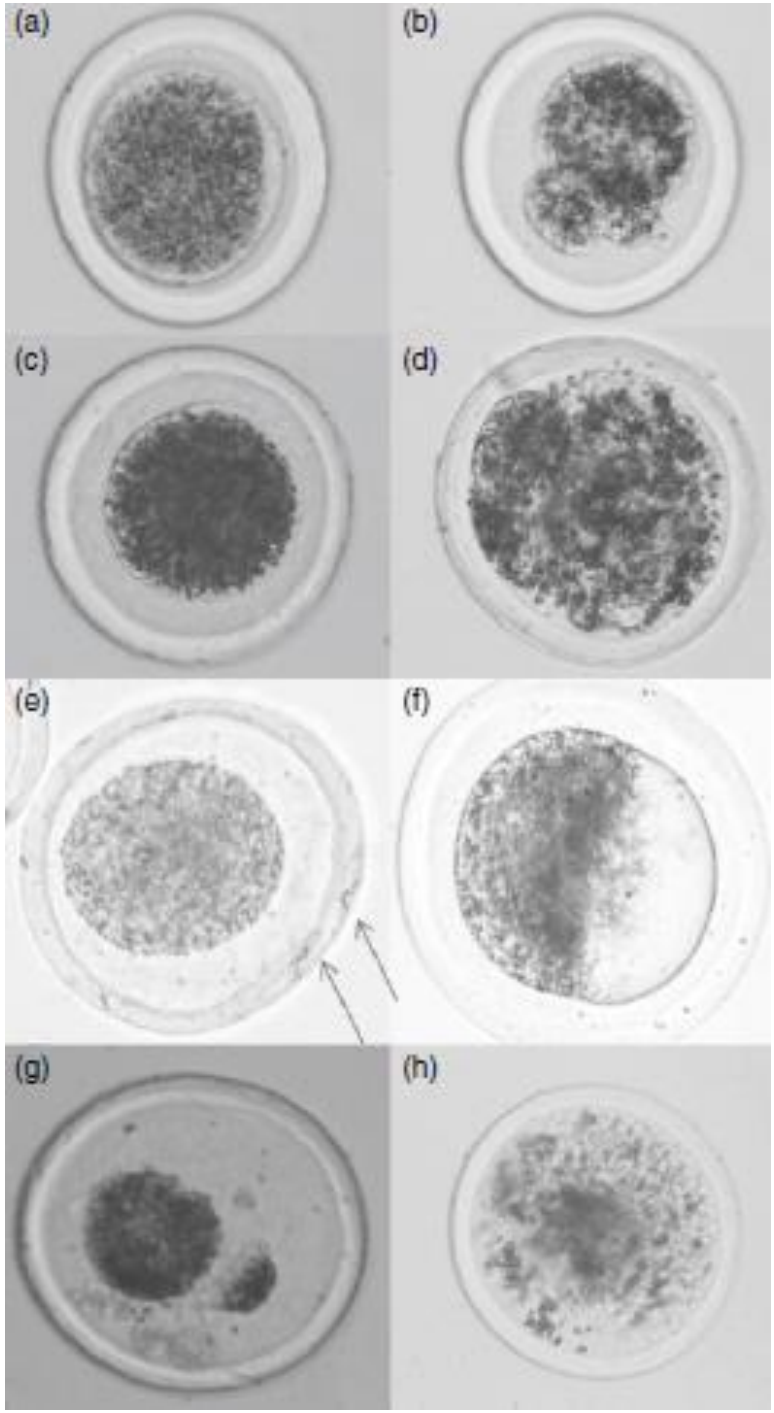




Comparison of developmental stages of *in vivo* derived bovine embryos collected on days 6–8 after estrus. At day 7, a stage code 1 embryo is considered an unfertilized ovum and a stage code 2 embryo is considered dead or degenerate



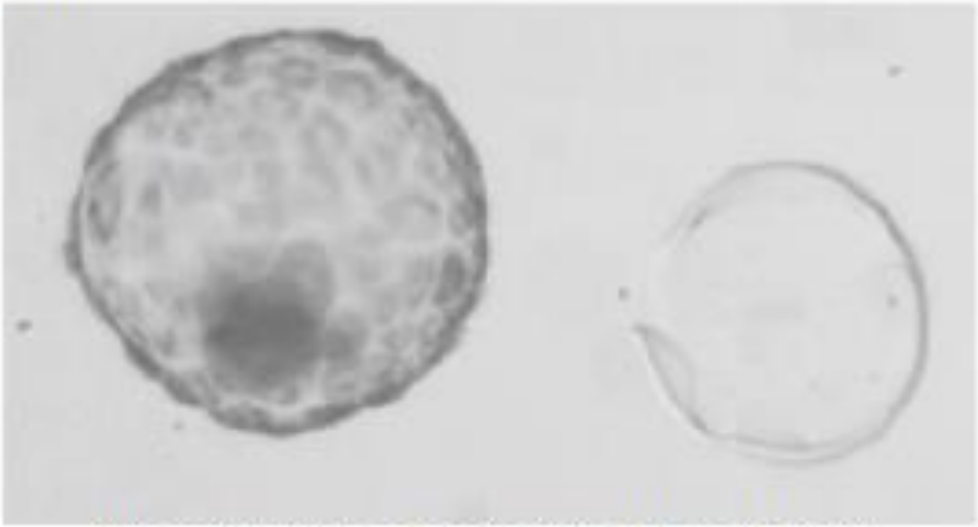
Degenerate bovine embryos collected on day 7: (a) and (b) are degenerate and are considered dead; (c) and (d) are not as developmentally advanced as expected for a day-7 embryo, and the cells are not fused tightly to one another, so are also considered degenerate



Unfertilized ova recovered non-surgically from superovulated donor cows six to eight days after the onset of estrus. Note the extremely wide variation in morphology which makes accurate identification difficult for inexperienced embryo technicians.

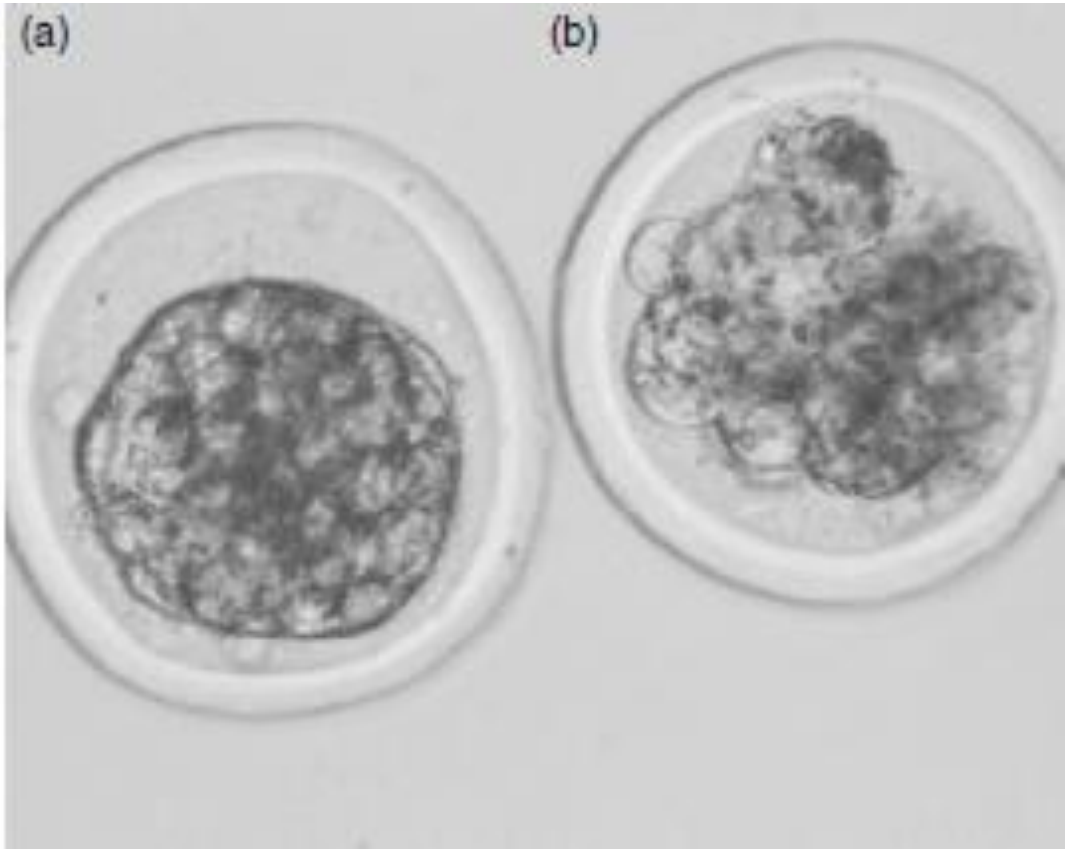
(a) Unfertilized ovum with smooth vitelline membrane and granular cytoplasm that can be mistaken for a compact morula. (b) Fragmented unfertilized ovum. (c) Unfertilized ovum possessing dark cytoplasm and a smooth vitelline membrane; can be mistaken for a compact morula. (d) Degenerate unfertilized ovum. (e) Unfertilized ovum with light-colored granular cytoplasm; note sperm (arrows) attached to the zona pellucida. (f) Unfertilized ovum with smooth vitelline membrane; ovum is granular in one half and clear in the other half; can be mistaken for a blastocyst-stage embryo. (g) Fragmented unfertilized ovum. (h) Degenerate unfertilized ovum; note "loss" of vitelline membrane

(b)

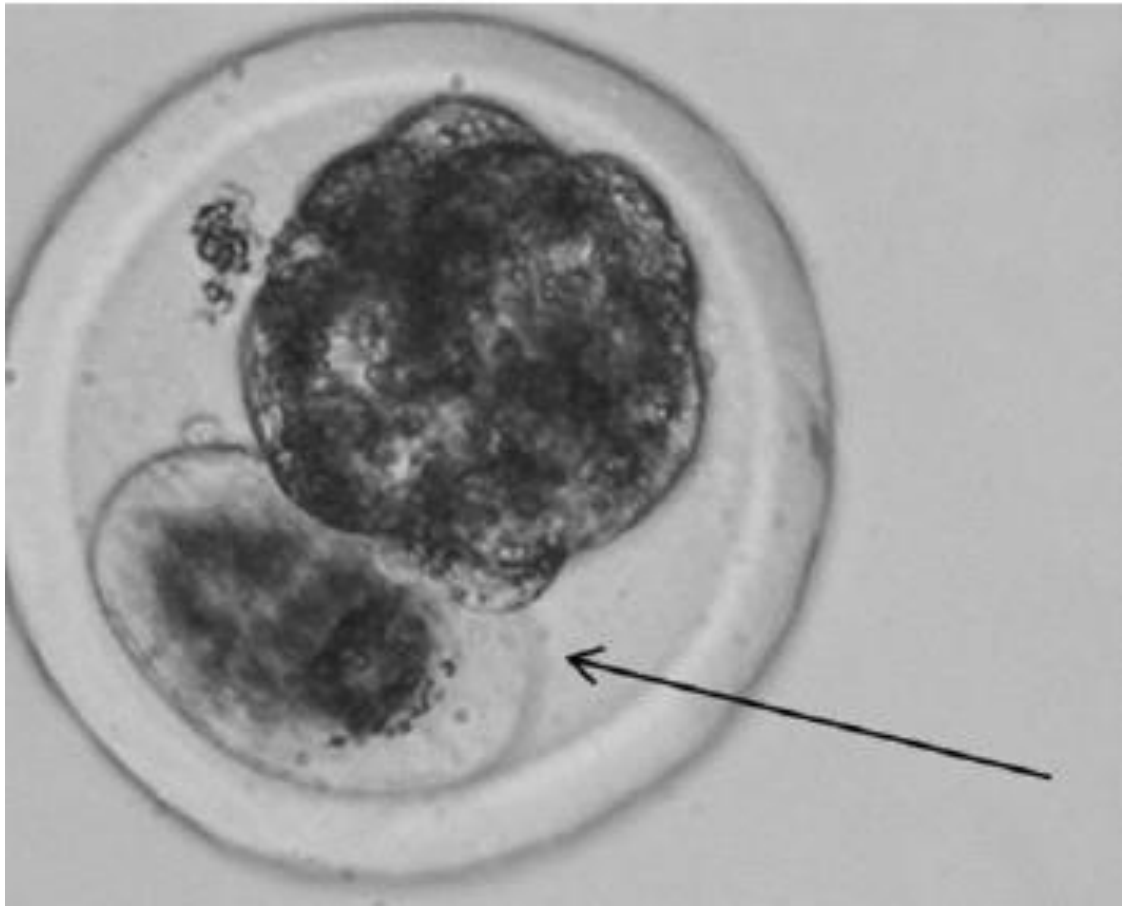


Stage 9 (expanded hatched blastocyst)

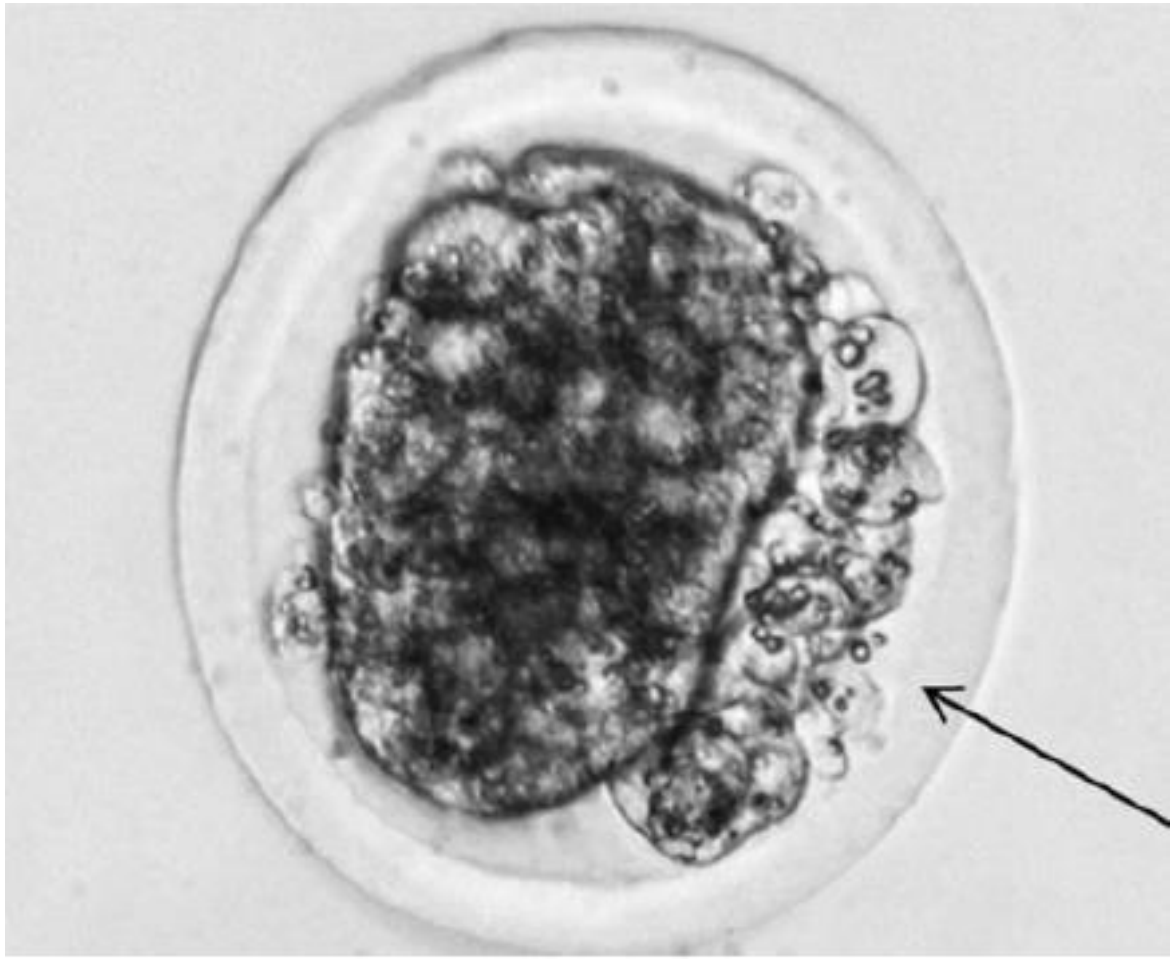
Expanded hatched blastocyst typically recovered on or after day 8 after estrus; note the empty zona pellucida on the right and the expanded embryo on the left



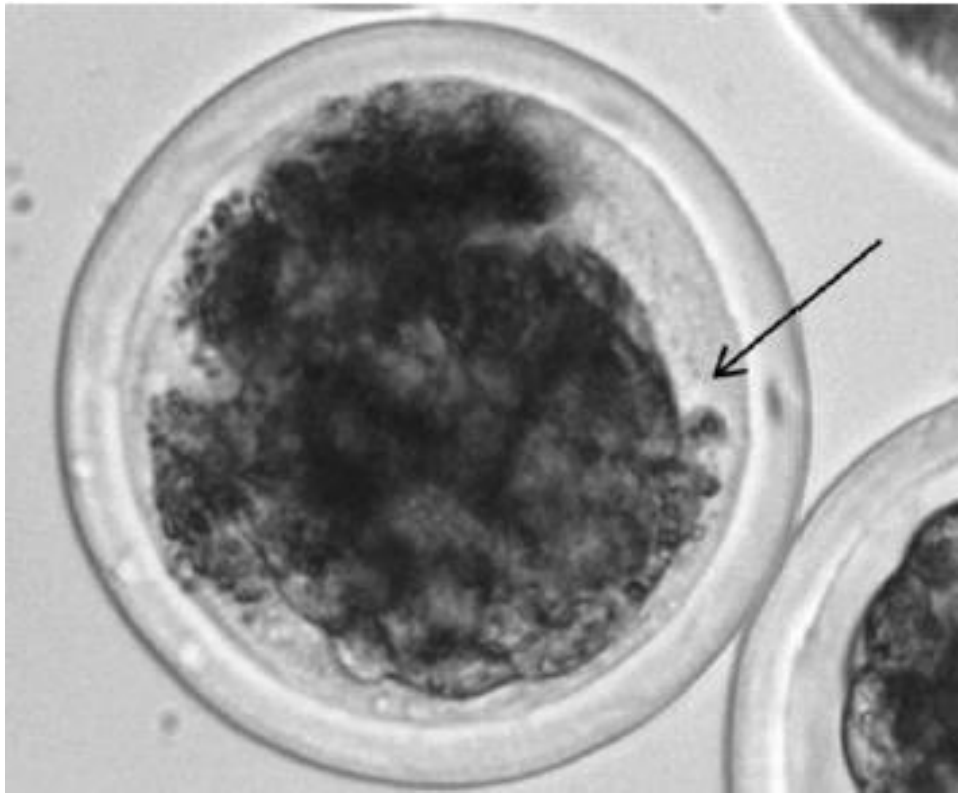
Day-7 compact morula (a) adjacent to a degenerate embryo



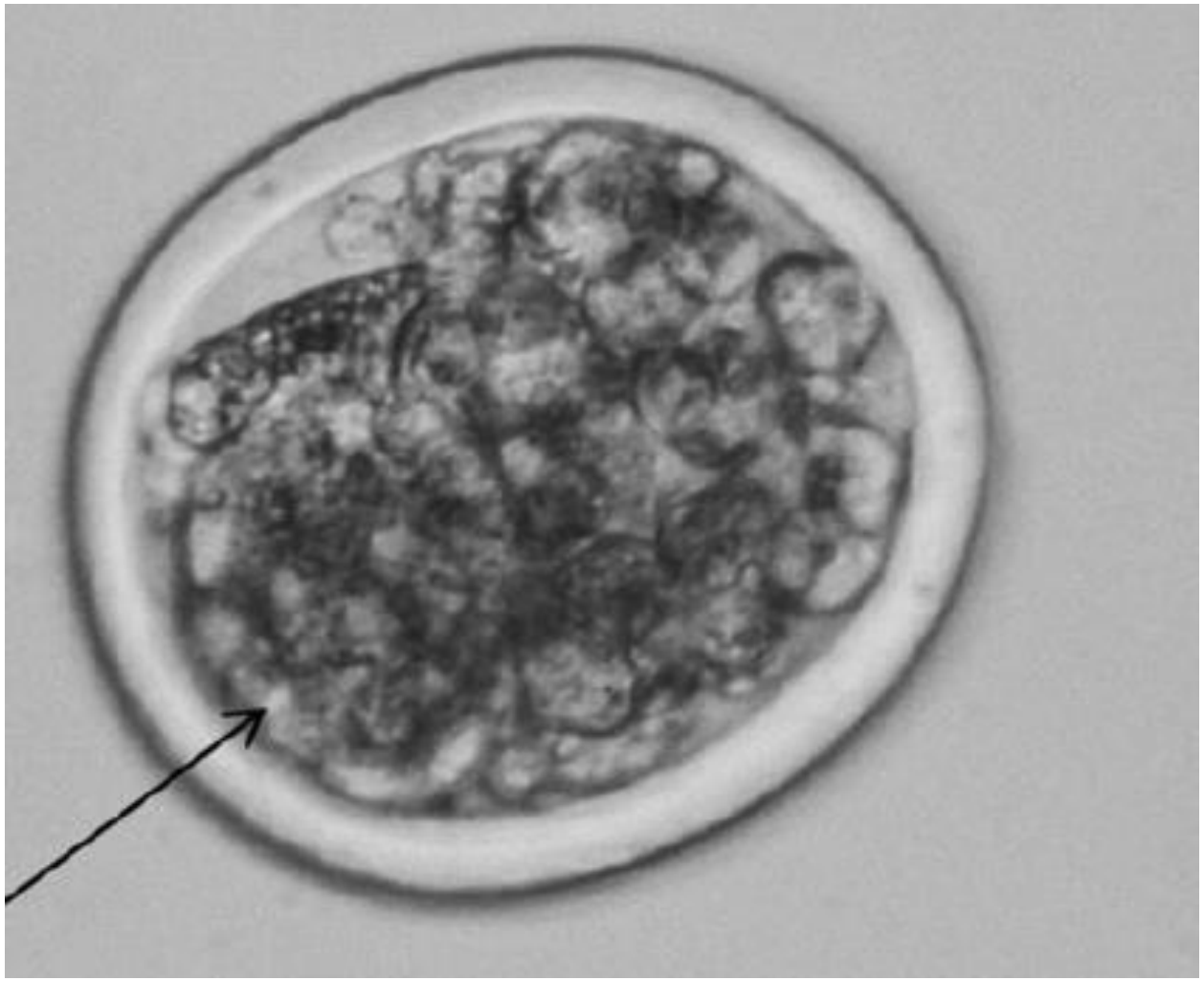
Grade 2 compact morula. Note one large blastomere (arrow) has stopped dividing, while others have formed a compact morula



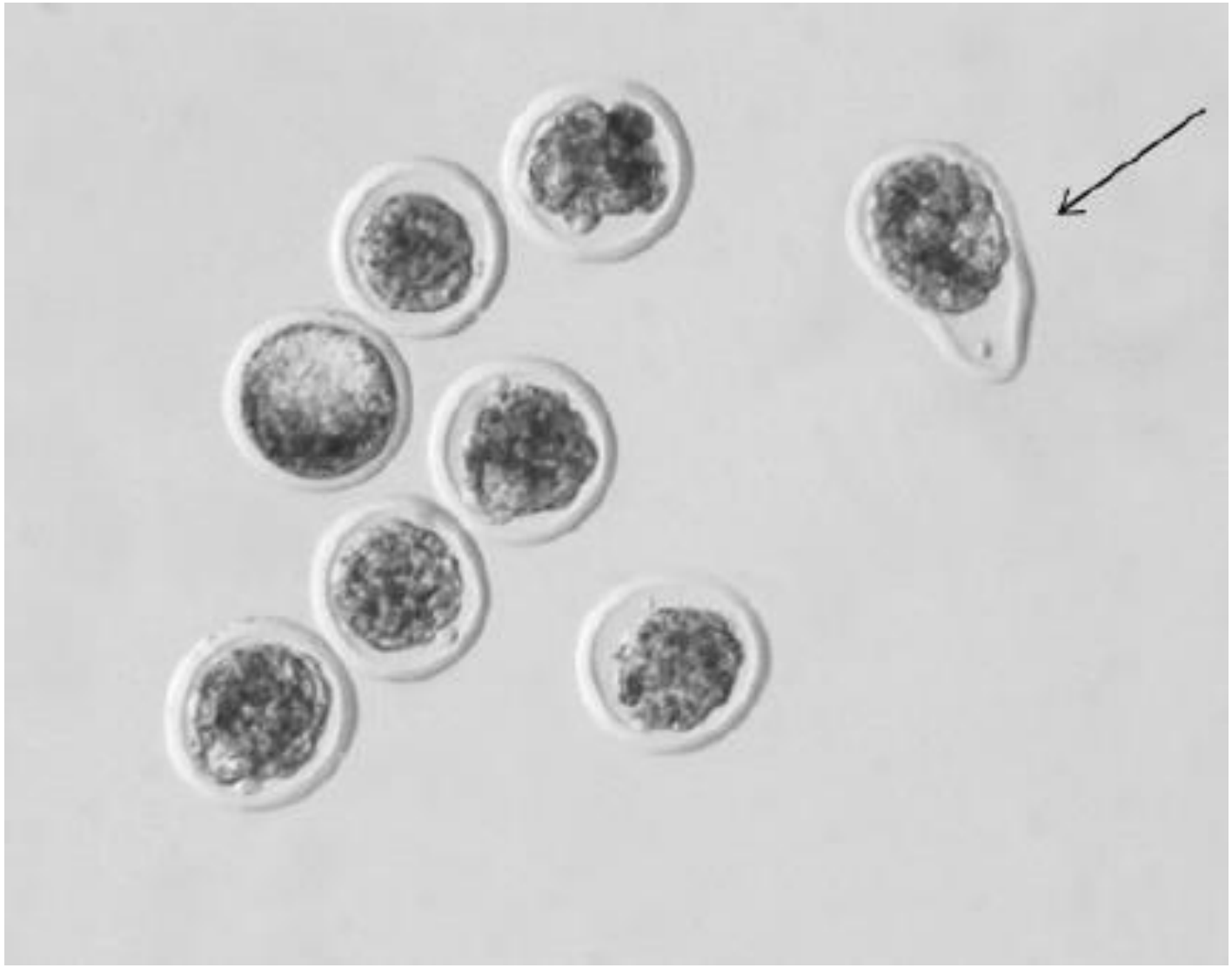
Grade 2 morula. Note several extruded blastomeres (arrow) to the right side of the embryo



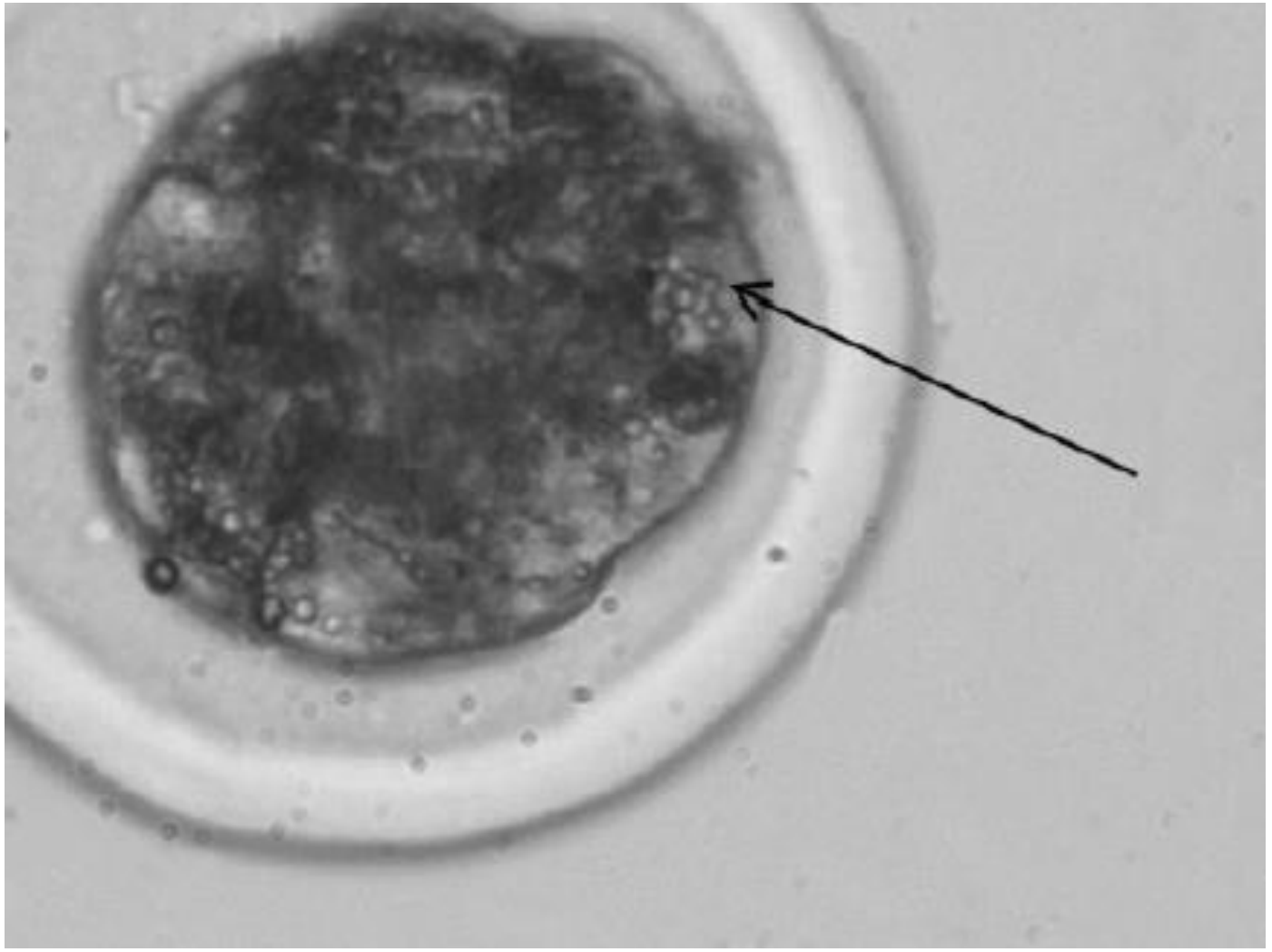
Grade 3 morula. Note embryonic cell mass on the right side (arrow) hidden by a large number of extruded cells in the upper left corner and left side. May be called grade 4 by some without rolling this embryo



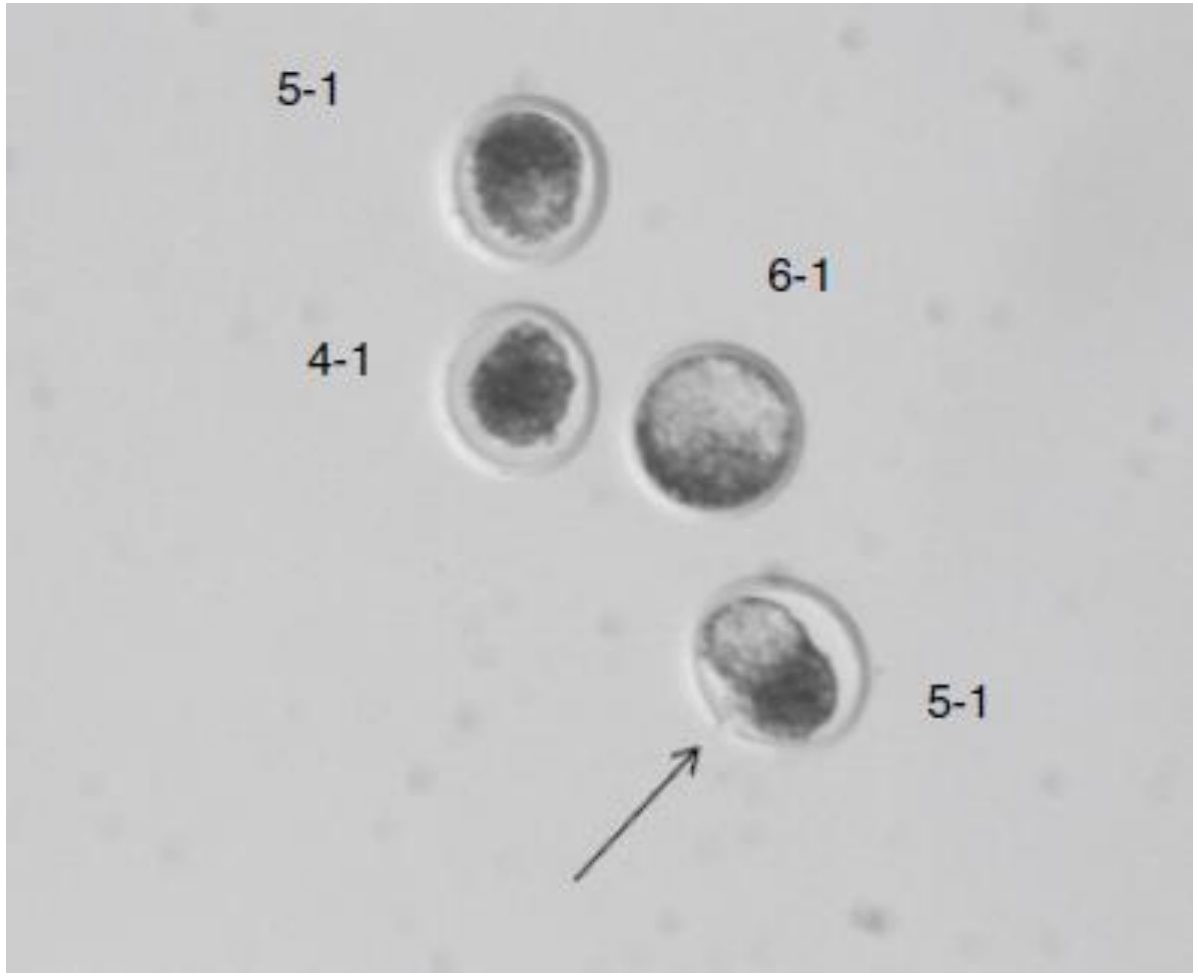
Grade 4 morula. Note the small group of cells on the left side (arrow) and very large number of extruded cells on the right. This embryo was identified as grade 4 by our laboratory but may be called grade 3 by others



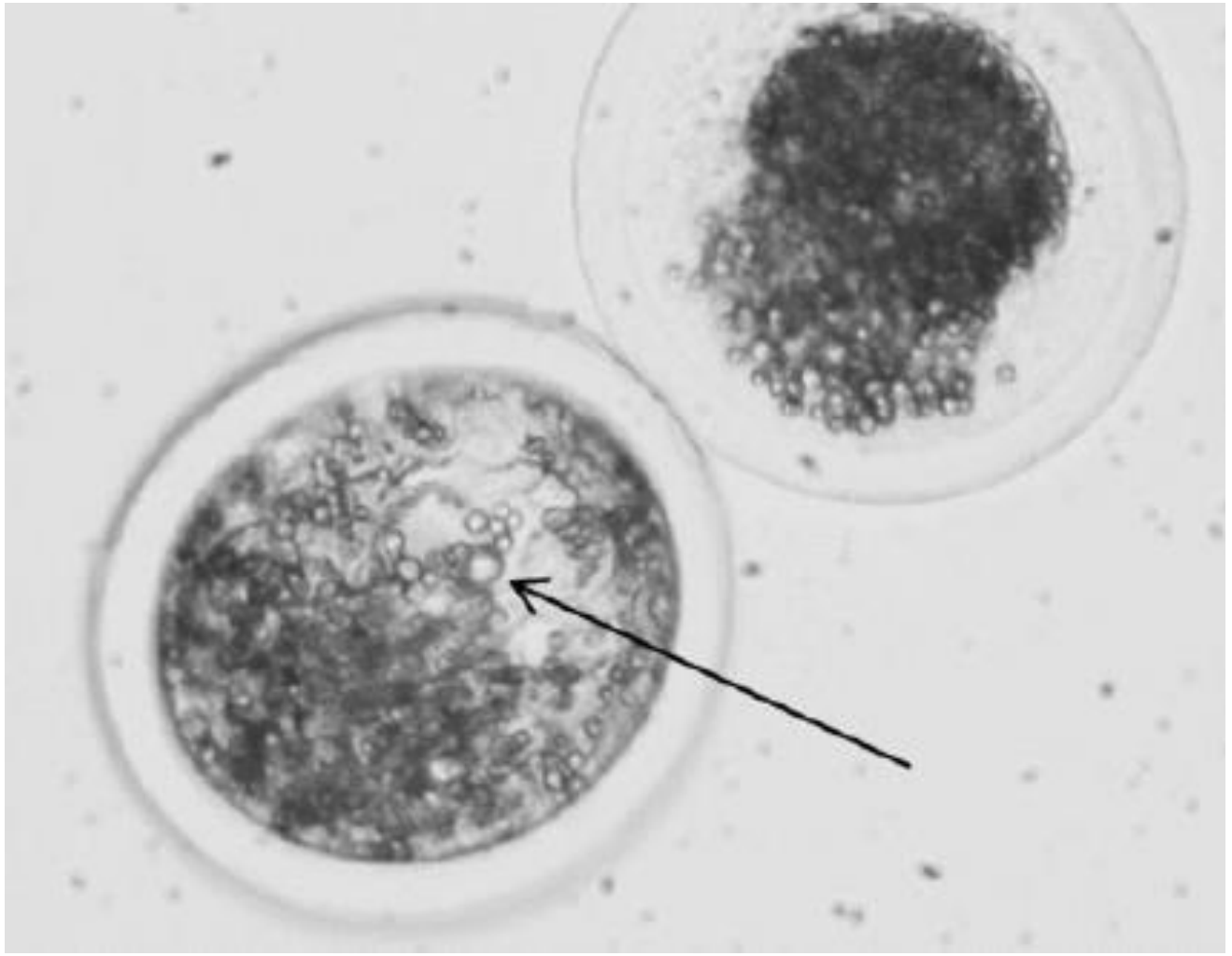
The arrow points to a day-7 grade 2 early blastocyst-stage embryo with a misshapen zona pellucida (50×).



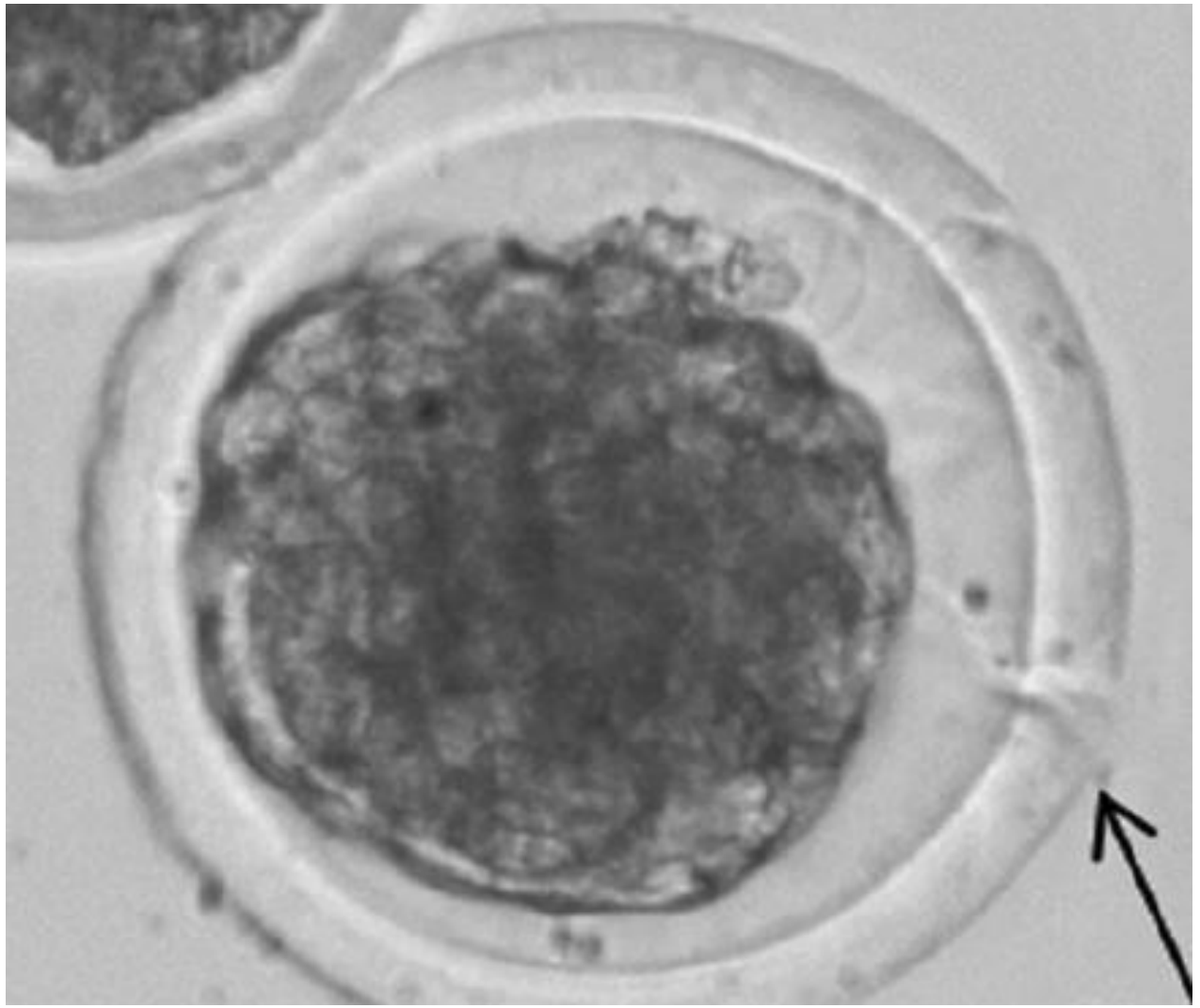
Grade 1 compact morula with vacuoles (arrow) in the cytoplasm



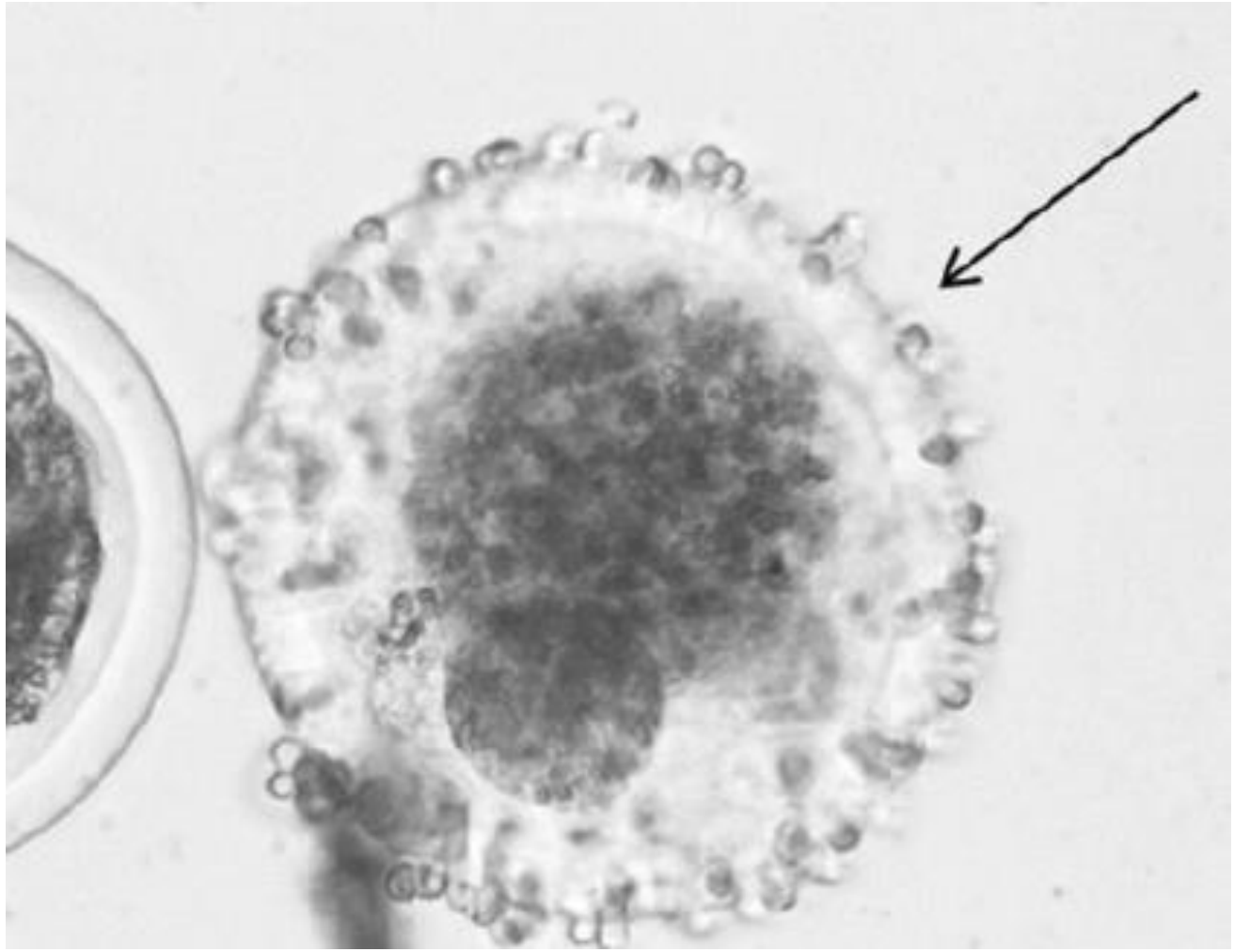
Collapsed early blastocyst with a cracked zona pellucida (arrow)



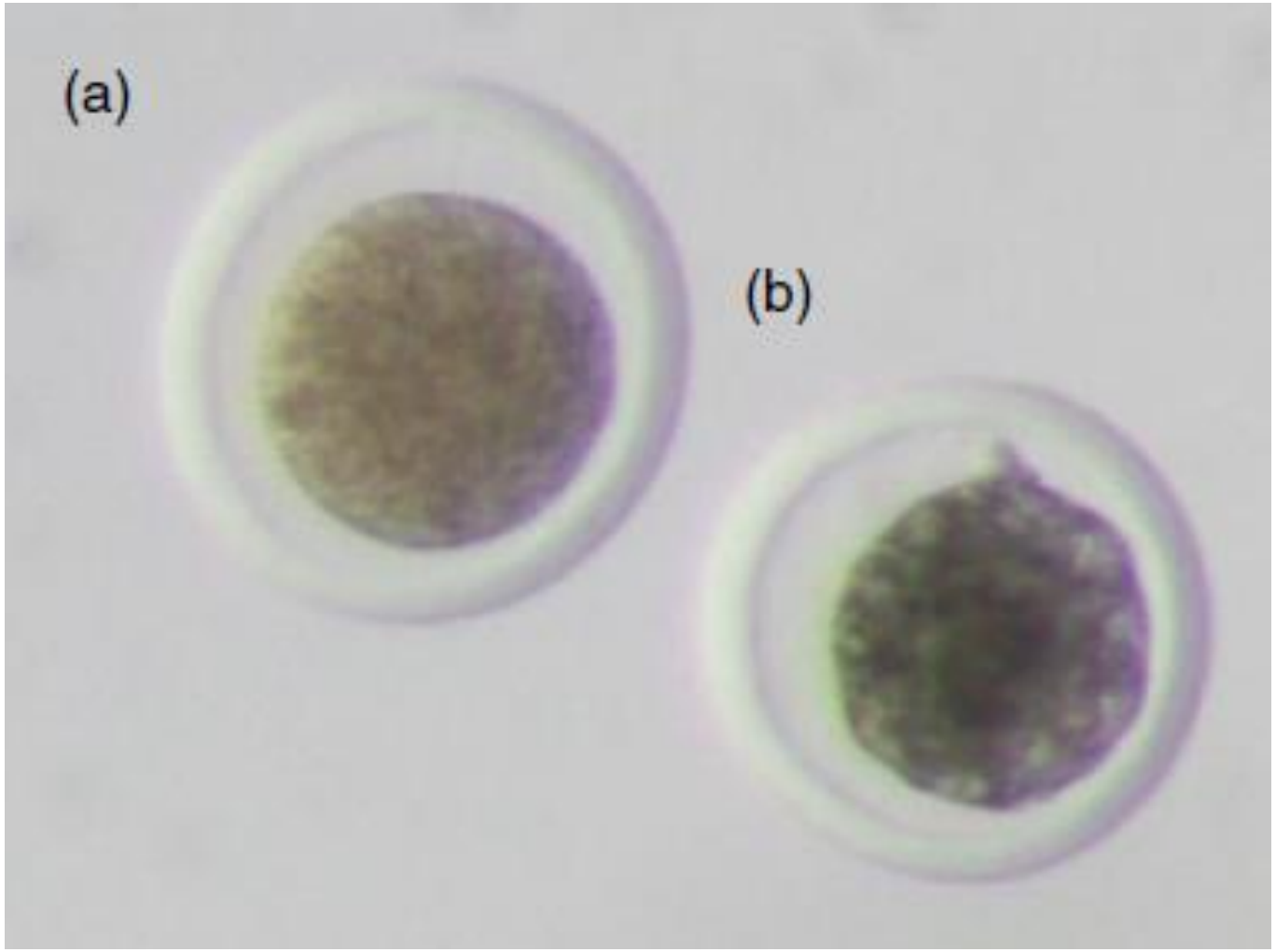
Grade 2 bovine blastocyst containing vacuoles (arrow) in the cytoplasm beside an unfertilized ovum. Note that it is difficult to identify the blastocyst embryo without rolling the embryo and having a good view of the blastocoele cavity. Could easily be mistaken for a degenerating unfertilized ovum



Day 7 compact morula. Note the crack in the zona pellucida (arrow)



Fragmented unfertilized ovum. Note the white blood cells (arrow) attached to the zona pellucida



An unfertilized ovum (a) and excellent-quality compact morula stage embryo (b)

Evaluation Criteria

1. Compactness
   - Compact rather than
   - Loose mass of cells

Evaluation Criteria

2. Shape

- Spherical better than oval

Evaluation Criteria

3. Variation in cell size

- Uniform blastomeres

Evaluation Criteria

4. Color and texture
   - Not too light
   - Not too dark

Evaluation Criteria

5. presence of vesicles
   - Agranular cytoplasm
   - Moderate size

Evaluation Criteria

6. Presence of Extruded Cells
   - No dissociated cells
7. Size
   - Normal
   - Learn with practice

Evaluation Criteria

8. Regularity of Zona Pellucida
   - Empty perivitelline space
   - Regular diameter
   - No wrinkling or collapse

Evaluation Criteria

9. Presence of cellular debris
    - No cell fragments

Embryo Classification

Grade 1	Excellent or good
G2	Fair
G3	Poor
G4	Dead or degenerating

Embryo Classification

- Excellent or good
  - Perfect embryo for its age
  - Oval ZP
  - Few small excluded cells
  - Slightly asymmetrical shape

Embryo Classification

- Fair
  - Definite
  - No severe abnormalities
  - Moderate number of excluded cells
  - Small size
  - Small amount of degeneration

Embryo Classification

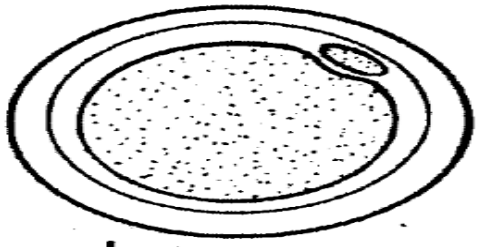
- Poor
  - Considerable degeneration
  - Vesiculated cells
  - Varying cell size
  - Failure of compaction

Embryo Classification

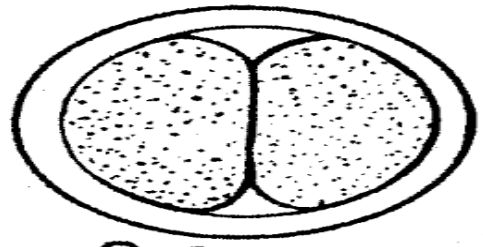
- Degenerate
  - Severe degeneration
  - Unfertilized or two- or three-cell

Stage of Development

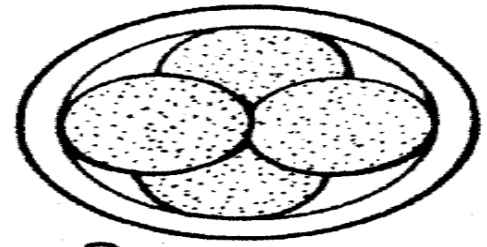
Stage 1	Unfertilized
S2	2-12 cell
S3	Early morula
S4	Morula
S5	Early blastocyst
S6	Blastocyst
S7	Expanded blastocyst
S8	Hatched blastocyst
S9	Expanded hatched blastocyst



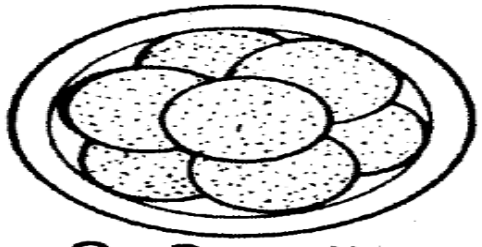
1. 1-cell
(day 1)



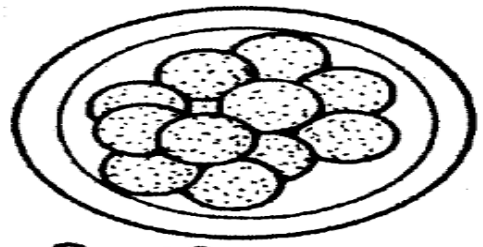
2. 2-cell
(day 2)



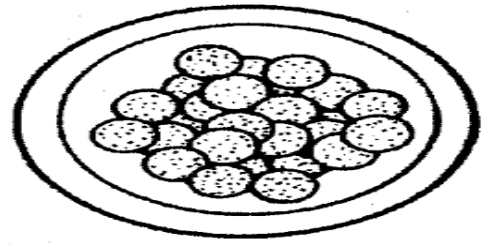
2. 4-cell
(day 3)



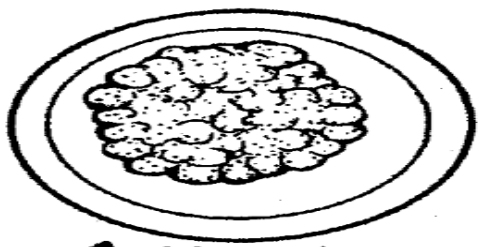
2. 8-cell
(day 4)



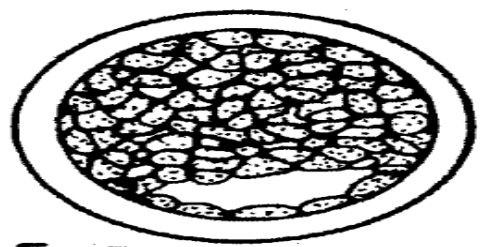
2. 16-cell
(day 5)



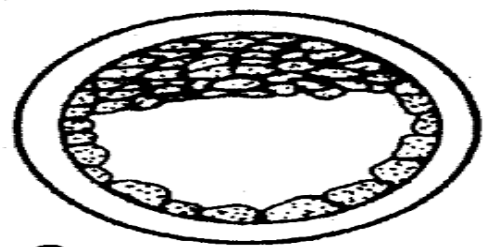
3. Early morula
(day 5-6)



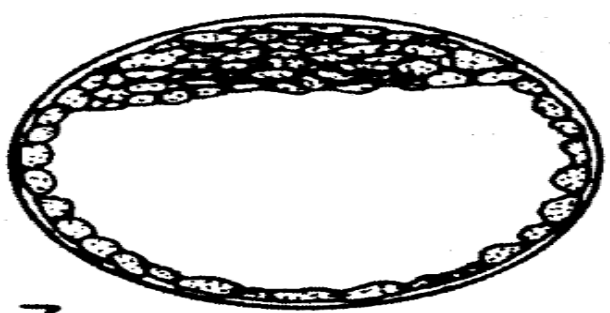
4. Morula
(day 6)



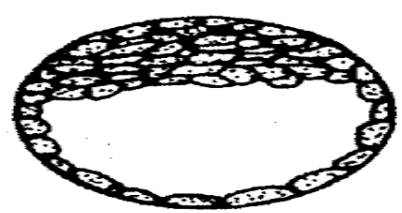
5. Early blastocyst
(day 7)



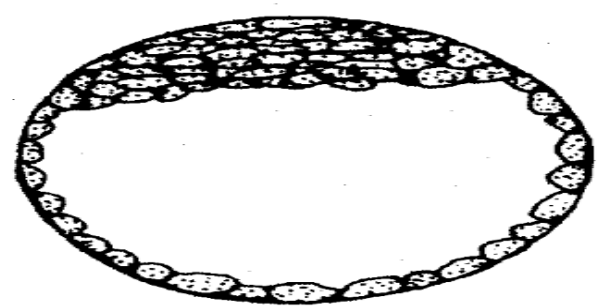
6. Blastocyst
(day 7-8)



7. Expanded blastocyst
(day 8-9)



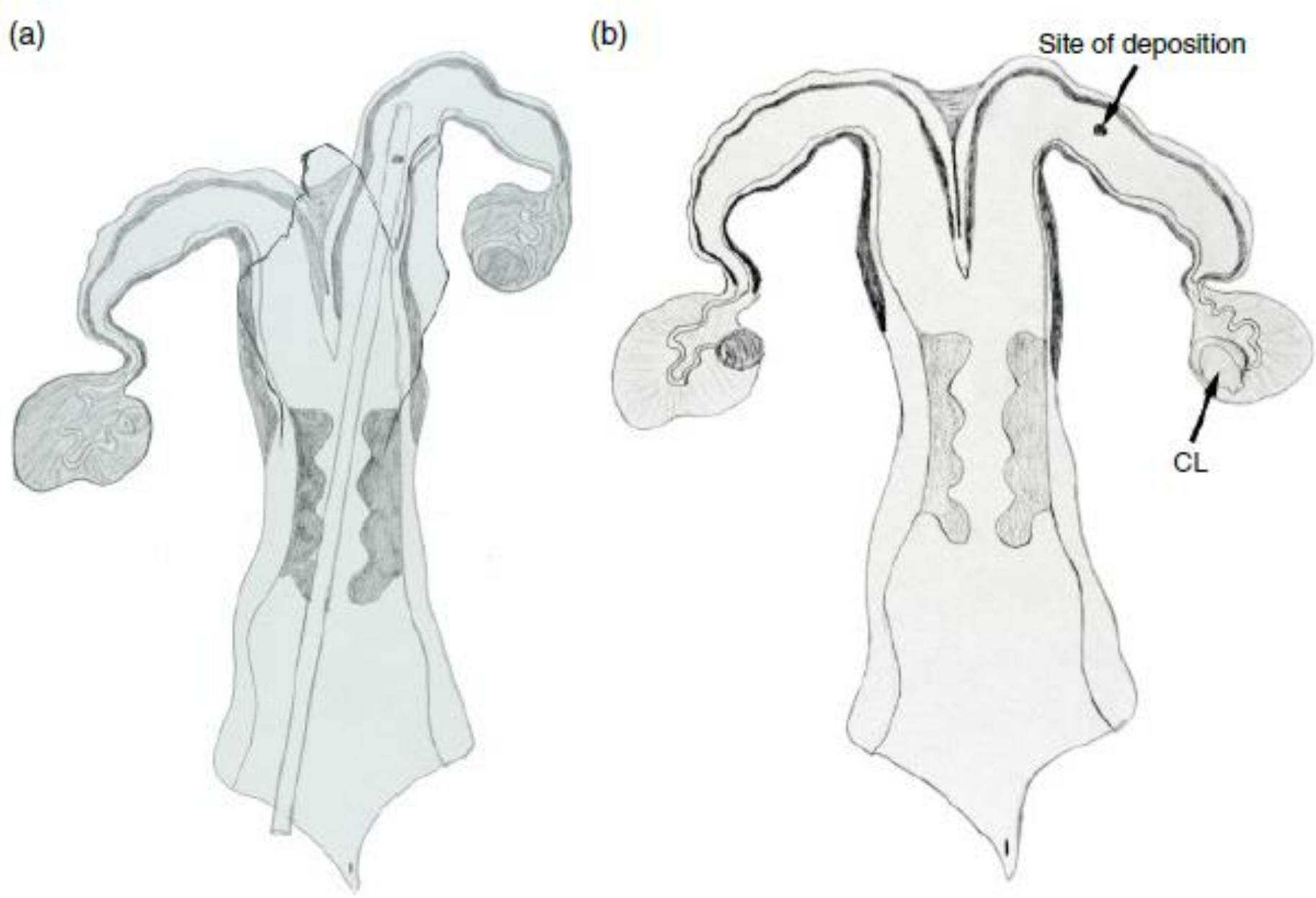
8. Hatched blastocyst
(day 9)



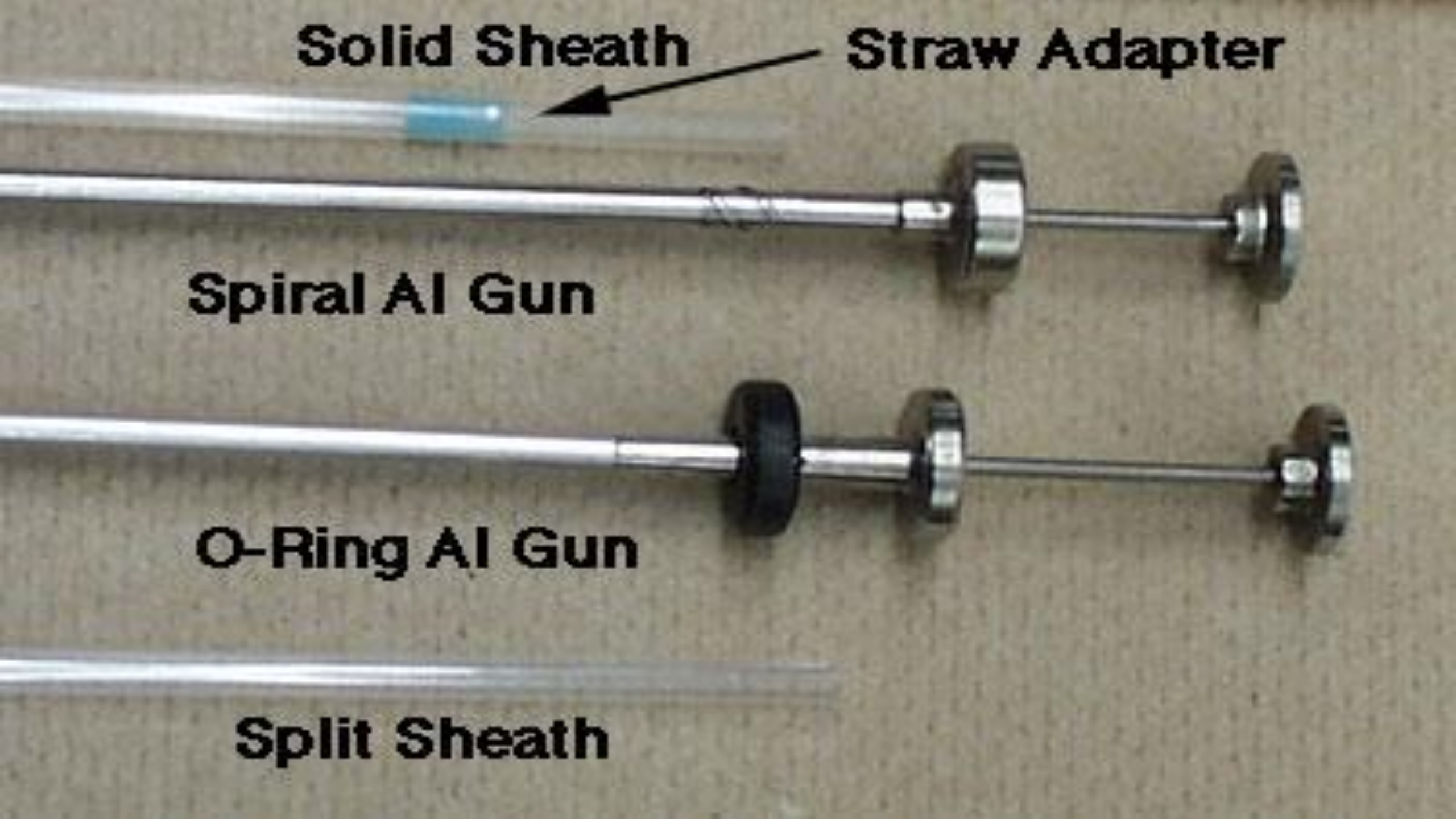
9. Expanding
hatched blastocyst
(day 9-10)

Transfer Embryos to Recipient

- Load in straws
- Non-surgical ET to recipient
- Transfer embryos 1 week after estrus
  - Closed cervix
  - Embryo in uterine horn on side of ovulation
  - AI straw gun



(a) and (b) Site of embryo deposition. CL, Corpus luteum


Solid Sheath
Straw Adapter
Spiral Al Gun
O-Ring Al Gun
Split Sheath

Pregnancy Diagnosis

- Uterine ultrasound
  - 30 days
- Rectal palpation
  - 45 days

Success rates of ET

- In vivo embryos
  - Fresh (60 %)
  - Frozen (50 %)

Success rates of ET

- In vitro embryos
  - Fresh (40 – 50 %)
    - ⇨ 60 % if transfer 2 embryos
  - Frozen (30 – 40 %)

Disadvantages of ET

- High cost
  - Professional fee
  - Drugs
  - Travel expenses
  - Recovery of embryos
  - Evaluation and storage of embryos
  - Transfer to recipient
- $ 700-2000

Disadvantages of ET

- 50 % of ET calves will be bulls
  - Limited market value unless from superior parents
- Variation in response to hormone treatment
- Careful selection of donor cows


Versana Active

EMBRYO PRODUCTION TECHNIQUES

IN VITRO EMBRYO PRODUCTION (IVP)

- In Latin, *in vitro* means **"in glass,"** and *in vitro* fertilization (IVF) is frequently used as a general term for the process of **generating embryos outside of the body**, which also includes ***in vitro* maturation (IVM)** and ***in vitro* culture (IVC)**

IN VITRO EMBRYO PRODUCTION (IVP)

- These procedures are conducted in the sequence :
  - **IVM–IVF–IVC** to produce embryos exclusively ***in vitro* (IVP)**

IN VITRO EMBRYO PRODUCTION (IVP)

- In vitro embryo production consists of three key steps that must be carried out in a specific sequence, ranging from **oocyte maturation** and **fertilization** to **embryo development**

IN VITRO EMBRYO PRODUCTION (IVP)

- **In 1959**, the rabbit was the first mammalian species in which live offspring were produced by IVF (Chang, 1968), followed by laboratory mice in 1968 (Whittingham, 1968)

IN VITRO EMBRYO PRODUCTION (IVP)

- The first successful IVF with cattle was in **1977** when sperm were capacitated in the oviduct or uterus of a cow or rabbit (Iritani and Niwa, 1977)
- The first live calf born in **1981** when a four-cell embryo was transferred into the oviduct of a recipient cow (Brackett et al., 1982)

IN VITRO EMBRYO PRODUCTION (IVP)

- During the 1990s, the bovine industry pioneered in vitro embryo production methods, which involve **fertilizing ova outside the body and then growing the resulting embryos in a lab**

IN VITRO EMBRYO PRODUCTION (IVP)

- Embryo transfers from in vitro-produced embryos have steadily increased, and by **2017**, around **1.5 million bovine embryos** (approximately **75%** of which are **IVP embryos**) were being transferred each year

IN VITRO EMBRYO PRODUCTION (IVP)

- **Glass** is rarely used for in vitro procedures today, having been **replaced** by disposable **plastics**, primarily made from **polystyrene** or **polypropylene**

Collection of Oocytes

1. Oocyte Collection from Living Cattle
2. Oocyte Collection from Excised Ovaries
3. Oocyte Collection from Slaughterhouse-Procured Ovaries

Collection of Oocytes

- Oocyte retrieval and in vitro embryo production may be practiced in **nonpregnant cows and heifers,** pregnant cows up to about **110 days of pregnancy**, and **postpartum cows not responsive to FSH treatment for superovulation**

Collection of Oocytes

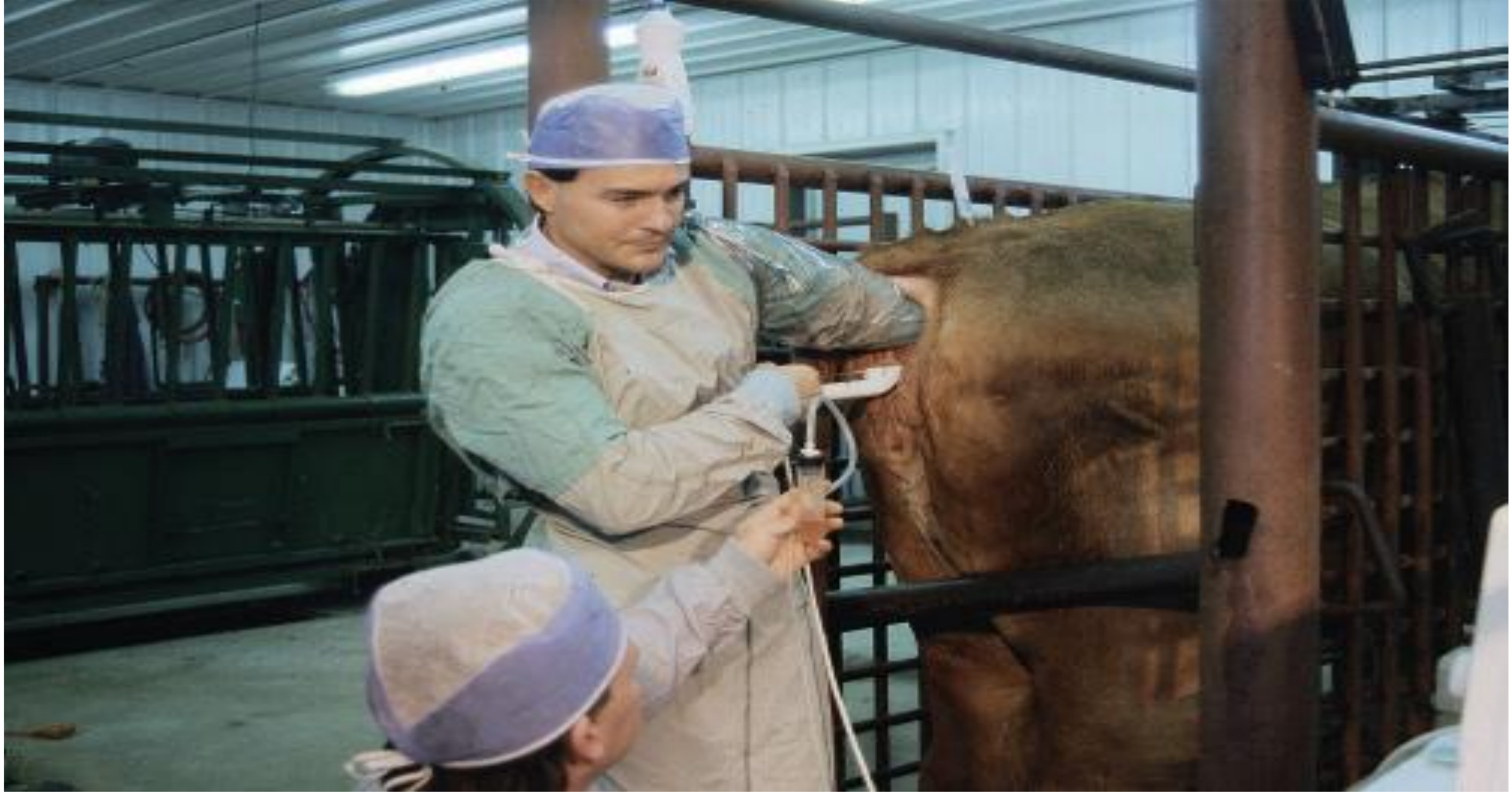
- Cattle producers often alternate between conventional **superovulation** and **IVP** in attempt to increase embryo yield from valuable donor cows

Applications of IVF technology

Commercial applications	Research applications
✓ Offspring from infertile cattle ✓ Offspring from pregnant cattle ✓ Offspring from young heifers prior to breeding age ✓ Salvage of genetics from terminally ill/injured cattle ✓ Efficient use of sexed semen ✓ Use of resorted semen (frozen–thawed and sexed after thawing) ✓ Use of multiple sires in a short period of time ✓ Utilization of slaughterhouse-derived oocytes for production of research and/or inexpensive embryos	✓ Improvement of IVF technology ✓ Improvement of IVC for cloning and transgenic procedures

1. Oocyte Collection from Living Cattle

- The primary method of collecting oocytes from live cattle is aspiration of ovaries manipulated per rectum and guided by a vaginally inserted ultrasound probe and needle



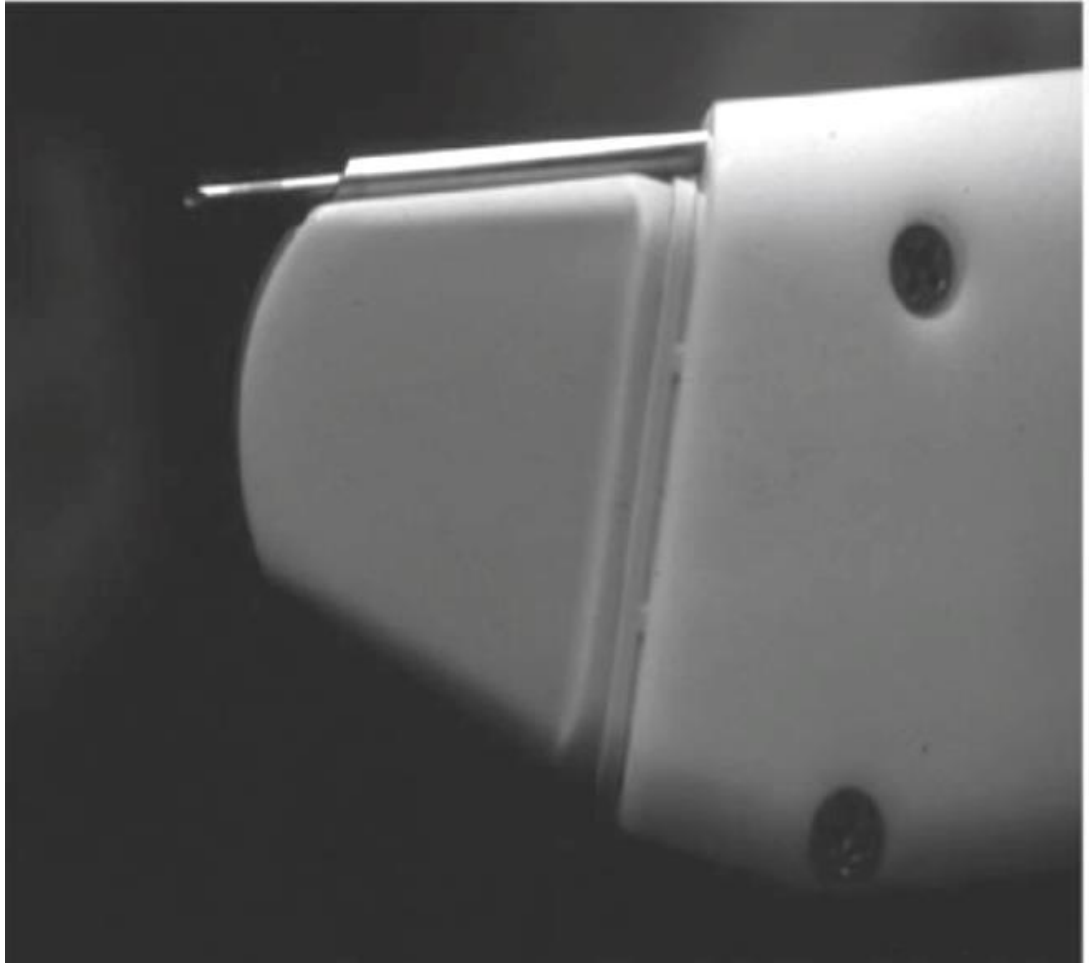
Demonstration of the technique to illustrate the ovum pickup procedure in a cow with a special curvilinear vaginal probe equipped with an elongated needle guide.
Please note that the ultrasonographer guides the vaginal probe to the ovary, which is secured via transrectal manipulation with the opposite hand

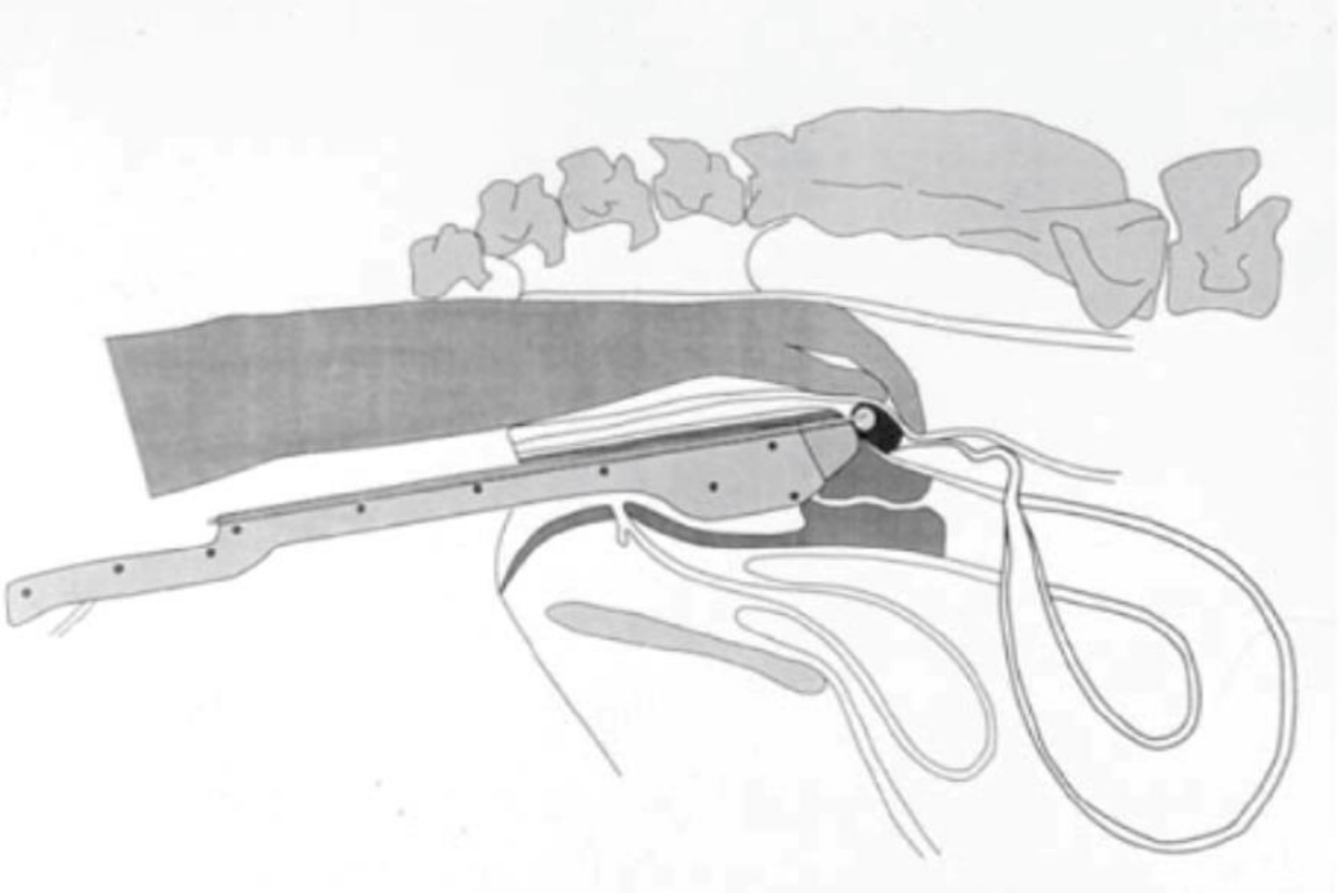
1. Oocyte Collection from Living Cattle

- Oocytes obtained surgically through a flank incision or laparoscopic procedures via the paralumbar fossa
  - **Expensive, inefficient, and risked the formation of adhesions with subsequent loss of fertility**

1. Oocyte Collection from Living Cattle

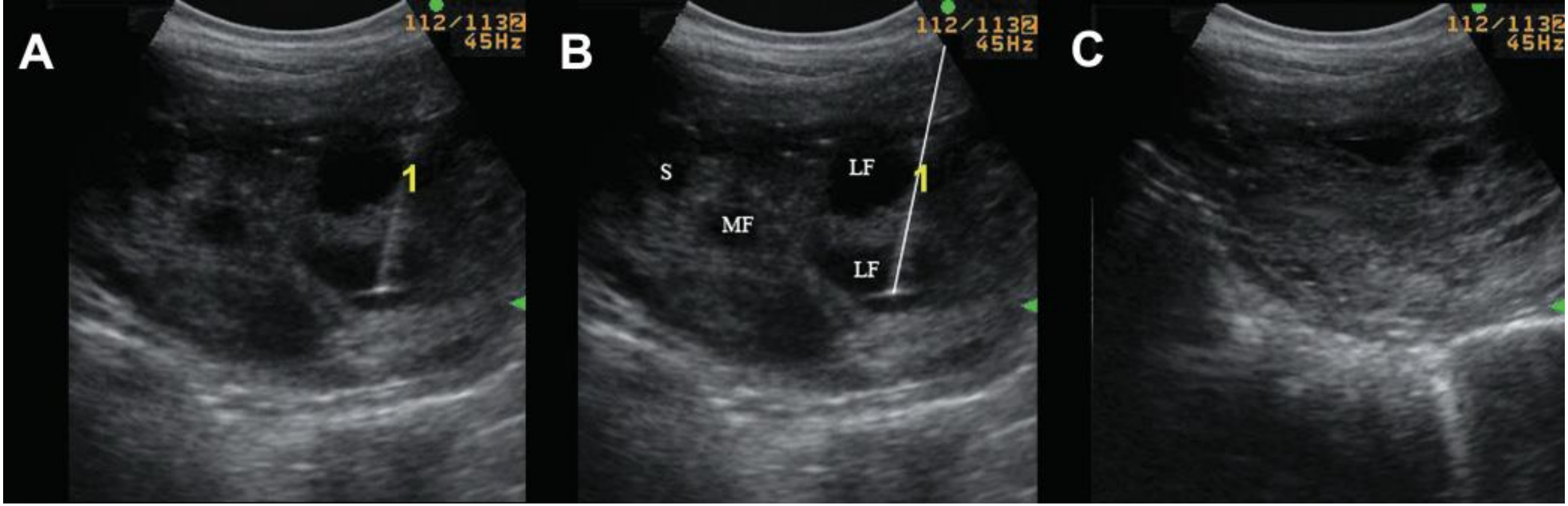
- The first repeatable, efficient technique involving **transvaginal ultrasoundguided aspiration** was developed in **1988**
- This technique has become widely known as **ovum pick-up (OPU) or transvaginal aspiration (TVA)**





a) Ultrasound transducer with oocyte aspiration needle protruding from needle guide.

b) Diagram showing position of ultrasound transducer pressed against vaginal fornix, with ovary manually manipulated per rectum and held up against vaginal wall



Ultrasonograms of the right ovary of a cow during and after an ovum pickup (OPU) procedure with the use of a special vaginal curvilinear probe equipped with an elongated needle guide. A and B: The elongated needle (1) passes through the vaginal wall and into the right ovary where the practitioner guides it into a follicle to retrieve a cumulus oocyte complex (COC). The tip of the needle has been slightly scratched at the factory to elicit a hyperechogenic effect to help guide the ultrasonographer. Once the needle enters the follicle, slight aspiration is applied to retrieve the follicular fluid and oocyte complex; C: Ultrasonogram immediately after the OPU procedure. The follicles are no longer present on the right ovary. However, frequently the larger aspirated follicles will fill back up with blood making them appear not to have been evacuated. An inexperienced OPU practitioner may reaspirate these blood-filled follicles creating a very bloody collection. LF: large follicle; MF: medium follicle; S: small follicle.

1. Oocyte Collection from Living Cattle

- The number of oocytes collected from a cow during a single session of OPU depends on a variety of factors
- On the mechanical side, the vacuum pressure used to remove the oocyte from the follicle, the gauge of the needle, and the length of the bevel on the needle

1. Oocyte Collection from Living Cattle

- Frequency of collection is another factor
  - OPU can be performed once or twice a week on the same cow without the use of exogenous hormones

1. Oocyte Collection from Living Cattle

- The number of oocytes collected per OPU can be increased by pretreatment of the donor with **gonadotropins**
- The use of FSH resulted in a higher number of usable embryos at the end of IVP than an injection of gonadotropin-releasing hormone (GnRH) or no pretreatment prior to OPU

1. Oocyte Collection from Living Cattle

- Breed of cattle
- **Age :** higher number of oocytes can be collected in cows 6–9 years old than in cows 14–18 years old

1. Oocyte Collection from Living Cattle

- The cow is restrained in a squeeze chute, and the external genitalia and perineal area are cleaned with a Betadine scrub, rinsed, and dried
- A standard epidural block of 2% lidocaine hydrochloride is administered to the donor cow, and a sedative may also be administered as needed

1. Oocyte Collection from Living Cattle

- The transvaginal aspiration system consists of a good quality ultrasound unit fitted with a 7.5MHz probe, a vacuum pump and regulator, a probe handle for housing the ultrasound probe, and aspiration needle

1. Oocyte Collection from Living Cattle

- The hub of the aspiration needle is attached by tubing to an oocyte collection container, such as a 50ml conical centrifuge tube or an embryo collection filter

1. Oocyte Collection from Living Cattle

- An additional piece of tubing connects the oocyte collection container to the vacuum pump
- The aspiration needle and tubing are first flushed with medium containing heparin
- The probe handle containing the ultrasound probe and aspiration needle is then inserted into the vaginal vault

1. Oocyte Collection from Living Cattle

- The operator's opposite hand stabilizes the ovary near the cranial vagina using per rectum palpation technique

1. Oocyte Collection from Living Cattle

- As ovarian follicles are visualized on the ultrasound screen, the operator carefully advances the aspiration needle through the vaginal wall and pierces follicles to be aspirated with the needle
- Follicular fluid and oocytes are aspirated into the collection tube or filter using a vacuum pressure of about 75mmHg

1. Oocyte Collection from Living Cattle

- Oocyte retrieval can begin in cows at approximately 30 days post partum, a time when cows are not usually responsive to superovulation treatment
- The TVOR procedure can be repeated as often as twice weekly until the desired number of oocytes is obtained from the cow

1. Oocyte Collection from Living Cattle

- Each TVOR procedure takes approximately 30 minutes to perform
- Oocyte yield following TVOR attempts is variable between donor cows; however, 4 to 6 oocytes suitable for IVP are commonly obtained from a healthy donor cow

1. Oocyte Collection from Living Cattle

- In a large study at a commercial ET facility, the mean number of oocytes per TVOR attempt in Holstein donor cows ranged **from 1.5 to 10.9 oocytes with 7.2% of oocyte collections yielding no oocytes.**

1. Oocyte Collection from Living Cattle

- Recovered oocytes in follicular fluid and medium should be maintained at 39° C
- Bovine oocytes may be shipped to an IVP facility using counter-to-counter or overnight services
- Oocytes may be shipped in oocyte maturation medium held in a temperature-controlled portable incubator

1. Oocyte Collection from Living Cattle

- Ovaries for shipment should be held in physiological saline with 0.75µg/ml penicillin in a sealed thermos bottle, and shipped in a cooler or Styrofoam box containing warm packs as needed
- After arrival at the IVP facility, oocytes are aspirated from follicles and placed in oocyte maturation medium

1. Oocyte Collection from Living Cattle

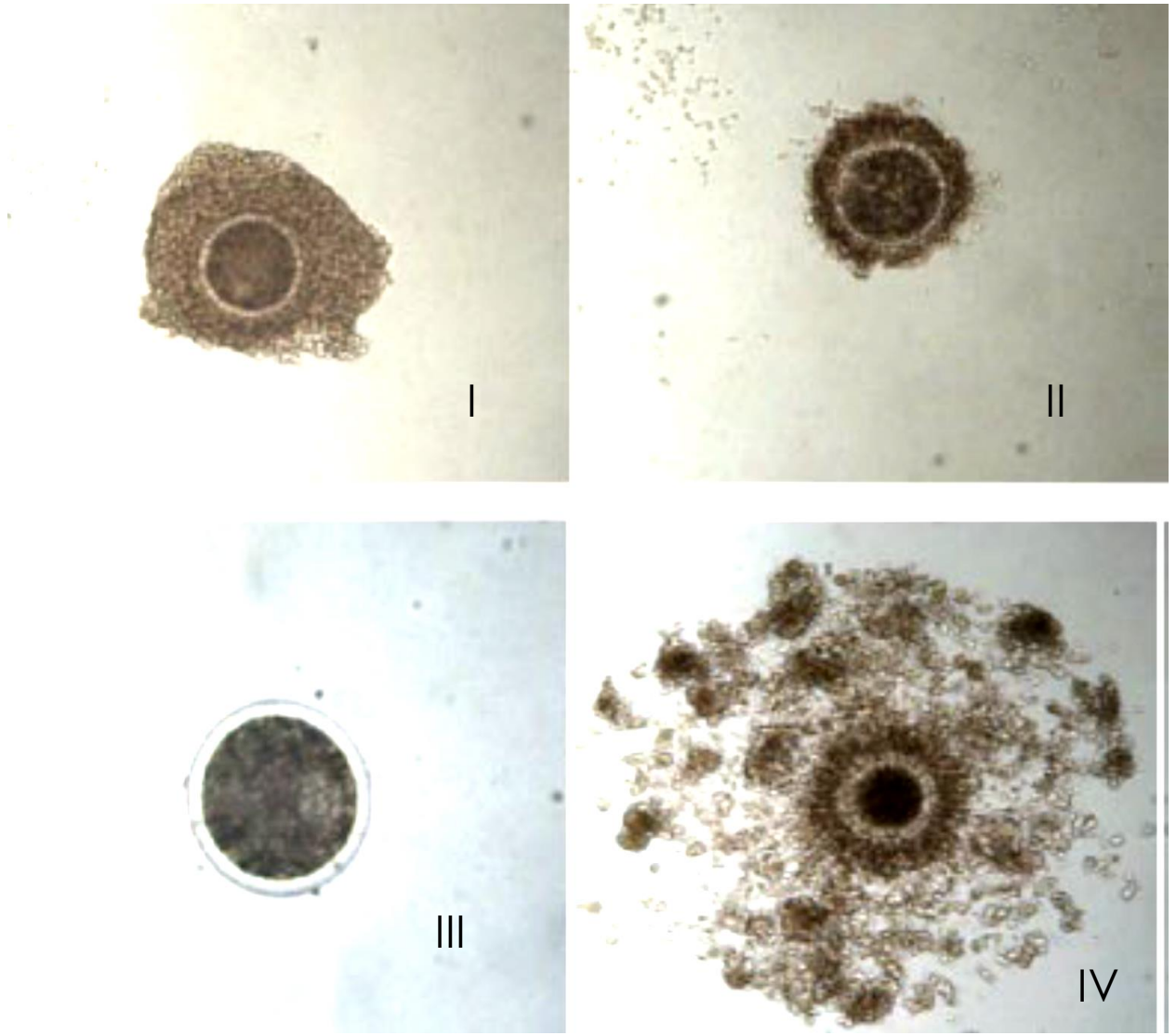
- Frozen semen to be used for IVP should be shipped to the facility either prior to or at the same time as shipment of the donor's oocytes or ovaries
- Ideally, the semen should first be tested in the IVP system using oocytes obtained at a slaughterhouse

2. Oocyte Collection from Excised Ovaries

- When cows develop terminal diseases or become crippled, ovaries can be removed via a flank laparotomy or through a vaginal incision and the oocytes recovered by follicular aspiration or slicing

3. Oocyte Collection from Slaughterhouse-Procured Ovaries

- All follicles between 3 and 8 mm in diameter are aspirated and the collected oocytes used to generate IVP embryos
- In addition to their commercial value, IVP embryos are produced in universities and labs around the world as a valuable source of material for bovine reproduction experiments



Types of bovine oocytes collected from cattle ovaries prior to IVM. I, >4 layers of cumulus; II, 1–3 layers of cumulus; III, nude oocyte with no cumulus; IV, expanded cumulus.

Potential for fertilization and embryonic development in different types of immature bovine oocytes

Oocyte type	Cumulus	No. of oocytes	Percentage cleaved	Percentage blastocysts	Percentage hatched
I	>4 layers	571	65^{a}	29^{a}	19^{a}
II	1–3 layers	228	40^{b}	8^{b}	6^{b}
III	Nude	289	38^{b}	$<1^{c}$	$<1^{c}$
IV	Expanded	151	38^{b}	7^{b}	5^{b}

^{abc} Values within a column with different superscripts differ significantly ($P < 0.025$).

In Vitro Procedures and Results

- In the laboratory, immature oocytes with their cumulus cells (cumulus oocyte complexes, COC) are washed in modified Tyrodes medium (TL-Hepes), and matured for approximately 22 hours in vitro using tissue culture medium-199 (TCM199) with supplements

In Vitro Procedures and Results

- At the end of the maturation period, COC are placed in fertilization medium (TALP medium with supplements) with thawed frozen spermatozoa selected for high motility using swimup or Percoll sperm separation procedures

In Vitro Procedures and Results

- Gametes are co-incubated for 18 to 20 hours after which time presumptive zygotes are washed in TL-Hepes and placed into culture medium

In Vitro Procedures and Results

- Bovine embryos have been successfully cultured to the blastocyst stage using undefined media (commonly, TCM199 plus co-culture cells, serum, and other components), semidefined media (e.g., modified synthetic oviductal fluid, SOF with BSA), and fully defined media (e.g., SOF with polyvinyl pyrrolidone, PVP)

In Vitro Procedures and Results

- The yield of transferable quality embryos after IVP varies from about 20% to 40% or greater
- Embryo yield from cows in poor body condition, terminally ill, or infertile is often low and more unpredictable than that from healthy cows

In Vitro Procedures and Results

- The consistent production of good to excellent quality morulae and blastocysts from an IVP system requires meticulous attention to detail

In Vitro Procedures and Results

- A number of factors can influence the survival of embryos produced using in vitro systems including medium composition, atmosphere, oocyte quality, and embryo genotype

In Vitro Procedures and Results

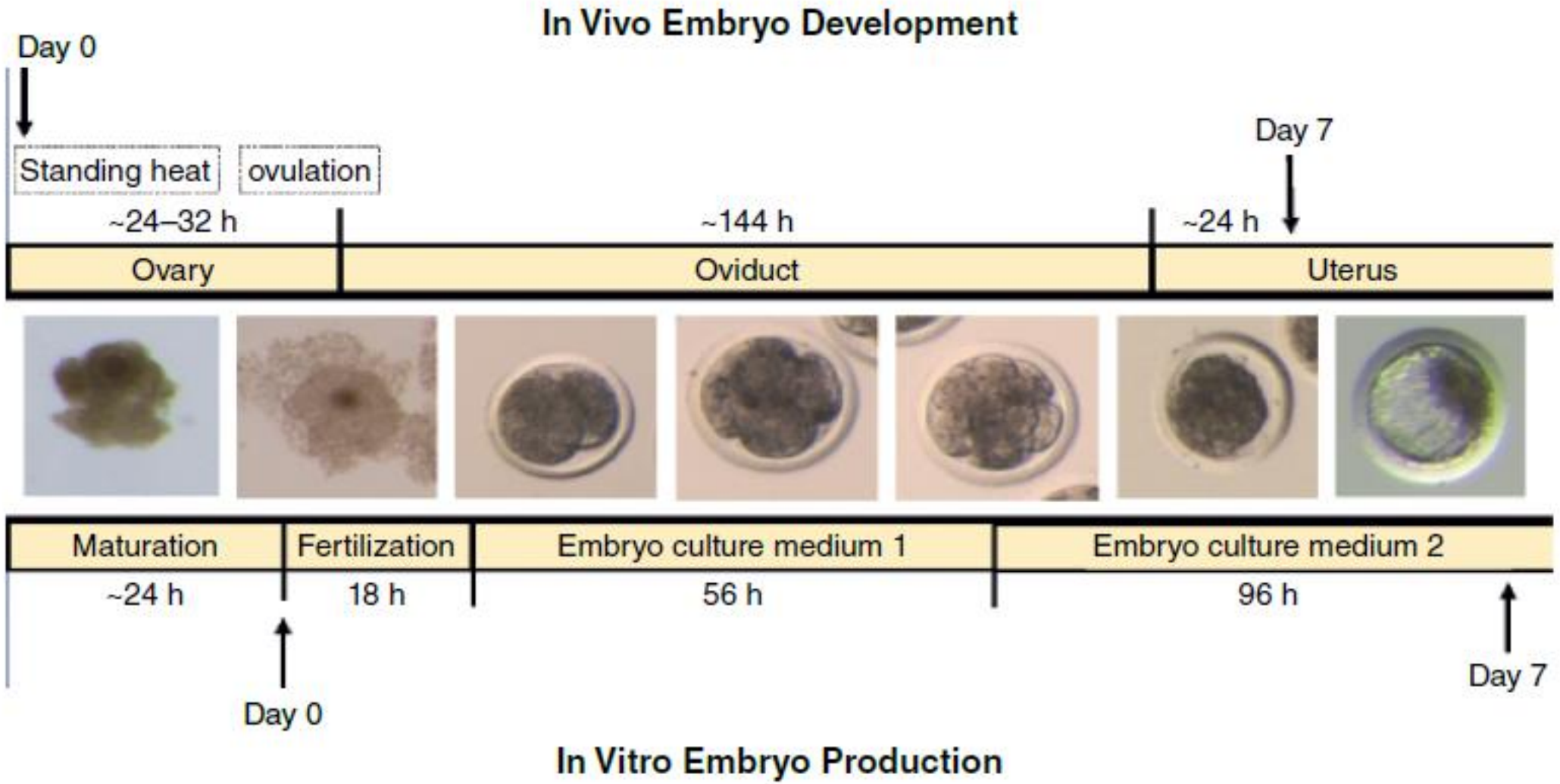
- Acceptable pregnancy rates can be achieved following transfer of in vitro–produced embryos; however, these pregnancy rates are often lower than those seen after transfer of in vivo–produced embryos

In Vitro Procedures and Results

- Pregnancy rates of recipients following transfer of in vitro–produced embryos of grade 1 (good/excellent) were greater than those for embryos of grade 2 (46.9% versus 35.6%; 60% versus 46%)

In Vitro Procedures and Results

- In addition to embryo quality, pregnancy rates after transfer of embryos produced in vitro are influenced by embry culture medium, stage of embryo development, fresh versus frozen embryos, and synchrony of embryo development and recipient's day of the estrous cycle



Comparison of early embryo development *in vitro* to *in vivo*

Cloning

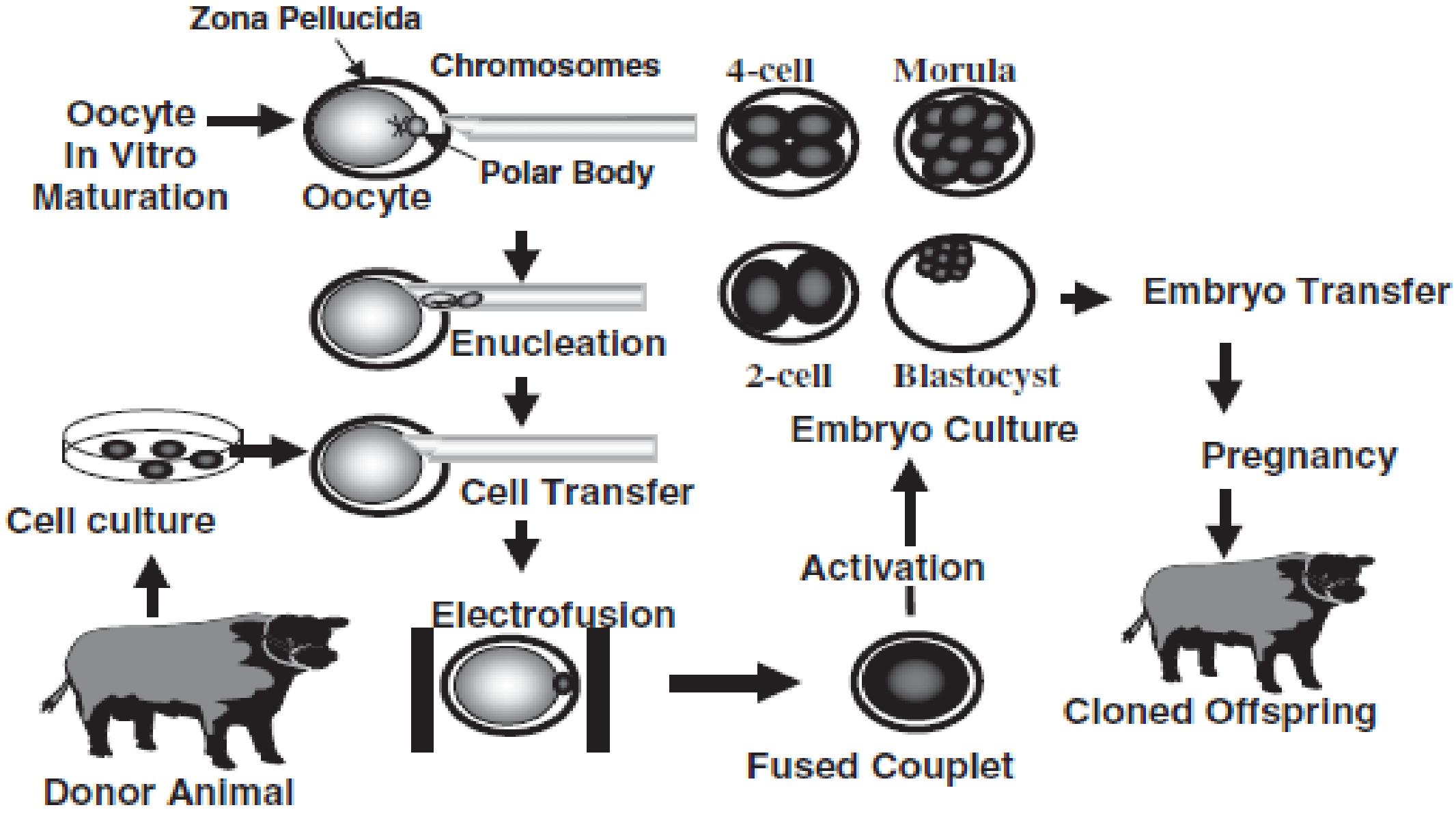
- Cloning is the process of creating genetically identical individuals
- Techniques like **somatic cell nuclear transfer (SCNT)**, which involves transferring a somatic cell's 2n nucleus into an enucleated ovum, can be used to accomplish this
- Cloning by **embryonic cell nuclear transfer** can also be used

Cloning

- Cloning is the production of a copy or copies of an individual and occurs in animals either naturally or artificially, when an embryo is split to produce identical twins
- The word “clone” has also been used to describe animals produced by nuclear transfer for the production of an unlimited number of genetically identical offspring

Cloning

- The first successes in cloning livestock were with sheep, by fusing a cell from a 16-cell embryo to an oocyte that had its chromosomes removed (enucleated oocyte)


Zona Pellucida
Chromosomes
Oocyte
In Vitro
Maturation
Oocyte
Polar Body
4-cell
Morula
Enucleation
2-cell
Blastocyst
Embryo Transfer
Embryo Culture
Pregnancy
Cell culture
Cell Transfer
Activation
Electrofusion
Cloned Offspring
Donor Animal
Fused Couplet

Cloning

- The biggest breakthrough in nuclear transfer came when it was demonstrated that a viable offspring could be produced by fusing cultured adult somatic cells with enucleated oocytes to produce Dolly the sheep in 1997

Factors influencing the efficiency of cloning

1. The cell cycle stage
2. Type of donor cell
3. The quality of recipient oocytes
4. Micromanipulation techniques
5. Activation protocols
6. The extent of nuclear reprogramming

Cloning by Somatic Cell Nuclear Transfer

- The reconstruction process comprising the fusion of an enucleated oocyte with a donor somatic cell, followed by oocyte activation represents a critical step, as it initiates the reprogramming of the somatic nucleus to a totipotent embryonic state

Cloning by Somatic Cell Nuclear Transfer

- The primary cause of SCNT's extremely low effectiveness to date, albeit 25 years after the first mammalian clone was created, is **incorrect or insufficient epigenetic reprogramming of the donor cell genome**

Cloning by Somatic Cell Nuclear Transfer

- Somatic cell nuclear transfer (SCNT) embryos continue to exhibit **elevated incidences** of **early embryonic loss**, **late-term abortion**, **abnormal organ development**, and **perinatal morbidity across all examined species**

Cloning by Somatic Cell Nuclear Transfer

- Despite advancements in in-vitro culture systems, **postnatal survival rates** remain **suboptimal** and require further optimization

Cloning by Somatic Cell Nuclear Transfer

- Developmental anomalies are frequently attributed to:
  - **epigenetic disruptions** induced by **culture conditions**, which can adversely **affect nuclear reprogramming** and **compromise the ability of the culture environment to adequately support the development of reconstructed embryos**