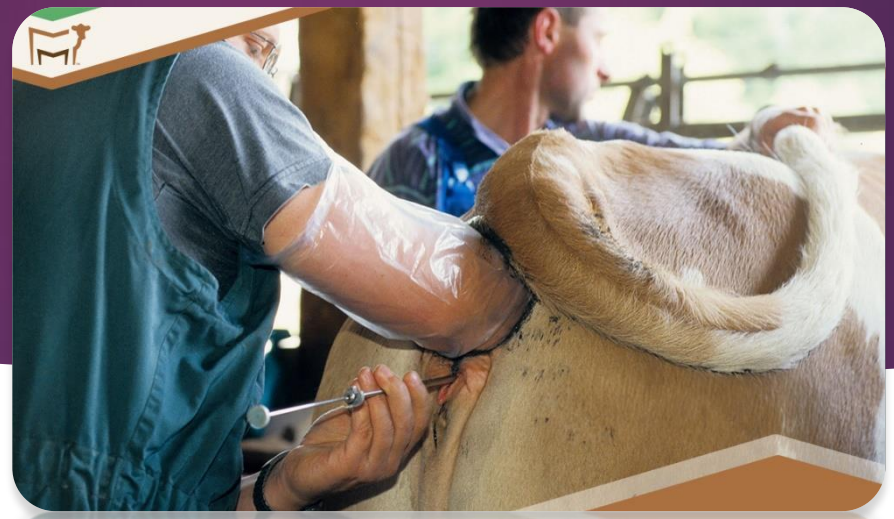
ARTIFICIAL INSEMINATION IN CATTLE



جامعة الإخوة منتوري قسنطينة
UNIVERSITE DES FRERES MENTOURI CONSTANTINE

THERIOGENOLOGY LECTURES – A5

Prof. Sana HIRECHE

History

- The arabs used artificial insemination for horses during the fourteenth century
- The first report regarding the successful use of AI was in 1780 by the Italian physiologist Lázaro Spallanzani, who inseminated a bitch that delivered three puppies 62 days later (Spallanzani 1776)

History

- It took >100 years until researchers established much of the foundation for using AI in other species
- 1890: the French veterinarian Repiquet used AI in horse breeding work

History

- Ilyá Ivanovich Ivanov (1870-1932) was the leading authority and pioneer investigator in the field application of AI in Europe
- Ilyá Ivanovich Ivanov (1899) developed semen extenders and established practical AI procedures in mares in Russia

History

- 1909, Russians began inseminating cattle
- 1928: Russians inseminated some 1.2 million cattle and 15.0 million sheep
- During World War I (1914-1918), thousands of mares were inseminated

History

- In 1914, Professor G. Amantea, University of Rome, constructed an artificial vagina for dogs
- From 1934 to 1938, the artificial vagina for cattle was developed by European and American workers

History

- In 1936, a large cooperative association for the artificial insemination of cattle was organized in Denmark. It had about 200 members, and over 1,000 cows were inseminated the first year
- Since 1936, the AI program has grown steadily and is now in use for livestock improvement throughout the world

History

- 1968 : Cassou further developed the straws by reducing their diameter for better freezing and named it as “mini French straw” (135 mm long and 2.8 mm diameter with 0.5 mL semen capacity)
- 1972: Simmet introduced a new straw called “mini tube” or “German straws” or “Landshut system” in Germany

Artificial Insemination Worldwide

- Dr. W. Bielanski, Agricultural College, Krakow, Poland, reported a survey completed in 1962 in which he estimated the total number of cattle in the worldwide AI program at about 60 million

Artificial Insemination Worldwide

- Milovanov (1938) developed artificial vaginas and established major projects for sheep and cattle breeding
- In Japan, Nishikawa (1912) began an AI program in horses that was later applied to cattle, sheep, goats, swine, and poultry

Artificial Insemination Worldwide

- In 1936, Gylling-Holm and Eduard Sørensen established the first cooperative dairy AI organization in Denmark, which enrolled 1070 cows with a 59% conception rate in the program's first year

Artificial Insemination Worldwide

- Dr. Perry visited the Danish cooperative and after returning to the United States, organized the New Jersey Holstein Breeders Cooperative Association (1938)

Artificial Insemination Worldwide

- Danish veterinarians developed the recto-vaginal technique of AI into the uterine body, enabling use of fewer sperm
- Straws for packaging semen were developed by Dr. Sørensen (1940) and modified by Robert Cassou (1964)

Advantages and disadvantages of artificial insemination

- AI in animals was originally developed to control the spread of disease, by avoiding the transport of animals with potential pathogens to other animal units for mating and by avoiding physical contact between individuals

Advantages and disadvantages of artificial insemination

- The use of semen extenders containing antibiotics also helped to prevent the transmission of bacterial diseases

Advantages

- AI helps prevents the spread of infectious or contagious diseases, that can be passed on when animals are in close contact or share the same environment
- The rate of genetic development and production gain can be increased, by using semen from males of high genetic merit for superior females

Advantages

- It enables breeding between animals in different geographic locations, or at different times (even after the male´s death)
- Breeding can occur in the event of physical, physiological or behavioural abnormalities
- AI is a powerful tool when linked to other reproductive biotechnologies such as sperm cryopreservation, sperm sexing
- AI can be used in conservation of rare breeds or endangered species

Herd improvement

- The use of selected proven sires through A.I. brings about genetic improvement in the herd.

Economical technique

- In A.I. programmes only selected bulls are kept for breeding purpose
- This reduces work load and expenditure on bulls

Maximum utilization of sire

- Several insemination doses are prepared from one ejaculate and thus proven sires are utilized to their maximum
- Through the use of A.I., a single bull in its life-time may easily cover up to I lac female cattle

Low transportation cost

- Through the export/import of semen, international transportation cost is reduced which otherwise would greatly exceed to actual transportation of the animals

Better safety

- Since only selected bulls are kept in A.I. programmes, the dangers are minimized
- Since males are not brought to the farm or females, it further leads to better safety

Early detection of diseases

- Since during semen collection or during artificial insemination, the genitalia of both males and females are closely approached and inspected, the genital defects are easily noted, which otherwise may remain undetected for longer period of time

Covering cows that refuse mounting

- Some females which are in good heat, but for some reason or the other refuse to be mounted, may be bred through AI

Overcoming physical inability

- The bulls which for some reason or other are unable to copulate, may be utilized in artificial
- insemination (e.g. from such bull semen may be collected using electro-ejaculator)

Overcoming different sizes of two partners

- Great variations in the size of male and female partners may prevent natural mating
- Cow with either extremely large or extremely small size may be covered through A.I. without any difficulty

Reduced risk of venereal diseases

- Bulls kept for A.I. purpose are regularily examined for their general as well as reproductive health
- Diseased bulls are either properly treated or culled. This reduces risk of spreading sexually transmitted diseases

Utilizing germplasm of dead animals

- The deep freezed semen of the bull, which has died, may be utilized in artificial insemination programme

Disadvantages

- Some males shed virus in semen without clinical signs of disease ("shedders")
- Some bacterial pathogens are resistant to the antibiotics in semen extenders or can avoid their effects by forming bio-films

Disadvantages

- There has been a decline in fertility in dairy cattle and horses associated with an increase in AI
- The focus on certain individuals may result in loss of genetic variation

Viruses in semen

- Cryopreserved semen doses can be “quarantined” until the male is shown to have been free of disease at the time of semen collection
- In contrast, the short shelf-life of fresh semen doses means that they must be inseminated into the female before the disease-free status of the male has been established

Viruses in semen

- Breeding sires used for semen collection are tested routinely for the presence of antibodies in serum as being indicative of past infection, but some viruses, e.g. equine arteritis virus, may be shed in semen for several weeks before there is evidence of sero-conversion

Viruses in semen

- In other cases, usually of congenital infection, individuals may be permanent virus “shedders” without ever developing antibodies
- Semen from these individuals represents a source of pathogens for disease transmission to naive females

Bacteria in semen

- Normally, in a healthy male, the ejaculate itself does not contain microorganisms, but contamination occurs at semen collection from the prepuce and foreskin, the male´s abdomen and the environment

Bacteria in semen

- Semen processing from livestock usually takes place without access to a laminar air flow hood, resulting in potential contamination from the laboratory environment

Bacteria in semen

- Antibiotics are added to semen extenders to limit the growth of these contaminants and prevent disease in the inseminated female

Antibiotics in semen extenders

- The addition of antibiotics to semen extenders is controlled by government directives, both nationally and internationally, which state the types of antibiotic to be used and also their concentrations

Antibiotics in semen extenders

- In general, there is a tendency to use broad spectrum, highly potent antibiotics in various combinations to reduce sperm toxicity

HACCP
Hazard Analysis & Critical Control Points

Critical Control Points

- Cow in estrus
- Reproductive Health
- Disease Free
- Optimal body weight

- Fertile Bull
- Calving Ease
- High Genetic Merit
- Disease Free

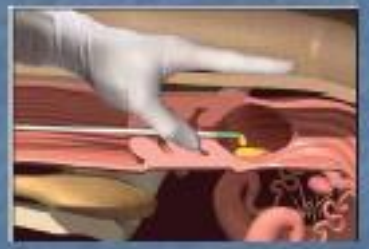
- Semen Storage
- Liquid Nitrogen (-196°C)
- Semen Identification
- Correct Handling

- Clean Equipment

- Thaw Semen
- 33°C to 35°C (95°F)
- 45 – 60 s
- Avoid Cold Shock

10 – 15 minutes

- Adequate restraint
- Work cleanly
- Work Gently
- Take your time



- Training and practice!!
- Be Gentle: Avoid force
- 2-step process
- Deposit semen just through cervix

Equipment required for artificial insemination

- **Tank**

Aluminium vacuum-insulated vessel used to hold semen and liquid nitrogen

- **Canister**

Removable cylinder with a mesh or solid bottom to hold semen in the tank. It has a long hooked handle to permit straw identification and access from the mouth of the tank

Equipment required for artificial insemination

- **Mini-goblet**

Plastic cylinder with a sealed base, and which fits into the canister. It will hold up to twenty-five straws in a bath of liquid nitrogen

- **Straws**

Each straw contains enough semen to inseminate a cow once. The volume of semen in the mini-straw is 0.25 ml, which normally contains 20 million sperm cells with a usual minimum of 40% live at thaw

HORMONAL SYNCHRONIZATION TECHNIQUES

- For AI and ET to be as successful as possible, hormonal synchronization techniques are essential
- By controlling the female's estrous cycle, these methods guarantee that insemination occurs at the ideal moment

HORMONAL SYNCHRONIZATION TECHNIQUES

- By guaranteeing ideal physiological conditions for fertilization and embryo implantation, synchronization procedures increase success rates

Typical protocols: Progestin-Based Protocol

- Begins with the administration of a progestin, such as a Controlled Internal Drug Release (CIDR) device or Melengestrol Acetate (MGA), on Day 0
- These progestins mimic the luteal phase of the estrous cycle, effectively suppressing estrus
- Between Days 7 and 9, the progestin device (CIDR) is either removed, or MGA administration is discontinued

Typical protocols: Progestin-Based Protocol

- Subsequently, a prostaglandin (PGF2a) injection, such as **dinoprost** or **cloprostenol**, is administered to induce luteolysis, leading to the regression of the corpus luteum and the initiation of estrus, typically between Days 8 and 10
- Insemination should occur during **standing estrus**

GnRH and Prostaglandin Protocol

- This protocol initiates with the administration of a GnRH (Gonadotropin-releasing hormone) injection, such as **Cystorelin** or **Factrel**, on **Day 0**

GnRH and Prostaglandin Protocol

- GnRH stimulates the release of luteinizing hormone (LH), which promotes follicular maturation
- Between Days 7 and 9, a PGF2a injection is given to induce luteolysis, resulting in corpus luteum regression

GnRH and Prostaglandin Protocol

- On **Days 10–12**, a **second GnRH injection** is administered to induce **ovulation**
- **Insemination** should occur **12–24 h following the second GnRH injection**, aligning with the expected time of ovulation, typically **between Days 12 and 14**

Ovsynch Protocol

- Starts on Day 0 with the administration of a **GnRH injection** (e.g., Cystorelin or Factrel), which synchronizes follicular development
- On **Day 7**, a **PGF2a** (prostaglandin) injection is given to induce luteolysis and corpus luteum regression

Ovsynch Protocol

- A **second GnRH** injection is administered on **Day 9** to stimulate ovulation.
- Insemination is carried out **12–16 h after the second GnRH injection**, corresponding to the expected time of **ovulation**, typically **between Days 10 and 12**

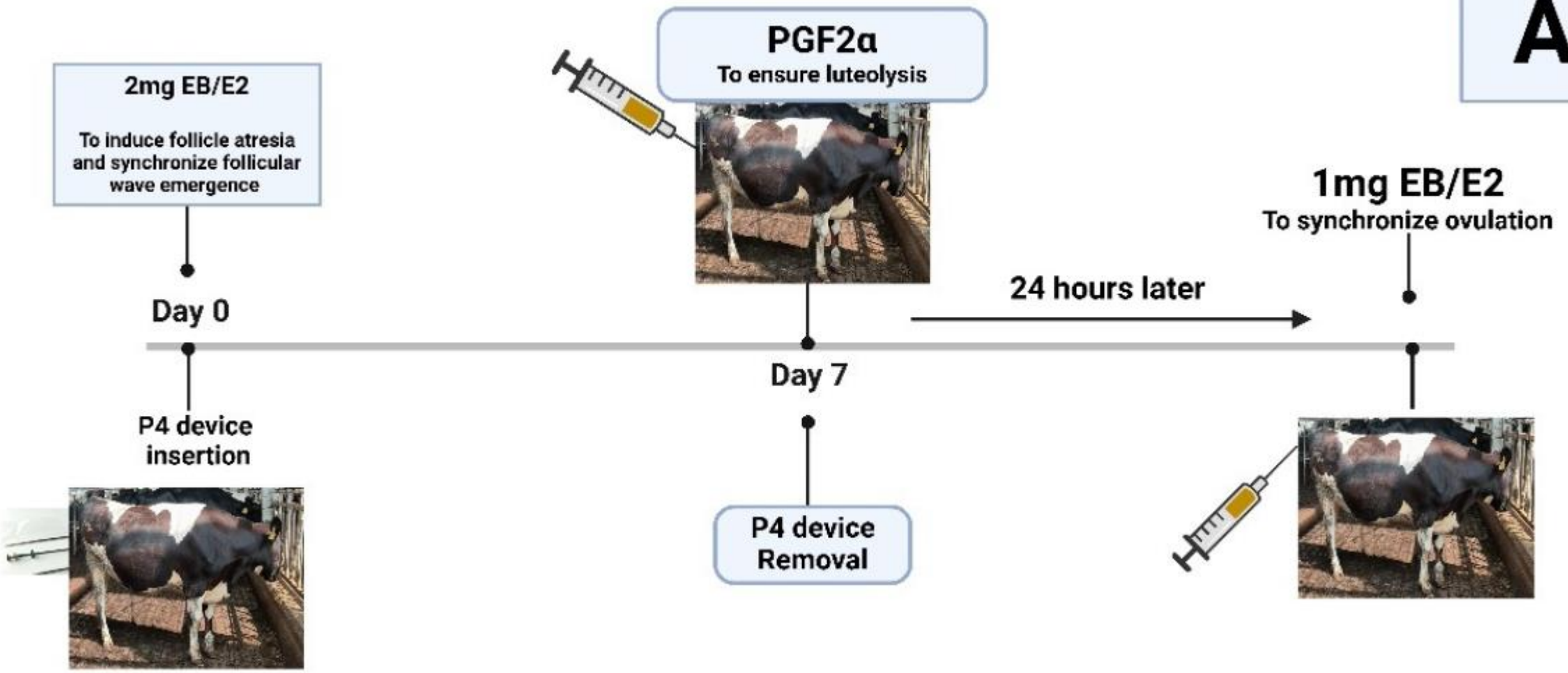
CIDR + PGF2a Protocol

- On **Day 0**, a **CIDR** (Controlled Internal Drug Release) device containing **progesterone** is inserted to regulate the estrous cycle
- On **Day 7**, the CIDR device is **removed**, and a **PGF2a injection** is administered to induce luteolysis, leading to the regression of the corpus luteum

CIDR PROGRAMS

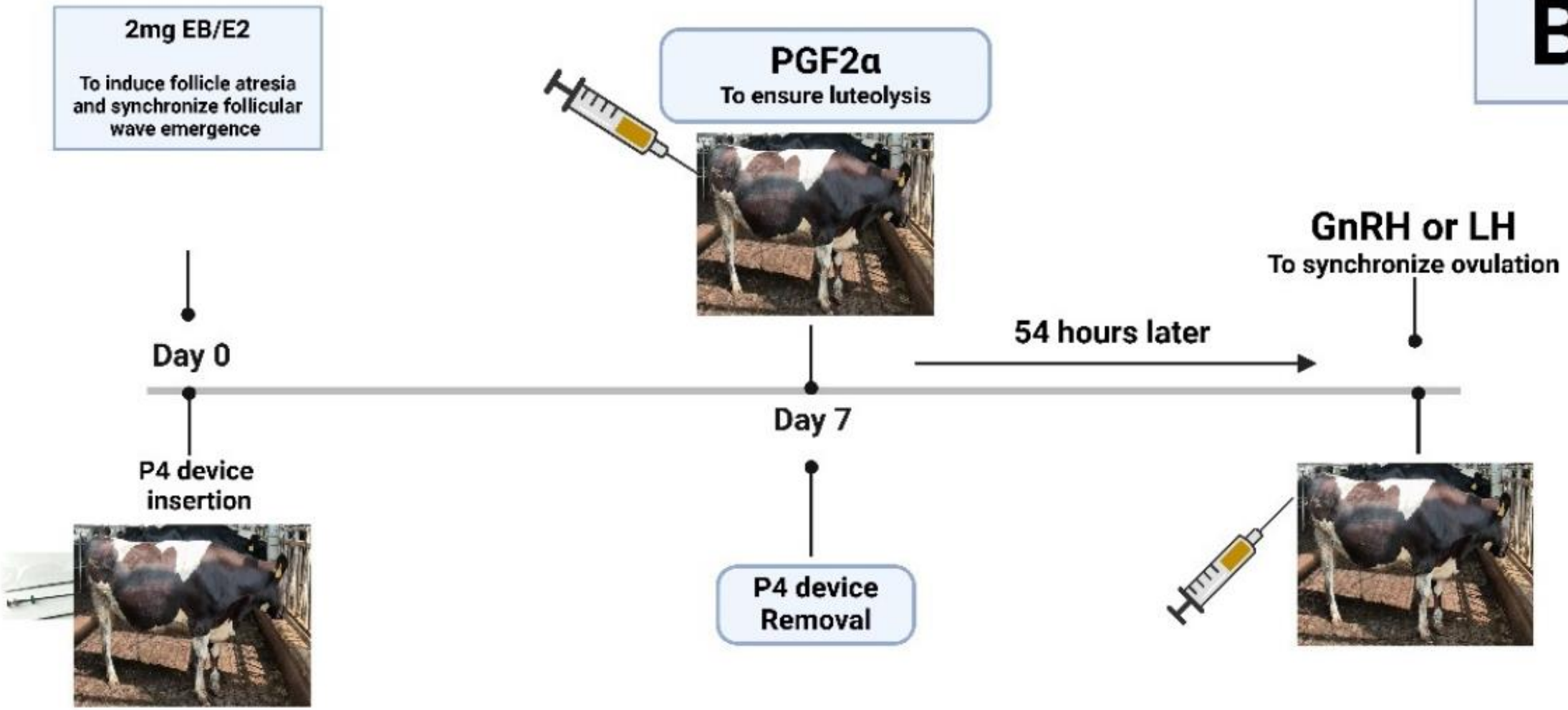
- Day 0 = CIDR In
- Day 6 = PgF2a
- Day 7 = CIDR Out
- Day 8 – 10 = Estrus Detection and AI

A



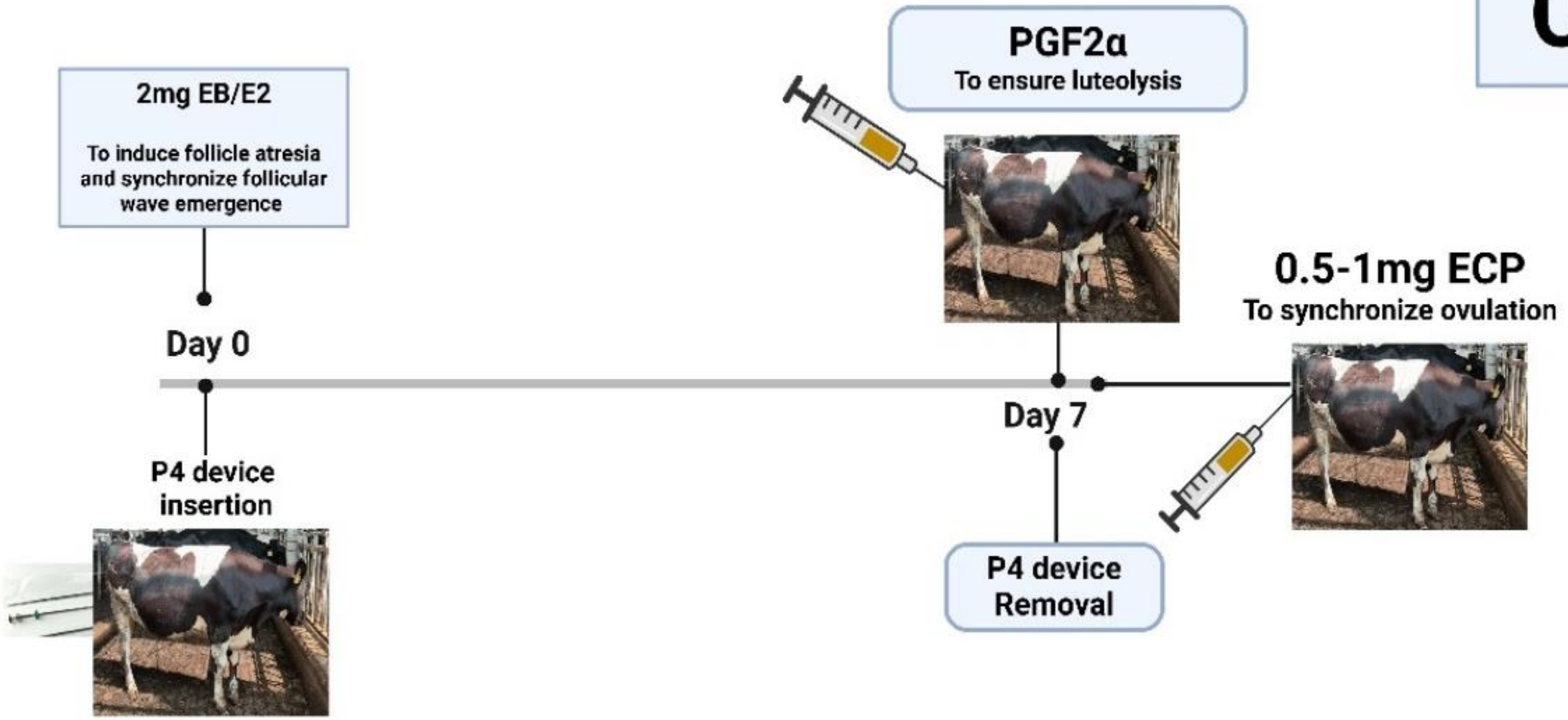
1 mg of EB/E2 24 h after PGF2α injection

B

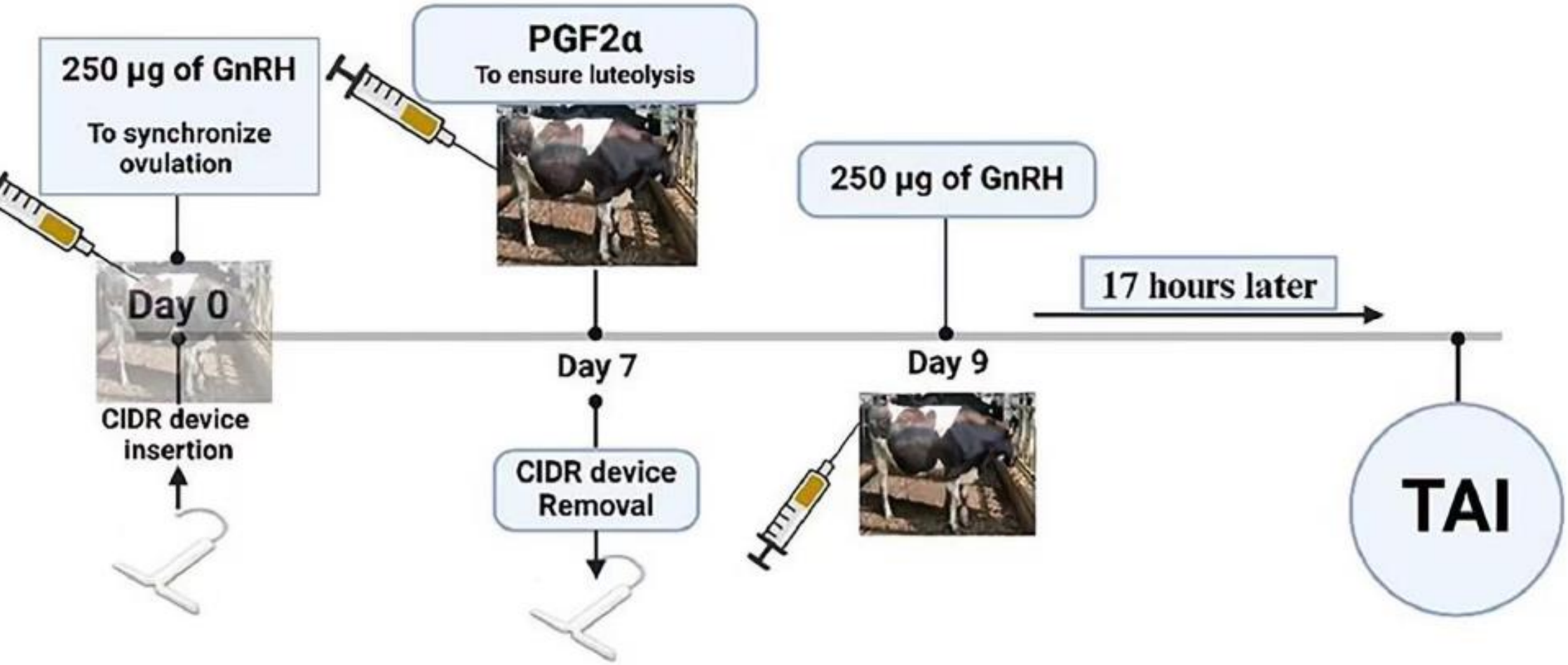


GnRH 54 h after PGF2α injection

C



PGF2α and ECP administered on Day 7 after removing the P4 device



Gonadotropin-releasing hormone (GnRH)-based protocols

When to inseminate a cow

- Best conception rates are achieved in cows inseminated **2–26 hours after first being observed on heat**
- The ideal time to inseminate is **12 hours after the onset of heat**
- The average cow is on heat for **18 hours** and releases the egg from the ovary about **12 hours after the end of heat**

When to inseminate a cow

- The egg must be fertilised within **ten hours of ovulation**, otherwise it dies

Twice-a-day insemination

- Some inseminators prefer to inseminate herds **twice daily**, thus reducing the number of cows requiring insemination at one time
- They do this by timing insemination as follows:
  - Cows first seen on heat during the day or at the evening milking are inseminated the next morning
  - Cows first seen on heat during the night or at the morning milking are inseminated in the evening

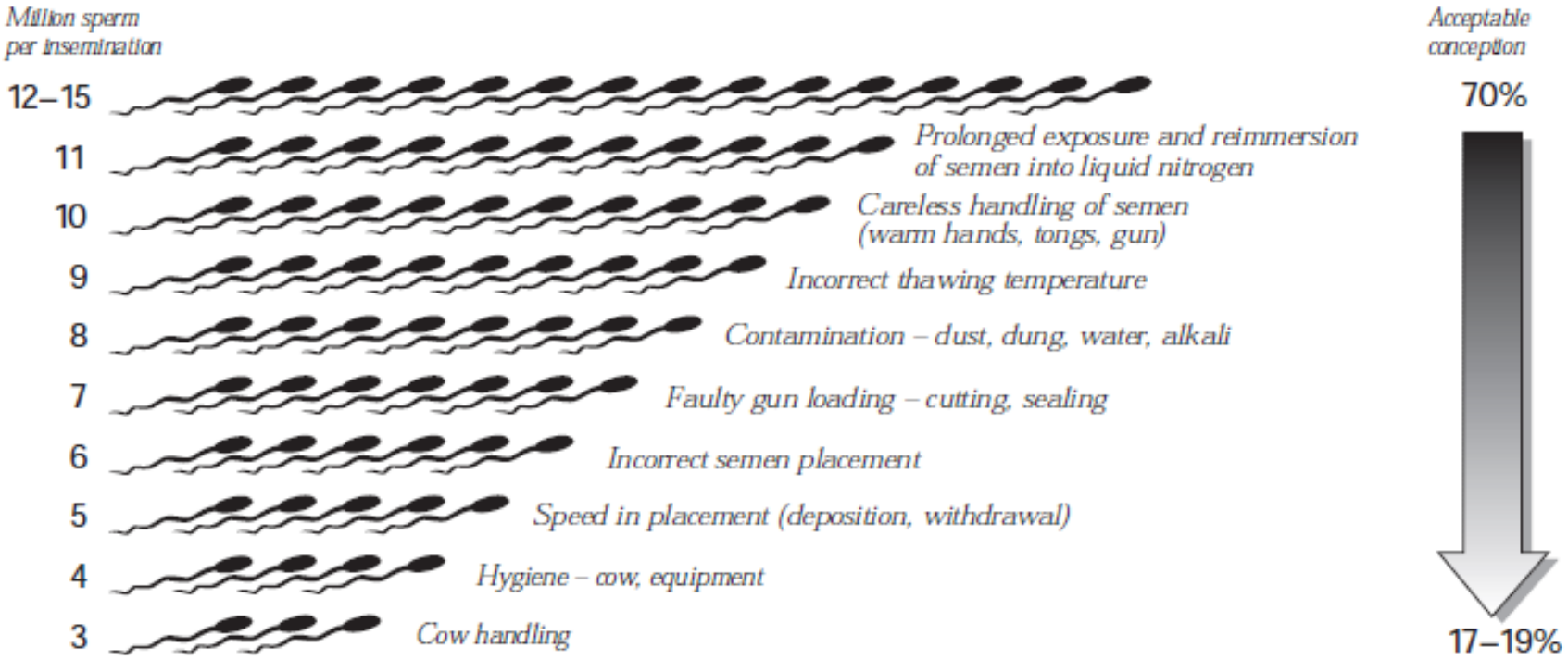
Once-a-day insemination

- Some inseminators prefer to inseminate all cows at one time during the day
- Cows on heat in the **last 24 hours are all inseminated at the one time**
- Inseminations should be done at a **similar time each day**
- Cows on a rising energy plane will give significantly higher conception rates

ARTIFICIAL INSEMINATION CONCEPTION CHART

Artificial insemination conception chart

Based on a cow receiving an average 12–15 million live sperm at time of insemination (natural mating 5–6 thousand million), this chart shows factors that can make the difference between success and failure with an AI programme.



Remember semen is fragile and highly perishable

Carelessness on one point may have little effect: carelessness on a number of points shown could seriously lower results. ***Attention to detail*** *with AI is the key to success.*

Thawing semen

- Frozen semen should not be exposed to air for more than two seconds during a transfer
- The recommended temperature for thawing frozen semen is **32°C** to **37°C** and any equipment that comes into contact with thawed semen should be kept warm to prevent cell damage
- Semen starts thawing at –130°C

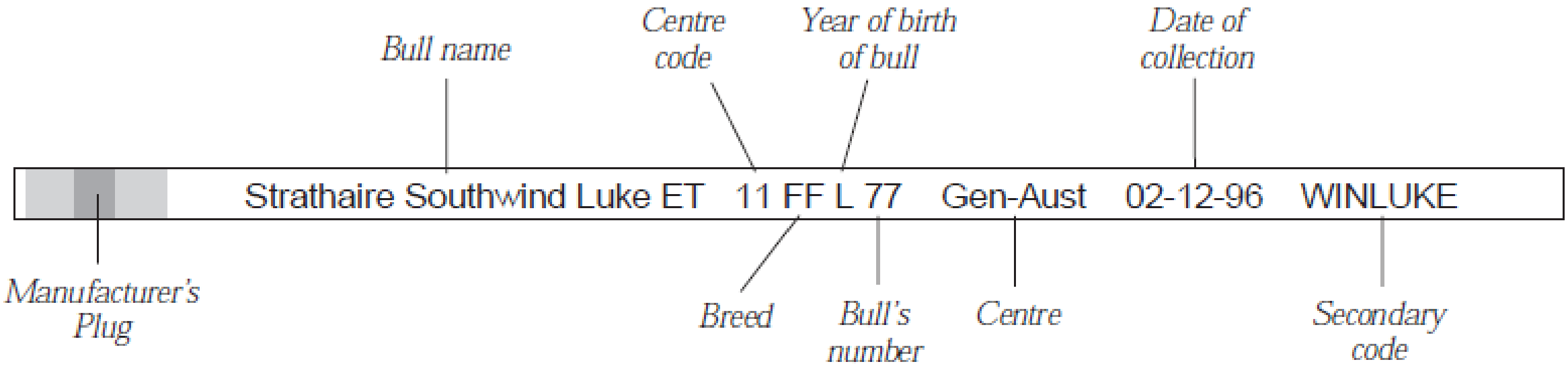
Quality control

- Following processing, sample straws from each batch of semen are thawed and visually appraised using a microscope with a specialised optical system that clearly shows living cells such as sperm cells
- Semen is checked for motility percentages and swimming characteristics
- Abnormal sperm cells and concentration are also assessed
- If the semen does not meet strict minimum quality standards, the batch is discarded
- The discard rate is approximately 10% of the semen processed

Straw identification

- Genetics Australia semen is packed in colored straws, coded according to individual breeds
- On each straw is printed the information required to correctly identify the individual bull

Apart from the bull's full name, there is a seven-character primary NASIS code, e.g. **11FFL77,** which is determined according to the following system



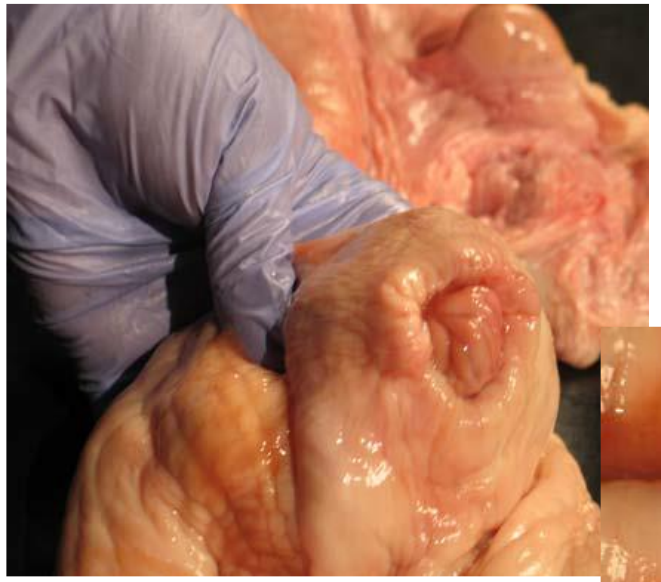
- The first two numbers identify the collection centre
- The next two letters identify the breed
- The next letter determines the year of the birth of the bull
- The final two numbers are specific to the individual bull

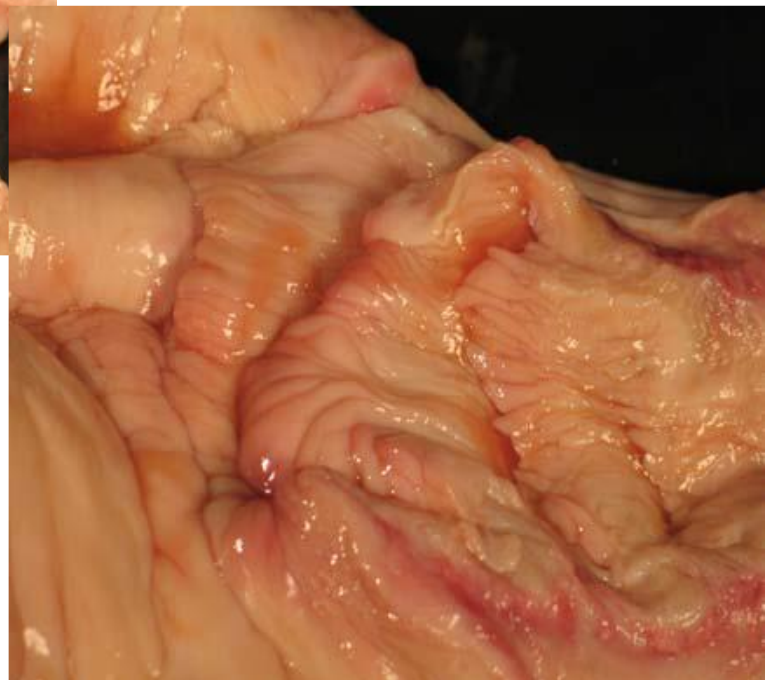
AI gun with protective sheath

REPRODUCTIVE ANATOMY OF THE COW

Cervix : 1 to 5 inches long and 1 to 3 inches in diameter. It is made up of fibrous tissue, which is dense and hard to the palpation.

- Position of the cervix may vary with age of cow

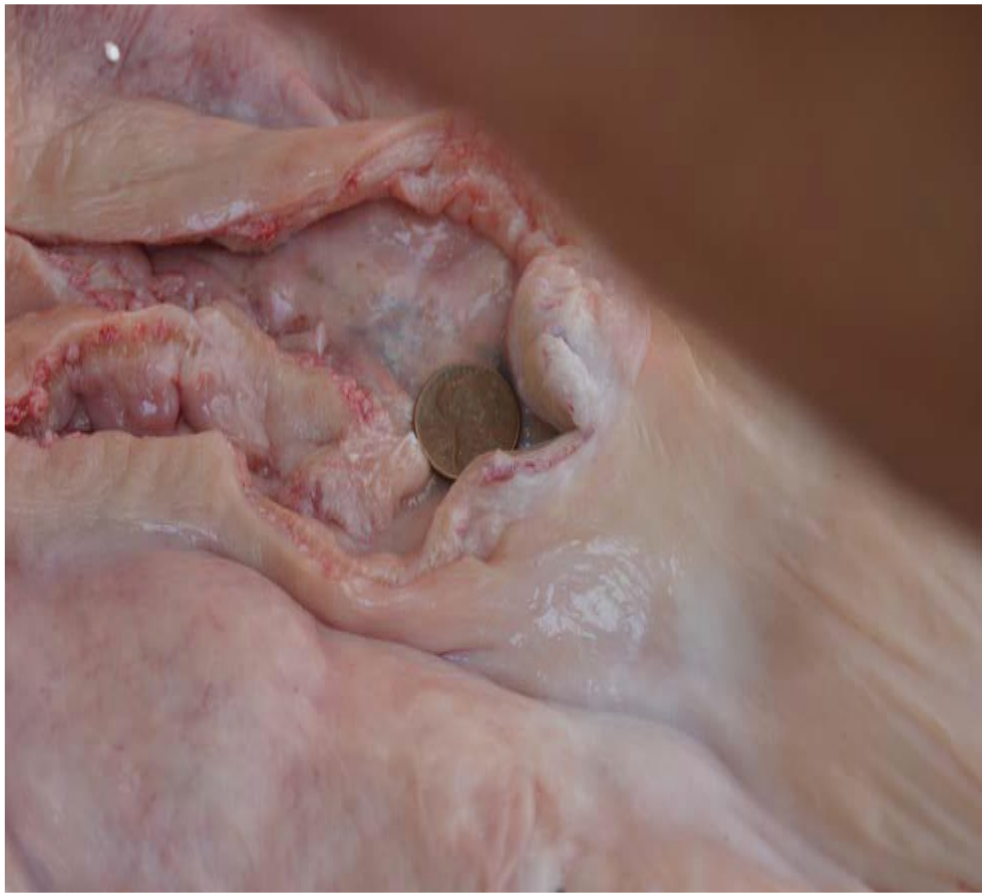




REPRODUCTIVE ANATOMY OF THE COW

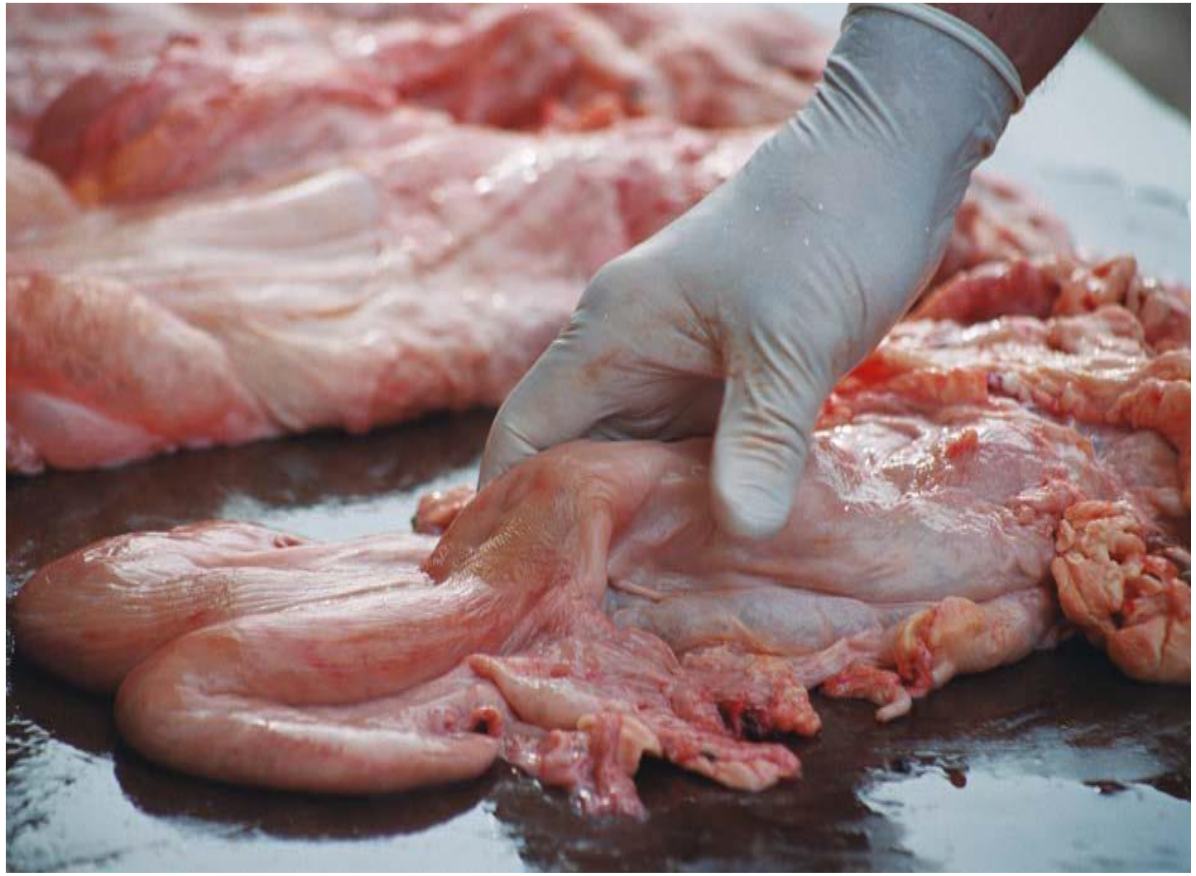
Body of the uterus :

Located anterior to the cervix, this structure is the place where the semen should be deposited (target site). It is made up of soft tissue and it is usually one (1) inch long



REPRODUCTIVE ANATOMY OF THE COW

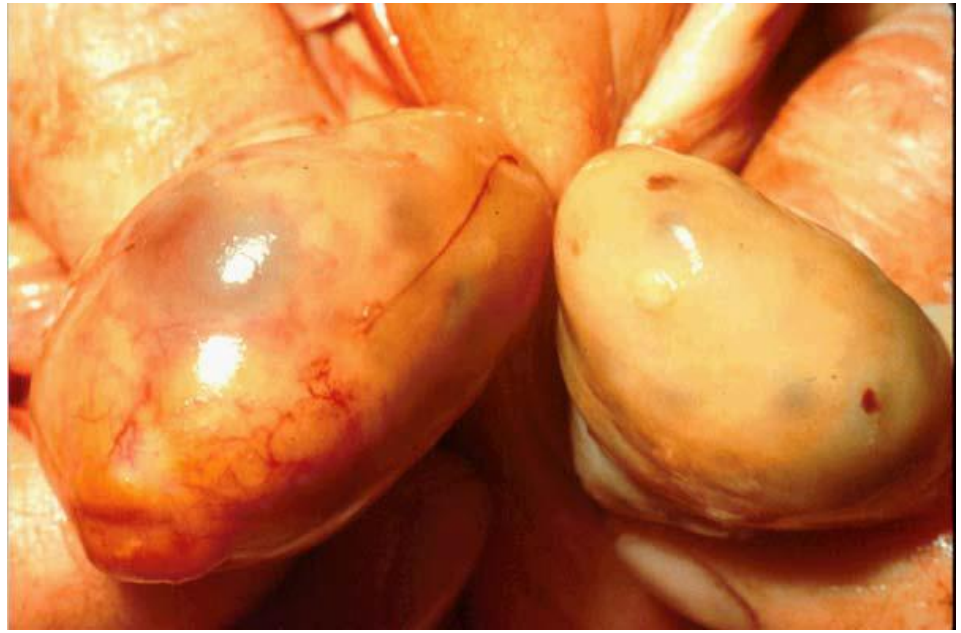
Uterine Horns: Each one is approximately from 8 to 16 inches of length and they are connected to their respective oviduct



REPRODUCTIVE ANATOMY OF THE COW

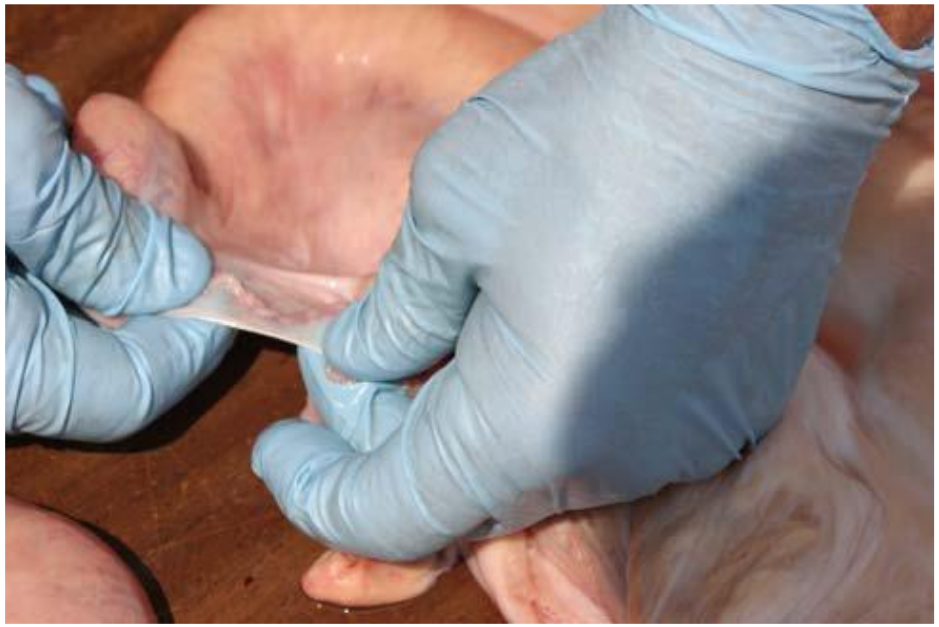
Ovaries: Approximately 1.5 inch in length, 1 inch in width and ½ inch in thickness

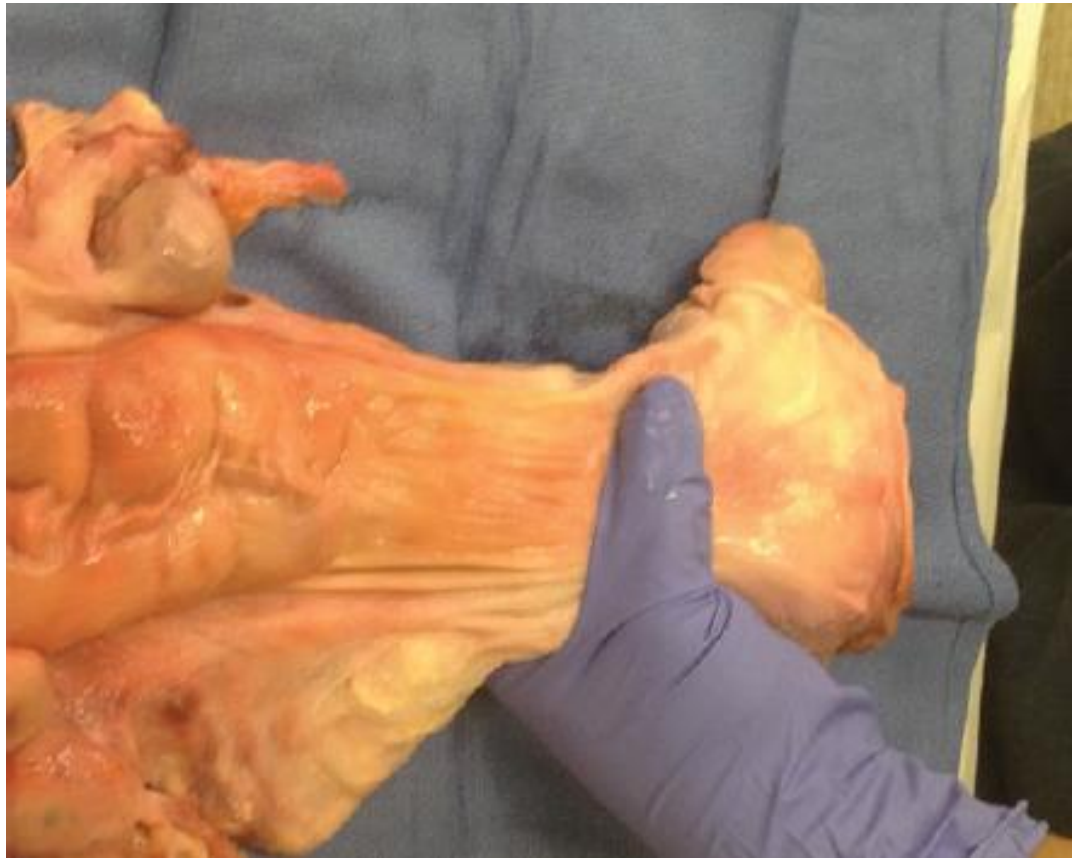
- Their main function is to produce eggs and the secretion of hormones (estrogen and progesterone)



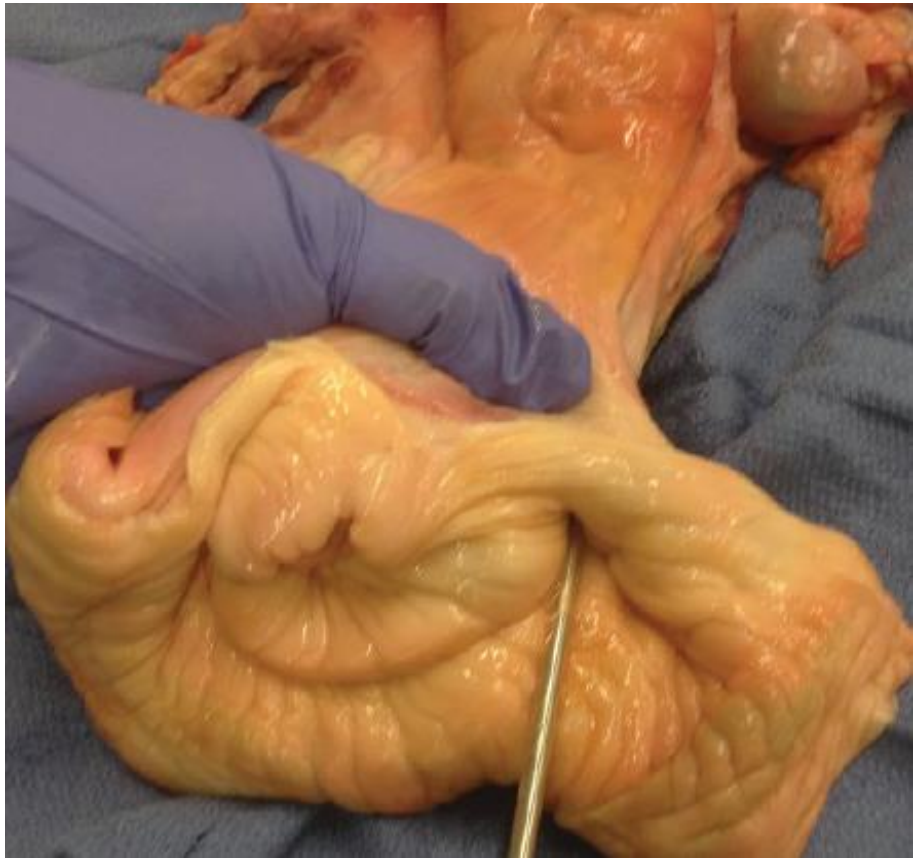
REPRODUCTIVE ANATOMY OF THE COW

Oviducts: Tubes that connect the ovaries with the uterine horns. It provides the site of encounter (fertilization) between the ovum and the sperm

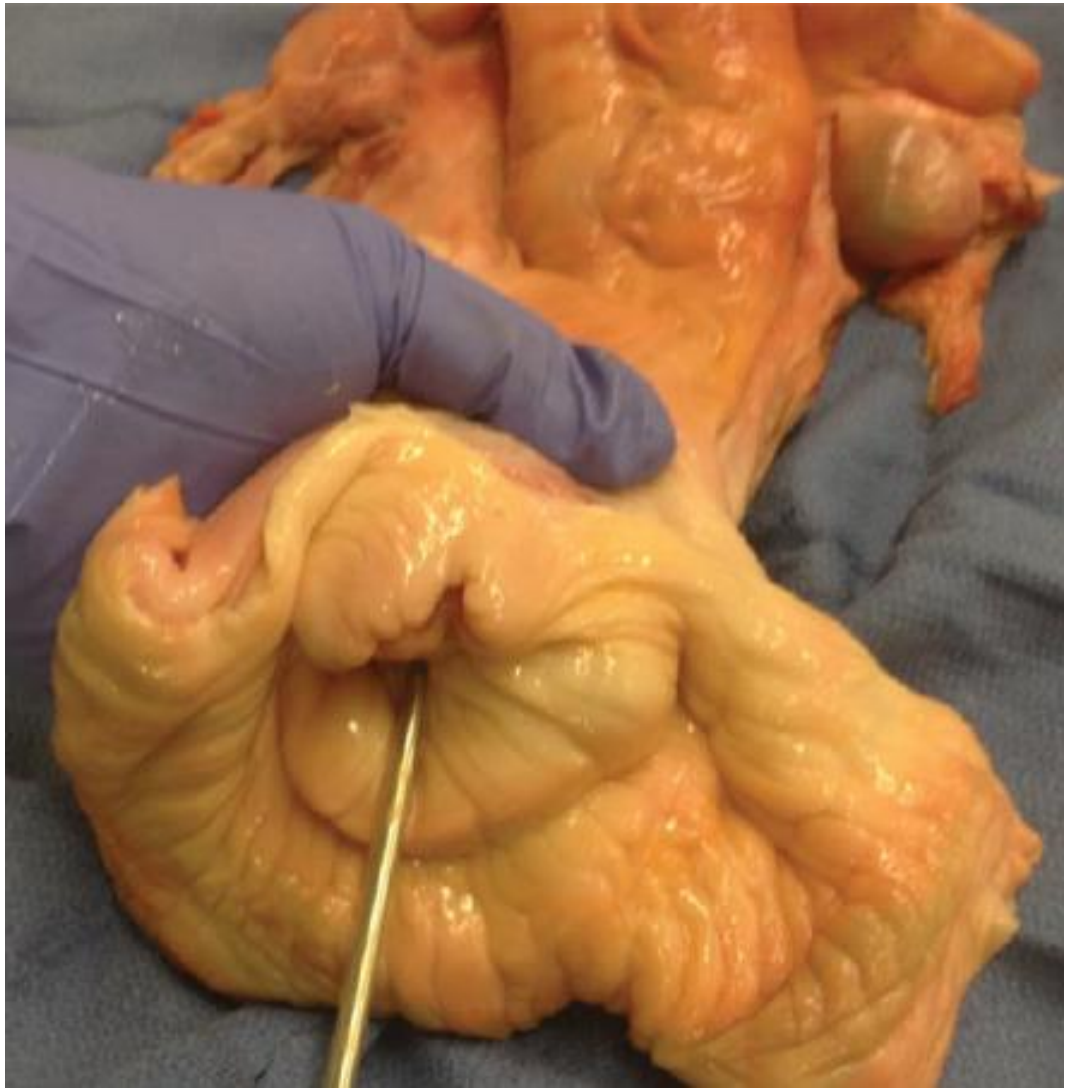




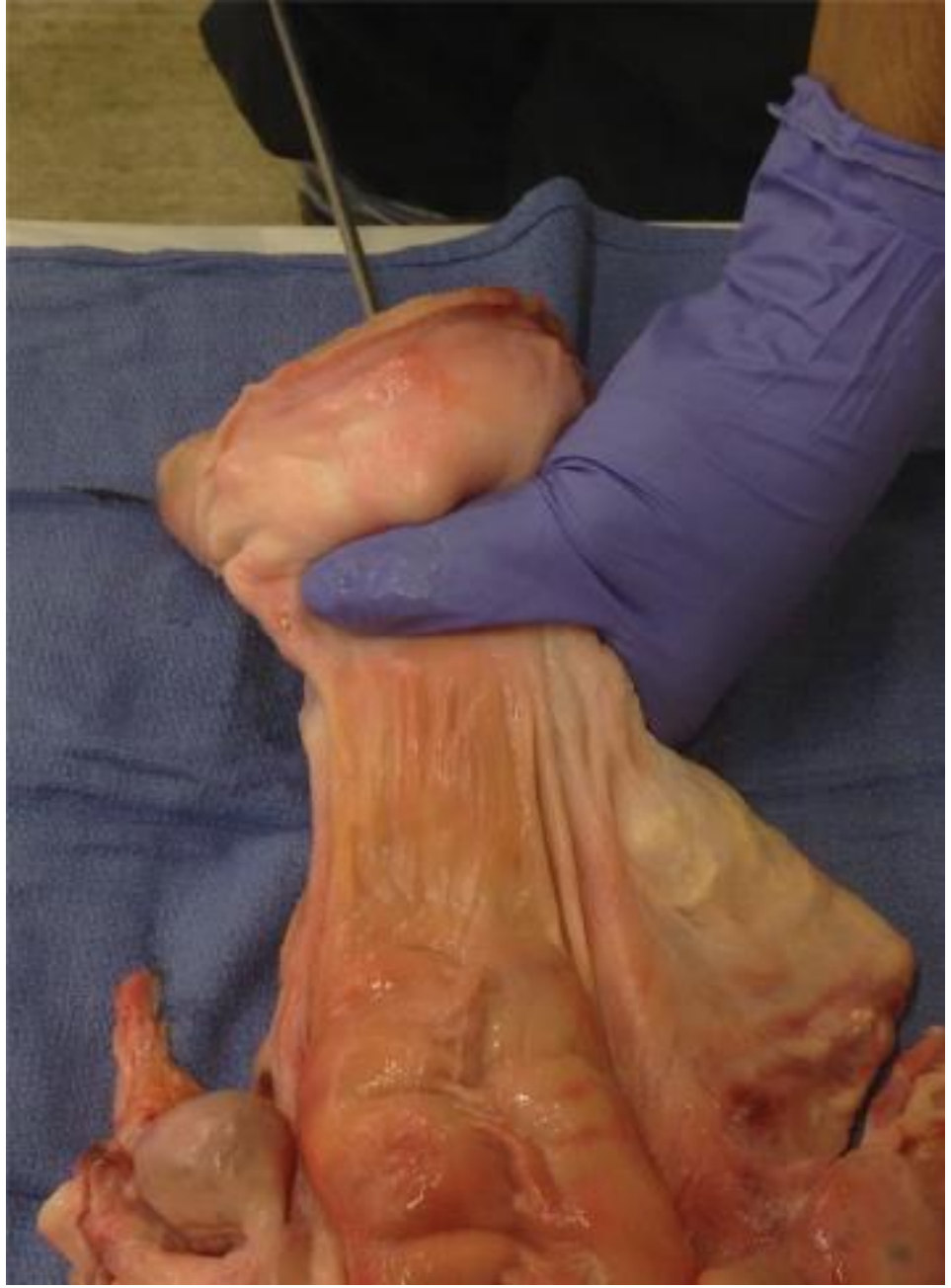
Locating and grasping the cervix



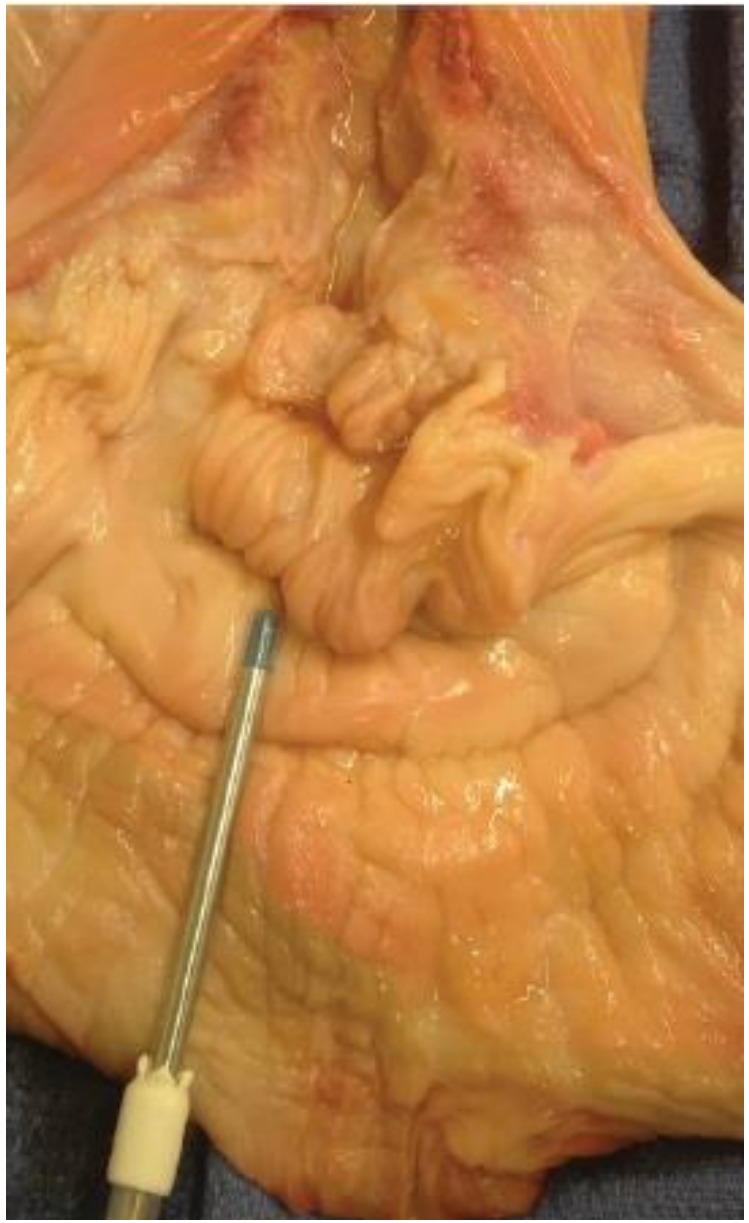
Avoiding the vaginal fornix



Locating the external os of the cervix

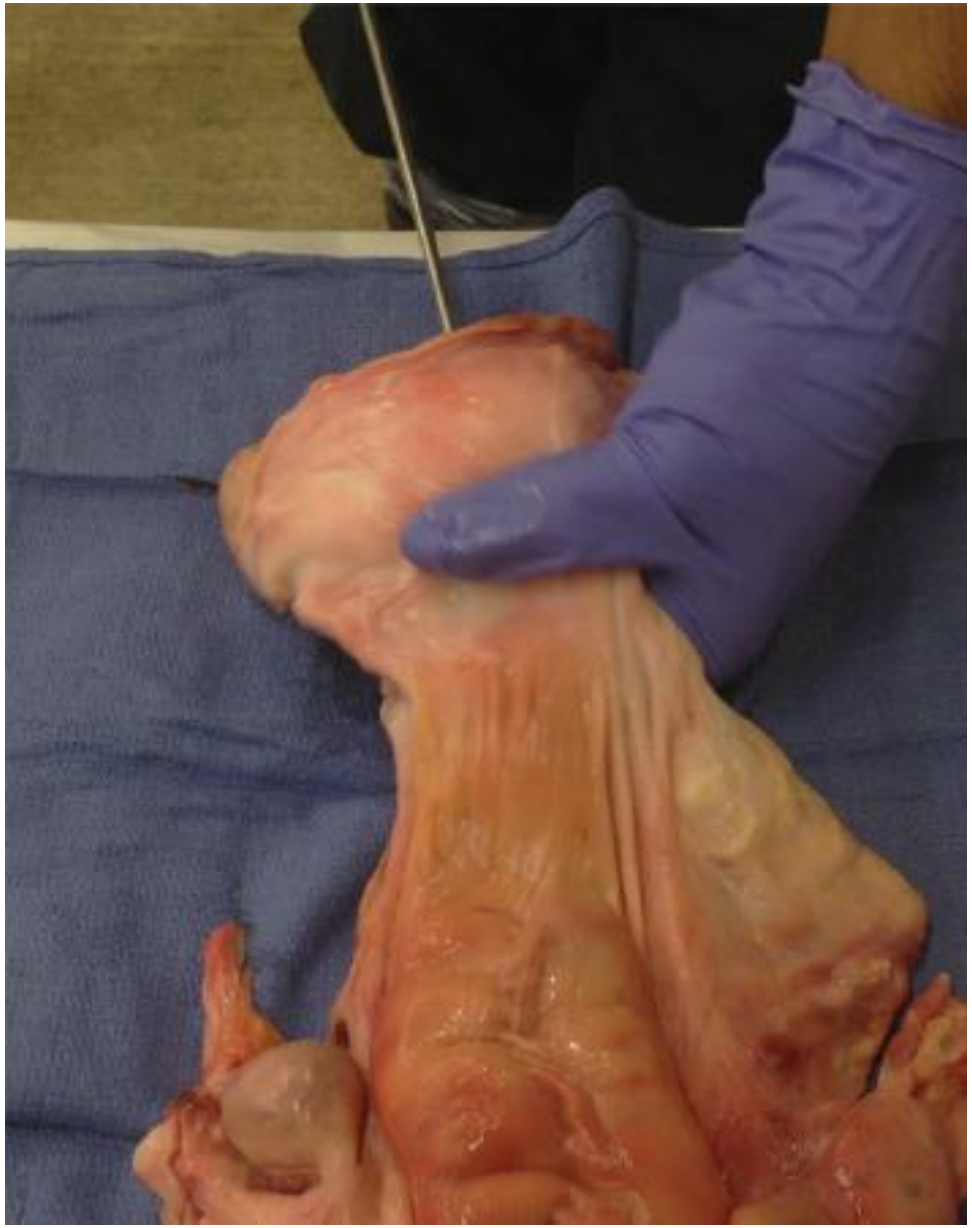


Locating the tip of the AI gun in the anterior vagina

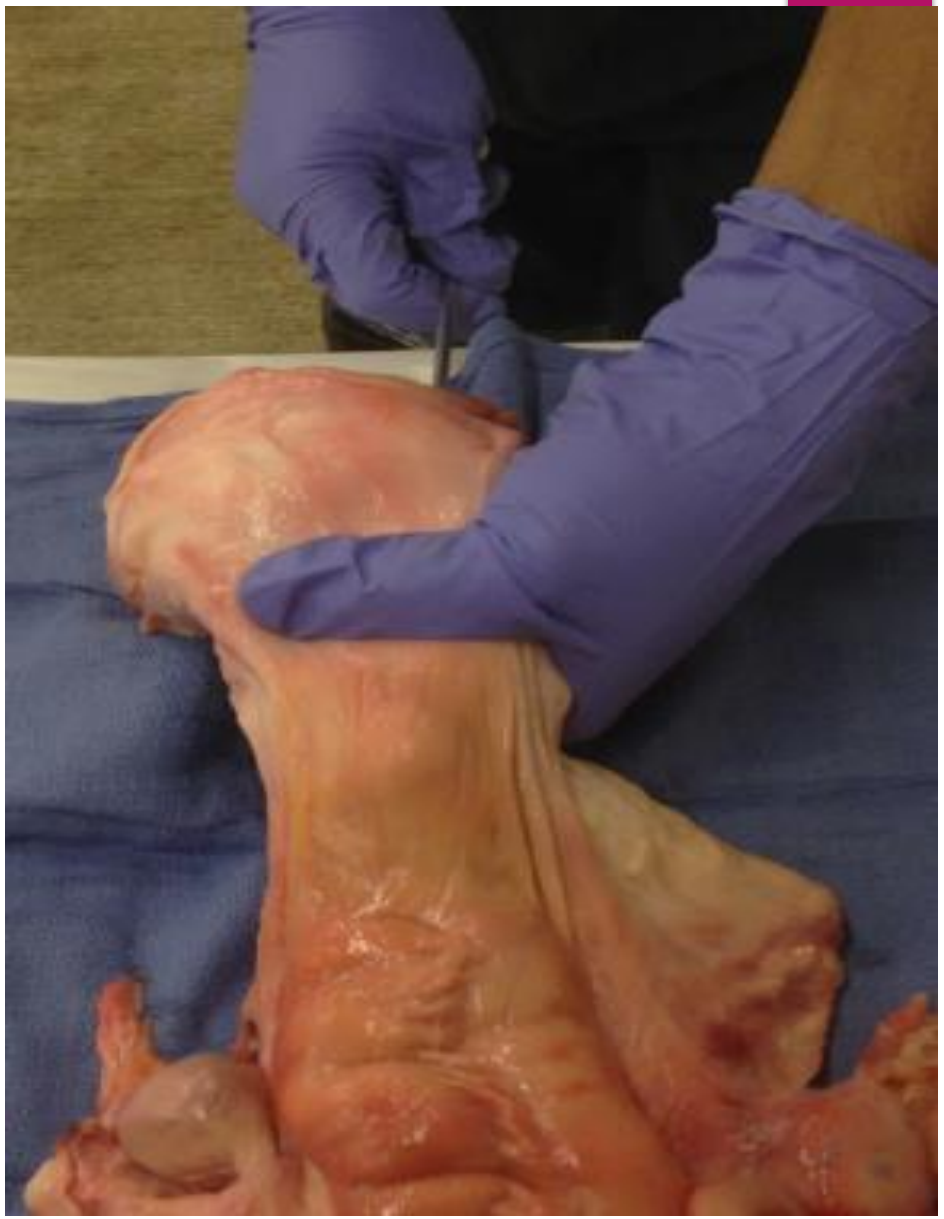


Puncturing the AI gun protective sheath at the external os of the cervix

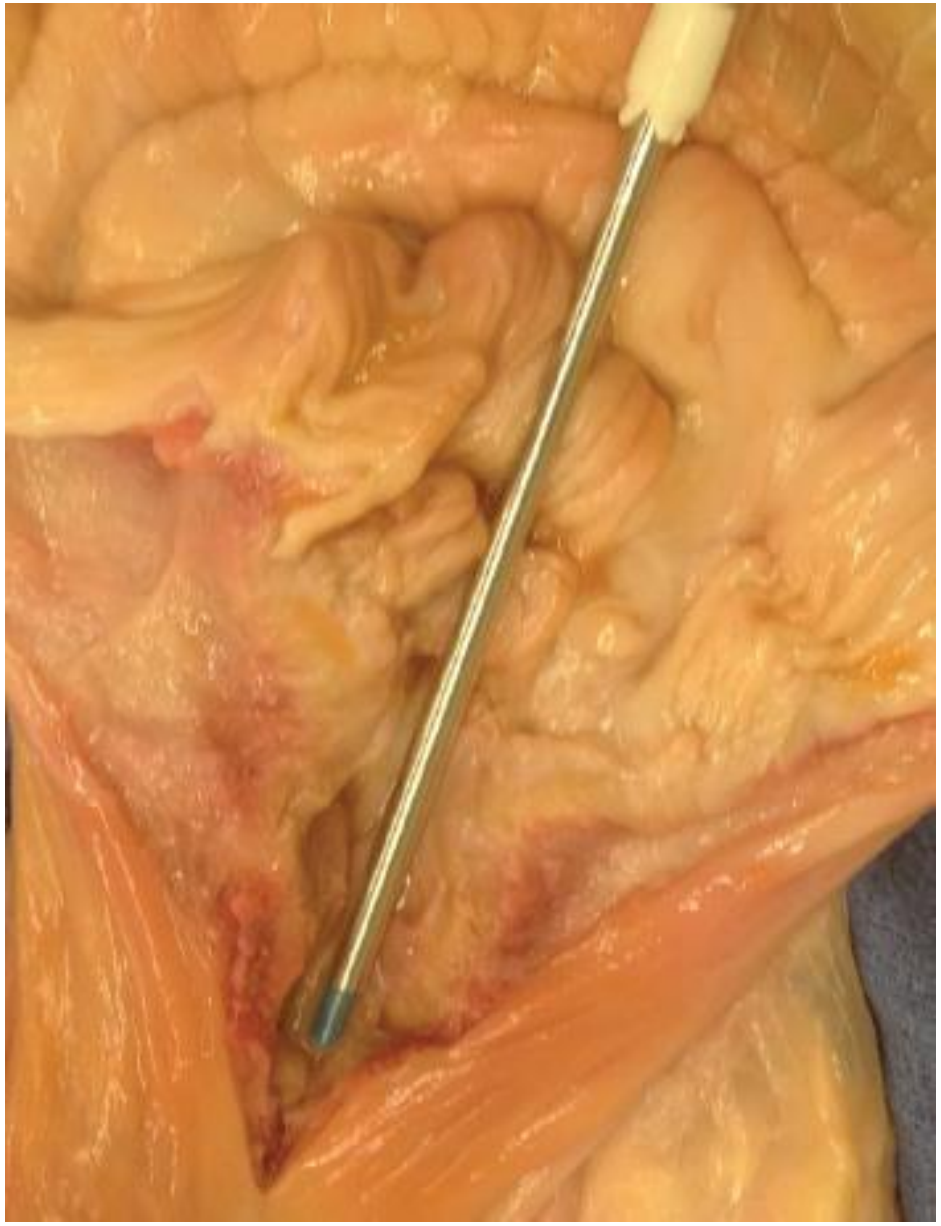
Feeling the AI gun in the uterine body



Passing the AI gun through the cervix



The tip of the AI gun in the uterine body



Semen deposition site (uterine body)

Types of Artificial Insemination

Intratubal insemination

The sperm is injected into the Fallopian tubes. The type of infertility will determine the site of injection of the sperm into the reproductive tract

Intrafollicular insemination

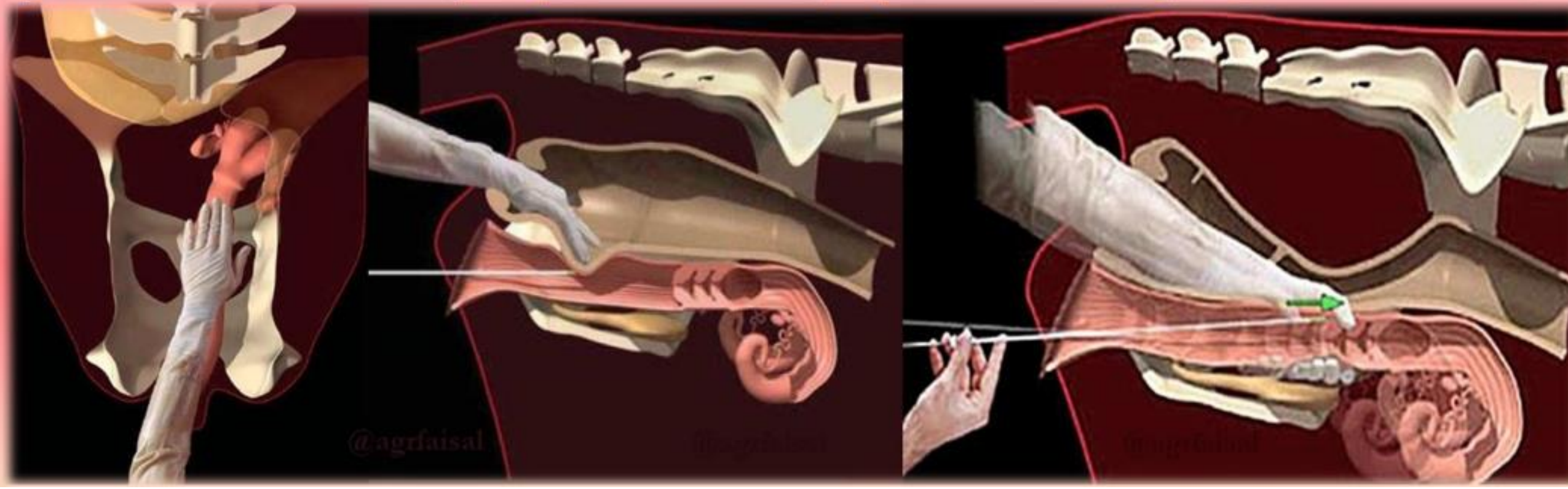
The sperm is injected into the ovarian follicle

Intrauterine insemination

The sperm is injected into the uterus

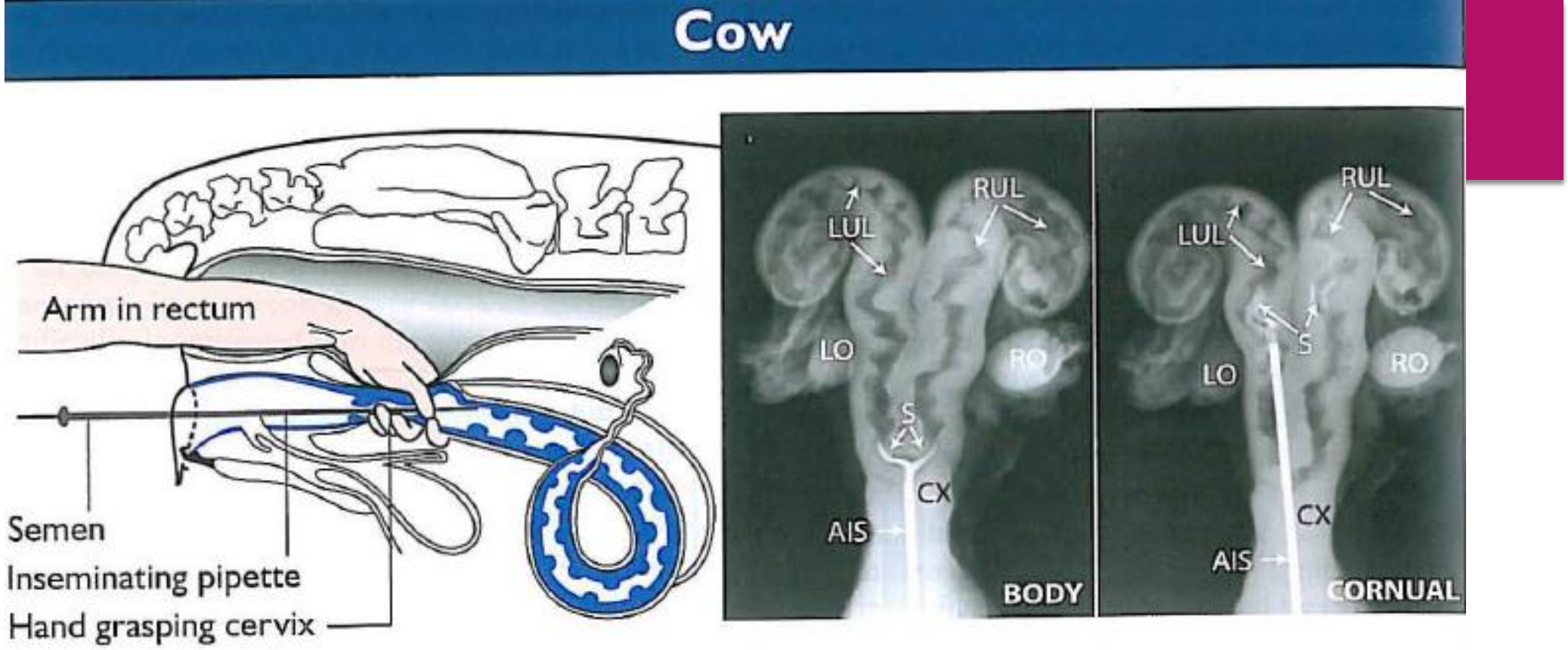
Intracervical insemination

The sperm is injected into the cervix, located at the opening of the uterus



The Main Factors Affecting AI

- Dose of Inseminated Semen
- Dose of Inseminated Semen
- Dose of PMSG Used
- Time of Insemination After Oestrus Synchronisation
- Labour
- Season
- Use of Fresh, Cooled, Chilled, Frozen Semen
- Age ♂♀
- Breed



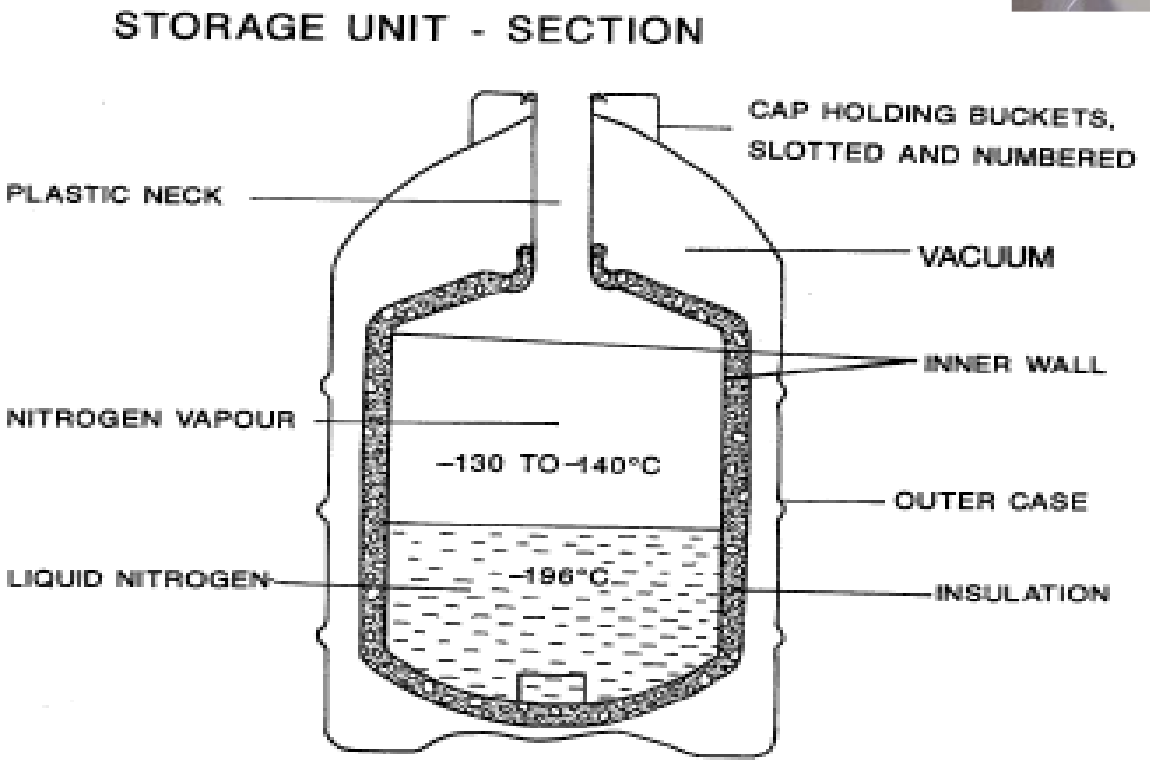
The radiographs above are from extirpated cow reproductive tracts (dorsal view). In cornual insemination, one-half of the semen is deposited in each uterine horn. In both examples, the inseminant volume is 0.5-ml. Cornual insemination minimizes the possibility of cervical deposition that results in significant retrograde loss of spermatozoa

RUL= Right Uterine Lumen; LUL= Left Uterine Lumen; RO= right ovary; LO= left ovary; S= semen; AIS= artificial inseminatio syringe; CX= cervix

AI EQUIPEMENT

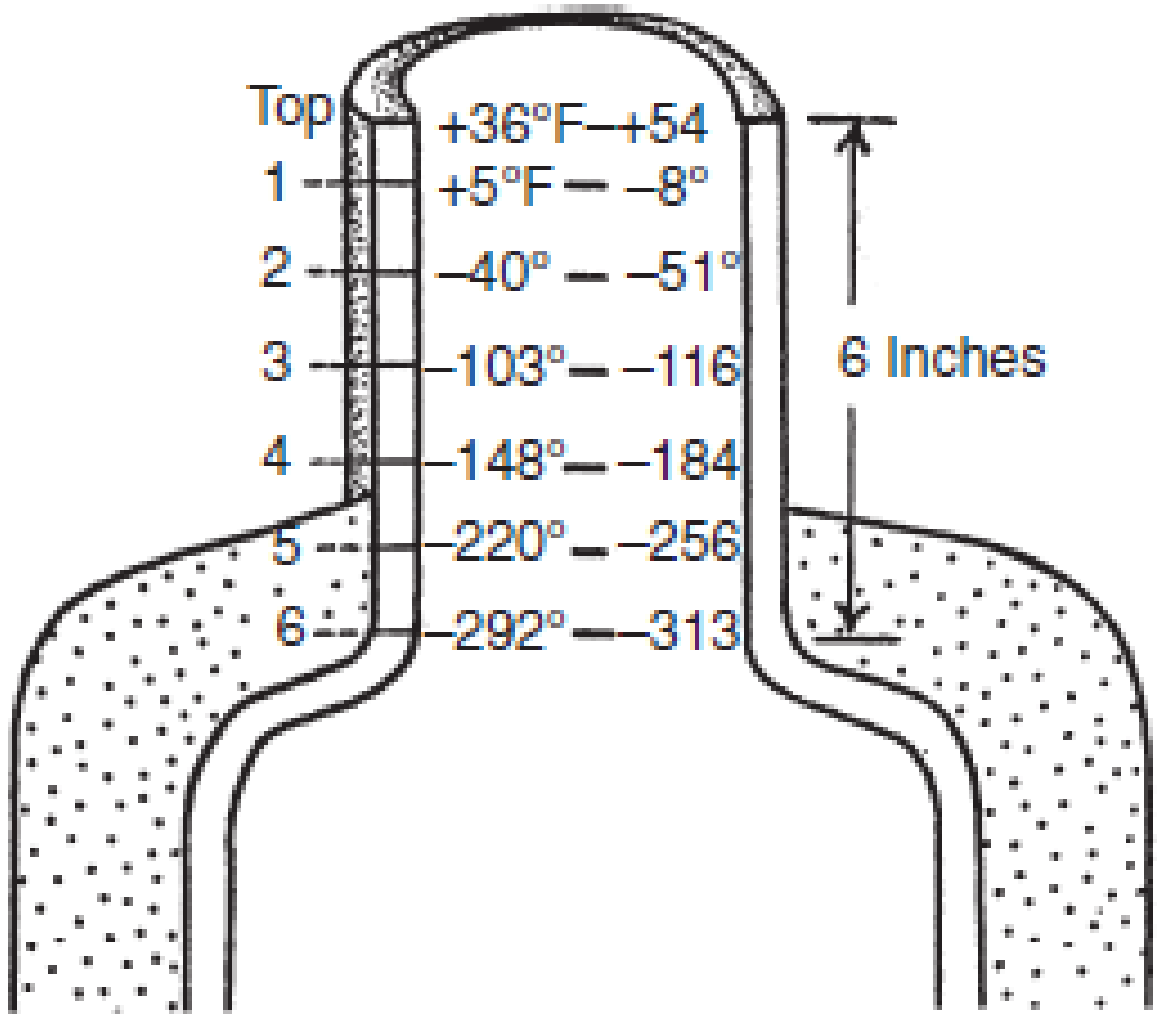
- Storage Tank

1. Clean and dry place
2. Always carry it in vertical position.
3. Check nitrogen periodically.



SEMEN HANDLING

- Wash hands prior to procedure
- Prepare thaw unit with clean water 35°C
- Place thaw unit close to the tank
- Keep canister below frost line
- Use tweezers to transfer straw from the tank to the thaw unit (< 5 seconds)
- Straw should be thaw in 35°C to 37°C water for a minimum of 40 seconds (10– 60 seconds)



Temperature range found in the neck of the semen storage nitrogen tank. (From Saacke RG: Concepts in semen packaging and use. Proceedings of the Eighth Conference of Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1974, p 15

Effect of Thaw Bath Temperatures and Cold Shock on Recovery by Spermatozoa in 0.5-ml French Straws

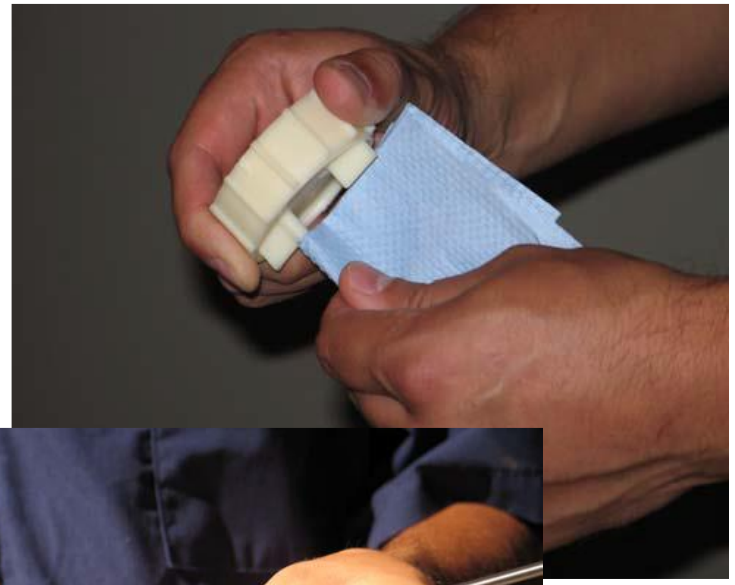
	AFTER INCUBATION FOR 3 HR AT 37°C	
Thaw Method	**% Motlle**	**% Intact Acrosomes**
5°C	30.3^{AB}	31.2^{A}
Air	21.4^{C}	26.4^{A}
35°C	51.4^{B}	61.0^{B}
35°C, then 5°C cold shock	41.1^{ABC}	44.6^{C}

^{ABC}Different superscripts designate significant differences ($P < 0.05$) in each column.

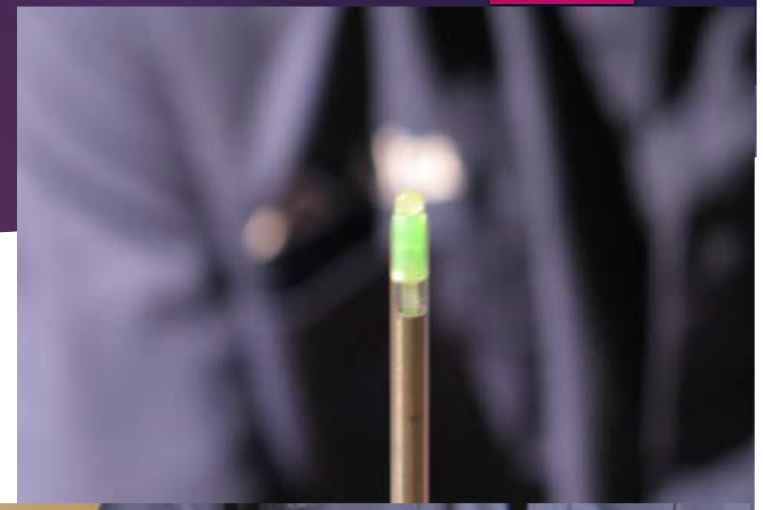
From Fleming WN, Olar TT, Mitchel JR: Techniques for evaluation of frozen bovine semen at Curtiss Breeding Service. Proceedings of the Sixth Technical Conference on Artificial Insemination and Reproduction of the National Association of Animal Breeders, 1976, p 90.

SEMEN HANDLING

- Once thawed, straws cannot be refrozen
- Pre-warm AI gun
- Dry straw completely with a paper towel
- Protect the straw from temperature fluctuations at all times
- Cut the sealed end of the straw squarely and cleanly
- Place plug end of straw into the gun
- Place a sheath over gun and secure firmly



SEMEN HANDLING



- Advance semen to fill airspace in straw
- Protect gun against temperature changes or contamination (gun warmer or shirt)
- Place semen into cow as soon as possible
- Prepare a maximum of 3 guns at a time

ARTIFICIAL INSEMINATION TECHNIQUE

- Once you are sure that the cow to breed is not pregnant and is in heat, check the identification number and the breeding records
- Put on a shoulder length disposable plastic glove, lubricate it and stand sideways behind the cow

ARTIFICIAL INSEMINATION TECHNIQUE

- Form a cone with yours fingers, and gently insert the hand through the anal opening
- Once the hand is fully in the rectum, open fingers from the cone position and remove the fecal matter if needed. Avoid excessive motion of your arm because it causes air to rush in the rectum, which will not allow you to grasp the cervix

ARTIFICIAL INSEMINATION TECHNIQUE

- Gently slide the hand from the upper part of the rectum to the lower part to identify the cervix
- Hold the cervix having your thumb on the top and the rest of your fingers on the bottom

ARTIFICIAL INSEMINATION TECHNIQUE

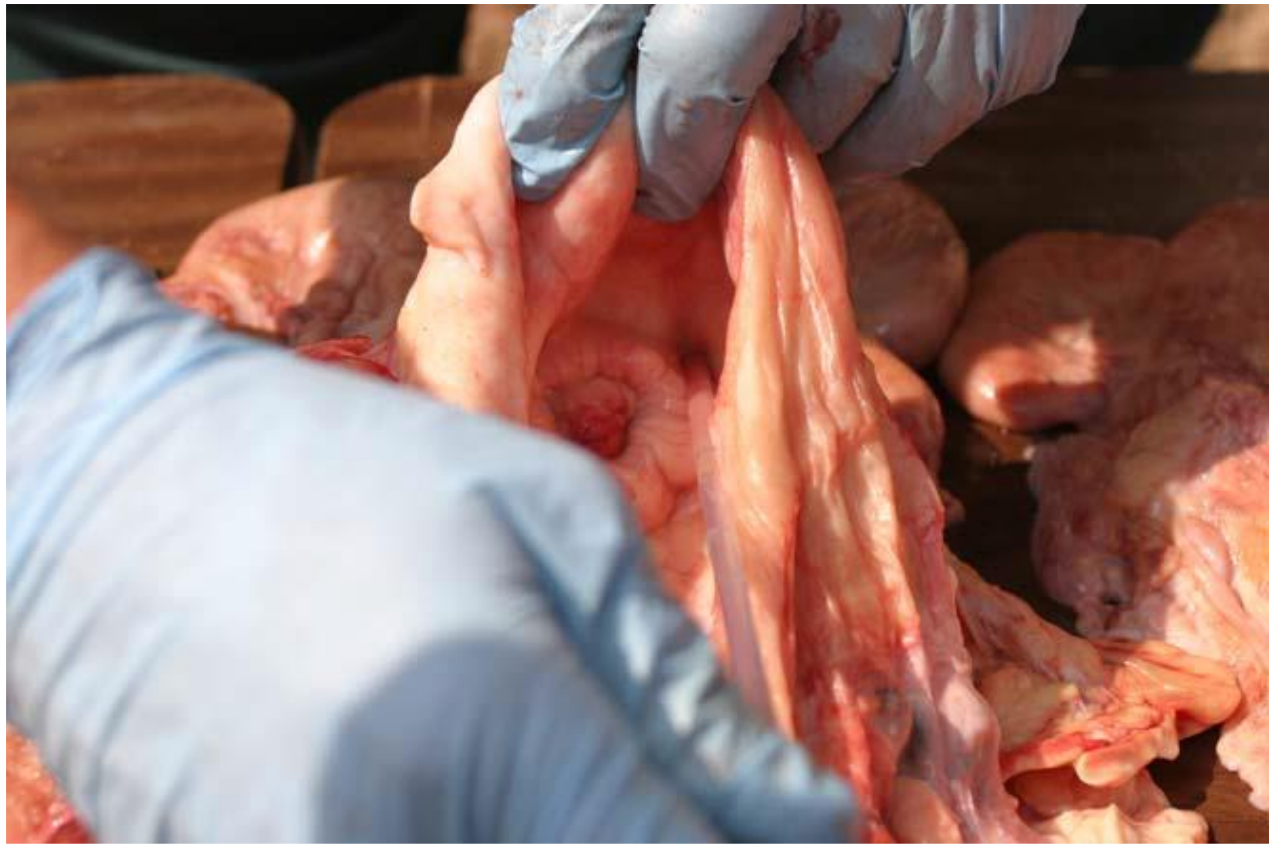
- Thoroughly wipe the vulvar area with a clean paper towel
- This helps to prevent the interior of the reproductive tract from becoming contaminated and possibly infected
- Insert the insemination gun through the vulva at a 40 to 45 degree angle until it touches the roof of the vagina

ARTIFICIAL INSEMINATION TECHNIQUE

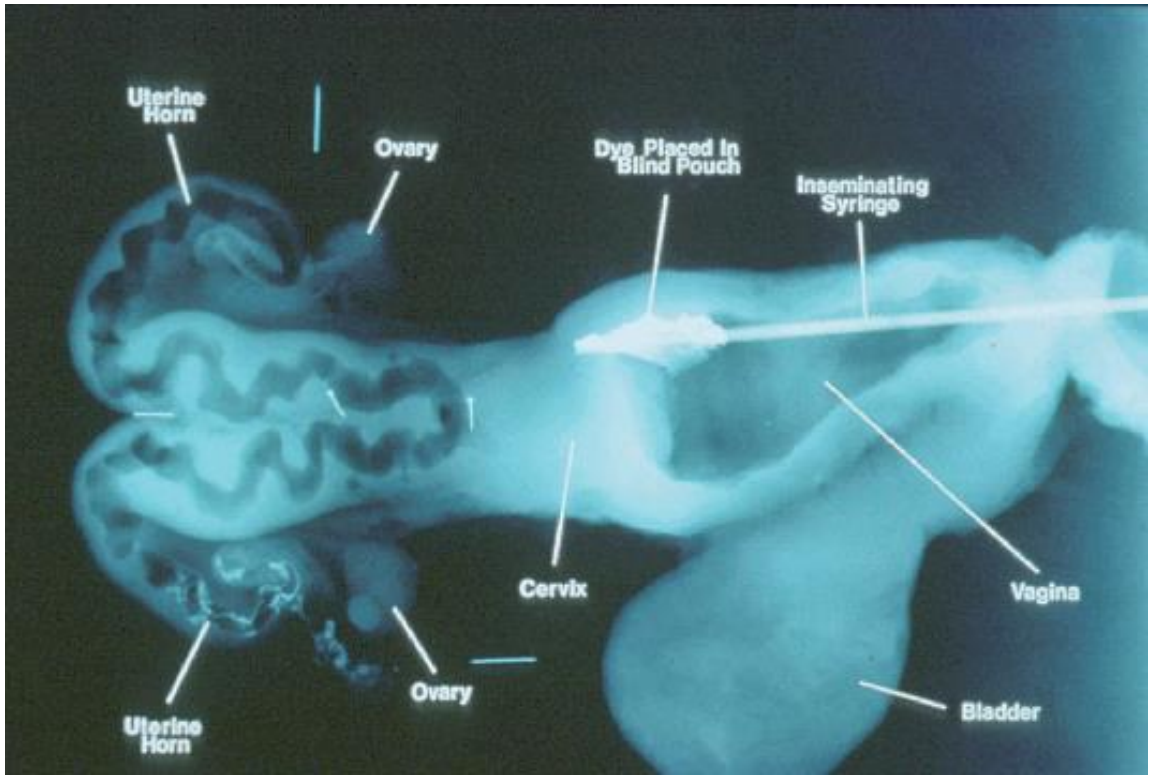
- Level the insemination gun to go through the passageway to the cervix
- This procedure avoids the possibility of entering the urethra located on the floor of the vagina

ARTIFICIAL INSEMINATION TECHNIQUE

- While passing the insemination instrument through the vagina, push the cervix forward with the hand holding the cervix
- This will stretch the vagina wall eliminating the possibility of the insemination gun getting caught in a vaginal fold or the blind pouch around the enter of the cervix



BLIND POUCH


Uterine Horn
Ovary
Dye Placed In Blind Pouch
Inseminating Syringe
Cervix
Vagina
Ovary
Uterine Horn
Bladder

ARTIFICIAL INSEMINATION TECHNIQUE

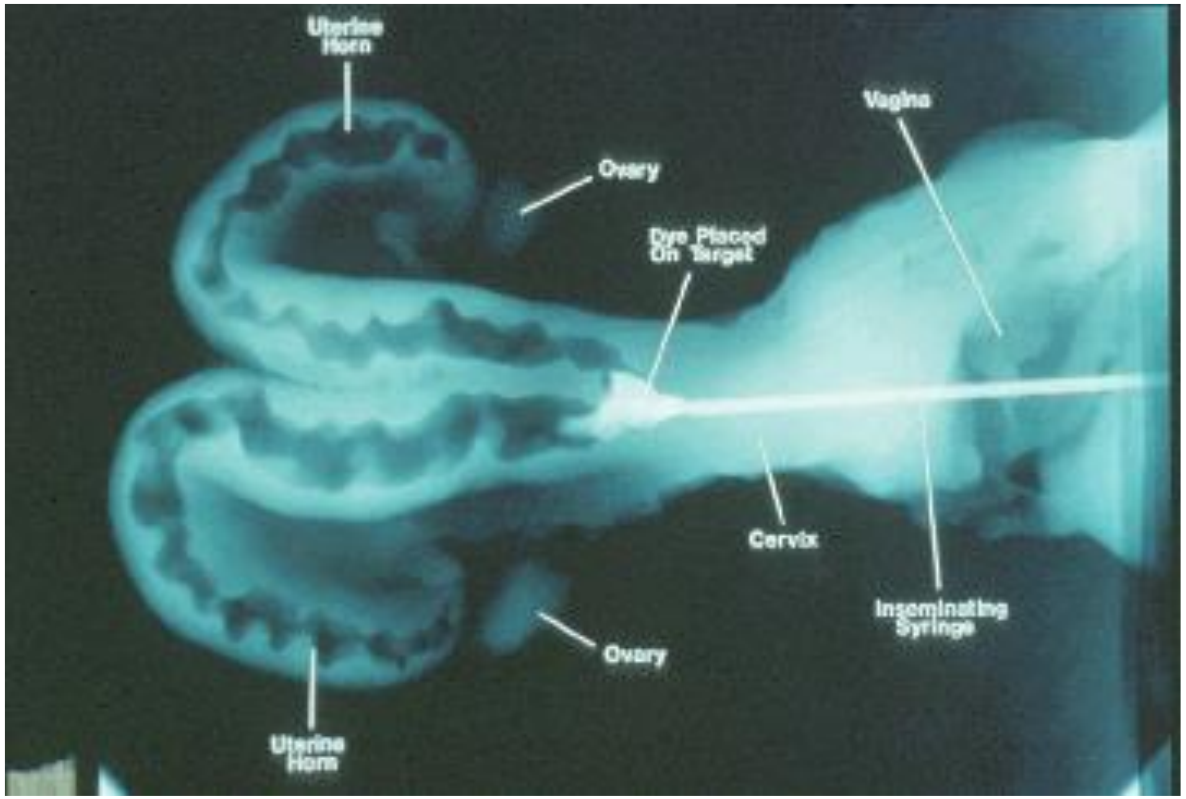
- At this point the tip of the gun can be guided to the cervical canal by the fingers of the hand holding the cervix
- Once the tip of the insemination gun is in the cervical canal, maintain slight forward pressure on the rod while manipulating the cervix ahead of the gun

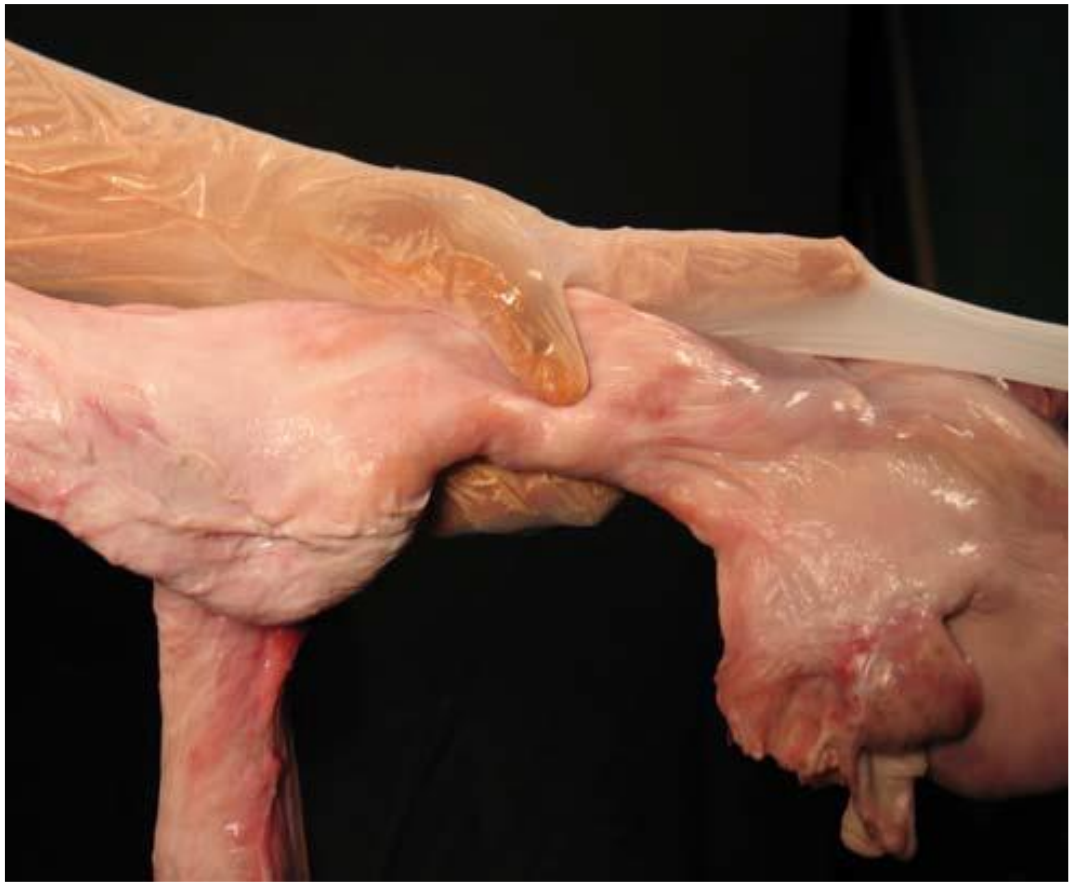
ARTIFICIAL INSEMINATION TECHNIQUE

- While you are passing the A.I. gun through the cervix keep your index finger at the end of the cervical canal, so you can feel the tip of the gun at the target site

ARTIFICIAL INSEMINATION TECHNIQUE

- Lift finger and slowly deposit the semen (this maximizes the amount and equal distribution of semen on the uterine body) make sure you are on the target at all times
- Depositing the semen in the cervix or in the uterine horns may result in lower pregnancy rates, and sometimes it may cause damage to the uterus


Uterine Horn
Ovary
Vagina
Dye Placed On Target
Cervix
Inseminating Syringe
Ovary
Uterine Horn



KEY PERFORMANCE INDICATORS (KPI)

- Palpated Pregnancy Rate (PPR) is an indirect measure of estrus detection efficiency and is calculated by dividing the number of cows found pregnant at pregnancy examination by the number of cows examined.
- 25 pregnant and 50 examined = PPR of 25/50 or 50%.

KEY PERFORMANCE INDICATORS (KPI)

- Extremely aggressive breeding decisions may lead to a high PPR but a lower Conception Rate (CR) because of a decline in estrus detection accuracy, servicing animals that are not truly in estrus
- Strive for a **PPR that is >65%**

KEY PERFORMANCE INDICATORS (KPI)

- Conception Rate (CR) is defined as the percent of animals that become pregnant to a single service.
- If 100 animals are serviced and 35 become pregnant the CR = 35/100 or 35%.
- Strive for a **CR >35%.**

KEY PERFORMANCE INDICATORS (KPI)

- **Service Rate (SR) is the percent of eligible animals serviced in a 21 day period.**
- An eligible animal is one that is past her voluntary wait period (VWP) and not pregnant.

KEY PERFORMANCE INDICATORS (KPI)

- If there are 100 eligible animals and 65 are serviced in a 21 day period the SR = 65/100 or 65%
- Strive for a **SR of >65%**

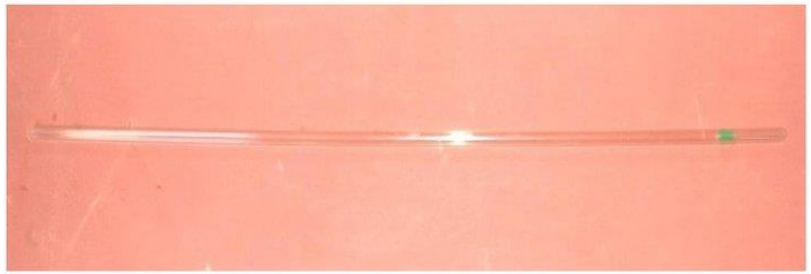
KEY PERFORMANCE INDICATORS (KPI)

- Hard Count Pregnancy creation can be measured as a percent of the adult milking herd made pregnant per time period

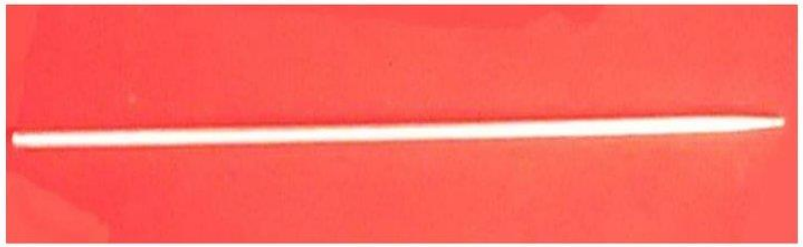
KEY PERFORMANCE INDICATORS (KPI)

- 10% of the milking herd made pregnant per month is an admirable goal
- Few herds achieve this number; many herds will achieve 9% made pregnant per month

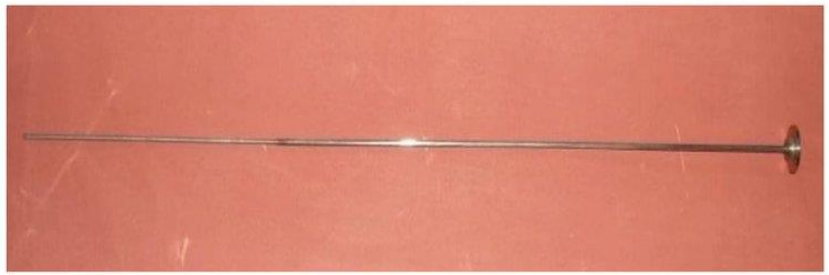
Al Sheath



Cervical Dilator

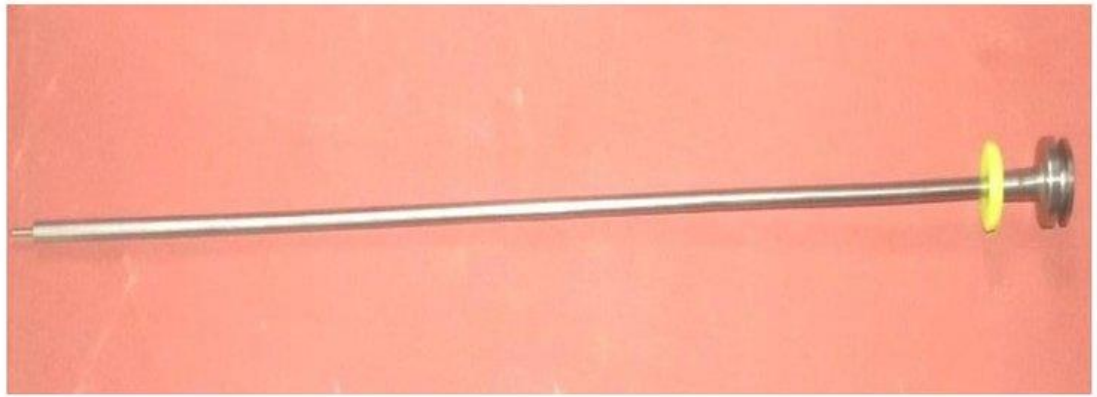


Stylet

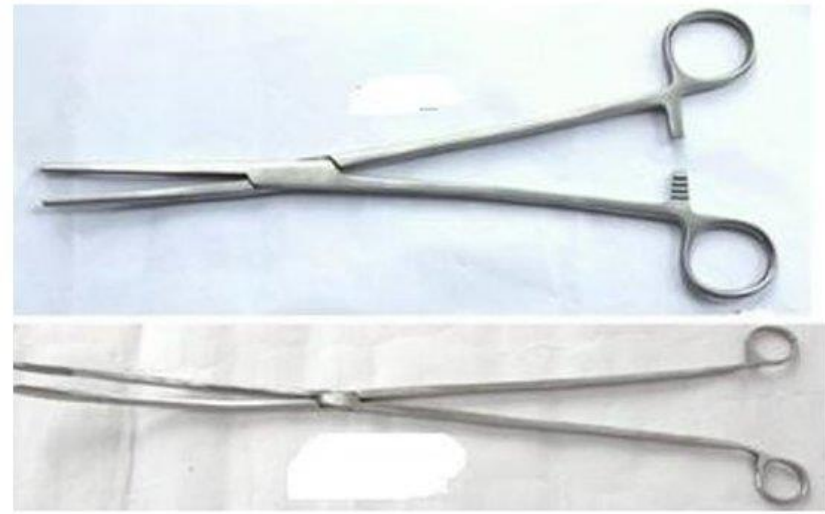


Hauptner Heat Detector With Re-Chargeable Battery

Cassou's AI Gun

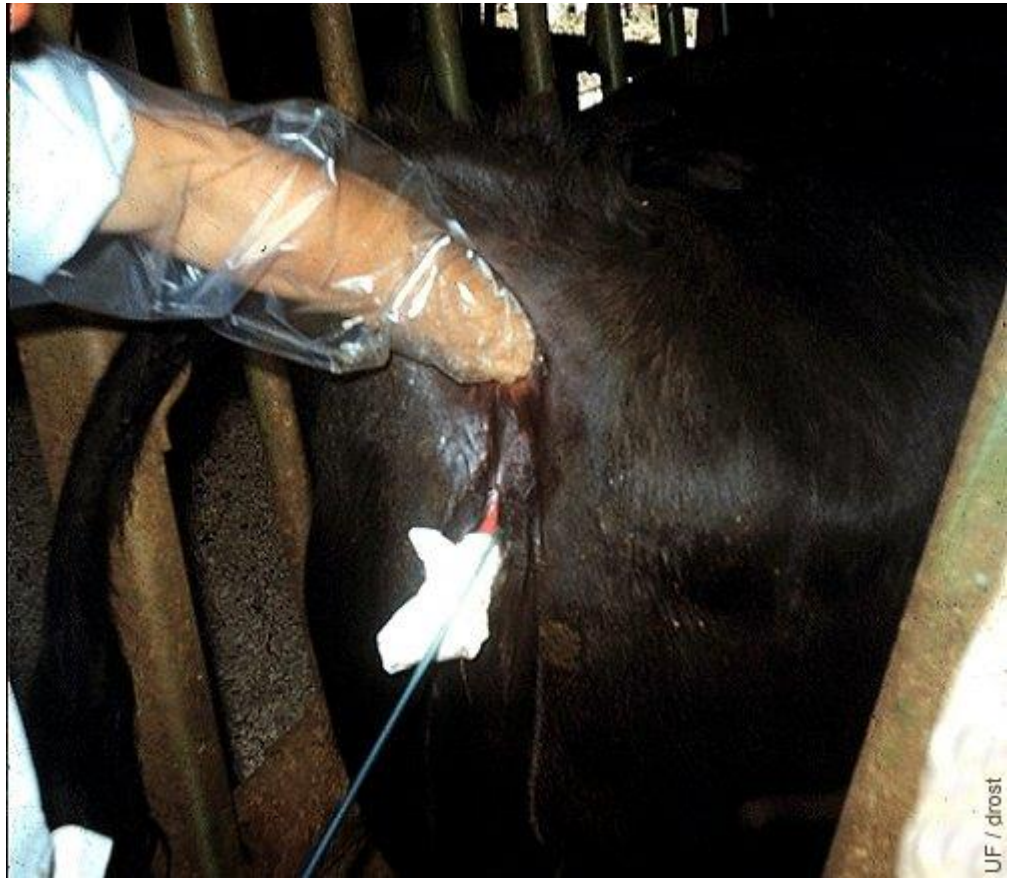


Straw Holding Forceps



Vaginal Speculum

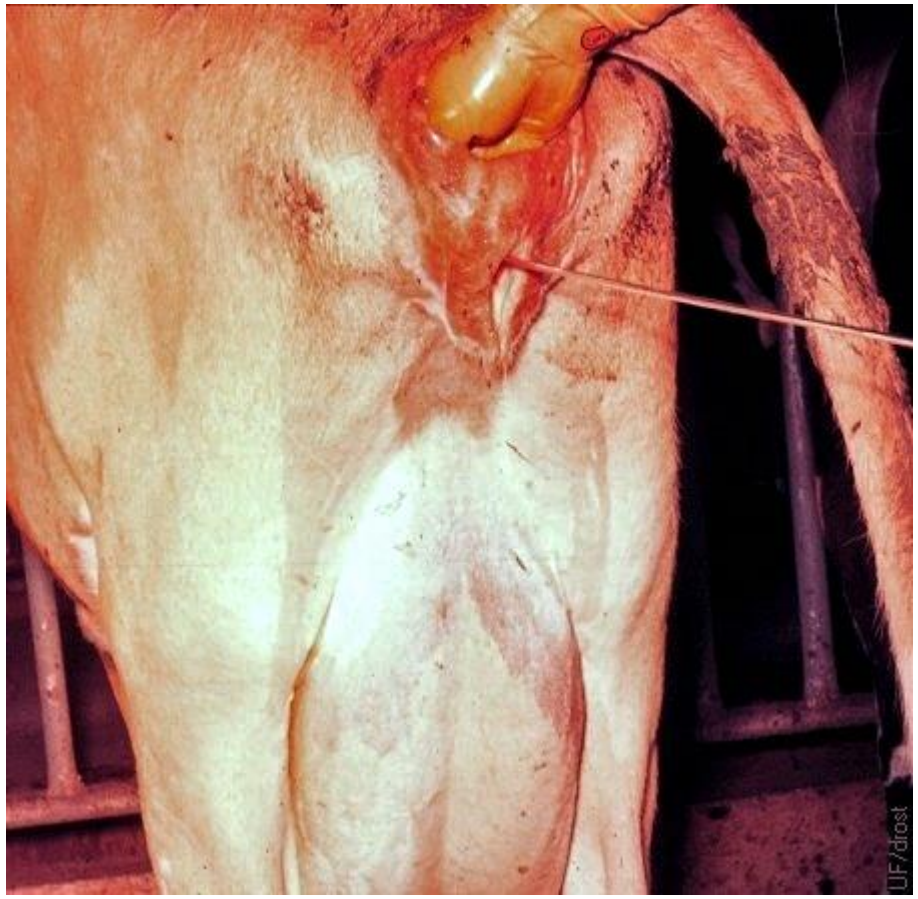
Rectal Gloves (Full Arm)



Clean Insertion of AI Pipet.

After wiping the vulva clean, a clean crumpled up paper towel may be placed in the ventral portion of the vulva to part the lips for a clean entry of the insemination syringe.

Drost M (1974)



Proper Technique for Artificial Insemination.

Cleanliness is important for success in artificial insemination. The fingers in the rectum can initially be used to spread the lips of the vulva by downward and backward pressure just in front of the anal sphincter.

Drost M (1974)