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Sustainable Development of Livestock for Livelihoods in Africa

EUROPEAN UNION



Guidelines for the effective utilization of Assisted Reproductive Techniques (ARTs) in dairy cattle

GUIDELINES FOR THE EFFECTIVE UTILIZATION OF ASSISTED REPRODUCTIVE TECHNIQUES (ARTS) IN DAIRY CATTLE

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ACKNOWLEDGEMENT

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We are grateful to the European Union for the financial support offered to African Union Inter-African Bureau for Animal Resources (AU-IBAR) Live2Africa Project. The manual attempts to provide basic information on the ARTs, along with illustrations. We sincerely hope that the manual will be useful to farmers, practitioners, extension agents and policy makers, and therefore, stimulate increased ARTs transfer and adoption across the region.

1. OESTRUS SYNCHRONIZATION OF CATTLE:

What is synchronization of oestrus?

- Manipulation of the oestrus cycle.
- Bringing about oestrus (heat) in a high percentage of a group of cows within a short, pre-determined time.

Advantages:

- The need for heat detection can be avoided.
- Insemination is scheduled at the convenience of the farmer.
- Calves are born close together, making management easier.
- Parturition is scheduled at an optimum time (e.g. rainy season with pasture available, good weather, etc.)

Types of Synchronization programs:

- Prostaglandin ($PGF_{2\infty}$) injections alone.
- The injected hormone causes lysis and regression of the Corpus luteum (CL) earlier than in the natural cycle.
- A functional CL must be present in an ovary (i.e. days 6-17 of the cycle).
- Two injections 10-12 days apart are then given.
- All cycling cows show heat about 2-4 days (most- 48 hours) after 2nd injection and are inseminated.





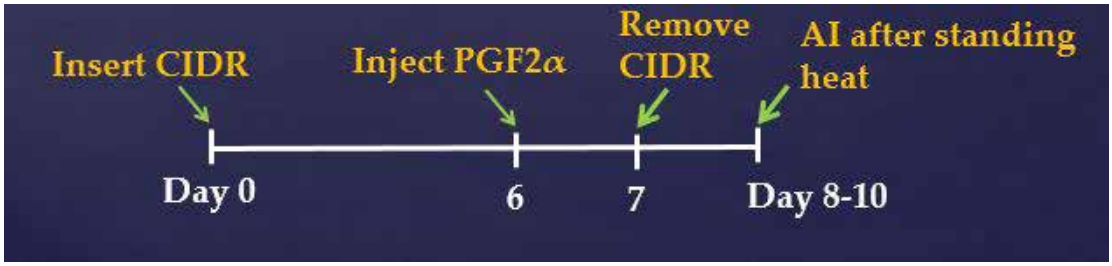
B) CIDR (Progesterone & PGF2□) program.

- CIDR = Controlled Internal Drug Releasing vaginal insert.
- The CIDR releases Progesterone into the body for 7 days.
- This keeps cows in the Luteal phase of the cycle.

- It's removal after $PGF_{2\alpha}$ injection causes a sudden drop in the body Progesterone levels provoking oestrus.



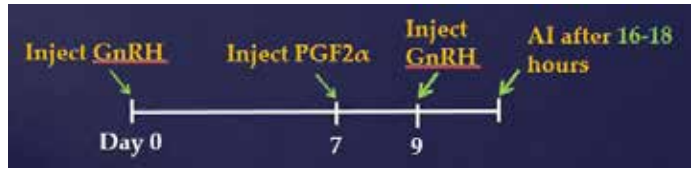




C) Ovsynch (GnRH & $PGF_{2\alpha}$) program.

- GnRH injection causes production of FSH which stimulates growth of Follicles, and LH that causes ovulation of the dominant Follicle.
- The growth of follicles is then synchronised.
- The $PGF_{2\alpha}$ is injected 7 days later to regress any CL present.
- The 2nd GnRH injection causes synchronization of ovulation.
- AI is done 16-18 hours after this 2nd injection, i.e. "Timed AI".





Choosing a synchronization program:

The $PGF_{2\alpha}$ and CIDR synchronization programs , though good, have their limitations, i.e. :-

1. Oestrus is not completely synchronised so animals come on heat over several (2-3) days.
2. Need to detect heat before AI. This is time consuming and some animals (e.g. on silent heat) may be missed.

The Ovsynch (GnRH) program is preferable as it:-

1. Synchronises Ovulation (rather than heat) which is more accurate.
2. Timed AI is done, 16-18 hours after the 2nd GnRH injection, removing the need for heat detection.
3. This protocol is best for management of large Dairy or Beef herds.

Ovsynch Synchronization program weekly schedule:

Day	Sun	Mon	Tue	Wed	Thus	Fri	Sat
Week 1		Day 0					
Week 2							

Insert the drugs to be administered on the appropriate day of the week, i.e. GnRH and $PGF_{2\alpha}$, as well as the AI.

Use of Ovsynch in the field:

Sites in Narok district	No. of cattle
Keringani	48
Kimintet	161
Moyoi	82
Olosentu	49

NB: All successfully inseminated 18 hours after 2nd GnRH injection.





Cost of Ovsynch program for use in oestrus synchronization:

- 20 ml bottle of Estroplan (PGF2□) = Ksh 3,000/-
- 20 ml bottle of Gonabreed (GnRH) = Ksh 4,200/-
- Each bottle is sufficient to synchronise 10 cattle.
- Therefore cost per cow = 720/-





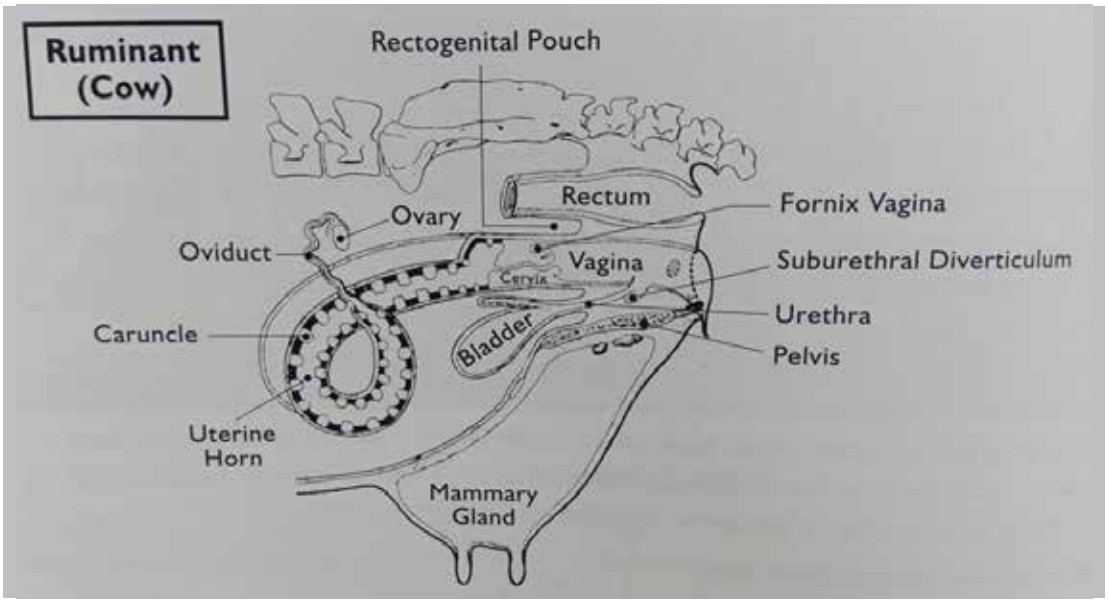
Take-home Message:

- Oestrus synchronization is a convenient way of facilitating AI of a group of cows, without the need for heat detection.
- The choice of synchronization method depends on the available drugs, as the conception rates are similar.
- For AI of a large dairy or beef herd, the method that synchronises ovulation (e.g. Ovsynch) is more accurate, therefore preferable.

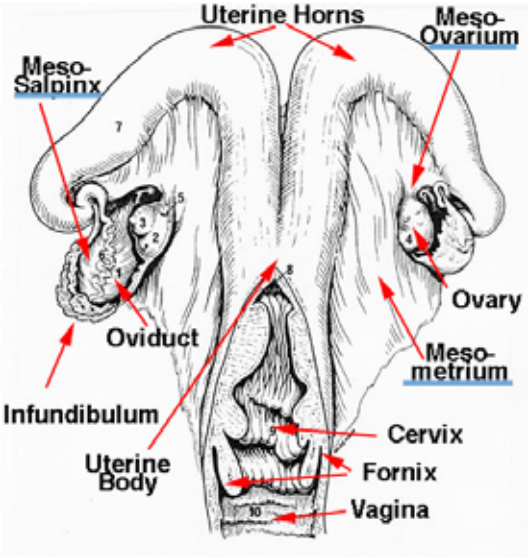
2. ANATOMY AND FUNCTION OF THE COW REPRODUCTIVE TRACT: PRACTICAL CONSIDERATIONS.

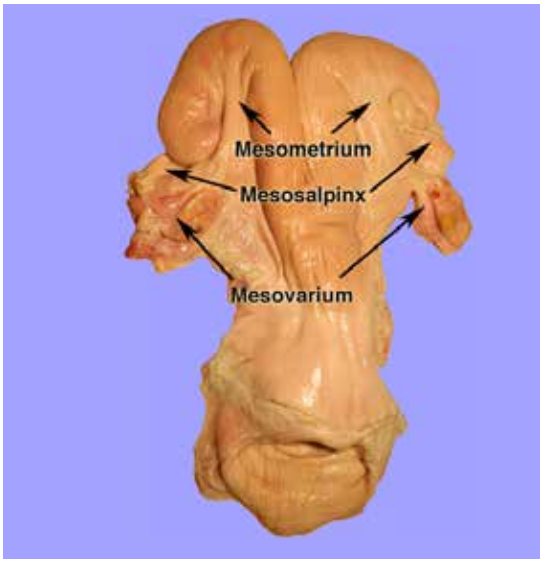
The Female reproductive tract:

- This includes the ovaries, oviducts, uterus, cervix, vagina and vulva.
- The reproductive tract lies below the Rectum and is attached to it at the Recto-genital pouch.
- This attachment allows for manipulation (e.g. rectal palpation) and examination (e.g. ultrasound) of the reproductive tract via the rectum.



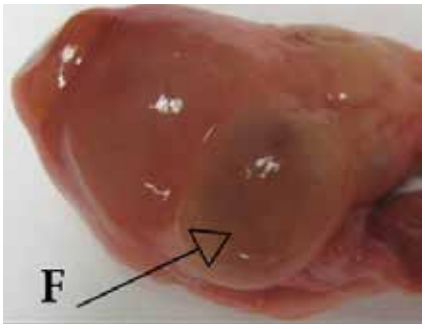
- The female tract is suspended and held in the abdominal cavity by the Broad Ligament.
- Its components are the Mesovarium (attached to ovary), Mesosalpinx (around the oviduct or "salpinx") and Mesometrium (supports the uterine horns).
- The broad ligament provides the blood supply, lymphatic drainage and nerves to the reproductive tract.
- The Mesosalpinx is particularly important as it directs the ova released at ovulation to the oviduct.

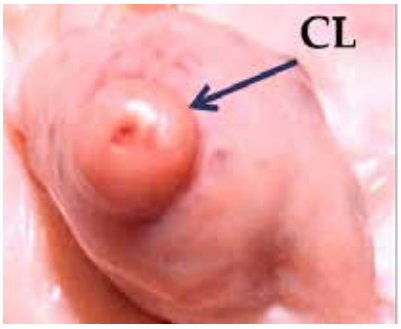


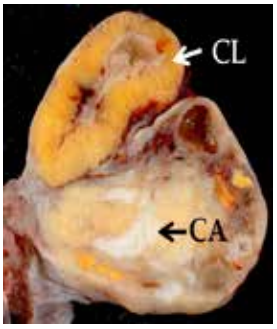


The Ovaries:

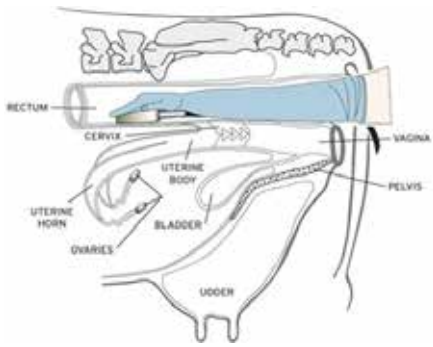
- Function is to produce the eggs (oocytes) and the hormones Estrogen and Progesterone.
  (NB: They also produce other hormones – Oxytocin, Relaxin, Inhibin and Activin).
- Several structures in the ovaries include:
- Follicles (F) – house the oocytes. Females are born with a lifetime supply of primordial (immature) follicles. Some mature and ovulate.
- Corpus Luteum (CL) – "yellow body" that produces Progesterone. As it grows it protrudes from the ovary surface and can be palpated.
- Corpus Albicans (CA) – white, scar-like structures representing degenerating corpora lutea from previous oestrus cycles.







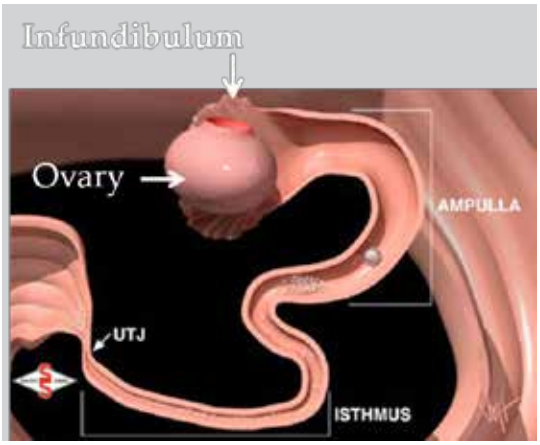
- The ovaries are dense, turgid structures that can be felt per rectum.
- Careful manipulation is done in front of the uterine horns.
- The functional status of the ovary (i.e. whether the cow is cycling) is determined by feeling for the CL and Follicles.
- Ultrasound technology accurately detects these structures.

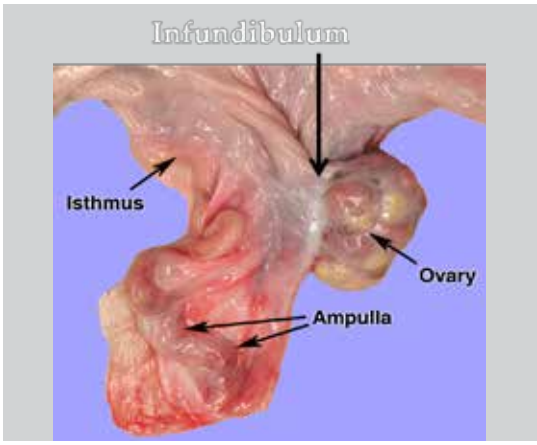


The Oviduct:

Consists of :

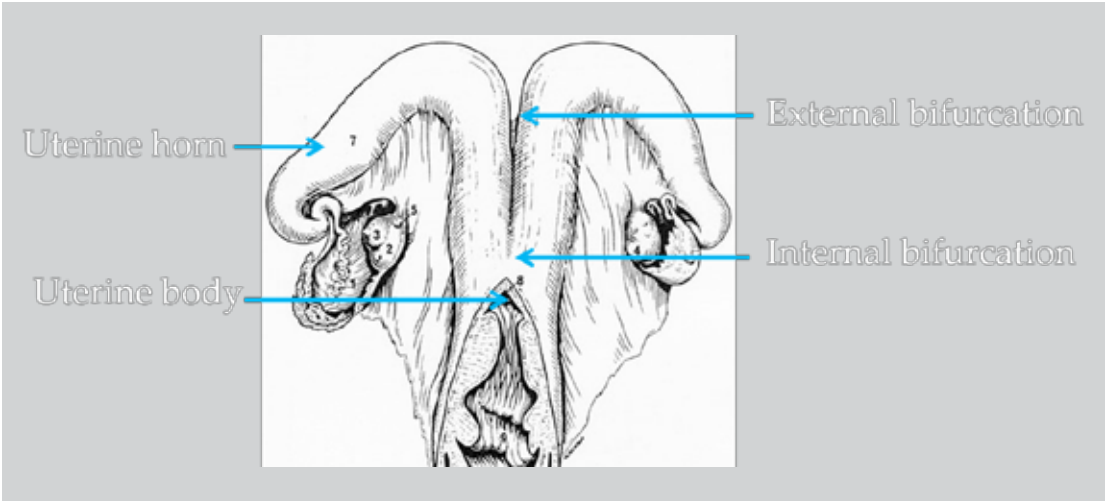
a. Infundibulum – funnel like opening that captures the ovulated egg;
b. Ampulla – longest part of oviduct and site of fertilization between egg and spermatozoa; and
c. Isthmus – smaller in diameter than ampulla and connects to the uterus via utero-tubal junction (UTJ).





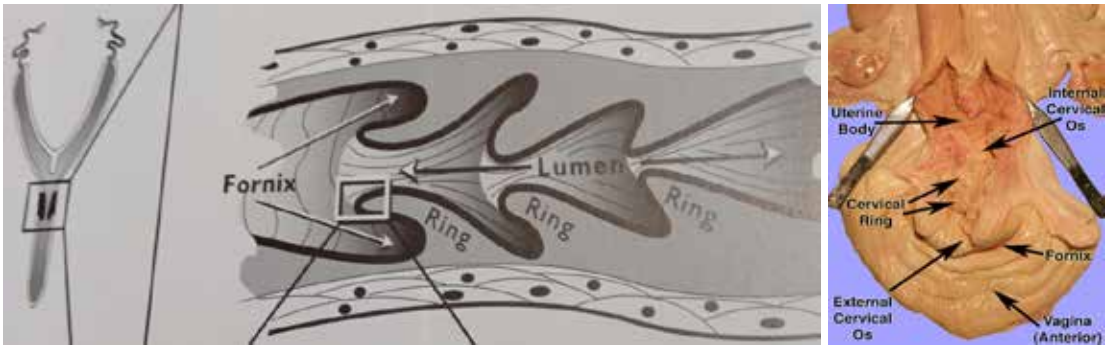
The Uterus:

- Organ of pregnancy.
- Consists of two uterine horns and a uterine body.
- Has an external and internal uterine bifurcation, important during uterine manipulation (e.g. in Embryo Transfer).
- It is turgid (firm tone) when Estrogen levels are high (e.g. Oestrus); and soft or flaccid when Progesterone levels are high (i.e. Luteal phase of cycle).
- The soft tone allows the embryo (fertilised egg) to attach to the uterine lining or endometrium.



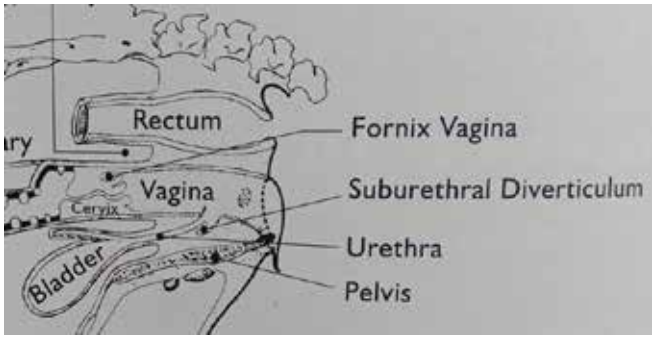
The Cervix:

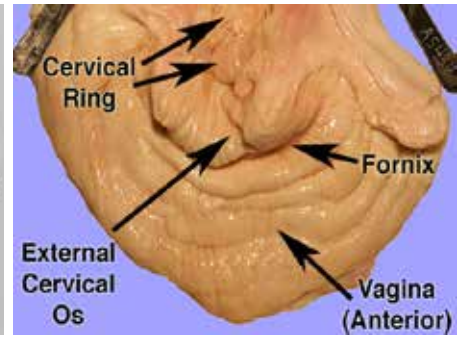
- A thick-walled organ that acts as a barrier between the uterus and external environment during pregnancy.
- Has a canal (lumen) with rings that protrude into it, important during AI.
- It produces mucus during oestrus that lubricates the vagina to assist copulation.
- During pregnancy the mucus becomes thick, preventing entry of microrganisms.



The Vagina:

- The organ of copulation.
- The cervix protrudes into the anterior vagina forming a pocket around it called the Fornix. Sperms are deposited into the fornix by the bull during natural service.
- The posterior vagina (or Vestibule) also serves the urinary system, ie. has urethral opening for expulsion of urine.
- Immediately below the urethral opening is a blind pouch- the sub-urethral diverticulum, where an inexperienced inseminator may put the AI gun.





The Vulva:



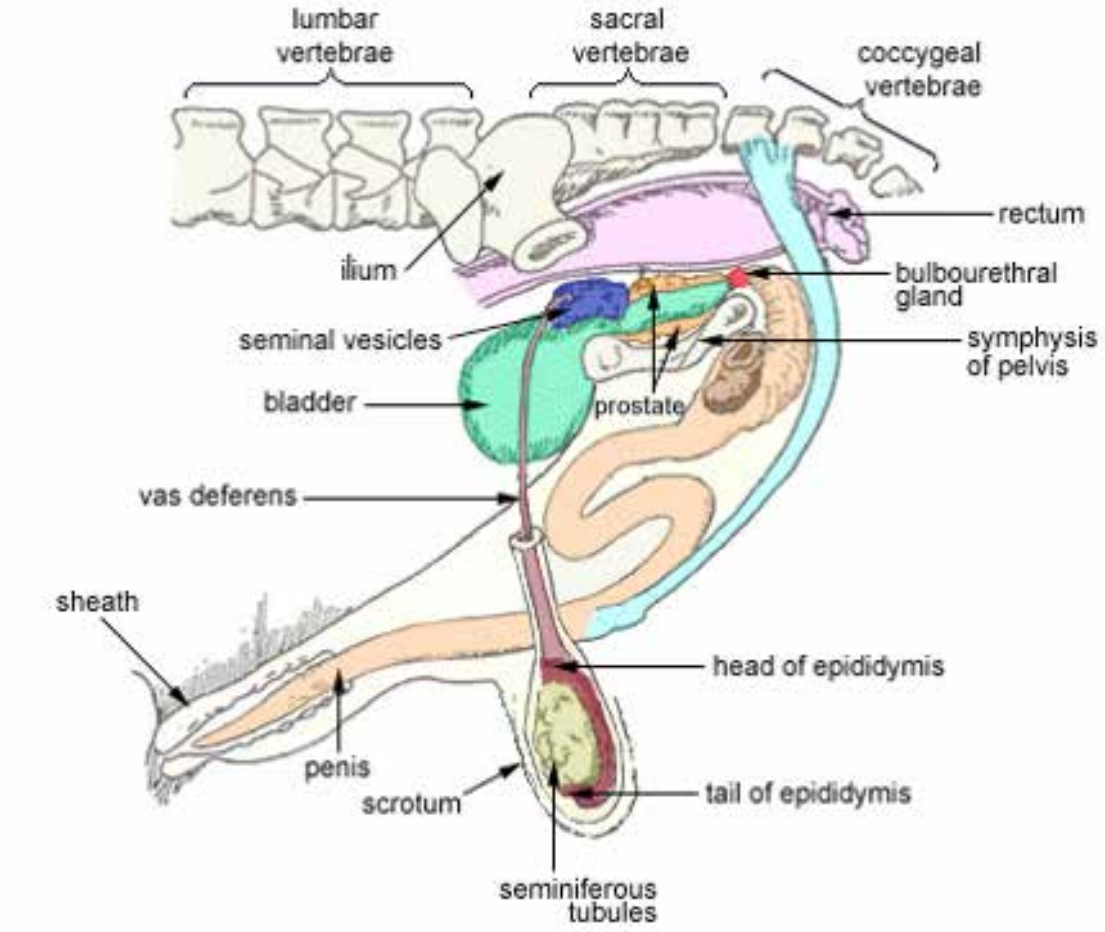
- Part of the external genitalia.
- Consists of two lips (Labia) which meet to form a dorsal and ventral commissure (site of union).
- The skin between the anus and dorsal commissure can get torn during parturition due to foetal oversize.
- The ventral commissure houses the Clitoris.
- Clitoral stimulation (by brief manual pressure at the ventral commissure) during AI can increase conception rates by up to 6% in beef cows.

Take-home Message:

- The Cow reproductive tract has several distinct structures.
- The Ovaries produce oocytes and several hormones.
- The Oviducts provide the optimal environment for fertilization and initial development of the embryo.
- The Uterus is the site for embryo attachment and growth.
- The Cervix secretes mucus during oestrus and produces a protective mucoid seal during pregnancy.
- The Vagina is the organ of copulation, with lubricating mucus to facilitate this during oestrus.
- The Vulva houses the Clitoris, whose stimulation can enhance conception rates during AI.

3. MALE REPRODUCTION ANATOMY AND PHYSIOLOGY

- Although the male sexual organs develop and begin to produce hormones before birth, the production of spermatozoa starts only at puberty.
- Spermatozoa are manufactured in two testes which are the primary sexual organs of the bull.
- The secondary sex organs are the ducts: epididymis, vas deferens and the penis which is traversed by the urethra and leads to the exterior a group of accessory sex glands(seminal vesicle,prostrate,bulbourethra)
- The urethra is also connected to the bladder. Thus the urethra is a common passageway for urine and sexual secretions.



The Testes

- Are the primary sex organs of the male and are formed in the abdomen but at birth descend into the scrotum.
- There are two of them each separated from the other in each scrotal sack by a septum (scrotal septum).

- Each is covered by a firm fibrous tissue called the TUNICA ALBUGINIA, along which blood, lymphatic vessels and nerves enter and leave the testicle.
- Tunica albugnia sends small fibrous tissues into the main testicular substance called the PARENCHYMA.
- These fibrous tissue material divide the parenchyma into small compartments, TESTICULAR LOBULES.
- Inside each LOBULE is found long-tortuous hollow structures (the SEMINIFEROUS TUBULES).
- These are lined with cells that form the sperms, called SPERMATOGONIA.
- The spermatogonia are surrounded by large cells called SERTORI CELLS, believed to basically NURSE the spermatogonia and play a big role in formation of sperms.
- Seminiferous tubules from each lobule, form straight tubules all of which interconnect in the centre of the testicle to produce a network of ducts, the RETE TESTIS.
- Rete testis Collect sperms and secretions of the seminiferous tubules, into other collecting tubules called the EFFERENT DUCTS.

View of testis

- Epididymis
- Rete testis
- Seminiferous tubules

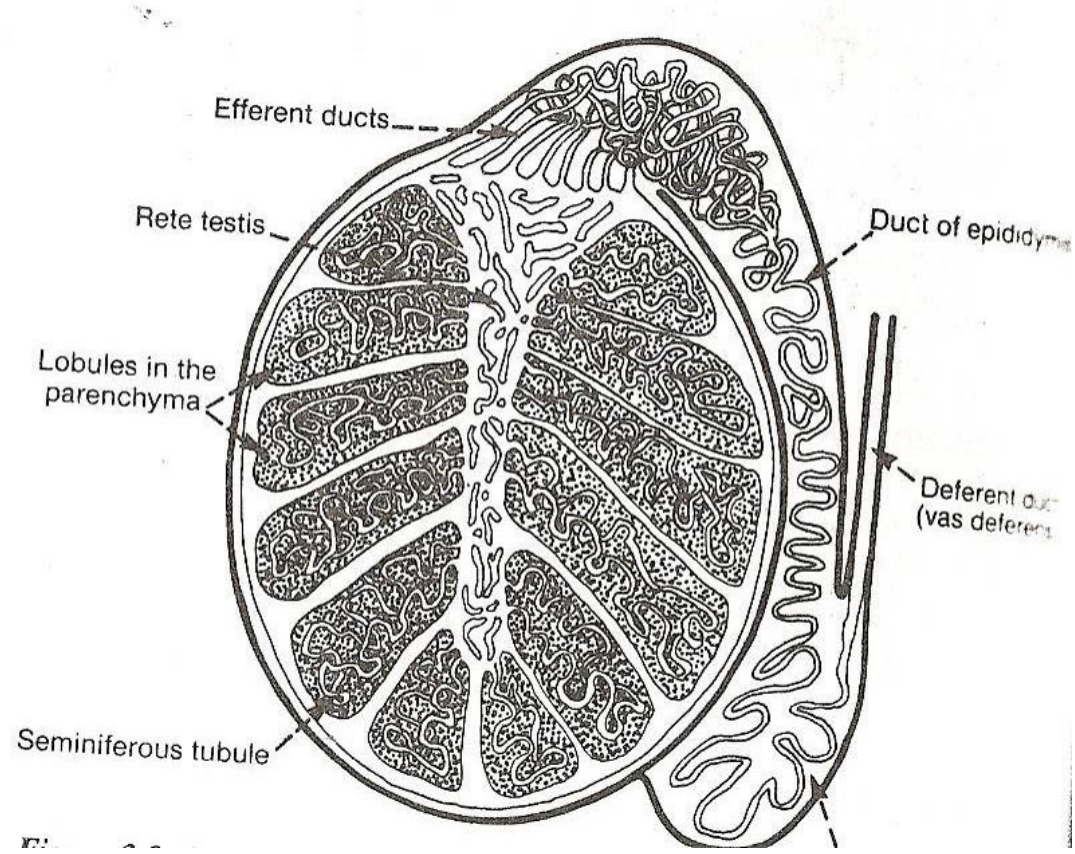


- The EFFERENT DUCTS drain into an enlarged portion where they unite and continue to form a single narrowing tortuous tube the EPIDIDYMIS which is attached to the surface of the testes.
- The first part of this structure where the efferent duct first join is the HEAD OF THE EPIDYDIMIS. The second narrowing part is the BODY OF THE EPIDIDYMIS and the last enlarged portion is the TAIL OF THE EPIDIDYMIS.
- Between each testicular seminiferous tubule is an INTERSTITIAL tissue of the testis.
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- Between each testicular seminiferous tubule is an INTERSTITIAL tissue of the testis.

- Special cells together with the blood vessels, nerves and lymphatic system are found here.
- These cells are the ones which are engaged in the formation of male hormone TESTOSTERONE. They are called LEYDIG CELLS.
- Their closeness with the blood and lymphatic tissue allows the male hormone after formation and secretion to be carried and transported to the rest of the body to allow the hormone to effect its "maleness" imparting action including male sexual behavior.

- From the tail of the epididymis, a thin tubular structure arises and makes its way into the abdomen passing and carrying stored sperms from the tail of the epidydimis during ejaculation and empting into the urethra through an opening where the sperms receives other secretory components from the Accesory sex glands.

- This execretory duct portion of the epidydimis is called the DUCTUS DEFERENS. Before entering its excretory duct in the urethra, it expands into a large portion which is purely for storage purposes (of sperms) and this portion is called the AMPULA.



Epididymis

- The epididymis is for storage and maturation of sperms.
- Wall composed of contractile muscles which on contraction expel stored sperms to ductus deferens.
- Sperm acquire motility and fertility from around the middle of the body to the tail region.
- Secretes and absorbs fluid and so contributes to sperm volume.

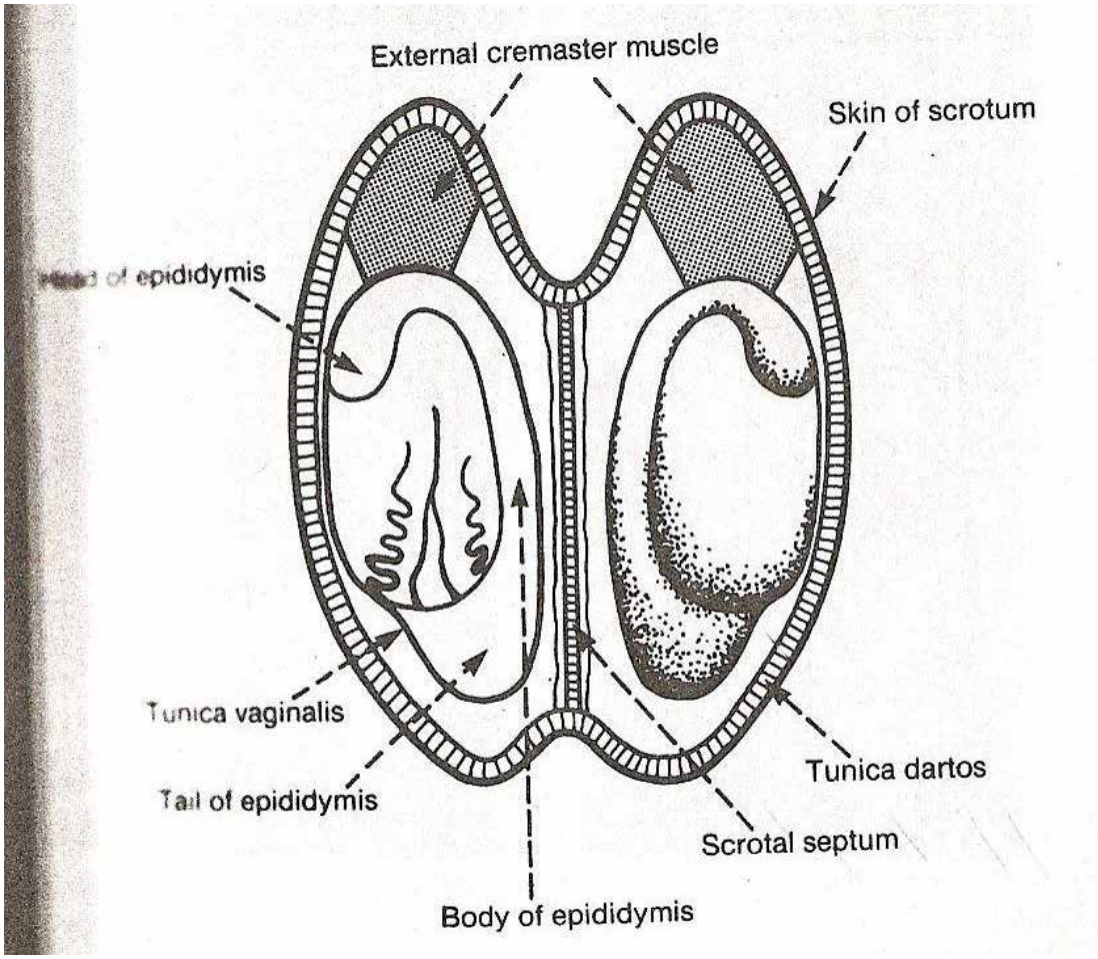
The scrotum

- Houses the testes and is removed from the abdomen (or body). It has a pouch-like appearance. It is in this pouch that the testes are carried.
- At its top it narrows to accommodate the entry and exit of blood vessels, nerves, lymphatic forming the so called PAMPINIFORM PLEXUS area.
- Here the blood vessel entering the testes (artery) is surrounded by the outgoing blood vessel (vein).

- The vein goes around the artery in a tortuous manner many times and in this way, the temperature of the arterial blood going to the testes is cooled by a COUNTER CURRENT HEAT EXCHANGE system.
- The scrotum has a skin covered with hair and containing several sweat glands and sebaceous glands involved in surface water evaporation when external temperatures become hot.

- Inside the skin are contractile muscle fibers, the TUNICA DARTOS which when contracted causes the skin to wrinkle and to withdraw towards the abdomen, slightly, the testicle also moves upwards. This occurs when the external temperature is too cold.
- At its narrow top end, there is a muscular tissue attachment, the CREMASETER MUSCLE which on contraction, carries the testicle closer to the abdomen, and so functions synergistically with the TUNICA DARTOS.

- The contractile functions of these two tissues occur when it is too cold and so aim to warm the testicles.
- All these anatomical arrangements (skin, glands, hair, the plexus etc) allow the testicular temperature to generally be kept lower than that of the body by 2-4°C.
- This is the temperature conducive to formation of sperms.

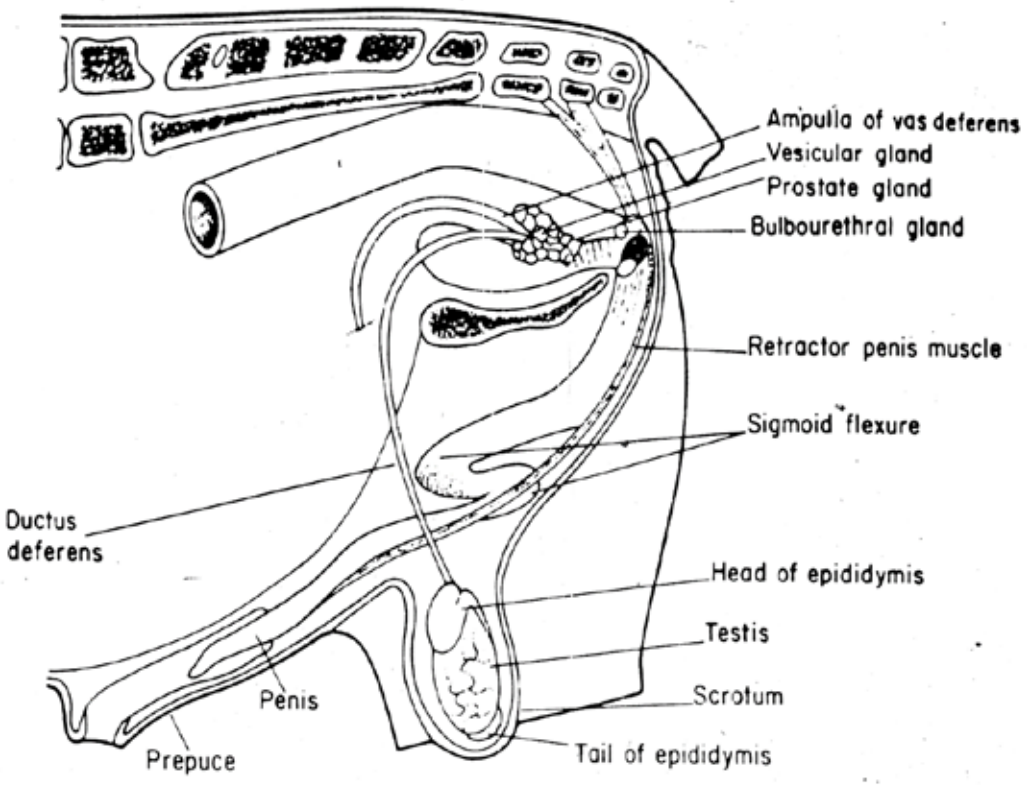


Accessory Sex Glands

- Ampula.
- Seminal vesicles
- Prostate,
- Bulbo – urethral glands

- Consist of the expanded portions of the ducts deferens as it approaches its opening into the urethra and is called AMPULA.
- This like the tail of the epidydimis is a semen storage organ essentially but also adds volume by way of secretions.
- The paired SEMINALVESICLES are the largest of the accessory sex glands and open into the urethra in same place as the opening of the ductus deferens . Contributes the largest portion of seminal plasma.
- The PROSTATE, completely spread all over the wall of the pelvic urethra, has very many openings into the urethra and so adds seminal plasma volume.

- The paired BULBO – URETHRAL GLANDS are in the pelvic surface of the urethra and each has its opening into the urethra.



Bull's genitalia (after Ashdown, R. R. and Hancock, J. L. in Hafez, *Reproduction in Farm Animals*).

The Penis

- Lies along the ventral abdomen housed in the smooth skin invagination, called the PREPUCE.
- It is Fibro – elastic, covered by the very tough tunica albuginia, enclosing the erectile tissue – which upon nerve stimulation and blood flow alterations, enlarge and erect allowing intromission into the vagina.
- Has an S-shaped "reserve" portion, the SIGMOID FLEXURE, held in this form by a contractile muscle, the RETRACTOR PENIS MUSCLE, running along the perineum, attaching at the sigmoid flexure.

Physiology of the Male Reproductive System

- The main or primary organ of the male reproductive system are the Testes whose role, like that of the ovary is

1. Production of sperms
2. Production of hormones

- All the other parts of the organs are supportive and ensure that semen is deposited into the genitalia of the female at oestrus.
- GnRH from the hypothalamus stimulates the anterior pituitary gland to secrete FSH and LH.
- FSH from anterior pituitary acts on SERTOLI CELLS of the testis in the process leading to formation of spermatozoa namely, growth by division of cells of the seminiferous tubules, their maturation and final release as spermatozoa. It also allows the sertoli cells to produce numerous secretions helping in the process of sperm formation maturation and release.
- LH from the anterior pituitary works on the LEYDIG cells to produce the Testosterone responsible for male sexual behavioral characteristics including sexual urge (LIBIDO).
- Testosterone also act on the accessory glands and the EPIDIDYMIS to cause secretions of the fluid that form the seminal plasma.

4. ARTIFICIAL INSEMINATION

INTRODUCTION

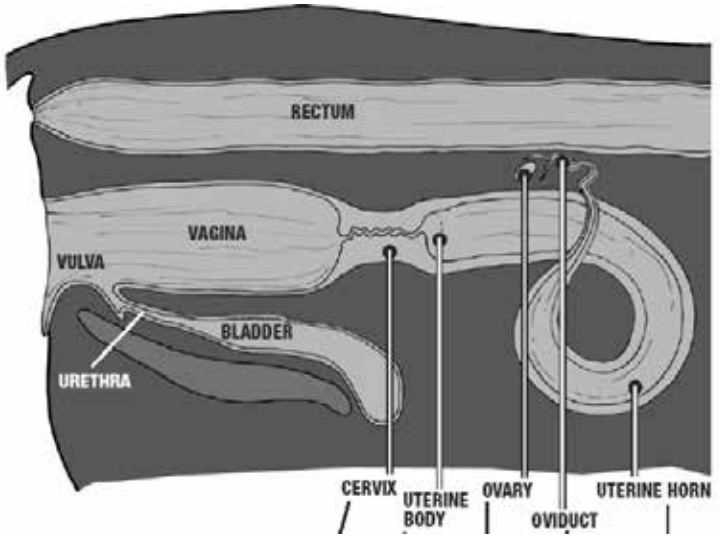
- Artificial Insemination (A.I) is a method in which semen is obtained from the male and introduced into female reproductive tract by means of instruments.
- It is the most important technique devised for genetic improvement in cattle.
- All efforts to make insemination successful by properly collecting, handling and processing semen are to no Vail if the final stage of insemination procedure is not properly carried.
- Sperm must be deposited within the reproductive tract best location and at the best time to enable the spermatozoa to meet an ovum.

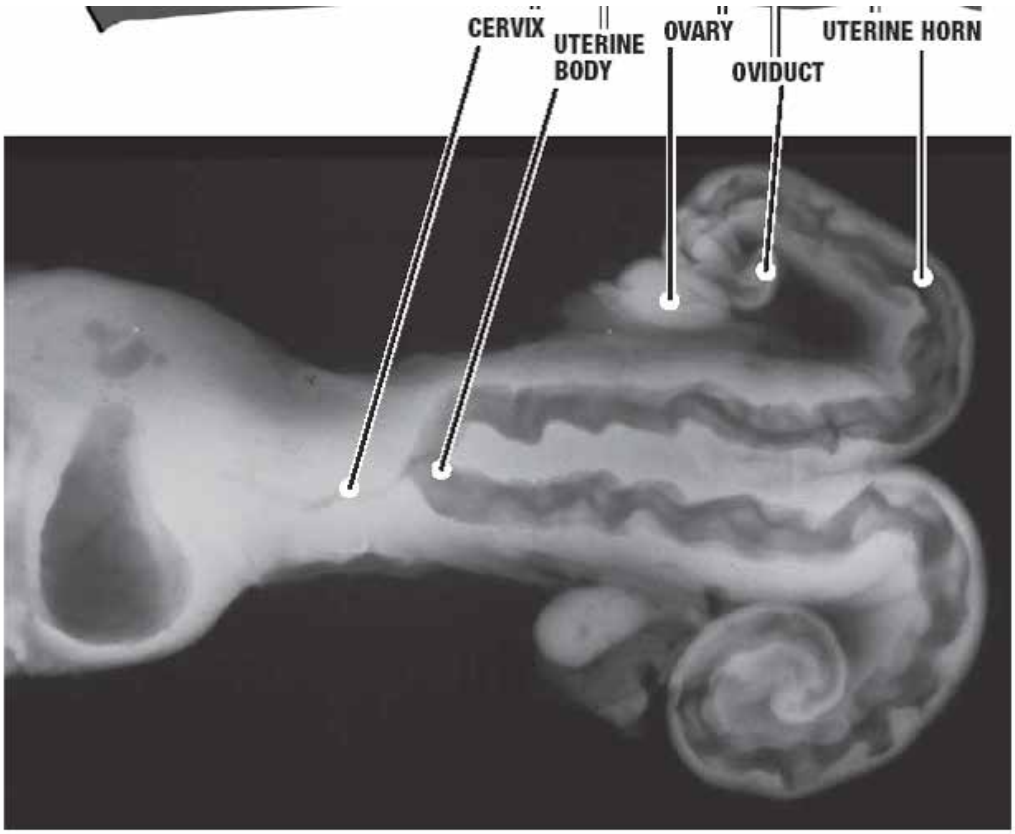
Early Methods of AI

- Deposition of the semen in the vagina, as would occur in natural mating.
- "speculum" method – Easy learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today.

Reproductive Anatomy

- In the early days of AI there was controversy among researchers about the optimum site for semen deposition.
- A studies conducted provided evidence that fertility was highest when semen was deposited in the uterine body.
- Failure to understand the anatomical and functional relationships among the various tissues and organs of the reproductive system may lead to consistent insemination errors.





Preparations for InseminationPre Insemination Checks

- Confirm Identity of the cow/heifer to be inseminated.
- Ensure that the cow to be bred is truly in heat.
- Ensure the cow is in good condition…physical and Health.
- Check for any abnormal discharges…Sign of infection – Need to treat before breeding.
- Blood Discharge?... May be too late to breed.
- The first step in the insemination process is to restrain the animal to be inseminated.
- There are several things to keep in mind when choosing a location for inseminating cattle:
  - Safety of both the animal and the inseminator.
  - Ease of use.
  - Shelter from adverse weather.

Reproduction History

- The farmer should give the following information:-
  - Date of last calving
  - Last date inseminated

- When cow started showing signs of heat and which ones
- When cow seen on standing heat
- Which breed/bull the farmer prefers.
- Sire of the cow to be inseminated

AI Equipment/Supplies

- Before setting off for the A.I., Technician should ensure he/she has the following items.
- Protective clothing
  - Gum boots, apron - these should be worn on arrival at the farm.
- A.I. equipment
  - Pistollette, Plastic socks, Forceps, thawing goblet, 2 thermos flasks (with one containing hot water) hand gloves, Thermometer, ,Paper towels,
- Semen
  - Deep Frozen, Semen in Liquid Nitrogen Container with enough liquid nitrogen

Records

- A.I. Records
  - Cow card
  - Pen
  - Receipt / Invoice book
- Restrain the cow first and then thaw the semen.
- The restraint area should be familiar to the cow and free of stressful conditions.
- Unnecessary excitement may interfere with physiological mechanisms important to achieving a good conception rate.

Hygiene

- Develop good sanitary procedures and practices throughout the Insemination process.
- It is easier to learn good habits than to break bad habits.
- Levels of hygiene in AI:
  - Bull
  - Semen Collection
  - Semen Processing
  - Instruments
  - Inseminator
  - Insemination
  - The Female

- Insemination supplies should be kept dry and clean at all times. Breeding sheaths should be stored in the original package until used.
- Once the insemination device is assembled it must be protected from contamination and cold shock temperatures.
- Materials used to lubricate the rectum should not come in contact with the vulva region. Some lubricants are spermicidal.
- Avoid using products that are irritating.
- The vulva region must be thoroughly wiped clean with a paper towel.
- This is important in helping prevent the interior of the reproductive tract from becoming contaminated and possibly infected.
- A folded paper towel can be inserted into the lower portion of the vulva. The insemination rod can then be placed between the folds of the towel and inserted into the vagina without contacting the lips of the vulva.

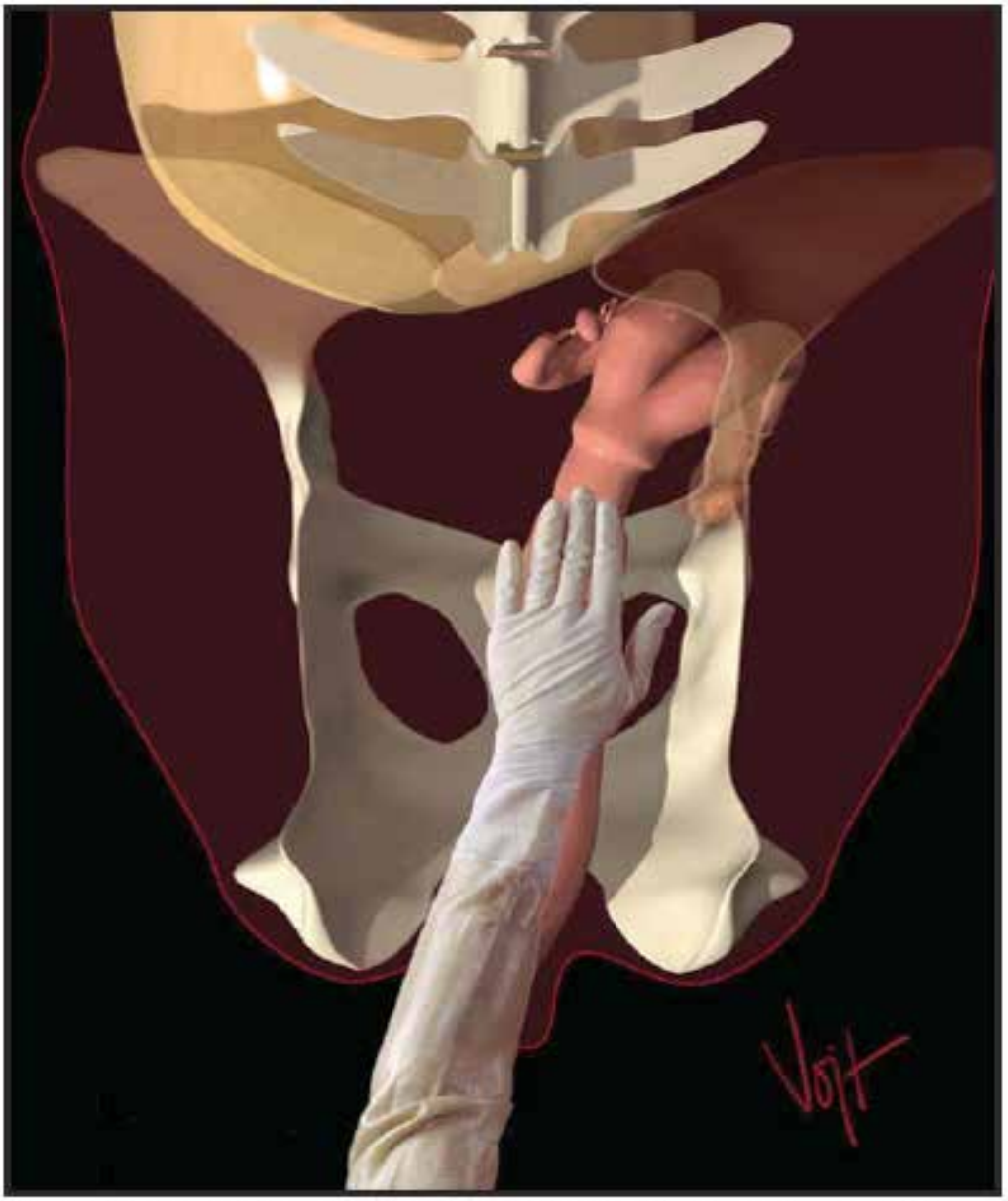
Thawing Procedure

- Open the container.
- Immerse the forceps into LN2 to cool it down.
- Bring the canister for straws carefully to the neck of the container and remove semen straw with a cool pair of forceps.
- Within less than 3 seconds place the straw into the thermos flask containing warm water at 34°C for 30 seconds.
- Remove the straw from the water and dry it properly on paper towel.
- Hold the straw upright, with the cotton end at the bottom and roll the bottom end between the thumb and the fore finger in order to free the cotton wool stopper.
- Tap the straw gently to allow air bubble to rise to the top.
- Using a pair of scissors, cut off the straw through the air bubble. Hold the scissors horizontally.
- Place the straw into the Pistollette – sealed end first.



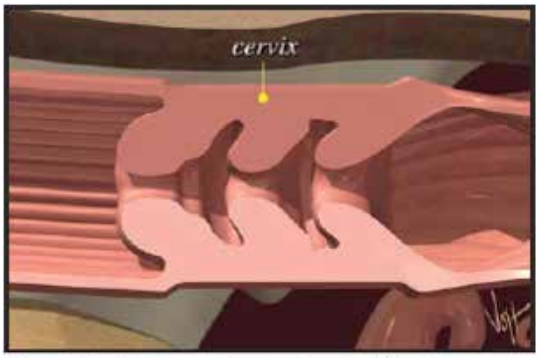
Insemination Technique – Rectovaginal Technique

- Regardless of whether you are left or right handed, it is recommended that you use your left hand in the rectum to manipulate the reproductive tract and the right hand to manipulate the insemination gun.
- This is because the rumen cow lies on the left side of the abdominal cavity, displacing the reproductive tract slightly to the right. Thus, you will find it much easier to locate and manipulate the tract with your left as opposed to right hand.

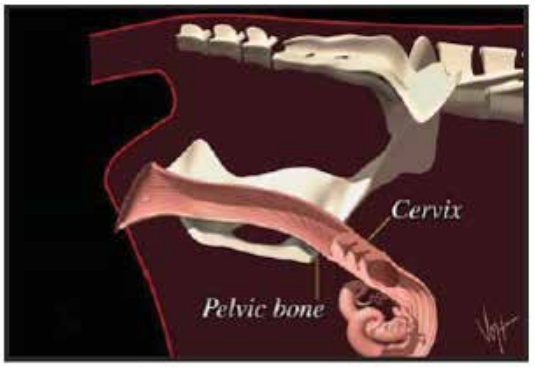


Because the rumen displaces the reproductive tract to the right, it is much easier to locate and manipulate the tract with your left hand.

- A gentle pat on the rump or a soft-spoken word as you approach for insemination, will help to avoid startling or surprising the animal.
- Raise the tail with your right hand and gently massage the rectum with the lubricated glove on your left hand.
- Place the tail on the back side of your left forearm so it will not interfere with the insemination process.
- Cup your fingers together in a pointed fashion and insert your hand in the rectum, up to the wrist.
- Gently wipe the vulva with a paper towel to remove excess manure and debris. Be careful not to apply excessive pressure, which may smear or push manure into the vulva and vagina.
- With your left hand make a fist and press down directly on top of the vulva. This will spread the vulva lips allowing clear access to insert the gun tip several inches into the vagina before contacting the vaginal walls.
- Insert the gun at a 45° upward angle to avoid entering the urethral opening and bladder located on the floor of the vagina.
- With the gun about 6 to 8 inches inside the vagina, raise the rear of the gun to a somewhat level position and slide it forward until it contacts the external portion of the cervix.
- You will note a distinct gristly sensation on the gun when it contacts the end of the cervix.
- The cervix your primary landmark for inseminating cattle.
- The size will vary, with post partum interval and age of the animal.
- The cervix usually has three or four annular rings or folds.
- The opening into the cervix protrudes back into the vagina. This forms a 360° blind-ended pocket completely around the cervical opening. This pocket is referred to as the fornix.
- In most cows, the cervix will be located on the floor of the pelvic cavity near the anterior end of the pelvic bone.
- In older cows with large reproductive tracts, the cervix may rest slightly over the pelvic bone and down into the abdominal cavity.
- It is very important that you always know where the tip of the insemination gun is located.
- The insemination gun can be easily felt with your palpating hand.
- As you insert the breeding gun into the vagina, keep your gloved hand even with the gun tip.
- Manure in the rectum can often interfere with your ability to palpate the cervix and gun tip. However, it is seldom necessary to remove all the manure from the bowel.

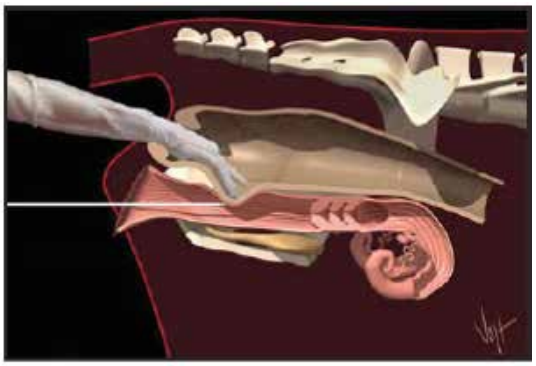


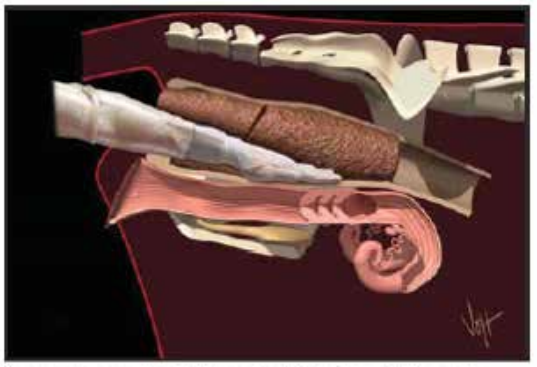
The opening into the cervix protrudes back into the vagina.



The cervix is located on the floor of the pelvic cavity near the anterior end of the pelvic bone.

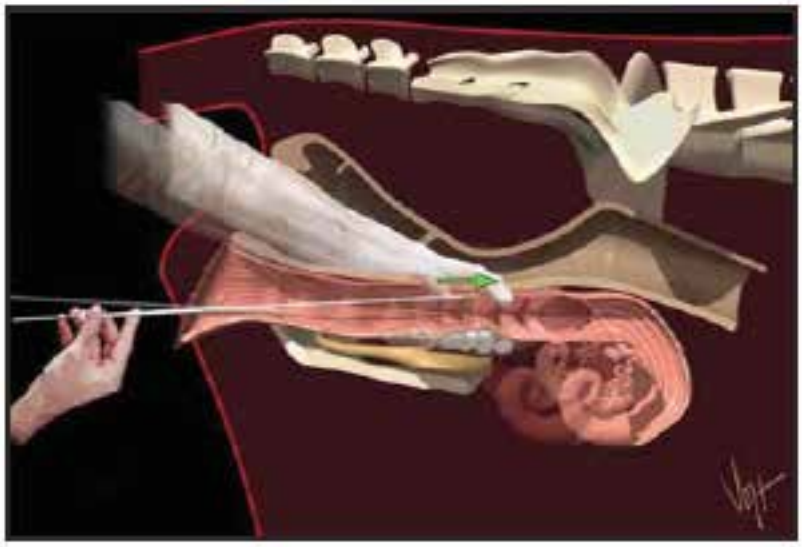
- Instead, keep your open hand flat against the floor of the rectum, allowing the manure to pass over the top of your hand and arm.
- While handling the cervix you may notice rectal constriction rings attempting to force your arm from the cow.
- To relax these rings, place two fingers through the center of a ring and massage back and forth. The constriction ring will eventually relax, pass over your hand and arm, and you can continue the palpation process.


As you insert the breeding gun into the vagina, keep your gloved hand even with the gun tip.


Keep your open hand flat against the floor of the rectum, allowing manure to pass over the top of your hand and arm.

- Because the reproductive tract is freely movable, cows that have strong rectal and abdominal contractions in response to being palpated may actually push their reproductive tract back into the pelvic cavity. This will cause many folds to form in the vagina.
- In such cases, the insemination gun will often get caught in these folds and little or no progress will be made until they are removed.
- If you can locate the cervix, grasp it and push it forward. This will straighten the vagina and the gun should pass freely up to the cervix.

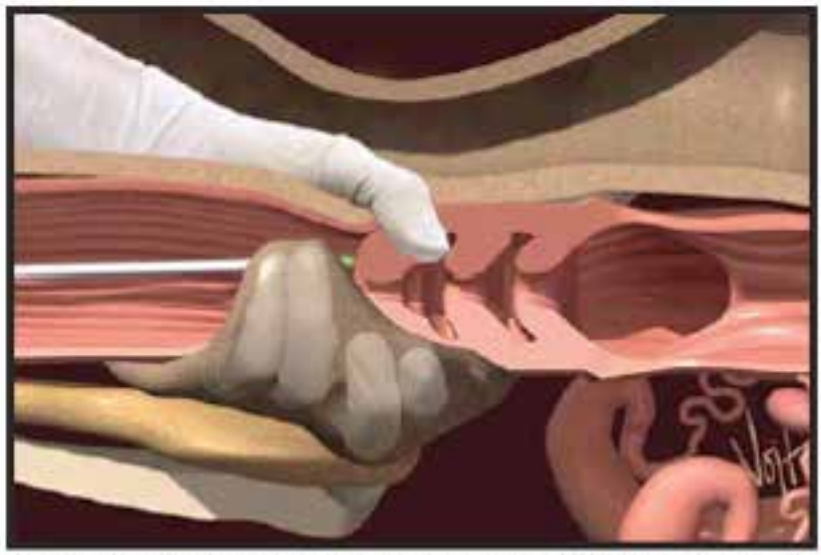
- If you cannot locate the cervix, encircle the gun tip with your thumb and forefingers. With a straightening motion of your wrist, gently "milk" the folds out of the vagina a little at a time.
- Slide the gun forward and repeat the process until the cervix is reached.



Grasp the cervix and push it forward to straighten vaginal folds.

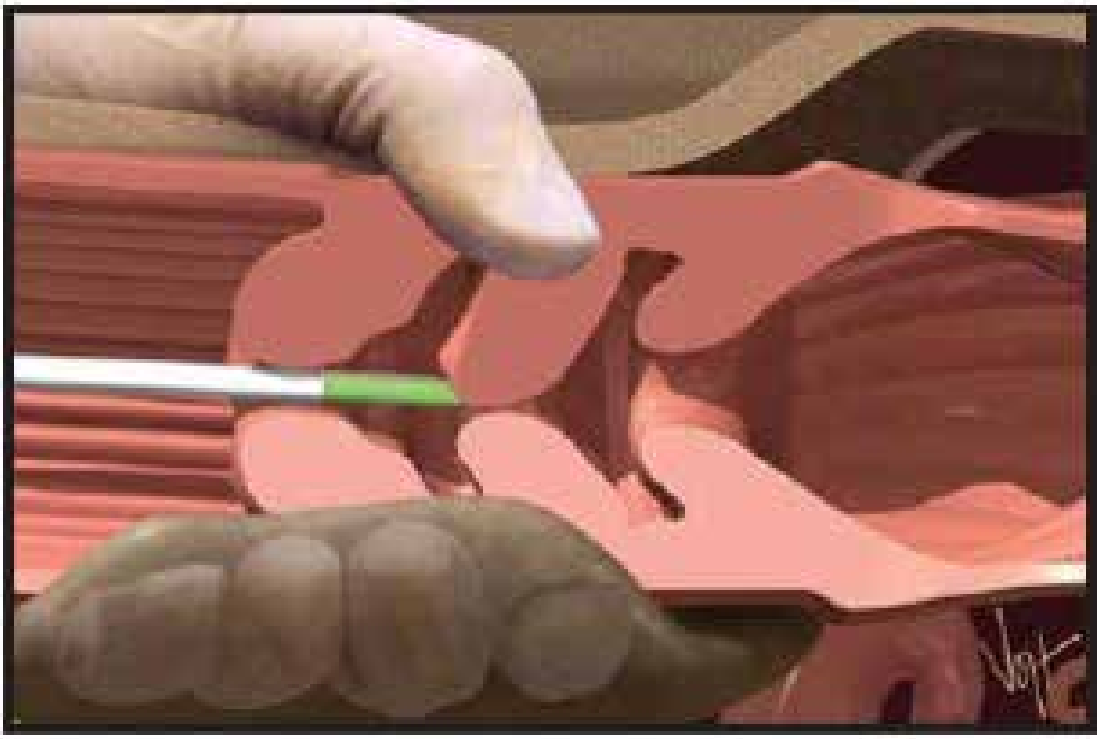
- Inseminating a cow is a two-step process.
- The first step is to get the gun tip to the cervix.
- To accomplish this you must work the vagina and cervix forward, away from you to straighten the vaginal folds.
- If you do not feel the gristly sensation of the cervix on the gun, you are still in step one of the process.
- Once the gun is in contact with the external surface of the cervix you are ready to begin step 2.
- In step 2, you place the cervix on or over the insemination gun.
- The cervix is placed on the gun, the gun is not passed through the cervix.
- Excessive movement or probing with the insemination gun during the second step is seldom productive and in fact, is very often counterproductive. Ground gained is often lost and we find ourselves back in a vaginal fold.
- The key to mastering step 2 of the insemination process is to know how to hold and manipulate the cervix and concentrating on doing the work with the hand inside the cow, not the one holding the gun.
- When the gun first contacts the cervix, you will usually find that the tip is in the fornix directly over top of the opening.

- Grasp the external opening to the cervix with the thumb on top and forefingers underneath. This closes the fornix at top and bottom.
- As in step 1 we must still know the location of the gun tip. This is accomplished with the palm and third and fourth fingers of your palpating hand.
- Use your palm and these two fingers to guide the gun tip to the cervical opening located between your thumb and forefingers.



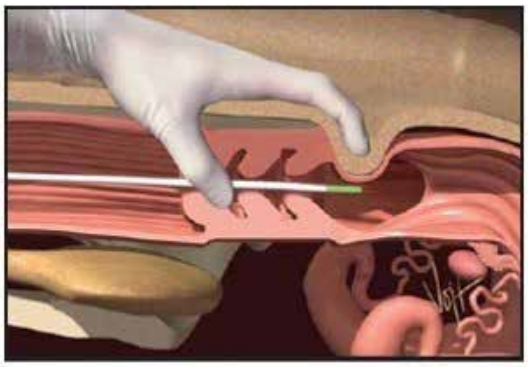
Grasp the external opening to the cervix with the thumb on top and the forefingers underneath to close the fornix and guide the gun tip into the cervix.

- With gentle probing the opening should be located. You will feel the gun slide forward until it contacts the second cervical ring.
- Maintain gentle but steady forward pressure on the gun and slide your thumb and forefingers just in front of the gun tip and re-grasp the cervix.
- Because the cervix is composed of dense connective tissue and muscle, it is difficult to clearly distinguish the gun tip when it is located within this structure. However, you can determine the approximate location by bending the cervix.
- Using the flexibility of your wrist, twist and bend the cervix until you feel the second ring slide over the gun tip.
- Repeat the process until all the rings have been passed over the gun tip.
- In some cases, it may be necessary to bend the cervix at a 90° angle to clear the cervical folds.
- Remember, you are placing the cervix over the gun, not the gun through the cervix.

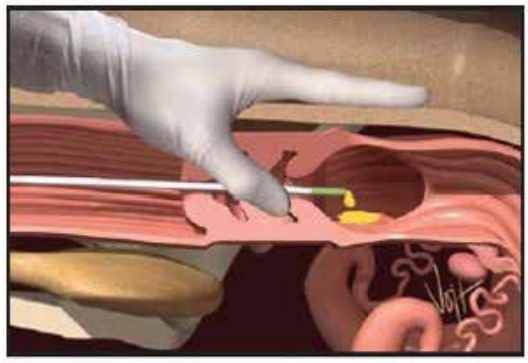


Using the flexibility of your wrist, twist and bend the cervix until you feel the second ring slide over the gun tip.

- When all rings of the cervix have been cleared, the gun should slide forward freely with little resistance.
- Since the uterine wall is very thin, you will once again be able to clearly feel the insemination gun. You are now ready to check your placement and deposit the semen.
- Rotate your gloved hand until it lies on top of the cervix. With your index finger, locate the far end of the cervix.
- Pull back on the gun until you feel the tip directly underneath your finger near the internal opening of the cervix.
- Raise your finger and slowly deposit the semen.
- Push the plunger slowly so that drops of semen fall directly into the uterine body.
- With proper A.I. technique and gun placement, semen will be deposited in the uterine body.
- Uterine contractions will then transport spermatozoa forward to the horns and oviducts with a good distribution of both sides.
- When the insemination gun is more than 1" through the cervix, all the semen will be deposited in only one horn.

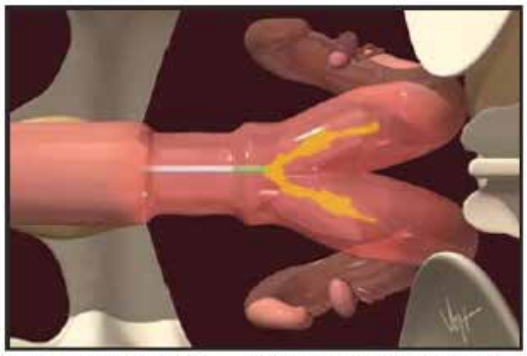


Use your index finger to check gun placement (1/4 inch past the end of the cervix) before depositing semen.

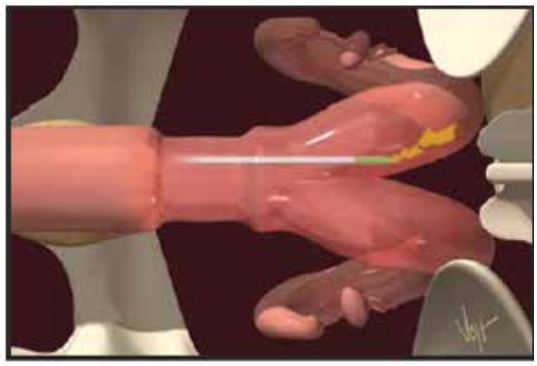


Push the plunger slowly so that drops of semen fall directly into the uterine body.

- This creates a situation of uneven semen distribution. Should the animal ovulate from the opposite horn, conception rates may be compromised.



With proper A.I. technique and gun placement, semen will be deposited in the uterine body and contractions will transport spermatozoa forward to the horns and oviducts.



If the gun is more than 1 inch through the cervix, all the semen will be deposited into only one horn.

- Make sure you push in with the plunger and do not pull back on the gun.
- Pulling back may result in much of the semen dose being deposited in the cervix and vagina instead of the uterine body.
- After properly depositing semen, slowly pull the gun from the reproductive tract.
- Remove the gloved hand from the rectum and shake off the excess manure.
- Check the gun tip for signs of blood, infection or semen leakage inside the sheath.
- Make notes for your veterinarian or future reference where appropriate.
- Remove the sheath from the gun and hold it in the gloved hand.
- For the final time, check to confirm which bull you have used.
- Remove the glove starting at the top of your arm by turning it inside out as you remove it.
- Remove air from the glove and tie a knot at the open end to trap manure, the sheath and dirt inside. Dispose of the used glove in a proper receptacle.
- Wipe the gun clean and dry and return it to the proper storage location.

Very Important

- Be gentle. Don't use too much force.
- Insemination is a two-step process. Get the gun to the cervix, and then place the cervix over the gun.
- Deposit the semen just through the cervix into the uterine body.
- Take your time.
- Relax.

5. ARTIFICIAL INSEMINATION RECORDS

COMPONENTS OF HERD RECORDING

i. Ownership and Animal Identification,
ii. Extended pedigree information,
iii. Breeding (AI)
iv. Registration Records
v. Reproduction,
vi. Production level at farm level,
vii. Health Report,
viii. Purchases and Sales Receipts.

1. IDENTIFICATION INFORMATION:

- Ownership details,
- Identification Name and/or Number of animal,
- Identification method (Ear Tag, tattoo etc),
- Birth date or date purchased,
- Breed Type,
- Registration Status.

2. PEDIGREE INFORMATION

- Extended Family (Names & ID Nos):
  - Dam
  - Sire
  - Sire`s Sire
  - Sire`s dam
  - Dam`s Sire
  - Dam`s Dam
- Registration Status:
  - Foundation,
  - Intermediate,
  - Appendix,
  - Pedigree or Pure

3. BREEDING INFORMATION:

- Dates heat observed,
- Service Dates,
- Sires ID and Breed,
- Technician`s ID,

- Type of Service (First or Repeat),
- Pregnancy Diagnosis Results and Dates.
- Follow-up comments

REPRODUCTION RECORDS

- Expected Date of Calving,
- Date of calving,
- Calving complications (more in cattle,
- Type of Birth:
  - Single /multiple,
  - Sex.

ARTIFICIAL INSEMINATION RECORDS

- What is an AI Record?,
- Importance of an AI Record?,
- Who should be responsible to keep AI Records?,
- Methods of keeping AI Records.

COMPONENTS OF ARTIFICIAL INSEMINATION RECORDS

i. Semen production,
ii. Semen Distribution,
iii. Insemination Records,
iv. Artificial Insemination Reports.

ARTIFICIAL INSEMINATION RECORDS

i. Semen Pruduction Records:
   - Semen Collection (Bull Identification Critical),
   - Semen Evaluation,
   - Semen Processing ,
   - Semen Storage (At Source and Field).

ii. Semen Distribution:
   - Semen doses and AI Accessories required,
   - Semen doses and accessories supplied,
   - Semen doses wasted.

iii. Insemination Records
   - Records on semen received, used and wasted
   - Animal Ownership:
     - Farm identification,
     - Owner`s Identification (Name, Location),

- Animal details:
  - Animal Identification (Name, Ear Tag , Tatoo No., Date of Birth, Sire, Breed),

iv. Insemination details :
- Date, Time, ID of Semen used ,
- Technician`s ID).
- Previous date of calving, sex and status of calf /(sold, dead or alive).

v. Repeat or First Insemination

vi. Remarks for follow-up),

vii. Insemination Fee charged.

EXPENDITURE AND REVENUE RECORDS

- Transport,
- AI Accessories,
- Revenue.

IMPORTANCE OF INSEMINATION RECORDS

i. Technical analysis for national planning and Budgeting:
- Semen production requirement,
- Number of Inseminations achieved,
- Expected number of pregnacies and births,
- Expected improvement of Dairy herd size,

ii. Monitoring of AI Service:
- Bull fertility,
- Repeat services,
- Avoiding inbreeding,
- Calf mortality,
- Technician`s efficiency.

iii. Revenue collection,

iv. Technical and Economic performance reports.

v. Sire selection for Genetic Improvement (Progeny Testing),

vi. Livestock Registration support
(Foundation, Intermediate, Appendix, Pedigree Status),

vii. Livestock showing:
- Champion class.
- Progeny class.

viii. Herd Management (culling etc),

ix. Evaluation of sires associated with calving deformities,

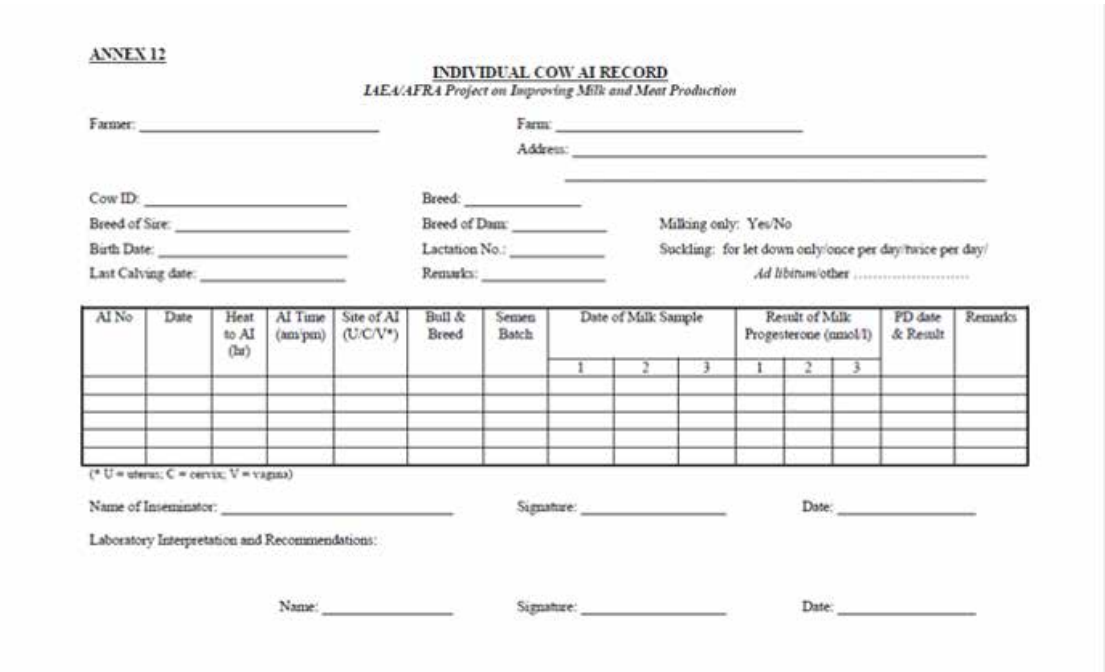
x. Education and Research,

xi. Value addition in marketing of Livestock.

RECORDING MATERIALS

- Notebooks for dairy events,
- Cards in Files,
- Papers in files
- Wall charts, especially for reproduction,
- Computer,

  NB: Head recording not viable in modern livestock keeping (Except pastoralists).



ANNEX 12

INDIVIDUAL COW AI RECORD

IAEA/AFRA Project on Improving Milk and Meat Production

Farmer: _____ Farm: _____

Address: _____

_____

Cow ID: _____ Breed: _____

Breed of Sire: _____ Breed of Dam: _____ Milking only: Yes/No

Birth Date: _____ Lactation No.: _____ Suckling: for let down only/once per day/twice per day/

Last Calving date: _____ Remarks: _____ *Ad libitum*/other .....

AI No	Date	Heat to AI (hr)	AI Time (am/pm)	Site of AI (U/C/V*)	Bull & Breed	Semen Batch	Date of Milk Sample			Result of Milk Progesterone (nmol/l)			PD date & Result	Remarks
							1	2	3	1	2	3		

(* U = uterus; C = cervix; V = vagina)

Name of Inseminator: _____ Signature: _____ Date: _____

Laboratory Interpretation and Recommendations:

Name: _____ Signature: _____ Date: _____

6. AI FAILURE: CAUSES AND MANAGEMENT

WHY IS GOOD REPRODUCTION PERFORMANCE?

- The level of reproductive performance of a herd closely reflects its reproductive health.
- To avoid losing the herd.
- To enhance the profitability of a farm in the short and long term.
- Improved efficiency of production through
  - Increased milk production per day of herd life;
  - Increased numbers of calves per cow;
- Minimization of costs associated with
  - Maintaining non-lactating cows
  - Loss of production due to reproductive problems at calving
  - Veterinarian consultations and breeding costs;
  - Culling cows for failure to reproduce.

  Increases the speed of genetic gain:
  - Culling of cows for low production rather than reproduction problems;

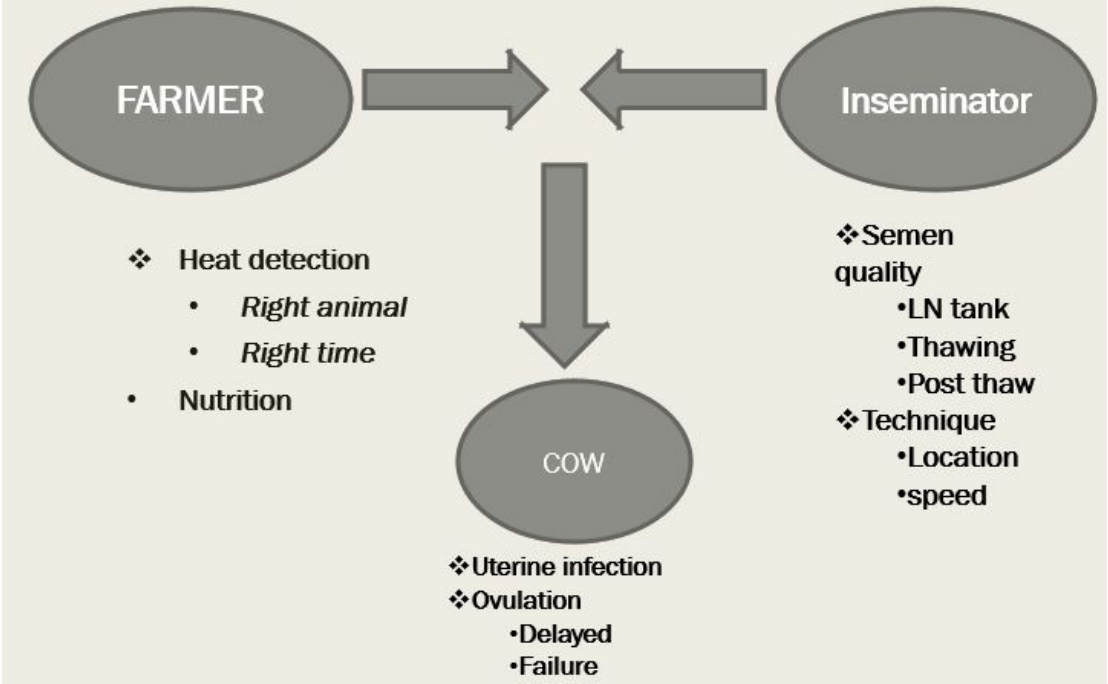
THE REPRODUCTIVE PROCESSES

<table>
<tr><td colspan="2">ESTROUS CYCLE</td><td rowspan="2">CONCEPTION</td><td colspan="2">PREGNANCY</td><td rowspan="2">PARTURATION</td><td>LACTATION</td></tr>
<tr><td>ESTRUS</td><td>OVULATION</td><td>EMBRYO</td><td>FETUS</td><td>NEONATE</td></tr>
<tr><td>ANAESTRUS</td><td colspan="3">INFERTILITY</td><td>ABORTION</td><td colspan="2">PERINATAL & NEONATAL DEATH</td></tr>
</table>

FERTILITY

- Is the ability of an animal to be able to produce a viable young within a stipulated period in the species .
- A cow should be able to produce at least one viable young within a year.
- Any day that passes with a cow not pregnant when she is supposed to be means money lost.
- The ultimate measure of AI success is production of a viable offspring
- This should be done with minimal attempts and therefore shortest possible period.
- For this to happen, there are several players, each with a specific role.
- Each of them must perform effectively and efficiently to achieve overall success.
- The major players in this activity are:
  The farmer, Inseminator and the cow.
- Each of these could contribute to failures.
- AI failure if any and the nature of it best identified if there are cow records.

THE AI TRIPARTITE



- Each of the "parties" are equally important and must play their role.
  - The farmer keeps the cow and wants her pregnant.
  - The cow has to show heat and be free of infections to enable her get pregnant and carry pregnancy to term.
  - The technician has to do what it takes to get the cow pregnant

1. The farmer

- The farmer or his/her appointed agent is in touch with the animal on regular basis.
- He is responsible for the cow management which includes record keeping and heat detection.
- Timing of AI - Based on proper record keeping and good observations the right cows should be presented for AI at the right time. Unless that happens cows will not get pregnant.
- Good Cow nutrition to enable them manifest heat. If this doesn't happen, heats will either be weak, missing or silent, such cows will not get pregnant.
- Any advice and follow-up should be strictly observed.

Mitigation

Farmer-education important on :

- How to identify a cow on heat.
  - Signs of heat
  - The cow on heat among several

- Times to present cows for service.
  - The right feeding.

2. The AI technician

He/she has replaced the bull, so has to do as well if not better. To achieve this, several areas need to be observed:

a. Maintain semen viability.

- The semen tank must always have enough liquid Nitrogen to ensure that spermatozoa do not die.
- Proper semen Handlin,
- The right semen thawing procedures must be strictly adhered to.
- Minimum time between thawing and insemination.

b. Semen delivery.

- Deposit at the right location in the uterus. The whole straw should be deposited at the uterine body and not partially.

Mitigation

Technician performance should be monitored by :

- Supevision, Impromptu visits.
- Results of performance through PD and records of non-return rates.

Regular refresher courses:

- To brush up the theory and catch up on any new information.

3. The cow

- Cows with infections will fail to either get pregnant or carry pregnancy to term.
- May come on heat, get served but fail to ovulate or ovulates late. In both cases conception fails.
- If not fed well will come on heat but fail to manifest the same (silent heat). In this case she will not be taken for service.

Mitigation

- Ensure that cows are free from any infectious reproductive diseases.
- Cows should be fed well to meet requirements for both milk production and estrus manifestation.
- If a cow returns to service for a third time when same bull semen is used, consider changing the bull.

TROUBLE SHOOTING IN AI FAILURES

COW NO.	ESTRUS INTERVALS (Days)		
	FARM A	FARM B	FARM C
1	20	26	22
2	21	30	42
3	22	32	40
4	20	28	63
5	21	31	41

- Three small scale dairy farms on an AI program have a problem with conception. Cows constantly come back to heat after service.
- The average cycle lengths in each of the affected cows are shown in the table.
- The farmers kept proper records, therefore it was possible to see the pattern of these repeat heats (repeat breeding).
- Can you appreciate the uniqueness of each case?
- Based on these observations, can you identify the problem each farm is facing?

Observations

In Farm A, heats are very close to a normal cycle, implying that cows behaved as if they were not served. Semen used was therefore either dead or not deposited in the right place. TECHNICIAN problem.

In Farm B, Cycle lengths are irregular but longer than the normal, implying that cows had conceived for a little while but lost. It would appear the uteri were not conducive to conception probably due to infection. A COW problem.

In Farm C, the cycles are long, but all a multiple of 20/21 days. This shows that cows came on heats that were not detected (silent heats) , either due to underfeeding or poor heat detection. FARMER problem.

7. SEMEN PROCESSING

Introduction

- Semen is processed with the aim of increasing its lifespan for extensive future use.
- Fresh Semen
- Chilled semen
- Frozen Semen

Process AIM

1. To produce a quality product (straw) according to the following criteria:
- Semen genetic quality: Genetics selection/Breeding programme
- Microbiologic quality (Hygiene)
- Sanitary quality: Zoo sanitary Conditions – free from major infectious diseases
- A clearly identified product (traceability)
- Biological quality: semen dosage with acceptable percentage of live and moving sperm

2. Adhere to and apply the mandatory tests or laws – DVS (Animal Disease Act Cap 364), OIE
3. Meet import/export requirements of potential international market- OIE

Bull Health

- Pre-Entry Health Testing for disease as stipulated by authorities. (US– Tuberculosis ≤60 days, Brucellosis ≤ 30 days, Leptospirosis ≤ 30days, Bovine Viral Diarrhea ≤ 30 days).
- Physical Examination/Breeding soundness examination)
- Resident bulls semiannual health testing– Tuberculosis, Brucellosis, Leptospirosis, trichomoniasis, Campylobacteriosis.

Bull Management

- Good quality semen starts in the barns.
- Bull feeding and management have influence on semen quality.
- Supplementation with vitamin, selenium and fatty acid.
- Proper bull housing, ensure comfort of the bull, clean adequate bedding, adequate quality feed and Water.
- Proper waste disposal.

Semen Disease Control

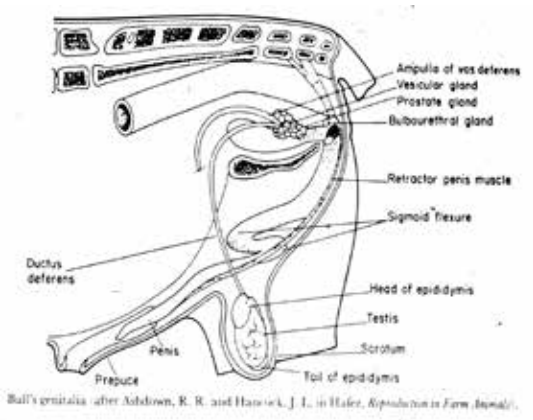
- Maintain general high hygiene standards from clean bulls, clean collection facilities and hygiene all through entire Process

Rules of Semen Production

- Sanitation and Environmental cleanliness.
- Clean equipments and thorough rinsing.
- Temperature control throughout during semen handling.
- Proper bull Identity.
- Quality control.

Semen Characteristics

- Semen is a secretion of the testis and the accessory sex glands.
- Consist of mature spermatozoa (sperm) from the epididymis and the seminal fluid (Seminal plasma) in which the spermatozoa are suspended.
- Spermatozoa consist of a head, neck, midpiece and tail.



Bull's genitalia (after Ashdown, R. R. and Hancock, J. L. in Hafez, *Reproduction in Farm Animals*).

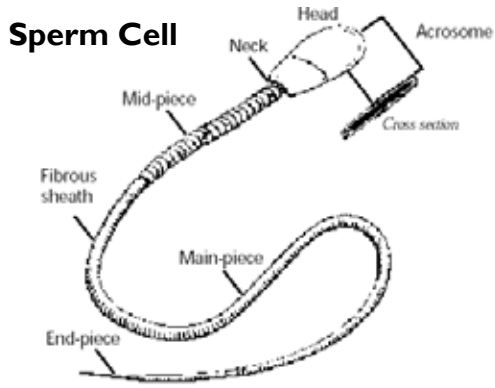


Figure 2.5: The components of sperm

Semen collection.

- Live mounts using a teaser (male or female) or dummy.
- Artificial Vagina (AV) most used.
- Electro-ejaculation is the preferred method for males that refuse to serve the AV, or when injuries make it impossible.
- Epididymal
- Massage











Electro Ejaculation

- Electro ejaculators are designed to stimulate the pelvic sympathetic and parasympathetic nerves with pulses of low voltage and amperage to induce penile erection and ejaculation.

Electro ejaculator





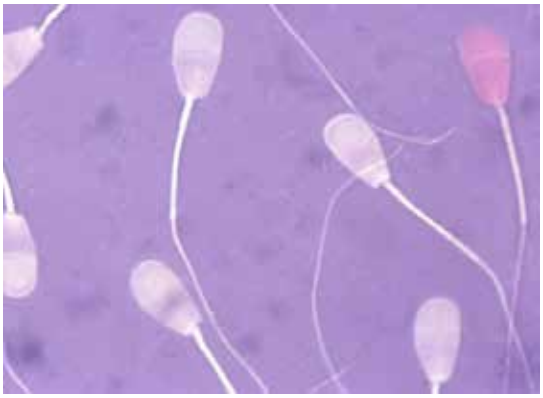
Semen Evaluation

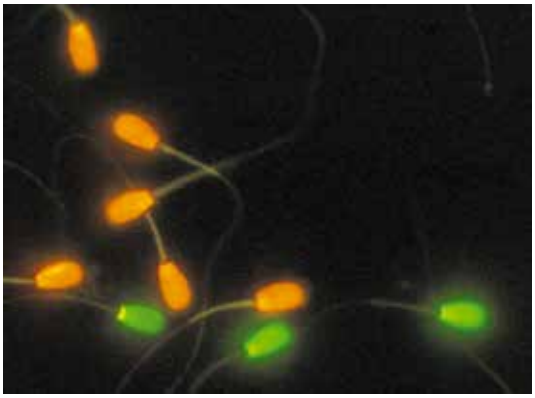
- Macroscopic evaluation.
- Volume (3-7ml)
- Colour (normal colour is milky-white, creamy, yellow).
- Smell – No smell
- Contaminants - abnormal semen may contain water, blood, preputial hair, pus and bad smell.
- Concentration – Photometer, CASA, Hemocytome



Microscopic Evaluation.

- Mass/Gross motility – Swirling pattern like a school of fish or heavy cloud (Wave Motion).
- Very Good: rapid dark swirls and eddies
- Good: slower swirls and eddies
- Fair: no swirls, but prominent individual cell motion
- Poor: little or no individual cell motion
- % individual progressive motility.
- Very Good: 80 - 100% motile
- Good: 60 - 79% motile
- Fair: 40 - 59% motile
- Poor: <40% motile
- Morphology. (Staining)
- Live - dead





CASA

Counts, concentration and percentages

- Total, static, motile progressive and slow cells



Doses

- Extender volume
- Final volume
- Number of doses

Morphometric characteristics

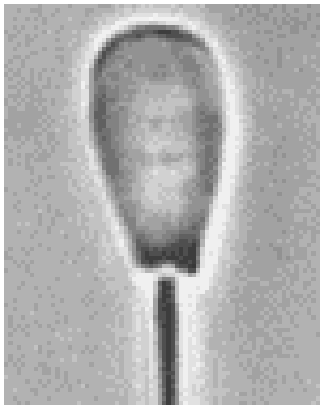
- Head length, width, perimeter and area. Tail length and straightness. Droplet distance.



Automated morphology

- Distal droplets
- Proximal droplets
- Distal Midpiece Reflexes
- Coiled tails
- Bent tails
-

Normal Sperm Cell



Pyriform

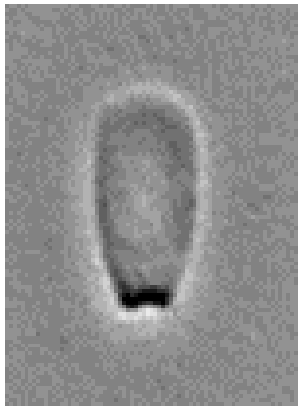


Tapered

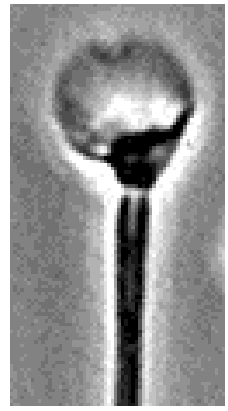


Primary Defects

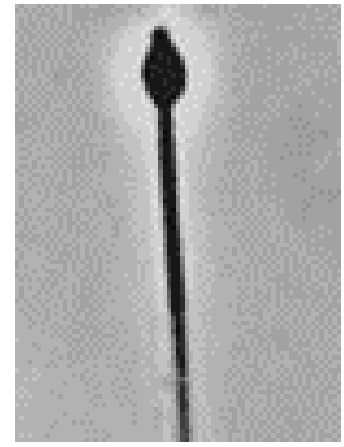
Decapitated



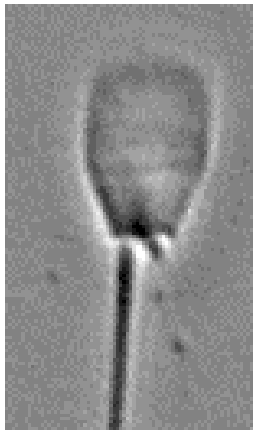
Round Head and Double Tail



Microcephalic



Macrocephalic



Round Head



Secondary Defects

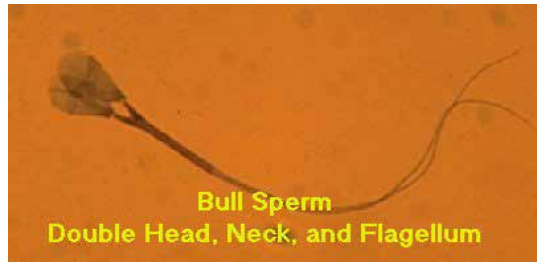
Proximal Cytoplasmic Droplet



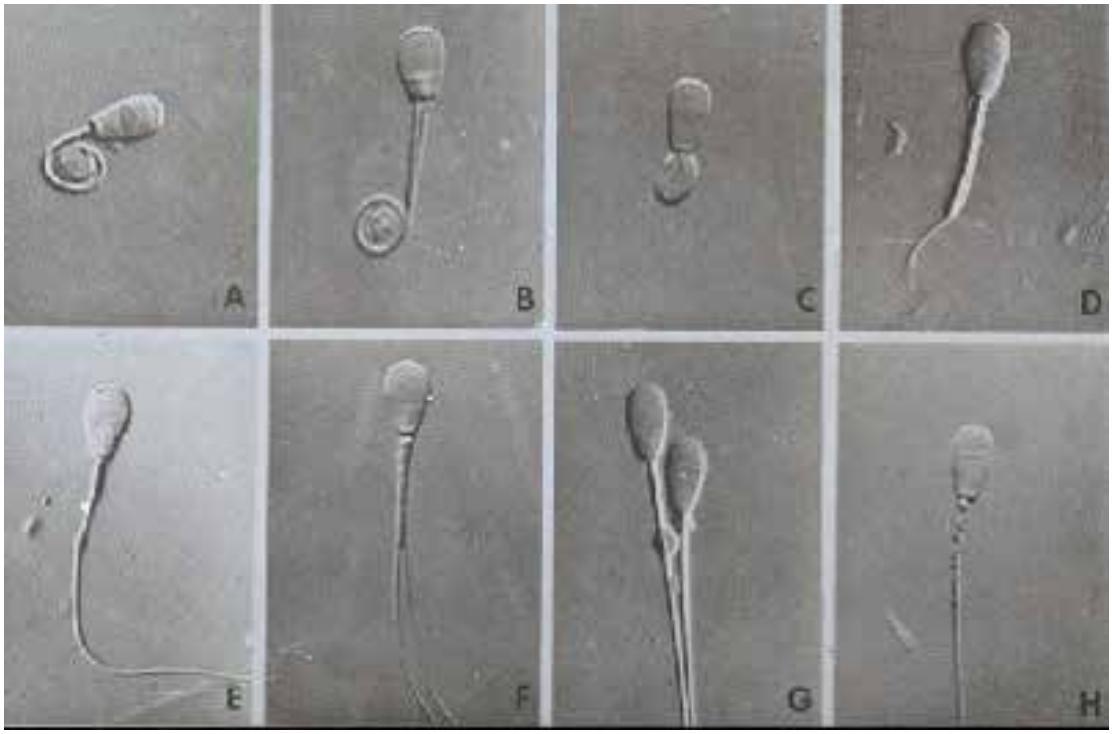
Cytoplasmic Droplet



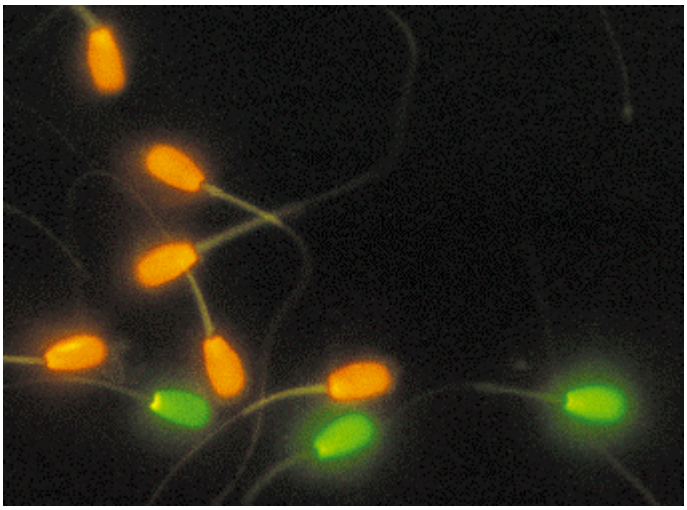




A -Coiled tail with droplet; B -Coiled double tail; C - "DAG" defect; D -Folded tail; E -Filamentous; F -Double tails; G - Corkscrew midpiece with droplet; H - Corkscrew midpiece



Live- Dead



Semen Dilution/Extension.

- One of the greatest advantages of the use of A.I is that valuable sires can be used to inseminate many more females than could be expected by natural mating.
- When AI is used both volume of the inseminate and the number of spermatozoa it contains are reduced in comparison to natural mating.
- Semen dilution enables reducing the number of spermatozoa to the required dose while maintaining inseminating volume convenient to handle.

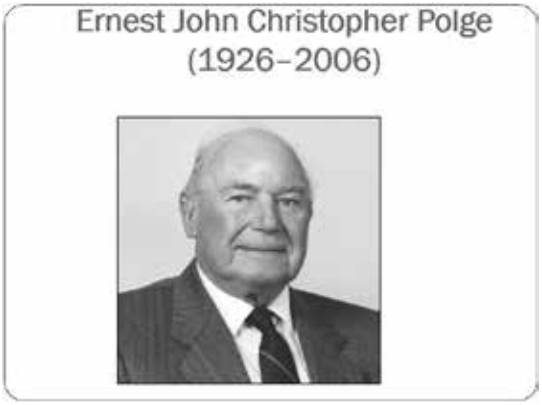
Functions of extenders.

- Provides nutrients as a source of energy.
- Protects against the harmful effects of cooling and freezing.
- Provides buffers to prevent harmful shifts in PH.
- Maintenance of proper osmotic and electrolyte pressure.
- Increase semen volume so it can be used for multiple inseminations.
- Provide an isotonic environment.

Components of extenders.

- Buffers - Control pH 6.7 to 7.0. Sodium citrate, egg yolk, tris buffers are commonly used.
- Lipids - Provides protection of sperm membranes from temperature changes. Skim milk and egg yolks are good sources of lipids.
- Nutrients - Provide energy for sperm. Fructose and glucose are typically used.
- Antibiotics - Prevent bacterial growth.

- Cryoprotective agent for freezing semen. It protects against the lethal effects of freezing to prevent crystallization of water within the sperm cells, which eventually allows sperm cells to be frozen rapidly. Formation of ice crystals results in puncture of cell membranes resulting in the decrease in membrane integrity – Glycerol, Ethylene Glycol.





Calculation of the Number of Doses and Dilution Rate

- Progressively Motile Morphologically Normal Sperm Cells (PMMN)
- The average required number of sperm cells per straw12 - 15 million progressively motile sperm cells post-thaw.
- Approximately 50% of sperm die in the freezing process.
- Pack double the number of sperm per straw; i.e., 25-30 million progressively motile sperm.

Extension/ Dilution

- One Step dilution
- Two Step dilution

Straw Identification.

- On each straw is printed the information required to correctly identify the individual bull.
- Bull name
- Bull code
- Collection centre
- Year of birth of bull.
- Bull registration code.
- Date of collection.
- Breed.
- Bar code
- (Colour combination).



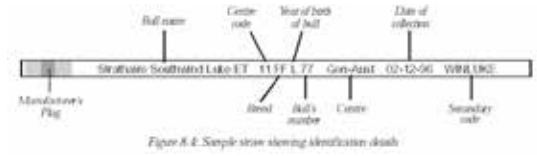
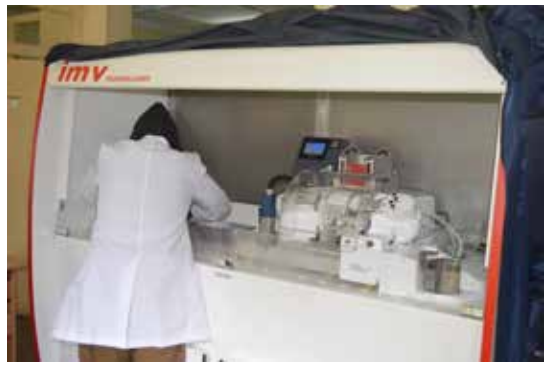


Figure 8.4. Sample straw showing identification details











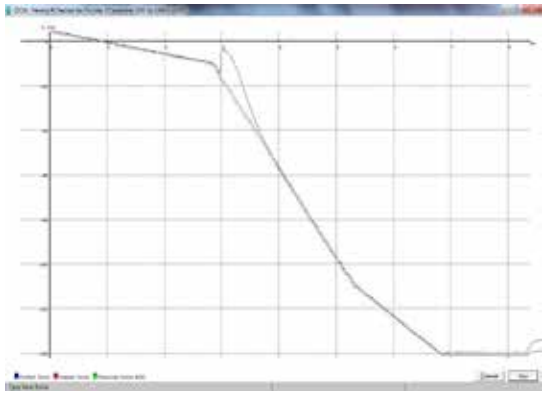
Freezing

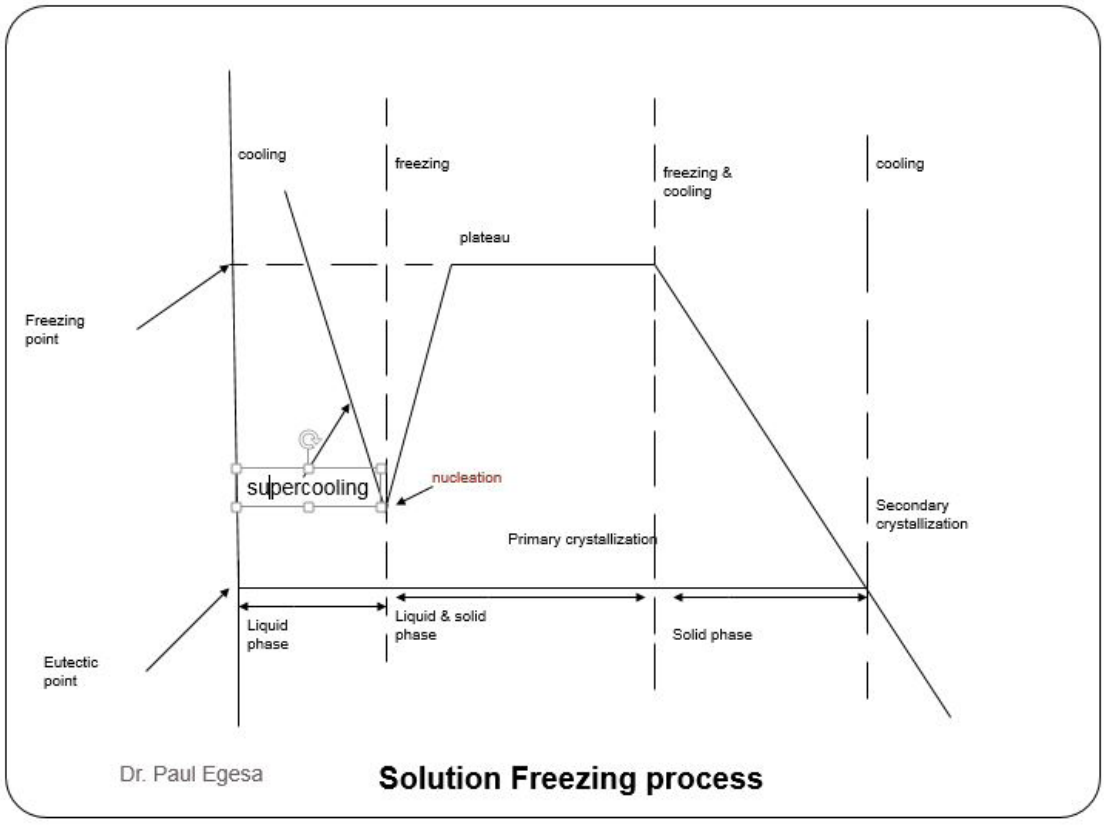
- Manual
- Automatic- Programmable Freezing











Quality Control

- Maintaining Standards in manufacturing by testing sample of products against specifications.
- Whose Responsibility is QC
- Role of different players
- QC along the Value Chain

Why Quality Control

- Optimum reproduction Performance (Fertility)

- Genetic gain
- Disease Prevention/control
- Uniform Product
- Happy Customers

Whose Responsibility is QC

- All of us in the industry
- AI centre
- Semen distributors
- Inseminators
- DVS
- LGSEA/CSS/NAAB



QC along the Value Chain

- Semen Producing Centre
- Semen Distributor
- Inseminator

AI Center

- AIM to produce a quality product from genetically superior Sires
- Sound Breeding Programme
- Maintain prescribed standards

AI Center

- Pre Entry Evaluation – One Month Old
- Physical Examination
- Genetic Defect
- Tests

Semen distributors/Inseminators

- Semen Production centers strive to deliver produce a quality product – genetics, microbiology, zoo sanitary, proper identity, viability.
- Unless the same quality is maintained, then all the efforts are in vain and the intended results can not be achieved.

Semen distributors/Inseminators

- Proper semen handling and storage.
- Liquid Nitrogen levels
- Proper semen Inventory

QC Watchdog

- OIE
- DVS
- LGSEA
- ISO 9000….
- CSS
- NAAB

Conclusion

- Quality semen is a prerequisite for the success of artificial insemination.



8. SIRE CATALOGUE INTERPRETATION

WHAT IS IT?

Provides the following information on the Sire/Bull:-

- The Identity of the Sire.
- The Attributes of the Sire.
- The Pedigree of the Sire.
- The Progeny (Production , Functional & Conformation Scores of the daughters)
- An Authorative linear / Conformation Traits Profile.
- Pictorial representation of Sire + Daughters.
- May contain Genomic data.

WHY NECESSARY:

- Reveals who the Bull/ Sire is. Eg: D. Birth, ID, Name, Reg. No, Bull Stud, Owner, Status.
- Reveals who the Bull/ Sire is. Eg: D. Birth, ID, Name, Reg. No, Bull Stud, Owner, Status.
- Underpins the strengths and weaknesses of the Bull.
- Ranks the Bull accordingly. (Australia)
- Indicates the Breeding potential (German).
- May estimate the BV (Kenya).
- May estimate LPI (Canadian)
- May point out strong attributes in each Sire. (USA, Holland).

SOME KEY TERMINOLOGIES:

TPI – Total Performance Index
PTA – Predicted Transmitting Ability
LPI – Lifetime Profit Index.
NM – Net Merit.
EBV – Estimated Breeding Value.
CM – Cheese Merit.
FM – Fluid Merit.
REL – Reliability.
CV – Complex Vertebral Malformation (CVM) Carrier.
TV – Tested free of CVM
TY – Tested free of brachyspina
BL – Bovine Leucocyte Adhension Deficiency (BLAD).
TL – Tested free of BLAD.
PL – Productive Life.

SCS – Somatic Cell Score.
CE – Calving Ease.
DPR – Daughter Pregnancy Rate.
SCR – Sire conception rate
FLC – Foot and Leg Composite.
UDC – Udder Composite.
PTAF – PTA Fat.
PTAP – PTA Protein.
PTAT – PTA Type.
STA – Standard Transmitting Ability
DCE – Daughter Calving Ease.
DSB – Daughter Stillbirth.
DF – Dairy Form.
MILKING SPEED – Milk letdown/milk Disposition – Temperament
aAa – Mating system that goes beyond traits that are measurable.
USDA/HA – US department of Agriculture/Holstein Association.

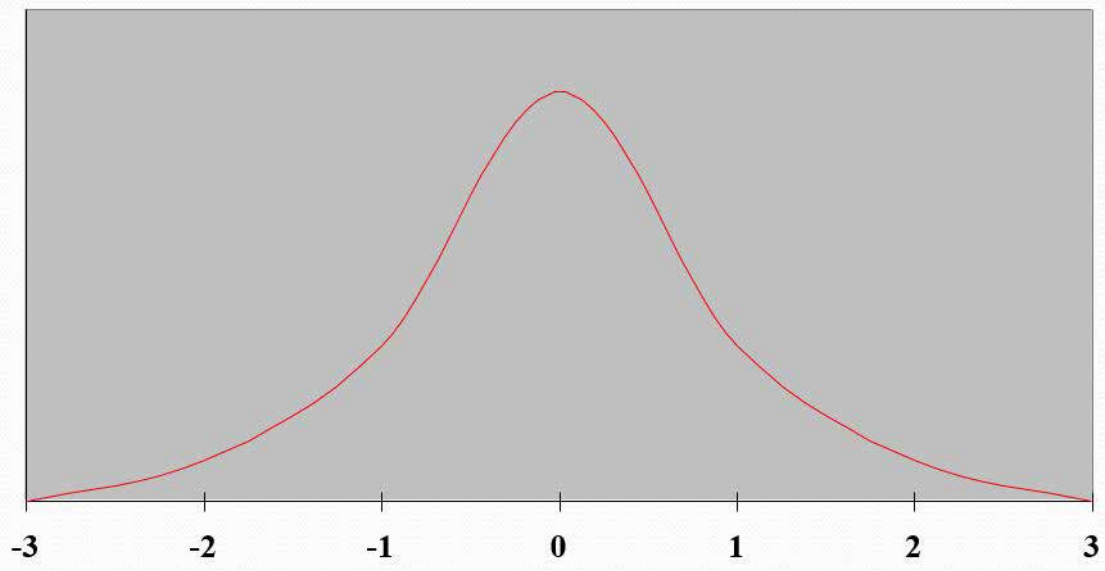
PREDICTED TRANSMITTING ABILITY (PTA)

- PTA is an estimate of genetic superiority that a bull or cow will transmit to its offspring for a given trait.
- PTAs are calculated for
  - Milk Pounds
  - Fat Pounds and Percent
  - Protein Pounds and Percent
  - Somatic Cell Score
  - Productive Life
  - Final Type Score
- This information can be used to rank bulls and cows by their genetic merit.

STANDARD TRANSMITTING ABILITY (STA)

- Genetic evaluations for the type traits are expressed as STAs.
- Standardized values for linear traits are used because each trait has a different average PTA, and the PTA ranges vary within traits.
- All linear traits have an average score of 0. STA values generally range from –3 to +3. Extreme values are near –3 or +3.
- The greatest number of bulls are at the average (STA=0).68% of bulls are within 1 STA in each direction of the average.

DISTRIBUTION OF STAs



RELIABILITY

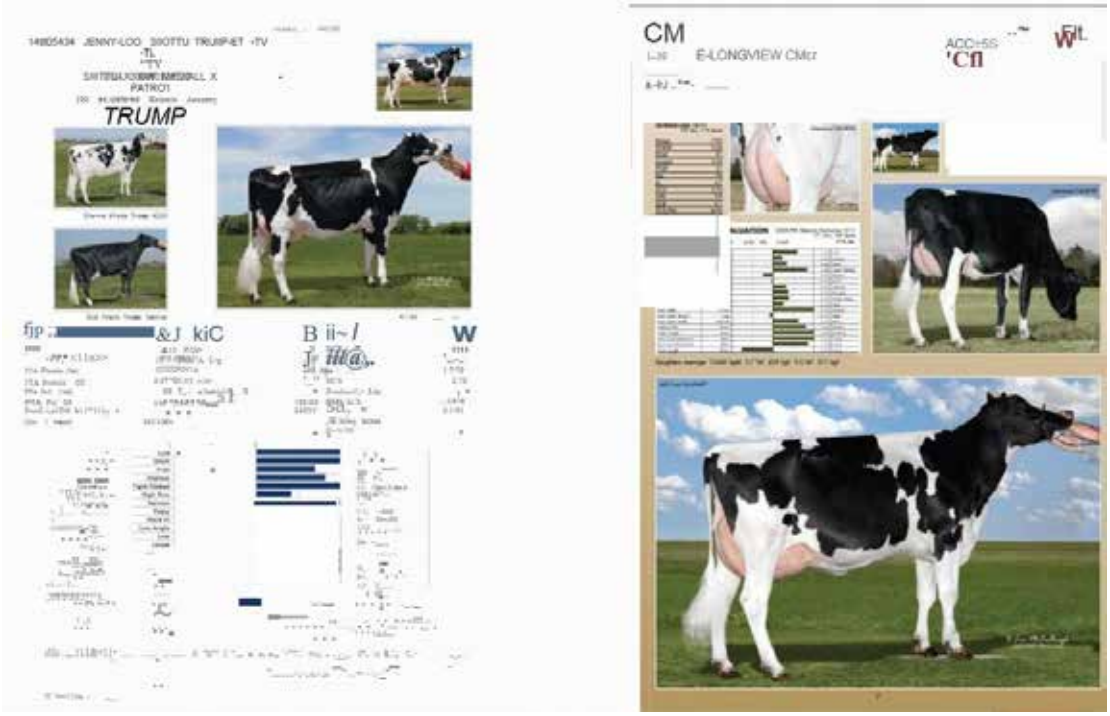
- Reliability measures the confidence you can place in ananimal's Predicted Transmitting Ability.
- The measure is based on the amount of information available in the evaluation.
- It considers animal, parent and progeny information.

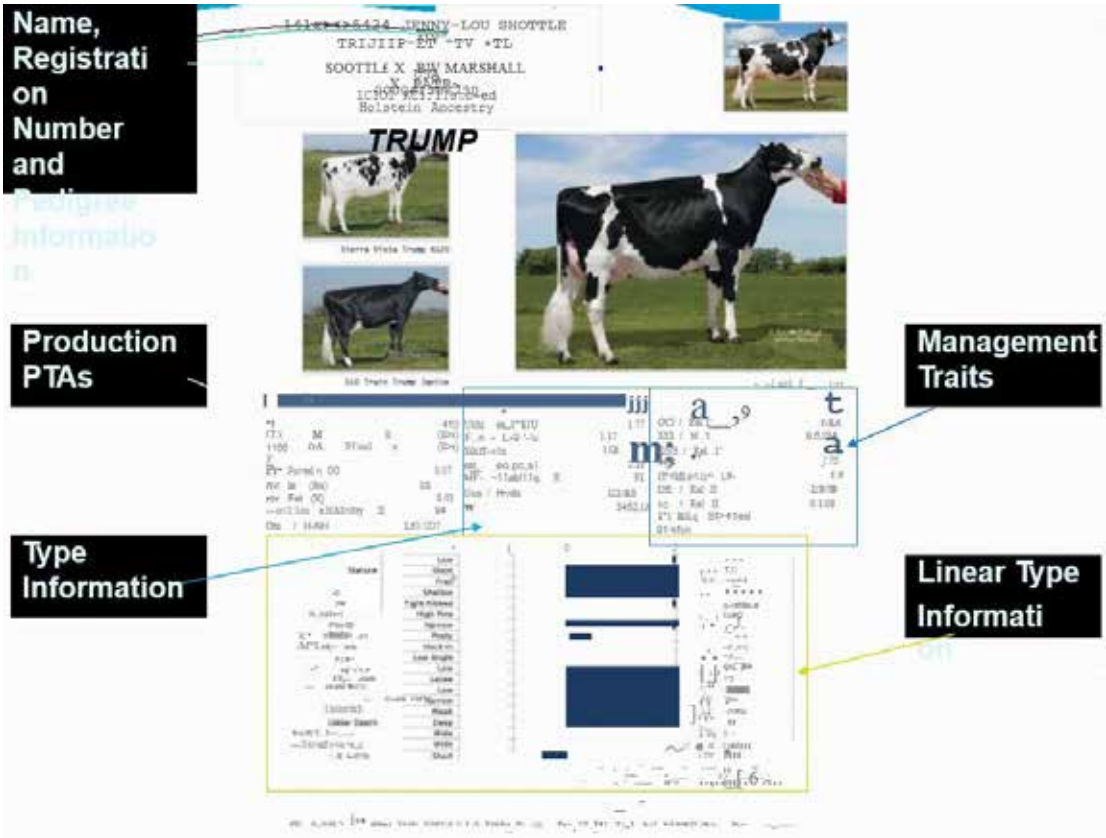
TYPE PRODUCTION INDEX (TPI)

TPI combines the following traits:

- PTA Protein
- PTA Fat
- PTA Type
- Udder Composite
- Feet and Legs Composite
- PTA Productive Life
- PTA Somatic Cell Score
- PTA Daughter Pregnancy Rate
- Dairy Form
- Daughter Calving Ease

SIRE SUMMARY EXAMPLE

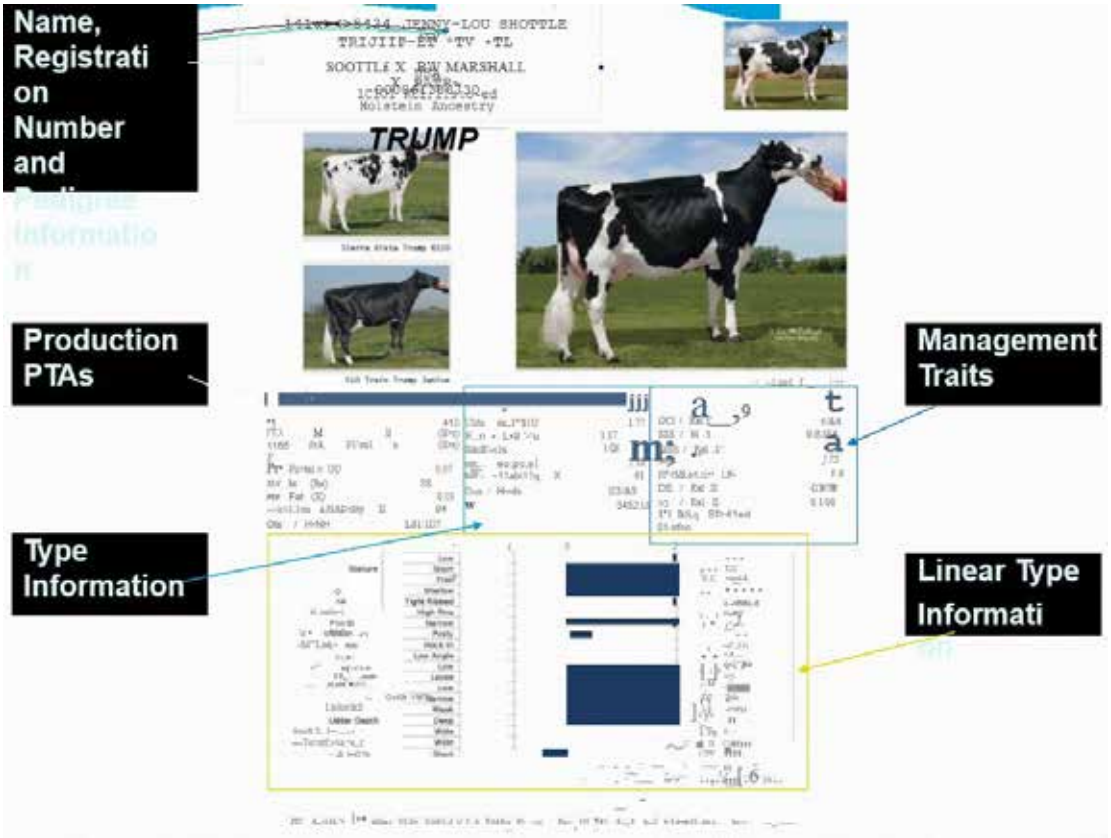




NAME REGISTRATION AND PEDIGREE INFORMATION

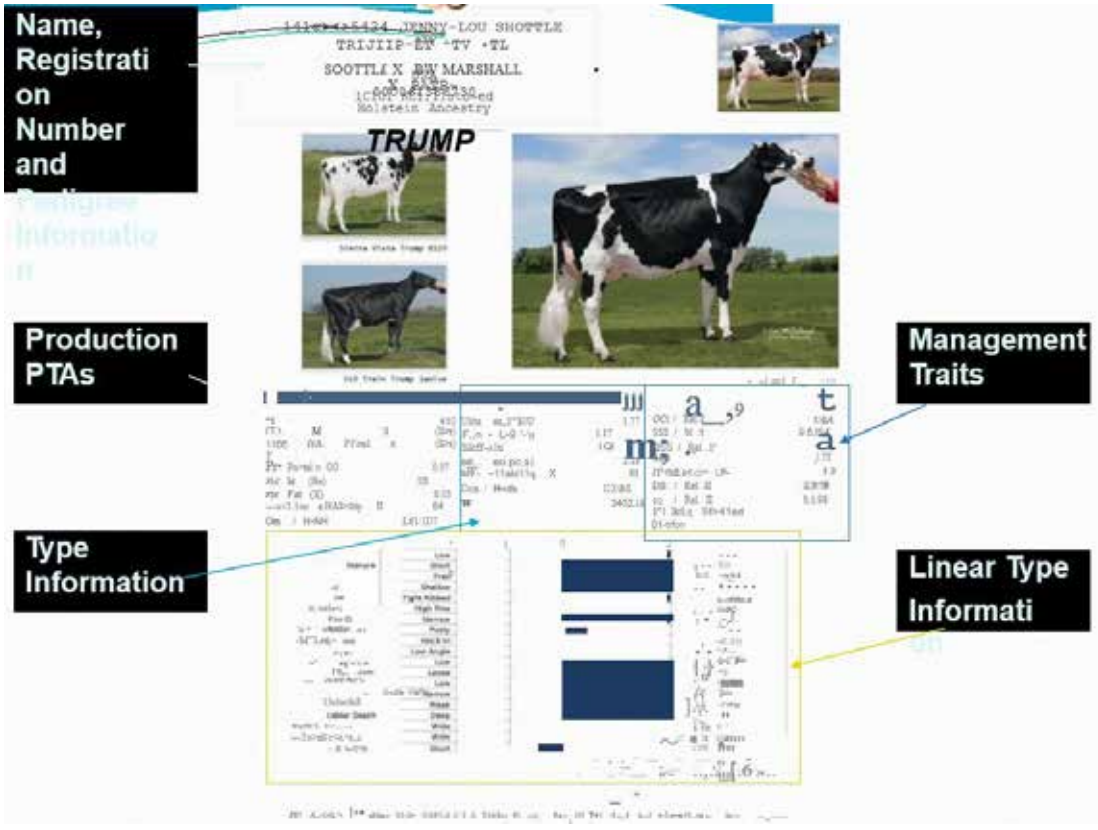
14H005434 JENNY-LOU SHOTTLE TRUMP-ET *TV *TL
*TY
USA 000061886730
SHOTTLE X BW MARSHALL X PATRON
100% Registered Holstein Ancestry

TRUMP



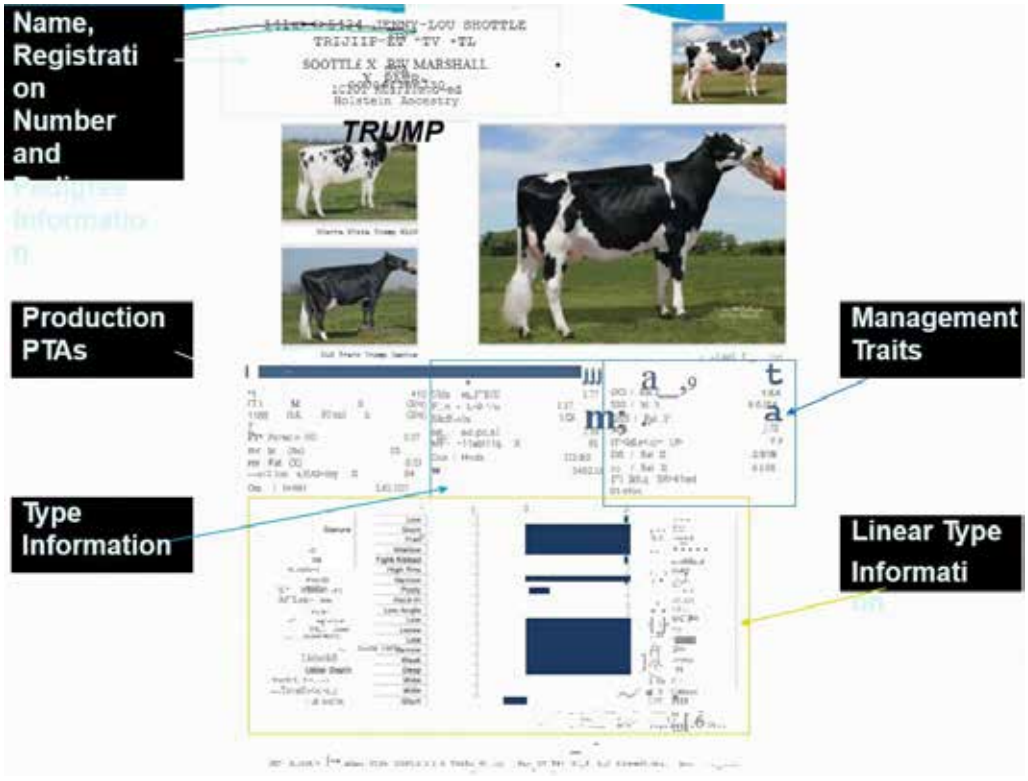
PRODUCTION PTA'S

Production	
TPI	1952
NM$	410
PTA Milk (lbs)	1766
PTA Protein (lbs)	34
PTA Protein (%)	-0.07
PTA Fat (lbs)	55
PTA Fat (%)	-0.03
Production Reliability %	94
Dtrs / Herds	168/107

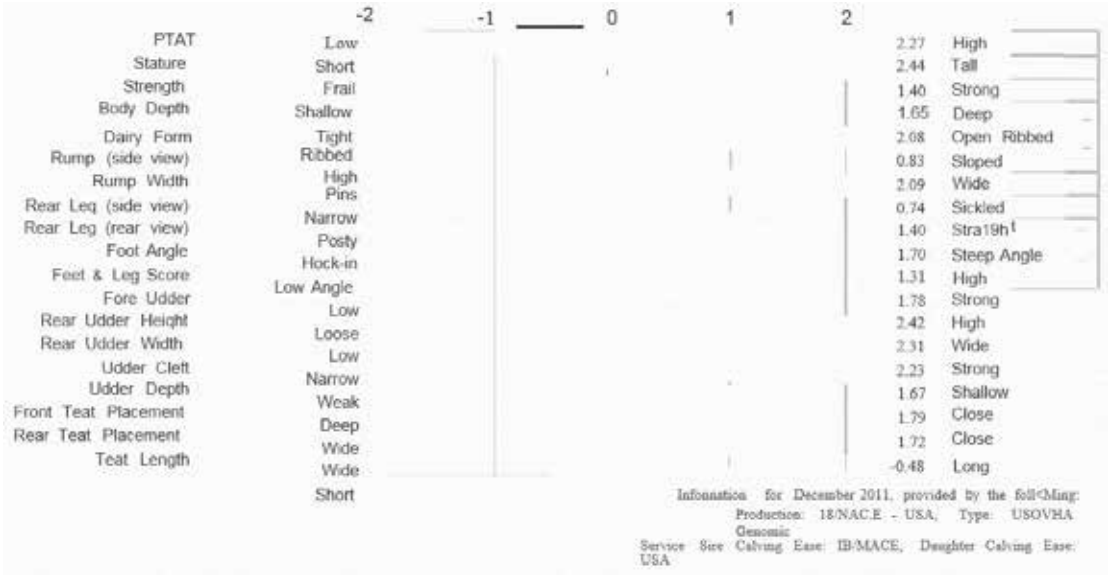


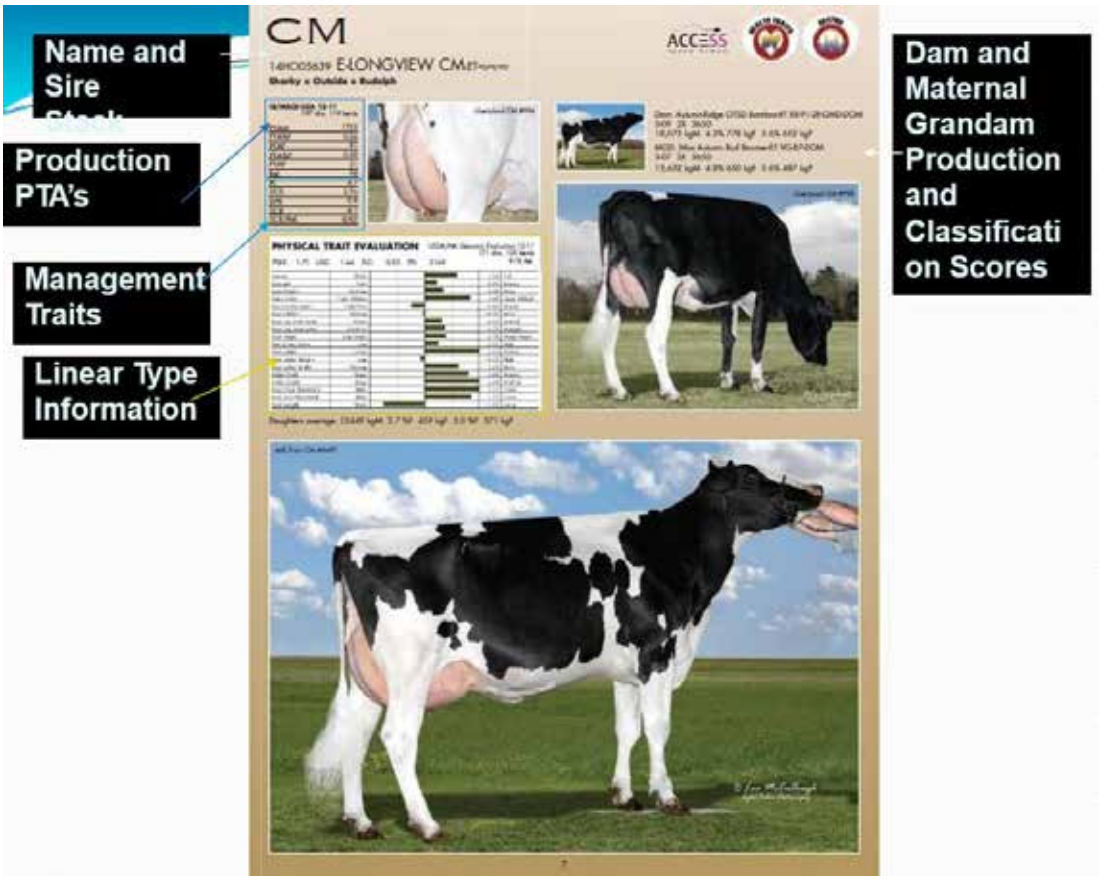
MANAGEMENT TRAITS

Management Traits	
SCE / Rel.%	9/98
DCE / Rel.%	7/84
SSB / Rel.%	9.6/94
DSB / Rel.%	8.7/79
SCS	2.75
Productive Life	2.9
DPR / Rel.%	-0.9/79
SCR / Rel %	-0.1/98
Milking Speed	3
Disposition	4

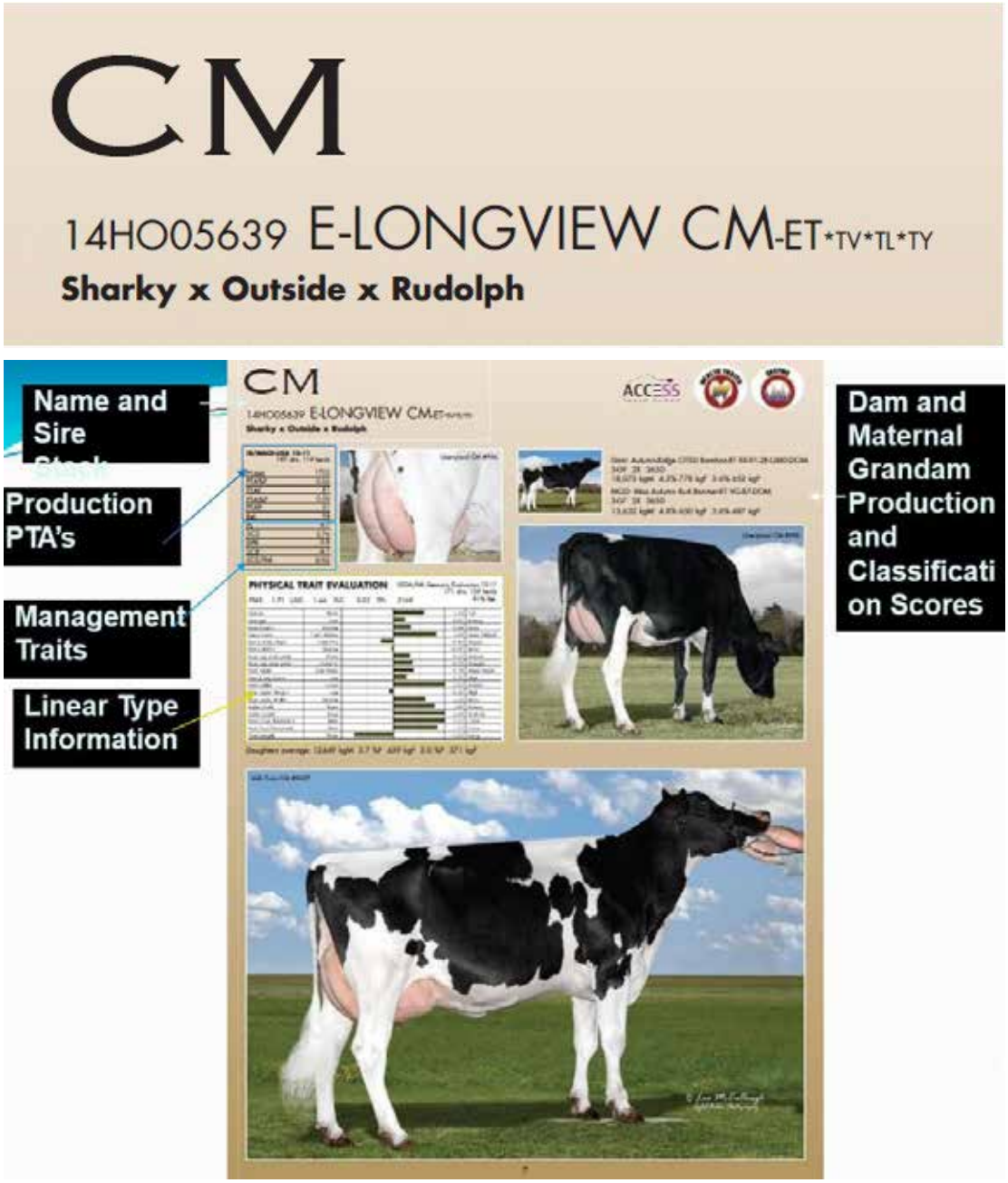


LINEAR TYPE INFORMATION



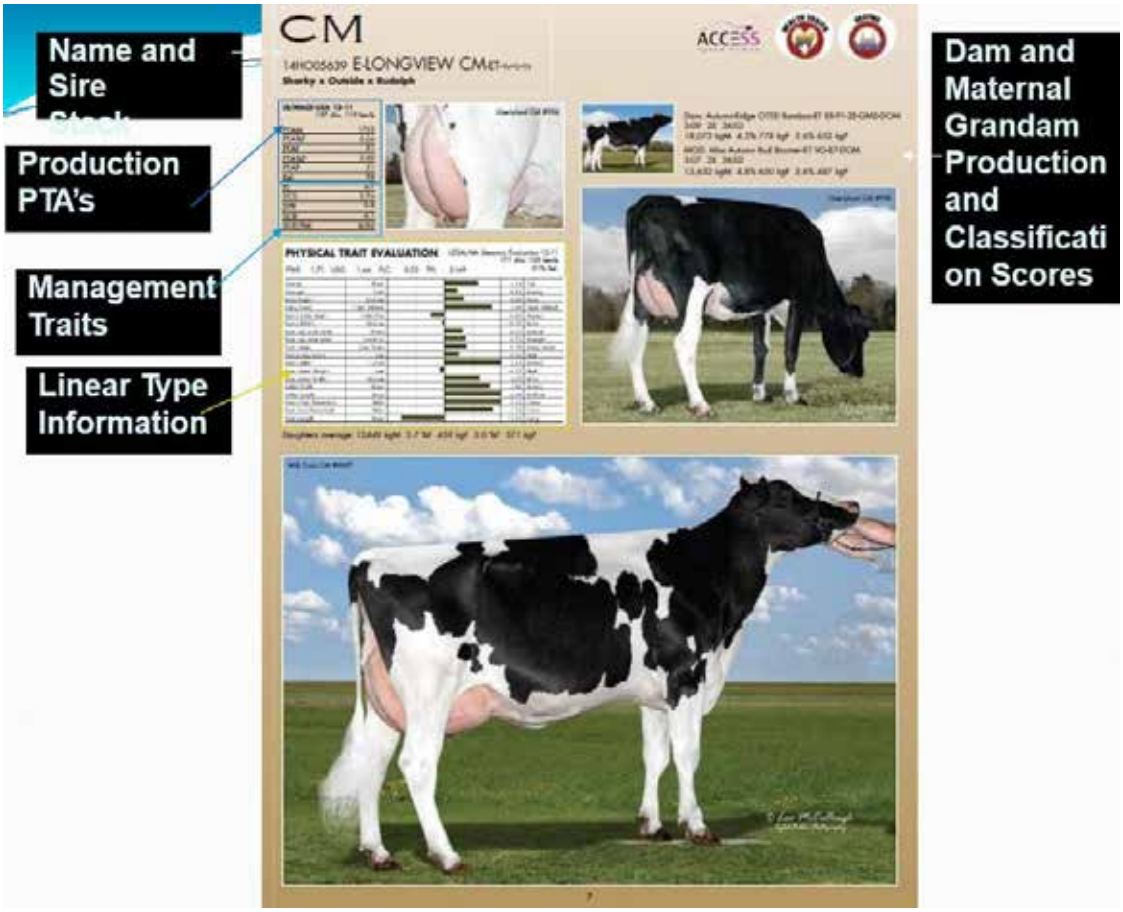


NAME AND SIRE STACK

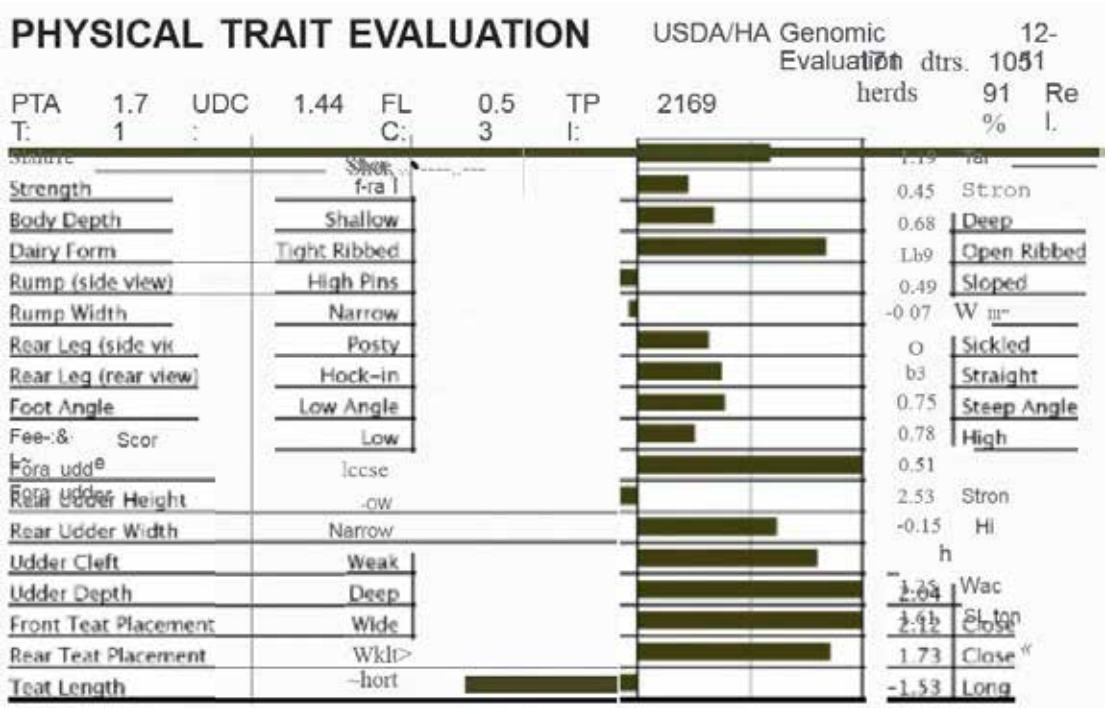


MANAGEMENT TRAITS

PL	4.7
SCS	2.76
DPR	0.8
SCR	-4.1
SCE/Rel.	8/82



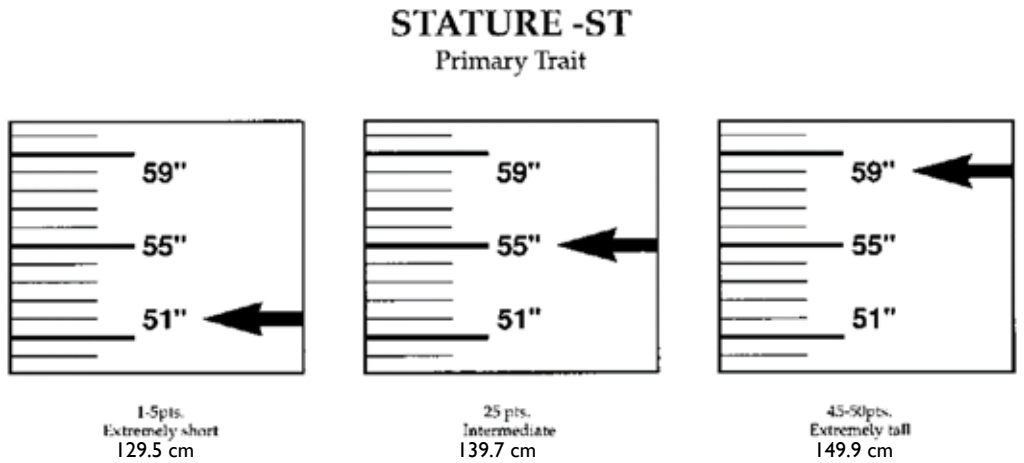
LINEAR TYPE INFORMATION



9. LINEAR TRAITS IN DAIRY CATTLE

Linear Trait Descriptions

- Evaluate all primary traits
- Use a 0-50 linear scale 25 being a biological midpoint, not the average
- Average linear for each trait varies by trait



DAIRYNESS

Head - clean cut; eyes large and bright; ears carried alertly; head with Holstein breed character

Neck - long and lean, blending smoothly into shoulder; clean cut about the throat, dewlap, and brisket

Withers - well defined and wedge-shaped, with the dorsal processes of the vertebrae rising slightly above the shoulder blades

DAIRYNESS

Ribs - wide apart; rib bones wide, f lat, and long

Flanks - deep and refined

Thighs - incurving to f lat from side view; from the rear view, wide apart, providing ample room for the udder and its rear attachment

Skin - loose and pliable; hair fine

Udder - soft and pliable, free from excess tissue or edema

STRENGTH - SR



1-5 pts
Extremely narrow & frail



25 pts
Intermediate



45-50 pts
Extremely strong & wide

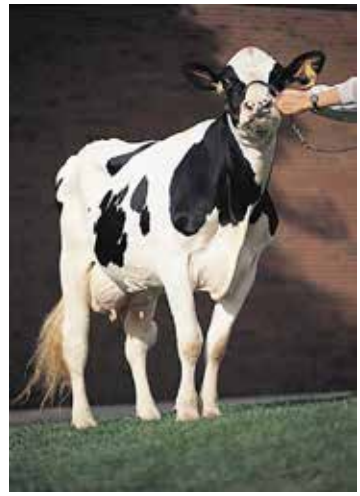
Strength = 9



Strength = 5



Strength = 1



BODY DEPTH - BD



1-5 pts
Extremely shallow body



25 pts
Intermediate



45-50 pts
Extremely deep body

Body Depth = 9



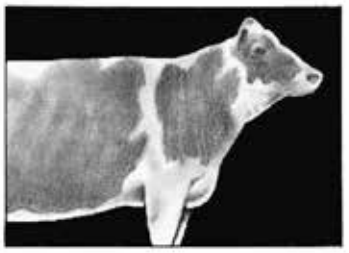
Body Depth = 5



Body Depth = 1



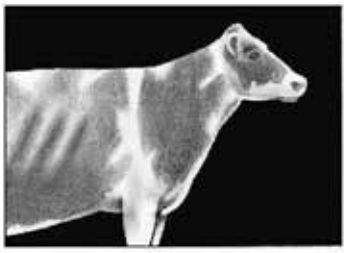
DAIRY FORM - DF



1-5 pts
Extremely tight



25 pts
Intermediate



45-50 pts
Extremely open

Dairy Form = 9



Dairy Form = 6



Dairy Form = 4



RUMP

Hips - wide but not prominent; slightly higher than pins

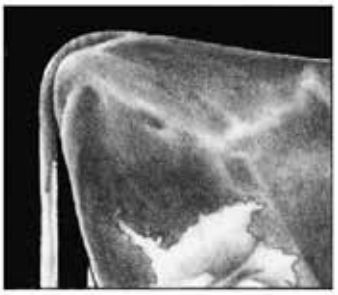
Pins - wide apart and free from patchiness

Thurls - high and wide apart, giving consideration to stage of lactation

Tail-head - refined, carrying out level with backline and set slightly higher than pins

Tail - long and slender

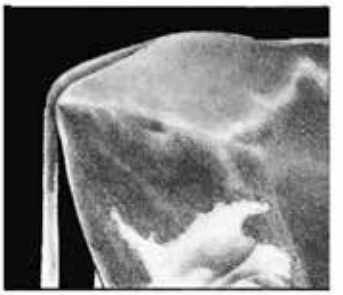
RUMP ANGLE - RA



1-5 pts
Pins higher than hooks



25 pts
Slight slope from hooks to pins



45-50 pts
Extremely sloped from hooks to pins

Rump Angle = 9



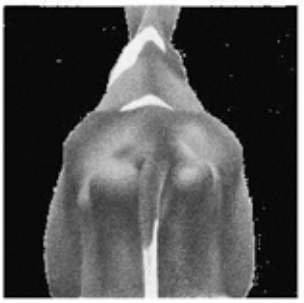
Rump Angle = 7



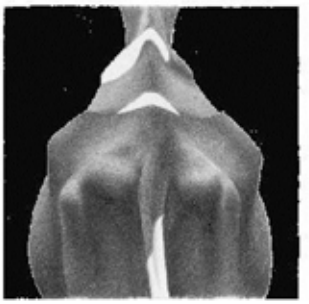
Rump Angle = 1



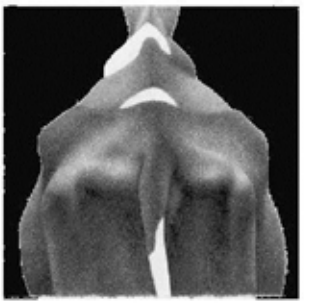
RUMP WIDTH - RW



1 pt = 2”
Extremely narrow



25 pts = 4 ½”
Intermediate width



50 pts = 7”
Extremely open

Rump Width = 9



Rump Width = 4



Rump Width = 6



FEET AND LEGS

Feet - short and well rounded, with deep heel; toes slightly spaced

Legs - pasterns strong, of medium length, and f lexible - fore legs straight and wide apart, with feet squarely placed - hind legs nearly perpendicular from hock to pastern from the side view, straight and wide apart from the rear view; hocks cleanly moulded - bone f lat, strong, and f linty, with tendons well done

REAR LEGS, SIDE VIEW - LS



1-5 pts
Posty and straight



25 pts
Intermediate set in hock



45-50 pts
Extremely sickled

LEGS REAR VIEW

Legs Side View = 9



Legs Side View = 6



Legs Side View = 2



Legs Rear View = 9



Legs Rear View = 4



Legs Rear View = 1



FOOT ANGLE - FA



1-5 pts
Extreme low angle



25 pts
Intermediate angle



45-50 pts
Extremely steep angle

Foot Angle = 9



Foot Angle = 5



Foot Angle = 1



MAMMARY SYSTEM

Udder - symmetrical, of moderate length, width, and depth; slight quartering on sides

Median Suspensory Ligament - strong, showing definite cleavage between halves

Udder Texture - soft, pliable, elastic, and well collapsed after milking

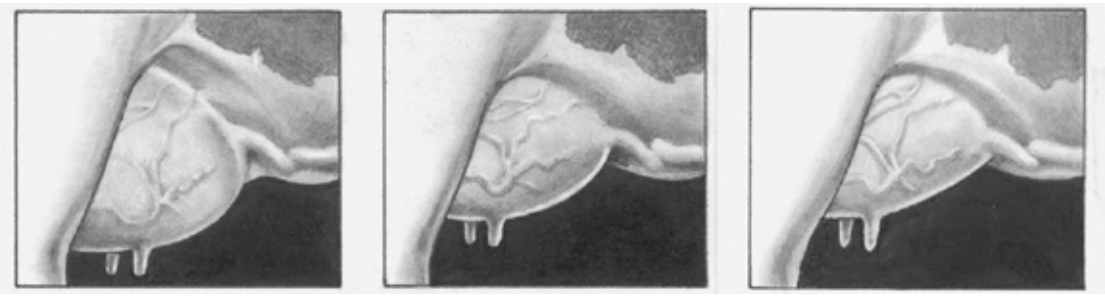
Fore Udder - firm and smooth attachment to body wall; of moderate length; quarters evenly balanced

MAMMARY SYSTEM

Rear Udder - attached high, wide, and strong; slightly rounded; uniform width from top to f loor; quarters evenly balanced

Teats - uniform size, of medium length and diameter, cylindrical, and plumb; from side view teats placed in centre of each quarter; from rear view teats slightly closer to inside than outside of each quarter

FORE UDDER ATTACHMENT - FU



1-5 pts
Extremely loose

25 pts
Intermediate strength

45-50 pts
Extremely snug & strong

Fore Udder = 1



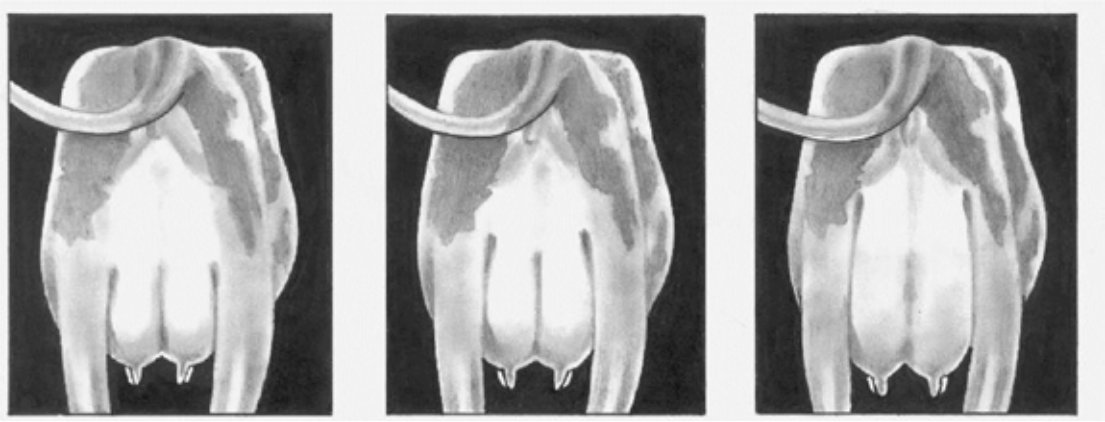
Fore Udder = 5



Fore Udder = 8



REAR UDDER, HEIGHT - UH



Extremely low

Intermediate height

Extremely high

Rear Udder Height = 9



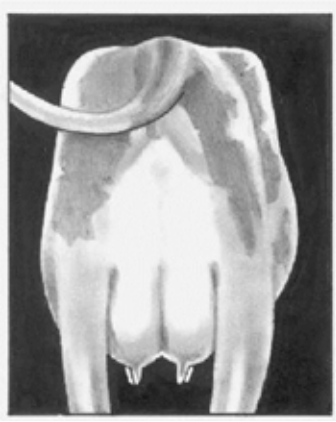
Rear Udder Height = 5



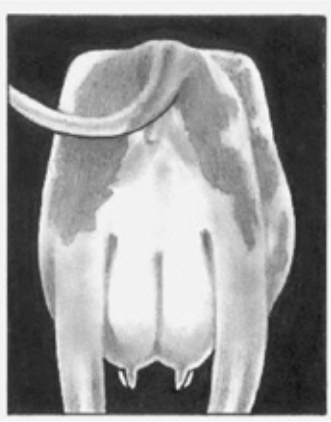
Rear Udder Height = 2



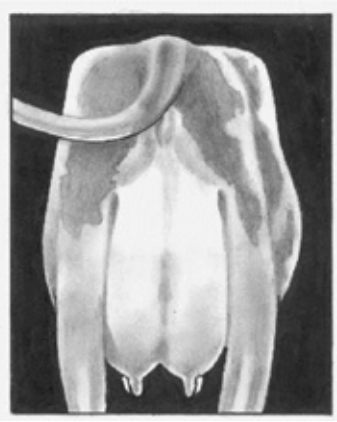
REAR UDDER, WIDTH - UW



Narrow rear udder



Intermediate width



Extremely wide rear udder

Rear Udder Width = 9



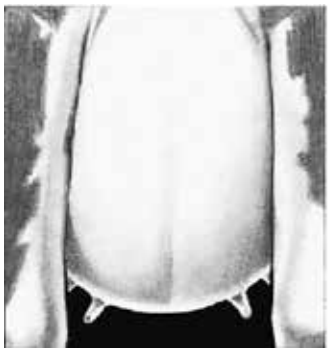
Rear Udder Width = 5



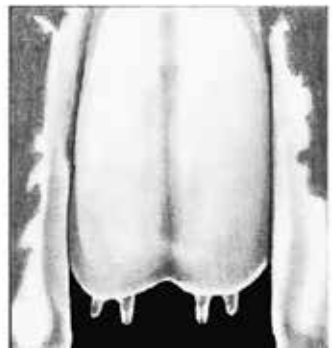
Rear Udder Width = 1



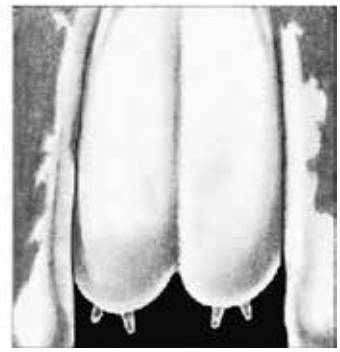
UDDER CLEFT - UC



1-5 pts
Weak cleft



25 pts
Intermediate



45-50 pts
Extremely strong cleft

Udder Support = 9



Udder Support = 6



Udder Support = 1



UDDER DEPTH - UD



Very deep udder floor
well below hocks



Udder floor
above hocks



Extreme height of udder
floor above hocks

Udder Depth = 9



Udder Depth = 4



Udder Depth = 1



TEAT LENGTH

Teat Length = 9



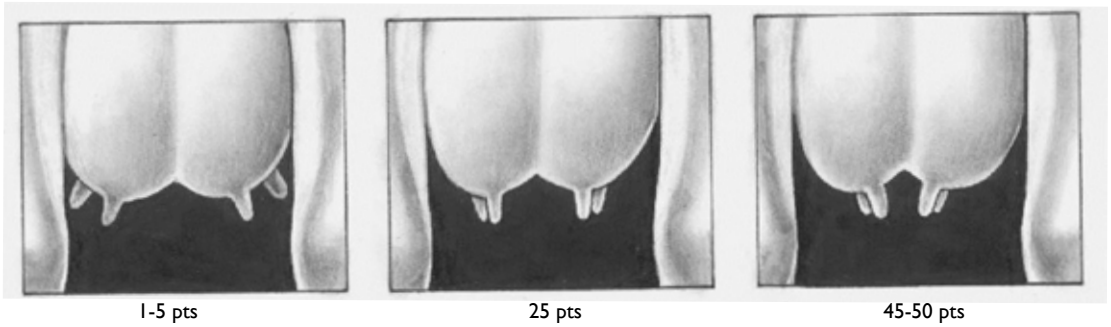
Teat Length = 5



Teat Length = 2



FRONT TEAT PLACEMENT - TP



1-5 pts
Extremely wide placement on outside of quarter

25 pts
Centrally placed on quarter

45-50 pts
Base of teats on extreme inside of quarter

Teat Placement = 9



Teat Placement = 5



Teat Placement = 1



FINAL SCORE

A cow final score is based on the major classification categories of:-

Cow *Frame (stature) - 15% *Dairy Character – 20% *Body capacity – 10% *Feet & Legs – 15% *Udder – 40%

Bulls *Frame (stature) - 30% *Dairy Character – 25% *Body capacity – 20% *Feet & Legs – 25%

The final score is expressed

*Excellent (E) 90 – 97 points *Very Good (VG) 85 – 89 points *Good Plus(G+) 80 – 84 points *Good (G) 75 – 79 points *Fair (F) 65 – 74 points *Poor (P) 50 – 64 points

BULL SELECTION

- Understand the Dam and the Sire very well.
- Establish what you want to improve. (Udders, Feet & Legs etc)
- Examine the breeding goals
- Choose the Bull to correct the faults. (The strong points of the sire)

MATCH MATING

1. Know the candidate (Dam) her shortcomings and her lineage.
2. Choose the Bull to correct the main faults.
3. Penalize inbreeding
4. Maximize Economic impact
5. Opt for quick mating programme (available as a soft ware)
6. Keep the breeding records.
7. Make sure you time the heat correctly.



10. SELECTION OF DONOR AND RECIPIENT CATTLE FOR MOET

Selection of Donor cattle:

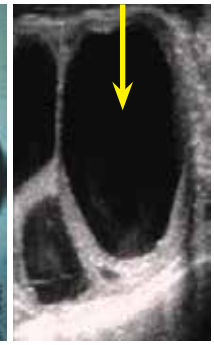
- Donors are the cattle from which embryos are harvested.
- Dairy cattle should be high milk producers.
- Must have other desirable physical features (e.g. good conformation; strong legs) and no genetic defects.
- Must be healthy.



Selection of Donor cattle:

- Cows with normal oestrus cycles, observed at least twice, should be chosen.
- Donors should Not have reproductive disorders, e.g.:
- Irregular oestrus cycle.
- Endometritis.
- Cystic ovaries.





Selection criteria- Age:

- Cows between the 3rd and 7th Lactation are the best producers of embryos.
- Heifers produce few embryos.
- 1st calver cows tend to be stressed from their new role so produce few embryos.
- Old cows vary from very good to very poor donors.

Selection criteria- stage of Lactation:

- Lactation stress (due to high milk production) is a major cause of low embryo production.
- Dairy cows 70-100 days after calving can be used as donors, i.e. after uterine involution and just before lactation stress.
- Between 100-200 days after calving, embryo production is low as milk production increases.
- The best donor is a Dry Cow (after 200 days) as milk production stress is removed.

Take-home message:

- All Donors react differently with regard to embryo production due to the factors mentioned.
- To determine whether a cow is a suitable donor it must be flushed at least 3 times.
- This takes into account potential problems in the uterus; those due to stress (lactation; environment-climate, rain); nutrition of cow; management on the farm; and semen quality.
- Use the knowledge at your disposal as well as that of recognised experts to choose the right donors.

Selection of Recipient cattle:

- Choose fertile cattle, i.e. those that are cycling regularly and can become pregnant by a bull or AI.
- Select animals that have reached the breeding weight and are big enough to carry the donor's calf.

- The body condition should be good (condition score of 2.5 to 3).
- It's best if they are in an increasing plane of nutrition, so improving in condition. Avoid overweight cows.



Selection of Recipient cattle:

- Heifers are good recipients as long as they have reached puberty (i.e. cycling) and breeding weight.
- 3rd to 6th Calvers are also suitable if having a good calving record and mothering ability.
- The worst recipients are 1st Calvers, which are the most stressed group so difficult to get pregnant.
- Do not use recipients that have been prepared unsuccessfully twice previously (i.e. poor response to synchronisation hormones).

Take-home message:

- A common misconception (i.e. not true) is that poor quality animals can be used as recipients.
- Beef breeds (e.g. Boran) make good recipients for Dairy cattle calves.
- Feeding, handling and condition of the recipients is vital for a successful ET program.
- It's best that a Vet checks both the donors and recipients before use, to ensure they are healthy, cycling and without reproductive problems.

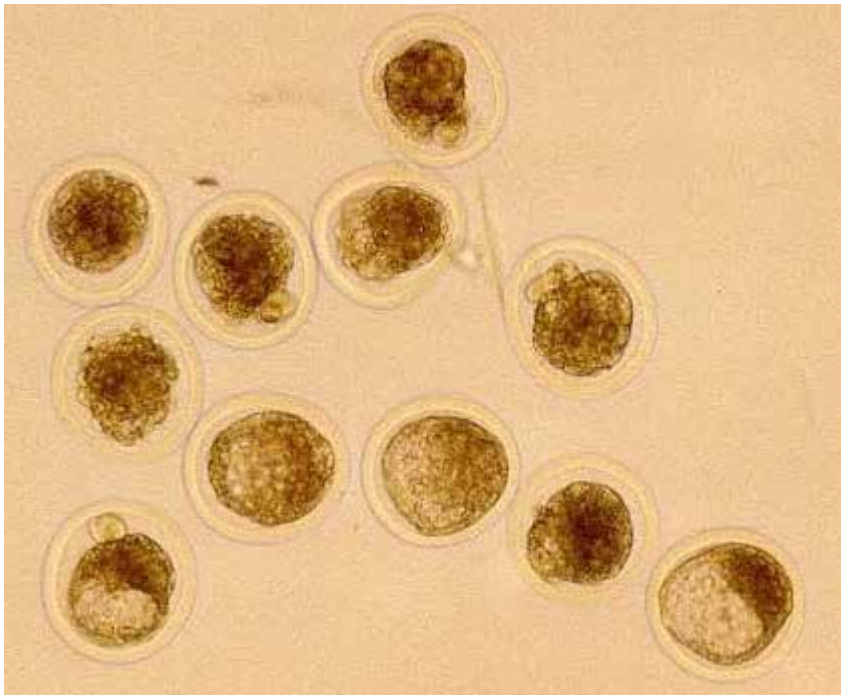




11. SUPEROVULATION OF DONOR CATTLE AND SYNCHRONIZATION OF RECIPIENTS FOR MOET.

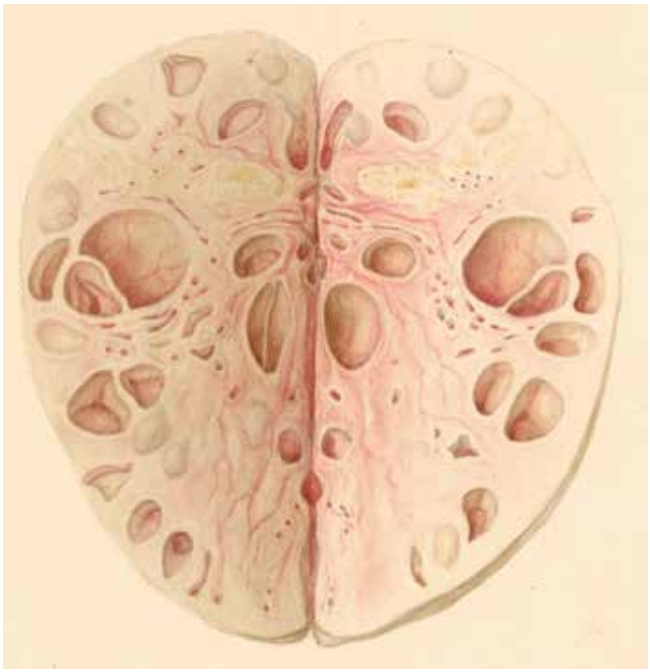
Superovulation of Donor cattle:

- A heifer is born with > 200,000 ova in the ovaries, formed when its a foetus. No new oocytes are made after birth.
- Many oocytes degenerate naturally with age and disappear from the ovary, a process called Atresia.
- A cow may ovulate about 25 times in her lifetime so the rest of the ova are lost, hence wasted.
- Superovulation and ET helps to reduce this wastage and improves the cow's reproductive potential.



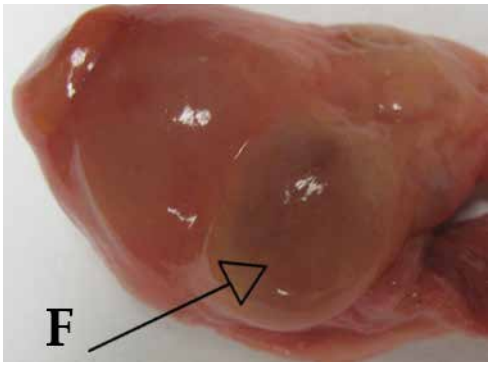
Superovulation of Donor cattle:

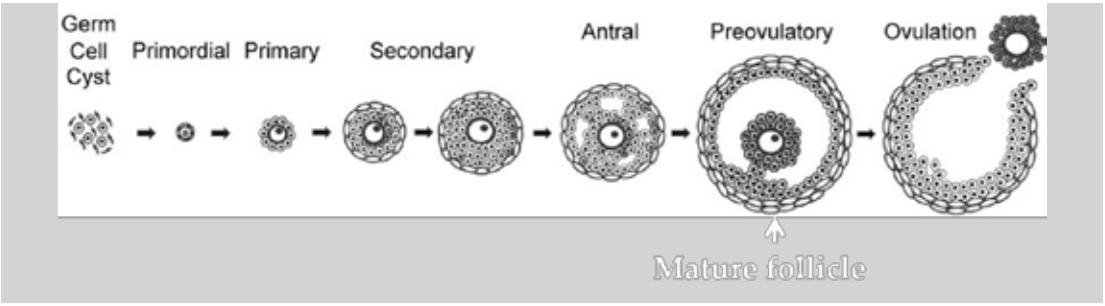
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Oocyte and Follicle development:

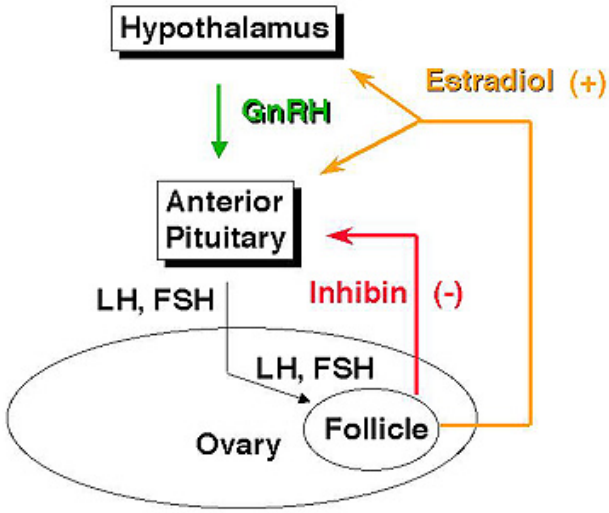
- In the ovaries, each oocyte is located within a Follicle.
- Each day, about 10-20 follicles start to grow. Most undergo atresia, but a few mature over a period of 4-5 months and then ovulate.
- The mature follicle is fluid filled, about 1 cm in size and can be felt through the rectal wall as a soft fluctuant area on the ovary.





Influence of hormones on the follicle:

- In the normal oestrus cycle a cow ovulates a single egg due to the interplay of 2 hormones- Follicle stimulating hormone (FSH) and Inhibin.
- FSH is secreted by the pituitary gland located at the base of the brain. Its function is to stimulate growth of follicles especially during the 4-5 days before ovulation.
- Inhibin is secreted into the blood by the fastest growing or dominant follicle. Its function is to stop FSH production by the pituitary gland.
- Therefore, no other follicles can mature and ovulate.

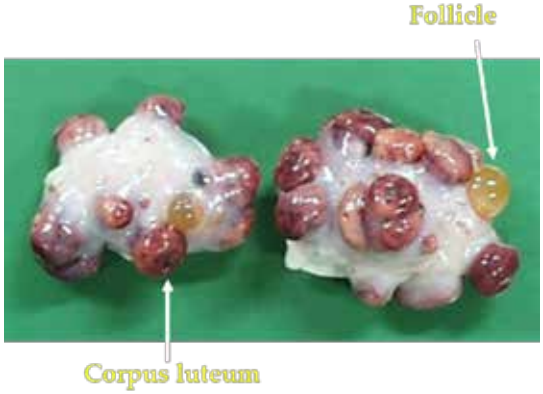


The process of Superovulation:

- During superovulation, extra FSH is injected to allow more follicles to ovulate.
- It is administered in two daily doses, reducing in volume over a period of 5 days, to obtain a total of 40-50 mg.
- Prostaglandin (PGF2∝) is also injected to cause lysis of the corpus luteum and rapid reduction of Progesterone hormone, which allows ovulation to occur.

Day	Time	Hormones injected
1	6 pm	4 ml Folltropin (FSH)
2	6 am 6 pm	4 ml Folltropin 3 ml Folltropin
3	6 am 6 pm	2.5 ml Folltropin 2 ml Folltropin
4	6 am 6 pm	1.5 ml Folltropin + 3 ml Estrumate ($PGF_{2\alpha}$) 1 ml Folltropin + 1 ml Estrumate
5	6 am 6 pm	1 ml Folltropin 1 ml Folltropin

Superovulated ovaries following use of an FSH product:





Synchronization of Recipient cattle:

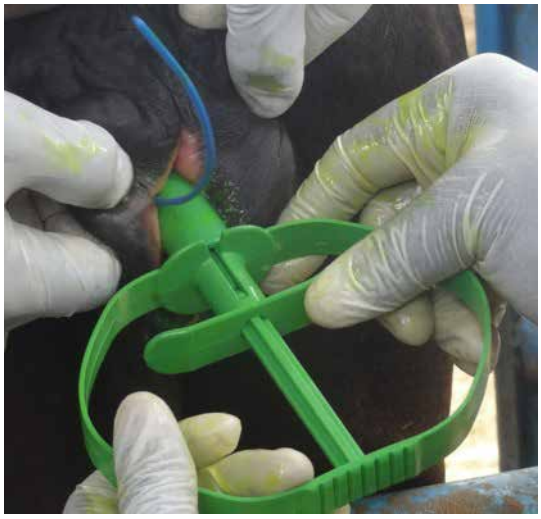
- ET is only successful if the uterine environments (especially hormonal) of the Donor and Recipient are similar.
- The oestrus cycles must then be synchronised with the demonstration of "standing heat" on or about the same day in both the donor and recipient.
- However, there is little difference in pregnancy rate in the recipients if both exhibit oestrus +/- 1 day of each other.



Preferred Synchronization method:

- Use of the hormone Progesterone in the form of a CIDR (Controlled Internal Drug Release) vaginal insert.




Abbreviated superovulation and synchronization protocol

Day	Time	Donor program	Recipient program
12	am		Insert CIDR
13	am	Insert CIDR	
17	6 pm	Inject 4 ml Folltropin (FSH)	Inject 2.5 ml Chronogest
19	6 am 6 pm	Inject 3 ml Folltropin Inject 2 ml Folltropin	
20	6 am 6 pm	Inject 1.5 ml Folltropin + 3 ml Estrumate Inject 1 ml Folltropin + 1 ml Estrumate	Remove CIDR & inject 2 ml Estrumate.
21		Inject 1 ml Folltropin + Remove CIDR Inject 1 ml Folltropin	Inject 1 ml Ciderol Observe for Heat

Day	Time	Donor program	Recipient program
22	6 am	1st Artificial Insemination (On standing Heat)	Observe for Heat
	6 pm	2nd AI	Observe for Heat
23	6 am	3rd AI	Observe for Heat
29	am	Flushing and Embryo collection	Transfer of embryos

NB: Chronogest – hormone (PMSG); Ciderol – hormone (Oestradiol).

Effect of the CIDR:

- The CIDR contains Progesterone that, after insertion into the vagina, is released continuously into the body for 8 days.
- When the CIDR is removed, there is a sudden drop in blood progesterone levels and oestrus occurs within 3 days.
- Prostaglandin ($PGF_{2\infty}$) is also injected a day before or on the same day of CIDR removal, to cause lysis of the corpus luteum and further rapid reduction of Progesterone.





Advantages of the CIDR:

- The percentage of cows coming on heat is higher than when synchronising with $PGF_{2\infty}$ alone.
- The synchronisation is closer after CIDR removal making heat observation easier.
- About 50-60% of the recipients on a synchronisation program are suitable for Embryo Transfer.
- A good recipient must have:
- An observed and recorded standing oestrus.
- A good sized (1-2cm) Corpus luteum on rectal examination.

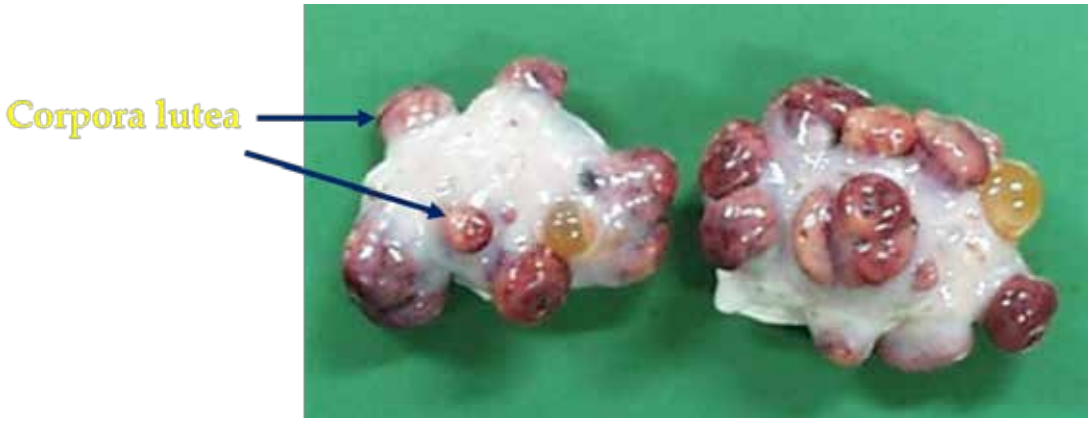
Take-home Message:

- The Superovulation drugs and program used are important factors regarding how many embryos are produced and recovered.
- The Synchronisation of the donors and recipients is critical for optimum conception and pregnancy rates after Transfer.

12. FLUSHING OF DONOR CATTLE AND TRANSFER OF EMBRYOS TO RECIPIENTS

Process of Flushing:

- After superovulation of the donors, AI is done 3 times at 12 hour intervals, when they show standing heat.
- The donor is then flushed (embryos harvested) 7 days later.
- Prior to flushing, the cow is examined by rectal palpation to check the response to superovulation, i.e. the corpora lutea on the ovaries are counted.



Process of Flushing:

- The cow, restrained in a crush, is then washed with water at the perineum.
- The area around the vulva is sprayed with 70% alcohol (surgical spirit) to minimise contamination.
- The equipment for flushing is also set up, including flush fluid, Y-junction tubing, Catheter, Embryo Filter and 60 ml syringe.











Process of Flushing:

- Just before flushing, the cow is given an Epidural injection using 5 ml Lignocaine, so that it does not strain during the process.
- The tail is then tied to one side, away from the perineum.
- The Catheter with a stylet is introduced into the vagina and manipulated through the cervix.





Process of Flushing:

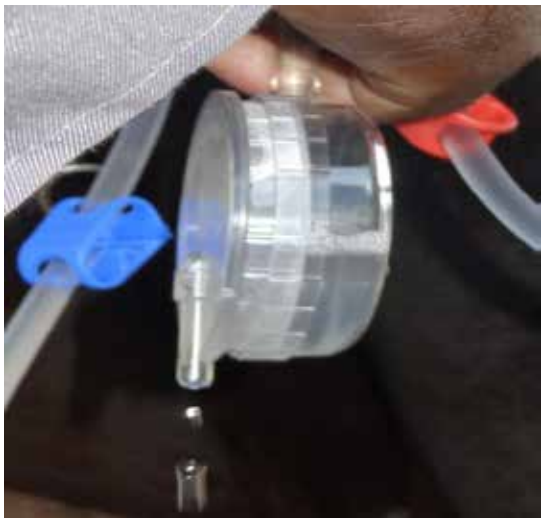
- The Catheter is passed up one uterine horn to just beyond the external bifurcation.
- The cuff (balloon) on the catheter is then inflated with some flush medium until it blocks the diameter of the horn.
- Some complete flush medium (fluid) is then introduced into the uterine horn above the cuff until it is distended.





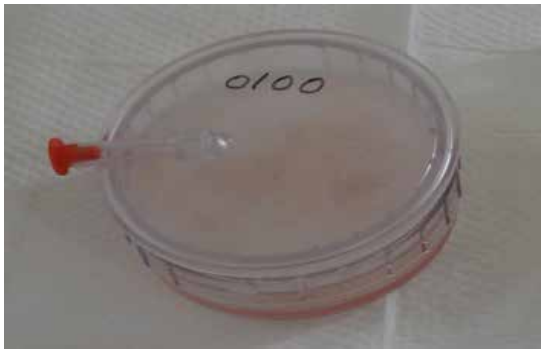
Process of Flushing:

- The flush fluid is then released using a valve to allow the fluid containing embryos to flow into the Filter.
- The excess fluid, but not the embryos, passes through the filter and is allowed to drain out.
- This process is repeated at least 5 times to complete flushing the horn.
- The other uterine horn is also flushed in the same way.

Searching for embryos:

- The embryo filter is labelled and taken to the laboratory.
- The top part of the filter is removed carefully and the surface rinsed using flush fluid. This is to wash off any embryos into the bottom half of filter.
- The bottom half is then examined under a Stereoscopic Microscope for embryos.



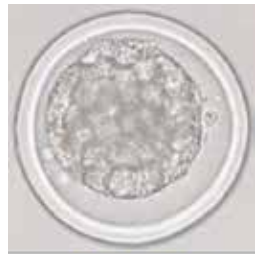




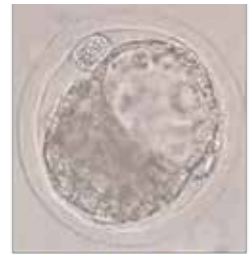
Handling of embryos:

- When embryos are observed, they are picked up using a micro-pipette.
- They are transferred to an embryo holding medium (fluid) in a 5 well plate.
- They are further examined using the microscope and graded with regard to the type (stage) of embryo and quality.
- The embryo types seen at 7 days of pregnancy are a Morula or Blastocyst.





Morula

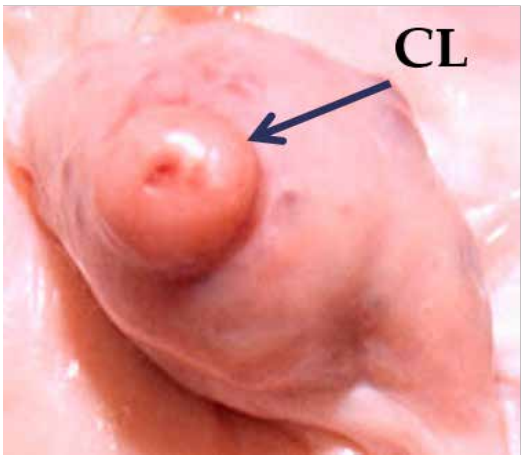


Blastocyst

Transfer of embryos to recipients:

- Only good quality embryos are transferred into the recipients.
- Prior to implantation, the ovaries of the recipient cattle are examined by rectal palpation.
- The Corpus Luteum (CL) size and the side (left or right ovary) is noted and marked on the animal.

- This is because the embryo will be transferred to the uterine horn on the same side as the ovary with the CL.





Transfer of embryos to recipients:

- A single embryo is picked up from the holding medium using a ¼ ml straw attached to a syringe.
- The straw is then disconnected and loaded into an embryo transfer gun.









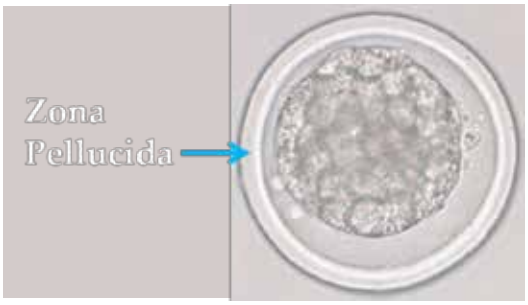
Take-home message:

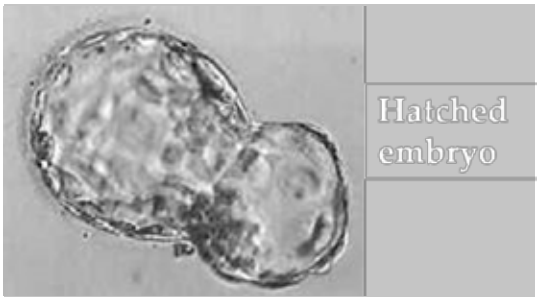
- Both the flushing of the donor and transferring to the recipient takes place 7 days after oestrus, when the cervix is closed.
- Manipulation of the Catheter or Transfer gun through the Cervix therefore requires skill and practice.
- Excess trauma with haemorrhage should be avoided as this affects embryo recovery and conception.
- Avoid trying to go too deep into the horn as this causes more trauma. The embryo can be deposited in the middle third of the uterine horn.

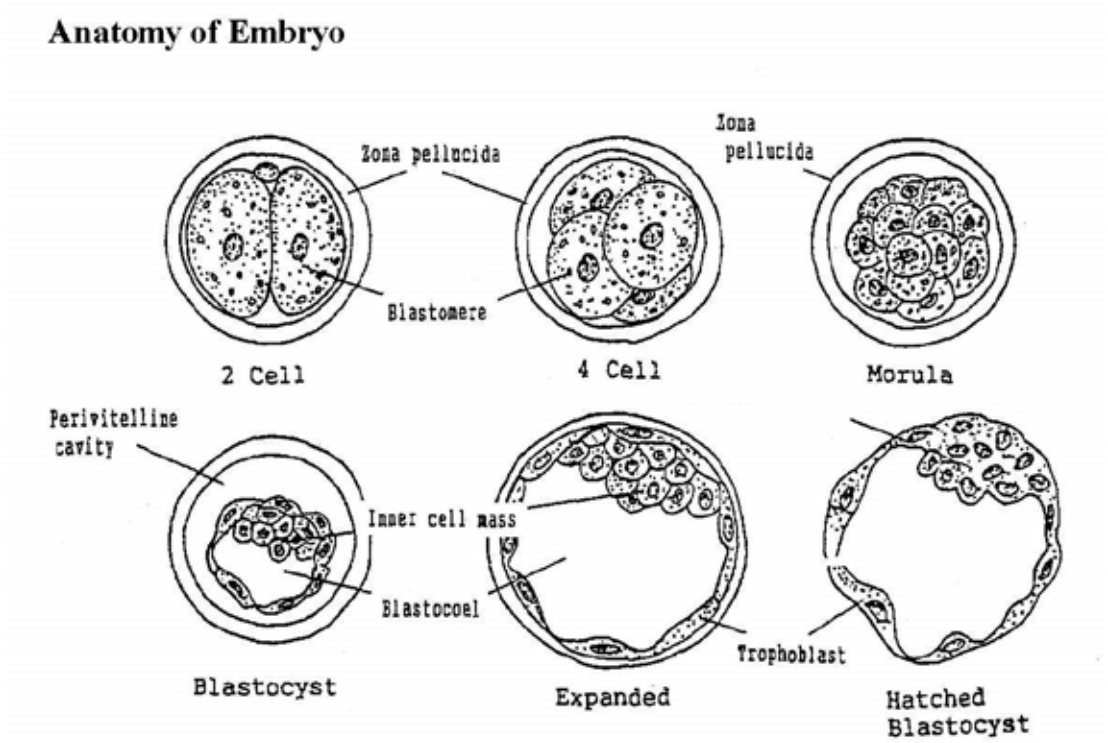
13. IDENTIFICATION AND GRADING OF EMBRYOS

Introduction:

- Embryos are commonly harvested 7 days after AI.
- Earlier than day 7 the embryos are within the oviduct or top of the uterine horn, where recovery using flushing is difficult.
- At about day 9 the embryos hatch and lose their protective covering (Zona Pellucida) so are prone to infections. Harvesting is then avoided at this time.



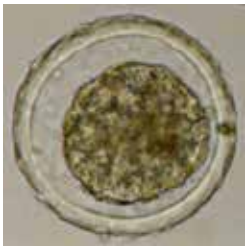


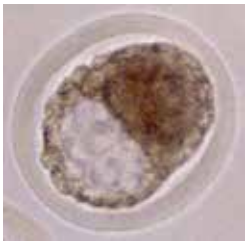


Developmental Stages at Flushing:

Day	Embryo type	Stage
5	16-32 cell	2
6	Early Morula (>32 cell)	3
7	Morula (Compacted M.)	4
	Early Blastocyst	5
	Blastocyst	6
8	Expanded Blastocyst	7
9	Hatched Blastocyst	8

NB: The stage is a code used to specify the embryo type (e.g. compact Morula = stage 4) in the "embryo export documents".







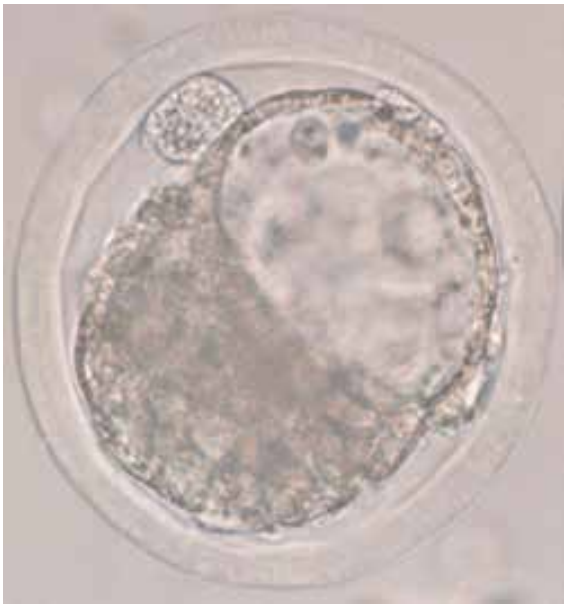
Physical characteristics of embryos to evaluate:

1. Morphology – e.g. Morula, Blastocyst, etc.
2. Age – based on morphology (previous slide).
3. Shape & symmetry – must be spherical (round) with sufficient nuclear mass cells. Roll the embryo to confirm the shape. Triangular or flat shape is an unfertilised ovum.
4. Extruded cells (Blastomeres) – cells outside the nuclear mass which are not viable.
5. Vessicles – spaces within the nuclear mass. If many, this is a low grade embryo.
6. Intact Zona pellucida – must not have cracks or evidence of hatching.

Criteria for classification of embryo quality:

Excellent or Good (Grade 1)

An ideal embryo, spherical, symmetrical with cells of uniform size, color and texture; or trivial imperfections such as few extruded blastomeres, irregular shape, few vesicles; At least 85% of the cellular material should be an intact, viable embryonic mass.



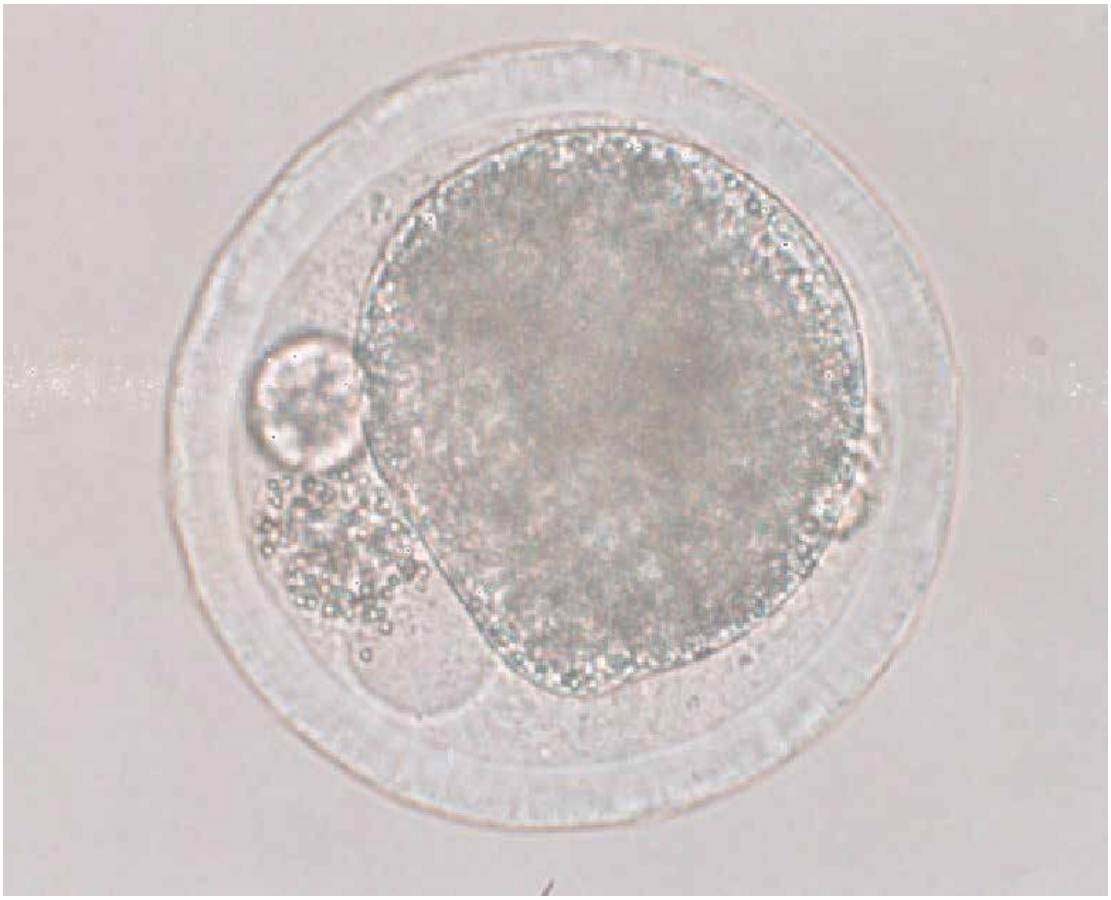
Criteria for classification of embryo quality:

Fair (Grade 2): Few more extruded blastomeres, vesiculation, and few degenerated cells; At least 50% of the cellular material should be an intact, viable embryonic mass.



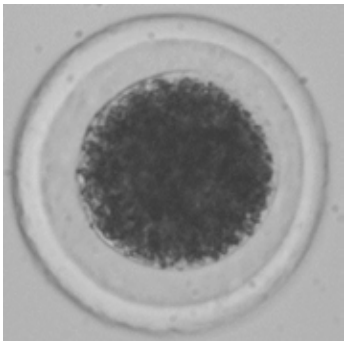
Criteria for classification of embryo quality

Poor (Grade 3): Numerous extruded blastomeres, degenerated cells, cells of varying sizes, large numerous vesicles; At least 25% of the cellular material should be an intact, viable embryonic mass.



Unfertilized Ovum:

- The nuclear mass is dark (black) and grainy in appearance.
- May appear round (spherical), flat or triangular in shape.
- Floats more quickly than an embryo (heavier) in holding medium.



Take-home message:

- Conception is only possible if a good quality embryo is transferred.
- The poor quality embryos and unfertilised ova must then be identified and discarded.
- Grade 1 embryos have the best conception rates.
- If there are more recipients than grade 1 embryos, then grade 2 can be used. This should be noted in the records.
- Grade 1 embryos are also best for Freezing, as there is some loss of viability during the process.

14. FREEZING OF EMBRYOS

- This is necessary in situations where there are insufficient numbers of recipient cattle for the embryos obtained.
- The good quality embryos are selected for freezing in liquid nitrogen.

Preparation for freezing of Embryos:

- Prior to freezing, the embryos are immersed in a cryo-protectant fluid (Ethylene glycol).
- This ensures they are not harmed by the very cold liquid nitrogen temperatures.





- The embryos are sucked into ¼ ml straws using a syringe.
- They are arranged in a receptacle placed in a Cryochamber containing liquid nitrogen.



- The freezing process is automated with a gradual reduction in temperature.



Storage of Embryos:

- Subsequently, the straws are put into a canister with liquid nitrogen (similar to that for frozen semen) for long term storage of the embryos.



15. IN VITRO EMBRYO PRODUCTION (IVEP)

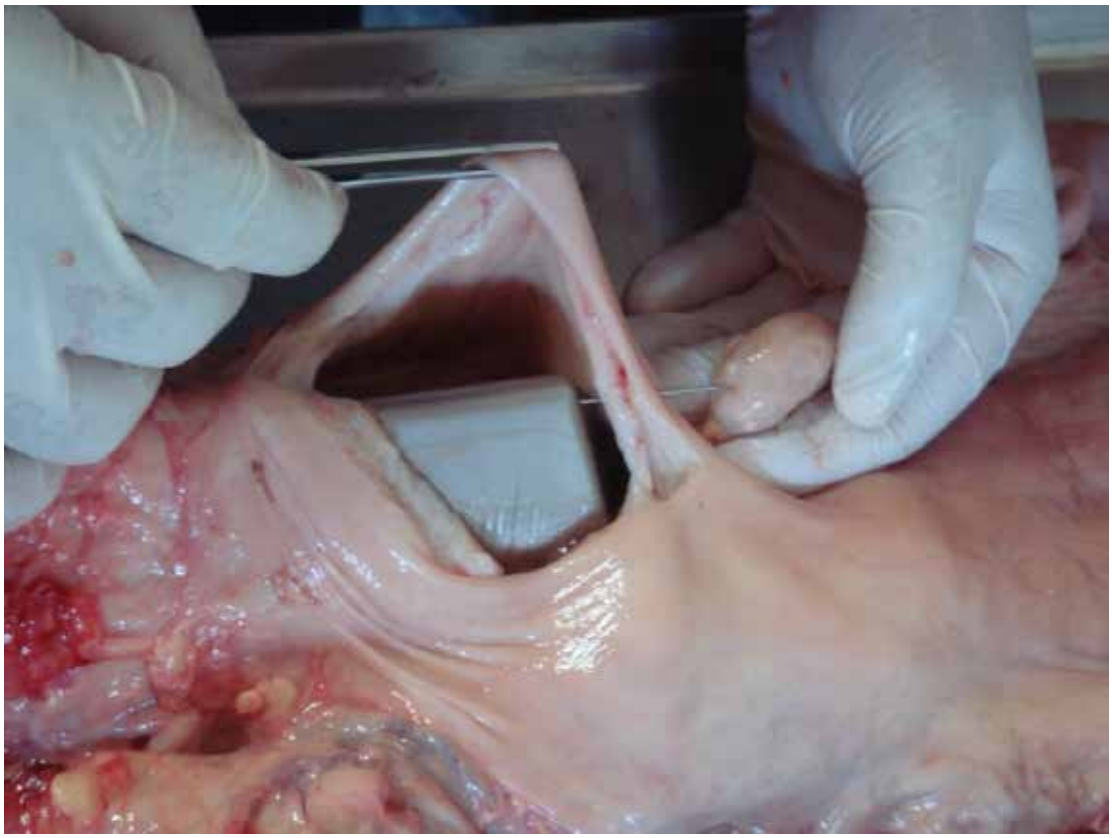
IVEP: Also know as Ovum pick up (OPU) or In vitro Fertilization (IVF).

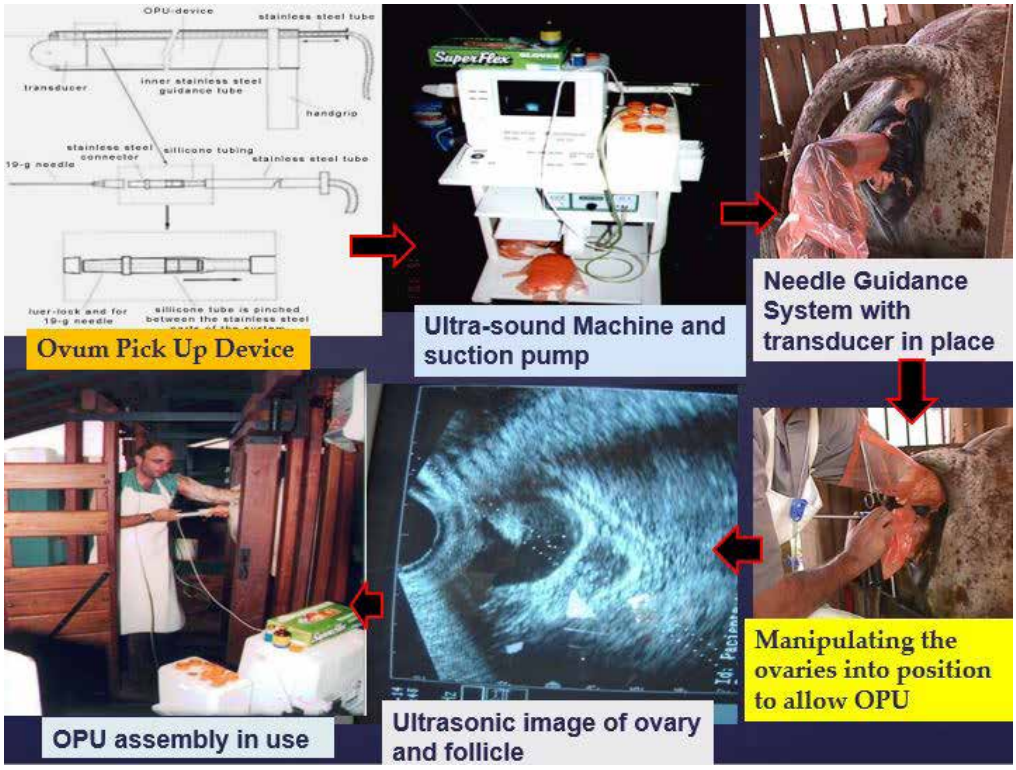
The steps involved in the process are:

1. Selection of donors
2. Selection of sires
3. Synchronization of donors
4. Collection of ova (Ovum Pick up)
5. Ova maturation
6. Decapitation of sperms
7. In-vitro fertilization
8. Maturation of embryos
9. Transfer of embryos.



Ovum pick up: device needle inserted into follicle:





Advantages of IVEP:

- Cheaper than MOET for production of embryos as superovulation hormones are not required.
- Suitable for Beef cattle that do not sell for as high as Dairy cows, so return on investment is better.

Disadvantages of IVEP:

- A high initial capital outlay is required in laboratory equipment (OPU machine; Incubator, etc)
- There's a tendency to get more bull calves than heifers using this technique (unless sexed semen is used).
- The frozen embryos have low survival, i.e. are not viable after the freezing process. Therefore all the embryos are transferred to recipients or wasted.

NB: In Kenya this technique has mainly been used as a research tool and not commercialised to the same extent as MOET.

16. COST-BENEFIT ANALYSIS OF MOET.

Introduction

- MOET programs are relatively expensive, mainly due to cost of the inputs.
- The cost-benefit ratio is best when the success rates are high, i.e. high embryo yields and good conception rates.
- The benefits include the potential sale of embryos and the value of the resulting heifers and bulls.

Costs of MOET:

1. Drugs for superovulation of donors and synchronization of recipients.
2. Semen and artificial insemination costs.
3. Labour, equipment and supplies to flush donors, collect and isolate embryos.
4. Labour, equipment and supplies to transfer embryos to the recipients.
5. Travel expenses for personnel.
6. Labour, equipment and supplies to freeze and store embryos.
7. Pregnancy diagnosis of implanted recipients.

Considering the Risks of MOET:

- In order to even out the risks of variable embryo production, a minimum of 3 donor cows are flushed.
- If one does not give embryos, the other 2 will make up for it.
- With an average production of 5-7 embryos per donor, at least 15 recipients are required for the transfers.
- Any excess embryos are frozen.
- If only one donor is flushed and she doesn't give embryos, all the money used and effort is wasted.

Estimated MOET costs

For 3 Donors and 15 Recipients:

Component	Item	Cost per unit	Total cost
Donors	Super ovulation drugs and Semen	40,000/-	120,000/-
Recipients	Synchronization drugs	5,000/-	75,000/-
Consumables	Flushing, Transfer and Freezing		55,000/-
Professional Fees	Flushing, Embryo searching and transfer / Freezing.	20,000/- per Consultant.	40,000/-
Travel	Minimum charged		10,000/-
Grand Total =			**300,000/-**

Income / Prices

Item	Sale Price (Ksh)	Sale Price (USD)
Locally produced embryo	20-30,000/-	200-300
Imported embryo	30-100,000/-	300-1000
ET heifer calf at birth	100,000/-	1000
ET bull at weaning- 3 months	120,000/-	1200
ET in-calf heifer	200-250,000/-	2000-2500

Cost-benefit analysis:

- The total costs, therefore, to prepare and flush 3 donors and transfer 15 embryos to the recipients, as well as freeze excess embryos, is Kshs 300,000/-.
- The expected income from the above process at a conception rate of 50% is 7-8 pregnancies, half of which are females.
- Assuming 4 in-calf heifers and 3 bulls are sold thereafter, the projected income would be Kshs 1,160,000/-.
- If Ksh 160,000/- is spent on raising (feed, treatment costs, etc) the7 cattle prior to sale, then the projected net profit is Ksh 1,000,000/-. This is within a 2 year period for the in-calf heifers.
- The expected income can be increased using economies of scale, i.e. increasing the numbers of donors flushed, hence embryos and offspring available for sale.

Challenges to overcome

- Relatively high cost of consumables, especially hormones.
  NB: EASETA has the capital equipment (e.g. Microscopes, Embryo Freezer, Transfer guns, etc.)
- Relatively high cost of consumables, especially hormones.
  NB: EASETA has the capital equipment (e.g. Microscopes, Embryo Freezer, Transfer guns, etc.)
- Marketing the MOET technology to ensure both small and large scale farmer uptake.
  NB: Most money is made from sale of breeding stock, while good management (e.g. optimum feeding) is required to maintain high milk production.

Conclusions

1. There is a place for Embryo Transfer in Kenya and beyond if all the stakeholders (farmers, ET practitioners, breed associations, etc.) are willing to pull together.
2. There is need, not only to train ET practitioners, but for them to obtain "hands on" experience in order to improve embryo yields and conception rates.

3. Commercial embryo transfer is viable in Kenya as there is an unsatisfied demand for high milk producing in-calf heifers and good quality bulls.
4. ET is not for the "faint hearted" as one experienced practitioner said, bearing in mind the costs and often variable success rates, even with the "experts".

17. EXPERIENCES OF MOET IN KENYA

History of Embryo Transfer in Kenya:

- 1982 - 1st batch of frozen embryos brought into Kenya and transplanted into recipients using surgical method – poor conception rate – 20 %. ADC and Wangu Embori involved.
- 1984/85 - 50 Ndama embryos brought in from Gambia for research at International Livestock Research Institute (ILRI) into trypanotolerance characteristics of Ndama Cattle.
- 1994 - 1st export of Kenyan Boran embryos to South Africa. Regular exports done from Ol Pajeta farm by Dr Morne.
- 1995/96 - Embryos imported from USA for ADC implanted by Dr. David Kennedy (ILRI). Good conception rates (50 %). His ET programs continued until 2002 for about 150 embryos.
- May 2006 - Registration of East Africa Semen and Embryo Transfer Association (EASETA).

EASETA activities:

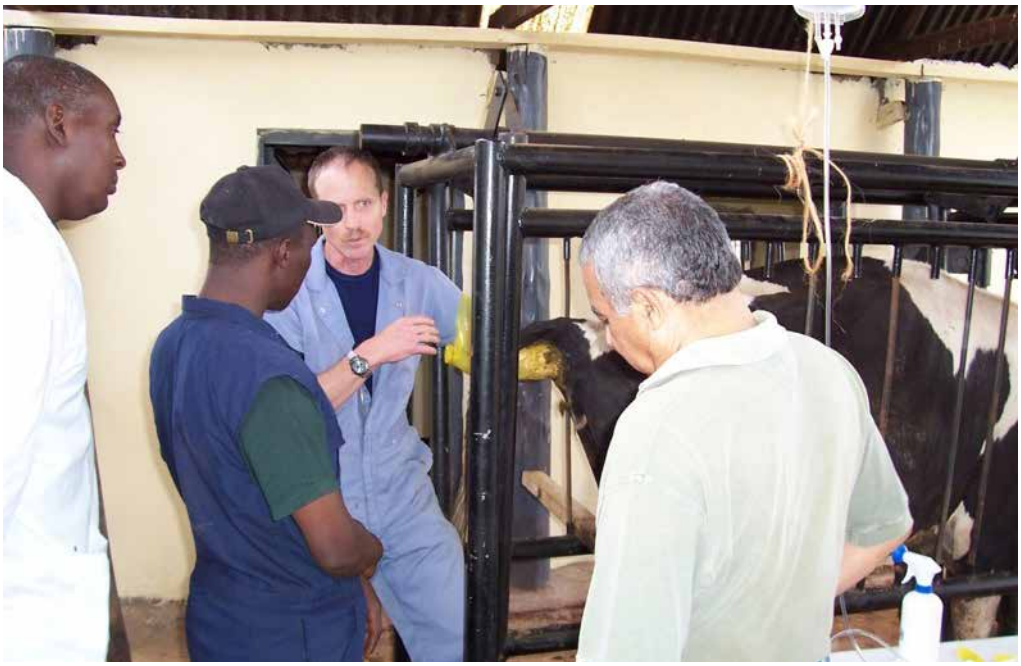
- 1st ET Training Workshop- November 2005
- ADC Namandala.
- Trainers - Dr Mubiru (Uganda) & Prof Kihurani.
- Participants from ADC, KARI, KAGRC.



Mr Kirwa (MD, ADC) presenting a certificate to Dr Mubiru

EASETA activities:

- 2nd ET Training Workshop- May 2006.
- ADC Namandala.
- Trainers: Drs Steel and Cuadra (USA), & Prof. Kihurani.
- 17 participants from ADC, KARI, KAGRC, WWS, Twiga, Katheju agencies.



Results - 2nd ET Workshop (2006):

- 9 donors flushed.
- 20 transferable fresh embryos harvested.
- 19 imported (USA) frozen embryos from WWS transferred also.
- 16 calves born.
- Conception rate = 41%.







EASETA activities:

- 3rd ET Training Workshop- October 2007.
- Trainers: Dr Steel and Prof Kihurani.
- 18 participants -13 from EASETA corporate institutes, 2 from National A.I Services (NAIS) Zambia and 1 from Spin Knit dairy.
- Other invited guests- 2 officials of Kenya Veterinary Board (KVB) and 18 (12 staff & 6 students) from 3 academic institutions - University of Nairobi, Egerton University and Moi University.





Results – 3rd ET Workshop (2007):

- 33 donors flushed.
- 84 embryos recovered (42 transferable)
- 34 embryos transferred to recipients (5 by trainees & 29 by facilitators).
- 8 embryos frozen for future use.
- 11 pregnancies resulted (NB: Only transfers by facilitators resulted in pregnancies).
- Conception rate = 38 %.





EAAPP supported ET Sessions in 2011-12:

Session	Date	Donors	Embryos	Degenerates/ oocytes	Transfers	Pregnancies
1.ADC Kitale	17-19-2/2011	11	45	23	21	
2.Skylink Nyahururu	23/3/2011	3	4	1	4	3 (75% conception)
3.ADC Kitale	5-7/5/2011	8	4	1	5	
4.Skylink Nyahururu	1/6/2011	4	12	109 Oocytes	None.	12 embryos frozen
5.ADC Kitale	13-15-7/2011	14	25	16	25	
6.Skylink Nyahururu	9/8/2011	4	12	38	12	3 (25% conception)
7.Skylink Nyahururu	6/11/2011	3	12	1	6	
8.Skylink Nyahururu	21/1/2012	3	16	28	6	10 embryos frozen
9.Skylink Nyahururu	27/2/2012	-	-	-	5 frozen embryos	
10.Dr Ngugi Nyahururu	27/2/2012	-	-	-	2 frozen embryos	
11.ADC Kitale	22-23/3/2012	7	35	10	18	17 embryos frozen
12.Skylink Nyahururu	26/4/2012	2	1	2	1	
13.Skylink Nyahururu	18/6/2012	2	15	1	14	1 (7% conception)
14.RDCoE Naivasha	21-22 /9/2012	9	10	1	10	
15.Makongi	27/9/2012	4	0	1	0	
TOTAL		**74**	**191**	**232**	**129**	

Summary of successes observed

- Highest conception rate (75%) - Session 1
- Best embryo yields (15 from 2 donors)- Session 13
- ET calves born :-
  1. Initial workshops = 27
  2. EAAPP – ADC = 12 calves.
  3. EAAPP – Skylink = 12 calves.

Challenges observed	Reasons
Large number of oocytes (109) – Session 4	Sexed semen used
Lowest conception rate (7%) – Session 13	Poor condition and nutrition of recipients
No embryo yields – Session 15	Inadequate concentrate feeding negative energy balance
Low embryo yields in Sahiwal breed- Session 14	Should the ET program used in Boran be used here also?

Commercialisation of MOET in small and large scale farms:

Large scale Farms (> 50 cattle):

1. ADC (Kitale)
2. Sasini (Nyeri farm)
3. Gicheha (Brookside- Juja)
4. Makongi (Eldoret)
5. KALRO (Naivasha)

NB: Research:

1. EAAPP funded Zebu project in KALRO – Makueni county.

Large scale Farms (5-20 cattle):

1. Skylink (Nyahururu)
2. Ololua Jerseys (Karen)
3. Gakindu (Nyeri) cooperative members.
4. Kanan Dairy (Machakos)
5. Dr Ngugi (Nyahururu)
6. Many others.

MOET Model used for Large Scale Farmers

1. Select the top 5-10% (e.g. in milk production) of the cattle herd.
2. Perform MOET to obtain as many calves as possible from this group.
3. The heifers are then bred at 14-18 months with top quality bull (including sexed) semen to increase the average herd production of milk.

ET Model for Large Scale Farmers (continued):

- The top 10% of this first generation is then bred again using MOET to further increase production.
- Excess embryos are frozen for sale.
- Excess in-calf heifers are sold at premium prices (> Kshs 200,000/-) due to high demand.
- Bull calves sold for breeding or as semen donors.
  NB: This model was used at Sasini Farm, Mweiga, Nyeri.
- Heifer calves born from the recipients are bred using MOET at 14-18 months. At calving, the owner now has a higher milk producer.
- Additional heifer calves born from this first generation are sold/ given to other Cooperative members for breeding.
- Bull calves sold for breeding or as semen donors.
  NB: This model was used by Gakindu Cooperative, Nyeri, using embryos bought from Sasini farm..

ET Model for Small Scale Farmers who cannot afford individual costs:

- Utilise the Cooperative movement to facilitate financing due to a large capital base.
- The Cooperative then purchases embryos from the large scale farmer at a fraction of the cost of in-calf heifers (e.g. 20-30,000/-).
- Embryos transferred to recipient cattle owned by Cooperative members.

Technical factors introduced to improve embryo yields:

- 3 way catheter.
- Mini-flush filter.

- Air insufflation of uterus after flush fluid administration.
- Insemination into the uterine horns when using sexed semen.



Activities carried out to improve conception rates:

- Emphasis to the farmer on the importance of adequate nutrition of both donors and recipients for the success of MOET programs.
- Consistent management of donors and recipients as per MOET program requirements.
- Joint MOET programs with external experts (e.g. Dr Morne of Embrio plus, South Africa.
- Appropriate delegation of responsibilities to ET team members in areas of flushing, embryo search, grading and transfer based on technical skills demonstrated.
- Continued practice to refine skills.

Way Forward:

- There has been tremendous interest in having the technology utilised in a more widespread manner, including regionally- Tanzania, Uganda, Zambia, Ethiopia.
- Individual small scale farmers can access the technology through their Cooperatives, hence making it more affordable.
- Capital equipment and materials have been sourced using funds from agencies like EAAPP (Eastern Africa Agricultural Productivity Project) to facilitate various MOET programs.

- Regional linkages have been created (i.e. Embrio plus (SA); EASETA Kenya, Uganda & Tanzania) for joint training and ET programs.

Incentives for MOET – calves:









18. PREGNANCY DIAGNOSIS BY RECTAL PALPATION IN CATTLE :

Scenario:

- Your cow was inseminated several months ago but you are not sure whether it is pregnant. Apart from calling a Vet/ Professional, what information and observations can you use to determine pregnancy?



Rectal palpation:

- The most commonly used method of detecting pregnancy in a cow.
- Can be done as early as 35-65 days after AI (commonly at 3 months).
- Advantage: an immediate result is obtained so non-pregnant cattle can be re-inseminated.
- Accuracy: up to 95%, but dependant on the experience of the practitioner.

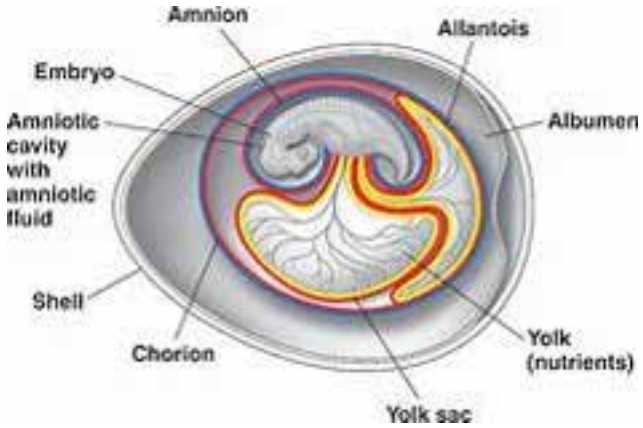
History prior to Rectal palpation:

- Exposure to a bull or AI done.
- Non-return to oestrus (heat no longer observed). The most common cause is pregnancy.
- Efficient heat detection is vital.

Cardinal (positive) signs of pregnancy :

A) Amniotic vessicle:

- This is the early developing foetus surrounded by amniotic fluid and the amniotic membrane.
- Detected by day 28 of pregnancy.
- It feels as a round, turgid, fluid filled structure within the pregnant uterine horn.



Cardinal (positive) signs of pregnancy :

B) Foetal membrane slip:

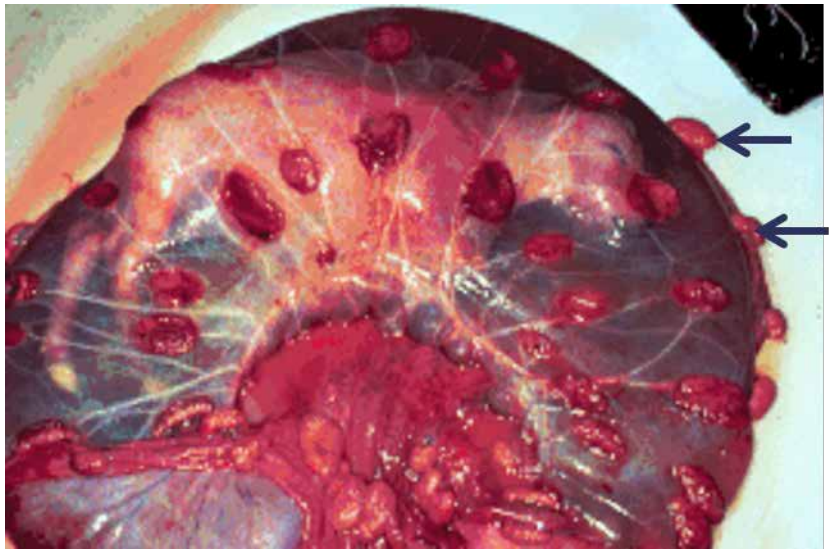
- The foetal membrane is the Chorioallantois, felt within the lumen of the uterus.
- Detected by day 35 in the pregnant uterine horn.
- Also detected by day 70 in the non-pregnant horn.



Cardinal (positive) signs of pregnancy :

C) Placentomes:

- Also called Caruncles
- Round and flat firm structures, arranged in 2 ventral and 2 dorsal rows.
- Palpable from day 75-80 of pregnancy.
- Can still be palpated for some time after foetal death so can give a false positive pregnancy result.



Cardinal (positive) signs of pregnancy :

D) Foetal Ballotment:

- The foetus is palpable from day 65 of pregnancy.
- Ballotment is the tapping of the distended uterus and feeling the foetus bounce and hit your hand.
- At 5 months of pregnancy the uterus is resting on the abdominal floor so the foetus is out of reach.

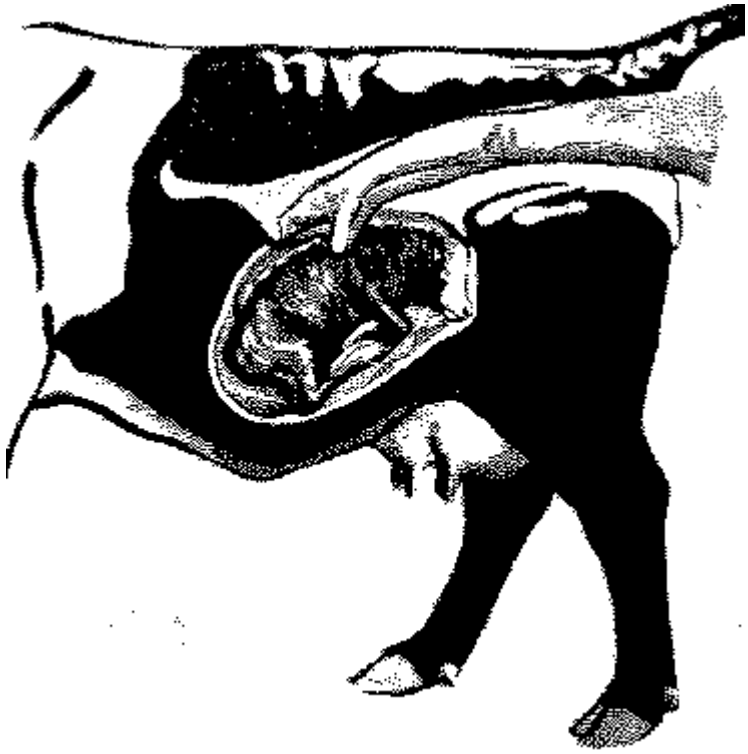




Cardinal (positive) signs of pregnancy :

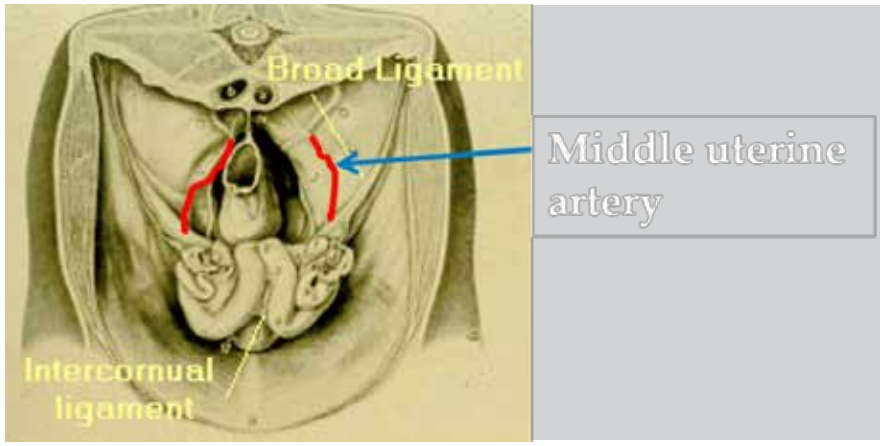
E) Foetal parts:

- The foetal parts (e.g. head, limbs) are palpable again at about 7½ months of pregnancy to term, when the foetus has grown and is within reach again.



Supporting signs of pregnancy:

- Asymmetry of the uterine horns.
- Enlargement and distinct blood flow (Fremitus) in the middle uterine artery.
- Presence of a Corpus luteum in the ovary on the pregnant horn.
- Fixation of the cervix due to the weight of the pregnant uterus.



Take-home message:

- Rectal palpation is an accurate method for pregnancy diagnosis of cattle, depending on the skill and experience of the practitioner, from as early as day 35 of pregnancy.
- However, very early palpation has the risk of causing foetal death, or missing early signs of pregnancy, so it's routinely done at 3 months after AI/ natural service.
- The distinct positive (cardinal) signs must be felt for confirmation of pregnancy.

19. PRINCIPLES OF ULTRASOUND

Ultrasound is a Diagnostic Imaging Technique. What other imaging techniques, that are used in animals, do you know?



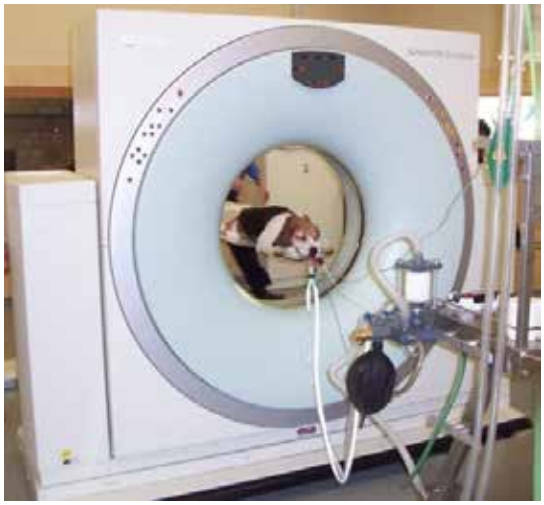
X-ray



Endoscopy



Scintigraphy or Nuclear medicine



CT scan

MRI

What is Ultrasound?

- Sound above the upper limit of human hearing so cannot be heard.
- Frequency of the sound is greater than 20 KHz.
- Used in Medicine to create images of body organs.
- Ultrasound used by Bats and Dolphins to locate prey and obstacles.





Production of Ultrasound

- In Medicine the sound is produced by a probe (Transducer).



Linear Array Transducer

Convex Array Transducer

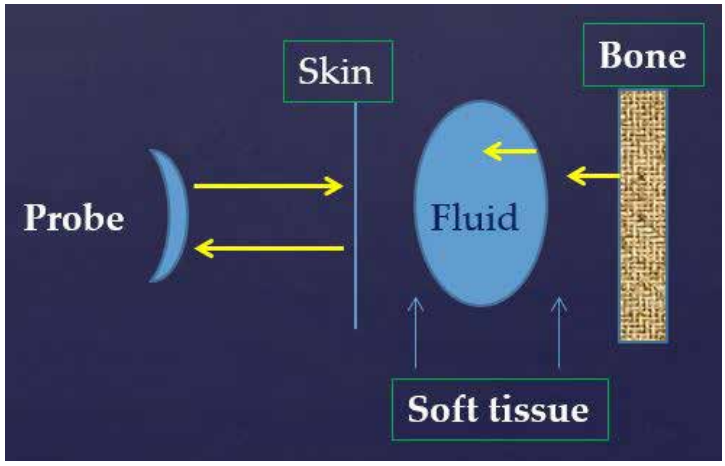
Ultrasound equipment.



Monitor, CPU , Coupling gel and laptop for transfer & storage of images.

How Ultrasound works:

- Transmission of sound pulses by probe.
- The sound travels until it hits a boundary, e.g. between fluid and soft tissue, or soft tissue & bone.
- Some waves are reflected back at each boundary.
- Reflected waves picked by probe and relayed to the machine.
- A 2-dimensional image is formed by the machine.



Ultrasound images

Tissues appear:

1. White (hyperechoic) - e.g. air or bone.
2. Grey (hypoechoic) - e.g. muscle.
3. Black (anechoic) – e.g. fluid.

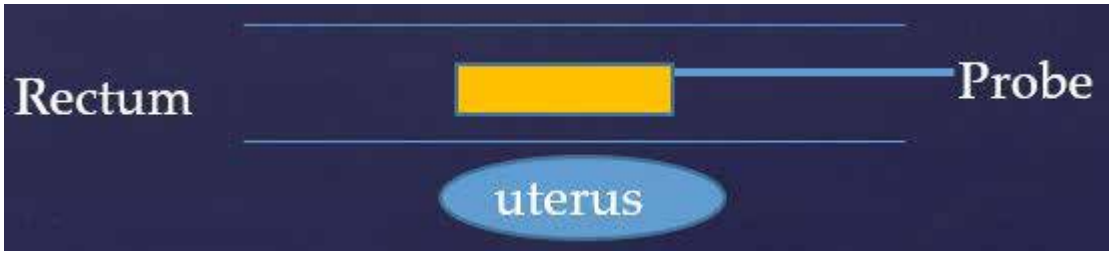


7 week old pregnancy in a cow.

Ultrasound Frequency

- For imaging a frequency range of 1-15 MHz is used.
- The lower the frequency the deeper you can scan:
- 3 MHz frequency scans up to 35 cm deep.
- 5 MHz frequency scans up to 10 cm deep.
- 7 MHz frequency scans up to 5 cm deep.

NB: 5 MHz frequency used for pregnancy diagnosis in cattle when the probe is placed in the rectum (i.e. Trans-rectal).



Uses of Ultrasound in Reproduction:

1. Evaluation of the female reproductive tract (Ovaries and Uterus) in selection of donors and recipients.
2. Diagnosis of diseases, e.g. Endometritis, Ovarian cysts, etc.
3. Pregnancy diagnosis.
4. Determination of the viability of the foetus by detecting the heartbeat.
5. Determination of the sex of the foetus.
6. Ovum pick up in In vitro embryo production (IVEP).

20. SEXED SEMEN, CONSIDERATIONS TO IMPROVE CONCEPTIONS

Introduction

- Semen Production Centres will always strive to give semen of the best quality possible.
- Highly Fertile Semen from High Genetic Value Sires for improved Productivity and Profitability of their offspring.

Why Proper Handling?

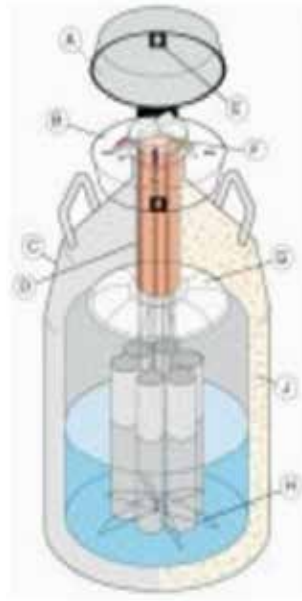
- Semen is an investment.
- Costs money
- Its your future calf
- To protect this investment, need handle it correctly

Stages to Consider

1. Temperature/Cold Chain
2. AI equipment
3. Semen storage
4. Thawing Procedure
5. Loading the gun
6. Insemination

1. Proper Temperature

- Deep Frozen semen is transported and storage in liquid nitrogen - Nothing else.
- ALWAYS Keep semen submerged in liquid nitrogen.
- Avoid exposing straws to elevated temperatures before they are actually needed for A.I.



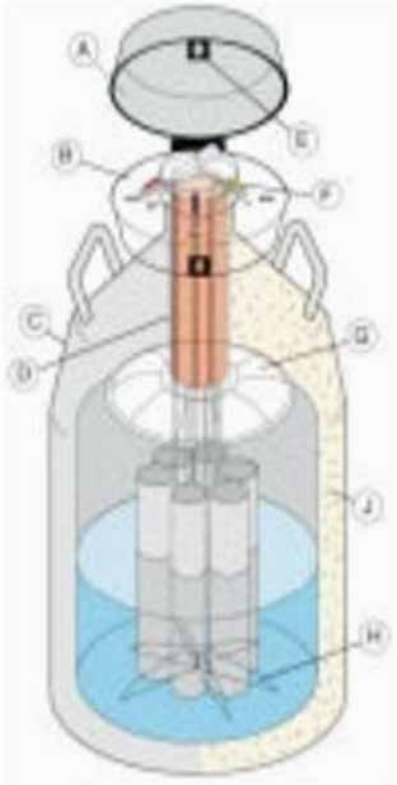
When Does the Damage Occur?

- Tank is allowed to run out of liquid nitrogen,
- Exposing straws for too long during transfer, or
- Too much time is taken when identifying straws in the neck of the nitrogen tank.
- Straw does not completely defrost/thaw.

2. AI Equipment

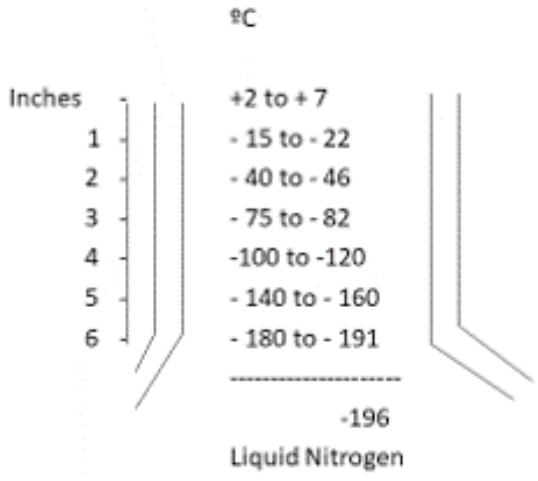
- Correct equipment
- Clean and in good working condition
- Tanks
- Canister
- Goblets
- Scissor
- Socks
- etc

- Most losses in semen are as a result of improper storage.
- Keep straws always submerged in LN2.
- Regularly Check LN2 levels with a calibrated plastic measuring stick.
- LN2 volume level minimum 1/3 high.





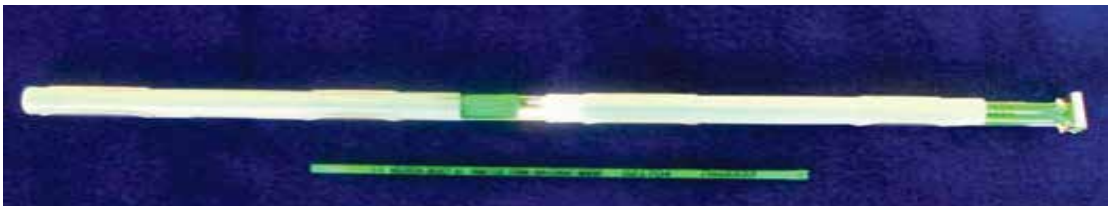
Dewar Temperatures



- Pack straws in canisters in a way which permits easy removal.
- Use canes and goblets.







Maintain a proper inventory for ease of removal of required straw.

Bull Name	Code No.	Breed	No Of Doses	Straw colour	Canister No.

Semen Accounting Form.

Canister Number	Bull code	Breed	Balance B/F	Semen purchased	Total used (Issued)	Balance C/F
2	108	A	15	0	2	13
3	1007	F	6	12	3	15
6	666	J	11	0	1	10

Always keep the canisters just below frost line while working with semen in container.







- Avoid putting too many straws in one canister/goblet.
- Any straw removed from the tank should be used immediately or discarded.
- Always replace the lid immediately.
- Take note of any dent or damage to the container.
- Keep containers in a clean well lit and ventilated place.
- Storage facility should be well secured.
- Only authorized personnel should have access to avoid unnecessary accidents.



4. Thawing

- Use Water bath deep enough to submerge the whole straw.
- 33 – 37°C for --- seconds.
- Use clean water.
- Dry the straw thoroughly.
- Regularly Recalibrate you thermometer.



- Avoid fluctuations in the temperature of thawed semen.
- Interval from thawing to insemination should be kept to a minimum.
- Only thaw the number of straws you can use within 10 minutes.

5. Loading

- Use quality gun and sheath
- Warm the gun before loading.
- Wipe the straw dry before loading.
- Do not expose the straw to direct sunlight.
- Gently tap the straw to locate the air space.
- Cut perpendicularly.

6. Insemination

- Check records to confirm the cow to be inseminated and the semen to use.
- Ensure proper restrain.
- Wipe the vulva area.

Conclusion

- All injuries to the sperm cell during processing and handling are cumulative and should be avoided.
- The viability and fertility of the final product depends on how well the semen was processed and handled.

21. USE OF HEIFER-PLUS® AND BULL PLUS® AS SEMEN SEXING AGENTS IN KENYA

Heifer-plus®

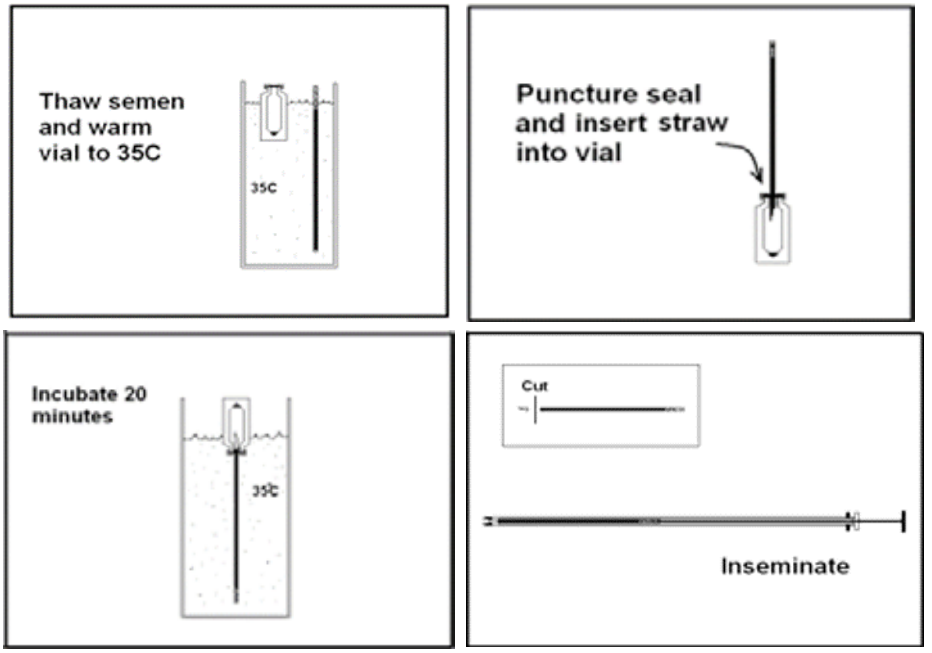


- Agent for sexing bull semen.
- Packaged as lyophilized vials in kit form.
- Works by enhancing the fertility of the X-chromosome bearing (female) sperm (by 5-20%) while reducing the fertility of the Y-chromosome bearing (male) sperm.
- More ova are fertilized by the X-chromosome bearing (female) sperm.
- The heifer sex ratio of calves is increased by 20-30%.

Delivery of Heifer-plus® (HP)

A) Cow side:

- Thawed semen introduced into the HP vial prior to artificial insemination (AI).



B) Lab - method used:

- Fresh semen extended with bovine extender.
- HP added to extended semen.
- Semen straws loaded & frozen.
- At AI, HP-semen straw thawed & inseminated.

NB:AI with HP-semen must be delayed for 16-20 hours after onset of standing oestrus.

ADC farms HP trial

- 40 cattle used (Friesians & Ayrshires) in 3 farms.
- Calving range = Heifers – 11th Calving (Most= H to 4th).
- Used Estrus Synchronization (Pre-synch and Ovsynch method)- 14 Cows; & Natural heats - 26 Cows.
- Semen from 3 bulls with good semen freezing ability was selected for mixing with HP & freezing, prior to AI.
- PD – rectal palpation & Ultrasound done at 2-3 months after AI.
- Foetal sexing – Ultrasound.



Results of ADC farms HP trial

Farm	No. of cattle	Calving range	Pregnant	Foetal sexing
Olngantongo	14	H-11	7	7-F
Namandala	17	H-8	13	6-F 1-M 6-U
Katuke	9	H-7	6	5-F 1-U
Total =	40	Most = H to 4th	26	18-F (72%)

Key: (1) H- heifer; (2) F- female; (3) Male; (4) U- undetermined (4 < 1.5 months old)

Results summary:

- 26 cattle out of 40 pregnant (Conception = 65%).
- Pregnancies occurred at various parities:-
- Heifers = 8/12 (67%).
- 1st-4th Calving = 16/21 (76%)
- 5th -11th Calving = 2/7 (29%)
- Foetal sexing- 18 female (72%); 1 male;
- 7 undetermined - 4 cows < 1.5 months old after AI; and 1 cow with a recto-vaginal fistula were not examined using ultrasound.
- The 5 undetermined cows later confirmed pregnant by rectal palpation. (NB: 2 undetermined but confirmed pregnant using Ultrasound).

Oversights and challenges:

- All cattle inseminated using the am-pm (12 hour) rule. (Recommended 16-20 hours, with heat detection up to 6 times a day).
- 2 cows not pregnant due to Endometritis (visible on Ultrasound).
- Some known difficult breeders (e.g. 5 year old heifer) were selected for the program. Understanding and reason was that Heifer-plus improves conception rates.

NB: Another trial is suggested considering the above and eliminating known problem cows.

Conclusions:

- Heifer plus (HP) improved the female: male calf ratio to 72% on Ultrasound.
- A conception rate of 65% with HP could be improved with consideration of previously stated challenges.
- A pre-mixed HP-semen preparation was used to ease the process of AI, i.e. direct thawing and insemination.
- Pregnancies occurred in cows with natural (17/26 - 65%) and synchronised heats (9 - 35%).

NB: Cow side mixing of HP with semen permits wider bull semen selection, but requires more time for AI and incubation facilities- e.g. 35.6 °C for 21 minutes.

Comparison between Sexed Semen (SS) & HP:

SS – Monsanto- Decisive brand

- High price – 3,500-7,000/- due to high production cost.
- Lower conception rate (85% of conventional) due to lower semen concentration (2.1 M Vs 30 M sperm cells / straw).
- Heifer sex ratio increased to 75-90% (higher cost for higher gender concentration)

HP – Emlab Genetics, USA.

- Lower price – proposed = 1,000/- per dose (HP alone); 1,500/- (Pre-mixed-semen).
- Higher conception – 5-20% above conventional semen.
- Heifer sex ratio increased to 70-80% at uniform cost. (NB: Individual farmers report up to 95% ratio).
- Challenge: Single distributor in Kenya

Other Applications for semen sexing agents:

- Bull plus (BP) for Beef breeds of cattle.
- Use of HP in Embryo transfer to maximise production of Heifer calves and make procedure more cost effective.





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