

Artificial insemination techniques in farm and pet animals

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Artificial insemination is one technique that has had the maximum impact on genetic improvement especially in **cattle and buffalo**; yet due to anatomic and physiologic reasons existent with other species such as small ruminants (sheep and goats), equines, camels and pets (dogs and cats) the progress has been slow and the techniques of insemination widely different.

Techniques- Each of the different techniques used for AI are mentioned and the insemination techniques are then discussed for each species

- **Recto-vaginal** (Semen deposited by trans-cervical passage of the catheter guided by one hand in the rectum)- **cattle, buffalo**
- **Vaginal** (Semen deposited intrauterine by passing the catheter to the uterus and guiding the catheter through a hand in the vagina and not in the rectum)- **mares.**
- **Trans-cervical-** Passing the catheter in the cervix without placing the hand inside- **sheep, sows and bitches**
- **Endoscopic-** Trans-cervical passage of endoscope- **mares, bitches**
- **Laparoscopic-** Trans-abdominal passage of the endoscope- **sheep and goats.**

Cattle and Buffalo

- In India, for first time, AI was done in 1939 by **Sampat Kumaran** at ‘Palace Dairy Farm Mysore’. He inseminated large number of **Halliker cows** with semen from Holstein Friesian bulls and got 33 cows pregnant.
- A pilot project was started in **1942** at Indian Veterinary Research Institute (IVRI) to study the feasibility of AI under the guidance of Dr. P. Bhattacharya. This team approved that this technique could be used under Indian conditions. Four regional centers were established at Bangalore, Calcutta, Patna and Montgomery (Now in Pakistan) by Govt. of India.
- Slowly since then, the scope of AI was widened and the technique has come into general use as a regular practice for breeding of cattle and buffaloes.

- **The first buffalo calf through AI was born at the Allahabad Agricultural Institute in 1943.**
- In 1951-56 the Government of India introduced the first five-year plan (1951-56) with 150 key village centers to perform AI in order to improve cattle and buffaloes in this country.
- 1956-61-The second five-year plan (1956-61) gave a boost to AI work by introducing AI in 400 key village centers. Since then many more AI centers came in operation in India.

- Government of India initiated a major program “**National Project for Cattle and Buffalo Breeding**” (NPCBB) from October 2000 over a period of ten years, in two phases each of five years, with an allocation of Rs 402 crore for Phase–I. In order to consolidate gains made during Phase-I, Phase-II was initiated from Dec 2006 with an allocation of Rs 775.87 crore. The NPCBB envisages genetic up gradation on priority basis with a focus on development and conservation of important indigenous breeds. The project envisages 100% grant-in-aid to Implementing Agencies.

- Artificial insemination in India using frozen semen was introduced during late 1960s. During the year 2010-11, India produced **63 million bovine frozen semen straws** including over **one million buffalo semen straws** through 49 semen stations. Artificial insemination services are provided through **71,341** AI stations clocking **52 million inseminations** with overall conception rate of 35% in bovine and buffalo population. The demand for semen doses in the country is projected by NDDDB to be around 140 million doses by 2021-22 in order to to achieve AI coverage of targeted 35% breedable female animals.

State-wise Artificial Inseminations* Performed ('000 Nos.) NDDDB report

State / UT	2017-18	State / UT	2017-18
Andhra Pradesh	4386	Manipur	13
Arunachal Pradesh	2396	Meghalaya	25
Assam	211	Mizoram	05
Bihar	2823	Nagaland	30
Chattisgarh	747	Orrissa	1424
Goa	34	Punjab	3937
Gujarat	7437	Rajasthan	4231
Haryana	3986	Sikkim	15
Himachal Pradesh	1024	Tamil Nadu	7026
Jammu & Kashmir	971	Telangana	1462
Jharkhand	728	Tripura	153
Karnataka	6672	Uttar Pradesh	12659
Kerala	1111	Uttaranchal	620
Madhya Pradesh	2690	West Bengal	3857
Maharashtra	2306		

Cattle and Buffalo

- The ease with which guiding the insemination pipette through the cervix via manipulation per rectum is possible led to the frequent use of this technique in species such as cattle and buffalo. The recto-vaginal method of insemination continues to be the method of choice worldwide although a new endoscope-guided artificial insemination has been developed for cows but offers little advantage over the traditional method of insemination in these two species.

- **Preparations for insemination and sanitation**
- Important steps to follow and points to remember:
- Ensure that the cow to be bred is truly in heat.
- 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.

Frozen semen must be taken out from the appropriate labelled goblet. The color codes for different species and breeds standardized by Govt of India

Breed	Code	Color
Holstein	HF	Pink/Rose
HF Crossbred	CB HF	Pistachio Green (Light green)
Jersey	JY	Yellow
Jersey Crossbred	CB JY	Salmon
Indigenous cattle	Sahiwal-SAH, Red Sindhi-RS, Tharparker-TAH, Gir-GIR, Kankrej-KANK, Rahi-RATHI, Haryana-HAR, Deoni-DEONI	Orange
Sunandini	SUN	Blue
Buffalo	Murrah Buffalo-MBF, Surti-SBF, Jaffarabadi-JBF, Nili Ravi-NLRVB, Bhadawari-BDBF, Banni-BBF	Grey

- Information pertaining to bull number, breed, name of the organization, year, batch number (as per the day of the year) are printed on the straw and should be recorded in insemination registers. The codes include name of semen collection center, production date, batch number, animal ID, name of the bull and breed code. The article code (N=Conventional semen, X and Y stand for sexed female and male semen). Many sperm stations are now using the Bar code which incorporates all the information that can be identified electronically.

Sequence of the imprinting on the straw

SEMEN CENTER CODE	Production date DDMMYY	Barcode	Article code	Batch number	Animal ID	Name of the bull	Breed code
CHAGAI02B	140514		N	046	CH 120.0917.3984.8	HARRY	BS
		19608425046					





Straw Package

The ABS Straw

Labeling found on 1/2ml or 1/4ml ABS white straws with the exclusive red, white and blue plug.



ABS Global Semen

Bull's full registered name with the common name **BOLD**

Country of registration and registration number

ABS stud code number.
HO = Holstein

CSS block: Logo.
Semen meets and exceeds the minimum standards of CSS (Certified Semen Services) a division of NAAB. Requirements include herd health and semen processing protocols. CSS is an internationally recognized standard.

Recessive testing code

Collection Code
1 = leading character.
02100 = The Julian calendar date, Year 2002, 100th day. This code can be cross-referenced on health documents with the day/month/year.

Thawing the straw

- Before thawing the straw, check the water temperature; it should be at 35⁰C-37⁰C, or as instructed by the semen company.
- The straw should be removed from the flask with forceps and submerged in the warm water. Leave it in for 20-30sec for a 0.25ml straw and 40sec for a 0.5ml one. Semen doses below 50% progressive motility should be discarded. Do not take out the goblet above the neck.
- After withdrawal, wipe it dry and place it in the gun, which should have been pre-warmed by rubbing between the hands. Only thaw one straw at a time.
- Cut the straw (lab plug) end at a 90 degree angle, then slide on to the plastic sheath and secure with the collar. Hold the gun vertically and gently press the plunger upwards, until the semen rises to the top. The gun is now ready.

- **Sanitary procedures and insemination practices.**

It is easier to learn good habits than to break bad habits.

- 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. Lubricants are generally spermicidal. Avoid using products that are irritating. Alternatively non spermicidal lubricants can be used.

– 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 gun can then be placed between the folds of the towel and inserted into the vagina without contacting the lips of the vulva. Alternatively one person can **hold the vulvar lips wide open**.





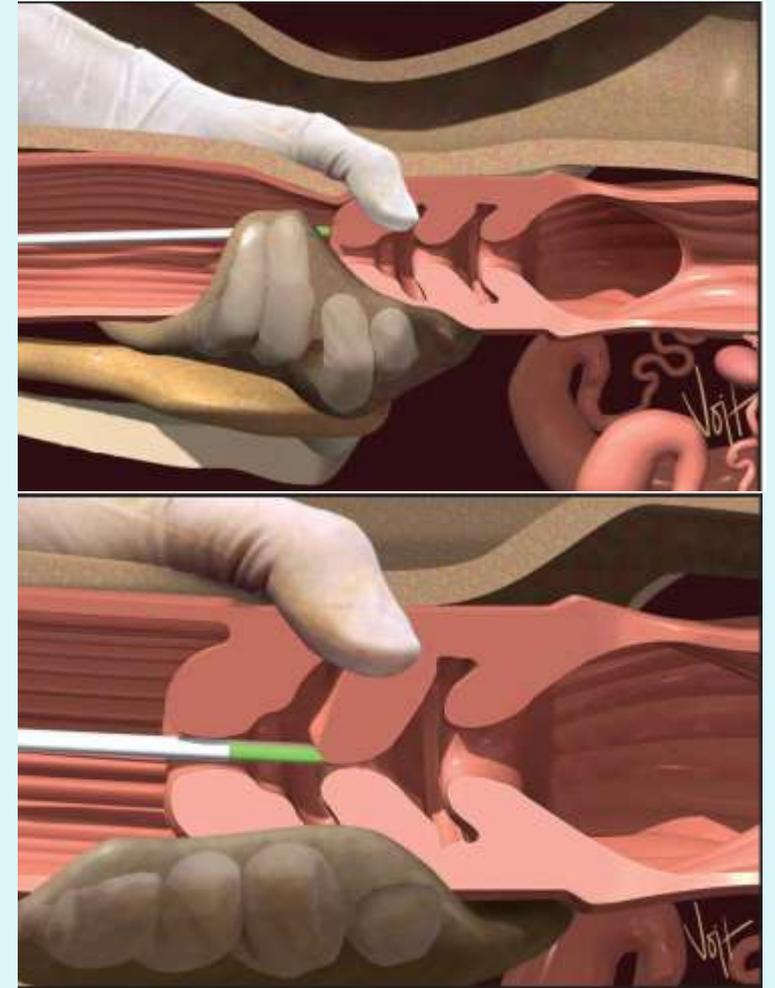
- **Protective sheaths** should be used in herds or for specific cows where vulvo-vaginal infection is a problem. When this system is used, the standard insemination rod and plastic sheath are inserted into the larger protective sheath. This double protection combination is passed through the vagina to the external cervical opening. At the cervix, the tip of the protective device is punctured by the insemination rod, which is then threaded through the cervix. This technique should only be used following the recommendations of a veterinarian, extension specialist, or AI representative -- and only when specific diseases have been diagnosed or suspected.

- **General tips for insemination**

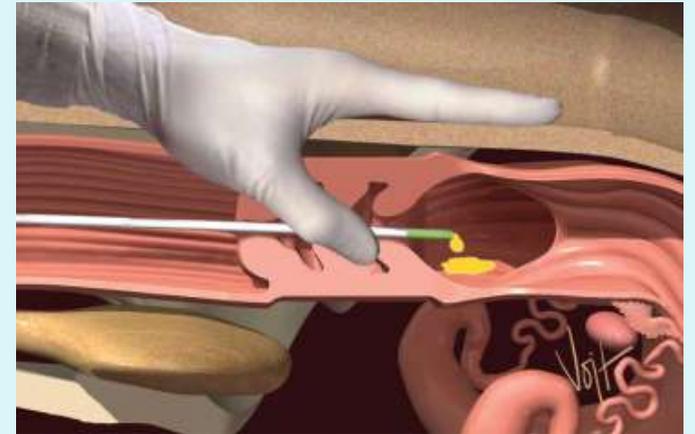
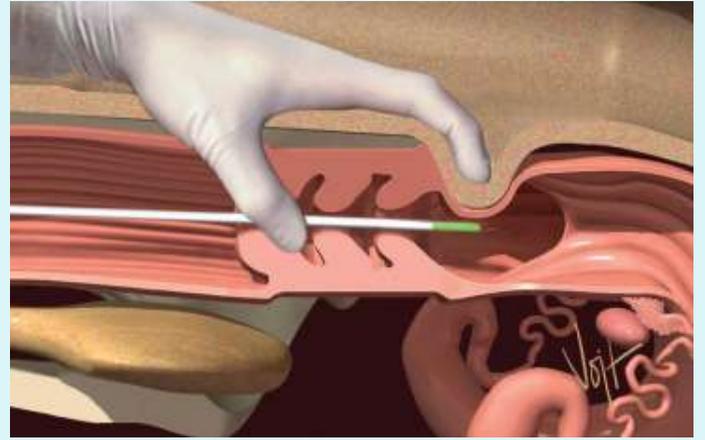


- To avoid the possibility of entering the urethral opening on the floor of the vagina, the insemination gun should be inserted into the vulva upward at a **30° to 40° angle**.
- The anterior portion of the vagina, termed the fornix vagina, tends to stretch rather easily when the insemination gun is pushed forward and beyond the cervix. This may give the false impression that the rod is advancing through the cervix, when indeed it is above, below, or to either side of the cervix. The inseminator should be able to feel the gun within the vaginal fold, but unable to feel the gun tip within the cervix.

- Place the cervix onto the insemination gun. Maintain slight forward pressure on the gun while manipulating the cervix slightly ahead of the gun.
- The target for semen deposition, the uterine body, is quite small. Accurate gun tip placement is probably the most important skill involved in the whole AI technique. Inseminators generally identify this target area by feeling for the end of the cervix and the tip of the gun as the gun emerges through the internal os or opening. Depositing the semen in the cervix or randomly in the uterine horns may result in lower conception rates.



- Once the AI gun tip is aligned with the internal cervical os, deposit the semen by pushing the plunger. Semen deposition should take about five seconds. Slow delivery maximizes the amount of semen delivered from the straw and minimizes the unequal flow of semen into one uterine horn.
- During the process of semen deposition, take care that the fingers of the palpating hand are not inadvertently blocking a uterine horn or misdirecting the flow of semen in some manner.
- Be careful not to pull the insemination gun back through the cervix while the semen is being expelled.
- If the cow has moved during semen deposition or you think the gun has moved, stop the semen deposition and correctly reposition the rod tip before continuing semen deposition.





The sequence of events in Artificial insemination of cattle and buffalo

- Minimum Standards and Standard Operating Procedures For Artificial Insemination issued by the Government of India should be followed.
- Results of depositing semen deep into the uterus using a Flexible telescopic insemination catheter (Ghent device) did not result in improvement in the conception rates in cattle.



Semen handling:

- 1. Keep the liquid Nitrogen container in a location that allows easy withdrawal of semen doses and replenishment of semen and liquid nitrogen. The surrounding should be well ventilated, dry and dust free.
- 2. Clean AI gun, scissors and other accessories whenever they get soiled or at least once a week with hot water and air dry them. Sanitize the AI gun and the scissor with Isopropyl alcohol after drying. The AI Gun piston and the scissors should be wiped clean with water after each insemination. Surgical spirit and soaps are lethal to semen, hence should not be used to clean equipments.
- 3. Maintain the liquid nitrogen level above the straw level in the portable container.

Semen Handling

4. Measure the liquid nitrogen level of 11 and 35 litre containers weekly with the help of measuring scale provided and refill with LN2 if the straws are not submerged. Maintain the record of measurements to monitor the evaporation rate of containers. The refill of other containers depends upon their frequency of use. A 3 liter container should be refilled twice weekly.
5. Carry the required semen doses in the portable liquid nitrogen container to farmer's door step. Never carry semen straws in pocket/thermos-flask / polythene bags filled with water/ice etc.
6. Maintain an accurate semen inventory to lessen the risk of semen exposure
7. Straws once thawed should never be refrozen.

Endoscopic AI for cattle



Artificial Insemination in Mares

- Artificial insemination in mares is still not allowed by many breed registries and the Jockey clubs in the Thoroughbred industry.
- Artificial insemination was probably permitted in the American Quarter horse, Standardbred horses and the Morgan horse (All these breeds derive from crosses of Thoroughbreds) a couple of years back and embryo transfer was legally permitted in American Quarter horse in 2001.



- Majority of the mares are still bred on premises, either by natural cover or by AI with freshly collected semen.



- In the Arabian breed, for example, Registry reports depict that only about 12% of the mares currently are being bred with transported cooled or frozen semen.
- Current figures demonstrate that there has been growth in the use of transported semen by the Arabian horse industry since its approval in 1995.
- In the Quarter Horse industry the number of mares bred by transported cooled semen is far lower than the number bred on premises by natural cover.

- Mares have been artificially inseminated by depositing the semen in the uterus. The inseminator places his hand in the vagina and guides the insemination gun in the other hand slowly in the uterus. Freshly collected and diluted semen is preferred by some owners but for the last more than 4 decades cooled transported semen has also been in practice.
- Cooled, transported semen (TS) was first successfully used in a 1983 field trial at Hamilton Farm (South Hamilton, MA). Results of the field trial (50 mares, 3 stallions) indicated that TS can achieve conception rates equal to those on the best farms

- Equine frozen semen came into existence when the semen of Abdullah the winner of 1984 Olympic games, 1985 World Cup and 1986 World Equestrian Games was frozen and exported to Europe from America in 1985.
- In India AI with liquid semen was started in 1994 and AI with frozen semen of Marwari and Kathiawari horses with moderate conception rates became available since 2003.

Problems with AI in mares

- The mare estrus continues from 4-7 days and mares ovulate 24-48 hours before the end of estrus.
- Most mares ovulate when the follicle size is above 2.5 cm and is pear shaped.
- Thus, inseminations have to be performed either every alternate day starting from Day 2 of estrus till the end of estrus or, when the follicle has been monitored ultrasonographically then a single or double insemination close to ovulation can be done
- The semen from many stallions do not freeze properly
- The minimum number of sperms required per insemination would be 250-500 million.
- Semen has to be deposited intra-uterine by manipulations in the vagina

When to inseminate a mare

- Mares should be inseminated closer to ovulation and this can be monitored by trans-rectal palpation of the follicle diameters or transrectal ultrasonographic visualization of the follicle characteristics and endometrial appearance.
- It is usual for the veterinarian at the stud to examine all mares in estrus daily by palpation or ultrasound in order to find the mares suitable for insemination.
- Cooled semen that has to be transported from elsewhere must be ordered at a time depending upon the size of the follicle on the ovary.
- It is also usual to administer a dose of hCG (3000 IU, IM or IV) 48 h before insemination to assure the ovulation but the follicle size must be at least 30 mm with endometrium showing folding at the time of hCG administration.

Pre-requisites of insemination in mares

Follicle shape change to pear shape (**A**)

Endometrial folding (**B**)

Follicle diameter at ovulation (**C**)



Table 1 Average diameter of pre-ovulatory follicles in various mare breeds.

Breed	Follicle Size at Ovulation (mm)	
Arabian	35–45	
Quarter Horse	35–45	C
Thoroughbred	45–55	
Warmblood	45–60	
Draft	50–60	

Insemination procedure

- After evaluation that the time for insemination is correct the tail of the restrained mare is bandaged and the rectum is evacuated of the feces. The perineum is washed with water and wiped dry with towel.
- The inseminator loads the semen in the syringe when using cooled semen and the insemination pipette when using frozen semen.
- The inseminator inserts the gloved hand in the vagina and the insemination pipette manually guiding the pipette through the cervix into the uterus and deposits the semen in the uterus. A non-spermicidal lubricant should be used for lubrication.

- The insemination is performed in the uterine body just inside the full length of the uterine cervix. A sterile insemination pipette (18–22 in.) is used.
- For cooled transported semen a 1 mL sample is evaluated for motility. Samples with greater than 30% progressive motility after appropriate warming can then be inseminated.
- It is not necessary to pre-warm the remainder of the insemination dose before use. Most mares are directly inseminated with the semen still chilled, which has no detrimental effect on either the sperm or the mare.



Insemination procedure

Washing the perineum

Tail Bandaging

Holding the AI Gun

Guiding the Gun

Insemination through the
vagina



Containers for transport of cooled semen and insemination pipette and lubricants available commercially. The volume of semen varies from 10-20mL.



Equitainer for transport of cooled equine semen



The semi-flexible Minitube insemination pipette for AI with cooled semen



Non Spermicidal jelly



AI with frozen semen

Typically straws of **stallion sperm** are warmed by immersion in 37°C water for 30 seconds or 70° C water for 7 seconds, and then transfer tem rapidly to another water bath at 35° C.

The **thawing** procedure recommended by personnel who froze the spermatozoa should be followed closely.

As sperm in the half ml straw is typically frozen at the concentration of 400 million per ml, each straw will hold approximately 200 million sperm. Depending upon post-thaw motility rates of the sperm, this will mean that thawing between four and eight straws will commonly be necessary to achieve the desired insemination dose (500 million) for mares.

- Once all required straws are thawed and added to the container, the semen should be evaluated and drawn slowly up into a warmed all-plastic insemination syringe, and inseminated as soon as possible into the mare.
- Minitube" produces a "**Universal**" insemination pipette, which will allow for the removal of an emptied straw from within the holder of the pipette, without removing the pipette from the mare's cervix. This means that multiple straws can be loaded into the pipette, and the semen deposited into the mare, without the need for pre-mixing the semen in a centrifuge tube or similar container.

Universal pipette with its stylet. The inverted cone (*arrow*) is located at approximately 10 cm from the tip so that it can pull back an empty straw.



Hysteroscopic low dose insemination

- Ensuring point of delivery as close to the site of fertilization or near the site of suspected sperm reservoir in the mare will decrease the insemination dose needed to achieve optimum conception rate.
- Mares inseminated near the UTJ using hysteroscopy with as few as 5 million progressively motile morphologically normal sperm have conceived at a rate similar to that for much higher sperm numbers when deposited in the uterine body by routine AI methods.

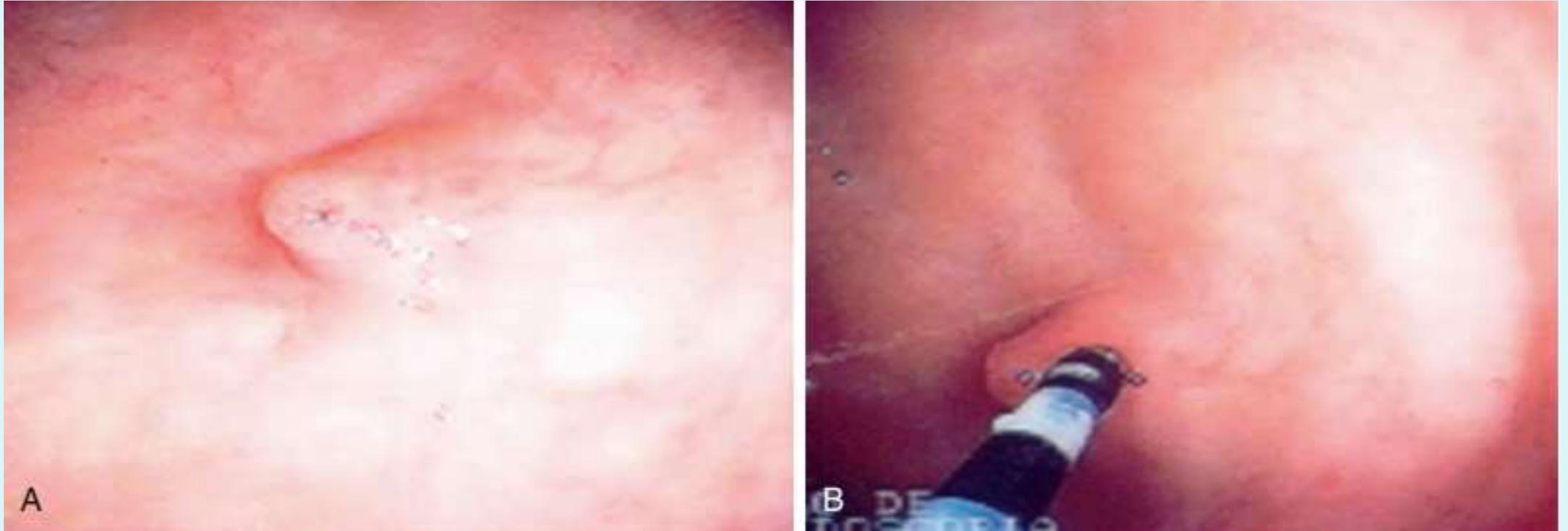
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- The mare is prepared for routine AI as above. The disinfected endoscope (videoendoscope preferred) is inserted through the cervix and directed up the uterine horn on the same side as the expected ovulation until the papilla of the UTJ is visualized.
- A sterile catheter preloaded with the insemination dose is directed into the biopsy channel of the endoscope and the semen is deposited onto the UTJ, or as close as possible next to it. An air flush through the catheter follows the semen deposition to ensure that all of the insemination dose is delivered from the catheter.

- The advantage of this technique is particularly important to stallions with limited sperm production. It is also applicable to use of frozen semen and sex-sorted semen.
- One straw of a stallion's frozen semen with less than the typical insemination dose needed for standard insemination may be delivered by this technique.

The advantage to the mare is that her uterus is exposed to a reduced sperm challenge. When frozen semen is used by standard AI, many mares react with an inflammatory response to the concentrated dose of spermatozoa inseminated. Use of the low-dose hysteroscopic insemination technique will circumvent this problem

Hysteroscopic insemination



A, Endoscopic view of the oviductal papilla. **B**, Endoscopic catheter approaching the papilla to deliver a small volume of semen.

- For problem mares, conventional insemination into the uterine body appears to be superior to hysteroscopic insemination and in normal mares, the highest pregnancy rates can be expected by hysteroscopic insemination.





AI in sheep and goats



- The sheep is less amenable to artificial insemination than is the cow because oestrus cannot be detected readily without the presence of rams, the technique of insemination is less straightforward than in cows, and ovine semen is less easy to freeze than bovine semen.
- However, the most important limitation of the use of AI in sheep and to some extent in goats is in the method of insemination, as intrauterine insemination is difficult to achieve because the cervical canal of the ewe is very tortuous. Because intracervical AI results in both a lower conception rate and a lower number of lambs per litter than natural service, a number of methods of insemination have been devised that try to bypass the cervix.



- The methods of AI in sheep and goats include the intravaginal, intracervical, transcervical intrauterine, and laparoscopic intrauterine routes.
- Around 50 to 100 million spermatozoa are needed in one dose for vaginal insemination, but 25 to 50 million are suggested for cervical insemination and 15 to 20 million are enough for laparoscopic AI.

Vaginal Route



- Vaginal insemination deposits semen into the cranial part of the vagina using a speculum without attempting to locate the cervix.
- Conception rates can be improved by inseminating the ewe twice daily
- However, conception rates with vaginal insemination are extremely poor for both sheep and goats

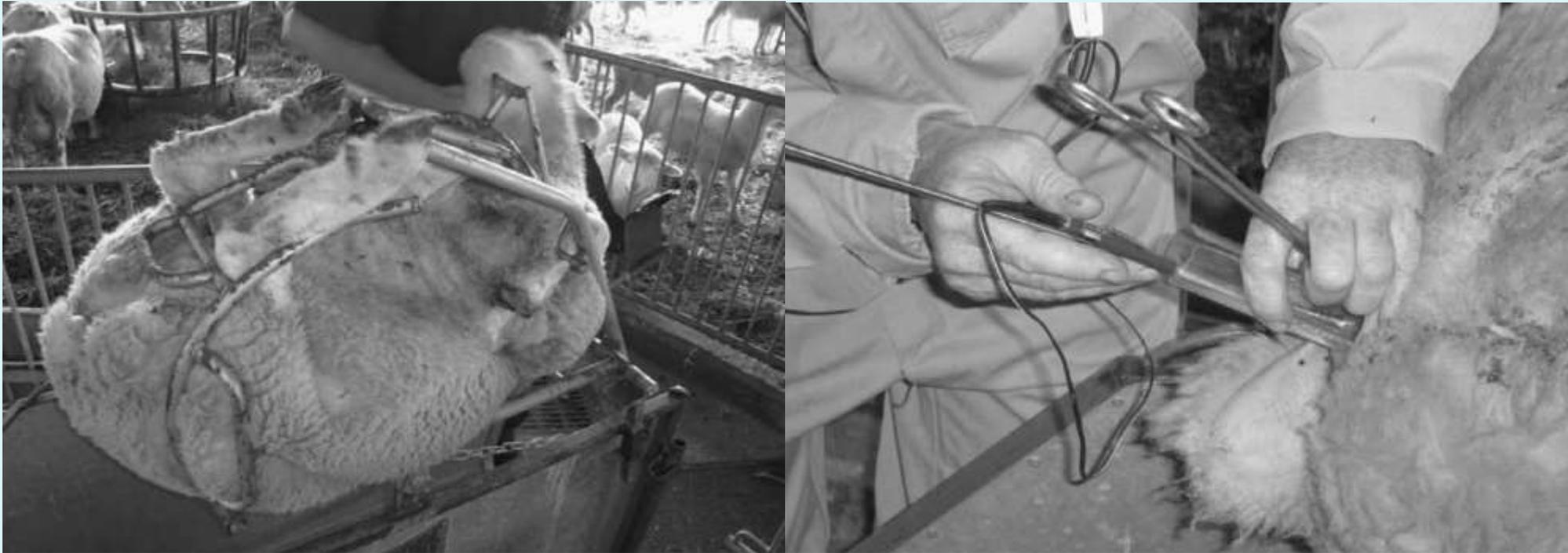
Intra-cervical insemination

- Intracervical insemination is best achieved with the hindquarters of the ewe elevated. After cleaning of the perineum, the vagina is opened with a duck-billed speculum, and the cervix is located.
- The insemination catheter is then inserted as far as possible into the cervix.
- Due to the anatomic barriers the intracervical insemination using chilled semen ($5-15^{\circ}\text{C}$) has moderate conception rates

Trans-cervical Intrauterine Insemination

- The difficulty in traversing the cervix led to attempts of inseminating sheep by grasping the cervix while placing the animal in special position (Guelph TCAI)
- Other approaches included the use of curved catheters (with/without cervical retraction)
- The use of cervical dilation pharmaceuticals such as PGE2 creams. However, the success with such approaches have been moderate.

The Guelph method of transcervical AI



The transcervical intrauterine insemination route in sheep, by using curved catheters and PGE2 creams



Trans-cervical insemination in goats

- Trans-cervical insemination is easier in goats compared to sheep as the number of cervical folds are lesser in number and less deep.
- Frozen semen is available in India for many sheep and goat breeds with the Central Institute for Research on goats, Makhdoom, CSWRI, Avikanagar, National Agricultural Research Institute and also other institutions.
- The transcervical inseminations in goats have resulted in acceptable conception rates.
- The procedure of AI utilizes speculum and raising of hind quarters.

Trans-cervical Artificial Insemination in goats



Laparoscopic Intrauterine Insemination in sheep

- The method of direct intrauterine, laparoscopic insemination was developed to overcome many of the difficulties of intravaginal and intracervical insemination.
- In this method, ewes are sedated and restrained in a cradle. Laparoscopy is performed close to the udder after administering local anaesthetic.
- The abdomen is insufflated with CO₂, the uterus is located and semen is injected into the uterine lumen via a small stab wound. The semen can be introduced to the uterus via a simple pipette or by the use of specialised insemination equipment.



1. Surgical sites are located cranial to the udder and medial or lateral to the mammary/superficial epigastric veins. Local anesthetic is infiltrated, and the sites are scored to identify them prior to the surgical procedure. Left of the image is cranial

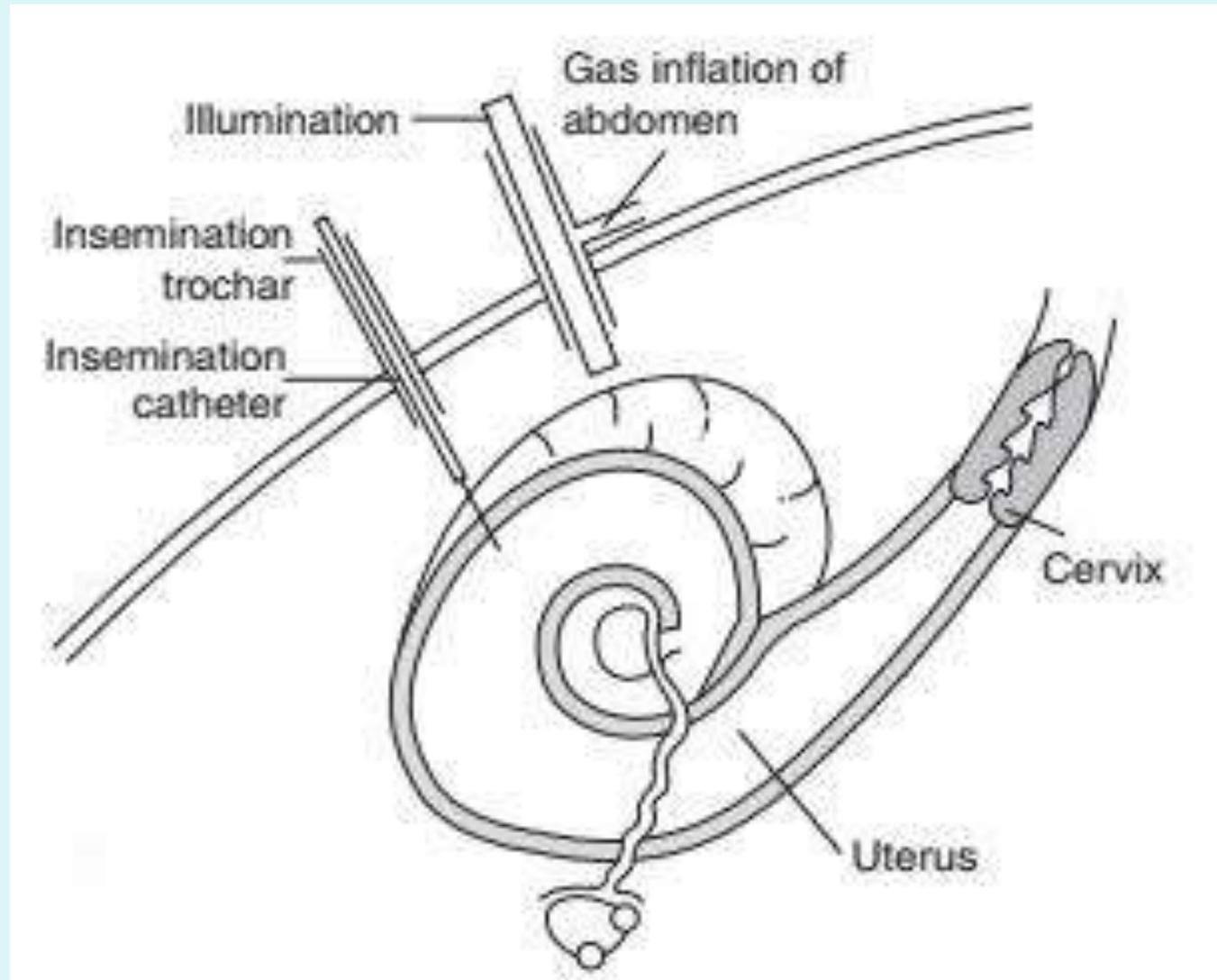


2. A 5 or 10 mm trocar and cannula pointing laterally, are inserted through the near incision into the abdominal cavity with a firm pressure and the trocar withdrawn to be replaced with a laparoscope.



3. A laparoscopic AI gun and needle apparatus are used to inject semen intra-uterine at the level of the mid-horn along the greater curvature.

Laparoscopic Intrauterine Insemination



AI techniques in sows

- Artificial insemination (AI) of swine is widely practiced in countries with intensive pig production. In Western Europe, more than 90% of the sows have been bred by AI for more than two decades.
- In practice, fresh diluted semen for intracervical insemination is mostly used in pigs.
- Frozen storage of boar semen still yields inferior fertility due to the loss of membrane integrity during freezing and thawing. Consequently, freshly diluted semen (liquid semen) is widely used for AI on the day of collection or in the following days.

- Ovulation occurs at the beginning of the last third of oestrus regardless of the overall duration of oestrus. Precise prediction of the time of spontaneous ovulation in individual pigs has not yet been achieved. However, prediction of oestrus duration by observing the onset of oestrus after weaning has found broad acceptance in AI practice for calculation of the expected time of ovulation. AI should be timed as close as possible to ovulation, preferably within 12 to 24 h before ovulation.

- In most cases, one insemination/day is adequate. Schedule in such a way that ensures repeat inseminations will occur less than 24 hours apart.
- For example, breed sows scheduled for a second service before the first-service sows each day. If doing a third-service mating, complete those before the second service, or 12 hours following the second mating

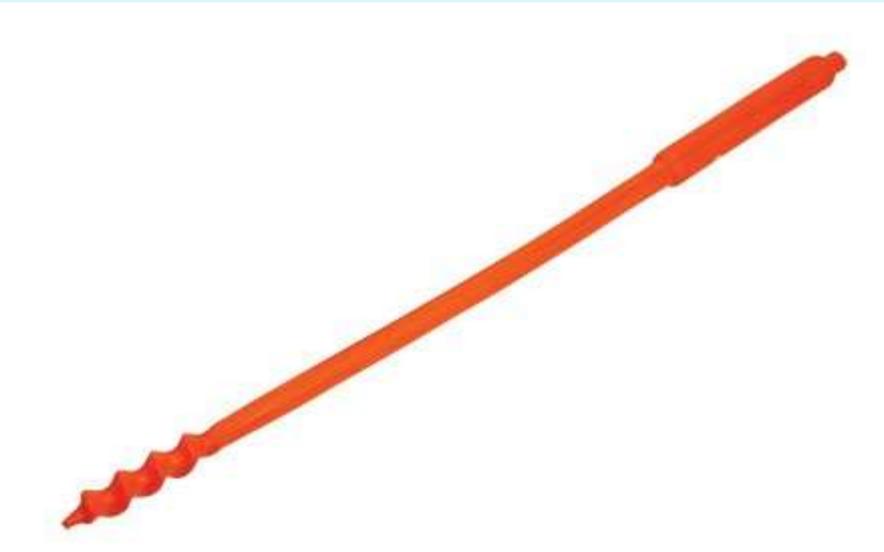
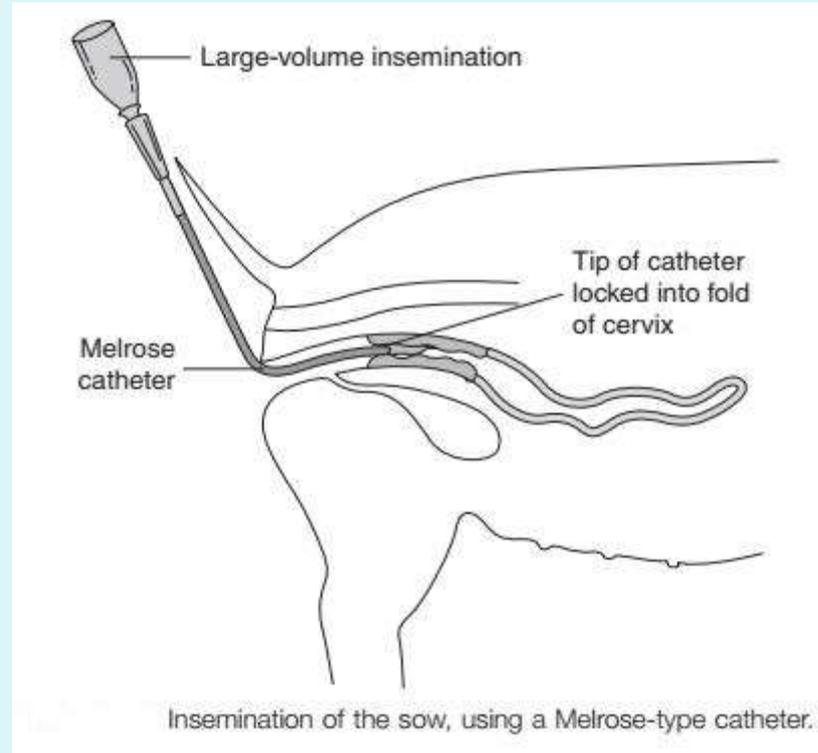
- Before using the semen, evaluate it under a microscope. Shipment, diluent, storage temperature, fluctuations in temperature and length of time since collection may all affect the shelf life, motility and viability of the semen.
- Before inseminating the female, use a paper towel to clean the vulva.
- Lubricate the tip of the spirette or catheter using any non spermicidal lubricant or a few drops of extended semen. Avoid getting lubricant in the opening of the spirette /catheter.

- Gently guide the spirette /catheter, with the tip pointed up, through the vagina to the cervix. The bottle of diluted semen is not attached to the spirette /catheter at this point. Keep the tip pointed up to reduce the chance of coming into contact with the bladder, which could cause a backflow of urine into the spirette /catheter. If this happens, use a new spirette /catheter because urine kills sperm . This is the primary reason the bottle of diluted semen should not be connected to the spirette /catheter until the cervix has been entered. Not connecting the bottle at this point also avoids exposing the semen unnecessarily to extremes of light or temperature . When using the cochette system instead of a bottle, it is common practice to attach the cochette before inserting the spirette/catheter because great dexterity is required .

- Use a counterclockwise rotation to insert the spirette into the cervix. Resistance can be felt by gently pulling back on the spirette .
- Gently invert the bottle of diluted semen two or three times to mix the semen. Attach the bottle to the end of the spirette and discharge the semen slowly. A gentle squeeze to start the process may be needed, but after that the semen should be allowed to be taken up by uterine contractions. This process takes at least 3 minutes. Because of the variation in intensity of uterine contractions, gilts often take longer to inseminate than sows. Depositing the semen too rapidly will cause a backflow of semen out of the vulva.

- Today, boars can be managed for production of 20 to 40 traditional AI doses containing 2.5 to 3.0 billion motile sperm in 75 to 100 mL of extender or 40 to 60 doses with 1.5 to 2.0 billion sperm in similar or reduced volumes for use in cervical or intrauterine AI. Storage temperatures range from 15° to 18°C for extended boar semen.
- Mixing or pooling semen from different boars (**Heterospermic Insemination**) has become a common technique for processing boar semen for AI. Pooled semen has been found to yield good fertility results in swine.
- A uniformly successful freezing and thawing protocol for porcine spermatozoa still eludes the industry. Certain boars and breeds appear to exhibit better sperm freezability than others, confounding results. However, use of frozen semen is still unpopular in pigs.

AI catheters for pigs



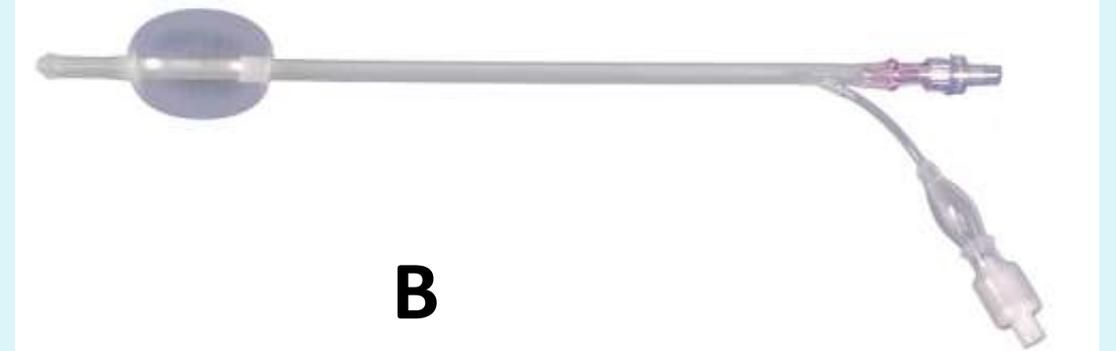
Artificial Insemination in Bitches

- Five forms of insemination of the bitch have been described, with only the first 4 being commonly performed today:
 - 1. Vaginal insemination
 - 2. Endoscope-assisted transcervical insemination (EIU)
 - 3. Non-Endoscopic Intrauterine insemination (Norwegian catheter)
 - 4. Laparotomy insemination (surgical insemination, surgical implant)
 - 5. Laparoscopic insemination

Transvaginal Insemination: Catheters

- The **Osiris catheter** is a rigid catheter with an inflatable balloon with a syringe port to blow up the balloon and a separate port for insemination. The main limitation to Osiris catheters is that they only come in one size. They are adequate for small and medium breeds; however, they are inadequate for toy, large, and giant breeds.
- Other catheters like **foley catheter or bovine insemination pipettes** have been used with limited success.
- The **Mavic catheter (Minitube)** is a plastic catheter with a malleable permanent stylet and a cuff located at the rostral end of the catheter, which is inflated with air once in situ. A separate insemination channel is provided, which has a valve to prevent backflow of semen. Mavic catheters come in 3 different sizes and most accurately emulate natural mating.

The Orisis (A) and the Mavic Catheters (B) for AI in bitch



Transvaginal Insemination: Technique

- The bitch should be in standing position. An assistant ensures she doesn't sit or collapse or specially designed crates may be used. Digital vaginal examination ensures no anatomic anomaly or obstruction to catheter passage, and provides a small amount of water-soluble lubrication. The catheter is passed along the dorsal surface of the caudal tubular tract, initially sharply dorsally through the vulva to avoid the clitoral fossa and the urethra, and then slightly ventrally as the catheter is advanced through the vestibulovaginal junction (**the singulum**) into the vagina with the tip of the catheter passing under the dorsal median post-cervical folds, coming to rest as close as possible to the vaginal fornix and the cervical opening.

- With the **Mavic catheter**, once the catheter is in position, the cuff is inflated to occlude and distend the vagina, emulating the glans penis. Semen can be inseminated as an extended total volume or by inseminating the sperm-rich fraction first, followed by prostate fluid/semen extender. Collectively, the insemination should occur slowly over 10 to 20 minutes. Rapid insemination distends the anterior vagina, which results in contractions of the abdomen and/or vagina and expulsion of the inseminate.

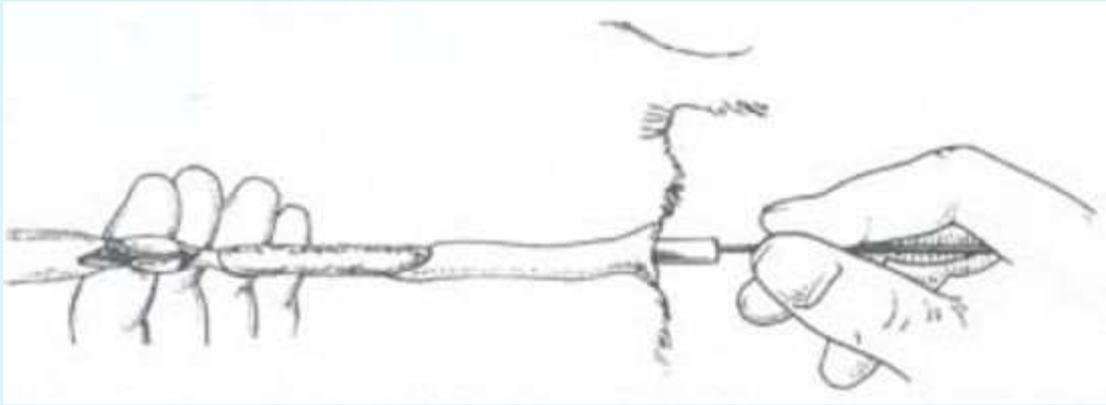


Intrauterine Insemination

- Intrauterine Insemination has been reported to be performed either by using the **Norwegian (Scandinavian) catheter** or **endoscopy**.
- Intrauterine insemination is indicated for poor-quality semen, chilled semen, frozen semen, if progesterone is > 90 nmol/L, and for some obstructions to the female tubular tract.
- The **Norwegian catheter** is a rigid catheter, passed transvaginally into the vaginal fornix. The cervix is palpable and fixed by transabdominal palpation, and the catheter is then threaded through the cervix using manipulation of both the rigid catheter and the cervix. This technique requires skill, practice, and dogs with the appropriately sized abdomen and is used in Scandinavian countries.

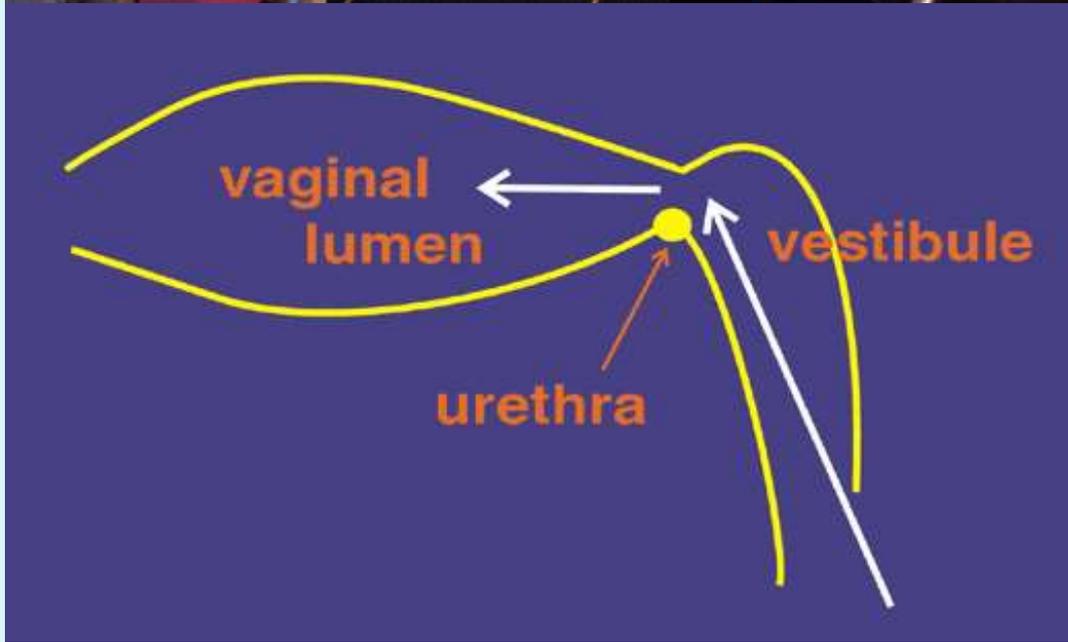
Scandinavian catheters and their tips





Endoscopic Intrauterine Insemination

- A cystoscope or a human utero-endoscope is used for the purpose.
- On introduction of the endoscope the vaginal mucosa and the cervical opening can be visualized prior to the passage of the catheter. The bitch is standing and, if receptive, is usually not sedated. The vagina is insufflated. A cuffed device can be used to occlude the caudal tubular tract and to hold the endoscope. The technique requires practice and skill and expensive equipment but is minimally invasive, allows visualization of the uterine lumen, and is predictable.
- The endoscope assembly ready for insemination in the bitch and the manner of introduction.



The endoscope assembly ready for insemination in the bitch and the manner of introduction.

Surgical Insemination

- A midline exploratory laparotomy incision is made under general anesthesia to permit exteriorization of the uterus. Each uterine horn is catheterized with 22-gauge catheters. The inseminate is inseminated into each of the catheters and the laparotomy incision is closed.
- Being invasive the technique is unpopular



Artificial insemination in camels

- Artificial insemination in camels has suffered from poor development on account of lack of clearly defined traits for propagation, poorly defined estrous cycle, induced nature of their ovulation presence of a thick gel in the semen and poor freezing of semen.
- Majority of studies report low post-thaw motilities and few, if any, pregnancies with AI using chilled or frozen semen



Semen collection

- Natural mating occurs in camel in a sitting position hence semen collection is done in the same position using female camel or artificial dummy for mounting.
- Semen is collected using the Artificial Vagina with rubber liners similar to bull, mounting over female camel in sitting position



The camel semen has a thick gel that liquefies in 1-8 hours making it difficult to evaluate and handle

- Cervical canal presents longitudinal folds which extend on annular muscular projections and form the cervical rings
- The external cervical orifice is surrounded by 1-2 circular indented rings of the cranial part of the mucosa of the vagina
- Consistency of cervix does not differ with that of the uterus which makes it difficult to identify by rectal palpation
- Thus insemination in the camel requires passing the catheter by placing the hand in the vagina and not the rectum



Insemination problems

- Exceptionally few pregnancies have resulted from AI using liquid extended semen and reports on pregnancies resulting from frozen semen in camel are not traceable.
- Due to induced nature of ovulation an hCG injection 2000-3000 IU administered IM or IV is suggested 24 hours before mating. An alternative suggested is mating with vasectomized males after AI
- The follicles in camels greater in diameter than 2.5 cm rarely ovulate only follicles in the size of 0.9-2.0 cm ovulate in response to a mating.

AI in Cats



- Insemination of domestic cats is undertaken relatively rarely because of the small volumes of semen obtained (0.05–0.25 mL) and the need for anaesthesia for collection and insemination.
- However, it is used in conserving rare breeds or for international trade and especially for the conservation of wild Felidae, many of these species being endangered.
- Ovulation has to be induced before insemination. The most commonly used hormone for this purpose is hCG: one or two doses are given and insemination performed 15 to 30 hours later.

- Intravaginal insemination has been reported, with poor to moderate pregnancy rates.
- In domestic cats, intrauterine insemination via a laparotomy is considered more convenient, yielding pregnancy rates of 80% for fresh semen and 0% to 20% for frozen semen.
- However, in some countries this procedure is considered to be invasive and unethical and is prohibited for normal breeding. Sperm doses are 50 to 80×10^6 for vaginal insemination and 20 to 50×10^6 for intrauterine insemination.

THANK YOU VERY
MUCH