ECVET Units as Initial or Additional Training to the European Veterinary Assistant Diploma
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Artificial Insemination

Insemination of cattle

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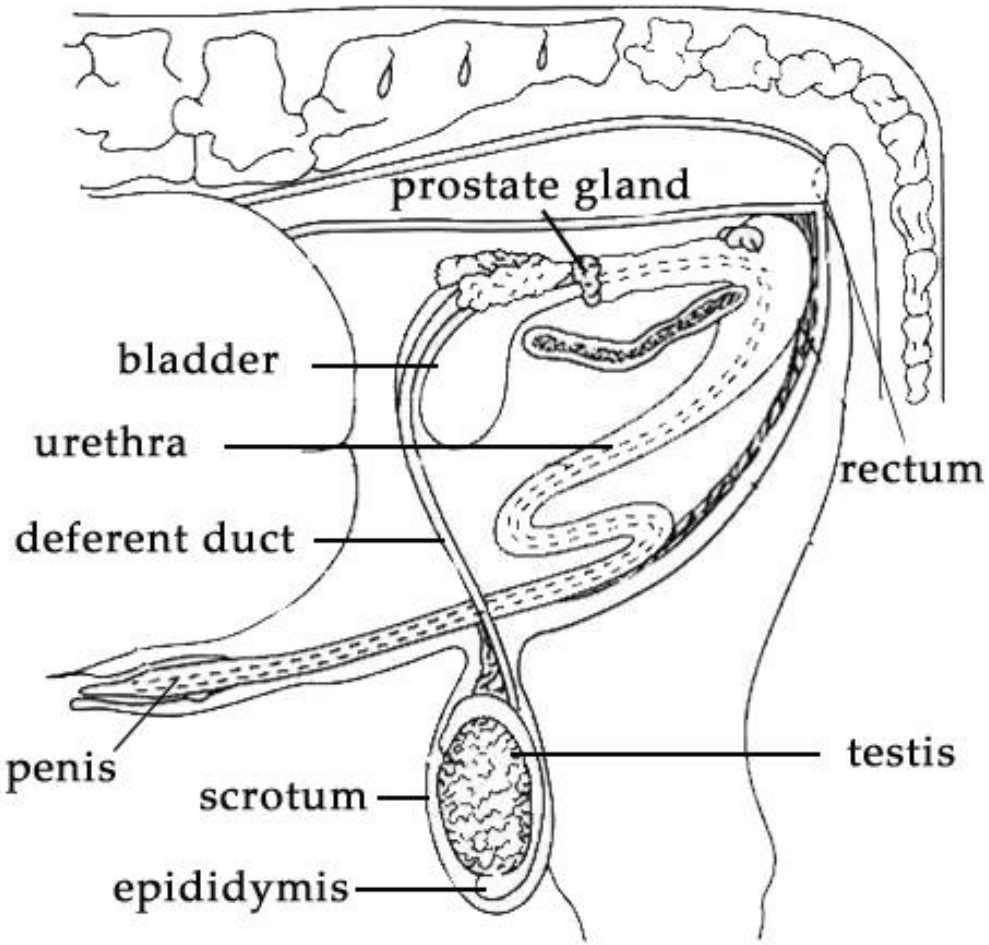
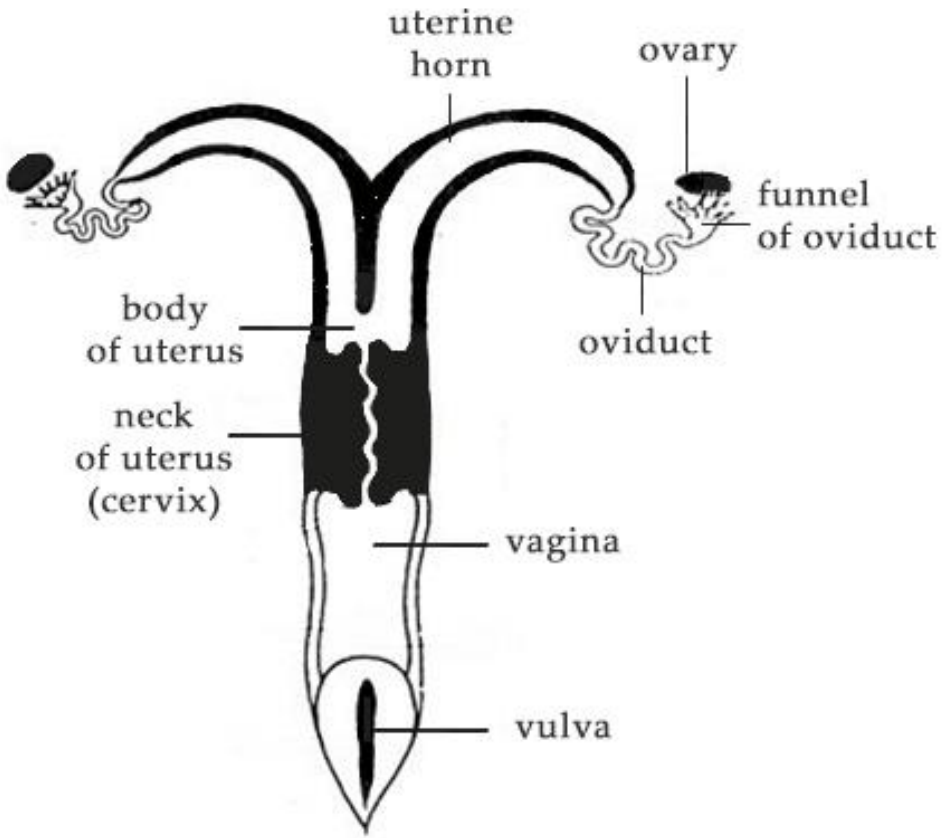
Anatomy of genitals of cattle - Male‘s genitals



Anatomy of genitals of cattle - Female‘s genitals



Finding out breeding - cows in heat

1. Visual detection: signs of individual phases

   one observer 3times a day for 15 min., perfect reproductive record

2. Detectors of jump: placed on cow‘s pelvis (wiping colours)

3. Testing bulls: also hormonally excited cows, marking aids

4. Continuous video recording of herd

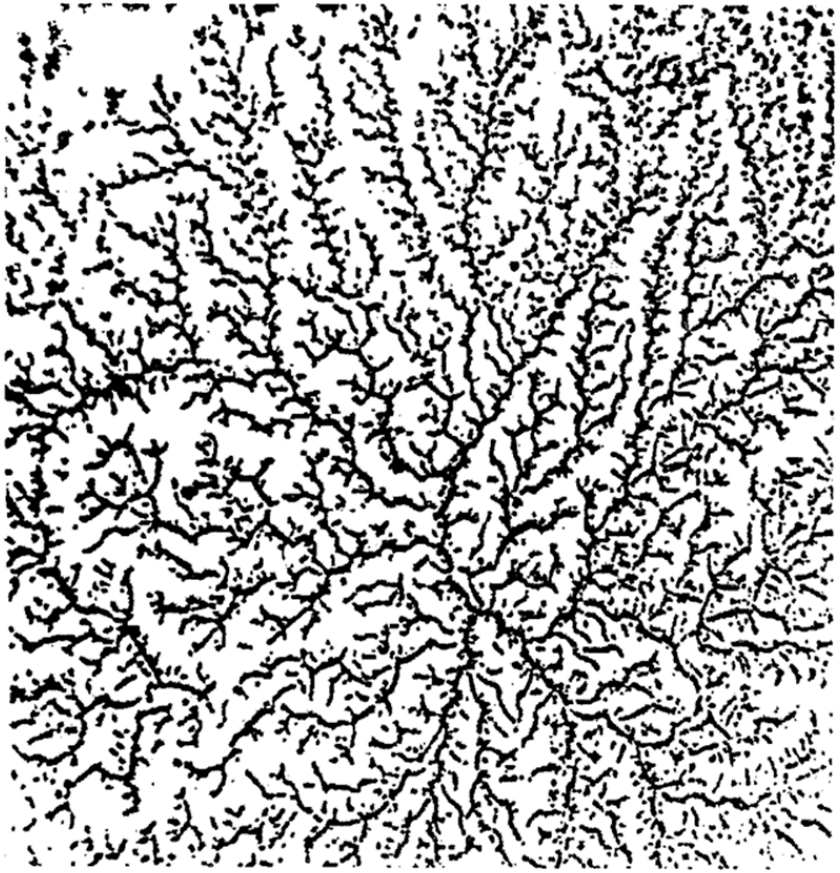
5. Changes in electric resistance: changed hydration of vulva, high resistance in luteal phase, lower in follicular phase, the lowest in estrus

6. Increase of intravaginal temperature and temperature of milk: increase of 0.2 - 0.4 °C

Finding out breeding - cows in heat

7. **Pressure sensors: subcutaneous implants, remote data transmission**
8. **Pedometers: increase in number of steps in heat twice – 4times, counting down the steps, data analysis system, transmitting data via antenna, computer processing, accuracy up to 92%**
9. **Progesteron test: concentration of progesterone in milk and blood, highest in luteal phase, lowest in estrus**
10. **Microskopic monitoring of cervical mucus – arborization: effect of estrogen in heat ⇒ specific cristalline forms, mucus is taken by pipette out of vagina, spread on slide, microscope viewing, it is possible to find out inflammation in genitals – atypical structures**

Twig crystallization of cow‘s cervical mucus in proestrus



Twig - lycopod crystallization of cow‘s cervical mucus at the beginning of heat

Lycopod crystallization of cow‘s cervical mucus in the first half of heat

Ferny crystallization of cow‘s cervical mucus in optimal time for insemination

Finding out breeding – cows in heat

- **Examination of uterus:**
  - **Breeding-cows tolerate insertion of hand into rectum without protest**
  - **During heat uterus horns react by expressive contraction**
  - **At the end of heat sensibility decreases**

Finding out breeding – cows in heat

- **Examination of Graafian follicles:**
  - **During heat repeated examination**
  - **At the beginning GF is small, tough, without follicular liquids → *insemination is premature***
  - **In the middle of heat GF is a bigger bump , contains liquid → *reinsemination is necessary***
  - **In the second half of heat and at the end of heat GF is about 15mm, protrudes above the surface of ovary → *the best time for insemination***
  - **Ovulation comes cca 8 hours after disappearance of external signs of heat, increase of liquid in GF → rupture of zona pellucida**
  - **Right ovary ovulates most often, 80% of ovulations happen between 4 p.m. and 4 a.m.**

Finding out breeding–cows in heat

- **Heifer:**
  - **Sexual maturity**
  - **Breeding maturity = age, weight**
- **Cows:**
  - **40-60 days after calving**
  - **Development of follicle for 60-80 days**
  - **Negative energy balance – most of all at 3rd a 4th ovulation, low-quality ovulation, lower chance to become pregnant between 45th-90th day of lactation, loss of bodyweight**

External manifestations of heat

- **Changes in behaviour:**
  - **Various intensity**
  - **It stands when other animals lie, watches surroundings**
  - **It is restless, smells neighbour**
  - **Moos, urinates, bends back**
- **Changes at genitals:**
  - **Since the 1st day before the start of heat**
  - **Slight swelling of vulva at the time of heat**
  - **Mucous membrane of vaginal atrium is shiny and filled with blood**
  - **Neck is swollen, flabby, without mucus stopper**
  - **Running mucus is clear at the beginning, watery, it sticks to tail and buttock**
  - **Mucus is thicker and cloudy at the end of heat**
  - **Biggest ductility is in the middle of heat**

Finding out breeding - cows in heat

http://www.zootechnika.cz/clanky/chov-skotu/porod---teleni-jalovic-a-krav/inseminace-a-plodnost-krav.html

Determination of time for insemination

- **Convenient time for insemination:**
  - At discharge of mucus from cervix
  - Duct of cervix is open
  - Breeding-cows stand still
- **High time for insemination:**
  - Mucus is getting thicker, it is cloudy
  - Vagina looses lubricity
  - Duct is difficult to access
  - Breeding-cow still stands
- **Inconvenient time for insemination:**
  - Little mucus, vagina is sticky
  - Mucous membrane is pale, no shine, cervix closed
  - Breeding-cow does not stand

Determination of time for insemination

- **Results of conception depend on time between insemination and ovulation**
- **Must be based on details about vitality of sperms and egg**
  - ***Vitality of sperms:*** **cca 12-24 hours**
  - ***Vitality of egg:*** **cca 4-6 hours**
  - ***Time necessary for transition of sperms:*** **optimum 1-2 hours (4-9 hours)**
  - ***Time necessary for capacitation of sperms:*** **on the average 4-6 hours**
  - ***Ovulation:*** **cca 8 hours after disappearance of external signs of heat**

⇒ **Artificial insemination should be carried out in the 2nd half of heat before disappearance of external signs. Reinsemination in case that heat lasts, no later than by 24 hours**

https://www.crvcz.cz/service/gravipro-2/

Placement of sperm

- **Intracervical insemination: mucus = biological filter, it lets off only progressive sperms, external (into the first half of cervix), depth (into the back part of cervix)**
- **Intrauterin insemination: into uterus, at breeding-cows with disappearing signs of heat, on the side where Graafian follicle is developed**
- **Intravaginal insemination: closed, deformed cervix, 2 – 3 inseminations**

Placement of sperm in breeding-cow‘s genitals

- **Various according to type of animals**
- **At heat increased contractility of uterus (effect of estrogens)**
- **Contractions of uterus increase at irritation of genitals → at sexual intercourse, insemination**
- **At saw transuterin transport of sperms (both horns)**
- **Big part of ejaculate disappears from the place shortly after insemination**
- **Big number of sperms excreted by vulva with mucus especially at cow‘s insemination**

Placement of sperm in genitals of breeding female

- **At sow sperm is in uterus for max. 1 hour, big amount of sperms go back through cervix (¼ to ½)**
- **Presence of sperms cause increase of number of leukocyte in uterus and cervix, some of them have absorbed sperms in cytoplasma**
- **Sperms are fixed at epithelial cells, cilia and epithelium close to uterotubal connection (tip of the end of uterine horn) – crossing between distal end of uterine horn and proximal end of oviduct**

Creation of sperm supply in genital tract

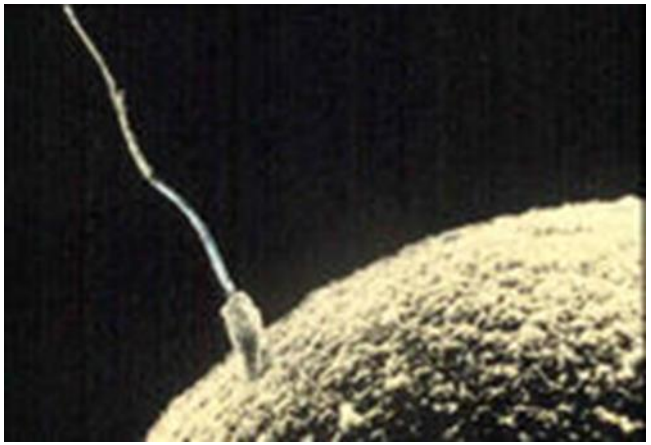
- **Fertilization ability of sperms for 24 – 48 hours (cattle, sheep, pig), 6 days (mare)**
- **In natural breeding the supply of sperms is cervix – sperms are carried from cervix for 24 hours**
- **Next supply is uterotubal connection (UTC) – sperms are kept in isthmus (the narrowest place,Ø 1mm)**
- **Supply of sperms in UTC is created in 6-12 hours**
- **After creation of supply there is direct relationship between number of sperms in UTC and isthmus**

Creation of sperm supply in genital tract

- **Sperm supplies are kept for 24 hours**
- **Sperms accumulate in uterotubal connection UTC in lengthwise cilia – these spaces do not succumb invasion of leukocyte**
- **Epithelium of UTC creates favorable environment for sperms**
- **Isthmus provides concentration of sperms in oviduct – surplus of sperms in oviduct would lead to polysperms fertilization**

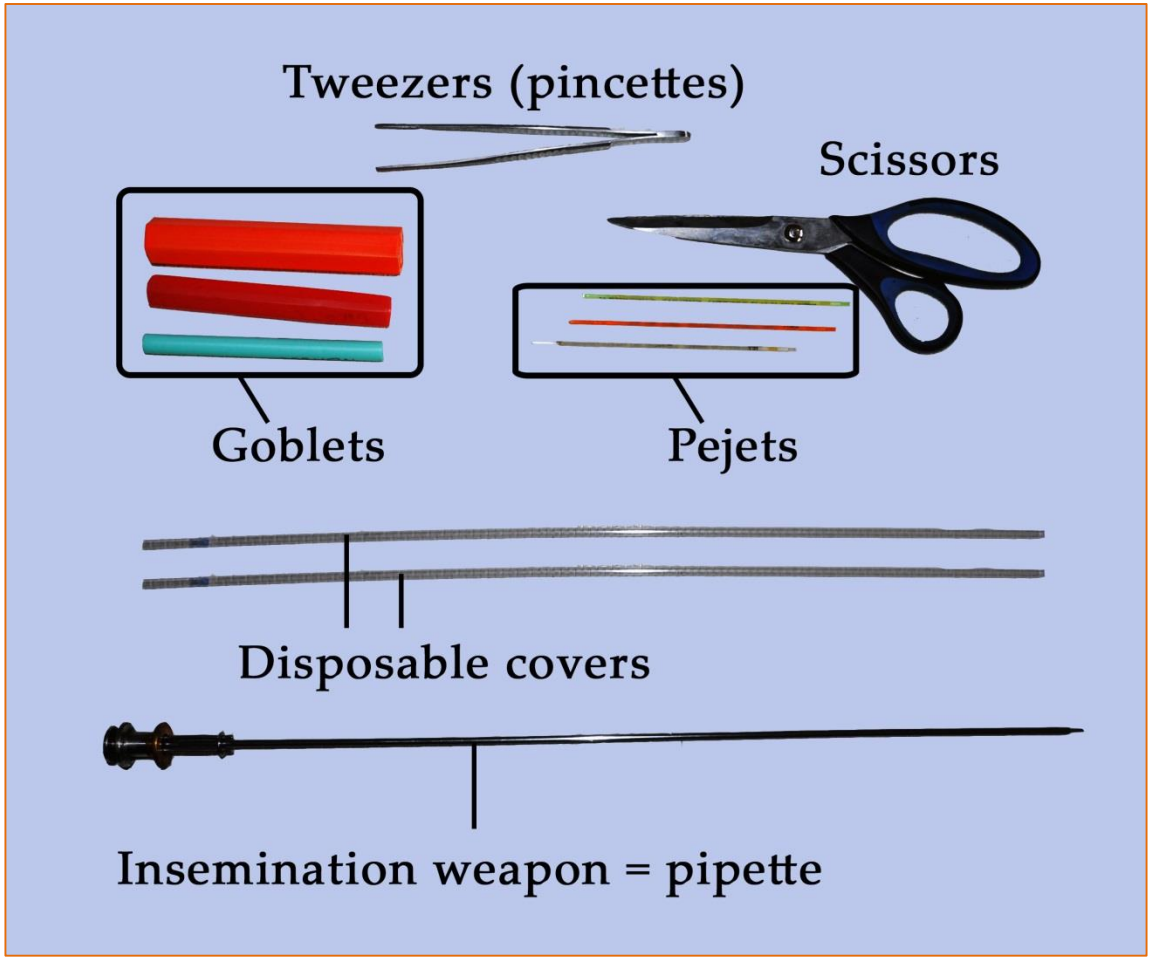
Stages of sperms‘ transport

- **Inicial fast transport**
- **Creation of reserves**
- **Gradual release of reserves**



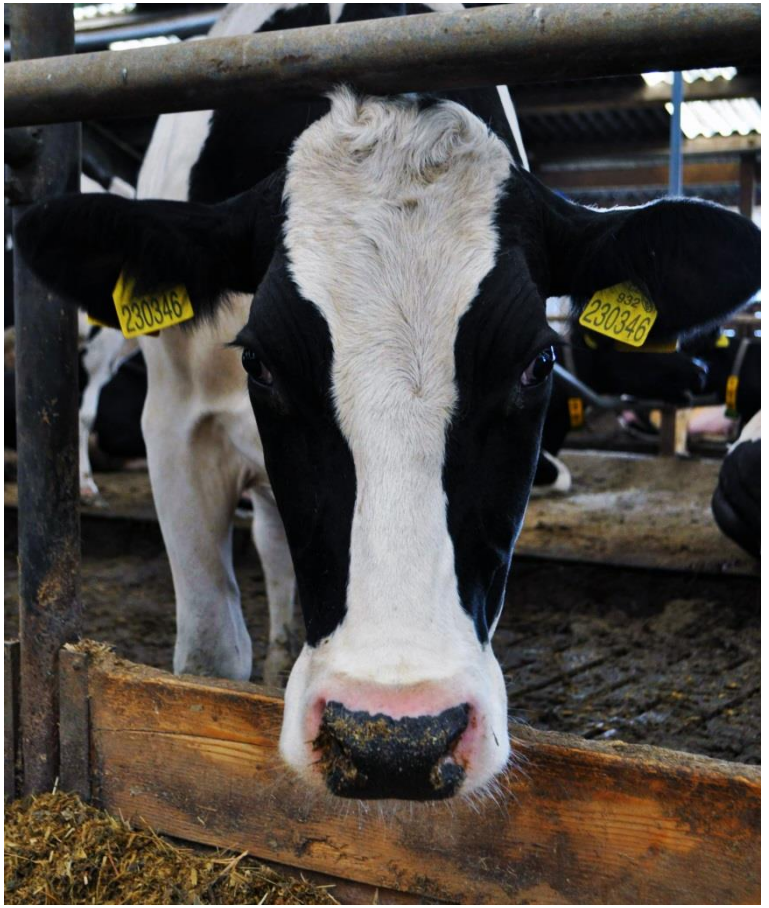
Instruments for insemination - Cooling box

Instruments for insemination



Methods of insemination of cows

1. Rectal
2. Vaginorectal
3. Vaginal
4. For trainees
5. With pulling out of cervix



Rectal method

- **Clean vulva, spread labia with left-hand fingers, with right hand insert insemination pipette at an angle of 450**
- **Left hand into rectum, remove excrements**
- **Examine genitals using palpation (uterine tone, Graafian follicle on ovary)**
- **Mild massage of uterine toward cervix**
- **Take cervix, find out gate with little finger, move on pipette with second hand, slide cervix onto pipette (3-5 cm), squeeze out the dose**
- **Prevent urging by grabbing skinfold with pressure on lumbar**

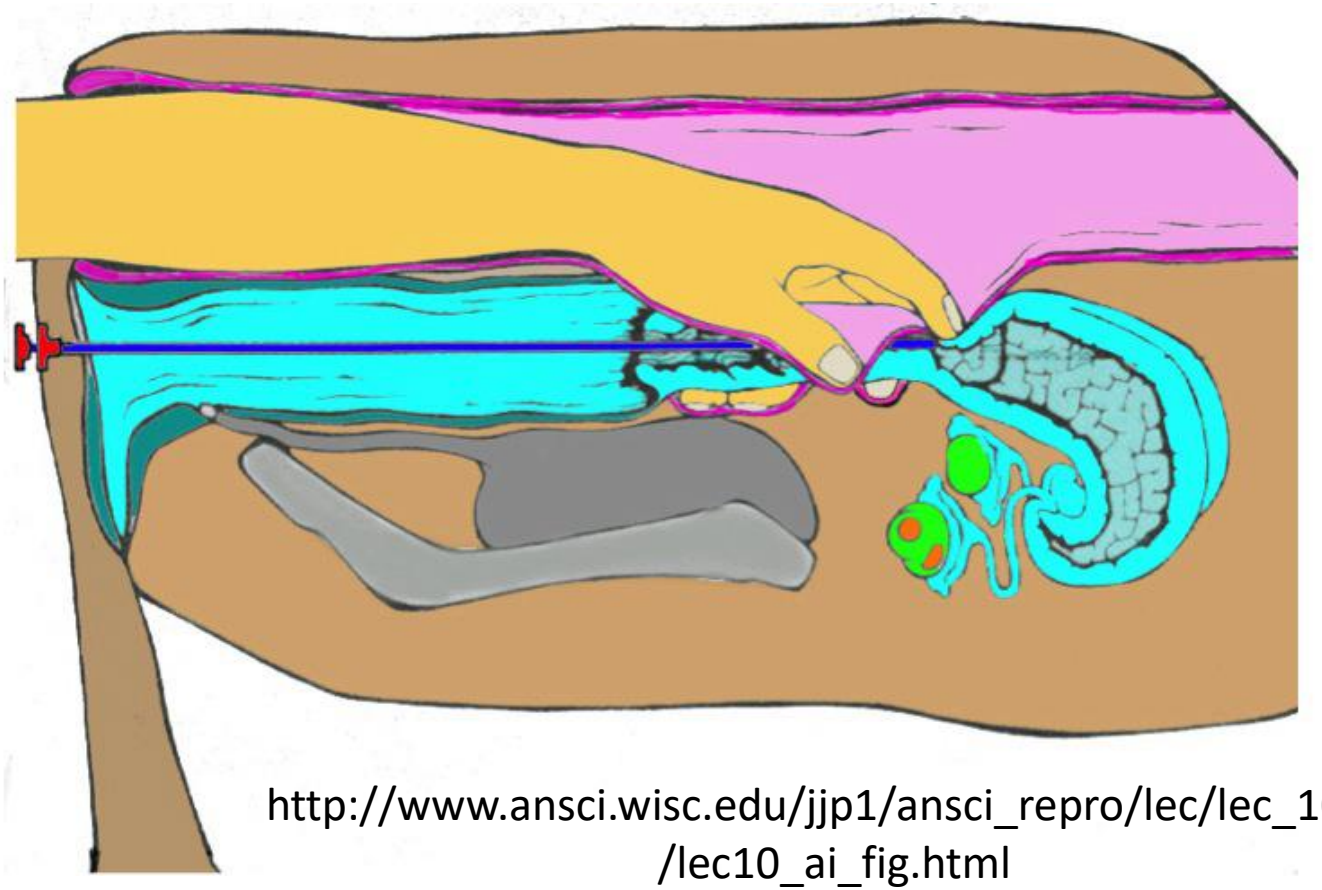
Rectal method

- **After pulling out of pipette assess quality of mucus**
- **Advantages:**
- **Vagina is not exposed the pressure of instruments, physiological conditions are kept**
- **Reduced risk of infection, possible examination of genitals through rectal wall**
- **Simple instruments, possible to put sperm into the given place**
- **The highest percentage of pregnancy**

Vaginorectal method

- **Clean vulva, left hand into vagina, slip in pipette under protection of the hand**
- **Right hand inserts pipette into vagina**
- **After inserting pipette the movement stops, pipette up to cervix**
- **Turn up mucus and assess its quality**
- **Next procedure like at rectal method**

Rectal and vaginorectal method



http://www.ansci.wisc.edu/jjp1/ansci_repro/lec/lec_10_ai/lec10_ai_fig.html

Vaginal method

1. **With manual control:**
   - **Under protection of hand insert pipette into ½ of cervix, squeeze out sperm**
   - **Used at mares**
2. **With use of vaginal mirror:**
   - **Speculum into vagina, after lighting assess quality of cervix, mucous membrane and present mucus**
   - **Insert pipette into cervix under visual control**

Method with pulling out of cervix (Italian)

- **Grasp cervix with Albrechtsen pliers and pull it out into vaginal atrium**
- **Sperm is inserted with short pipette into one third of cervix**
- **Visual control of mucus and mucous membrane**
- **Disadvantage : it is impossible to assess other parts of genital tract , risk of injury of cervix**
- **It is not used in the Czech Republic**

Method for trainees

- **The same like at vaginorectal method**
- **Pipette under protection of hand is inserted into cervix (2 - 3 cm),**
- **Pull out hand of vagina, turn up mucus and assess it**
- **Insert hand into rectum, next procedure like at rectal method**

Insemination with frozen sperm

- **Preparation:**
  - **Right identification of breeding-cows**
  - **Carry out anamnesy, assess records**
  - **Examination of mucus, genitals**
  - **Breeding-cows with symptoms of veneral diseases, nonphysiological discharge or breeding-cows out of heat are removed of insemination**
  - **Preparation of aids**
  - **Preparation of breeding-cows: fixation, cleaning genitals**

Insemination with frozen sperm

- **Preparation of dosage at Japanese method of conservation:**
  - **Fast manipulation with pellets**
  - **Taking out of container using desinfected pincettes**
  - **Into 1cm3 of citrate, 45oC, then into capillary**
- **Preparation of dosage at French method of conservation:**
  - **Defrost pejets in water bath (38-40oC) for 12 – 25 seconds**
  - **Dry pejets, rub the end with cotton wool plug between fingers (easier moving)**
  - **Into equipment, cut the end without the cotton wool plug, put on cover pipette, squeeze out a drop of sperm**
- ***Treat animals gently and softly: adrenalin = antagonist of smooth muscle contractions → prevent suck of sperm into genital tract***

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