Cryoconservation of Ram Semen and Artificial Insemination: Kazakhtani Experience

Kazakh Research Institute of Animal and Fodder Production

Cryoconservation of Ram Semen:

1. Maximally realizes reproductive potential of the ram. According to Maxwell, up to 15 thousand lambs can be produced from one sire if his sperm was frozen for one year and then was laparoscopically inseminated.
2. Allows international export/import of genetic material in the form of frozen semen providing low transport costs and low risk for import of infectious diseases.
3. Allows wide use of the best elite sires, for example breed champions.

Cryoconservation of Ram Semen. Main Steps:

1. Collection of sperm from rams with the aid of an artificial vagina.
2. Dilution of semen:
   400 million motile cells / ml or
   100 million motile cells / 0.25 ml straw.
3. Equilibration at room temperature for at least 20 minutes.
4. Cooling of diluted semen in the fridge at 5°C for at least 2 hours in the water or fabric jacket (to prevent cold shock).
5. Freezing in the liquid nitrogen (LN) vapor.
6. Storage in LN.
7. Thawing and artificial insemination
   (thawing of straws in a water bath at 38°C for 20 seconds,
   thawing of pellets on warm 38°C stage for 30 seconds).

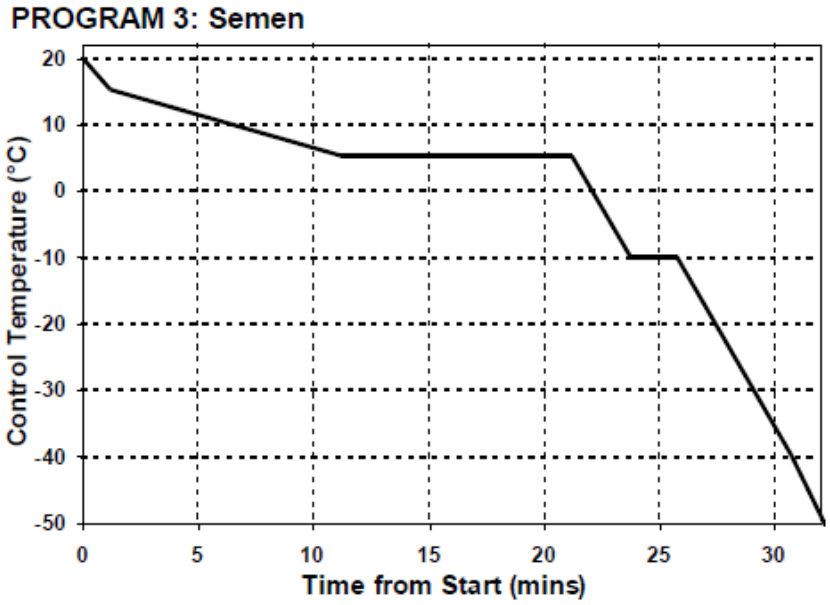
Freezing of semen in the LN vapor 5 cm above LN surface:

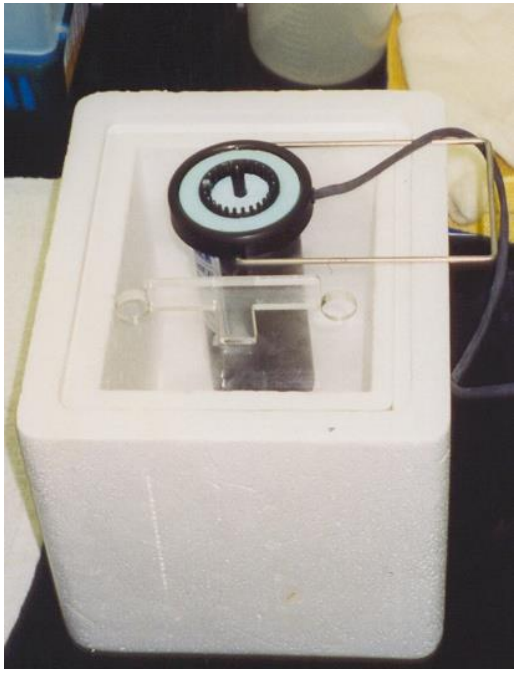
on polymer plate in the form of pellets

with Minitube freezing unit (Germany) for 90 straws.
Metal bath filled with LN, floating rack keeps straws 5 cm above LN surface. During freezing process LN evaporates, but distance between LN surface and straws does not change, stays constant due to catamaran or cork principle.

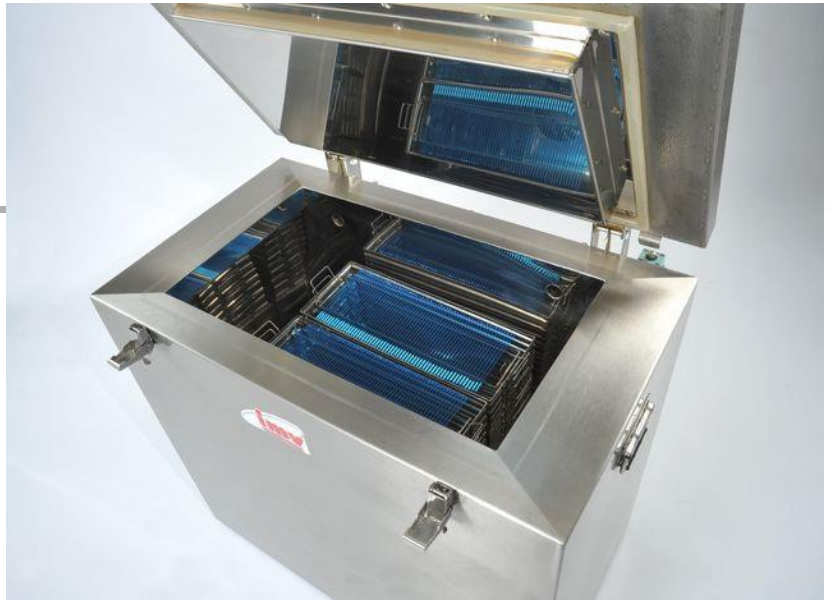
Floating rack for straws (Minitube)

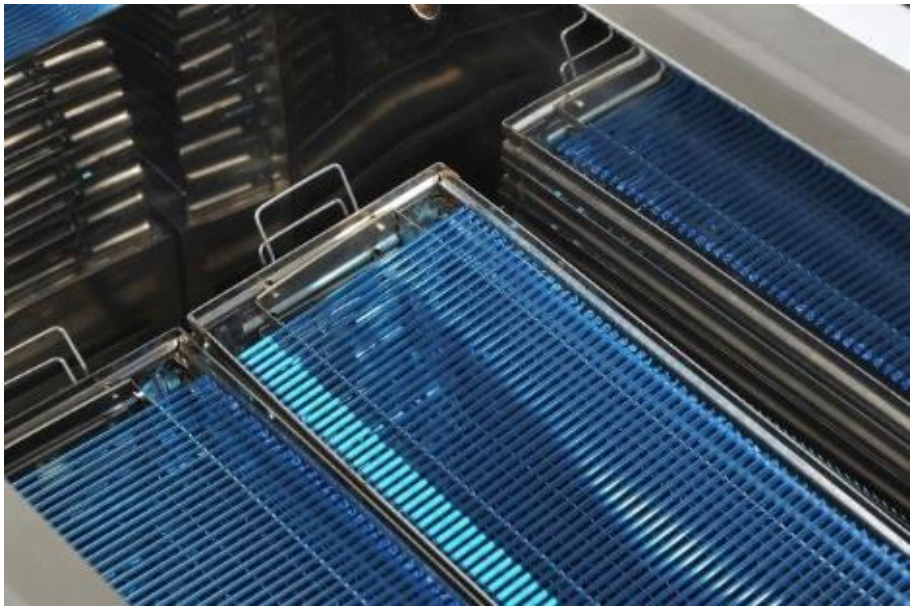
Computerized programmable freezer CL-8800 Cryologic
with 23 x 0,5 ml straws or 46 x 0,25 ml straws capacity





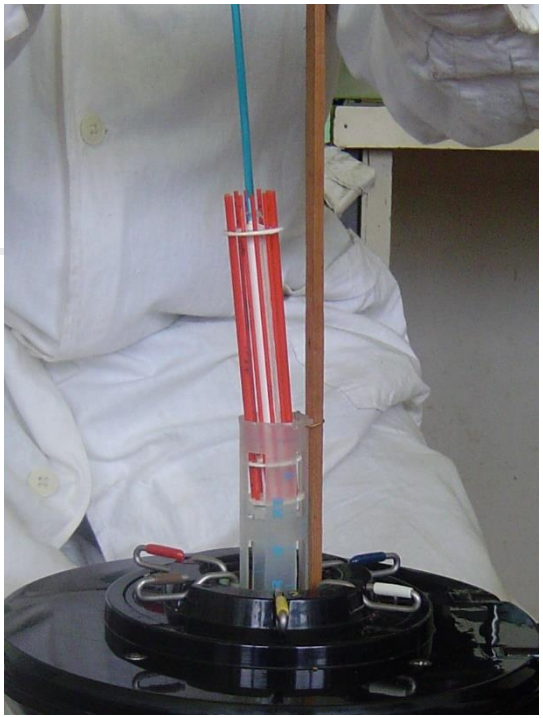
IMV (France) computerized programmable freezers



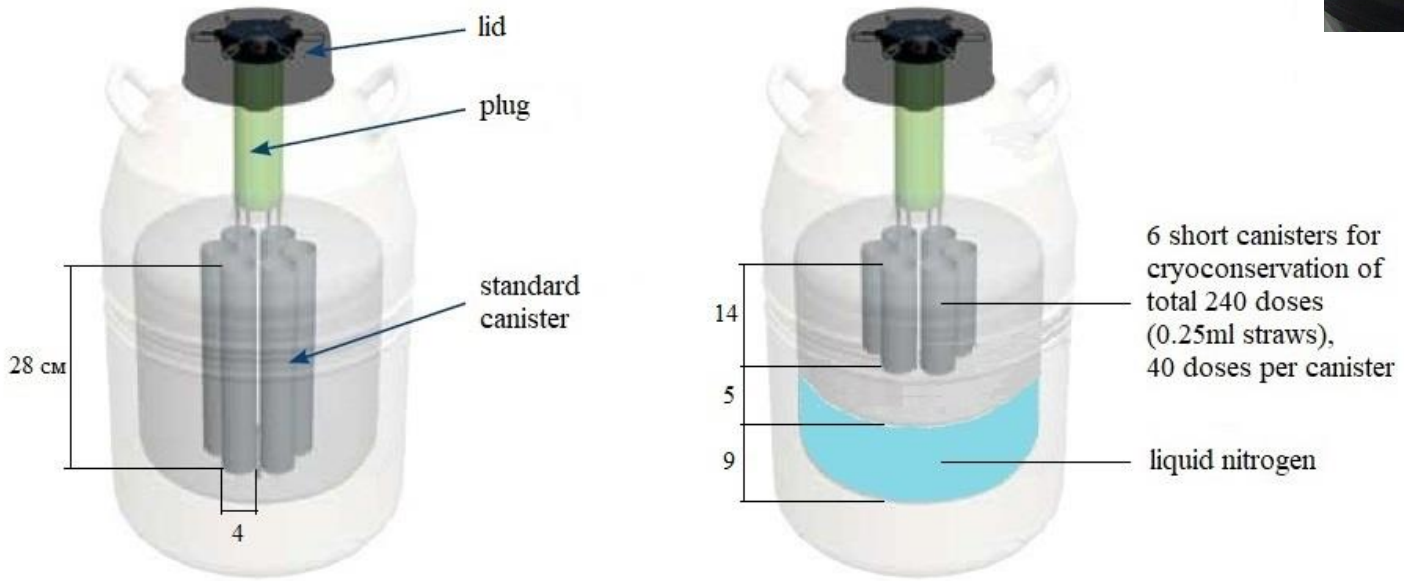


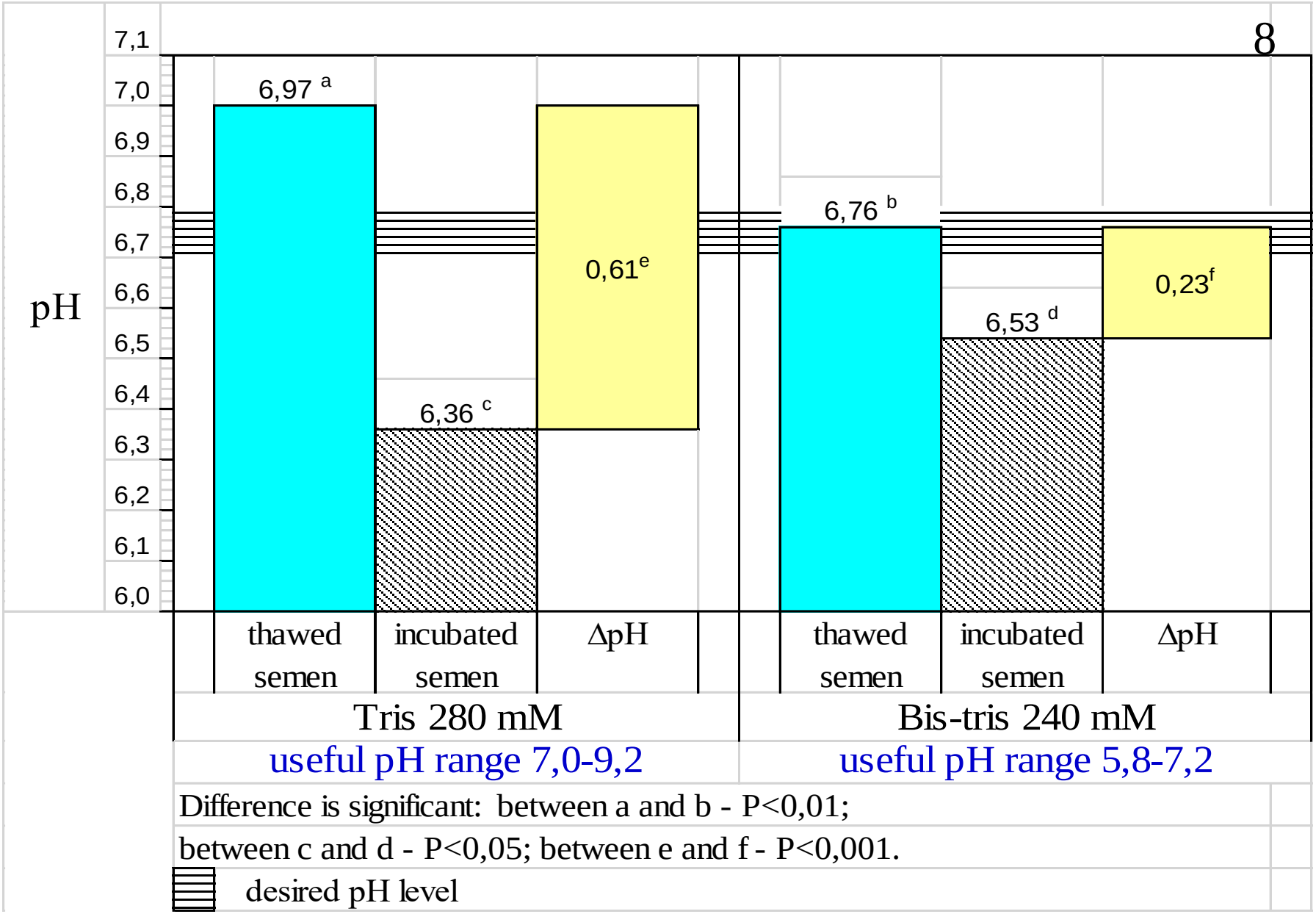
1. Digitcool – 2 400 ministraws on 24 shelves (100 ministraws / shelf),
2. Mini-Digitcool – 450 ministraws on 9 shelves (50 ministraws / shelf).

Freezing of semen in the Dewar MVE ET20 vessel in the canister and rack made from 50-ml plastic syringe for 16 mini-straws 0.25 ml



Freezing of semen in the Dewar MVE ET20 vessel with a capacity of 240 doses





pH of ram semen frozen in tris- or bis-tris-based diluents, thawed and incubated at 37°C for 7 hours (n=15)

Motility of semen frozen twice in the diluent with glycerol or frozen once in the glycerol-free diluent

Group	Diluent	Motility (%)						
		fresh semen	semen frozen in the diluemt with glycerol (n=14)				semen frozen in the glycerol-free diluemt (n=15)	
			once	%	twice	%	once	%
I	Tris-based (control)	80,7	50,4	62,4	13,4	16,6	27,3	33,8
II	Tris + arginine	80,7	55,4	68,6	16,0	19,8	34,0	42,1
III	Bis-tris-based	80,7	56,4	69,9	23,7 [b]	29,4	38,0 [d]	47,1
IV	Bis-tris + arginine	80,7	60,0 [a]	74,3	26,9 [a]	33,3	41,0 [c]	50,8
V	Sucrose-based	80,7	55,0	68,2	11,3	14,0	2,2	2,7

a. Difference between IV and I, IV and V groups is significant, P<0,01;

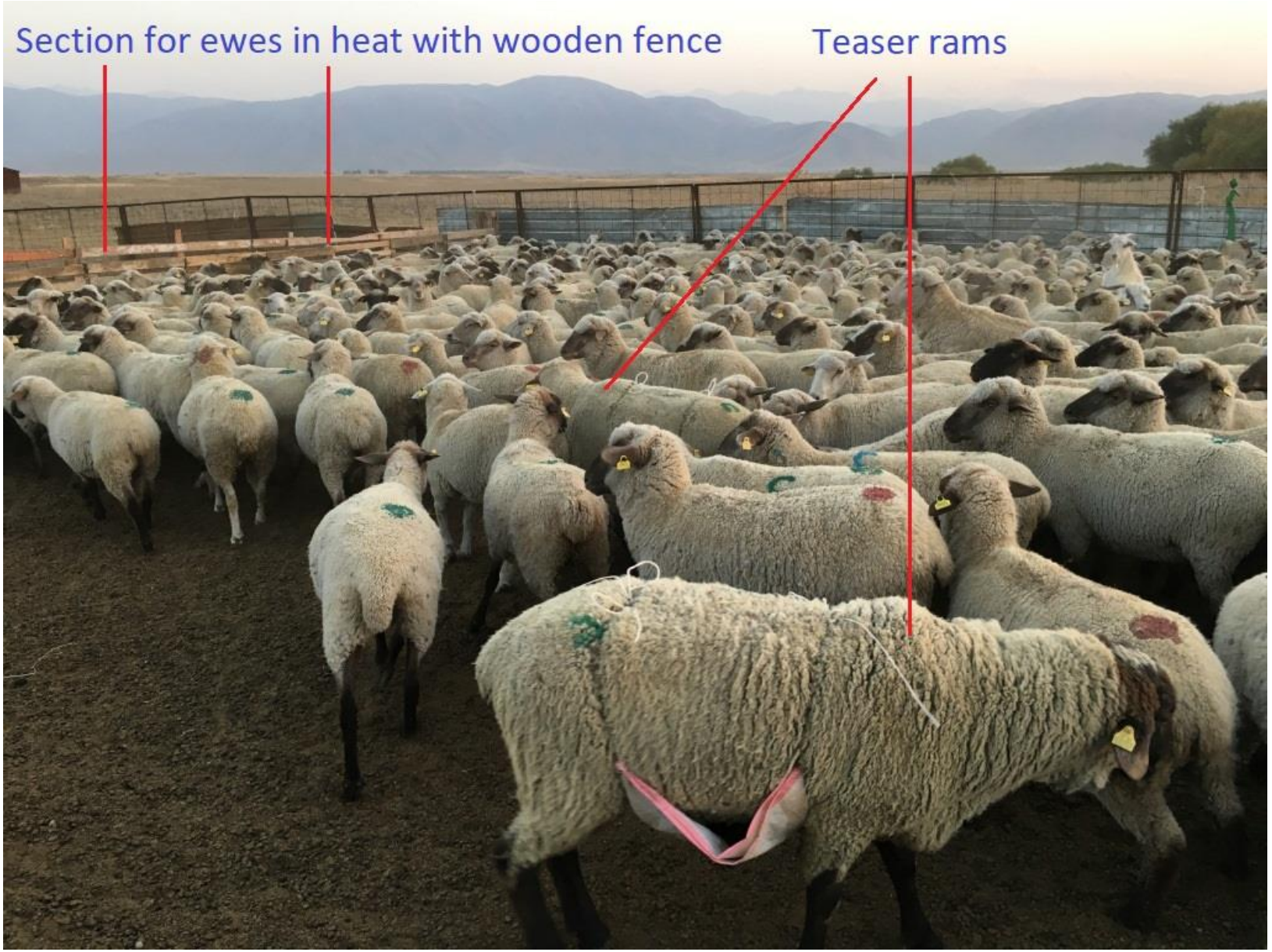
b. Difference between III and I, III and V, II and IV groups is significant, P<0,05;

c. Difference between: между IV and I, IV and V groups is significant, P<0,001;

between IV and II groups, P<0,01.

d. Difference between III and I groups is significant, P<0,01; between III and V groups, P<0,001.

Detection of ewes in heat with the aid of teaser rams (100 ewes/ram). Prepuce is covered with sackcloth.



Signs of ewe in heat:

- Ewe in heat stay close to a ram and wags her tail (like dog wags tail when sees owner). If ram does not pay attention to her, she follows him everywhere.
- Ewe in heat stands motionless when a ram courts and jumps on her to mate, while a ewe not in heat runs away from ram if he courts or jumps on her.
- The external genitalia are reddened and hyperemic, there may be a discharge of vaginal mucus, according to which it is possible to determine the stage (beginning, middle or end) of oestrus.






Collection of semen

The technician sits on a low stool or crouches to the right of the ewe and holds the artificial vagina with the receptacle facing upwards at a 35° angle, holding it with the index finger and thumb. As the ram jumps on the ewe, the technician guides the ram's penis into the artificial vagina with his left hand.

Keep in the separate section or “blind” the most active and strong rams by covering their head with sack so that they do not interfere in the collection of semen from other rams in row.

Assessment of sperm motility in the field

Sperm motility is assessed at a temperature of 36-37°C (body temperature). At room temperature, the assessment is biased, as the mobility slows down. Morozov's metal table with hole in the center filled with warm water is used in the field conditions to keep warm glass slide with sperm sample at 37°C. Nowadays such Morozov's table is not available, we suggest to take 50 ml transparent plastic culture flask fill it with warm water and use instead.

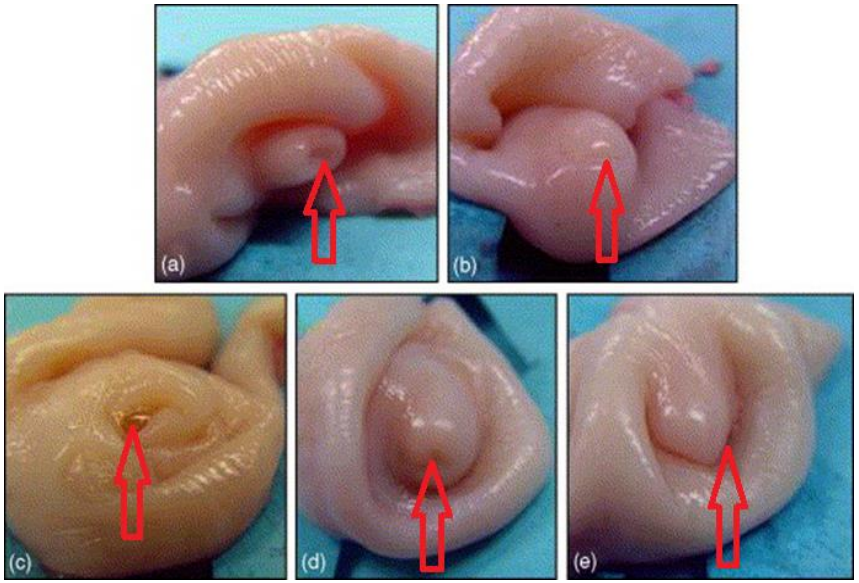
Cervical Insemination

Minimal dose is 200 million motile spermatozoa per ewe with single insemination or 2x100 million motile spermatozoa with double inseminations.

Reasons for low results:

1. Poor quality of sperm.
2. Cold room. Temperature in the room must be 20-25°C.
3. Inaccurate and shallow introduction of sperm into the cervix. Figures show entrance in the cervix.
4. Wrong posture of the ewe at the time of insemination. It is desirable that back part of the body was raised by 10-15 cm.
5. Poor lighting. The right choice of location - window should be behind the back of the technician.

Different kinds of the cervix, arrow shows it's entrance



Cervical Insemination

We recommend to use:

1. Metal thermos with 0.5 liter capacity for moisturizing the vaginal mirror (speculum) with warm water. Usually a stainless steel bath is used for this purpose (photo 1). Due to the large open area the solution in the bath cools quickly and lot of debris and dust get into it. Unlike a bath, a thermos (photo 2) keeps water warm for a longer time, collects less debris and dust. Thermos is quite massive and does not tip over when a vaginal speculum is immersed in it.
2. A portable LED-flashlight (photo 3), attached to the body of the AI-gun with electric insulating tape (blue on the photo) or scotch. Flashlight helps to quickly locate the cervix and accurately insert the tip of AI-gun into it's entrance and channel. The energy of one high-quality Duracell AAA battery is enough to inseminate 100 sheep twice daily for three days.


1


2


3

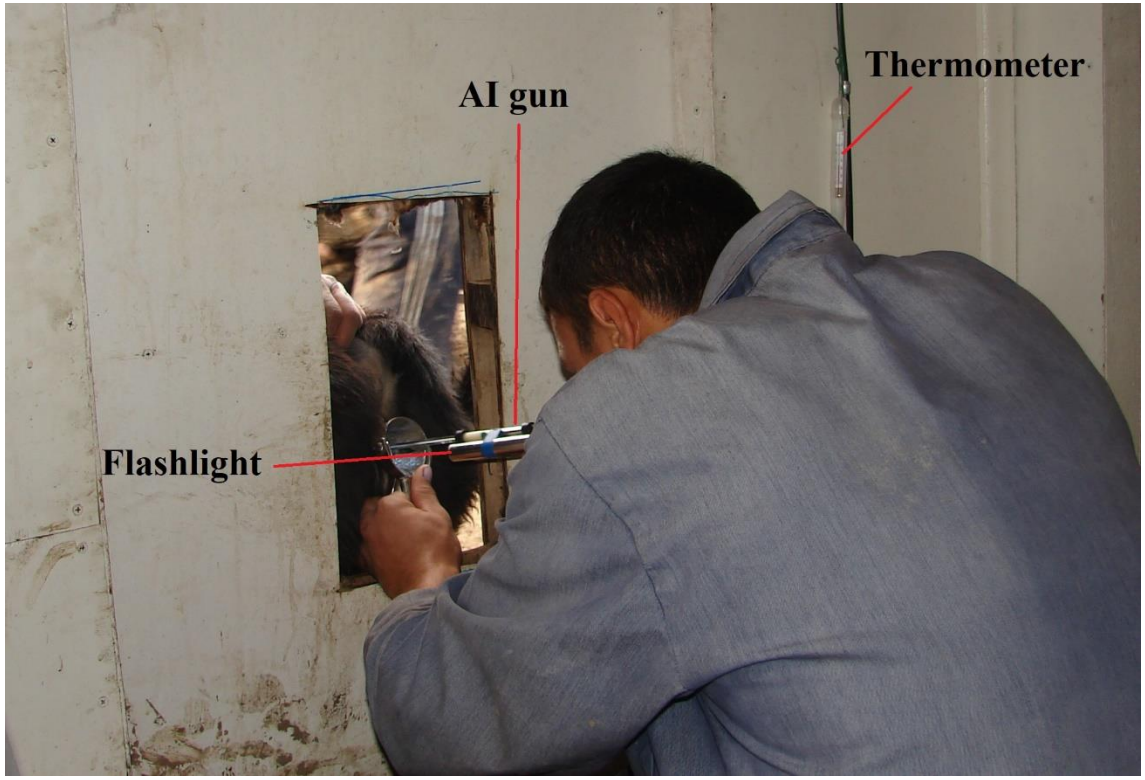
Cervical insemination through the window in the door of the mobile AI facility

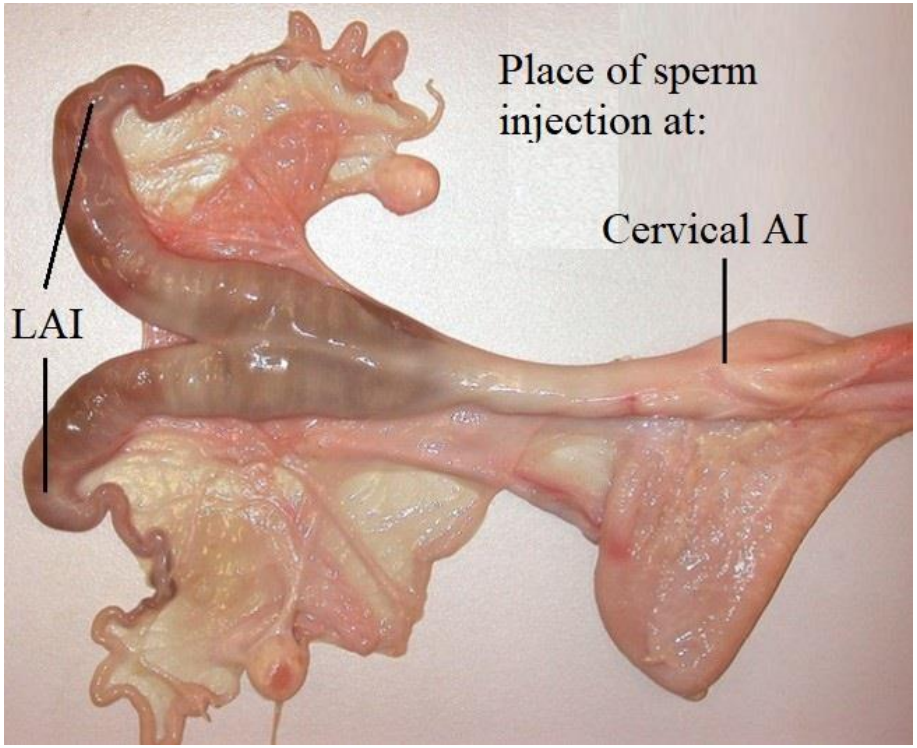
Youtube video

performed if ambient temperature is above 0°C.

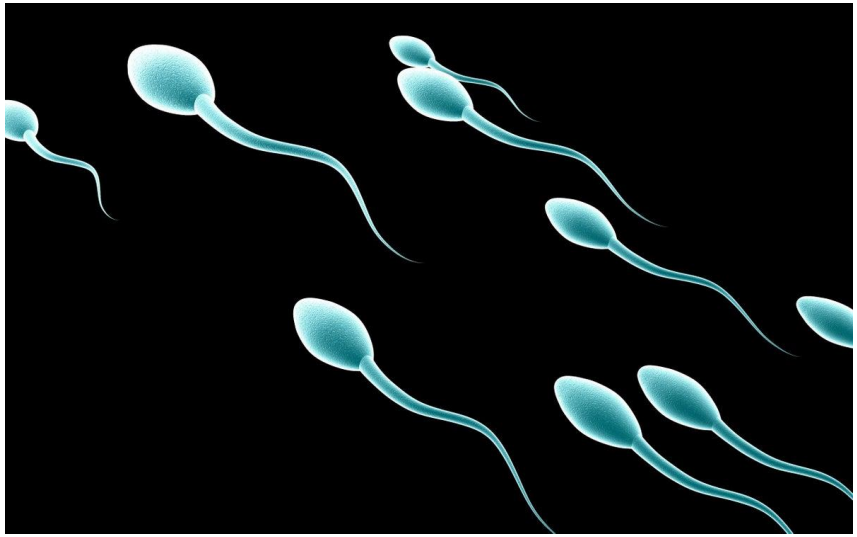
This method:

- reduces the time and labor required to move animals to and from the AI facility,
- reduces stress in animals,
- reduces time for cleaning the facility after completion of work.



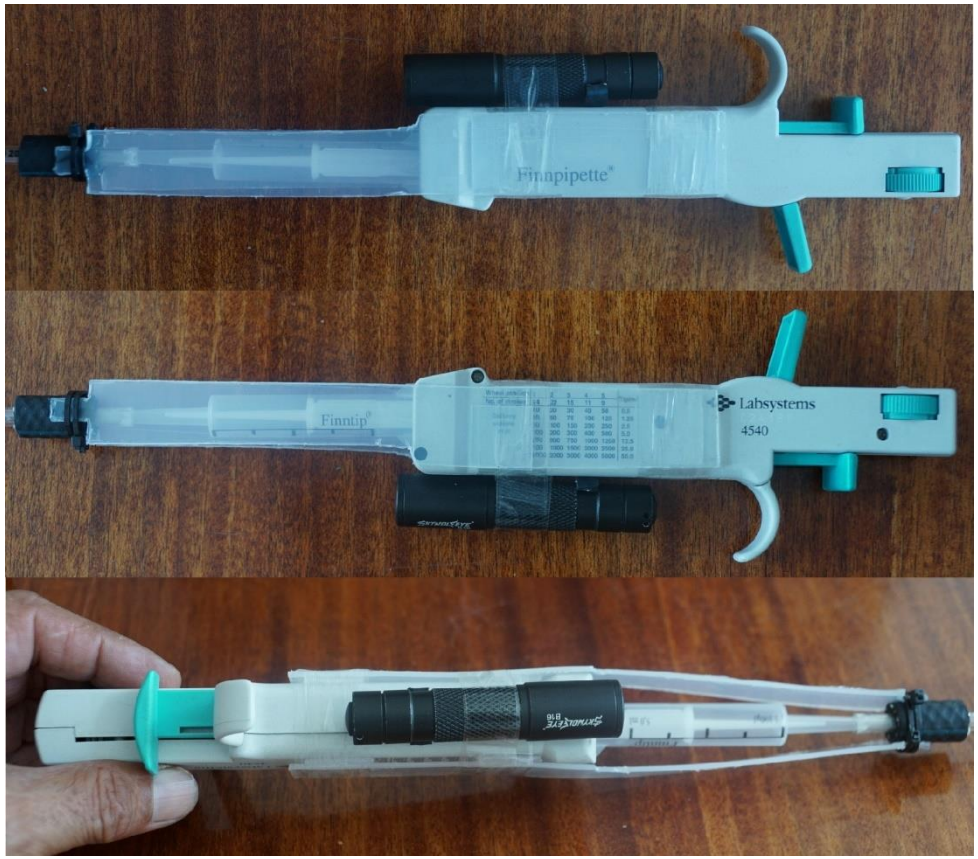


At mating or AI the transport of spermatozoa from the cervix to the oviducts can be associated with the migration of fish from the lower to the upper parts of the river for spawning. There are two depots of spermatozoa in the: 1) cervix and 2) utero-tubal junction. During the sexual heat (oestrus), the female produces vaginal mucus. The higher quantity or flow of mucus, the better transport of spermatozoa.



AI-gun for Cervical Insemination:

Finnpipette pipettor, 5ml syringe, 2ml plastic serological pipette, flashlight

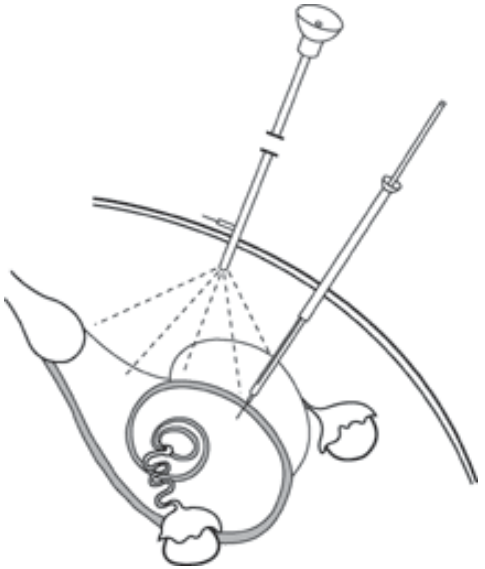


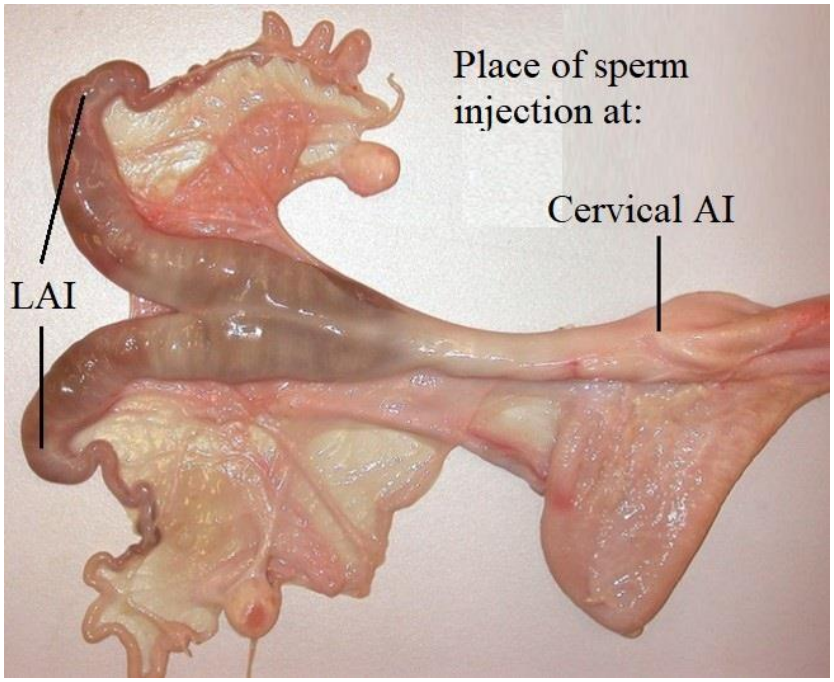
Figures important in the artificial insemination of sheep

№	Indicator	Value	
		mean	limits
1	**Duration of sexual cycle**	**17 days**	**14-19 days**
		16-17 days	**14-19 days**
2	**Duration of sexual heat (oestrus):**	**30 h**	**3-79 h**
			18-72 h
	in Merino ewes		**24-42 h**
	in Merino hoggets		**24-36 h**
	in fine-wooled ewes	**38 h**	
3	**Time of oocyte ovulation post onset of sexual heat (oestrus):**	**28 h**	**12-40 h**
	in Merino		**25-30 h**
	in Soviet Merino ewes		**30-32 h**
	at synchronized oestrus	**60 h**	
4	**Duration of time post ovulation the oocyte is capable to be fertilized:**		**12-24 h**
			5 h
5	**Duration of time the fresh semen is viable:**		
	in the mucus of cervical canal:		**≥ 24 h**
		36 h	**≤ 47 h**
	in the uterus and oviducts	**10-12 h**	
	in the oviducts:	**5-7 h**	
		9 h	
		9-10 h	
6	**Duration of time the frozen semen is viable:**		
	in vitro at 38°C	**10-11 h**	
	in the uterine horns	**8-10 h**	
	in the oviduct when semen is injected into oviduct	**до 15-16 h**	
	in the oviducts	**4-5 h**	**6 h 40 min**
		up to 10 h	
7	**Time required for sperm to move into the oviducts and accumulate in sufficient quantities:**		**several minutes**
	at mating	**5-6 h**	
	at cervical insemination	**5 h**	
		7-8 h	

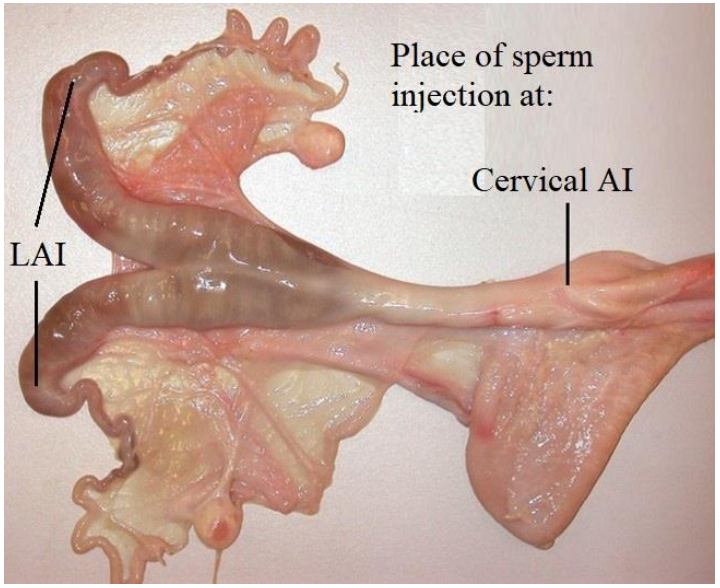
Laparoscopic artificial insemination (LAI) with frozen semen

LAI was developed in 1984 in Australia. It's main purpose is efficient and rational use of frozen semen collected from valuable sire-rams. Minimal dose of semen with LAI is 20 million motile spermatozoa.





Disadvantages and advantages of laparoscopic AI vs cervical AI



Disadvantages :

- Expensive equipment and instruments – price of laparoscope starts from USD6000.
- Necessity in highly qualified personnel.
- Cost, time and work consuming.
- Requires source of electric energy.

Advantages:

- Economic use of semen: minimal dose is 20 million and 200 million motile spermatozoa per ewe respectively.
- Higher fertilization rate due to place of semen deposition: semen gets oviducts in few minutes after laparoscopic AI and in 7-8 hours after cervical AI.
- Possibility to produce progeny from sires with low quality of semen expressing short lifespan in the female reproductive tract.
- Higher fertilization rate with frozen semen.
- It is possible to produce up to15000 lambs in one year from one valuable sire with laparoscopic AI and 1000-2000 (few thousand) lambs with cervical AI.

Flashlight as a light source for a laparoscope

10 mm diameter laparoscope with light cable, stationary “Carl Storz” light source and steel rod 35 cm long 5 mm dimeter

Flashlight, size 100x13 mm, price $1, powered by battery AAA size

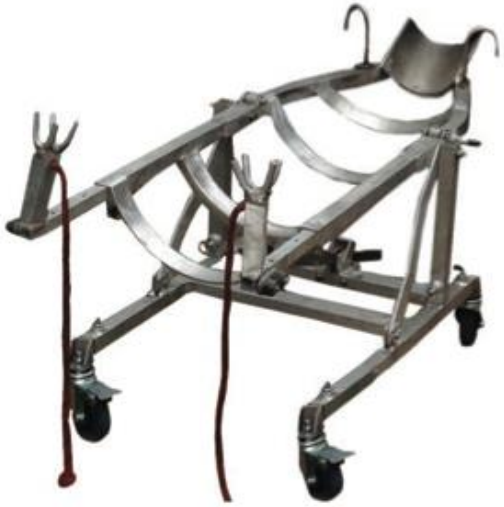
Laparoscopic insemination with the aid of a flashlight attached to the laparoscope using rubber tube with inner diameter 12 mm

Disadvantages of a stationary light source for laparoscope:

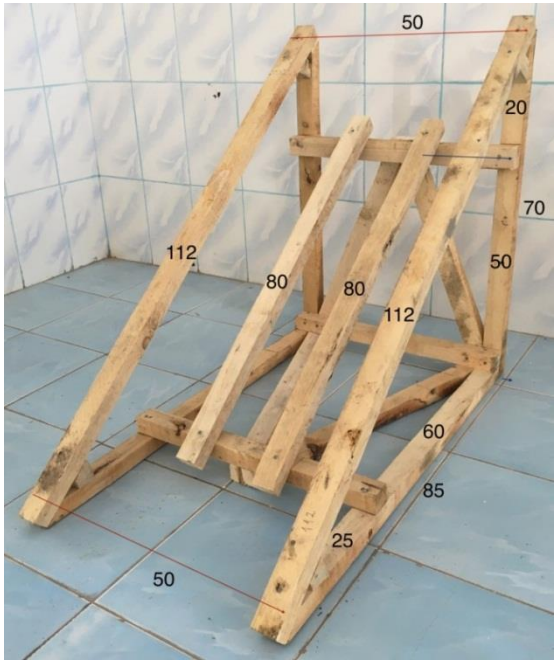
- very expensive (from $2000);
- in the field conditions, it is necessary to have benzine generator and benzine, price of benzine grows constantly;
- generator voltage surges burn out expensive and rare halogen or xenon lamps.

Cradles for laparoscopic AI

Australian

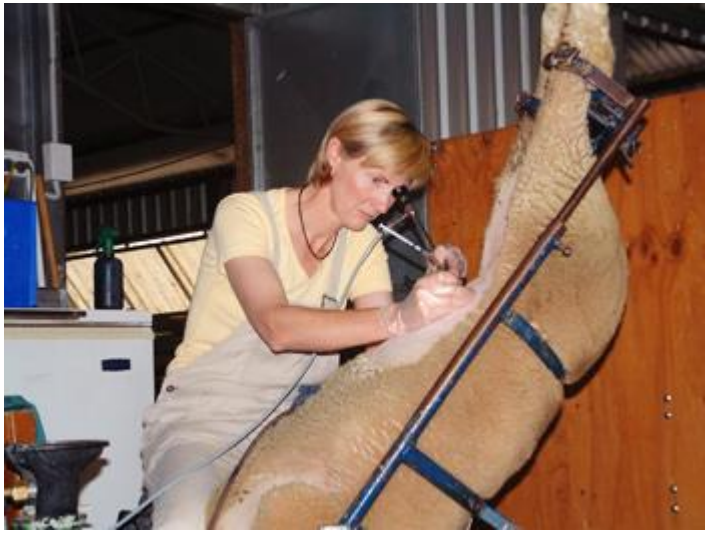


German (Minitube)



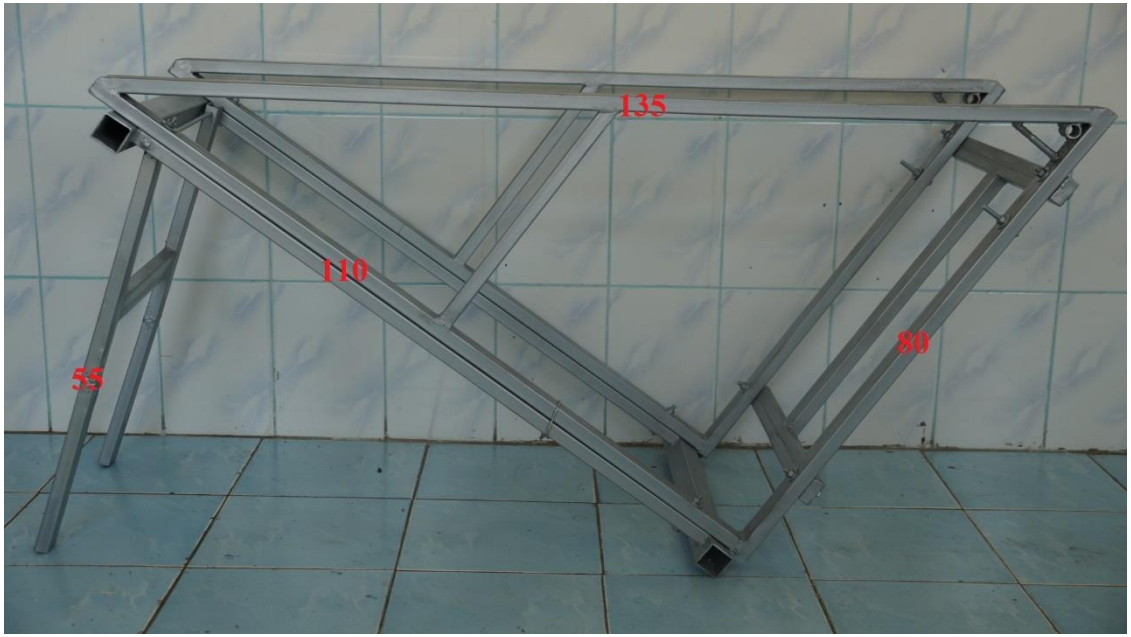
Cradle made from wooden blocks 5 x 5 cm.

Leg is fixed to a cradle with a red rope ring (like lasso) in 2-3 seconds.

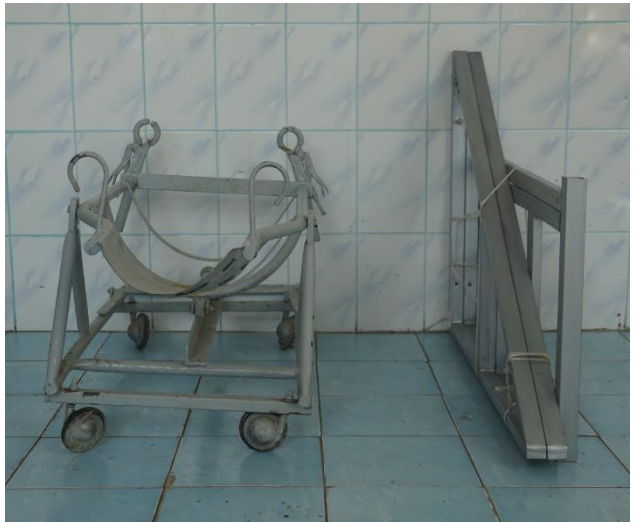


American

Dismountable cradle for laparoscopic AI



Australian cradle is on the left side, dismounted cradle is on the right side



Laparoscopic insemination with Robertson's pipette and applicator

Robertson's applicator and pipette

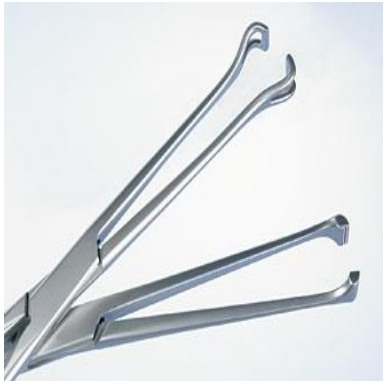
Robertson's pipette needle

Semen straw with metal ball inserted, paper cork is cut off

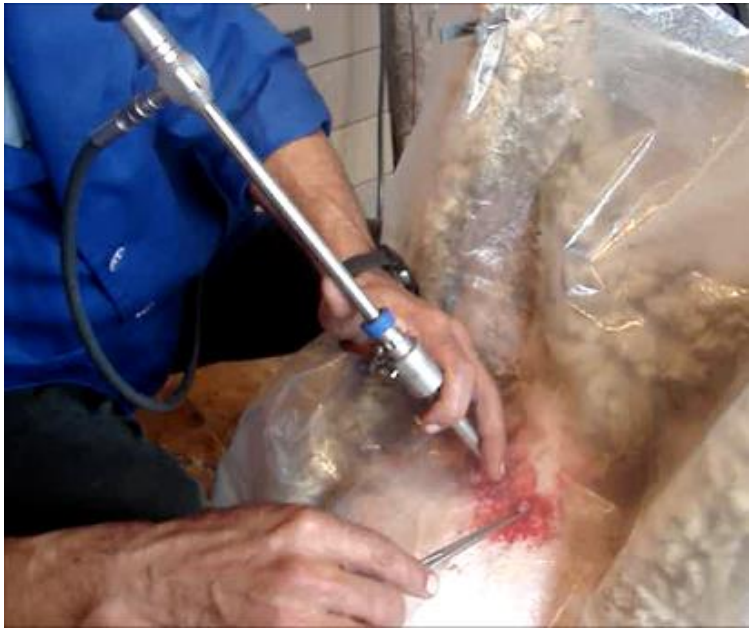
Robertson's applicator with inserted Robertson's pipette with straw of frozen semen inside

LAI with the aid of Robertson's applicator and pipette

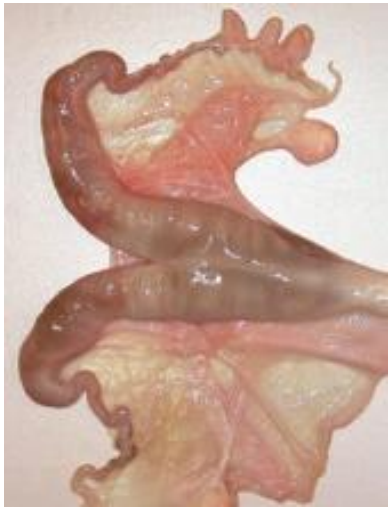
Laparoscopic insemination with surgical abdominal forceps



Surgical abdominal forceps

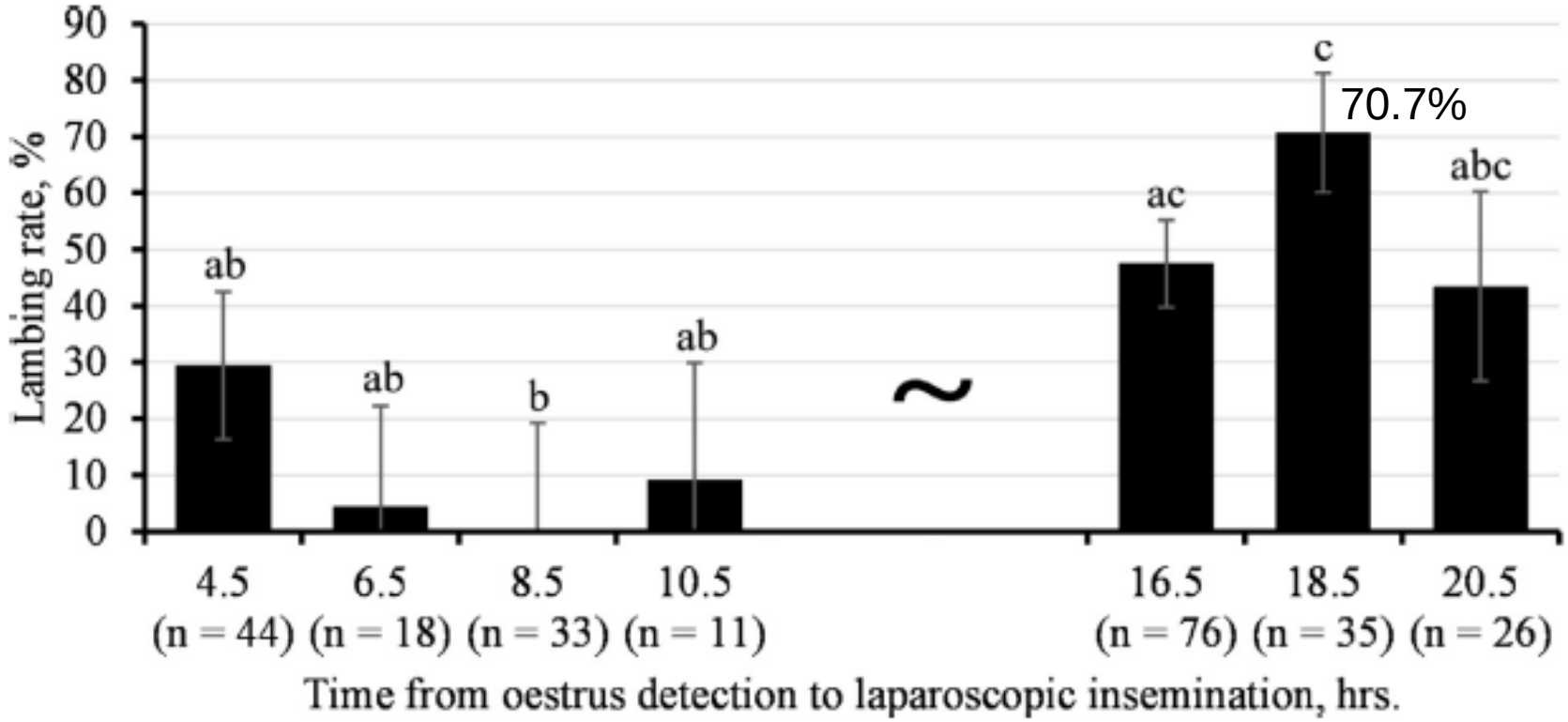


Exteriorization of the uterine horn using a laparoscope and abdominal forceps



Injection of semen into uterine horn

Lambing rate (LSM values ± SE) of sheep laparoscopically inseminated at time interval 4.5–20.5 h after the heat detection
(Source: Malmakov et al., 2022. Saudi J Biol Sci)



a,b,c – Means within the columns with differing superscripts differ significantly at $P < 0.05$.
LSM – least squares means. SE – standard error.


Thanks for attention!