Methods (poultry semen, ram epididymal semen, stallion semen)

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Freezing of semen

- Horse
- Poultry
- Epidymal ram semen

Freezing of Stallion semen

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Developing a new Stallion extender

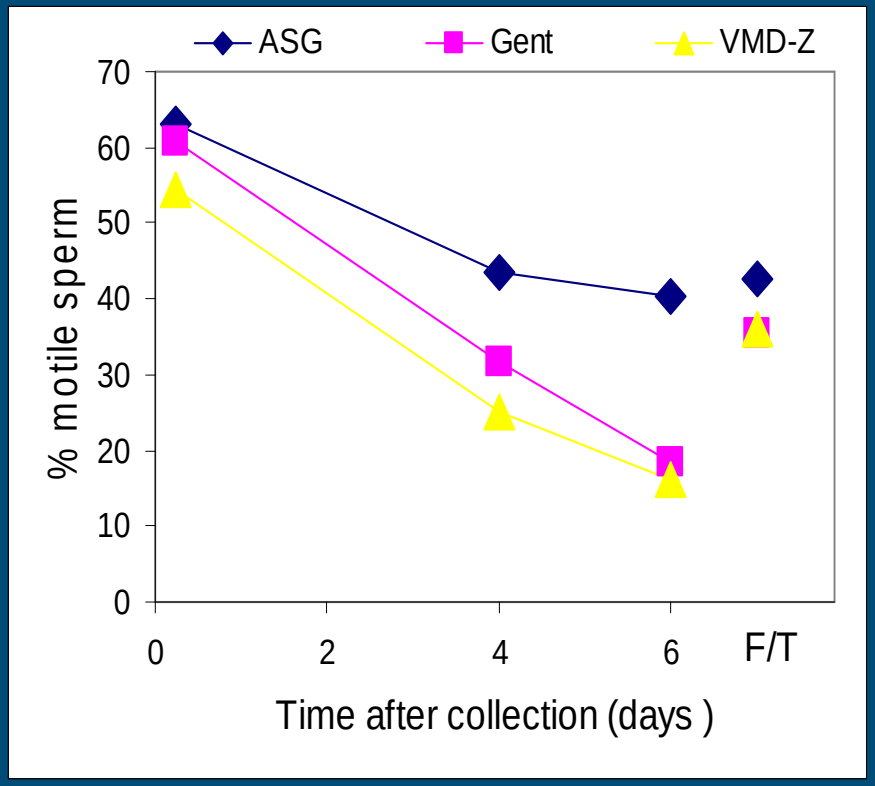
- We have tested numerous combinations of
  - Sugars
  - Salts
  - pH buffers
  - Milk
  - Egg yolk
  - Equex
  - Soy
  - Etc.
- And have tested both fresh storage and freeze thaw

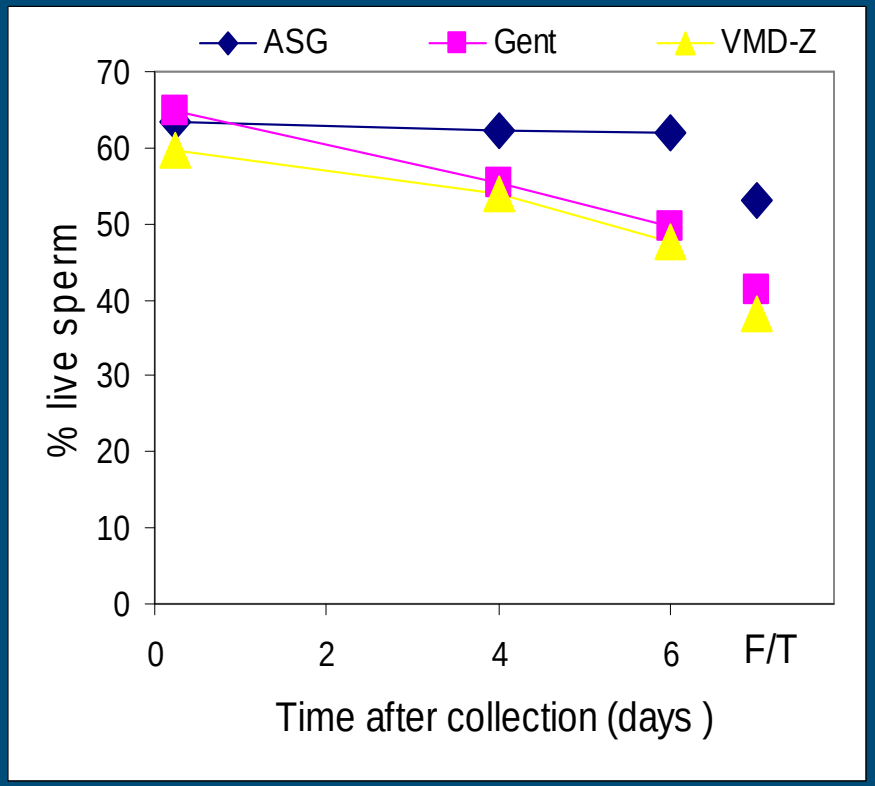
Developing a new Stallion extender

Washing medium

Clear! No milk or egg yolk

Comparison of 3 media for fresh and freezing





The Dutch gene bank (CGN) is currently using this ASG medium for gene banking of semen of Dutch rare horse breeds.

Freezing of Poultry semen

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Dutch rare breeds

Number of Dutch rare domestic animal breeds and their status

(SZH and ID-Lelystad, 2002)

	Cattle	Horse	Goat	Sheep	Poultry	Duck	Rabbit	Goose	Pigeon
Critical, declining								1	2
Critical, stable					1	3	2		2
Critical, growing							1		1
Endangered, declining		1					1		2
Endangered, stable			4		23	1	4		6
Endangered, growing	2	1	1		4				
Vulnerable, declining		1							
Vulnerable, stable	1		1	1	4		3		2
Vulnerable, growing	2		3			1	1		1

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Dutch Uilenbaard

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Drente Fowl

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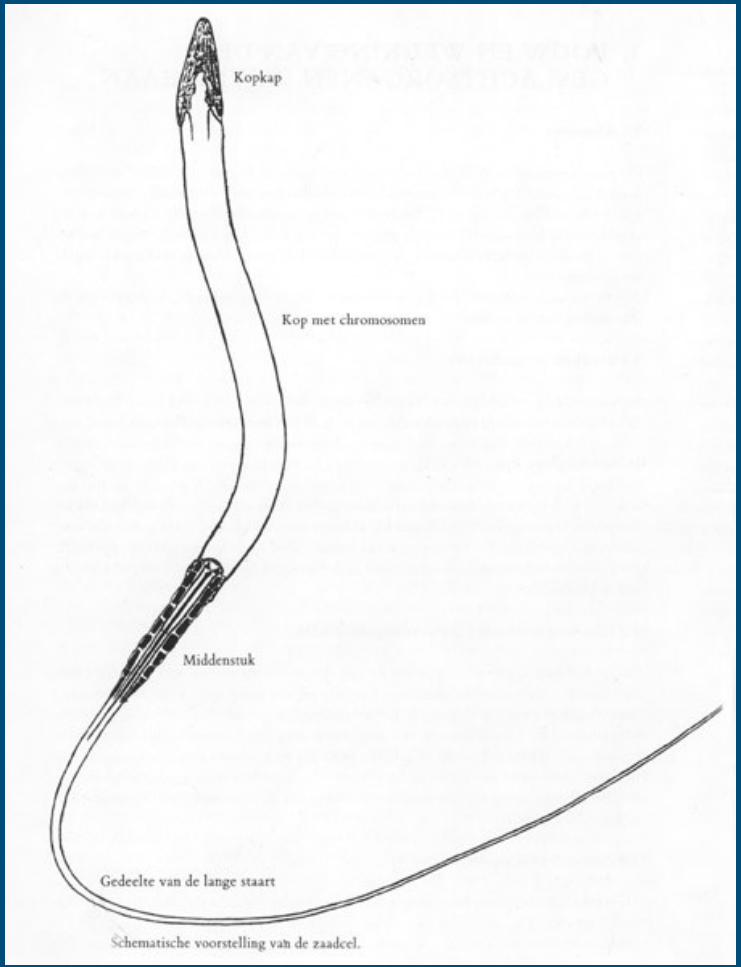
Collection of semen

Collection of semen

Sperm cell



Schematische voorstelling van de zaadcel.

Genital tract of the hen

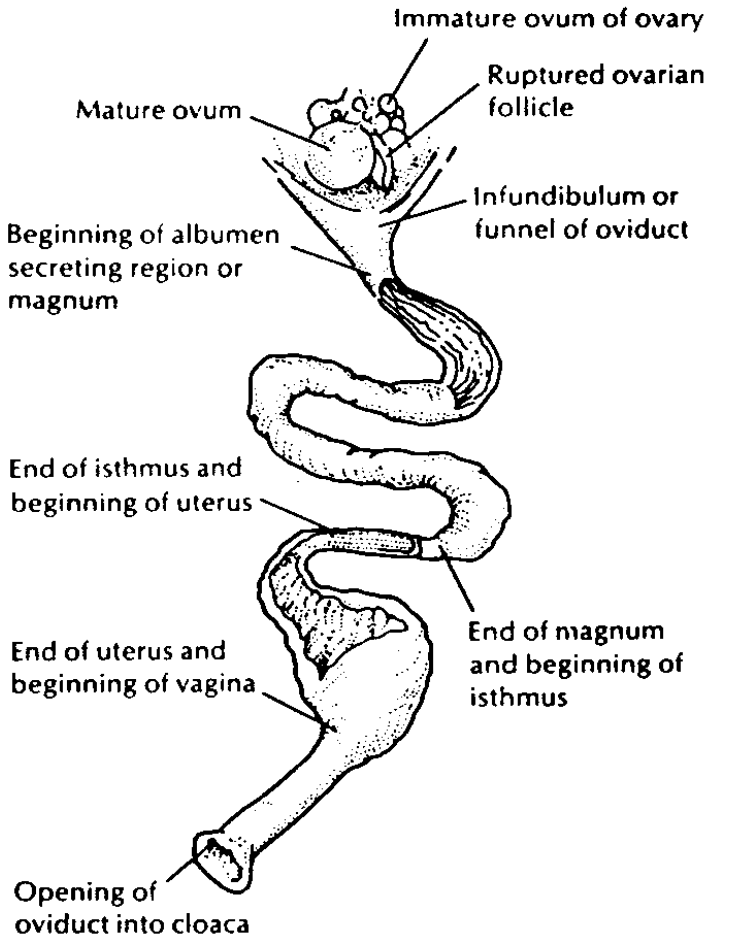


FIG. 1. Anatomical outline of the female reproductive tract of the hen. Taken from Johnson (31).

Freezing methods

- Freezing semen is successful:
  - Chicken
  - Turkey
  - Goose
  - Duck
- With good sperm survival up to 60%, but...

Freezing methods

“The enigma of Glycerol”

- Glycerol is generally seen as the better cryprotectant

  Hammerstedt en Graham 1992; Tselutin et al 1999; Phillips et al. 1996

- But glycerol acts as a contraceptive.

Freezing methods

- Other cryoprotectants have been tried
  - DMSO (o.a. Van Voorst en Leenstra, 1995)
  - DMA
  - DMF
  - ….

Freezing methods

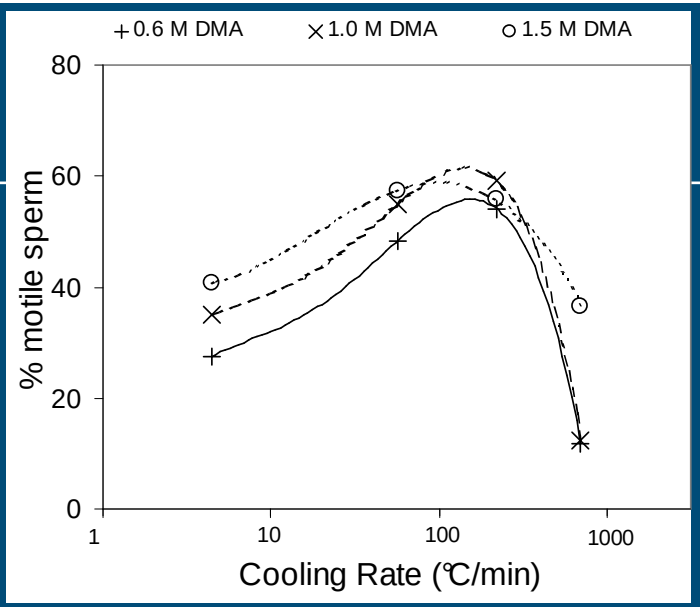
- Tselutin et al.1999

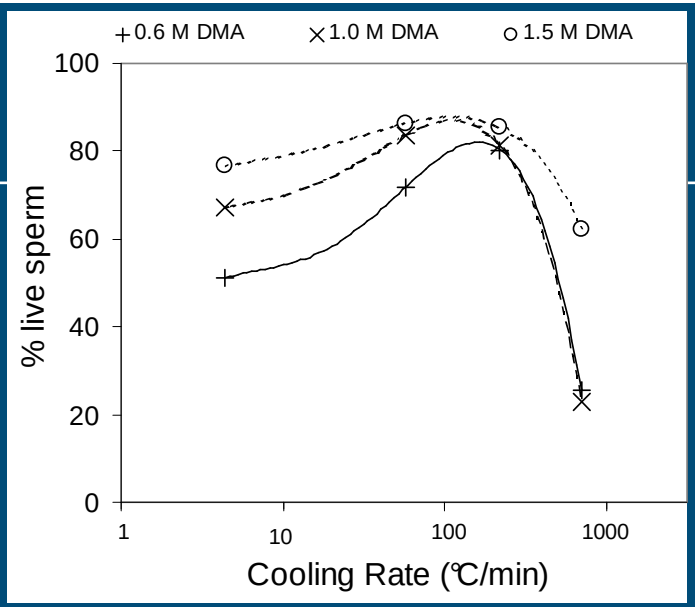
CPA	package	% fertilized eggs
glycerol	straws	63.9
DMA	pellets	84.7
DMA	straws	26.7

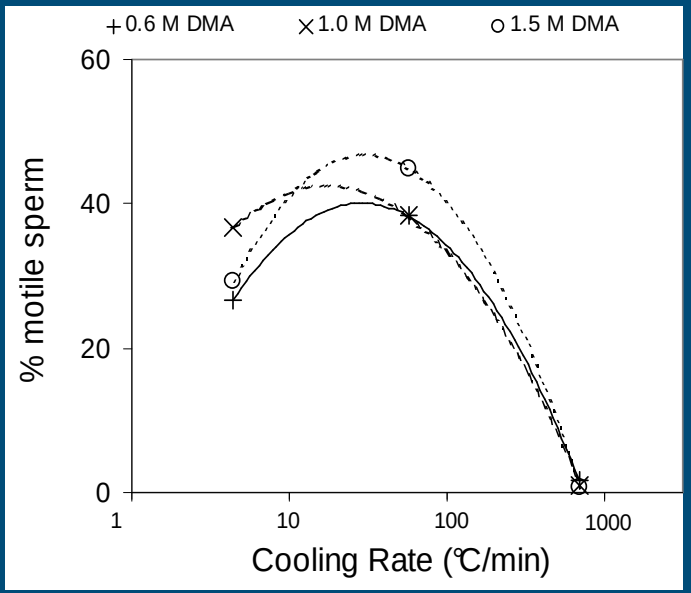
Cryoprotectants

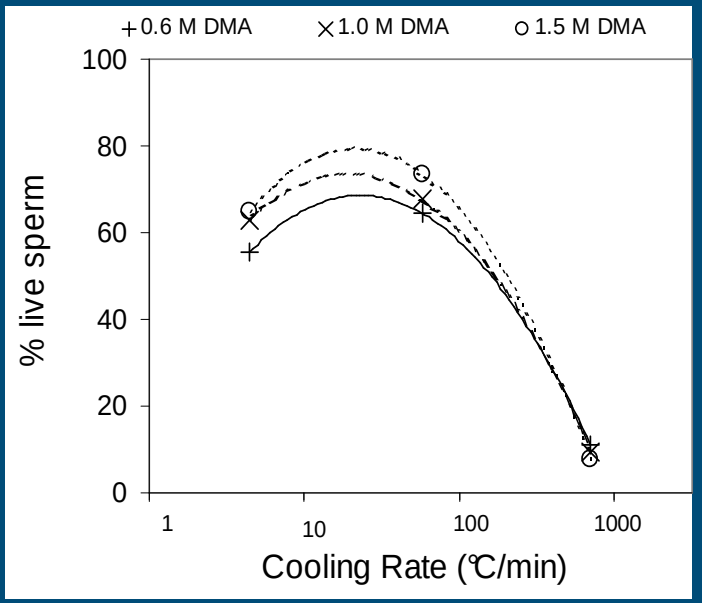
We have tried

- Glycerol (= propanetriol
- Ethylene glycol (= ethanediol)
- DMSO (dimethylsulfoxide)
- Ethanol
- Methanol
- DMA (dimethylacetamide)
- Glycerol or DMA in combination with sucrose









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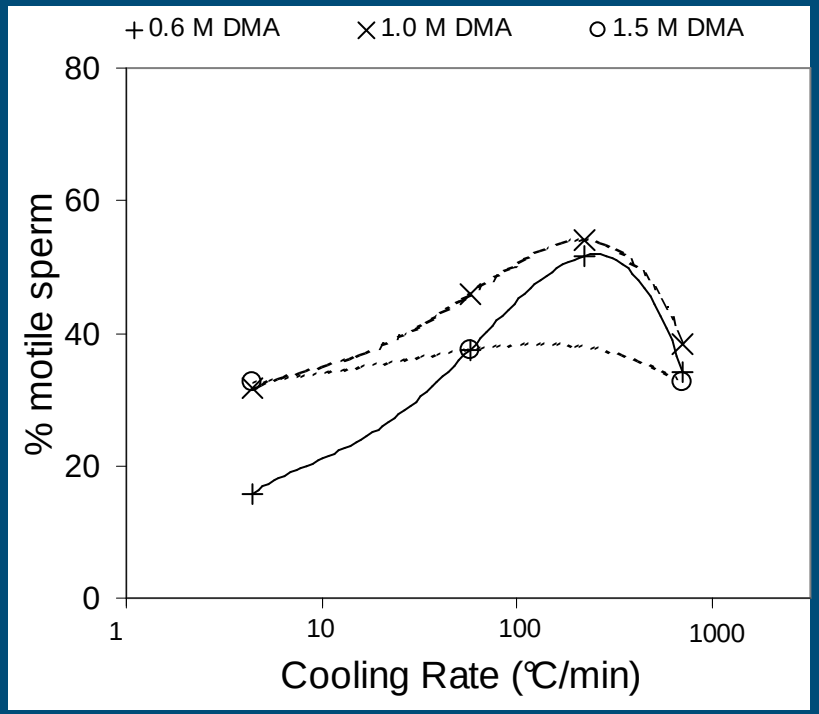
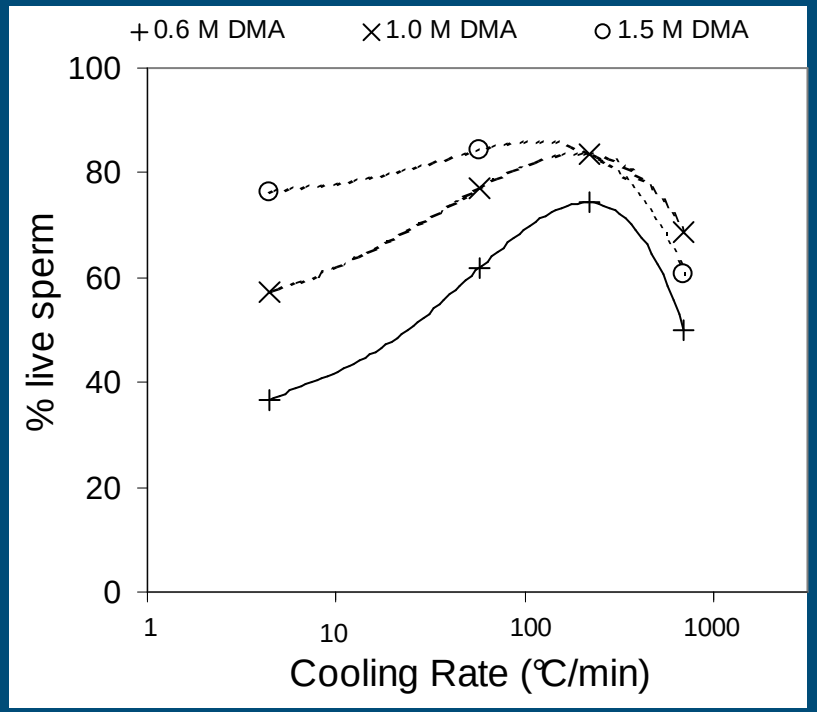
Frozen poultry semen





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Insemination experiment

	Fresh	Straws	Pellets
Lake		x	x
ASG	x	x	

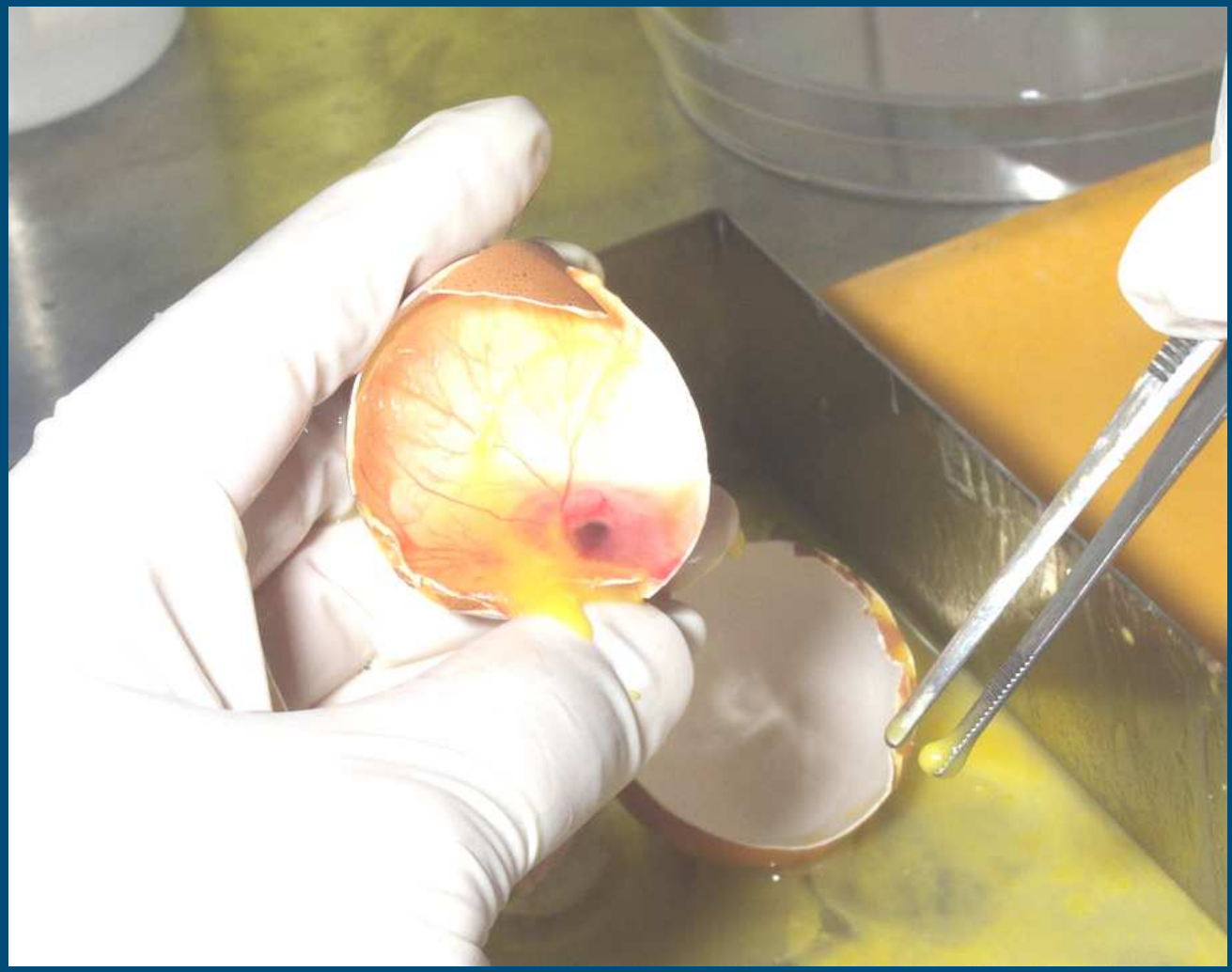
	Medium	Package	Freezing method
1. Fresh	ASG		
2. Frozen	ASG	Straw	Controlled rate
3. Frozen	Lake	Straw	Controlled rate
4. Frozen	Lake	Pellets	Plunge in LN2

Insemination experiment

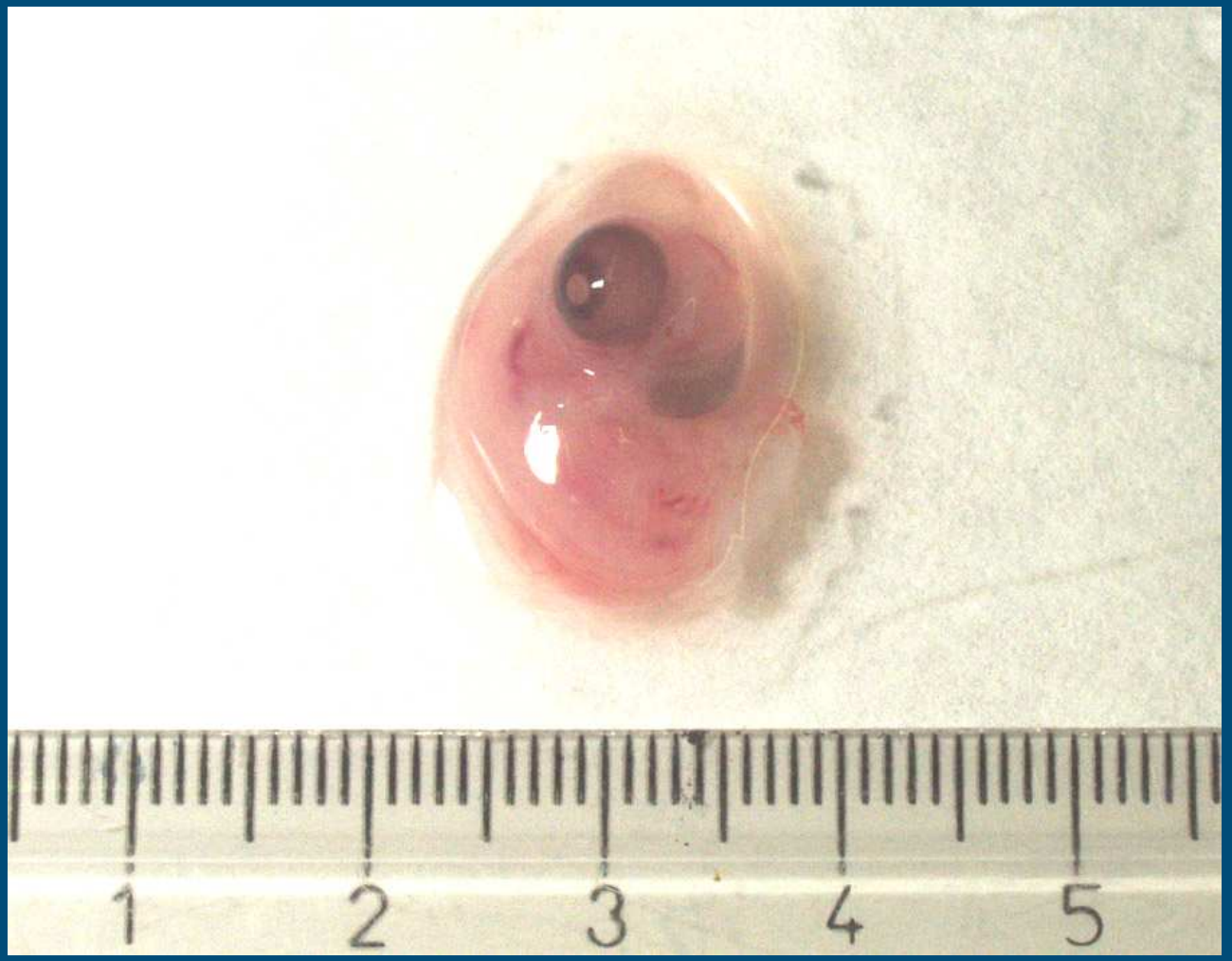
- Semen was collected from cocks from a broiler breed.
- The semen from 8 cocks was pooled, and used for the four treatment groups on the same day.
- 4 x 23 hens (Isa brown) were inseminated twice weekly, with 0.3 x 10^9 spermatozoa.
- Approximately 625 eggs per group were collected, and inspected after 1 week incubation.

Insemination experiment

Insemination experiment



Insemination experiment



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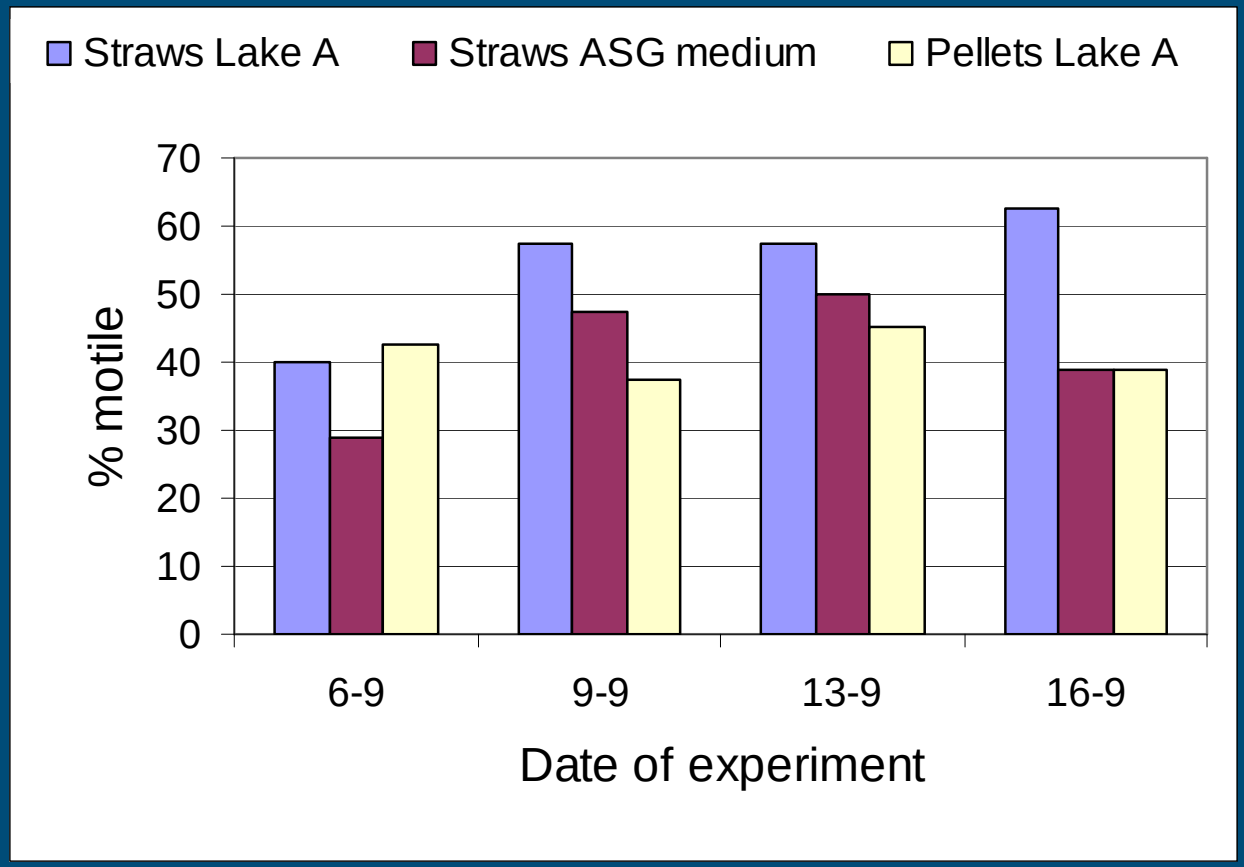

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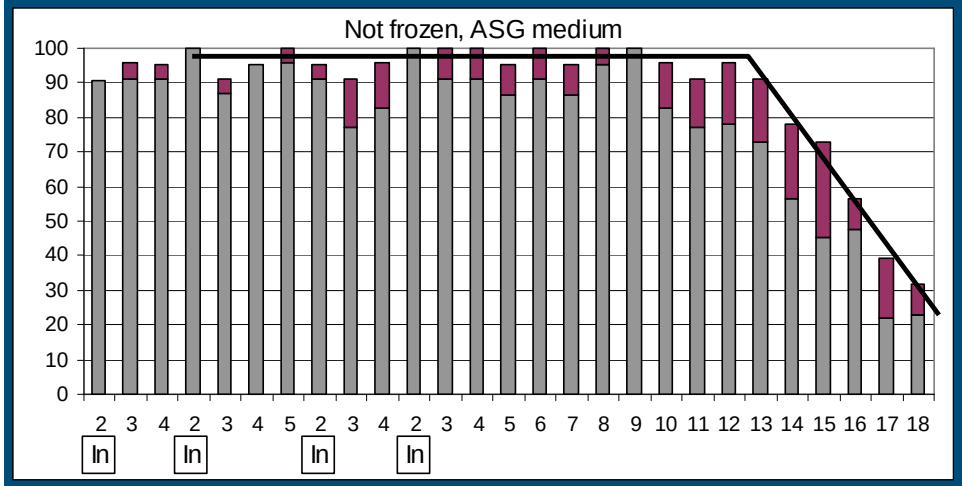
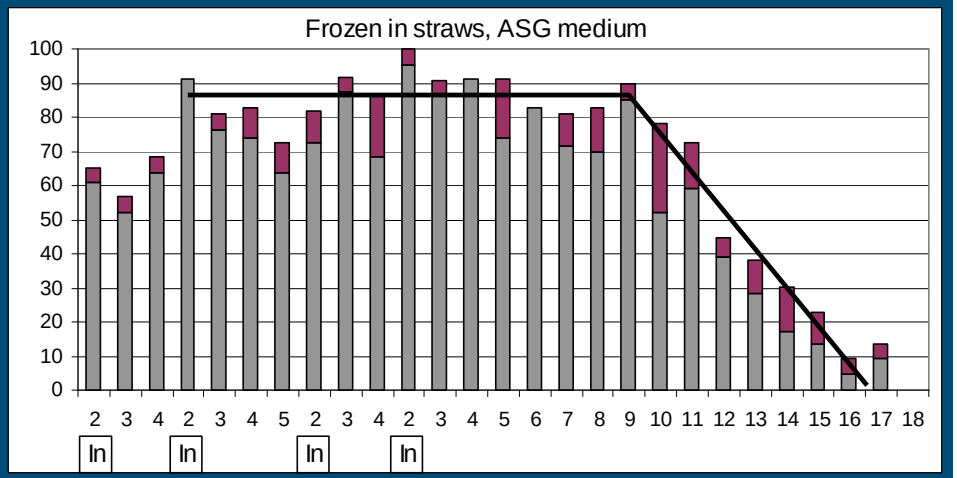
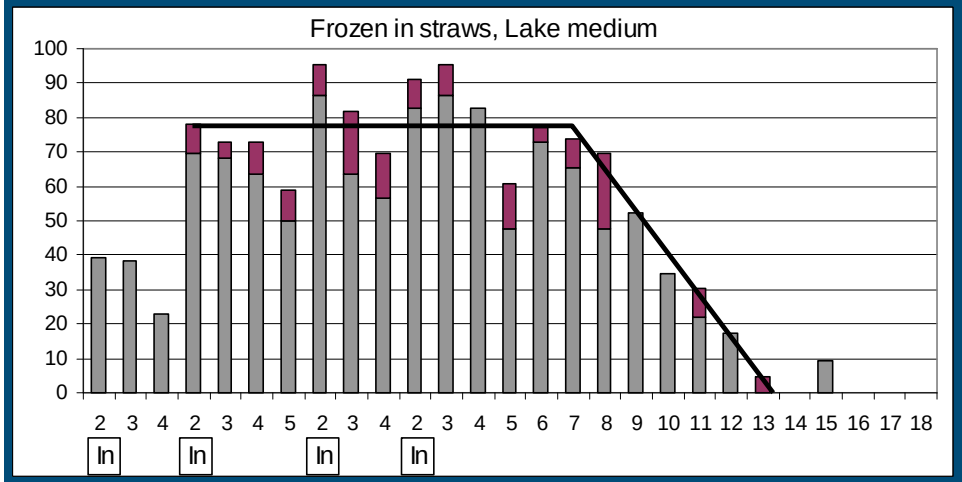
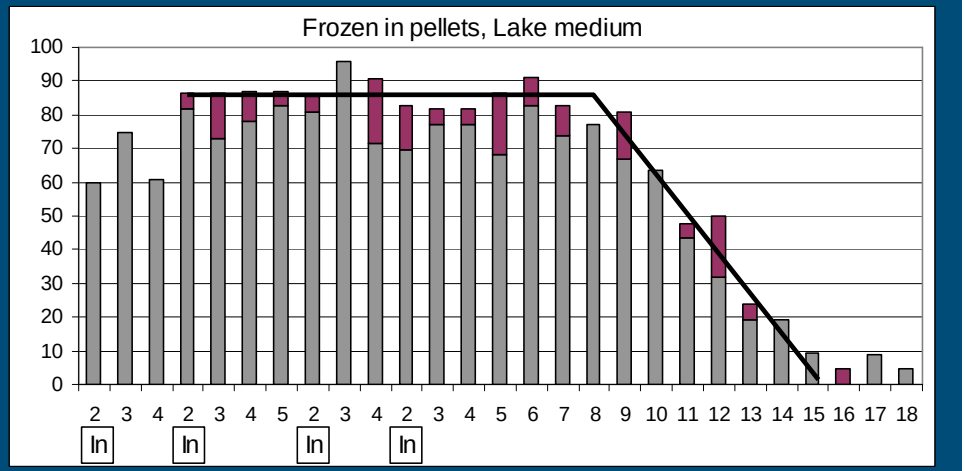
Insemination experiment









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Insemination experiment

	Treatment	% fertilized eggs	% eggs embryos	Mean day of last fertilized egg after last insemination
1	Fresh semen in ASG medium	96.6[a]	90.5[a]	16.4[a]
2	Frozen in straws in ASG medium with DMA	87.6[b]	80.4[b]	12.7[b]
3	Frozen in straws in Lake's medium with DMA	78.1[b]	68.9[b]	9.9[c]
4	Frozen in pellets in Lake's medium with DMA	85.9[b]	77.8[b]	12.3[b]

Conclusions

- Good fertility of semen frozen with the pellet method can also be obtained using freezing in straws with optimised freezing programme.
- The new carrier medium gave better fertility than Lake's freezing medium
- We now use this medium and straw freezing method for continuation of our poultry cryopreservation programme in the gene bank.

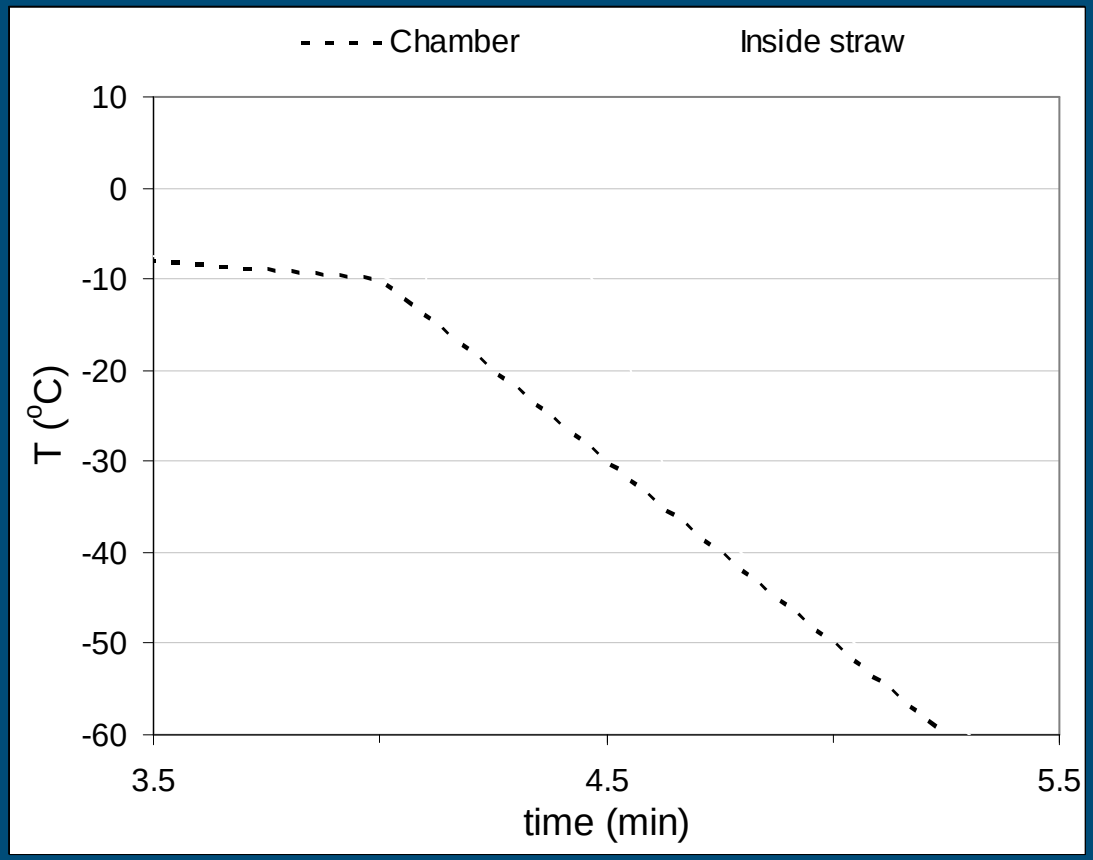
Present collection of Dutch gene bank

Breed	Nr cocks	Nr doses	Breed	Nr cocks	Nr doses
Barnevelder	13	1034	Ned. Baardkuifhoen	11	862
Drents Hoen	12	578	Noordhollands Hoen	14	1489
Twents Hoen	13	902	Groninger Meeuw	10	865
Ned. Uilenbaard	12	858	Assendelfter Hoen	11	651
Welsumer	12	820	Schijndelaar	5	500
Hollandse Kriel	30	803	Hollands Hoen	12	850
Lakenvelder	9	890	Chaams Hoen	10	1004
Brabanter	13	1211	Hollands Kuifhoen	10	834
Fries Hoen	13	754	Sabelpoot Kriel	19	564
Kraaikop	10	992	Eikenburger Kriel	7	206

Turkey, Geese, Ducks

- Done experiments with turkey semen
  - Various media, cryoprotectants and freezing rates
  - Results expected shortly
- Will start with geese and ducks this year

Programmable freezers?



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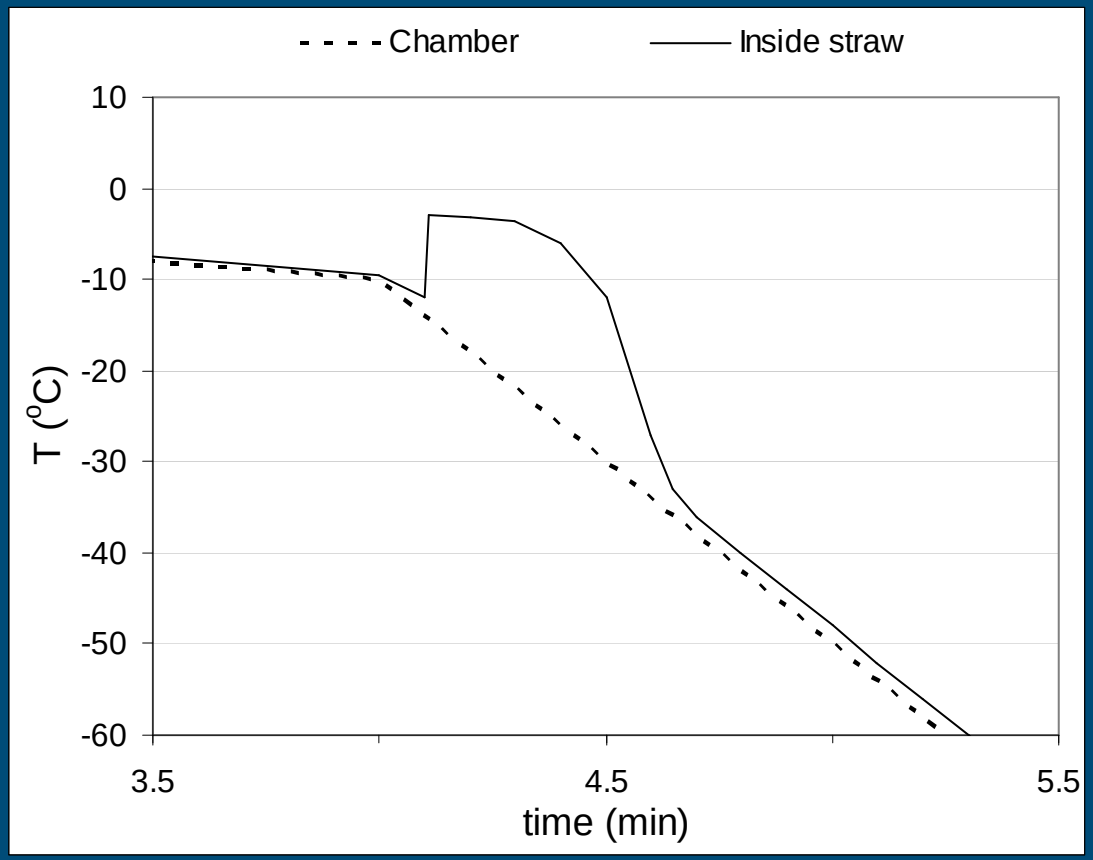

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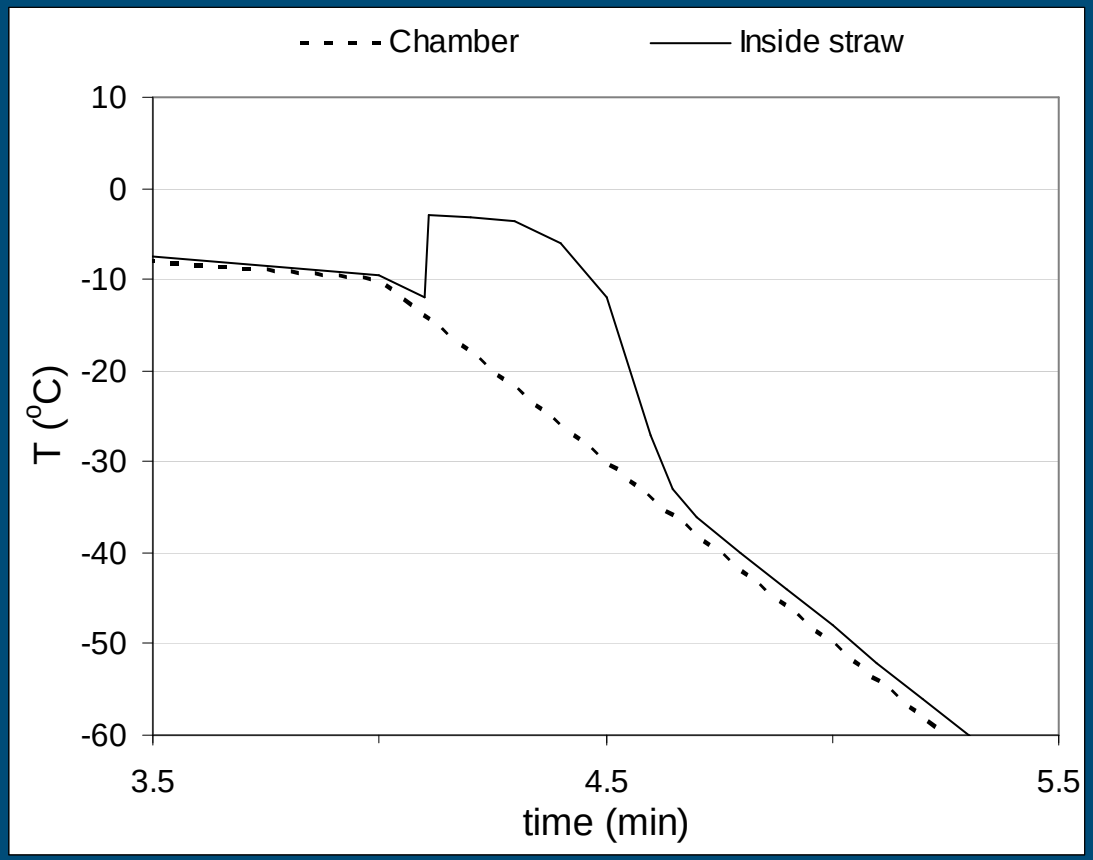

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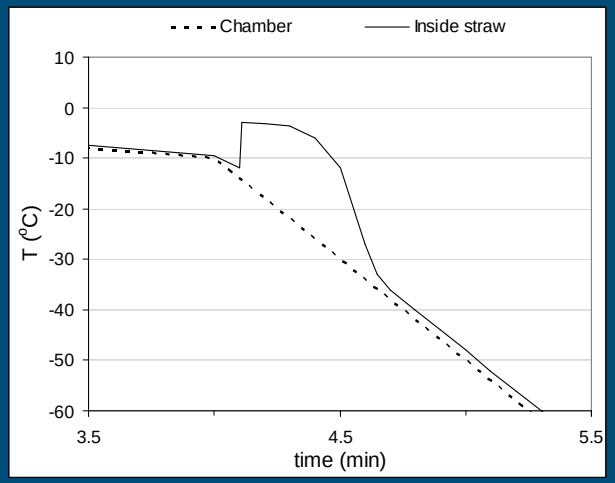

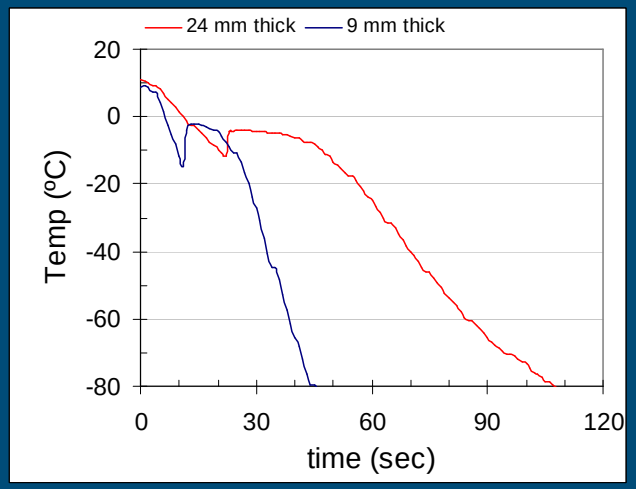

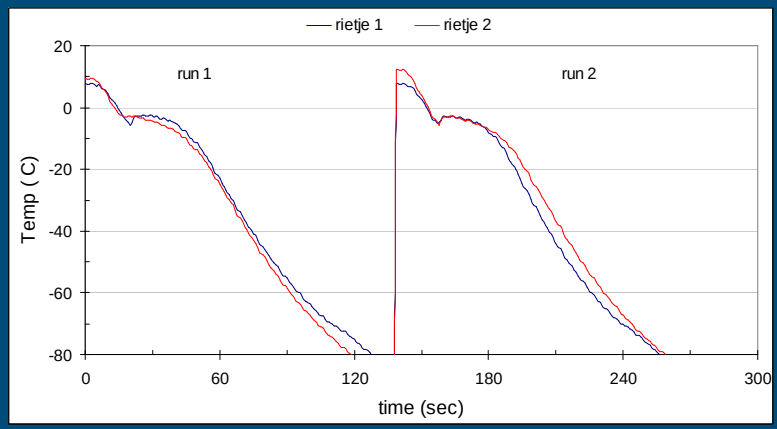

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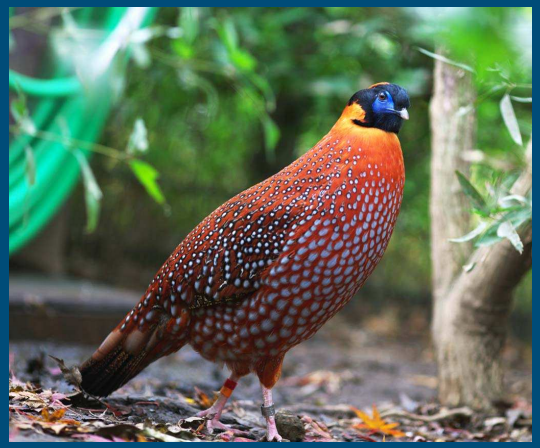
Programmable freezers?

- Compared programmable with not-programmable for various species
- Post thaw sperm quality results are equal

Tragopan pheasants and Cranes

- Our ASG extender improved longevity
- Semen frozen on site, to prevent transport time
- Portable freezer. Freezing on the kitchen table!
- Successful cryopreservation of semen from these species is considered to be a world first.



Freezing of epididymal ram semen

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Epididymal ram semen

- We developed method for semi-quantitative collection from the caudae epididymidis of slaughtered rams.
- Motility of epididymal semen was good
- Freezability seemed to be better than that of ejaculated semen.

- On average **20 billion epididymal spermatozoa per ram**. = 108 doses of 0.2 billion sperm/dose.

Epididymal ram semen:

Will it fertilise ewes?

Our insemination study, 2004

Insemination experiment

Ejaculated semen:

- Cervical AI: 10 animals
- Laparoscopic AI: 10 animals

Epididymal semen:

- Cervical AI: 10 animals
- Laparoscopic AI: 10 animals

Semen from Veluwe rams

	% motile sperm	% live sperm
Ejaculated semen	42 ± 4.5	48.8 ± 2.1
Epididymal semen	60 ± 0	62.3 ± 5.6

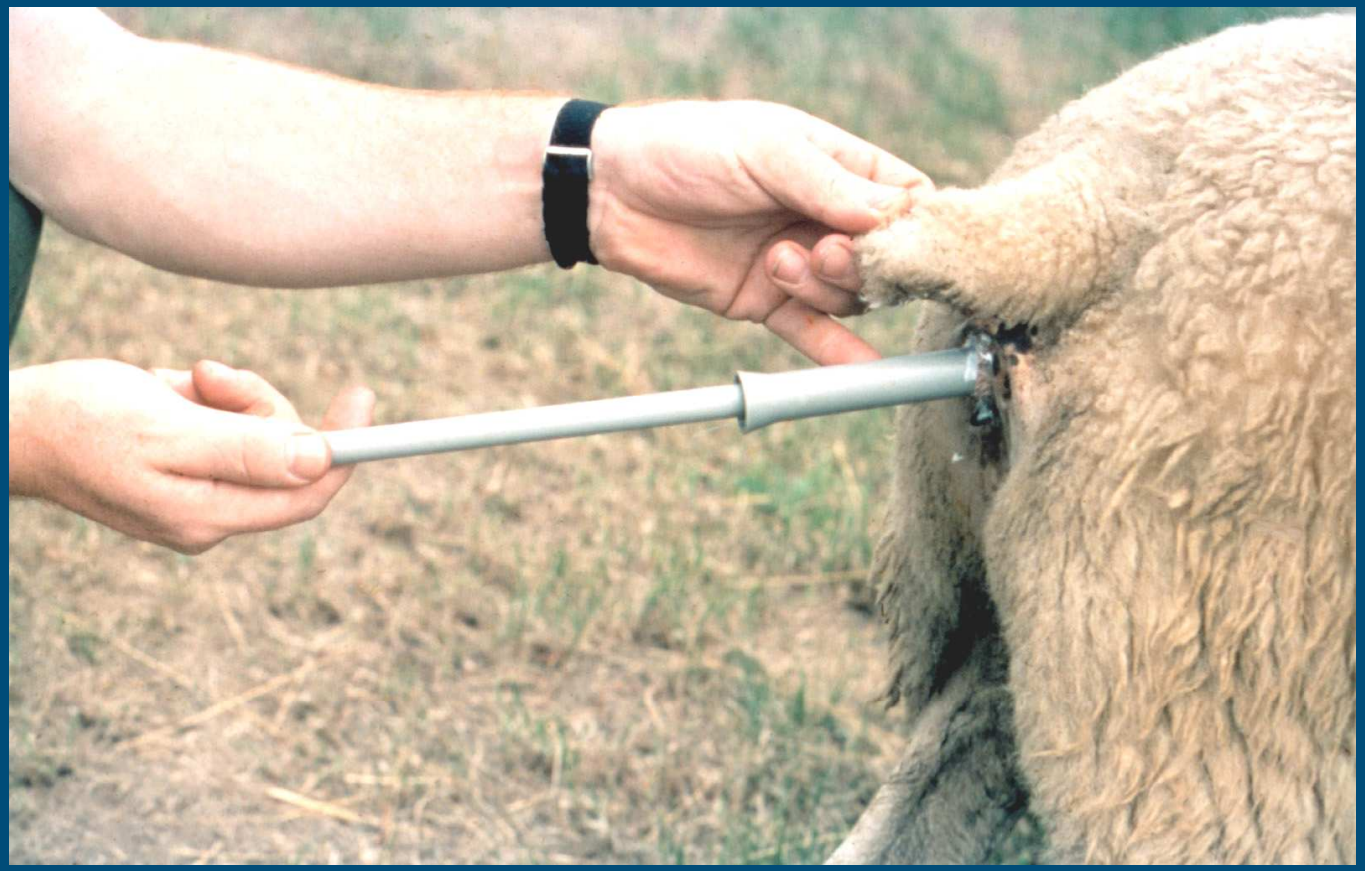
Synchronisation protocol

- **Day 0**. Progesterone sponge for 12 days
- **Day 10**. Prostaglandins
- **Day 12**. Sponges removed + eCG injection
- **Day 14**. HCG + antibiotics (52 ± 1 hours after removal)
- **Day 14**. Cervical AI after 57 ± 1 hours
- **Day 14**. Laparoscopic AI after 59 ± 1 hours

Drugs and devices

- Sponges: Chronogest®
  (30 mg flugest.acet.= Cronolone)
- PG: Prosolvin®
  1 ml (7.5 mg/ml Luprostiol) im.
- eCG: Folligonan®500 IU im.
- HCG: Chorulon® 500 IU im.

Synchronised Swifter ewes



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Insemination moment

* Cervical AI

  57 ± 1 hours after sponge removal

* Laparoscopic AI

  59 ± 1 hours after sponge removal

Close to ovulation!

The semen master

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Insemination dose

- Cervical AI: 0.5 ml/straw

  $200x10^6$ spermcells inseminated

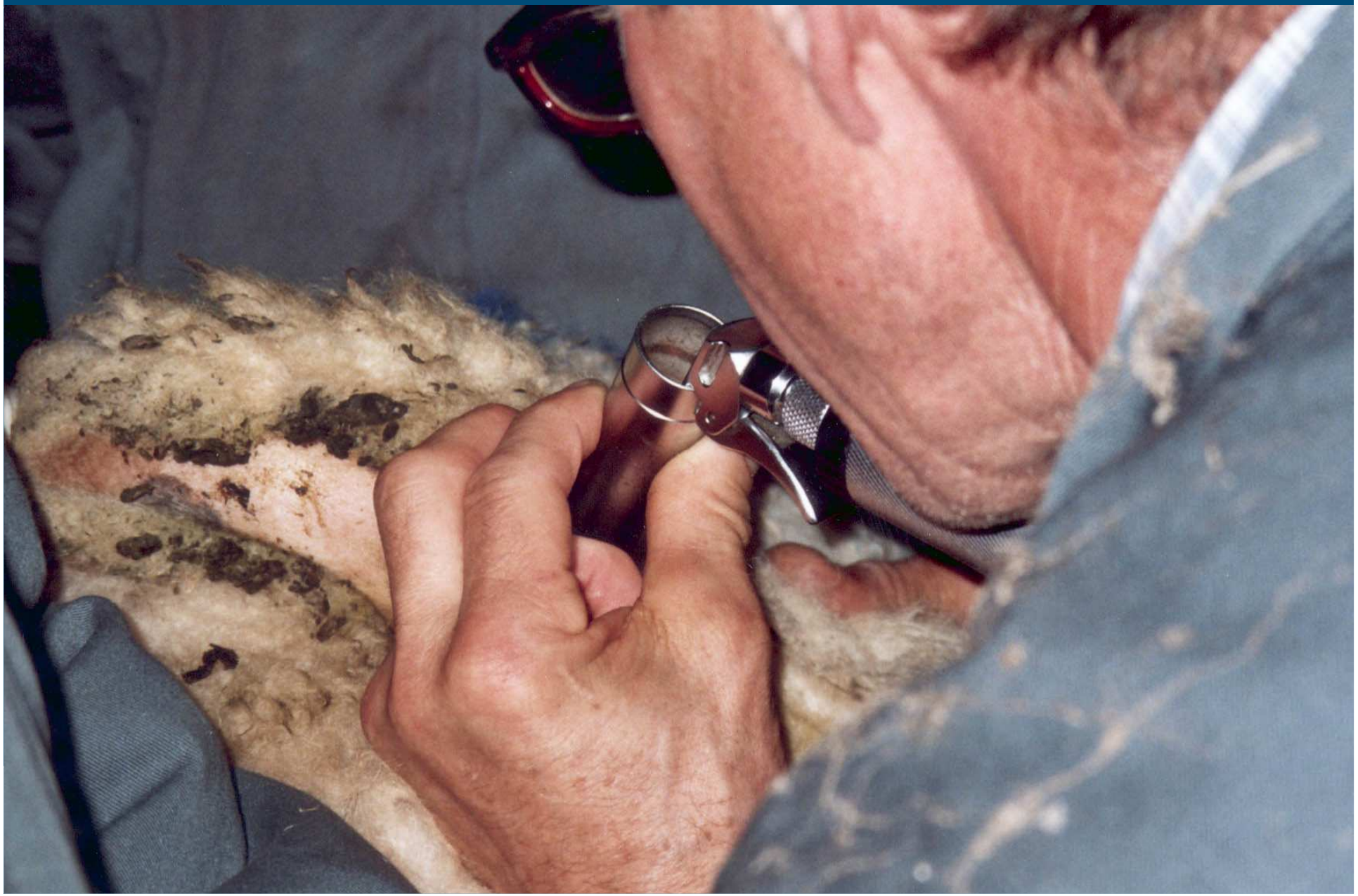
- Laparoscopic AI: 0.25 ml/straw

  $80x10^6$ spermcells

  $40x10^6$ cells inseminated per horn

Cervical AI

Where is the cervix?



The workers

The project leader

Laparoscopic AI

38 days pregnancy

Lambs

Results

	Ejaculated semen		Epididymal semen	
	pregnant	lambs/ewe	pregnant	lambs/ewe
Cervical AI	0/11		4/10	2.0
Laparoscopic AI	6/10	2.3	7/10	3.1

Conclusions

- Pregnancies in sheep with epididymal semen is possible!
- Cryopreservation of epididymal semen is an easy, quick and low cost method for the preservation of e.g. endangered mammal breeds.