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Cryopreservation and insemination of
ejaculated and epididymal semen from
Dutch rare sheep breeds

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Genetic diversity of farm animals

- Decline of genetic diversity in livestock species
 - Breed diversity
 - Genetic diversity within breeds
- (Potential) loss of genetic diversity is generally seen as a threat
- Many countries have taken measures to maintain genetic diversity

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How can we maintain genetic diversity

- Sustainability of breeding programs
- Maintain variety in animal production systems
- Stimulate *in situ* conservation
- Set up gene banks: *ex situ* conservation

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A gene bank: Why?

- Conserve genetic diversity
 - Breeds or lines
 - Alleles, genes or traits
- From different perspectives
 - To save our cultural heritage
 - 'Insurance' for disease threats
 - Support breeding programs (avoid inbreeding or use in case of genetic problems)
 - Flexibility to re-orient breeds (change production systems or markets)

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Ex-situ conservation

- Cryopreservation: "suspended animation" in liquid nitrogen (-196 °C)
- All chemical and physical processes arrested
- Only Cosmic background radiation is a possible source of DNA damage.
- This is estimated to become relevant only after several thousands of years (Mazur, 1985)
- Consequently: Storage is safe for several thousands of years, possibly longer

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FMD, scrapie

- Stamping out/culling of a region in the case of FMD (e.g. Veluwe) would mean extinction of rare breed
- Selection program on scrapie sensitivity genotype
 - Low ARR frequency/small population → loss of genetic diversity
 - Rare breeds can be exempted, but even then, breeders will use the ARR/ARR rams as much as possible
- In 2001 LNV commissioned CGN to freeze semen of rams of 5 rare breeds

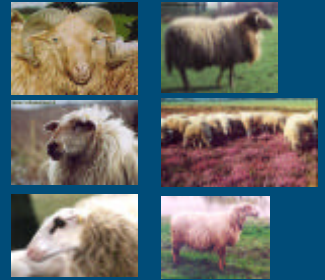
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5 Dutch rare sheep breeds

Sheep

- Drente Heath Sheep
- Schoonebeeker Sheep
- Mergelland Sheep
- Kempen Heath Sheep
- Veluwe Heath Sheep



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2001/2002

- Selection of rams within breeds
- Transport of rams, housing in Lelystad and Utrecht
- Training rams for semen collection
- Collection and freezing of semen
- Objective:
25 rams/breed x 100 doses per ram

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Semen collection

	# rams acquired	# rams ejaculated	# doses frozen	mean dose/ram
Breed				
Mergelland	26	21	2676	127
Kempen heath sheep	29	15	2370	158
Veluwe heath sheep	34	5	768	153
Schoonebeeker	19	12	1144	95
Drente heath sheep	21	2	45	22
Total	129	55	7003	127

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Problem

- Some Heath sheep (breeds) were too wild for semen collection
- Only 5 males of Veluwe heath sheep
- Only 2 rams of Drente Heath sheep, a few doses

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Problem ? Ejaculation

Solution ? Epididymal semen of slaughtered rams

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Collection of epididymal ram semen

- We developed a method for semi-quantitative collection from the caudae epididymidis of slaughtered rams
 - Transport after slaughter at -5 Celcius
 - Processing within a couple of hours
 - Preparation of cauda from body
 - Cut cauda in pieces after preparation from body
 - Preparation in freezing media

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Results

- Extremely high concentration of sperm cells
- Motility of epididymal semen was good
- Freezability seemed to be better than that of ejaculated semen
- 20 billion epididymal spermatozoa per ram (34 rams)
- >100 doses per ram of 0.2 billion sperm/dose

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Epididymal ram semen:

Will it fertilise ewes?

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One earlier study

- Suzanne Fournier-Delpech et al. 1981 (C.R. Acad Sc. Paris)
 - Laparoscopic Insemination of ewes
 - Fresh (not frozen) epididymal sperm
- | | % cleaved eggs |
|-------------------------------------|----------------|
| ■ With caudal or ejaculated sperm : | 85% |
| ■ With corpus sperm : | 30% |

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A recent study

- T.A.Schmidt et al. 2004 (EAAP abstract 409)
- Laparoscopic AI with frozen/thawed epididymal semen
- 8 ewes → 7 pregnancies

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Our study

Insemination experiment

Ejaculated semen

- Cervical AI 10 animals
- Laparoscopic AI 10 animals

Epididymal semen

- Cervical AI 10 animals
- Laparoscopic AI 10 animals

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Post-thaw semen quality

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

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Synchronisation of ewes

- **Day 0** Progesterone sponge for 12 days
- **Day 10** Prostaglandins
- **Day 12/13** Sponges removed + PMSG injection
- **Day 14/15** CG + antibiotics
- **Day 15** **Cervical AI: 57 hours after PMSG**
Laparoscopic AI: 59 hours after PMSG

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Insemination dose

- **Cervical AI:** 0.5 ml/straw
200x10⁶ spermcells inseminated
- **Laparoscopic AI:** 0.25 ml/straw
90x10⁶ spermcells
45x10⁶ cells inseminated per horn

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Results

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

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Conclusions

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

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Collaborators

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 - René van den Brom
- Animal Health Service
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Thank you for your attention



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