

# Latest developments in cryobiology and reproductive technologies

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# Semen collection

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## ■ Cattle

- On farm collection. derogation is now official.

## ■ Ducks (and geese)

- Collection more difficult than in chicken
- There are few publications regarding collection of epididymal semen of birds
- Birds different from mammals in morphology and function of the epididymis.
- Most 'extragonadal' sperm are found in the vas deferens.
- But not more than one ejaculate worth of sperm.
- → Collection of extragonadal semen of slaughtered drakes is not cost-effective for gene banking of duck, or even rooster semen.

# Semen collection, Ducks

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- In the mean time we have obtained experience in collecting ejaculates from drakes (and ganders)
- Seems to be working reasonably fine now
- We now are collecting routinely 500 million sperm/ejaculate
- e.g. 5 straws with 400 million sperm/straw
- Movie on collection and freezing will be available on CGN website







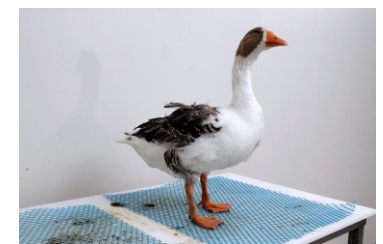
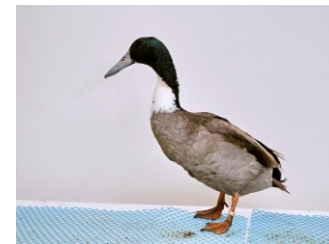
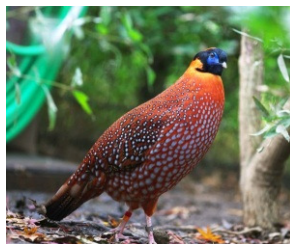
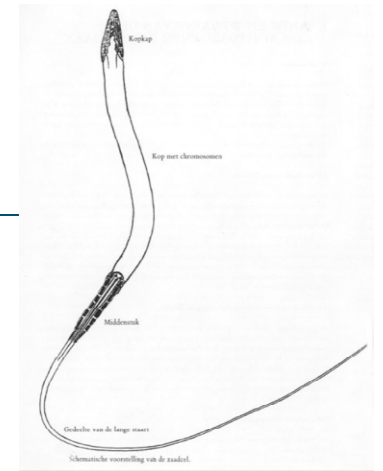
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For quality of life

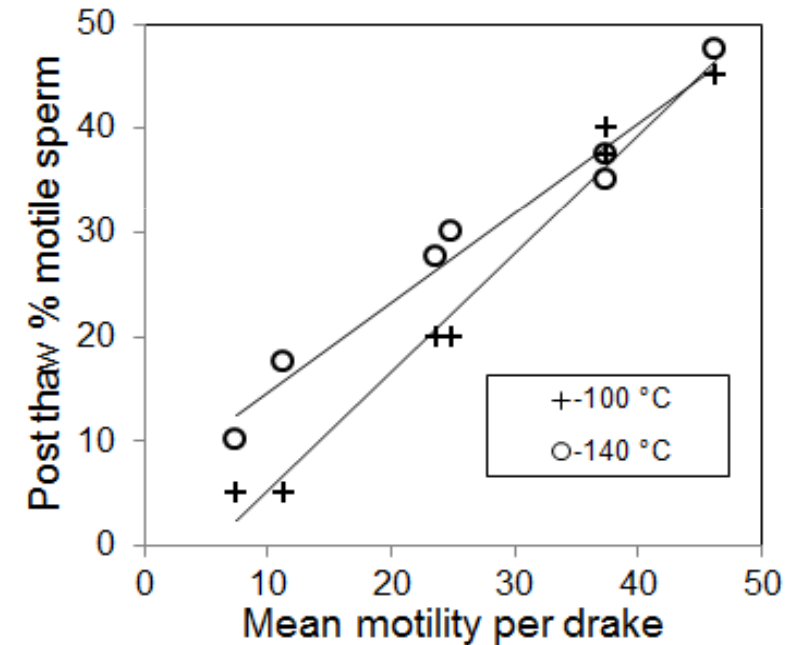
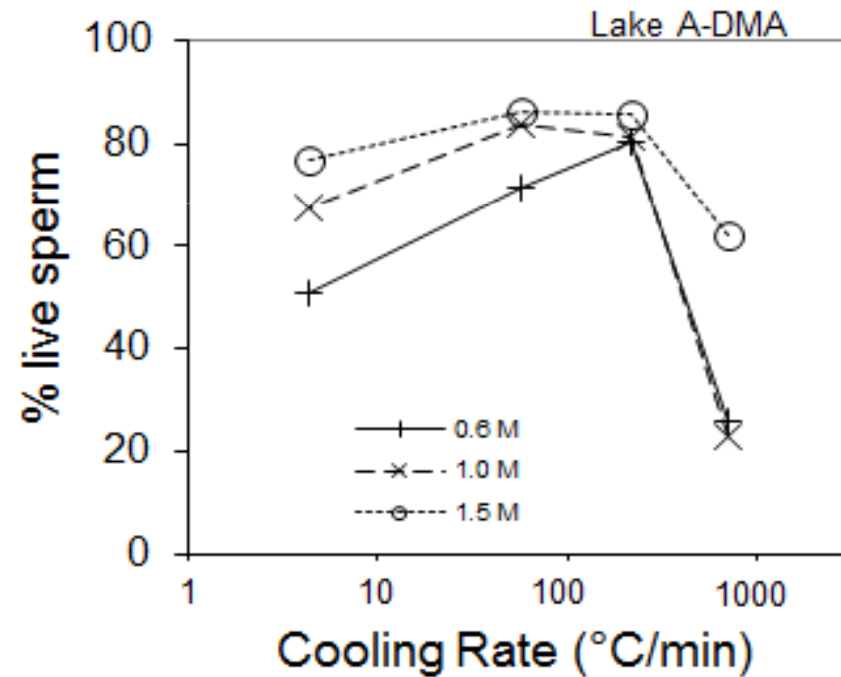


# Cryopreservation of poultry semen

- Our method gives high post-thaw fertility.
- Important factors are
  - DMA as cryoprotectant, 0.6 M
  - Use of our ASG extender → better post-thaw fertility
  - High cooling rate is necessary (200°/min)
  - High cooling rate enable the use of lower [CPA]
- These factors also beneficial for other species
  - Tragopan pheasants and cranes
  - Turkey
  - Ducks and geese



# Cryopreservation of poultry semen



# CGN collection Chicken, Duck, Goose breeds

Breed	cocks	doses	Breed	cocks	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
Hooked Bill Duck					
North-Holland White Bibbed Duck			Twentse Landgans goose		

# AI in sheep with frozen/thawed semen

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- Most countries use laparoscopic (surgical) AI
  - Give high pregnancy rate (70%)
  - But not allowed in NL
- Non-surgical method of intrauterine semen deposition.
  - Three approaches have been tried
    - retract the cervix into the vagina to align the funnel-shaped rings of the cervix to some degree
    - agent to relax the cervix (e.g. PGE-2 or PGE analogues, oxytocin, interleukin-8, misoprostol, or hyaluronan, or the beta-adrenergic blocking agent carazolol
    - designing appropriate transcervical AI and ET equipment to overcome the physical difficulties associated with the ovine cervix
  - 100% penetration of the cervix is achieved
  - but lambing data missing or not dramatically improved.



# AI in sheep with frozen/thawed semen

- Norway approach: Vaginal or cervical insemination
- Yet 70% lambing rate (most other studies report 17-23%)
  - Ewes inseminated in natural oestrus
  - Fertility of Norwegian crossbred ewes is very high
  - Farmers are well trained and experiences
- Method is
  - Simple
  - Cost effective
  - Animal welfare

Paulenz et al. 2005

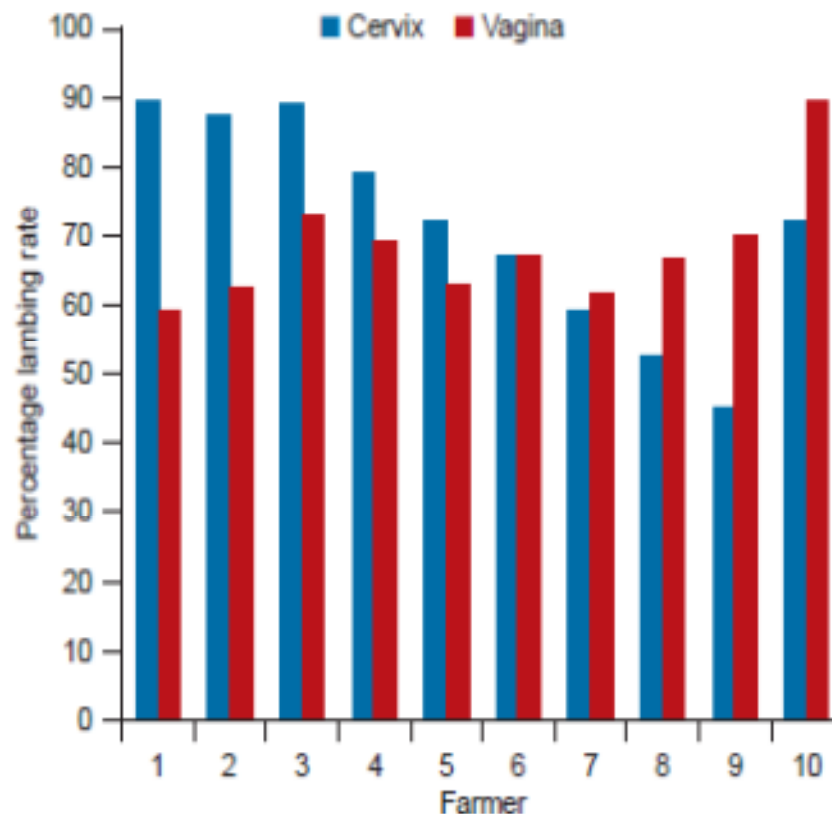


FIG 1: Percentage lambing rates achieved by 10 farmers after inseminating 543 ewes either into the vagina or the cervix with frozen-thawed semen collected from six mature rams. Only the results from farmer 1 showed a significant difference between the results of the vaginal and cervical inseminations

# Horse

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- Oocytes/embryos, to preserve genotypes of females
  - Ovum pick up is possible throughout oestrous cycle.
  - 70 oocytes per year per mare
  - 50 oocytes of Gelderlander mare frozen as pilot.
- Cryopreservation of horse oocytes (or embryos) is very difficult.
- Setting up a PhD project
  - Focus on fundamental cryobiology
  - Minimal volume vitrification
  - Increased cooling and warming rate
  - Reduced or even zero permeant cryoprotectant concentration

# Ovaries of mice and birds

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- **Vitrification of juvenile mouse ovaries.**
  - Using similar protocols as for embryos/oocytes
  - At first glance a less obvious option
  - But, grafting of vitrified/warmed ovaries into young animals is a relatively simple and successful procedure.
  - Efficiency demonstrated by banking a large series (22) of mutant mouse strains with various genetic backgrounds (C57BL/6, FVB, BALB/c) to recover the genotype by orthotopic grafting of the vitrified ovaries in recipient mice
  
- **Similar approach successful in bird species**
  - Group of Fred Silversides at Agassiz Canada



# Other option in birds: PGCs

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- Alternative approach for gene banking birds: PGCs
  - Isolate PGCs from embryos
  - Propagate clonal cell lines, and freeze them
  - Inject into embryo to produce chimaeric embryos
  - No backcrossing needed provided that male and female PGCs can be cloned and propagated.
- Several publications show it can be done
- But in reality it may not always work or be more difficult than it seems.

# Somatic cells

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- CGN has considered cryobanking of somatic cells to preserve the genotypes of unique, old mares.
- The ethical acceptability of nuclear transfer is debated in Dutch society and government
  - Animal health and welfare?
  - Also other ethical considerations
- Currently, CGN is not applying gene banking of somatic cells.

Thank you for  
your attention

