Lab course: Preparation of native chicken frozen semen

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Purposes of semen cryopreservation in poultry

- Increase the diffusion and measurement of genetic progress.
- Conservation of genetic biodiversity.
- Improve the management of artificial insemination (AI).
- The only efficient method of *ex-situ* management available for avian species.

Review of semen cryopreservation in poultry

Development

Years	Researcher	Fact
1909	Atkins	Freezing points and the effects of low temperatures on hen's eggs
1925	Moran	
1941	Shaffner *et al.*	Fertile eggs could be obtained from hens inseminated with frozen chicken semen
1949	Polge *et al.*	Glycerol protected spermatozoa against the effects of low temperatures
1978	Lake and Stewart	The first successful method: Glycerol／Slow cooling rates／Glass ampoules
1980	Sexton	Dimethyl sulfoxide (DMSO)／Slow cooling／Straws
1991	Schramm	Dimethyl formamide (DMF)／Rapid cooling／Pellets
1995	Tselutin *et al.*	Dimethyl acetamide (DMA)／Rapid cooling／Straws

Current conclusion

The highest fertility rates after AI with frozen semen were obtained either with

Glycerol/Slow cooling/Straws or **DMA/Rapid cooling/Pellets.**

Methods

Cryoprotectants	Glycerol/Dimethyl Acetamide (DMA)/Dimethyl sulfoxide (DMSO)
Procedures	Slow/Rapid freezing-thawing
Packages	Pellets/Ampoules/Straws

Procedures of semen cryopreservation in poultry

Animals

Taiwan Native Roosters include L7, L9, L11, L12 stains were used.

Semen collection

- Semen was collected to a glass beaker by abdominal massage.
- Semen quality tests.

Pre-freezing

- Dilute semen (1:1) with IMV extender at room temperature.
- Diluted semen was cooled to 4℃ over 20 mins.
- The diluted and cooled semen was then mixed with the same diluent (2:1) containing glycerol (33%, v/v) to reach final concentration 11%.
- The diluted semen and cryoprotectant were equilibrated at 4℃ for 30 mins.
- Semen was transferred to 0.5 mL plastic straws, and the straws were sealed.

Freezing

- The semen straws were placed in a programming freezer.
- 2 steps-cooling curve
  A. From 4℃ to -35 ℃ at 7℃/min.
  B. From -35 ℃ to -140℃ at 20℃/min.
- The semen straws were then plunged into liquid nitrogen.

Thawing

- The frozen semen straws were thawed in a water bath at 4℃.
- The straws were quickly opened and the semen was transferred to a microtube.
- The semen was rediluted and glycerol was removed by centrifugation.
- The supernatants were then discarded.
- Semen quality tests/Inseminate immediately after thawing.

Future needs

- The management of genetic diversity which is decreasing mainly due to specific selections of commercial lines.
- Increasing risks of epidemic avian influenza.
- The development of cryobanks to complete the *in-situ* management of avian genetic resources by *ex-situ* management.

Reference

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