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# EFFECTS OF FREEZING ON RAM AND BOAR SPERM

a thesis submitted by

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## Declaration

I declare that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge it does not contain any material previously published or written by another person except where due reference is made in the text.

Betty Y.M.Cheng (nee Ju)

# 論文摘要

程朱友梅

觀查冷凍精子存活的最重要因素，是在冷凍及解凍之後，測定其細胞內酵素的遺失量。本文資料解釋，不同的稀釋液，溫度，甘油的含量，及蛋黃的添加量對 GOT 酵素遺失的影响。上述的影响是與稀釋液中的滲透壓，離子傳導性及酸鹼度有相關性的。GOT 酵素的重要性是測知細胞破損的程度，以及在 30 分鐘內有效的鑑定冷凍精子的品質。

膠質電泳法在本文用來探測蛋白質的遺漏，在冷凍的精液中顯示有特別加深的蛋白質帶。由此可見蛋白質會從受傷的精子遺漏出來。

電子顯微鏡的照片上也表示出在冷凍之後，豬的精虫有非常嚴重的細胞膜破損現象。

由實驗結果可以綜合結論，最適合羊和豬精子冷凍的情況是：

| | 溫度 | 稀釋液 | 酸鹼度(7.4) |
|---|---|---|---|
| 豬 | 37°C | Tris 緩衝液加果糖 | |

| | | |
|---|---|---|
| | 5°C | TES 緩衝液加雞黃及甘油 |
| | 解凍 40°C | Tris 緩衝液加果糖 |
| | 冷凍 −196°C | 不適合 |
| 羊 | 37°C | 精子林格液加果糖 |
| | 解凍 40°C | 精子林格液加果糖 |
| | 冷凍 −196°C | 檸檬酸緩衝液加葡萄糖 |

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Summary

Sperm survival in frozen semen appears to be determined largely by the amount of intracellular enzymes during freezing and thawing. Data are presented to show the effects of different diluents, temperature and glycerol as well as egg yolk concentrations, on glutamic-oxaloacetic-transaminase (GOT) release. The influence of different diluents has been related to their osmolality, ionic conductivity and pH. The importance of the enzyme GOT is that it indicates the degree of cell damage, and also efficiently shows the quality of frozen semen in a 30 minute test.

Polyacrylamide gel electrophoresis has been used to detect extra dense protein bands which appear in frozen samples of semen. It seems that the protein leaks from damaged cells.

Electron microscopy of boar and ram spermatozoa yields a picture of severe disruption of the frozen cell membrane.

It is concluded that the optimal conditions for freezing ram and boar spermatozoa are:

| Animal | Temperature | pH | Diluents |
|---|---|---|---|
| Boar | 37°C | 7.4 | Tris buffer fructose |
| | 5°C | 7.4 | Tes buffer with egg yolk and glycerol |
| | -196°C | 7.4 | Tes buffer with egg yolk and glycerol or BF3 extender |
| | Thawing 40°C | 7.4 | Tris buffer with fructose |
| Ram | 37°C | 7.4 | Sperm Ringer phosphate with fructose |
| | -196°C | 7.4 | Sperm Ringer phosphate with fructose |
| | Thawing 40°C | 7.4 | Citrate glucose |

These diluents which minimize GOT release.