



EFFECTS OF FREEZING ON RAM AND BOAR SPERM



a thesis submitted by

Betty Y. M. CHENG

to the University of Adelaide  
towards the degree of Master of  
Agricultural Science

Department of Animal Physiology  
Waite Agricultural Research Institute  
University of Adelaide  
July 1976

Table of Contents

	1
1. Table of Contents	1
2. Declaration	
3. Acknowledgement	
4. Summary	2
5. Introduction	3
Thesis	
The advantage of frozen semen	
Chapter One Literature Review	
Storage of sheep spermatozoa	3
Experiments with pig spermatozoa	5
Freezing, storage and thawing injury	
The biochemical function of GOT	7
The release of GOT and GPT from ram and boar spermatozoa	7
Protein leakage from frozen semen	8
Electron micrograph description of ram and boar frozen sperm	8
General characteristics of ram and boar semen	9
A. Boar semen	
Constituents of semen	9
Gelatinous material	10
Chemical components	10
a. Choline	11
b. Ergothioneine	
c. Lipids in the seminal plasma	
d. Fructose	12
e. Inositol and sorbitol	
f. Citric acid	13
g. Proteins	
B. Ram semen	14
(1) Grading ram semen	
(2) Quantity of semen and number of spermatozoa	15
Chemical composition and metabolism	15

(3) Protein constituents of ram semen	15
Sperm motility and evaluation of semen quality	15
(A) Evaluation of semen quality	
(i) General methods	
(ii) Histological methods	16
(iii) Chemical and biochemical methods	
(B) Factors which affect sperm motility in <u>vivo</u>	17
(i) Management	
(ii) Temperature and light	
(iii) Nutrition	18
(C) Sperm motility in <u>vitro</u>	19
(1) pH	
(2) Diluents	20
(3) Temperature and storage	20-21
(4) Osmotic pressure	22
(5) Electrolytes	
(6) Female genital tract fluid	23
(7) Toxin	
(8) Nutrients	23-24
 Material and methods	
1. Animal Training	25
2. Semen collection	25
3. Semen evaluation steps	26
4. Live and dead staining	
5. Motility tests	26
a) Wave motion	
b) Oxygen consumption	27
6. Freezing and thawing procedures and media	
a) Ram semen diluents	
b) Boar semen diluents	
7. pH, osmolality and ionic conductivity measurements	28
8. Glutamic-oxaloacetic-transaminase analysis	

Removal of spermatozoa from seminal plasma	
Sample treatment	
Colorimetric reaction for determination of GOT	29
9. Polyacrylamide gel electrophoresis	29
Separation of seminal plasma proteins	
Stain	
10. Electron microscopy of spermatozoa	30
Procedure of washing spermatozoa	
Fixation	
Dehydration	
Embedding	
Sectioning	
Staining	
Results	
1. Oxygen consumption of ram and boar spermatozoa	31
Effects of diluents	31-32
Comparison with solution of different osmolality	33
2. GOT release of ram and boar spermatozoa	
Effects of different diluents on GOT release	34
Ram spermatozoa	35-36
Boar spermatozoa	37-39
3. Disc electrophoresis	40
Boar spermatozoa	40
Ram spermatozoa	41
4. Electron microscopy of ram and boar spermatozoa before and after freezing	42
Ram spermatozoa	
Boar spermatozoa	
Discussion	43
Section 1. Frozen semen evaluation by GOT enzyme method	

Section 2. Protein leakage and role of reaction and low temperature	44
Section 3. Ultrastructure of frozen spermatozoa	45
Section 4. Protein leakage and polyacrylamide gel electrophoresis	46
Conclusion	
The best diluent for ram spermatozoa	47
The best diluent and temperature for storage boar spermatozoa	48
Appendix	
References	

### Acknowledgements

I hereby wish to thank all persons connected with the production of this thesis. In particular I wish to acknowledge the guidance and supervision of Professor W. V. Macfarlane during the experimental work and writing of this thesis. Thanks are due also to Dr. D.E. Brooks for his inspiration.

Further I wish to thank Dr. R. Bais, Mr. R. Miles and Mr. B. Palk for technical assistance, Mr. P. Heap and Mr. B. Stone of the Pig Research Unit, Department of Agriculture for their cooperation in the boar semen collection and many others. To my husband Charles C.K. Cheng with my sincere thanks for encouragement and company throughout the experiment.

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)

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 酵素遺失的影響。上述的影響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 緩衝液加蛋黃及甘油

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

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			
Ram	40°C	7.4	Tris buffer with fructose
	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.