

Benha University, Faculty of Veterinary Medicine, **Department of Theriogenology**

Study of Some Factors Affecting Efficiency of Frozen Buffalo Semen

A Thesis Presented By Mohammed Ismail Abd El-Kader

(B. V. Sc. 2004, South Valley University)

For M. V. Sc. Degree of Theriogenology

Under Supervision of Prof.Dr. Abd El-Salam I. EL-Azab

> **Professor of Theriogenology Faculty of Veterinary Medicine Benha University**

and

Prof.Dr. Reda I. El Sheshtawy

Professor of Artificial insemination Dept. Animal Reproduction &A.I **National Research Center**

2010

Acknowledgment

First of all, Prayer of full thanks to our merciful God.

I wish to express my deep thanks to Prof. **Dr. Abd El-Salam I. EL-Azab**, Professor of Theriogenology, Faculty of Veterinary Medicine, Benha University for his supervision, continuous support, criticism and valuable assistance throughout the course of this study.

I want to express my sincere gratitude and thanks to Prof. **Dr. Reda I. El-Sheshtawy**, Professor of Artificial insemination, Department of Animal Reproduction &A.I, National Research Center; for his valuable supervision, ideal guidance, continuous advice, great financial support and encouragement.

My deep gratitude to Dr. **Gamal Attia Abdel-Hamid El-Sisy**, Assistant professor of Animal Reproduction and Artificial Insemination, National Research Center, for his kind help, great cooperation and valuable advice.

My deep thanks to all staff members of Theriogenology Department, Faculty of Veterinary Medicine, Benha University and all staff members of Animal Reproduction and Artificial Insemination, National Research Center, Dokki – Giza, and my deep precious thanks for all members of the Center of the frozen semen production for Buffaloes, General Organization for Veterinary Services, Ministry of agriculture, Abbasia.

То Му	Parents.	• • • • • •	• • • • •	••••	• • • •	• • • • •
То Му	Family.	• • • • • •			• • • •	• • • • •

السيرة الذاتية للباحث

الإسم: محمد إسماعيل عبد القادر محمد شهبة

تاريخ الميلاد: 1982/9/20

محل الميلاد: قرية سرابيوم - الإسماعيلية

المؤهلات الدراسية:

*الشهادة الإبتدائية عام 1993 من مدرسة عمر بن الخطاب الإبتدائية بقرية سرابيوم.

*الشهادة الإعدادية عام 1996 من مدرسة سعد زغلول الإعدادية بنين بقرية سرابيوم.

*الثانوية العامة عام 1999 من مدرسة سرابيوم الجبل المشتركة بقرية سرابيوم.

*بكالوريوس العلوم الطبية البيطرية عام 2004 من جامعة جنوب الوادى- فرع قنا .

*الباحث مسجل لدرجة الماجستير في العلوم الطبية البيطرية تخصص الولادة وأمراض التناسل

والتلقيح الاصطناعي في عام 2007.

الوظيفة الحالية: مساعد باحث بالمركز القومي للبحوث

shahbavet@yahoo.com : البريد الإلكتروني

VITA

- *The reasercher **Mohammed Ismail** was born at 20/9/1982 in Serabium, Ismailia.
- *Primary education was completed in Omar Ibn El-Khatab primary school in 1993.
- *Preparatory education was completed in Saad Zaghlol preparatory boys school in 1996.
- *Secondary education was copmpleted in Serabium secondary scool in 1999.
- *Undergraduate and professional education was completed in faculty of veterinary medicine, South valley university, Quena branch in 2004.
- *The reasercher had been occupying the assisstant researcher degree in the national research center since 2007.
- *The researcher enrolled for M.V.Sc degree in 2007.

Contents page	ge
Introduction	1
Review of Literature	2
1. Effect of low density lipoprotein (LDL)	5
2. Effect of glycerol	7
3. Effect of disaccharides	11
3.1. Effect of trehalose on freezability	. 13
3.2. Effect of sucrose on freezability	. 17
4. Effect of some commercially available extenders	. 19
Materials and Methods	25
1. Procedures	26
2. Experimental design	27
3. Semen quality assessment after chilling and post-thawing	31
4. Statistical analysis	33
Results	34
1. Effect of different concentration of LDL on buffalo	
semen freezability	34
2. Effect of disaccharides (trehalose and sucrose) with different	
glycerol concentrations on buffalo semen freezability	44
2.1-Effect of trehalose with different glycerol concentrations	
on buffalo semen freezability	44
2.2-Effect of sucrose with different glycerol concentrations on	
buffalo semen freezability	54
3. Effect of glycerol with different concentrations of disaccharides	
(Trehalose and sucrose) on buffalo semen freezability	64

3.1- Effect of glycerol with different trehalose concentrations on	
buffalo semen freezability	64
3.2- Effect of glycerol with different sucrose concentrations on	
buffalo semen freezability	74
4. Effect of some commercially available extenders containing	
egg-yolk (Triladyl®)or milk (Laiciphos®) or soybean-based	
(Bioxcell®, IMV, France) extender on sperm characteristics	
of frozen buffalo bull semen	84
Discussion	94
Summary	102
References	105
Arabic Summary.	

List	of	Tab	les
	-	_ •• ~	

Tables	page
1. Diluents with different glycerol concentrations	. 29
2. Diluents with different sugars concentrations	. 30
3. Effect of different LDL concentrations on sperm motility (%)	
of chilled and frozen buffalo semen (Means ± SEM)	. 35
4. ANOVA showing the effect of different LDL concentrations on	
sperm motility (%) of chilled and frozen buffalo semen	. 35
5. Effect of different LDL concentrations on sperm liveability (%)	
of chilled and frozen buffalo semen (Means ± SEM)	. 37
6. ANOVA showing the effect of different LDL concentrations on	
sperm liveability (%) of chilled and frozen buffalo semen	37
7. Effect of different LDL concentrations on sperm abnormalities (%)	
of chilled and frozen buffalo semen (Means ± SEM)	. 39
8. ANOVA showing the effect of different LDL concentrations on	
sperm abnormalities (%) of chilled and frozen buffalo semen	. 39
9. Effect of different LDL concentrations on sperm membrane integrity (%)
of chilled and frozen buffalo semen (Means ± SEM)	41
10. ANOVA showing the effect of different LDL concentrations on sper	m
membrane integrity (%) in chilled and frozen buffalo semen	41
11. Effect of different LDL concentrations on sperm DNA integrity (%)	
of chilled and frozen buffalo semen (Means ± SEM)	43
12. ANOVA showing the effect of different LDL concentrations on sper	m
DNA integrity (%) of chilled and frozen buffalo semen	43
13. Effect of Trehalose with different Glycerol concentrations on sperm	
motility (%) of chilled and frozen buffalo semen (Means \pm SEM)	45

25. Effect of Sucrose with different Glycerol concentrations on sperm	
liveability (%) of chilled and frozen buffalo semen (Means ± SEM)	.57
26. ANOVA showing the effect of Sucrose with different Glycerol	
concentrations on sperm liveability (%) of chilled and frozen	
buffalo semen	57
27. Effect of Sucrose with different Glycerol concentrations on sperm	
abnormalities (%) of chilled and frozen buffalo semen (Means \pm SEM)	59
28. ANOVA showing the effect of Sucrose with different Glycerol	
concentrations on sperm abnormalityies (%) of chilled and frozen	
buffalo semen	.59
29. Effect of Sucrose with different Glycerol concentrations on sperm	
membrane integrity (%) of chilled and frozen buffalo semen	
(Means ± SEM)	61
30. ANOVA showing the effect of Sucrose with different Glycerol	
concentrations on sperm membrane integrity (%) of chilled and	
frozen buffalo semen	61
31. Effect of Sucrose with different Glycerol concentrations on sperm DNA	
integrity (%) of chilled and frozen buffalo semen (Means ± SEM)	63
32. ANOVA showing the effect of Sucrose with different Glycerol	
concentrations on sperm DNA integrity (%) of chilled and frozen	
buffalo semen	63
33. Effect of Glycerol with different Trehalose concentrations on sperm	
motility (%) of chilled and frozen semen (Means ± SEM)	65
34. ANOVA showing the effect of Glycerol with different Trehalose	
concentrations on sperm motility (%) of chilled and frozen buffalo semen	65
35. Effect of Glycerol with different Trehalose concentrations on sperm	
liveability (%) of chilled and frozen buffalo semen (Means \pm SEM)	67

36. ANOVA showing the effect of Glycerol with different Trehalose
concentrations on sperm liveability (%) of chilled and frozen
buffalo semen
37. Effect of Glycerol with different Trehalose concentrations on sperm
abnormalities (%) of chilled and frozen buffalo semen (Means \pm SEM) 69
38. ANOVA showing the effect of Glycerol with different Trehalose
concentrations on sperm abnormalities (%) of chilled and frozen
buffalo semen
39. Effect of Glycerol with different Trehalose concentrations on sperm
membrane integrity (%) of chilled and frozen buffalo semen
(Means \pm SEM)71
40. ANOVA showing the effect of Glycerol with different Trehalose
concentrations on sperm membrane integrity (%) of chilled and
frozen buffalo semen
41. Effect of Glycerol with different Trehalose concentrations on sperm
DNA integrity (%) of chilled and frozen buffalo semen (Means \pm SEM) 73
42. ANOVA showing the effect of Glycerol with different Trehalose
concentrations on sperm DNA integrity (%) of chilled and
frozen buffalo semen73
43. Effect of Glycerol with different Sucrose concentrations on sperm
motility (%) of chilled and frozen buffalo semen (Means \pm SEM)
44. ANOVA showing the effect of Glycerol with different Sucrose
concentrations on sperm motility (%) of chilled and frozen buffalo semen75
45. Effect of Glycerol with different Sucrose concentrations on sperm
liveability (%) of chilled and frozen buffalo semen (Means \pm SEM)
46. ANOVA showing the effect of Glycerol with different Sucrose
concentrations on sperm liveability (%) of chilled and frozen

buffalo semen
47. Effect of Glycerol with different Sucrose concentrations on sperm
abnormalities (%) of chilled and frozen buffalo semen (Means \pm SEM) 79
48. ANOVA showing the effect of Glycerol with different Sucrose
concentrations on sperm abnormalities (%) of chilled and frozen
buffalo semen
49. Effect of Glycerol with different Sucrose concentrations on sperm
membrane integrity (%) of chilled and frozen buffalo semen
(Means ± SEM)
50. ANOVA showing the effect of Glycerol with different Sucrose
concentrations on sperm membrane integrity (%) of chilled and frozen
buffalo semen
51. Effect of Glycerol with different Sucrose concentrations on sperm DNA
integrity (%) of chilled and frozen buffalo semen (Means ± SEM)83
52. ANOVA showing the effect of Glycerol with different Sucrose
concentrations on sperm DNA integrity (%) of chilled and frozen
buffalo semen
53. Effect of some commercial available extenders on sperm motility (%)
of chilled and frozen buffalo semen (Means \pm SEM)
54. ANOVA showing the effect of some commercial available extenders on
sperm motility (%) of chilled and frozen buffalo semen
55. Effect of some commercial available extenders on sperm liveability (%)
of chilled and frozen buffalo semen (Means \pm SEM)
56. ANOVA showing the effect of some commercial available extenders on
sperm liveability (%) of chilled and frozen buffalo semen87
57. Effect of some commercial available extenders on sperm abnormalities
(%) of chilled and frozen buffalo semen (Means ± SEM)89

58. ANOVA showing the effect of some commercial available extenders
on sperm abnormalities (%) of chilled and frozen buffalo semen89
59. Effect of some commercial available extenders on sperm membrane
integrity (%) of chilled and frozen buffalo semen (Means \pm SEM)
60. ANOVA showing the effect of some commercial available extenders on
sperm membrane integrity (%) of chilled and frozen buffalo semen 91
61. Effect of some commercial available extenders on sperm DNA
integrity (%) of chilled and frozen buffalo semen (Means \pm SEM)93
62. ANOVA showing the effect of some commercial available extenders on
sperm DNA integrity (%) of chilled and frozen buffalo semen

List of Figures Figures page 35 1. Effect of LDL on sperm motility of chilled and frozen buffalo semen 37 2. Effect of LDL on live sperm (%)of chilled and frozen buffalo semen 3. Effect of LDL on percentage of sperm abnormalities of chilled and 39 frozen buffalo semen 41 4. Effect of LDL on intact sperm % of chilled and frozen buffalo semen 5. Effect of LDL on sperm DNA integrity of chilled and frozen buffalo 43 semen 6. Effect of Trehalose with different Glycerol concentrations on sperm 45 motility of chilled and frozen buffalo semen 7. Effect of Trehalose with different Glycerol concentrations on live 47 sperm% of chilled and frozen buffalo semen 8. Effect of Trehalose with different Glycerol concentrations on sperm 49 abnormalities% of chilled and frozen buffalo semen 9. Effect of Trehalose with different Glycerol concentrations on sperm 51 membrane integrity of chilled and frozen buffalo semen 10. Effect of Trehalose with different Glycerol concentrations on sperm 53 DNA integrity of chilled and frozen buffalo semen 11. Effect of Sucrose with different Glycerol concentrations on sperm 55 motility of chilled and frozen buffalo semen 12. Effect of Sucrose with different Glycerol concentrations on live 57 sperm % of chilled and frozen buffalo semen 13. Effect of Sucrose with different Glycerol concentrations on sperm 59 abnormalities% of chilled and frozen buffalo semen 14. Effect of Sucrose with different Glycerol concentrations on sperm 61 membrane integrity of chilled and frozen buffalo semen