

شبكة المعلومات الجامعية







شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأفلام قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار في درجة حرارة من ١٥-٥٠ مئوية ورطوبة نسبية من ٢٠-٠٠% To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%



بعض الوثائـــق الإصليــة تالفــة



بالرسالة صفحات لم ترد بالإصل

Cyon

Cairo University

Faculty of Veterinary Medicine

Department of Theriogenology

In Vitro Maturation and fertilization of Camel oocytes

_ _

Thesis presented
by
Adel Reda Moawad
(B.V. Sc. 2000; Cairo University)

for

M.V. Sc. (Theriogenology)

Under Supervision

Prof. Dr. Abou Bakr A. El-Wishy
Prof. of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Gamal M. Darwish

Head Researcher in A.I. and E.T. Dept.

Animal Reproduction Research Institute
Agriculture Research Center

بنيالتالخرالجي

قَالُواْ سُبْحَانِكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآ إِنَّكَ

أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ اللهِ

صدق الله العظيم (سورة البقرة آية : ٣٢)

To Whom I Love FATHER, MOTHER, BROTHER, SISTER

Cairo University

Faculty of Veterinary Medicine Department of Theriogenology

Approval Sheet

This is to approve that the dissertation presented by:

Adel Reda Moawad

To Cairo University

Faculty of Veterinary Medicine

Entitled:

In Vitro Maturation and fertilization of Camel oocytes

For M.V.Sc. Degree (Theriogenology)

Is approved by the Examination Committee

Dr. Bahy Hussein Mohamed Sorror

Prof. and Head of Theriogenology

Faculty of Vet. Med., Kafr El-Shaikh, Tanta University Sonerin E(wishu)

Dr. Ibrahim Mostafa Ghoneim

Prof. of Theriogenology, Faculty of Vet. Med.

Cairo University

Dr. Abou Bakr Abdel-Kader El-Wishy

Prof. of Theriogenology, Faculty of Vet. Med.

Cairo University

Cairo University

Faculty of Veterinary Medicine Department of Theriogenology

Name : Adel Reda Moawad

Date of birth : 27/8/1977

Place : Giza

Nationality : Egyptian Degree : M.V. Sc.

Specialization: Theriogenology

Title of Thesis : In vitro maturation and fertilization of camel

oocytes

Supervisor: Prof. Dr. Abou Bakr A. El-Wishy Prof. of

Theriogenology, Faculty of Veterinary

Medicine, Cairo University

Prof. Dr. Gamal M. Darwish Head

Researcher in A.I. and E.T. Dept. Animal

Reproduction Research Institute, Agriculture

Research Center.

Abstract

A total number of 1225 ovaries of pregnant and non-pregnant dromedary camels slaughtered at Cairo and Giza slaughter houses were collected during the period between March 2003 and April 2004. The oocytes were aspirated from 2-6 mm follicles. The oocytes were classified into 4 grades, then cultured in TCM-199 + 10% FCS for 24, 30, 36 or 48 hours. Mature oocytes were inseminated with caffeine (5mM) capacitated epididymal spermatozoa at concentration of 1, 2, 3 or 4X10⁶ sperm cell / ml. Presumptive zygotes were cultured in H-TCM-199 at 39°C in 5% Co₂ for 9 days. The results indicated that, gravid organs resulted in significantly lower (P<0.05) oocyte yield, quality and maturation rate than non-gravid organs. Collection of oocytes during autumn and winter significantly increase the percentage of selected oocytes and maturation rate than spring and summer. Maturation rate of camel oocytes was highest (72.30%) for 36 hours culture period. The fertilization rate was highest (29.68%) for oocytes inseminated with caffeine capacitated camel epididymal spermatozoa at 2 X 10⁶ sperm cell /ml in BSA containing F-TALP medium. The cleavage of camel oocytes up to 16 cell stage was noted 18 to 24 hours after IVF, while the morula and blastocyst stages began to appear after 4 and 5 days, respectively.

Key words: Camel, Oocyte, IVM, Epididymis, IVF, IVC

Contents

	Page
Introduction	1
Review of literature	5
1- Reproductive physiology of female camels	5
1-1- Morphological features of the ovaries and ovarian	contents 5
of camels.	
1-2- Seasonality of reproduction	7
1-3- Oestrous cycle and follicular waves	8
1-4- Ovulation	9
1-5- Development of the corpus luteum	10
2- In vitro fertilization (IVF) Technology	11
2-1- Historical overview	11
2-2- In vitro production of embryos in farm animals	
3- Oocyte collection	14
3-1- In vivo methods for oocyte recovery	15
3-1-1- Surgical method for oocyte collection	
3-1-2-Transvaginal ultrasound-guided follicular as	spiration 16
3-2- Oocyte recovery from slaughtered animals	17
4. In Vitro maturation (IVM)	24
4-1- Factors affecting IVM	
4.1.1. Media used in IVM	25
4.1.1.2. Effect of media supplements	26
4.1.1.3 Effect of serum and albumin on IVM	27
4.1.1.4. Effect of PVA or PVP, (defined medium)	on IVM 29
4.1.15. Effect of hormone supplementation	31
4.1.2. Role of cumulus cells in IVM	38
4.1.3. Time required for in vitro maturation	42

	Page
5. In vitro fertilization (IVF):	46
5.1. Sperm Capacitation	46
5.1.1. High ionic strength medium	48
5.1.2. Heparin and / or Caffeine	49
5.1.3. Uses of calcium ionophore A23187	54
5.1.4. Uses of theophylline	56
5.2. Hyperactivation	57
5.3. Camel epididymal sperm and IVF	58
Materials and Methods	60
1. Collection of ovaries	61
2. Oocyte recovery and quality	62
3. Oocyte maturation in vitro	64
3.1. Staining of oocytes	65
4. In vitro fertilization of oocytes (IVF)	66
4.1. Recovery and Preparation of epididymal sperm for	66
IVF	
4.1.1. Technique of epididymal flushing	66
4.1.2. Processing of epididymal sperm	69
4.2. In vitro insemination of oocytes	69
5. In vitro culture	72
Results	74
1. Oocyte yield and quality	74
2. Camel oocyte maturation in vitro	83
3. In vitro fertilization using epididymal sperm	97
4. In vitro culture of camel embryos	104
Discussion	119
Summary	139
Conclusion	145
References	147
Arabic summary	

List of Tables

	Page
Table 1: Effect of reproductive status (pregnant vs. non-pregnant)	75
on oocyte recovery and quality.	
Table 2: Effect of month of collection on camel oocyte yield and	78
quality.	
Table 3: Effect of season on camel oocyte yield and quality.	80
Table 4: Influence of post- slaughter storage time of ovaries on	82
recovery rate and quality of camel oocytes.	
Table 5: Effect of maturation media on camel oocyte maturation	84
in vitro.	85
Table 6: Effect of reproductive status on maturation rate of camel	63
oocytes in vitro.	0.7
Table 7: In vitro maturation of camel oocytes during different months.	87
Table 8: Seasonal variation in camel oocyte maturation rate.	88
	90
Table 9: Effect of post – slaughter storage time of ovaries on camel oocyte maturation in vitro.	, ,
	91
Table 10: Influence of cumulus cell layers on in vitro maturation	71
of camel oocytes.	
Table 11: Effect of culture medium supplementation on in vitro	93
maturation of camel oocytes.	
Table 12: Effect of different concentrations of FCS on in vitro	94
maturation of camel oocytes.	

	Page
Table 13: Effect of supplementation of the maturation medium	95
containing 10% FCS with gonadotrophic hormones on in vitro	
maturation of camel oocytes.	
Table 14: Influence of culture duration on maturation rate of	96
camel oocytes.	
Table 15: Effect of different capacitating materials on motility of	97
camel epididymal spermatozoa.	
Table 16: Effect of sperm cell concentration on in vitro	99
fertilization of camel oocytes.	404
Table 17: Effect of different capacitating materials on rates of	101
sperm penetration and fertilization of camel oocytes.	
Table 18: Effect of post-slaughter flushing time of epididymal	102
spermatozoa on camel oocyte fertilization in vitro.	
Table 19: Effect of defined and undefined F-TALP medium on in	103
vitro fertilization of camel oocytes.	
Table 20: Effect of sperm capacitating materials on camel embryo	105
development in vitro cultured in H- TCM-199+5% FCS.	
Table 21: In vitro maturation of camel oocytes compared with	126
other species.	
Table 22: In vitro fertilization of camel oocytes compared with	134
other species	
Table 23: In vitro development of camel embryos compared with	138
other species	

List of figures

	Page
Fig. 1: Oocyte aspiration technique	63
Fig. 2: Testis and epididymis of mature camel	67
Fig. 3: Epididymis of mature camel	67
Fig. 4: Needle inserted in the body of the epididymis before	68
pushing of the flushing media	
Fig. 5: Note the engorged body of the epididymis after pushing of	70
the flushing media (S-TALP) into the epididymal tract	
Fig. 6: Receiving driblets of flushed spermatozoa in a tissue	71
culture dish	
Fig. 7: Non pregnant camel ovaries	106
Fig. 8: Ovaries with CL of pregnancy	106
Fig. 9: Camel oocyte (grade I) surrounded by mutlilayres of	107
cumulus cells	
Fig. 10: Partially denuded camel oocyte	107
Fig. 11: Completely denuded camel oocyte (Grade III)	108
Fig. 12: Grade IV camel oocyte with broken zona pellucida	108
(discarded oocyte)	
Fig. 13: In vitro matured camel oocyte with expanded cumulus	109
cells	
Fig. 14: In vitro matured camel oocyte with extrusion of first	109
polar body	
Fig. 15: In vitro matured camel oocyte with metaphase spindle	110
(stained with orcein stain)	
Fig. 16: Camel oocyte with metaphase II stage (stained with	110