

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Massey University Library
Palmerston North
Turitea

A STUDY OF COMMERCIAL EMBRYO TRANSFER PROGRAMMES

CONDUCTED WITH TEXEL AND AWASSI SHEEP

A thesis presented in partial fulfilment of the requirements for
the degree of Master of Agricultural Science
in Animal Science at Massey University

ANDREW EDWARD DRUMMOND PAVITT

1997

Abstract

The results of two commercial embryo transfer (ET) programmes conducted in Central Hawkes Bay, comprising 93 Texel and 78 Awassi donor ewes, were analysed to identify variables that affect the success of commercial ET programmes. The production of high quality embryos for export was the primary objective in the Texel programme, while the rapid multiplication of the Awassi was the sole purpose of the Awassi programme. Reproductive parameters such as; ovarian response to exogenous gonadotrophins, recovery and fertilisation rate of ova, yield of good quality embryos and embryo survival rate to scanning and birth were evaluated. The influence of age, ovulatory response, repeated flushing, the number of corpora lutea in recipient ewes and donor surgeon, on the reproductive parameters, were assessed.

The Texel ewes were all purebred consisting of two-tooth and four-tooth animals. The Awassi ewes were either three-quarter or purebred, and included both ewe hoggets and mixed age ewes.

Synchronisation of oestrus was attempted using a double CIDR-GTM regime. The super ovulatory programme differed in each breed but consisted of a series of FSHp injections in combination with PMSG. The ewes were inseminated *intra-uterine* with fresh diluted semen from a ram of the same breed after detection of oestrus. Embryo recovery was attempted 6-6.5 days after insemination using a standard flushing technique in which the oviducts and uterus were exposed by mid-ventral laparotomy. Two or three embryos were transferred into each recipient ewe within 1.5 hours (Texel) and 4 hours (Awassi) of recovery.

Ovarian response to superovulatory treatments was not significantly affected by any of the variables recorded in this study, although the Texel programme provided a higher ovulatory response than the Awassi programme (8.89 CL vs 7.08 CL). The embryo recovery rates were 71.8% for the Texel and 78.1% for the Awassi sheep. Age of the donor ewes significantly affected ($p=0.006$) recovery rate of ova in the Awassi sheep, adult ewes and ewe hoggets recorded recovery rates of 90.7% and 65.2%. Fertilisation

rate was not affected by any of the factors studied with 94.6% and 77.6%, of the Texel and Awassi ova, being fertilised. The overall yield of good quality embryos was 91.6% for the Texel and 80.1% for the Awassi. The yield of good quality embryos was significantly affected ($p=0.03$) by age in the Awassi, 87.5% of adult embryos and 64.4% of ewe hogget embryos were of good quality. Survival rates in the Awassi data set were significantly affected by the lower scanning and birth rates of the Awassi ewe hoggets than Awassi ewes (43.8% vs 65.9%: $p=0.02$) and (39.3% vs 59.4%: $p=0.03$) respectively. Each Texel donor ewe produced, on average, 3.89 embryos that were of sufficient quality to be preserved and therefore not transferred, resulting in 0.70 lambs being born per donor ewe in the Texel programme. The Awassi programme produced 2.10 lambs donor per ewe, however, this was significantly affected ($p=0.03$) by the age of the donor ewes, with adult ewes produced an average of 2.7 lambs per donor, and ewe hoggets, 1.2 lambs per donor.

This work demonstrated the variable nature of embryo transfer programmes as well as the difficulty in achieving acceptable results from ewe hoggets. The Texel and adult Awassi results compared favourably to the other published reports and illustrate that on-farm commercial embryo transfer can provide acceptable results.

Acknowledgements

I wish to thank my supervisor Associate Professor M.F. McDonald for his guidance and assistance during the preparation of this manuscript.

Special thanks also to Trevor Cook and his ET staff at Manawatu Veterinary Services for their invaluable assistance.

Thanks also to Angela and Jamie Molloy of Awassi New Zealand Ltd and Richard Lee of Vet Services, Hawkes Bay for sharing their knowledge and information.

I am grateful to all the staff and students of the Animal Science Department who have assisted me during my studies. In particular I would like to acknowledge the support of Richard Spelman and Paul Charteris who have provided a great deal of assistance.

Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	v
List of Tables	ix
List of Figures	xi
List of Abbreviations	xii

Chapter One

<u>Introduction</u>	1
---------------------	---

Chapter Two

<u>Literature Review</u>	3
2.1 Oestrus Synchronisation	3
2.2 Superovulation	5
2.2.1 Physiology of the oestrous cycle and ovulation	5
2.2.2 Manipulating ovulation rate using exogenous gonadotrophins	8
2.2.3 LH/ FSH ratios	11
2.2.4 Dose of gonadotrophin	11
2.2.5 Timing of administration	12
2.2.6 On-farm hormonal regimes	12
2.3 Embryo Recovery	13
2.3.1 Day of flush	14
2.3.2 Ovulation rates	15
2.3.3 Repeated flushing	15
2.4 Embryo Survival	16
2.4.1 Donor-recipient oestrus synchrony	16
2.4.2 Number of embryos transferred	17
2.4.3 Site of transfer	18

2.4.4	Effect of supplementary progesterone	18
2.5	Technical Factors Influencing the Success of Embryo Transfer	19
2.5.1	Artificial insemination technique	19
2.5.2	Timing of insemination	20
2.5.3	Embryo recovery technique	21
2.5.4	Embryo transplant technique	22
2.6	Embryo Preservation	23
2.6.1	Freezing medium	23
2.6.2	Process of freezing	24
2.6.3	Thawing process	24
2.6.4	Cryoprotectant removal	24
2.7	Implementation of a MOET Programme	25
Chapter Three		
	<u>Materials and Methods</u>	30
3.1	Texel Programme	30
3.1.1	Donor and recipient ewes	30
3.1.2	Rams	30
3.1.3	Synchronisation of oestrus	31
3.1.4	Superovulatory regime	31
3.1.5	Artificial insemination technique	31
3.1.6	Embryo recovery	32
3.1.7	Embryo classification	33
3.1.8	Transfer to recipients	34
3.1.9	Management of recipients	34
3.1.10	Embryo preservation	35
3.2	Awassi Programme	36
3.2.1	Donor and recipient ewes	36

3.2.2 Rams	37
3.2.3 Synchronisation of oestrus	37
3.2.4 Superovulatory regime	37
3.2.5 Artificial insemination technique	38
3.2.6 Embryo recovery	38
3.2.7 Embryo classification	39
3.2.8 Transfer to recipients	39
3.2.9 Management of recipients	39
3.3 Analysis of Data	40
3.3.1 Ovulation rate and recovery rate	40
3.3.2 Fertilisation rate, yield of good quality embryos and embryo survival to scanning and birth.	40
Chapter Four	
<u>Results</u>	42
4.1 Ovulation Rate	42
4.2 Recovery Rate	43
4.3 Fertilisation Rate	45
4.4 Embryo Quality	47
4.5 Embryo Survival to Scanning	48
4.6 Embryo Survival to Birth	50
Chapter Five	
<u>Discussion and Conclusions</u>	52
5.1 Ovulation Rate	52
5.2 Recovery Rate	54

5.3 Fertilisation Rate	56
5.4 Embryo Quality	58
5.5 Embryo Survival to Scanning and Birth	59
5.6 Number of Lambs Produced	60
Chapter Six	
<u>References</u>	62
Chapter Seven	
<u>Appendix I</u>	77
<u>Appendix II; Breed descriptions</u>	
Texel	80
Awassi	82

List of Tables

2.1	Effect of superovulatory regime on the number of embryos transferred per donor ewe programmed	13
2.2	Embryo development rate	14
2.3	Effect of timing of insemination after CIDR withdrawal on the number of embryos obtained	21
3.1	Embryo classifications	33
3.2	Dates of embryo collection in the Awassi programme	36
3.3	Superovulatory regime for Awassi hoggets and ewes	38
4.1.1	Overall ovulation rate for each breed	42
4.1.2	Ovulation rate for each donor breed by donor age combination	42
4.2.1	Overall recovery rate for each breed	43
4.2.2	Recovery rate for each donor breed by donor age combination	43
4.2.3	Effect of repeated flushing on recovery rate in the Awassi data set	43
4.2.4	Effect of ovulatory response on recovery rate in the Awassi data set	44
4.3.1	Overall fertilisation rate for each breed	45
4.3.2	Fertilisation rate for each donor breed by donor age combination	45
4.4.1	Yield of good quality embryos for each breed	47
4.4.2	Yield of good quality embryos for each donor breed by donor age combination	47
4.5.1	Embryo survival rate to scanning for each breed	48
4.5.2	Embryo survival to scanning for each donor breed by donor age combination	48
4.5.3	Effect of the number of corpora lutea on the survival rate to scanning for the Awassi data set	48
4.6.1	Embryo survival to birth for each breed	50
4.6.2	Embryo survival to birth each donor breed by donor age combination	50
4.6.3	Effect of the number of corpora lutea on the survival rate to birth in the Awassi data set	50
7.2.1	Individual recovery rate for each breed	77
7.2.2	Percentage of embryos recovered relative to surgeon in the Texel data set	77

List of Tables

2.1	Effect of superovulatory regime on the number of embryos transferred per donor ewe programmed	13
2.2	Embryo development rate	14
2.3	Effect of timing of insemination after CIDR withdrawal on the number of embryos obtained	21
3.1	Embryo classifications	33
3.2	Dates of embryo collection in the Awassi programme	36
3.3	Superovulatory regime for Awassi hoggets and ewes	38
4.1.1	Overall ovulation rate for each breed	42
4.1.2	Ovulation rate for each donor breed by donor age combination	42
4.2.1	Overall recovery rate for each breed	43
4.2.2	Recovery rate for each donor breed by donor age combination	43
4.2.3	Effect of repeated flushing on recovery rate in the Awassi data set	43
4.2.4	Effect of ovulatory response on recovery rate in the Awassi data set	44
4.3.1	Overall fertilisation rate for each breed	45
4.3.2	Fertilisation rate for each donor breed by donor age combination	45
4.4.1	Yield of good quality embryos for each breed	47
4.4.2	Yield of good quality embryos for each donor breed by donor age combination	47
4.5.1	Embryo survival rate to scanning for each breed	48
4.5.2	Embryo survival to scanning for each donor breed by donor age combination	48
4.5.3	Effect of the number of corpora lutea on the survival rate to scanning for the Awassi data set	48
4.6.1	Embryo survival to birth for each breed	50
4.6.2	Embryo survival to birth each donor breed by donor age combination	50
4.6.3	Effect of the number of corpora lutea on the survival rate to birth in the Awassi data set	50
7.2.1	Individual recovery rate for each breed	77
7.2.2	Percentage of embryos recovered relative to surgeon in the Texel data set	77

7.2.3	Effect of ovulatory response on recovery rate for the Texel data set	77
7.2.4	Effect of previous flushing on recovery rate in the Awassi data set	77
7.3.1	Effect of the sire on the fertilisation rate in the Texel data set	78
7.3.2	Effect of the sire on the fertilisation rate in the Awassi data set	78
7.3.3	Effect of ovulatory response on fertilisation rate in the Texel data set	78
7.3.4	Effect of ovulatory response on fertilisation rate in the Awassi data set	78
7.3.5	Effect of previous flushing on fertilisation rate in the Awassi data set	79
7.4.1	Effect of ovulatory response on embryo quality in the Texel data set	79
7.4.2	Effect of ovulatory response on embryo quality in the Awassi data set	79

List of Figures

Figure I	Laparoscopic examination of the ovaries	32
Figure II	Flushing of the uterus	32
Figure III	Transfer of embryos into recipient ewes	34
Figure IV	Texel ram	80
Figure V	Awassi ram	82

List of Abbreviations

ACP	Acetylpromazine
AI	Artificial insemination
BSA	Bovine serum albumin
°C	Degrees Celsius
CIDR	Controlled internal drug release
CL	Corpus luteum
CO ₂	Carbon dioxide
DMSO	Dimethyl sulfoxide
FGA	Flurogestone acetate
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
HAP	Horse anterior pituitary extract
hMG	Human menopausal gonadotrophin
i.u	International units
LH	Luteinizing hormone
MAP	Medroxyprogesterone acetate
mg	Milligrams
ml	Millilitres
MOET	Multiple ovulation embryo transfer
No.	Number of
pFSH	Porcine follicle stimulating hormone
PMSG	Pregnant mares' serum gonadotrophin
PBS	Phosphate buffered saline
SE	Standard error