

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.



γίγονται δὲ τῶν ἵππων αἱ μὲν καὶ ἄτεκνοι
ὄλως, αἱ δὲ συλλαμβάνουσι μὲν, οὐ δύναται
δ' ἐκφέρειν.

*Some mares are completely sterile; some
conceive, but cannot complete their
pregnancy:...*

*Aristotle
Historia Animalium, VI. xxii*

AN INVESTIGATION OF MOSAIC/CHIMAERISM
IN THE DOMESTIC HORSE (Equus caballus caballus)

A thesis presented in partial
fulfilment of the requirements for the degree of
Doctor of Philosophy in
Cytogenetics at
Massey University

Karen Sheila Walker

1980

81152.58

ABSTRACT

This study involved three aspects of the field of Cytogenetics applied to the domestic horse (E. caballus caballus). The first aspect, the identification and investigation of individuals with cytogenetic anomalies, is fundamental to Cytogenetics. The individuals discovered, however, pointed to the close interrelationship of Cytogenetic and Genetic research. Thirdly, questions of fertility, and in one mare the tentative demonstration of an application of cytogenetics in veterinary clinical evaluation, were concerned with functions of Clinical Cytogenetics.

The primary hypothesis of this study was that individuals with abnormal sex chromosome constitutions, the majority of which are either mosaics or chimaeras, form a significant proportion of some E. c. caballus breeds, in particular the Thoroughbred Breed. Subsidiary to that hypothesis was that these mosaic/chimaeras as a group were disproportionately responsible for the sub-fertility of some horse populations, through infertility, sub-fertility and generating abnormal offspring.

Five horses were investigated. One, a XY/XYY/?XO mosaic/chimaeric stallion was fully fertile. However, he sired an XO still-born filly. Limited studies of three of his relatives and three of his other progeny, suggested the possibility of cytogenetic anomalies in that kinship. He was the first reported stallion with an XYY line, and his XO filly was the first reported still-born foal with a chromosome abnormality.

Two 64,XX/65,XXY mares with no physical signs of intersexuality were studied. One was infertile, however, there was insufficient information to assess the other mare's fertility. An infertile mare was discovered and investigated in depth, which had 65,XXX/65,XXY/64,XX/66,XXY/66,XXXX mixoploid mosaic/chimaerism, although she was primarily a triple-X mare. The fourth mare was primarily thought to

be a proliferative mosaic, which exhibited mitotic instability. Post mortem evidence, however, confirmed that she had been cytogenetically abnormal and that she probably was a mosaic/chimaera.

Aside from one XX/XXY mare, which had been born with congenital defects, there was nothing either in the history or phenotype of these five horses to distinguish them from other Thoroughbreds. Only one of the mares was presented for analysis because of infertility, although two of the other mares might have been termed "shy breeders".

The discovery of all the major sex chromosome aneuploid conditions infrequently found in humans, XO, XXY, XYY and XXX, in the mosaic/chimaeric condition among less than 200 Thoroughbreds evaluated, suggested that chromosome anomalies are prevalent in the Thoroughbred population.

ACKNOWLEDGEMENTS

I am indebted to the Faculty of Veterinary Science, Massey University, for providing the opportunity and facilities for this investigation.

In particular I would like to thank my supervisor Prof. A. N. Bruère and acknowledge the patient assistance and guidance of both the academic and technical staff of the Department of Veterinary Science, with mention of: Prof. E. D. Fielden, Dr. B. E. Goulden, Dr. R. E. Harris, Dr. R. J. Holmes, Dr. I. L. Anderson, Dr. H. G. Pearce, Mrs. R. B. Foote, Ms Karen Armitage, and the staff of the Large Animal Unit.

Recognition is also due to the Department of Veterinary Pathology and Public Health staff and faculty, with special mention of: Ms L. Frey, the Histology Preparation Unit, the Post-mortem Examination Facility, Dr. A. C. Johnstone, Dr. M. R. Alley, Dr. K. M. Moriarty, Dr. A. J. Robinson and P. Ramadass, Ph.D. candidate; and Prof. R. E. Munford and Mr. M. J. Birtles of the Department of Anatomy and Physiology.

This study would not have been possible without the help and cooperation of horse owners and breeders in New Zealand, to whom the author expresses gratitude. In addition, the active participation of members of the veterinary profession was an invaluable help, especially Mr. H. Dewes, Mr. J. Hope, Ms G. Verkerk, Ms J. Francis, Mr. P. Williams and Mr. C. Kelly. I should also like to acknowledge the financial assistance of The New Zealand Equine Research Foundation.

I would like to express my appreciation to the Librarians and Mr. T. Law (Photographic Unit) for their tolerance and professionalism, as well as to the Department of Botany and Zoology for their support and sufferance.

For their help, encouragement and enduring faith, a very special thankyou to my parents.

Many people, known and unknown, have made this study possible. To them, οἶδα ὅτι οὐκ οἶδα. -Mahalo.

TABLE OF CONTENTS

	<u>Page</u>
Acknowledgements.....	iv
Contents	v
List of Figures	xii
List of Tables	xv
CHAPTER I - <u>Introduction</u>	1
1.1. Infertility in the domestic horse (<i>E. caballus caballus</i>): the aims of this study	3
1.2. Mosaicism and chimaerism: definitions and possible relation to infertility	5
1.2.1. Mosaicism, chimaerism and "mosaic/chimaerism": definitions	5
1.2.2. Chimaerism: types, possible relation to fertili- ty and possible predisposing factors	6
1.2.2.1. Haemopoietic chimaerism	6
1.2.2.2. Whole body chimaerism	7
1.2.3. Mosaicism: possible implications in infertility and possible predisposing factors	7
1.2.3.1. Factors which might predispose to formation of mosaicism	8
1.2.3.2. The formation of mosaicism	9
1.3. Factors affecting mosaic/chimaeric composition and their interpretation	10

1.4.	Aspects of the cytogenetic evaluation of mosaic/chimaerism ...	10
1.4.1.	Chromosome preparations from cultured cells in relation to the <u>in-vivo</u> mosaic/chimaeric condition ...	10
1.4.1.1.	Representativeness of the sample	11
1.4.1.2.	Differential viability and growth of cell lines in culture	12
1.4.1.3.	Natural, artifactual and symptomatic aneuploidy and hyperploidy	12
1.4.1.4.	Mitogenic stimulation and mitotic inhibition	13
1.4.2.	Mosaic/chimaerism and sex-chromatin studies	13
1.5.	Atypical development and cytogenetic abnormalities	15
1.6.	The family <u>Equidae</u>	16
1.6.1.	The karyotypes of Equidae	16
1.6.2.	Equine hybrids	17
1.7.	Cytogenetics of <u>E. caballus caballus</u>	19
1.7.1.	The normal karyotype of <u>E. c. caballus</u>	19
1.7.1.1.	Chromosome identification	19
1.7.1.2.	Chromosome polymorphisms	20
1.7.1.3.	The karyotype of the domestic horse	20
1.7.1.4.	Arrangement of the karyotype of the domestic horse	21
1.7.2.	Abnormal karyotypes in the domestic horse	21
1.7.3.	Infertility and chromosome anomalies in the domestic horse	22
1.7.3.1.	Infertility: comments on terminology	22
1.7.3.2.	Chromosome anomalies in the domestic horse.....	26
CHAPTER II - <u>Materials and Methods</u>		28
2.1.	Subjects	29
2.2.	Cytogenetic techniques	29
2.2.1.	Leucocyte cultures	30

2.2.2.	Cell cultures other than leucocyte cultures	30
2.2.3.	Harvesting cell cultures and slide preparation	30
2.2.4.	Staining	30
2.2.5.	Sex-chromatin	30
2.2.6.	Evaluation of cultured cells	31
CHAPTER III - <u>H-11, A fertile XY/XYY/?XO Mosaic/Chimaeric Stallion</u>		32
3.1.	Introduction	33
3.2.	Materials and methods	36
3.2.1.	Sources of information	36
3.2.2.	H-11	36
3.2.2.1.	Description	36
3.2.2.2.	General history	36
3.2.2.3.	Reproductive performance	37
3.2.2.4.	H-11's sire and dam	38
3.2.3.	Relatives and progeny investigated	38
3.2.4.	Cytogenetic materials and methods	41
3.3.	Results of cytogenetic analysis of H-11	44
3.4.	Extended cytogenetic study of H-11: his relatives and progeny	50
3.5.	Discussion	58
3.5.1.	H-11's fertility	58
3.5.2.	H-11's XO filly	59
3.5.3.	The abnormal chromosome arrangements in cultured cells of H-11's family	60
3.5.4.	H-11's size and behaviour in view of his XYY line.....	61
3.5.4.1.	H-11's size	61
3.5.4.2.	H-11's behaviour.....	63
3.5.5.	Possible manners of formation of H-11's mosaic/chimaerism	64
3.5.6.	Summary	65

CHAPTER IV - <u>H-52, A Mare of Distinction</u>	66
4.1. Introduction	67
4.2. Materials and methods	69
4.2.1. Description	69
4.2.2. History	69
4.2.3. Cytogenetic materials and methods	69
4.2.3.1. Cell cultures	69
4.2.3.2. Sex-chromatin investigations	71
4.2.4. Examinations and observations	71
4.3. Results	73
4.3.1. Results of cytogenetic investigations	73
4.3.1.1. Lymphocyte cultures	86
4.3.1.2. Cultured cells other than lymphocytes	90
4.3.1.3. Sex-chromatin studies	96
4.3.2. Reports of examinations and observations	99
4.3.2.1. Physical examinations of reproductive organs	99
4.3.2.2. General medical history at Massey University	99
4.3.2.3. Serum hormone analysis	101
4.3.3. <u>Post-mortem</u> findings	102
4.3.4. Observations on H-52's behaviour	111
4.4. Discussion	112
4.4.1. Cytogenetic aspects of H-52's mixoploidy	112
4.4.1.1. The chromosome constitution of H-52	112
4.4.1.2. The possible zygote/embryonic chromosome constitution of H-52	113
4.4.1.3. Possible predisposing factors to H-52's mixoploidy	117
4.4.2. Physical and behavioural aspects of H-53's mixoploidy	117
4.4.3. Summary	121

CHAPTER V - <u>Two 64,XX/65,XXY Mosaic/Chimaeric Mares</u>	122
5.1. Introduction	123
5.2. Materials and methods	124
5.2.1. Sources of information	124
5.2.2. H-46	124
5.2.2.1. Description	124
5.2.2.2. Feminine structures	124
5.2.2.3. Reproductive history	124
5.2.2.4. Behaviour	127
5.2.3. H-47	127
5.2.3.1. Description	127
5.2.3.2. General history	127
5.2.3.3. Reproductive history	129
5.2.3.4. Behaviour	131
5.2.4. Cytogenetic materials and methods	131
5.2.4.1. H-46	131
5.2.4.2. H-47	131
5.2.4.3. Evaluation of cells	131
5.3. Results	132
5.3.1. H-46	132
5.3.2. H-47	136
5.4. Discussion	140
5.4.1. Errors of development and H-46 and H-47	144
5.4.2. Summary	147
CHAPTER VI - <u>H-27, An Enigma</u>	148
6.1. Introduction	149
6.2. Materials and methods	151
6.2.1. H-27	151
6.2.1.1. Description	151
6.2.1.2. General information	151

6.2.1.3.	Pre-admission history	151
6.2.1.4.	History at Massey University.....	151
6.2.1.5.	Behaviour.....	152
6.2.1.6.	<u>Post-mortem</u> report	152
6.2.2.	Cytogenetic materials and methods	155
6.2.2.1.	Cultured cells	155
6.2.2.2.	Sex-chromatin investigations	156
6.2.2.3.	Lymphocyte transformation test	156
6.3.	Results	157
6.3.1.	Results of cytogenetic investigations.....	157
6.3.1.1.	Cultured cells	157
6.3.1.2.	Sex-chromatin investigations.....	167
6.3.2.	Lymphocyte proliferation test	169
6.4.	Discussion.....	172
6.4.1.	Cytogenetic abnormality	172
6.4.2.	Some implications of the failure of lymphocyte cultures	173
6.4.3.	Possible implications of mitotic instability in cell cultures	174
6.4.4.	Infertility	176
6.4.5.	Summary	177
CHAPTER VII - <u>General Discussion</u>		179
7.1.	Prevalence of the mosaic/chimaeric condition in <u>E. c. caballus</u>	180
7.2.	Possible predisposing factors to the mosaic/chimaeric condition and the horses in this study.....	182
7.2.1.	Parental age effect	182
7.2.2.	Birth order and dam's obstetric history	183
7.2.3.	Possible influences of metabolism and physiology....	184
7.2.4.	Inbreeding and relationships between the mosaic/ chimaeras in this study	185

7.2.5. Genetic predisposition to masked karyotype change	186
7.3. Mosaic/chimaeric individuals and breed sub-fertility.....	188
7.3.1. Mosaic/chimaerism and fertility	188
7.4. Cytogenetics and the Domestic Horse	191
Appendix I - The 1978 Pilot Survey	193
Appendix II - Unpublished XO Mares Discovered in New Zealand and Other Horses Investigated for which there was a Suggestion of a Cytogenetic Anomaly	200
Appendix III - Ancillary Information to Chapter IV	
A. H-52's Dimensions	203
B. H-52 <u>Post-Mortem</u>	204
C. Estimates of Primordial Cell Pool Size ...	207
Appendix IV - H-27 <u>Post Mortem</u>	209
Appendix V - Materials	
A. Supplies	211
B. Tissue culture materials	212
C. Cell culture media	214
D. Staining solutions	214
E. Equipment	215
Appendix VI - Methods	
I. Leucocyte culture system	216
II. Cell culture techniques	217
III. Slide preparation	219
IV. Sex-chromatin	220
V. Differential staining	220
VI. Photographic methods	221
VII. Lymphocyte transformation test	222
Bibliography	224

LIST OF FIGURES

		<u>Page</u>
III.1	H-11 as a four year old	35
III.2	Mare "B" with three day old 1979 filly by H-11.....	40
III.3a	H-11, the sire of H-58.....	42
3b	H-58 age nine months.....	42
III.4	A normal 64,XY metaphase from H-11.....	46
III.5	A metaphase with 65 chromosomes from H-11's cultured skin (designated XYY).....	47
III.6	A metaphase with 65 chromosomes from H-11's cultured lymphocytes (designated XYY).....	48
III.7	An example of a 63,XO cell from H-11.....	49
III.8	Metaphase with 65 chromosomes and tri-radial (non-homologous chromatid interchange) involving chromosome 1 and possibly 9 (from H-11's sire).....	52
III.9	Examples of abnormal chromosome arrangements found in one of H-11's half brothers and H-11's 1978 colt..	53
III.10	C-banded cell from H-68, an XO filly sired by H-11...	55
III.11	G-banded cell from H-68, an XO filly sired by H-11...	56
IV.1	Left lateral view of H-52.....	68
IV.2	Metaphase containing 65 chromosomes (designated XXY).	75
IV.3	G-banded metaphase containing 65 chromosomes, confirming the 65,XXY cell line.....	76
IV.4	A metaphase with 65 chromosomes (designated XXX).....	77
IV.5	G-banded metaphase containing 65 chromosomes, confirming the 65,XXX cell line.....	78
IV.6	A metaphase with 64 chromosomes (28 non-acrocentric) containing two X-chromosomes.....	79
IV.7	Another example of a 64,XX cell from H-52.....	80
IV.8	One of the metaphases with 66 chromosomes (designated 66,XXXY).....	81
IV.9	Another metaphase with 66 chromosomes designated 66,XXXY.....	82
IV.10	One of three metaphases with 66 chromosomes (30 non-acrocentric) (designated 66,XXXX).....	83
IV.11	Another of the three metaphases designated 66,XXXX...	84

IV.12a	An example of a neuron with two sex-chromatin bodies, confirming the presence of either XXX or XXXY cells.....	85
12b	An example of a neuron with one sex-chromatin body, which could represent either an XX or XXY cell.....	85
IV.13	Histogram of cultured lymphocytes analysed showing the proportions (%) of XX, XXX and XXY cells.....	89
IV.14	Proportions (%) of the cells evaluated from solid tissues with 64,XX, 65,XXX and 65,XXY chromosome constitutions.....	95
IV.15	A section of uterine tissue from H-52 showing endometrial hypoplasia.....	100
IV.16	External (ventral) view of H-52's genital organs.....	104
IV.17	External view of the uterine horns, ovaries, and body of the uterus.....	105
IV.18	Internal view of the uterus.....	106
IV.19a	Left <u>cornu uteri</u> and ovary.....	107
19b	Sagittal bisection through the left ovary's ovulation fossa.....	107
IV.20	Drawings of Pearl's Iron and Sudan Black positive areas of left ovary sections and two photographs of a region of the area (probable <u>corpus albicans</u>).....	108
IV.21a	Right <u>cornu uteri</u> and ovary.....	109
21b	Sagittal bisection through the right ovary's ovulation fossa.....	109
IV.22	Drawings of Pearl's Iron and Sudan Black positive areas of right ovary sections and two photographs of a region of the area (probable <u>corpus albicans</u>).....	110
IV.23	Diagram of the possible mosaic manner of formation of H-52's mixoploidy.....	116
V.1	H-46 as a nine year old.....	125
V.2	Vulvar cleft and anus of H-46.....	126
V.3	H-47 as a two year old maiden mare.....	128
V.4a	Left lateral view of H-47's rump.....	130
4b	The appearance of H-47's artificially formed continuous rectum and "cloaca" when she was two years old.....	130
V.5	An example of a cell from H-46 which was designated 65,XXY.....	134
V.6	A normal 64,XX cell from H-46.....	135
V.7	An example of a cell from H-47 which was designated 65,XXY.....	138
V.8	A normal 64,XX metaphase from H-47.....	139

VI.1a	Dorsal view of H-27's internal genitalia.....	153
1b	Ventral view of H-27's internal genitalia.....	153
VI.2	Internal view of H-27's cervix, uterus and right uterine horn.....	154
VI.3a	Example of a neuron with two sex-chromatin bodies from H-27	159
3b	Example of a neuron with one sex-chromatin body from H-27	159
VI.4	The only 63,XO cell found in H-27's cultured cells....	160
VI.5	One of the three cells which suggested, initially, that H-27 might have a 66,XXXX cell line	162
VI.6	One of the subsequent metaphases with 66 chromosomes, suggesting that H-27's cells were mitotically unstable <u>in-vitro</u>	163
VI.7	An example of a 62,XX cell.....	164
VI.8	An example of an abnormal chromosome arrangement seen in two of H-27's cultured cells.....	165
VI.9	An example of endoreduplication in H-27's cultured cells.....	166
VI. <u>i</u>	Lymphocyte transformation test experimental design ...	156

LIST OF TABLES

	<u>Page</u>
1-1 Extant members of the family <u>Equidae</u>	16
1-2 Equine hybrids	18
1-3 Summary of published reports of XO mares	23
1-4 Summary of published reports of horses with non-mosaic/ chimaeric sex chromosome abnormalities, other than XO mares	24
1-5 Summary of published reports of horses with mosaic/ chimaerism	25
3-1 Reproductive performance of H-11 (1977-1980)	39
3-2 The distributions of chromosome counts from H-11's cultured cells.....	45
3-3 Results of cytogenetic investigations of H-11's relatives and progeny.....	51
4-1 Summary of cell culture techniques and culture periods of non-lymphocyte cultured cells.....	70
4-2 The distribution of gross chromosome counts from H-52's cultured cells.....	74
4-3 The distribution of gross chromosome counts in H-52's cultured lymphocytes.....	87
4-4 Condensed distributions of differential chromosome counts from H-52's cultured lymphocytes.....	88
4-5 The distributions of gross chromosome counts in cultured cells other than lymphocytes.....	91
4-6 The distributions of differential chromosome counts in cultured cells other than lymphocytes.....	92
4-7 Percentages of artifactual aneuploidy and cells with 64/28 non-acrocentric chromosome counts.....	93
4-8 The numbers and distribution of sex-chromatin bodies in neurons of H-52's central nervous system.....	97
4-9 The proportions of cells with sex-chromatin bodies and their distribution in H-52's central nervous system.....	98
4-10 Results of serum hormone analysis' I and II.....	102
5-1 The distributions of chromosome counts from H-46's cultured cells.....	133
5-2 The distributions of chromosome counts from H-47's cultured cells.....	137

6-1	The distributions of chromosome counts from H-27's cultured cells.....	158
6-2	Summary of recorded abnormal differential chromosome counts from cultured cells from H-27.....	161
6-3	The numbers and distributions of sex-chromatin bodies in the central nervous system of H-27.....	168
6-4	Thymidine incorporation of <u>Equine</u> peripheral blood lymphocytes cultured with PHA for three days.....	170
7-1	Comparison of the ages at conception of parents of XO mares and the mosaic/chimaeras in this study.....	183
7-2	Approximate coefficient of relationship.....	185
A-I-1	The distributions of chromosome counts in the 1978 pilot survey.....	195
A-II-1	Summary of unpublished cases of XO mares in New Zealand.	201
A-II-2	Summary of cytogenetic investigations of horses which had a suggestion of an anomaly, but for which there was insufficient information to verify.....	202
A-III-1	Estimated primordial cell pool sizes.....	207