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.
THE MEASUREMENT OF PLASMA PROGESTERONE LEVELS IN
THE NORMAL MARE AND ITS APPLICATION TO SOME EQUINE
BREEDING PROBLEMS

A thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Veterinary Science
at Massey University

CHRISTOPHER MORTON KELLY
November, 1977
A radioimmunoassay system was developed to measure plasma concentrations of endogenously produced and exogenously administered progesterone in non-pregnant and pregnant mares in normal and some abnormal reproductive states. Assay sensitivity was 0.5 ng/ml, with a between assay coefficient of variation of 16.8% for a high progesterone sample, estimated over 24 assays, and 8.5% for a low progesterone sample, estimated over 15 assays; the within assay coefficient of variation was 7.3, 10.1 and 6.9% respectively for six replicates of one sample, estimated in three separate assays.

Plasma progesterone concentrations of six normal non-pregnant cycling mares followed regular cyclic changes, with levels less than 0.5 ng/ml during oestrus and ranging between 8-22 ng/ml at peak values in dioestrus. The first oestrus following the winter non-breeding period was longer than the following oestrus and the period from ovulation to progesterone decline tended to be less variable than the rest of the cycle.

There was a large between-mare variation in plasma progesterone concentrations in mares at both early and late stages of gestation with levels varying from 4.9 to 15 ng/ml in the former and 5.2 to 16.9 ng/ml in the latter group. No significant effect was noted between stage of gestation and progesterone concentration.

A group of five mares all had plasma progesterone concentrations greater than 9.5 ng/ml within 24 hours prior to parturition; two of these mares sampled within eight hours prior to parturition had plasma progesterone concentrations of 4.3 and 3.9 ng/ml. The first post partum sample was taken within 24 hours of foaling; by this time plasma progesterone concentrations had fallen to less than 0.5 ng/ml and remained low until sampling ceased at the first post partum oestrus.
Prostaglandin F2α (THAM salt) was effective in causing luteolysis in 13 mares with active corpora lutea before treatment. By three days post-injection 12 of the 13 mares had plasma progesterone concentrations of less than 0.5 ng/ml and by five days post-injection 12 of the 13 mares were exhibiting oestrus. Of the ten mares bred at the induced oestrus, seven became pregnant to that mating.

Plasma progesterone concentrations were measured on 16 non-pregnant mares in anoestrus. Six of eight mares sampled early in the breeding season (September) had plasma progesterone concentrations of less than 0.5 ng/ml, the other two mares had plasma progesterone concentrations of 0.6 ng/ml. Eight of eight mares sampled later in the breeding season (November and December) had plasma progesterone concentrations greater than 0.5 ng/ml, the levels ranging from 6.2 to 13.1 ng/ml.

Concentrations of plasma progesterone in normal dioestrous mares were measured half and one hourly (three mares) for 24 hours and four hourly (two mares) for 120 hours. There were large apparently random variations, with more than 100% differences being recorded between a number of consecutive samples. Plasma progesterone concentrations varied from 7.8-23.0, 3.2-21.9 and 4.2-12.9 ng/ml for the three mares sampled half hourly and hourly, and from 6.8-24.6 and 0.8-11.0 ng/ml for the two mares sampled four hourly.

Radioactive progesterone, administered by venepuncture to a mare with no detectable endogenous plasma progesterone, disappeared from the plasma within 40 minutes; 85% of the injected steroid had left the plasma by 2.5 minutes post-injection.

Two and 25 mg of progesterone in 16% alcohol in saline was administered by venepuncture to mares with plasma progesterone concentrations of less than 0.5 ng/ml. For the mare given 2 mg, the plasma half life of injected progesterone was 1.75 minutes for the initial "fast" component, and for the mare given 25 mg the plasma half life was 2.75 minutes. There was a second peak of plasma progesterone at from 8 to 19.5 minutes for the former and from 9.5 to 17 minutes for the latter mare. A third much smaller peak was recorded at about 50 minutes post-injection for the mare given 2 mg progesterone.

A mare with no detectable endogenous plasma progesterone was administered by intramuscular injection a total of 600 mg progesterone
in arachis oil over a period of seven days. Plasma concentrations of the steroid reached a maximum of 4.3 ng/ml at one day post-treatment and were maintained at this level for only a maximum of 24 hours. A second mare, again with no detectable plasma progesterone, was administered by intramuscular injection a total dose of 2 g of hydroxyprogesterone capronate in castor oil over a period of ten days. Maximum plasma progesterone concentrations of 1.2 ng/ml, maintained for less than 24 hours, were reached nine days after the first injection.

Wide variation in plasma progesterone levels within and between mares over relatively short time periods suggest that there are many difficulties in identifying "progesterone insufficiency" as a cause of embryonic absorption or abortion in this species. Moreover the short half life of this steroid in the plasma of the mare, together with the sustained high dose levels that would be required to elevate plasma concentrations of progesterone to a level equivalent to that produced by normal secretory corpora lutea, indicate that current levels of administration of this drug are likely to have little effect in overcoming such a breeding problem unless the progesterone is acting at a local level. A definitive answer in respect to this vexed question concerning the existence or not of "progesterone insufficiency" as a cause of prenatal loss in the mare, together with an appropriate method of treatment, still remains to be found.
I wish to record my thanks to many people without whom this thesis could not have been completed.

To my supervisors, Professor E.D. Fielden, Department of Veterinary Clinical Sciences and Dr K. Lapwood, Department of Physiology and Anatomy, Massey University, I extend my sincere thanks for their unflagging interest, valued advice and continued encouragement during this study.

For assistance in establishing and running the radio-immunoassay I wish to thank Mr P. Wilson, Department of Physiology and Anatomy, Massey University, Dr G. Burrell, Lincoln College, Mrs H. Carter and Mrs H. Walker.

I appreciate the facilities and equipment put at my disposal for this study.

To the New Zealand Thoroughbred Breeders' Association and the trustees of the Norman Cunningham Fellowship, I wish to express my thanks for the financial support given me.

I wish to thank Upjohn Ltd., N.Z. and Tasman-I.C.I. Ltd. for supplying the prostaglandin F2α, and Dr R. Fairclough for the generous gift of progesterone antiserum.

Finally I wish to extend special thanks to Erin Temperton, Mrs H. Harker, Miss Carol Black, Mrs Angela Low and my wife Kathy for the arduous tasks of typing drafts and final copy and drawing figures.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Abstract</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>v</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
</tbody>
</table>

**INTRODUCTION**

**CHAPTER I LITERATURE REVIEW**

**A. ENDOCRINE CONTROL OF REPRODUCTION IN THE MARE**

1. The Oestrous Cycle
   a. Puberty
   b. Hypothalamus
   c. Pituitary
      i. Follicle Stimulating Hormone
      ii. Luteinizing Hormone
      iii. Thyroid Stimulating Hormone
   d. Ovary
      i. Anatomy
      ii. Follicular Phase
      iii. Oestrus
      iv. Ovulation
      v. Luteal Phase
   e. Uterus
2. Pregnancy
   a. Blastocyst, Embryo and Foetus
   b. Placenta and Ovary
   c. Initiation of Parturition

**B. METABOLISM OF PROGESTERONE IN THE MARE**

1. Synthesis of Progesterone
   a. Ovary
      i. Follicular Phase
      ii. Luteal Phase
   b. Placenta
2. Transport and Breakdown of Progesterone
C. MEASUREMENT OF PLASMA PROGESTERONE IN THE MARE 40
   1. Non-pregnant Mare 40
   2. Pregnant Mare 43
D. RADIOIMMUNOASSAY OF PLASMA PROGESTERONE 45

CHAPTER II GENERAL MATERIALS AND METHODS 49
A. ANIMALS 49
B. BLOOD COLLECTION 51
C. RADIOIMMUNOASSAY 52
   1. Reagents 52
   2. Extraction Procedure 53
   3. Radioimmunoassay procedure 53
   4. Calculation of Results 54
   5. Validation of Assay 54
      a. Antibody Specificity 54
      b. Dextran Coated Charcoal 54
      c. Extraction 54
      d. Parallelism 57
      e. Assay Specificity 57
      f. Between Assay Precision 57
      g. Within Assay Precision 57
      h. Biological Validation 57

CHAPTER III RESULTS AND DISCUSSION 59
A. PLASMA PROGESTERONE LEVELS OF NORMAL MARES 59
   IN VARIOUS REPRODUCTIVE STATES 59
   1. Cycling, Non-pregnant Mares 59
   2. Pregnant Mares, Gestation Length 59
      less than 70 Days 59
         a. Special Methods 59
         b. Results 59
   3. Mares, 240 or More Days Pregnant 61
      a. Special Methods 61
      b. Results 61
   4. Plasma Progesterone Levels in Mares from 61
      Immediately Prior to Parturition Until the 61
      First Day of Oestrus Post Partum 61
   5. Discussion 61
B. PLASMA PROGESTERONE LEVELS IN MARES WITH MODIFIED REPRODUCTIVE ACTIVITY

1. Use of Prostaglandin F2α to Induce Luteolysis
   a. Special Methods
   b. Results

2. Termination of Pregnancy of an Approximately 85 Day Pregnant Mare

3. Measurement of Plasma Progesterone Levels in an Ovariectomized Mare
   a. Special Methods
   b. Results

4. Measurement of Plasma Progesterone in Anoestrous Mares
   a. Special Methods
   b. Results

5. Plasma Progesterone Concentrations in Mares in Advanced Pregnancy Prior to Abortion of Twins or Premature Parturition of Dead Foals

6. Discussion

C. REPEATED MEASUREMENTS OF PLASMA PROGESTERONE CONCENTRATIONS IN NORMAL MARES AND IN MARES GIVEN EXOGENOUS PROGESTERONE

1. Four Hourly Plasma Progesterone Measurements on Normal Dioestrous Mares

2. Plasma Progesterone Measurements at Hourly or Half-hourly Intervals on Normal Dioestrous Mares

3. Measurement of Radioactive Progesterone Administered to a Mare in Anoestrus
   a. Special Methods
   b. Results

4. Plasma Progesterone Levels in 2 Anoestrous Mares Given Exogenous Progesterone
   a. Special Methods
   b. Results
5. Plasma Progesterone Levels in a Mare Given Progesterone in Oil
   a. Special Methods
   b. Results
6. Discussion

CHAPTER IV SUMMARY AND CONCLUSIONS
REFERENCES
TABLE I: Plasma progesterone levels in non-pregnant mares measured by RIA

TABLE II: Plasma progesterone and progestogen levels in pregnant mares by RIA

TABLE III: Plasma progesterone levels of mares less than 70 days pregnant

TABLE IV: Plasma progesterone levels of mares greater than 240 days pregnant

TABLE V: Plasma progesterone levels in mares from immediately prior to parturition to first post partum oestrus

TABLE VI: Results of treatment of mares with prostaglandin F2α

TABLE VII: Plasma progesterone levels of 16 anoestrous mares

TABLE VIII: Concentration of plasma progesterone in mares in advanced pregnancy having stillborn foals

TABLE IX: Plasma progesterone levels in a mare given progesterone in arachis oil

TABLE X: Plasma progesterone levels in a mare given hydroxyprogesterone capronate in oil
LIST OF FIGURES

FIGURE 1: Steroid synthesis in the equine graafian follicle facing 15

FIGURE 2: Hormone concentrations of follicular fluid in various reproductive states facing 16

FIGURE 3: Ovaries from a mare in anoestrus 55
FIGURE 4: Ovaries from a mare in oestrus 55
FIGURE 5: Ovaries from a mare in dioestrus 56

FIGURE 6: A typical sensitivity curve (± S.E. mean counts) showing "best fit" of progesterone standards facing 57

FIGURE 7: Concentration of plasma progesterone in normal cycling mares facing 60

FIGURE 8: Concentration of plasma progesterone in normal cycling mares 60

FIGURE 9: Concentration of plasma progesterone in a pregnant mare given intra-muscular prostaglandin P2± facing 67

FIGURE 10: Concentration of plasma progesterone in two mares sampled four hourly facing 75

FIGURE 11: Concentration of plasma progesterone in three mares sampled at 30 or 60 minute intervals 75

FIGURE 12: Disappearance of radioactivity from the plasma of a mare given (H3)1,2, progesterone intravenously facing 77

FIGURE 13: Concentration of plasma progesterone in a mare given 2 mg progesterone intravenously facing 78

FIGURE 14: Concentration of plasma progesterone in a mare given 25 mg progesterone intravenously 78