The Synchronisation of Oestrus and Ovulation in the Mare

— Current knowledge, future direction and a practical regimen —

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The Synchronisation of Oestrus and Ovulation in the Mare

Current knowledge, future direction and a practical regimen

by S.T. Norman and J.E. Larsen

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Foreword

The synchronisation and scheduling of oestrus and ovulation is a management tool that is useful at all levels of equine reproductive management. Improving synchrony regimens will aid the efficiency and safety of the equine stud industry due to anticipated reductions in the need for teasing mares, palpating and scanning mares, and improved timing of inseminations. At the commencement of this study there was no single regimen which achieved the goal of tight synchrony, with the current “gold standard” regimen requiring daily injections for 10 days of a formulation which is not commercially available.

All members of the equine stud industry may benefit from information contained in this report. In the day-to-day management of a stud utilising natural service, synchronisation or scheduling of ovulation can reduce the labour and risks associated with teasing. It can also assist stud managers to gain more efficient use of stallion services. At a higher level, some artificial breeding technologies are dependent on well timed synchronisation or scheduling of ovulation to the point where technologies such as frozen semen insemination or embryo transfer are not commercially viable if suitable control of the reproductive cycle is not available.

This report identifies a regimen for the synchronisation of ovulation which appears as effective as the current industry “gold standard” as described by Loy (1981). This provides the potential to significantly reduce the teasing and palpation requirement for preparing breeding mares. Importantly, while achieving the efficacy of the current industry “gold standard” this new regimen is practical (requiring only two mare handlings) and is based on commercially available drugs. This makes it readily available, via veterinary distribution, to all members of the equine stud industry.

This report provides scientific information that can assist stud breeders and veterinarians in making informed decisions on suitable methods for the synchronisation of oestrus and ovulation in the mare. A Fact Sheet is included (Appendix 1) outlining the logistics of applying the specific protocol identified in this study which provided the best synchrony.

The information in this report will also inform further research in this field and could be used by the equine breeding industry to encourage the development of synchrony pharmaceuticals registered for use in the mare.

The project was funded in part from industry revenue which is matched by funds provided by the Australian Government. Additional financial support was provided by Charles Sturt University and Bioniche Animal Health A/Asia Pty Ltd.

This report is an addition to RIRDC’s diverse range of over 2000 research publications and it forms part of our Horse R&D program, which aims to assist in developing the Australian horse industry and enhancing its export potential.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

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Managing Director
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Abbreviations

AB  Artificial breeding
AI  Artificial insemination
CID Ciderol™
CL  Corpus luteum
CM  Cue Mare™
eCG Equine chorionic gonadotrophin
ET  Embryo transfer
ECP Oestradiol cypionate (estradiol cypionate)
FSH Follicle stimulating hormone
g gram
GnRH Gonadotrophin releasing hormone
hCG Human chorionic gonadotrophin
HPG Hypothalamic-pituitary-gonadal
IGF-1 Insulin-like growth factor
IM Intramuscular
IU International units
KP Kisspeptins
LH Luteinising hormone
mg milligrams
mL millilitres
mm millimetres
ODB oestradiol benzoate
ODD oestradiol dipropionate
ODV oestradiol valerate
OV Ovuplant
P&E Progesterone and oestradiol
PGF2α Prostaglandin F2α
PMSG Pregnant mare serum gonadotrophin
SC Subcutaneous
STI Short-term implant
µg microgram
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Executive Summary

What the report is about

This is a report on investigations into the synchronisation and scheduling of oestrus and ovulation in the mare.

At the commencement of this study there were no practical techniques to synchronise oestrus and ovulation in the mare. The technique considered to be the industry “gold standard” for synchronisation requires daily injections for 10 days of a formulation that is not commercially available. Ovulation synchronisation is essential if artificial breeding technologies such as frozen semen artificial insemination (AI), embryo transfer, and oocyte transfer are to become efficient and reliably successful procedures. The overall aim of this project was to develop a novel and practical protocol for the synchronisation of oestrus and ovulation in the mare.

This investigation was addressed in two phases:

- Phase 1 consisted of a review of the literature associated with the synchronisation of oestrus and ovulation in the mare.
- Phase 2 consisted of six field trials to assess the efficacy of selected methods for the synchronisation of oestrus and ovulation in the mare.

Who is the report targeted at?

This report is targeted at members of the equine breeding industry with scientific training. A Fact Sheet (Appendix 1) has been produced targeting the general members of the industry.

Where are the relevant industries located in Australia?

The equine breeding industry covers the majority of populated Australia with areas of larger financial interest situated in the Darling Downs region of Queensland, the Hunter Valley region of New South Wales, multiple sites throughout Victoria and multiple sites mainly in the south-west of Western Australia. Within the equine breeding industry, the breeding of Thoroughbreds generates the majority of economic activity.

Anyone associated with any form of equine breeding may benefit from this research. This includes stud owners, stud managers, stud farm employees, and veterinarians. Members of the Thoroughbred industry may find the use of the ovulation scheduling capabilities of the treatments more suitable to their management situation rather than synchronising ovulation in groups of mares.

Background

Current methods used for the synchronisation of oestrus can, with reasonable accuracy, predict the onset on oestrus. However the accurate timing and prediction of ovulation is not at a level suitable for most natural or artificial breeding requirements. When the timing of ovulation cannot be accurately predicted there is a requirement to frequently assess mares using ultrasonography (colloquially known as ‘scanning’) in order to determine the best time to breed. Mares may need to be ‘scanned’ twice daily until ovulation is imminent at which time they may be scanned as often as every three hours. This is particularly common in cases of artificial insemination with frozen-thawed semen. The need to frequently scan mares increases costs to breeders and increases the risks to the mare associated with trans-rectal examination. Accurate synchronisation or scheduling of oestrus and ovulation would reduce the labour associated with managing the breeding of mares, improve the use of a veterinarian’s time and ensure stallion services are used to maximum efficiency.
Current methods of synchronisation have generally been developed using empirical testing in the field. A problem with this 'best guess' method of investigation is that it doesn't take into account the effect of the stage of the oestrous cycle at commencement or completion of treatment. In most domestic species, there are follicles waxing and waning throughout the oestrous cycle. This is often referred to as 'follicular dynamics', or 'follicular waves'. If a large, mature follicle is present at the time the corpus luteum (and hence progesterone) is removed, it can rapidly progress towards ovulation. However, if the follicle is only small at the time of progesterone removal, it may take close to a week to mature and ovulate. In other species, there is evidence that resetting this follicular wave (ie. making all of the females in the group have follicles of similar size at commencement of treatment), may assist with the synchronisation of ovulation. This project aims to investigate the influence of follicular wave dynamics on ovulation synchrony to allow informed drug selection and administration regimens to be developed.

Aims/objectives

The objective of this project was to develop a practical protocol for the synchronisation of oestrus and ovulation in the mare. The implication for the protocol to be practical was that there should be minimal handling and injection requirements during treatment and drugs should be commercially available. An additional objective was to assess effects of the treatment regimens on mares for adverse outcomes.

The goals of this research were to:

- Provide a literature review pertaining to follicular dynamics in the mare and the relationship with oestrus and ovulation synchrony.
- Examine mares before, during and after synchrony treatment to assess possible adverse outcomes.
- For the different synchrony treatment regimens, investigate:
  - follicular dynamics during the treatment and ovulatory periods
  - the duration from treatment completion to the attainment of a 35 mm follicle
  - the duration from treatment completion to the attainment of ovulation
  - the interovulatory interval between the first and second post-treatment ovulations.

Methods used

The practical aspects of the study ran over three breeding seasons (September to December 2007, 2008, 2009), with literature review, statistical analysis and project write-up at other times.

Phase one comprised of a literature review investigating equine follicular dynamics, and the synchronisation of oestrus and ovulation in the mare. Papers were identified using a computerised literature search, library searches of relevant journals and searches for relevant conference proceedings or newsletters. Databases searched included, CAB Abstracts, Medline and Biological Abstracts. Search terms included "oestrous synchronisation", "estrous synchronisation" and "follicular dynamics", with boolean operators associated with the words "mare", "horse" and "equine".

Papers were considered suitable for the review if they met the criteria of:

1. being published between 1960 and 2009;
2. being printed in English;
3. it being clearly stated whether the mare population investigated included pony mares or horse mares;
4. as a minimum, oestrus was detected using behavioural assessment (teasing), or ultrasonography;

5. if ovulation was reported, it was detected by a minimum of alternate-day palpation or ultrasonography during the periovulatory period. Information was drawn from other species as considered relevant to the topic.

Phase two comprised of a randomised, controlled, blocked design to investigate five selected treatments. Treatment selection was informed by the literature review with a focus on treatments that had potential to reset the follicular wave. The chosen regimens would ideally cause atresia or ovulation of dominant follicles present at the commencement of treatment allowing a new follicular wave to emerge. The literature review identified a seminal study utilising follicle ablation techniques as a synchronisation tool (Bergfelt and Adams, 1996). This protocol was not included in the treatments for this study as it had been well investigated and was not considered a practical tool. However, it did highlight the value in resetting the follicular wave with regard to ovulation synchrony.

There were five replicates of the study and within each replicate 12 mares ranging in age from 3 – 12 years old were assigned to one of the five treatments (2 mares/treatment) and 2 mares served as untreated controls. Each treatment mare received an intravaginal progesterone-releasing device in conjunction with the assigned additional drug treatment for a period of ten days. Mares were assessed by palpation and ultrasonography every second day during treatment and then until ovulation occurred.

The treatments for the five groups and the Control are summarised in Table 1.

Table 1  The five synchrony treatments administered to the mares
CID = Ciderol; OV = Ovuplant; CM = Cue Mare intravaginal device

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>DAY 0</th>
<th>DAY 5</th>
<th>DAY 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1 (CID10D0,5)</td>
<td>Device insertion, 10 mL oestradiol benzoate</td>
<td>10 mL oestradiol benzoate IM</td>
<td>CM removal, 1 mL prostaglandin F2α IM</td>
</tr>
<tr>
<td>Treatment 2 (OVD0,5)</td>
<td>Device insertion, deslorelin implant SC</td>
<td>Deslorelin implant SC</td>
<td>CM removal, 1 mL prostaglandin F2α IM</td>
</tr>
<tr>
<td>Treatment 3 (OV2D0)</td>
<td>Device insertion, 2 x deslorelin implant SC</td>
<td>No treatment</td>
<td>CM removal, 1 mL prostaglandin F2α IM</td>
</tr>
<tr>
<td>Treatment 4 (CID20D0)</td>
<td>Device insertion, 20 mL oestradiol benzoate</td>
<td>No treatment</td>
<td>CM removal, 1 mL prostaglandin F2α IM</td>
</tr>
<tr>
<td>Treatment 5 (CM Only)</td>
<td>Device insertion</td>
<td>No treatment</td>
<td>CM removal, 1 mL prostaglandin F2α IM</td>
</tr>
<tr>
<td>Control</td>
<td>No treatment</td>
<td>No treatment</td>
<td>No treatment</td>
</tr>
</tbody>
</table>

The trial ran by testing each of the six groups in five replicates in order to remove possible seasonal and year influences on the synchrony result as could occur if each treatment was trialled individually against the Control consecutively throughout the season.

Statistical analysis utilised a non-parametric model and reports on median follicle diameter at commencement of treatment, median follicle diameter at completion of treatment, duration from completion of treatment to the development of a 35 mm follicle, and duration from completion of treatment to ovulation.
Results/key findings

In Phase 1, the literature review provides a contemporary overview of current techniques used to synchronise oestrus and ovulation in the mare. The review highlights the fact that the control mechanisms of follicular dynamics in the mare and their role in influencing synchrony treatments still require significant research. The review also describes a recently identified hormone known as kisspeptin, which may play an important role in controlling follicular wave emergence.

With regard to Phase 2, Treatment 4 (CID20D0) listed in Table 1 provided ovulation synchrony comparable to the current industry “gold standard” described by Loy et al 1981. For this treatment, the median duration from treatment-end to ovulation was 8 days. The lower and upper quartiles were 6 and 9 days respectively with an overall range of 5 to 10 days. There were no adverse effects noted in the mares associated with the treatment regimen. It is noted that the addition of an ovulation induction agent to the Treatment 4 regimen would provide commercially valuable synchrony and scheduling. This option is outlined in the Fact Sheet in Appendix 1.

The CID20D0 treatment achieved the goal of resetting the follicular wave (causing regression of the dominant follicle at treatment commencement) in all cases. This provided a similar outcome to the follicle ablation technique (Bergfelt and Adams, 1996) without the need for an invasive procedure.

If adopted, the CID20D0 regimen has potential to reduce the managerial effort currently required to tease, palpate and scan mares.

Implications for relevant stakeholders

For industry, findings contained within this report will assist in the making of informed decisions with regard to the synchronisation of oestrus and ovulation in the mare. This report confirms that commercially valuable synchrony regimens have been available for a number of decades, yet there is a lack of commercially available products that are registered for use in horses. Therefore, the report should provide stimulus for the industry to encourage commercial products to be developed, and appropriately registered.

With regard to policy makers, there is a need for more basic and applied scientific investigation into factors controlling follicular maturation in the period immediately prior to ovulation. This report identified the value of resetting the follicular wave as part of a synchrony protocol and identified a pharmaceutical regimen with the capability to achieve this. A knowledge deficit which emerged was the need for further understanding of the processes regulating follicle growth and maturation in the period immediately prior to ovulation. This type of information is not readily extrapolated from other species due to the comparatively long duration of oestrus and follicular phase in the mare. Improved understanding of these processes may lead to the development of protocols which can provide enhanced control over the timing of ovulation. Research funding in this area needs to be provided.

Policy makers may also assist industry to encourage the development of commercially available and registerable products to allow broader use of current technologies. Oestrogen pharmaceuticals are currently sourced from products designed for use in cattle and there is a risk that production of these may be reduced or discontinued. The equine breeding industry could be proactive in securing synchrony pharmaceuticals specifically designed for use in the mare.

Recommendations

Treatment 4, investigated in this research, and described in detail in the Fact Sheet in Appendix 1, is suitable for commercial application. It can be expected to provide a degree of synchrony equivalent to the current industry “gold standard” with significantly less labour input and drug costs.
The equine stud industry could encourage the development of commercial synchrony products specific to the equine industry and have them suitably registered. Slow-release injectable formulations utilising microsphere technology appear to have significant potential.

The results should be used as the basis for further research into the development of pharmaceuticals suitable for controlling follicular growth and maturation in the immediate pre-ovulatory period.
Introduction

Current oestrous synchronisation protocols can, with reasonable accuracy, predict the onset of oestrus. However the accurate timing and prediction of ovulation is not at a level suitable for most natural or artificial breeding requirements. When the timing of ovulation cannot be accurately predicted there is a requirement to frequently assess mares using behavioural assessment (teasing), and ultrasonography (colloquially known as ‘scanning’) in order to determine the best time to breed. Mares may be ‘scanned’ twice daily until ovulation is imminent at which time they may be scanned as often as three-hourly. Scanning in this manner is particularly common in cases of artificial insemination with frozen-thawed semen. The need to frequently tease and scan mares increases costs to breeders and increases the risks to the mare associated with animal interaction and trans-rectal palpation respectively. Accurate synchronisation of oestrus and ovulation would reduce the labour associated with managing the breeding of mares, improve the efficiency of stallion and semen use, and improve safety and time management for stud managers and veterinarians. In the specific situation of the Thoroughbred industry, where artificial breeding technologies are currently not utilised, the ability to reliably schedule oestrus and ovulation in individual mares can assist with managing stallion bookings.

Of the current methods for synchronising oestrus in the mare, the one considered to be the “gold standard” requires a regimen of injecting a combination of oestradiol 17β and progesterone daily for 10 days (Loy et al., 1981). This is not particularly practical as it is labour intensive, mares can become difficult to handle after repeated injections, there is risk of infection at the injection sites and importantly, there is no commercial preparation available. In addition, the synchrony is only at a level where mares ovulate over a three to five-day period, which is only just reaching the threshold of being a commercially viable protocol.

The development of synchronisation protocols have generally been based on empirical testing in the field. A problem with this 'best guess' method of investigation is that it doesn't take into account the effect of the stage of the oestrous cycle that the mare is at when treatment commences. In most domestic species, there are follicles waxing and waning throughout the oestrous cycle. The follicular development pattern is often referred to as 'follicular dynamics', or 'follicular waves'. If a large, mature follicle is present at the time the corpus luteum (and hence progesterone) is removed, it can rapidly progress towards ovulation. However, if the follicle is only small at the time of progesterone removal, it may take a number of days to mature and ovulate. In other species, there is evidence that resetting this follicular wave (ie. making all of the females in the group have follicles of similar size at commencement of treatment), may assist with the synchronisation of ovulation. In the mare it has been found that the ablation of follicles greater than 10 mm in a group of randomly cycling mares can assist with ovulation synchrony without the need to administer drugs (Bergfelt et al., 2007). However the commercial application of this technique is questionable due to the expense of the required equipment, the invasiveness of the procedure and the fact that synchrony is still imprecise.

The aim of this project was to review the literature, design a practical pharmaceutical-based treatment regimen for oestrus and ovulation synchrony in mares and investigate its influence on follicular wave dynamics.
Objectives

The objective of this project was to develop a novel and practical protocol for the synchronisation of oestrus and ovulation in the mare. The implication for the protocol to be practical is that there should be minimal handling and injection requirements during treatment, and products used should be commercially available. Additional objectives were to assess effects of the treatment regimens on follicular wave patterns, and to assess mares for any adverse outcomes that may have been associated with the treatments.

To provide the best opportunity to achieve this objective, the goals of this research were to:

- Review the literature regarding oestrous cycle control, follicular dynamics and synchrony techniques.
- Examine mares before, during and after synchrony treatment to assess possible adverse outcomes.
- For the different treatment regimens, investigate:
  - follicular dynamics during the treatment and ovulatory periods
  - the duration from treatment completion to the attainment of a 35 mm follicle
  - the duration from treatment completion to ovulation
  - the duration from first post-treatment ovulation until the second ovulation.
Methodology

The data collection stage of the study ran over three breeding seasons (September to February 2007, 2008, 2009), with literature review, statistical analysis and project write-up proceeding throughout.

Phase one comprised of a literature review investigating follicular dynamics, and the synchronisation of oestrus and ovulation. With regard to the mare, papers were identified using a computerised literature search, library searches of relevant journals and searches for relevant conference proceedings or newsletters. Databases searched included, CAB Abstracts, Medline and Biological Abstracts. Search terms included "oestrous synchronis(z)ation", "estrous synchronis(z)ation" and "follicular dynamics", with boolean operators associated with the words "mare", "horse" and "equine". Papers were considered suitable for the review if they met the criteria of: 1) being published between 1960 and 2009; 2) being printed in English; 3) it being clearly stated whether the mare population investigated included pony mares or horse mares; 4) as a minimum, oestrus was detected using behavioural assessment (teasing), or ultrasonography; 5) if ovulation was reported, it was detected by a minimum of alternate-day palpation or ultrasonography during the periovulatory period. Information was drawn from other species as considered relevant to the topic.

Phase two comprised of a randomised, controlled, blocked design to investigate five selected treatments. Treatment selection was informed by the literature review with a focus on treatments that had potential to reset the follicular wave without the need for a high frequency of mare handling or injections. The regimens would ideally cause atresia or ovulation of dominant follicles present at the commencement of treatment in order to allow a new wave to emerge. The literature review identified a seminal study utilising follicle ablation techniques as a synchronisation tool (Bergfelt and Adams, 1996). This protocol was not included in the treatments for this study as it had been well investigated and was not considered a practical tool. However it did highlight the value in resetting the follicular wave with regard to ovulation synchrony. Readers should refer to the literature review for detail in relation to follicular development. However, relevant findings from the literature review relating to follicular dynamics are summarised in Figure 2. In particular, it was considered ideal for the treatments to:

- Induce atresia, luteinisation, or ovulation of any dominant follicles present at the time of treatment commencement
- Allow timely commencement of a new follicular wave during the treatment process
- Not allow ovulation to occur within five days of completion of the treatment process
- Have an adequate duration to allow a new wave of follicles to progress through the common growth phase and deviation by the time of treatment withdrawal

Based on these requirements, treatments were formulated using varied regimens of oestradiol benzoate, or a GnRH agonist, in conjunction with an intravaginal progesterone releasing device (Cue-Mare™, Bioniche, Australia, Figure 1) and prostaglandin F₂α (PGF₂α). While Cue-Mare™ devices are not yet registered for use in mares in Australia, the decision to use an intravaginal progesterone-releasing device was based on previous work finding it to require minimal labour input while being a safe and effective method of progesterone delivery to the mare (Norman et al., 2006). It is our understanding that the regulatory requirements for registration of this product are in progress. While it is apparent that other hormonal treatments such as inhibin may have suitable merit, a major goal of this study was to work with treatments which are commercially available. For this reason, treatments as outlined in Table 2 were used.
Figure 1 The Cue-Mare intravaginal progesterone releasing device used in this research

Note that the blue nylon cord was removed prior to insertion into the vagina of the mare to ensure none of the device compromised the vulva or vestibulovaginal seals.

There were five replicates of the study in order to remove possible seasonal and year influences on the synchrony result, as could occur if each treatment was trialled individually against the control consecutively throughout the season. In each replicate, 12 mares ranging in age from 3 – 12 years old were randomly assigned to one of the five treatments (2 mares/treatment) and 2 mares served as controls. This resulted in a total of 60 mares in the study with 10 mares in each group. Each treatment mare received the intravaginal progesterone releasing device for a period of ten days in conjunction with the assigned additional drug treatments. The intravaginal device containing 1.72 grams of progesterone was administered using the following procedure:

A tail wrap was applied to the mare before cleaning the anus and vulva using what we refer to as the ‘clean-hand-dirty-hand’ technique. Iodine surgical scrub was used for the initial preparation of the peroneal region, with thorough rinsing using clean water, followed by drying the anus and vulva. A requirement of this technique was to ensure the vulva was always given a final scrub and rinse using fresh water and towel after the anus was cleaned and dried. It was considered important to ensure that the anus did not become wet during this final stage of preparation, otherwise water contaminated with faeces may have passed over the vulval lips. A Cue-Mare™ progesterone releasing device was then loaded into the applicator after removing the nylon extraction cord.

A small amount of clean obstetrical lubricant or KY-gel was then applied to the applicator prior to insertion into the anterior vagina. Once the applicator was placed into the anterior vagina the plunger on the applicator was depressed to place the progesterone device deep into the vagina. The applicator was then removed and washed in correctly diluted disinfectant prior to use in the next mare. After device application, the mare was checked to ensure no portion of the device protruded from the vulva. If so, small adjustments to its position were made by applying forward pressure to the device with a clean hand. In most instances, the posterior portion of the device was cranial to the vestibulovaginal sphincter. This ensured two of the physical barriers to contamination (the vulva and vestibulovaginal sphincter) were closed behind the device. If more than 3 cm of the device protruded from the vulva, the device was removed, washed in correctly diluted disinfectant, rinsed with water and re-inserted using the applicator.

For each mare, follicle size and location were assessed by palpation and ultrasonography every second day during treatment and until ovulation occurred. Follicles were measured by freezing the image on the ultrasound machine to allow the inbuilt callipers to be utilised. One operator performed all palpations and measurements to remove the possibility of poor reproducibility. Mares were followed through to the ovulation subsequent to the treatment ovulation to monitor longer term effects of the treatments on cyclicity. The five treatments are outlined in Table 2 below.

Table 2 The five synchrony treatments administered to the mares

<p>| CID = Ciderol; OV = Ovuplant; CM = Cue Mare intravaginal device |</p>
<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>DAY 0</th>
<th>DAY 5</th>
<th>DAY 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Device insertion, 10 mL oestradiol benzoate IM</td>
<td>10 mL oestradiol benzoate IM</td>
<td>CM removal, 7.5 mg prostaglandin F2α IM</td>
</tr>
<tr>
<td>(CID10D0,5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Device insertion, deslorelin implant SC</td>
<td>Deslorelin implant SC</td>
<td>CM removal, 7.5 mg prostaglandin F2α IM</td>
</tr>
<tr>
<td>(OVD0,5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 3</td>
<td>Device insertion, 2 x deslorelin implant SC</td>
<td>No treatment</td>
<td>CM removal, 7.5 mg prostaglandin F2α IM</td>
</tr>
<tr>
<td>(OV2D0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 4</td>
<td>Device insertion, 20 mL oestradiol benzoate</td>
<td>No treatment</td>
<td>CM removal, 7.5 mg prostaglandin F2α IM</td>
</tr>
<tr>
<td>(CID20D0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 5</td>
<td>Device insertion</td>
<td>No treatment</td>
<td>CM removal, 7.5 mg prostaglandin F2α IM</td>
</tr>
<tr>
<td>(CM Only)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No treatment</td>
<td>No treatment</td>
<td>No treatment</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis utilised a non-parametric model reporting on median follicle diameter at commencement of treatment, median follicle diameter at completion of treatment, duration from completion of treatment to the development of a 35 mm follicle, and duration from completion of treatment to ovulation.

Data for evaluation of the duration (days) to 35 mm follicle development and ovulation was skewed with a tendency for high values to be more common than low values. To assess for a difference between treatments for these variables, the Brown-Forsythe test was used which is particularly robust with respect to skewness.
Literature Review

An understanding of folliculogenesis, the formation of oocytes, their arrangement in follicles, their growth and maturation and the associated processes involved is required to establish a basis for the requirements of oestrous synchronisation.

The initiation of meiosis in the oocyte coincides with the onset of folliculogenesis in the mare. The follicle is the functional unit of the ovary and is responsible for the maturation of the oocyte and the production of reproductive androgens such as testosterone, oestrogens and inhibin, peptide sex hormones including insulin-like growth factor 1 (IGF-1) and other substances such as angiogenic factors (Gordon, 2003; Pineda, 2003; Senger, 2003). There are approximately 35000 primordial follicles in the mare ovary, of which 100 are maturing at any given time (Driancourt et al., 1982). Only one and sometimes two follicles ovulate during each oestrous cycle therefore the great majority of follicles undergo atresia (Pedersen et al., 1998). In most mammals, the majority of follicles undergo atresia during the late pre-antral to early antral phase of development. Continued development from the early antral phase is then dependant on gonadotrophic stimulation via gonadotrophin-releasing hormone induced increases in FSH and LH (Tilly, 1996).

The process of folliculogenesis occurs in waves midway between inter-ovulatory intervals of 18 – 23 days. It has been proposed that follicular waves emerge at 8 to 10 day intervals resulting in two to three follicular waves during each oestrous cycle involving different cohorts of follicles. Cycles with two follicular waves are considered most common (Ginther, 1993). The first wave of follicular growth, (referred to as the secondary wave), begins at or just before ovulation and generally culminates in atresia of all follicles during dioestrus. The diameter of the largest follicle from this cohort may occasionally attain ovulatory size before regressing. Although rare, ovulations do occur during dioestrus and these ovulations may reflect maturation and ovulation of follicles from this cohort. The second wave of follicular growth, (paradoxically described as the primary wave, since it is the one which leads to the primary, ovulatory, follicle), involves a second cohort of follicles and becomes apparent approximately 10 days prior to ovulation. Final maturation of this cohort of follicles is characterised by the selection of a dominant follicle that goes on to ovulate. The period from follicular recruitment to ovulation is between 11 and 14 days, suggesting that recruitment of the ovulatory follicle probably occurs approximately 8 to 11 days after the preceding ovulation, in a normal cycle.

Following follicular recruitment, the primary wave of follicular growth is characterised by an initial increase in the number of follicles greater than 10 mm. This period is described as the common-growth phase and continues for a mean of 7.4 (± 0.5) days (Ginther O.J., 1992; Ginther, 1993), or until approximately 6 to 7 days before the next ovulation. After this time there is a decrease in the number of large follicles (>20 mm) in association with selection of the dominant follicle(s) destined to ovulate. Emergence of the dominant follicle is referred to as deviation and is preceded by an increase in IGF-1, oestradiol, inhibin-A and activin-A in the future dominant follicle (Beg and Ginther, 2006). Inhibin produced by the granulosa cells of the dominant follicle inhibits continued growth of other follicles in the same cohort. The remaining subordinate follicles grow at a reduced rate, regress and then become atretic. This process of follicular deviation begins when the two largest follicles in any cohort have a mean of 22.5 and 19.0 mm in diameter respectively (Gastal et al., 2004; Ginther, 1993; Ginther et al., 2002). The future dominant follicle emerges earlier on average than the future largest subordinate follicle. A consequence of the rapid growth of the selected, dominant follicle is that whereas several follicles greater than 25mm are generally present 5 days before ovulation, follicular atresia generally results in ovulation of only one. Following ovulation the follicle undergoes luteinisation, forming a corpus luteum, and this remains as the dominant ovarian structure until initiation of luteolysis at around day 14 post-ovulation.

Current understanding of follicular wave dynamics are presented graphically in Figure 2.
Figure 2 Graphical representation of follicular wave patterns in the mare based on current understanding

The timeline represents two oestrous cycles of approximately 21 days between ovulations. Ov = ovulation. The 8 to 11 days parenthesis represents the duration from ovulation to the recruitment of the primary follicular wave. The 7.4 days parenthesis represents the common growth period from emergence of the primary wave to deviation of the dominant follicle at approximately 20mm diameter. The 11 to 14 days parenthesis represents the duration from follicle emergence to ovulation of the dominant follicle arising from the primary follicular wave.

In heifers the relationship between the two largest follicles within a wave has been given some attention, the two largest follicles increase in parallel during the 16 hours before the end of the common-growth phase and smaller follicles increase at a slower rate (Ginther et al., 2001). There has been very little research done on interfollicular relationships in mares with the exception of Ginther in the early 1990’s and Gastal et al in the late 1990’s and again in 2004. The available information is based on the two-follicle model; two follicles were retained and other follicles were ablated as they developed (Gastal et al., 2000). As the two largest follicles grew however, there was no interchange with smaller follicles. In heifers, many follicles within any wave have the capacity to become the dominant follicle. This potential for any follicle to become dominant at the expected beginning of deviation has been shown by ablating various follicles according to rank (Ginther et al., 2001). Ginther et al (2001) were able to demonstrate a hierarchical position, with the largest retained follicle being in the most favoured position for dominance. It was also shown that the second largest follicle retains the capacity for dominance for approximately 24 hours after the beginning of deviation. In mares, there has been very little work done in the area of potential for dominance among follicles at the beginning of deviation. In the studies that have been done the two-follicle model was again used. When the largest follicle was ablated at the expected beginning of deviation, the second largest follicle continued to grow without interruption, became dominant and ovulated in most mares (Gastal et al., 1999). Work by Ginther et al showed that if all the follicles of the post-ablation wave were retained and the largest follicle ablated 24 hours after the expected commencement of deviation, the second largest follicle typically did not become dominant (Ginther et al., 2004). It is possible these findings were influenced
by the puncturing of the second largest follicle for the purposes of sampling. This may have affected its ability to become the dominant follicle. These findings demonstrate some significant differences between bovine and equine folliculogenesis and that knowledge gained from bovine research cannot necessarily be applied to the horse in relation to follicular dynamics. Later work by Gastal showed the largest remaining follicle after ablation of the balance of the cohort became dominant in 26 of 34 mares (76%). In 10 of 15 mares (67%) the second largest follicle became dominant after the ablation of the largest follicle 1 – 2 days after the beginning of deviation. Essentially this work showed that the first follicle to emerge maintained its diameter advantage in most mares and went on to ovulate. The capacity for dominance is similar among the four largest follicles at the beginning of deviation. However, dominance by a smaller follicle is prevented when a larger follicle is present on the ovary. Gastal et al also showed that the second largest follicle retains the capacity for dominance in most mares for up to 2 days after the beginning of deviation (Gastal et al., 2004).

Studies on Manipulation of Ovarian Function

Methods to control the oestrous cycle of the mare have been developed over many years. There have been multiple reasons for this but commonly it is to try to cause the mare to ovulate earlier than the natural start of the breeding season. This brings about an earlier foaling date, therefore an older and possibly more mature foal when compared to foals born later in the season. Since the use of artificial breeding techniques has become common in veterinary practice, breeders have sought to manipulate the oestrous cycle so as to be able to predict when oestrus and ovulation will occur. This allows the timing of insemination to closely coincide with ovulation thereby improving pregnancy rates. There is also the requirement to prevent athletic mares from entering oestrus prior to sporting events as oestrous behaviour is considered to have a negative effect on performance and handling.

Many studies have been conducted to devise improved methods of manipulating the oestrous cycle of the mare. Progesterone and oestradiol 17β has been administered in late winter in conjunction with artificially increased hours of daylight (Taylor et al., 1982). This was done in an attempt to cause mares to ovulate earlier in the breeding season, closer to the start of spring and to determine what degree of control over ovulation could be achieved in late winter by the combination of steroid hormones, photoperiod and prostaglandin F₂α. This work demonstrated that a 15 day protocol of treatment was able to give satisfactory control over ovulation in the early breeding season (spring) however it did not show the same response for mares treated in summer. For mares treated in spring, there was a conception rate of 72% compared with a conception rate of 50% in untreated mares. The study does not identify whether these figures relate to a single service to the controlled ovulation or whether mares were bred by the stallion a number of times. It does however demonstrate that the oestrous cycle of the mare can be readily manipulated by progesterone, oestradiol 17β, increased photoperiod and prostaglandin F₂α. This trial was repeated using the same treatment for ten days, on a larger group of mares (n=128). This work showed that 80% of mares ovulated within eight days of the final treatment (Taylor et al., 1982). This was considered ‘reasonably precise’ by the authors however this level of control would be impractical if being used for the purposes of synchronising ovulation for artificial insemination. However, these results were an improvement on earlier attempts to control ovarian function in the mare.

A number of hormones or their analogues have been used in an attempt to accelerate (induce) ovulation in the mare. The use of a gonadotrophin releasing hormone (GnRH) analogue or Human chorionic gonadotrophin (hCG) is now a common equine breeding practice. A GnRH analogue such as deslorelin acetate administered in the form of a short-term implant (STI) has been shown to successfully shorten the duration to ovulation once a 30 mm diameter follicle is present. Without the use of an ovulation-inducing agent the mean duration from 30 mm follicle to ovulation is 69.5±25.48 hours compared with 42.7±12.35 when deslorelin acetate (2.2 mg STI) is administered (Ganheim et al., 1995). There have been numerous studies indicating that deslorelin is effective in shortening the duration to ovulation in non-lactating, cyclic mares (Hemberg and Lundehem, 2006; Jochle and Trigg, 1994; Squires et al., 1994). It is of interest that Hemberg et al (2006), gives no indication as to why the deslorelin was not administered until a follicle of 42 mm in diameter was reached in each of the 11
mares in their study. In all other studies, results on the use of deslorelin acetate has shown that it is effective if administered after a follicle greater than 30 mm in diameter has been detected on the ovary. Varied dose rates have been trialled ranging from 1.7 mg to 2.7 mg (Squires et al., 1994). Larger doses, in the form of multiple implants have been described and it has been shown that larger doses of deslorelin acetate are effective in shortening the duration to ovulation (Johnson et al., 2002). Unfortunately, they can have a negative effect on the time taken for a mare to return to oestrus if pregnancy does not occur subsequent to the ovulation of treatment. The use of multiple implants of deslorelin acetate (4.4mg dose) increased interovulatory intervals by 14.4 days (36.8 versus 22.0±3.2 days) (Johnson et al., 2002), indicating a significant down-regulatory effect on the hypothalamus. If deslorelin implants are removed 48 hours after administration, mares will have a normal interovulatory period. The STI is most easily removed if inserted in the vulvar mucosa however this location seems to find little favour with horse breeders who appear to prefer placing the implant under the skin of the neck (Wendt et al., 2002). It seems apparent that the prolonged release of deslorelin from the implant was responsible for the lengthened interovulatory intervals (McCue et al., 2002; Wendt et al., 2002). This information is useful for future research requiring prolonged deslorelin acetate secretion.

In recent years, kisspeptins (KP), a peptide product of the \textit{KiSS-1} gene, have been identified as likely major players in reproduction (Caraty and Franceschini, 2008; de Roux et al., 2003; de Tassigny et al., 2007; Gianetti and Seminara, 2008; Roa and Tena-Sempere, 2007). A significant amount of research has gone into determining their precise mode of action by examining different sections of the endocrine pathway and their actions on GnRH and gonadotrophins. The \textit{KiSS-1} system is central in the control and timing of puberty and the regulation of gonadotrophin releasing hormone (GnRH) and gonadotrophins (Kinoshita et al., 2005; Magee et al., 2009; Navarro et al., 2005; Roa et al., 2006; Roseweir and Millar, 2009; Seminara et al., 2003). It has been established that it exerts this effect via interaction with receptors belonging to a protein group known as G protein-coupled receptors (GPCR’s). GPCR gene expression is widely distributed throughout the placenta, pancreas, pituitary, spinal cord, and throughout the brain. Environmental cues and metabolic signals control the onset of cyclicity in seasonal breeders such as horses, and a feedback mechanism from the gonads (sex steroids) regulates the flow of hormones within the hypothalamic-pituitary-gonadal (HPG) axis during the months of normal cyclicity (Revel et al., 2006; Smith, 2008; Tena-Sempere, 2006). It is apparent from this work that kisspeptins may have a significant role in the initiation of follicular wave emergence and follicle maturation. With regard to areas for further research, there would be value in future investigations focusing on the influence of kisspeptins on GnRH and luteinising hormone release.

**Final Follicular Maturation and Ovulation**

Ovulation is a physiological process with an inflammatory component that depends on the coordinated activity of gonadotrophins and steroid hormones, as well as inflammatory mediators such as cytokines, prostaglandins, leptin, nitric oxide (NO) and matrix metalloproteases (Conti et al 2006; Khodaei et al 2009). The LH surge causes major remodelling of the ovarian follicle in preparation for the ovulatory process. These changes include reprogramming of granulosa and theca interna cells to differentiate into luteal cells, changes in cumulus cell secretory properties, and oocyte maturation. Published data supports the concept that LH stimulation of ovarian follicles involves activation of a local epidermal growth factor (EGF) network (Conti et al, 2006). According to this theory, LH activation of granulosa cells stimulates cAMP signalling which, in turn induces the expression of the EGF-like growth factors epiregulin, amphiregulin, and betacellulin. These growth factors function by activating EGF receptors within the granulosa cells (which are epithelial cells), or they may diffuse through the follicular fluid to act on cumulus cells surrounding the oocyte. Activation of EGF receptor signalling in cumulus cells, together with cAMP priming, reactiveates meiosis leading to nuclear maturation and the acquisition of developmental competence within the oocyte, as well as cumulus expansion. As a very specific point, it is worth noting that the matrix metalloprotease (MMP) inhibitor GM6001 completely blocks LH-stimulated oocyte maturation and ovulation in rats (Conti et al, 2006). The specificity of this inhibitor is suggested by the finding that epiregulin-induced oocyte maturation is not affected by the MMP inhibitor. These findings strongly suggest that LH action requires MMP activity, and when
this activity is blocked, it can be bypassed by the addition of epiregulin. Both Epiregulin and MMP therefore are prime candidates for further investigation into the pharmaceutical control of the final stages of ovulation. Due to the wide and potentially harmful effects of MMP’s, it would appear that epiregulin would be the most suitable for initial studies.

LH acts via interaction with its associated LH receptor (LHR), a member of the large family of G protein-coupled receptors (GPCRs). Perhaps importantly and as previously noted, kisspeptins also function through GPCR’s, which provides the possibility of a local ovarian effect of the KiSS-1 system. It is noted that all kisspeptins are able to bind to, and activate GPCR’s specific to kisspeptin (Gianetti & Seminara, 2008; Ohtaki et al., 2001; Roseweir & Millar, 2009; Tena-Sempere, 2006). It would be interesting to investigate whether kisspeptins can activate the LHR group of GPCR’s, as this may provide information regarding the trigger for mares emerging from seasonal transition.

Oestrous Synchronisation in the Mare

Efficient horse breeding programmes require synchronisation or scheduling of oestrus, and ideally ovulation, in the mare. This may be the scheduling of ovulation in a single mare, or it may be the synchronisation of oestrus and ovulation in a group of mares. Labour requirements, both veterinary and on-farm can be better managed if oestrus and ovulation can be accurately predicted. Current protocols for the synchronisation of oestrus can, with reasonable accuracy, predict the onset of oestrus. However, accurate timing and prediction of ovulation is not possible. When the timing of ovulation cannot be accurately predicted there is a requirement to assess mares frequently using ultrasonography (colloquially known as ‘scanning’). Mares may be ‘scanned’ twice daily until ovulation is imminent at which time they may be scanned as often as every three hours. This repeated scanning is particularly common in cases of artificial insemination with frozen-thawed semen. The frequent need to scan mares increases costs to breeders and increases the risks to the mare associated with trans-rectal palpation. The synchronisation or scheduling of oestrus and ovulation improves the time efficiency of personnel involved as well as ensuring efficient use of the stallion and semen.

Current methods of synchronising oestrus in the mare

There are a number of pharmaceuticals and regimens currently used by practitioners to attempt to synchronise or schedule oestrus in the mare.

Prostaglandin

The oestrous cycle of the mare may be manipulated by the administration of exogenous hormones or their analogues. The most commonly used is prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}). Prostaglandin is used to cause luteolysis of mature corpora lutea (CL) during the luteal phase of the oestrous cycle. This luteolysis causes a shorter return to oestrus than would occur in the untreated mare. Prostaglandin is administered intra-muscularly and is effective from five days post-ovulation. Prostaglandin is less effective in causing luteolysis if given prior to five days after ovulation. The specific mechanism for this reduced efficacy on the immature CL is not known, but is postulated to be due to reduced receptors and luteolytic enzymes within the CL. Prostaglandin may also be administered to a group of randomly cycling mares (5 mg IM) on two occasions fourteen days apart. This protocol ensures that by the second injection, all mares will have received a luteolytic dose of prostaglandin that will be effective regardless of the stage of the mare’s cycle at the time of the first injection. Mares treated with this protocol will be reasonably expected to commence oestrus three to five days after the second injection of PGF\textsubscript{2α}. Using this regimen, as with a single injection of prostaglandin, only allows oestrus to be synchronised. The time to ovulation from the administration of prostaglandin will vary significantly, with mares still requiring frequent palpation or scanning to gauge when ovulation will occur. Studies in the 1970’s sought to synchronise oestrus and ovulation by the administration of two courses of treatment with a prostaglandin analogue (Equimate™) and hCG (Palmer and Jousset, 1975). Thirty-three mares received a single IM injection of Equimate (250μg) followed six days later by a single IM injection of hCG (2500 i.u.) This treatment was repeated eight days later. The first treatment was designed to remove all luteal tissue on the ovary and induce ovulation of any follicles
that developed as a result of falling progesterone concentrations. The second treatment after eight days was an attempt to synchronise oestrus and ovulation after luteolysis of the induced CL in each of the mares. Plasma progesterone levels were determined by radioimmunoassay to provide an estimate of when ovulation occurred. This work showed that 79% of the mares had ovulations synchronised over a four day period (Palmer and Jousset, 1975). This study was done prior to the use of shipped chilled or frozen-thawed semen being common. The synchronisation of ovulations over four days may be useful if inseminating mares with fresh semen that will survive in the reproductive tract of the mare for up to 48 hours, however this is too broad a duration when using chilled or frozen-thawed semen or when synchronising mares for an embryo transfer programme.

**Progesterone and oestradiol**

Progesterone has been used to treat anoestrous cattle and horses for many decades. The use of progesterone or progestogens to control the follicular stage of the mare’s oestrous cycle has been commonly used at studs in the USA and UK where mares may be more intensively managed. Altreonogest (Regumate™) is added to the feed of the animal in either a liquid or powder form daily for 10 to 14 days. This requires mares to be given a daily ration of concentrated feed with which the progesterone is mixed. After the cessation of treatment there is normally follicular growth, with subsequent ovulation generally occurring within 10 days. Unfortunately, mares are able to ovulate dominant follicles while under the influence of progesterone (Hughes et al., 1980; Vandeplassche et al., 1979) meaning that as a sole treatment, progesterone will not provide reliable control of the follicular phase of the cycle.

Oestrogen most likely acts by stimulating oestrogen receptor-α in kisspeptin-producing neurones within the medial-basal hypothalamus (Smith 2008; Tena-Sempere 2006; Norman unpublished data). It has also been postulated that oestrogen disables the inhibitory effect of gonadotrophin inhibitory hormone (GnIH) on the GnRH neurones. Thus, as oestrogen concentrations rise as the follicle matures, kisspeptin neurones stimulate GnRH neurones to produce and release GnRH, while they are no longer inhibited by GnIH. With regard to selecting an oestrogen product for use in synchrony programs, it is necessary to understand that oestradiol and its different esters have distinct durations of action (Anonymous, 1983). Oestradiol cypionate (ECP) is formulated to be a long-acting pharmaceutical. It has a duration of action of 14 to 28 days. Oestradiol benzoate (ODB) has a duration of action of 2 to 3 days, oestradiol dipropionate (ODD) 7 to 14 days, and oestradiol valerate (ODV) 14 to 21 days. Oestradiol 17β is one of the natural forms of oestrogen and is metabolised relatively quickly by the liver. Its plasma half-life is measured in minutes, meaning that daily administration is necessary to maintain significant blood concentrations (National Library of Medicine, 2010).

A seminal study in mares used intramuscular injection of a combination of 150 mg progesterone and 10 mg oestradiol 17β (described as a P&E program, being influenced by the American linguistic form with removal the “o” from oestradiol) administered for 10 days to synchronise oestrus and has been considered the “gold standard” for level of synchrony achieved ever since (Loy et al., 1981). In this study there was a 5 day range for ovulations from days 9 to 13 after the completion of treatment. Further studies have been performed in mares using this protocol for 15 days (Taylor et al., 1982). The 59 mares used for this latter study then received 10 mg of prostaglandin F₂₀ on the last day of treatment. This work showed that no significant increases in follicle size (for follicles greater than 20 mm in diameter) occurred during the treatment. In a more recent study, comparisons were made between synchronisation using the P&E program and the follicle ablation technique described subsequently (Bergfelt et al., 2007). In this study, there was an 8 day range for ovulations from days 20 to 27 after the completion of treatment for the P&E program and a 7 day range for ovulations from days 11 to 17 after the completion of treatment for the follicle ablation treatment. These studies, utilising either P&E or follicle ablation, give a good indication of the level of synchrony that is currently achievable in the mare. It can be concluded that the ten day P&E administration protocol is effective in synchronising oestrus but does not allow the accurate prediction of the day of ovulation. However, the level of synchrony is still useful in commercial applications as it allows managerial
attention to be focussed on a time-period during which the mare should be closely assessed for ovulation.

An alternative to the use of oestradiol 17β is the daily injection of progesterone and oestradiol cypionate (ECP) for ten days. However, the use of oestradiol cypionate in animals has been banned in Australia and this protocol also requires daily handling of the mare and is therefore labour intensive. There has been some use in the United States of America of a single “long-acting” injection of combined progesterone and oestradiol 17β, however this product has not been approved for use in Australia. Anecdotal evidence suggests it may be less effective in synchronisation of oestrus than the protocol requiring daily injection (Motteshead, 2005). Two studies have investigated the use of microspheres as a delivery vehicle for the pharmaceuticals (Blanchard et al., 1992; Burns et al., 1990). Microspheres designed to deliver 1.25 g of progesterone and 100 mg of oestradiol 17β at a controlled rate over a 12 to 14 day period were injected intramuscularly. Results showed that progesterone used in combination with oestradiol 17β and prostaglandin F2α gave synchrony of ovulation in 24.1 ± 2.2 days after completion of treatment. However, mare numbers throughout the trials were relatively low, ranging from nine in control and treatment groups to seven in the progesterone only trial (Blanchard et al., 1992). The 2.2 day variation in the progesterone, oestradiol and prostaglandin F2α compares favourably with a 6.6 day variation between mares in the control group. This result demonstrates the usefulness of progesterone, oestradiol and prostaglandin F2α to synchronise oestrus and ovulation. However, a window of 2.2 days is still not ideal when preparing mares for artificial breeding programmes such as artificial insemination and embryo transfer. One interesting aspect of this trial not considered in similar trials is the incidence of oestrous behaviour displayed by mares in the oestrous period following treatment. More mares showed oestrous behaviour in the subsequent trial after treatment with progesterone and oestradiol than mares treated with progesterone alone (p<0.05). This trial also demonstrated a more uniform inhibition of follicular development in the mares receiving the progesterone, oestradiol 17β and prostaglandin F2α. The addition of oestradiol 17β to the protocol reduced variation in the number of days to ovulation, this being the primary goal of synchronisation programmes (Blanchard et al., 1992). The inhibitory effect of oestradiol 17β on follicular development has been demonstrated with the administration of the hormone for 31 days post ovulation (Burns and Douglas, 1981). Mares showed a prolonged interovulatory interval, suppressed follicular development and ovulation. Luteal function did not appear to be affected by daily administration of 10 mg of oestradiol 17β, with normal plasma progesterone concentrations of > 1 ng/mL throughout treatment, falling to < 1 ng/mL at the expected time of days 14 to 20. The reduction in plasma progesterone concentrations coincided with commencement of oestrous behaviour as would occur in untreated animals.

Artificial lighting

The horse breeding industry and particularly the Thoroughbred and Standardbred breeding industries require that the pre-seasonal oestrous transition phase commences as early as possible and be short in duration. One of the most commonly used methods of manipulating the oestrous cycle of the mare is via artificial lighting. Mares are housed indoors, or small holding yards and exposed to extended hours of light to modify the production of melatonin and its negative influence on GnRH production. The range of artificial light treatment required to induce ovulation is 50 to 65 days (Johnson and Becker, 1993). The use of additional lighting enables the advancement of the first fertile oestrus by 6 ± 2 weeks (Guillaume et al., 2000). The use of artificial light has been shown to be a successful method of manipulating the oestrous cycle of the mare and can bring forward the first ovulation of the breeding season but it has not been shown to effectively synchronise oestrus or ovulation.

Prolactin

Acute hyperprolactinaemia, induced by the infusion of the dopamine antagonist metoclopramide has been investigated to determine whether prolactin affected timing of ovulation in mares. These studies failed to show that prolactin had any effect on timing of the end of the luteal phase or the interval to the next ovulation (Johnson and Becker, 1993). From these results Johnson and Becker concluded that
the pharmacological manipulation of serum prolactin concentrations was unlikely to assist in the synchronisation of ovulation. However, further studies may be justified utilising improved dopamine antagonists such as sulphiride or domperidone.

**Follicle ablation**

Generally, oestrous synchronisation is attempted using pharmaceuticals, including steroid hormones as previously described. However, after successful ultrasound-guided oocyte retrieval in mares, a study was performed to determine the efficacy of follicle ablation as an alternative to protocols utilising hormones (Bergfelt and Adams, 1996). Ablation involves the aspiration of follicular fluid until the collapse of the follicle is noted ultrasonographically. Prior to ablation, randomly cycling mares were sedated and given epidural anaesthesia. All follicles ≥ 10 mm in diameter were ablated. A control group of mares received PGF2α only. While the methods used for the statistical analysis of the results is questionable, this work indicated that although the control group had a shorter duration to ovulation, there was less variance in the interval to ovulation in mares that had follicles ablated. An even greater synchrony (75%) occurred in mares with the addition of hCG to the protocol. However, the authors do not state at what follicle diameter hCG was administered. This work was repeated comparing synchrony due to follicle ablation and the progesterone/oestrogen protocol described by Loy et al (1981) (Bergfelt et al., 2007). While statistical analysis of the results was again questionable, there was a suggestion that the ablation technique provided synchrony results similar to the Loy “gold standard” protocol. Although successful, commercial application of follicular ablation is problematic as the equipment required for follicle ablation is specialised and relatively expensive. Its uptake in routine equine veterinary practice is unlikely to be high given these factors. However, it does demonstrate the value for any treatment regimen to induce regression or ovulation of any dominant follicle at the commencement of treatment.

**Intravaginal progesterone-releasing devices**

There have been a number of studies utilising pharmacological treatments to attempt to shorten or remove the transition period as well as synchronise oestrus and ovulation. These studies have used hormones including GnRH and its analogues (Hyland and Jeffcott, 1988; Johnson and Becker, 1993), progesterone administered parentally and progestins orally (Wiepz et al., 1988), PGF2α analogues (Loy et al., 1981), and dopamine antagonists (Besognet et al., 1996). The results of all of these trials have failed to identify a protocol that is reliably able to synchronise oestrus or ovulation.

Intravaginal progesterone-releasing devices have been trialled in an attempt to induce anoestrous mares to return to cyclicity. Acyclicity is sometimes seen in the breeding season due to stress caused by insufficient nutrition, disease, social interactions or high workloads, for example performance horses or lactation. Research has demonstrated that progesterone impregnated controlled intravaginal drug release (CIDR-B) devices can be successfully used to treat mares with persistent anoestrus that is not caused by persistence of the corpus luteum (Newcombe, 1998; Newcombe and Wilson, 1997). The devices were inserted for a variable duration, generally ten to fourteen days, or until a follicle of at least 30 mm in diameter was detected. When this follicle was considered mature (criteria for maturity not listed in the study) the mares were inseminated and 2500 i.u. of hCG was administered intravenously. If no 30 mm follicle had developed after twelve to fourteen days of progesterone treatment the original CIDR-B was removed, another device inserted and the mare monitored until a follicle of sufficient size to allow administration of hCG was detected. Of the mares used in this trial 65.5 % ovulated within ten days of CIDR-B device withdrawal (n=87) (Newcombe, 1998). During this study no comment was made on any signs of vaginitis in the mares from CIDR-B use and the author recommended that all acyclic mares be treated with progesterone until a follicle of 30 mm or greater had developed.

Progesterone in conjunction with equine chorionic gonadotrophin (eCG), formerly known as pregnant mare serum gonadotrophin (PMSG) has been trialled in an attempt to synchronise oestrus (Dinger et al., 1981). Progesterone (1g) was administered via an intravaginal sponge for seven days after which it
was replaced for an additional seven days. On day 12, five of the ten mares were administered 1000 IU of eCG IM. No significant difference in oestrous synchronisation was found between the two groups. The retention of sponges in this trial was 75%. A second trial was conducted using double the dose (2g) of progesterone however no difference in synchrony was found between mares receiving 1g or 2g of progesterone. Retention of the intravaginal sponges was higher in the second trial, possibly due to their larger size. It was also noted by the authors that upon removal, the sponges were saturated with blood and mucus with no permanent damage found to the vaginal mucosa at necropsy. Subsequent research has shown eCG to be ineffective in manipulating the oestrous cycle of the mare.

Intravaginal progesterone releasing devices have been used in cattle for many years for the synchronisation of oestrus and ovulation (MacMillan and Peterson, 1993). The devices were soon used ‘off-label’ in horses as soon as their efficacy in cattle became known. Progesterone releasing intravaginal devices have been used to attempt to induce oestrus and ovulation in anoestrous mares during the transition periods of Spring and Autumn and during lactation (Jochle, 1991; Newcombe et al., 2002; Newcombe and Wilson, 1997). These works have shown that intravaginal progesterone releasing devices may be successfully used to induce oestrus and ovulation during transition and anoestrus. The body of research attempting to synchronise oestrus and ovulation by the use of intravaginal progesterone-releasing devices is quite limited. One study showed reasonable efficacy of CIDR-B used in conjunction with 10 mg oestradiol benzoate given at day 1, with 5 mg prostaglandin $F_{2\alpha}$ analogue dinoprost trometamol (Lutalyse™) administered at device removal day 10 (Norman et al., 2006). This trial showed a mean time to oestrus of 2 days from device removal and 2.9 days to the presence of a 35 mm follicle. In 2004 the efficacy and safety of CIDR-B was investigated by comparing pregnancy rates between three groups of mares (n = 17/group) (Card and Green, 2004). Group 1 received no treatment. Group 2 received 5 mg prostaglandin $F_{2\alpha}$ on day 0 and mares in Group 3 had a CIDR-B device inserted into the vagina, on day 8 the device was removed and 5 mg of prostaglandin $F_{2\alpha}$ administered. Mares in Group 3 were turned out with a stallion after device removal. During treatment, all devices were retained in the mares of Group 3. All Group 3 mares had vaginal discharge at the completion of treatment, with 13 of 17 mares classified as mild discharge and the remaining four classified as moderate. None were found to have severe discharge. There were no differences ($p = 0.7522$) between groups in pregnancy rates. Overall pregnancy rates were 94% for Group one (16 of 17), 100% for Group 2 (17 of 17) and 88% for Group 3 (15 of 17).

**Ovulation Induction**

There have been a number of studies on hastening ovulation in the mare. These began with Nishikawa in the 1940’s (Nishikawa, 1959). Ovulation induction allows the timing of ovulation to be co-ordinated with routine insemination, or when breeding mares with a stallion of low fertility, poor longevity, or with frozen-thawed semen. Two pharmaceuticals have been mainly used for this purpose; gonadotrophin releasing hormone and Human chorionic gonadotrophin.

**Gonadotrophin-releasing hormone (GnRH)**

In many species, exclusive the mare, ovulation is preceded by a surge in LH in the systemic circulation known as the ‘pre-ovulatory LH peak’. Synthetic GnRH has been shown to induce a similar LH surge. After the synthesis of GnRH factors in the early 1970’s (Schally et al in 1971 and further work by Geiger et al 1971) it was shown that GnRH can cause sufficient release of FSH and LH to induce follicular growth and ovulation in domestic animals and rodents. It was considered reasonable that the same effect may occur in the mare and trials were commenced in the breeding seasons of 1973 and 1974 (Heinz and Klug, 1975). Of relevance from this study are the results from group 4, a group of 39 mares having a mature follicle at the time of a 4.0 mg injection of GnRH. All but one of the mares ovulated within 48 hours of administration of the hormone. This preliminary study as well as subsequent studies (Irvine et al., 1975) showed that exogenous GnRH had the ability to induce ovulation and may therefore be useful in synchronisation programmes to either induce ovulation of naturally developing follicles, or perhaps be used in conjunction with other synchrony treatments.
Human Chorionic Gonadotrophin

Human chorionic gonadotrophin has been used to induce ovulation in equine reproduction for over 40 years (McCue et al., 2004). It has LH like effect that acts as an ovulation inducing agent on the dominant follicle. Administration of 1500 – 3300 iu of hCG advances the time of ovulation and the onset of the endogenous LH surge (Evans et al., 2006). hCG has been shown to induce ovulation in greater than 80% of cases 36 – 48 hours after administration to mares with a follicle greater that 30 mm but no greater than 40 mm (Duchamp et al., 1987). Trials conducted by Evans et al (2006) have shown the profile of the LH surge that occurs prior to ovulation after administration of hCG is identical to that associated with non-induced spontaneous ovulation. During the breeding season hCG is often used more than once. It may be administered during subsequent oestrus cycles if the mare fails to become pregnant after the initial ovulation. The production of antibodies against hCG has been reported (Roser et al., 1979) and has caused controversy for many years. Some maintain it causes a reduced efficacy of hCG (McCue et al., 2004) while others have found no effect (Evans et al., 2006; Roser et al., 1979). The recent work (Evans et al., 2006) has shown that perceived loss of functionality may not be the case. The ability of hCG to induce ovulation was found to be maintained after its administration for three cycles during the breeding season. This may suggest that the production of antibodies that has been shown to occur after repeated administration may not be functionally significant. It has also been hypothesised that an ovulatory dose of hCG induces the cessation of follicle growth thereby reducing the follicle diameter during the preovulatory period (Gastal et al., 2006).

Summary

There are several pharmaceuticals which can be used for the synchrony of oestrus and ovulation in the mare. They can be broadly divided into products with PGF2α activity which shorten the luteal phase by removing the CL; products lengthening the luteal phase by providing exogenous progesterone; products hastening ovulation or luteinisation by delivering GnRH or LH-like activity; and products containing steroid hormones or GnRH agonists which directly influence the hypothalamus and pituitary to modify gonadotrophin release. Non-pharmaceutical means of providing synchrony involving a process of follicle ablation using ultrasound-guided transvaginal techniques have also been described.

Of the techniques currently used, 10 daily injections of 150 mg progesterone and 10 mg oestradiol 17β provide the best results, with ovulations clustering from 9 to 13 days after completion of treatment. There is an indication that administering progesterone and oestrogen utilising microsphere technology may provide equivalent results with less handling. The follicle ablation technique also provides similar synchrony results, and while not considered practical, provides an indication of the value in controlling the follicular wave for achieving ovulation synchrony.

The delivery techniques for pharmaceuticals include injections of solutions or suspensions; subcutaneous implants; and intravaginal devices. From the point-of-view of commercial practicality, any delivery technique which reduces the number of mare handlings is desirable. In this regard, the use of microsphere, implant, or intravaginal device technologies are ideal.

For synchrony programs to be practical, there is a need for pharmaceuticals to be commercially available and for the labour involved in handling mares and administering pharmaceuticals to be kept to a minimum. Products which currently fit these requirements while having the potential to effectively influence follicular wave dynamics include the GnRH agonist deslorelin; prostaglandin F2α; and the steroid hormones progesterone and oestrogen. With regards to oestrogen, there are a number of esters available, with varying half-lives. In administering oestrogen, a balance is required between over-stimulation of the hypothalamus with subsequent down-regulation, or too little stimulation failing to provide the desired follicular control. For these reasons, the medium-acting oestradiol benzoate ester is considered most suitable for use in synchrony regimens.
Results

There were a total of 60 mares included in the study which ran over three breeding seasons from 2007 to 2009.

Assessment of mares for possible adverse effects of treatments

Particular attention was paid to the influence on the mare of inserting an intravaginal, progesterone-releasing device. No significant abnormalities were detected in the vaginal vault of any of the mares at the commencement of the treatments. Immediately after insertion of the device, all mares raised the tail, with 17% urinating. After this initial response, the only indication of the presence of the device was a slight, intermittent elevation of the tail in the period within 30 minutes of treatment followed by no further visible signs. All mares grazed normally for the duration of the treatment and no clinical signs of systemic disease were noted during or at the completion of the trial. In 63% of treated mares, a slight discharge was noted at the vulval lips while the intravaginal device was in place. At the time of removal of the device, a small amount of purulent material on the device and at the vulval lips was observed in 85% of the mares. The discharge resolved spontaneously within two days after device removal in all mares. No mares expelled the device prematurely. During the final speculum examination, at the time of ovulation, no significant abnormalities were detected within the vaginal vault.

No untoward effects associated with any of the other treatments were noted other than well documented mild discomfort associated with the prostaglandin injection in some mares.

Important Points

- Attention was paid to ensuring clean insertion of the device into the mare
- The blue nylon cord was removed from the Cue-Mare device to reduce the potential for bacterial contamination of the reproductive tract.

Investigation of follicular dynamics during the treatment and ovulatory periods

The aim of the treatments was to gain control of the follicular wave. Evidence of this control is the regression of dominant follicles present at the commencement of treatment allowing emergence of a new follicle. For each of the treatments, Table 3 shows the percentage of cases where the initial follicle at commencement of treatment regressed and a new follicle emerged.
Table 3  Percentage of cases (mares) where treatments resulted in regression of the dominant follicle present at the commencement of the regimen and emergence of a new dominant follicle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Follicle regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70%</td>
</tr>
<tr>
<td>10CIDD05</td>
<td>70%</td>
</tr>
<tr>
<td>CM Only</td>
<td>80%</td>
</tr>
<tr>
<td>OVD05</td>
<td>80%</td>
</tr>
<tr>
<td>2OVD0</td>
<td>80%</td>
</tr>
<tr>
<td>CID20D0</td>
<td>100%</td>
</tr>
</tbody>
</table>

In addition to regression of the initial follicle, it is necessary for treatments to allow a new follicular wave to emerge in a timely manner. In this regard, timeliness was considered to be a new follicular wave emerging within the treatment period in order for it to be ready to mature and ovulate soon after completion of treatment.

For each mare, the size of the largest follicle at the commencement of treatment and the size of the ovulatory follicle were documented over time. In some instances these were the same follicle. The progressive size of the ovulatory follicle was determined retrospectively from the records once ovulation had occurred. For each of the treatment groups, selected individual graphs of the follicle dynamics over the treatment period and up until ovulation are presented.

**Follicle dynamics for the Control group**

For three of the ten mares in the Control group, the follicle present at the commencement of treatment was also the follicle which went on to ovulate. This indicates that the follicular wave pattern was not influenced by the procedure. An example of this pattern is shown in Figure 3.

![Mare 1 - Control](image)

**Figure 3**  An example of a Control mare where the largest follicle at the commencement of the treatments was the same one which became dominant and ovulated

The follicular wave was not modified.
Figure 4 shows the wave pattern of a normally cycling mare in the Control group where the follicle present at the start of treatments ovulated nine days later, with the second follicular wave emerging and ovulating in another 20 days.

**Figure 4** An example of a Control mare where the largest follicle at the commencement of the treatments ovulated allowing a new follicle to emerge and ovulate 20 days later.

The follicular wave was not modified.
Follicle dynamics for treatment groups 2 and 3

Treatment groups two and three are considered together as they were both based on the use of a GnRH agonist. While 55% of these two groups showed useful response to treatment, there were significant abnormalities in the follicular waves and cycles of the remainder. The graphs shown in Figure 5 and Figure 6 are examples of abnormal wave patterns resulting from the treatments. It is postulated that once a suitable injectable formulation of deslorelin becomes available, there may be potential to customise the dose in a continuum based on bodyweight. This may reduce abnormal responses associated with the current restrictions imposed by the need to use implants.

Figure 5  An example of the follicular response after the administration of 4.2 mg deslorelin (2 implants) at the commencement of treatment

Note ovulation of the dominant follicle at treatment commencement followed by a 25 day interovulatory interval.

Figure 6  An example of the follicular response after the administration of 4.2 mg deslorelin (one implant day 0 and one implant day 5)

In this case, treatment commenced during dioestrus and the dominant follicle progressively reduced in size over a period of 21 days prior to ovulating. It is postulated that the oocyte contained within this follicle would have reduced fertility.
Follicle dynamics for treatment groups 1 and 5 (CID10D05 and CM Only)

These two treatments provided good synchrony, with 65% of cases in these two groups showing good control of the follicular wave pattern. However, there were indications of incomplete control of the follicular wave pattern. This leaves doubt as to the repeatability of the synchrony resulting from these treatments. Examples of wave patterns indicating incomplete control are shown in Figure 7 and Figure 8.

**Figure 7**  An example of poor control of the follicular wave pattern in mares treated with progesterone only (CM Only group)

Development of the initial follicle continued throughout treatment resulting in ovulation the day after treatment was completed at day 10.

**Figure 8**  An example of poor control of the follicular wave pattern in mares treated with progesterone and two 10 mg doses of oestradiol benzoate

Note that the development of the initial follicle appears to be suppressed, but not halted as a result of the treatment. The initial follicle went on to ovulate 10 days after completion of the treatment. This indicates the 10 mg dose is not high enough to reliably reset the follicular wave.
**Follicle dynamics for treatment group 4 (CID20D0)**

Figure 9 provides an individual example of the follicular dynamics associated with the CID20D0 treatment. In all cases for this treatment, the dominant follicle at the commencement of treatment underwent atresia in a pattern similar to the one shown.

![Figure 9](image)

**Figure 9  Follicle dynamics typical of treatment 4 (CID20D0)**

Note the atresia of the dominant follicle at treatment start, followed by emergence of the ovulatory follicle approximately 9 days after the commencement of treatment. This pattern indicates that the 20 mg oestradiol benzoate dose reliably resets the follicular wave.

Figure 10 shows the mean follicle size for this group from the start of treatment for both the initial follicle and the ovulatory follicle. The initial follicle regressed progressively, to become less than the size considered necessary to be steroidogenically active (< 19 mm) by approximately 7 days after the commencement of treatment. The follicle destined for ovulation emerged (>10 mm) a mean of 7.8±3.2 days after the commencement of treatment and reached deviation size (approximately 19 mm diameter) a mean of 10.4±4.3 days after the commencement of treatment. This coincided with the end of the treatment regimen.

In Figure 11, the progress of the ovulatory follicle can be assessed with follicle size standardised to the day of ovulation. This demonstrates that the follicle emerged and reached dominance at approximately 11.5 and 8.5 days prior to ovulation respectively.
Figure 10  Mean (±SE) follicle size from treatment start for both the initial follicle and the ovulatory follicle in the CID20D0 treatment group

An outlier was removed from the dataset. Treatment was completed on day 10.

Figure 11  An ovulation-centric depiction of follicular dynamics for the CID20D0 treatment group.

With measurements standardised around the day of ovulation it can be seen that the ovulatory follicle emerged (>10 mm) approximately 11.5 days prior to ovulation and reached dominant size (>19 mm) approximately 8.5 days prior to ovulation.
**Interovulatory interval between the first and second post-treatment ovulations**

Mares were monitored until their second post-treatment ovulation to assess whether the treatments had any adverse effects on cycle length. The summary of the results for each group are shown in Table 4.

**Table 4  Mean and standard deviation for the cycle length following the first post treatment ovulation**

Mares were not monitored for second ovulation for one of the replicates due to a communicable disease outbreak. This resulted in eight cases per group for this analysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (Days)</th>
<th>SD (Days)</th>
<th>Mares failing to ovulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVD0</td>
<td>21</td>
<td>2.07</td>
<td>2</td>
</tr>
<tr>
<td>2OVD0</td>
<td>21.14</td>
<td>2.12</td>
<td>1</td>
</tr>
<tr>
<td>10CID05</td>
<td>20.5</td>
<td>2.67</td>
<td></td>
</tr>
<tr>
<td>CM Only</td>
<td>20.88</td>
<td>3.68</td>
<td></td>
</tr>
<tr>
<td>CID20D0</td>
<td>20.88</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23.29</td>
<td>4.15</td>
<td>1</td>
</tr>
</tbody>
</table>

For the three groups producing tight post-treatment synchrony (10CID05, CM Only, CID20D0), all mares cycled at standard interovulatory intervals in the cycle following treatment. This indicates that these treatments did not have an adverse long-term effect on follicle emergence and development.

It is interesting that despite failing to provide good synchrony for the first post-treatment ovulation, both treatments containing the GnRH agonist deslorelin (OVD05 and 2OVD0) provided the smallest variation in cycle length subsequent to the first ovulation. Unfortunately, three of the four mares failing to ovulate for a second time were also in these two groups. This may indicate that further work on the use of GnRH agonists to manipulate the follicular wave may be warranted provided dosages can be fine-tuned by using an injectable formulation. It is possible that pre-synchrony treatments utilising these pharmaceuticals may be useful. At the time of these trials, deslorelin was only available in implant form. Therefore, dosing of mares could only be done in multiples of the implant dose of 2.1 mg. This does not allow for body weight based dosing, as would occur with an injectable formulation. Body weights of the mares ranged from 477 kg to 572 kg, meaning that on a dose-rate per kilogram basis, there would have been variation from 7.3 µg/kg to 8.8 µg/kg in the OVD05 and 2OVD0 groups. Intuitively, higher dosages are more likely to result in anovulatory responses associated with down-regulation of the hypothalamus.

Also of interest is that it is common anecdote within the veterinary profession that 4.2 mg deslorelin (two implants), will stop mares from cycling for several months. That was not the finding in this study, where only three (18%) of the mares receiving two implants failed to ovulate for a second time within the study period.
Investigation of the duration from treatment completion to the attainment of a 35 mm follicle

For each treatment, the durations from treatment completion until the attainment of a 35 mm follicle are presented in Figure 12. The Box and Whisker plot shows the median value, quartiles, plus minimum and maximum values for each treatment.

![Figure 12 Box and Whisker plot of days from treatment completion to the emergence of a 35 mm follicle for each of the treatments and Control animals](image)

Upper and lower ends of the whiskers indicate the upper and lower values. Upper and lower margins of the box represent the upper and lower quartile respectively. Medians are indicated by the dark line. Circles indicate possible outliers.

Application of the Brown-Forsyth analysis shows a significant difference in variability of time to 35mm follicle between treatments (p-value = 0.03599). From examination of the boxplot, it is clear that the lowest variances are for the two treatments incorporating oestradiol benzoate (CID10D05 and CID20D0) and the treatment using only the intravaginal progesterone-releasing device (CM Only). These treatments also have the lowest medians indicating the shortest time-period from treatment-end to ovulation. The treatment with the least amount of variation is the CID20D0 protocol where median duration from treatment end to 35 mm follicle is 5 days. The lower and upper quartiles were 3 and 6 days respectively with an overall range of 1 to 8 days. An important aspect to this result is that none of the mares in this group ovulated prior to day 5. Therefore, if they had been bred on day 5 (if teased in oestrus), and given an ovulation induction agent such as hCG at the time of breeding, then 80% would have ovulated while the inseminate was still viable.

It is noted that one of the mares in the CID20D0 group was listed as an outlier. On following up on her history, it was revealed that she had been administered two deslorelin implants approximately 12 months earlier in an attempt by her trainer to prevent oestrus during competition. While it cannot be confirmed whether this administration may have adversely influenced the outcome, it has been apparent from these studies that high doses of deslorelin can result in long-term suppression of follicular development in a small percentage of mares. If the outlier mare is removed from the data set,
then 89% of the group would have ovulated while the inseminate was viable from a single breeding on day 5 post-treatment, provided ovulation induction was utilised.

**Investigation of the duration from treatment completion to the attainment of ovulation**

For each treatment, the durations from treatment completion to ovulation are presented in Figure 13. The Box and Whisker plot shows the median value, quartiles, plus minimum and maximum values for each treatment.

![Box and Whisker plot](image)

**Figure 13** Box and Whisker plot of days from treatment completion to ovulation for each of the treatments and Control animals

Upper and lower ends of the whiskers indicate the upper and lower values. Upper and lower margins of the box represent the upper and lower quartile respectively. Medians are indicated by the dark line. Circles indicate possible outliers.

Application of the Brown-Forsyth analysis shows a significant difference in variability of time to ovulation between treatments ($p$-value = 0.01264). From examination of the boxplot, it is clear that the lowest variances are for the two treatments incorporating oestradiol benzoate (CID10D05 and CID20D0) and the treatment using only the intravaginal progesterone-releasing device (CM Only). These treatments also have the lowest medians indicating the shortest time-period from treatment end to ovulation. There is an indication that the treatment with the least amount of variation is the CID20D0 protocol where median duration from treatment end to ovulation is 8 days. The lower and upper quartiles were 6 and 9 days respectively with an overall range of 5 to 10 days.

The result from this treatment regimen is very positive when compared to the currently held “gold standard” reported by Loy et al (1981). After completion of their regimen of daily injections for 10 days, all mares ovulated over the five-day period from 9 to 13 days later. The value in gaining a one-day reduction in the spread of ovulations compared to the CID20D0 protocol seems limited when the logistics of each programme are considered. The Loy protocol requires the handling and injection of
mares daily for 10 days in contrast to the CID20D0 protocol requiring two handlings and two
injections. In addition, the drugs required for the Loy protocol are not available commercially and
would need to be compounded. The drugs for the CID20D0 protocol are all commercially available.
Discussion

In specifically addressing the goals of this research, we have provided a literature review of follicular dynamics in the mare and how it relates to oestrus and ovulation synchrony. Current techniques for the synchronisation of oestrus and ovulation have been discussed and their expected outcomes documented.

There were no long-term adverse effects on mare health or subsequent cyclicity of the most effective treatment regimens (Treatments 1, 4 and 5). Previous reports on the use of intravaginal progesterone-releasing devices suggest they have no long-term adverse influences on fertility, with pregnancy percentages subsequent to their use being similar to industry standards (Norman et al., 2006). The treatments incorporating GnRH agonists adversely affected cyclicity in 18% of mares. Although not successful when utilised in the administration regimens in this study, it is suggested that GnRH agonists may still have potential value in synchrony programs once dose rates can be more precisely administered with injectable formulations.

From this research it is apparent that progress has been made in understanding and controlling follicular wave dynamics and follicle emergence. In treatment four, involving the injection of 20 mg of oestradiol benzoate on the day of insertion of the progesterone releasing intravaginal device, all mares underwent resetting of the follicular wave to the point where the dominant antral follicle at the time of treatment commencement became atretic prior to the emergence or maturation of the eventual ovulatory follicle. Within this treatment group the follicle destined for ovulation reached deviation (dominance) a mean of 10.4 days after the start of treatment and eight days prior to ovulation. Taking into account studies suggesting a mean of approximately 7.4 days for the duration of the common growth phase of a cohort of follicles after follicle emergence (Ginther O.J., 1992; Ginther, 1993), extrapolation suggests that emergence of the follicle cohort most likely occurred a mean of three days after treatment commencement rather than 7.8 days which was based on ultrasonographic identification of a follicle 10 mm in diameter or greater. This indicates that the combination of a 20 mg dose of oestradiol benzoate and intravaginal progesterone-releasing device has a profound suppressive effect on post-deviation follicles, yet does not interfere with the emergence of subsequent follicular waves. Despite a comparable degree of synchrony associated with the treatment utilising the 10 mg doses of ODB, the fact that they failed to reliably suppress the dominant follicle at the commencement of treatment leaves a degree of uncertainty regarding their ability to reliably produce repeatable results. It appears that a single 20 mg ODB dose is most suitable for follicle control and investigation of slightly higher doses would be valuable to determine if synchrony can be improved.

Despite some progress in the control of follicular wave patterns and follicular wave emergence, there is still an undesirable degree of variance in the duration from the end of treatment to ovulation. From this research it becomes apparent that in addition to controlling follicular wave patterns and follicular wave emergence, there is also a need for the development of pharmaceuticals suitable for controlling follicle growth and maturation during the period immediately prior to ovulation. By combining the results provided in Figures 10 and 11, there appears to be the possibility that an increase in the duration of progesterone treatment may provide improved synchrony. If follicle development can be suppressed by progesterone for another 48 hours after deviation, it may be possible to have improved synchrony in the final maturation stage prior to ovulation. The literature review also identified the possibility of further investigation into the role of epiregulin in the final stages of follicle development and its potential to be harnessed as a pharmaceutical aid to the synchronisation of ovulation.

It has been determined that for the most effective treatment identified in this study (Treatment 4), mares developed a 35 mm follicle from one to eight days after the completion of treatment. The significance of a 35 mm follicle is that at this size, they have adequate receptors to respond to luteinising hormone or its analogues. Importantly, none of the mares ovulated prior to day five post-treatment. This provides opportunity for the addition of an ovulation induction agent such as human chorionic gonadotrophin (hCG) to the synchrony regimen on day five post-treatment to ensure follicle
maturation and ovulation. In this study, 89% of mares in Treatment 4 would have ovulated while the inseminate from a single breeding on day five was still viable if hCG had been added to the regimen on day five post-treatment.
Implications and Recommendations

Findings contained within this report will assist the equine breeding industry in the making of informed decisions with regard to the synchronisation of oestrus and ovulation in the mare. This report confirms that commercially viable synchrony regimens have been available for a number of decades, yet there is a lack of commercially available products that are registered for use in horses. Therefore, the report should provide stimulus for the industry to encourage commercial products to be developed, and appropriately registered. In particular, slow-release injections utilising microsphere technology appear to have significant potential and could be developed for the delivery of progesterone and oestrogen pharmaceuticals to provide synchrony with minimal handlings and injections.

There is still further work required to achieve the ideal regimen for the synchronisation of oestrus and ovulation in the mare. In particular, there is a need for more investigation into methods of controlling follicular maturation in the period immediately prior to ovulation. This type of information is not readily extrapolated from other species due to the comparatively long duration of the follicular phase and oestrus in the mare. However, this report identified an EGF-like growth factor called epiregulin, which may be worthy of further investigation. It will also be valuable to reassess the use of GnRH agonists utilising recently available injectable formulations.

It is noted that products utilised in many synchrony regimens were originally developed for use in cattle. With particular regard to the use of oestrogen pharmaceuticals, there is growing pressure from major markets to reduce their use in food producing animals. This will see a progressive decline in their use within the cattle industry and may result in manufacturers discontinuing production. The value of oestradiol benzoate for the manipulation of the oestrous cycle of the mare has been confirmed by results contained in this report. Unless the equine breeding industry is proactive in securing formulations specifically for use in the mare, there is a real risk that they will soon become unavailable.

Treatment four described in this report, and outlined in detail in the Fact Sheet in Appendix 1, is suitable for commercial application. It can be expected to provide a degree of synchrony (or accuracy in ovulation scheduling) equivalent to the current industry “gold standard” with significantly less labour input and drug costs.
Appendix 1

Synchronisation of oestrus and ovulation in the mare

Applies to: Synchronisation; oestrus; ovulation

Authors: Scott Norman, Jennifer Larsen

Last updated: Thursday, November 04, 2010

Introduction

The synchronisation of oestrus and ovulation can be a valuable tool for assisting the reproductive management of the mare and stallion. There is still the need for teasing or ultrasound evaluation with current synchrony programs, however their requirement is greatly reduced. While there are a number of products and regimens that can be used, the protocol described in this Fact Sheet is one which requires minimal labour input, yet is not surpassed by any other technique with regards to ovulation synchrony.

Note: This procedure description is designed to be used under the guidance of a registered veterinarian. No guarantee regarding the suitability, accuracy or timeliness of this information is given or inferred (refer to page ii).

Procedure

1. Place the mare in a veterinary crush or other form of dependable restraint.

2. Apply a tail wrap before cleaning the anus and vulva of the mare. Ensure the vulva is always cleaned with fresh water and towel after the anus is cleaned and dried. We recommend the ‘clean-hand-dirty-hand’ technique.

3. Select a clean CIDR-B™ or Cue-Mare™ progesterone releasing device and remove the nylon extraction cord prior to loading into the applicator.

4. Apply a small amount of clean obstetrical lubricant or KY-gel to the applicator and insert it into the anterior vagina. Depress the plunger on the applicator to place the device deep into the vagina and remove the applicator. Ensure no portion of the device protrudes from the vulva. If so, small adjustments can be made by applying forward pressure to the device with a clean hand. If more than 3 cm of the device protrudes from the vulva, remove the device, wash it in correctly diluted disinfectant, rinse with water and re-insert using the applicator.

5. Inject 20 mg oestradiol benzoate (20 mL Ciderol™) intramuscularly and release the mare. Most will attempt to urinate immediately after the procedure. They may have the tail slightly raised for a few hours after the procedure, after which there is no further indication of the presence of the device.

6. Ten days after insertion of the device, repeat Steps 1 and 2 above. While wearing a disposable glove, insert a hand through the vulval lips, grasp the back of the progesterone releasing device and remove it. Be prepared for a small amount of cloudy discharge associated with the device removal. This discharge is normal and clears within 36 hours. Inject a luteolytic dose of prostaglandin F2α intramuscually. For example: Lutalyse™ at 15 µg/kg, or approximately 1.5 mL for a 500 kg mare.
7. For ovulation scheduling of an individual mare, organise palpation and scanning 48 hours after device removal. Take the advice of your veterinarian regarding further treatments and time to breed.

8. For the synchronisation of a group of mares, tease and palpate 72 hours after device removal. Take the advice of your veterinarian regarding further treatments and the timing of ovulation. In the majority of instances, ovulations can be timed between 5 to 8 days after device removal.

9. You have completed the procedure.
References


Palmer, E., Jousset, B., 1975. Synchronisation of oestrus in mares with a prostaglandin analogue and hCG. In: pp. 269-274.


The synchronisation and scheduling of oestrus and ovulation is a management tool that is useful at all levels of equine reproductive management. Improving synchrony regimens will aid the efficiency and safety of the equine stud industry due to anticipated reductions in the need for teasing mares, palpating and scanning mares, and improved timing of inseminations. At the commencement of this study there was no single regimen which achieved the goal of tight synchrony, with the current “gold standard” regimen requiring daily injections for 10 days of a formulation which is not commercially available.

All members of the equine stud industry may benefit from information contained in this report. In the day-to-day management of a stud utilising natural service, synchronisation or scheduling of ovulation can reduce the labour and risks associated with teasing. It can also assist stud managers to gain more efficient use of stallion services.

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