Reproductive performance and the transition period of Thoroughbred mares in New Zealand: Evidence and implications for future alternative management strategies

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David William Hanlon

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Abstract

The aims of this research were to investigate the reproductive performance of Thoroughbred mares in New Zealand, to examine the use of intravaginal progesterone to manage transitional mares and to develop a potential model for haemorrhagic anovulatory follicle (HAF) development in the mare.

Firstly, a prospective cohort study was performed involving five stud farms in the Waikato region of New Zealand during three consecutive breeding seasons (2006-2008). A total of 1482 individual mares contributed 2007 mare years and 3402 oestrous cycles over the three breeding seasons. The mean first-cycle pregnancy rate was 53.6%, the end-of-season pregnancy rate was 85.3% and the foaling rate was 80.2%. The length of the breeding season was relatively short with 87% of services occurring in the 91 day period between 1st September and 30th November. Multivariable analyses revealed that reproductive performance was influenced by two main mare-related factors; the age of the mare and her reproductive status (dry or foaling). Increasing mare age significantly reduced the first-cycle pregnancy rate, reduced the end-of-season pregnancy rate and increased the interval from the start of the breeding season to conception. In terms of reproductive status, dry mares had a significantly higher first-cycle pregnancy rate and end-of-season pregnancy rate compared with foaling mares. The majority of variation in reproductive performance was associated with mare-level factors and the contribution of the stallion and stud farm was relatively minor.

Transitional mares treated with intravaginal progesterone at the start of the breeding season were served two weeks earlier than untreated control mares. In the first 21 days of the season, 95% of treated mares were served compared with 43% of control mares. Treated mares also conceived earlier and had a higher end-of-season pregnancy rate than control mares. Follicle development in response to intravaginal progesterone treatment

appeared to be mediated through a close temporal association between progesterone, FSH and LH during treatment.

In the last study, transplantation of chorionic girdle into non-pregnant mares resulted in elevated eCG concentrations in all mares and the development of multiple HAFs in the treated mares over a prolonged time-span.

In conclusion, this research has, i) identified the most important factors associated with the reproductive performance of New Zealand Thoroughbred mares, ii) determined that intravaginal progesterone treatment is a suitable management tool for transitional mares and iii) created a model for HAF development in the mare.

Preface

Each chapter of this thesis is set out in the style of the journal to which it has been submitted. Therefore there is some repetition, and there are stylistic differences between chapters in terms of spelling (USA vs New Zealand) and in the layout of each chapter. The submitted manuscripts include other authors. For each chapter, I designed the research, undertook the fieldwork, analysed the data and wrote the manuscripts. I was, however, assisted by my co-authors in the final revision of each manuscript prior to submission.

Approval for the research conducted in this thesis was granted by the Massey University Animal Ethics Committee (Chapters 5 and 6) and the guidelines set forth by the Institutional Animal Care and Use Committee of Cornell University (Chapter 7).

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Chapter 1: Introduction

The New Zealand Thoroughbred breeding industry is one of the largest in the world, producing around 4500 foals per year, representing 4% of the total global Thoroughbred foal crop (http://www.jockeyclub.com). The industry employs around 23,500 people and is worth $1.1 billion annually to the nation's economy (Anonymous 2004). Activities associated with the breeding of Thoroughbreds account for approximately half of the income generated by the industry and it is estimated that around 7000 people directly derive their income from Thoroughbred breeding (Anonymous 2004).

Historically, New Zealand-bred Thoroughbreds have always performed well on the world stage, particularly in Australia. In recent years, horses such as Sunline, So You Think, Seachange, Starcraft, Might and Power, Octagonal, and Bonecrusher have dominated Australian racing. In the 2010/2011 racing season, the New Zealand bred horse **So You Think** (High Chaparral (Ire) ex Triassic (NZ)) was rated the Champion Stayer of the World by the International Federation of Horse Authorities in London. In the same year, **Black Caviar** (Bel Esprit (Aus) ex Helsinge (NZ)) was officially rated the Champion Sprinter of the World. New Zealand-bred horses won 21 of the 68 (31%) Group 1 races in Australia in the 2010/2011 racing season despite comprising only 5.2% of the Australian racing population in that year (Anonymous 2011a).

In the 2009/2010 season the New Zealand Thoroughbred Stallion Register listed 52 individual commercial stud farms, 6200 broodmares and 166 stallions that served more than 10 mares (Anonymous 2011a). The New Zealand Thoroughbred breeding industry is predominantly based in the North Island with 51% of broodmares and 52% of stallions located in the South Auckland and Waikato regions (Anonymous 2011a). The structure of the breeding industry in New Zealand is similar to that in many countries with stallions residing at larger stud farms and mares boarding at those farms for the duration of the breeding season. Mares may also visit stallions for service from outside boarding farms ("walk-ins"). In recent years there has been an increase in the number of "shuttle stallions" used in New Zealand. These stallions breed mares in both the Northern and Southern Hemispheres in consecutive breeding seasons. Most shuttle stallions are from the UK and USA (Anonymous 2011b).

New Zealand is unique in that pasture is the predominant source of nutrition throughout the year for all horse classes on a commercial stud farm (Rogers et al 2007). The maintenance of horses outside, on pasture, means that large numbers of mares can be efficiently managed by relatively few people and this contributes to low production costs compared to other major Thoroughbred breeding countries (Anonymous 2010a). Most commercial stud farms derive most of their income from selling yearlings at annual sales in New Zealand and Australia. Around 65% of yearlings are purchased by Australian owners and trainers (Anonymous 2011a). On stud farms that stand stallions, the majority of income is generated by the sale of service fees to resident stallions. A service fee is payable by the mare owner to the stallion owner on the diagnosis of a 42 day positive pregnancy test.

The breeding season commences on 1st September in New Zealand and Australia. A horse's birth date (regardless of its actual date of birth) is 1st August in the Southern Hemisphere and 1st January in the Northern Hemisphere. The universal birth date for all horses dates back to 1751 when the English Jockey Club decreed that the birth date of all horses in the UK would be 1st May (Ginther 1992). However, the 1st May birth date occurred right in the middle of the summer racing season and therefore caused considerable difficulties for racing administrators in regards to age-restricted races. In 1833 the English Jockey Club decided to change the birth date to 1st January as this was prior to the summer racing season (Ginther 1992). In the Southern Hemisphere, 1st July was initially chosen as the birth date for all horses because it was exactly 6 months after the 1st January. However, in 1859 the Australian Jockey Club decreed that the universal birth date in Australia would be 1st August because this coincided with the start of the spring racing season (Anonymous 2010b). New Zealand racing administrators followed suit to be in line with the Australian racing calendar and this official birth date has remained unchanged since 1859. Historically, this meant that if a foal was born prior to 1st August in the Southern Hemisphere, it would turn 1 year of age in the same calendar year in which it was born. In 2000, the New Zealand and Australian Stud books modified the rules related to determining the age of horses, such that the birth date is now calculated in relation to when a mare is covered (served) as opposed to when the foal is born (Anonymous 2011b). Rule 401 from the New Zealand Rules of Racing:

The age of each horse shall be determined as follows:

(a) in the event that the mare was first covered before 1 September in a calendar year, then the produce of that mare which is born in the following calendar year (regardless of its date of birth) will be deemed to have commenced its second year of life on 1 August of the calendar year in which it is born (i.e. it will become a yearling on that day); or

(b) in the event that the mare was first covered on or after 1 September in a calendar year, then the produce of that mare which is born in the following calendar year (regardless of its date of birth) will be deemed to have commenced its first year of life on 1 August of the calendar year in which it is born, provided that date of birth is consistent with such covering.

Whilst the changes to this rule alleviate the problem of foals becoming yearlings within days or weeks of birth, it has introduced another logistical problem for the industry in that now all mares start the breeding season on the same day of the year (1st September). Mares are seasonal breeders, and the artificially imposed start of the breeding season occurs at a time of the year when most non-pregnant (dry) mares are in the transition phase from winter anovulatory anoestrus to normal, regular oestrous cycles (Donadeu and Watson 2007). The transition period is associated with erratic oestrous behaviour, the growth and regression of ovarian follicles which fail to ovulate, and lasts between 30 and 90 days (Nagy et al 2000; Donadeu and Watson 2007). The natural tendency of mares to produce one or more large anovulatory follicles during the transition period means that enormous amounts of time, effort and money are wasted from attempts to breed mares before normal ovulatory cycles are established (McCue and Squires 2002).

In addition to the artificially imposed start of the breeding season, Thoroughbred stud books throughout the world stipulate that a foal can be registered only if it is the product of natural service. Therefore, the use of techniques such as artificial insemination and embryo transfer are forbidden within the industry. The First Appendix of the New Zealand Thoroughbred stud book states:

2 (1) The following horses and no others shall be eligible for entry in the New Zealand Stud Book, viz:

(a) horses which authentically trace in all their lines to horses recorded in that Stud Book;

(b) horses which are recorded in a recognised Stud Book of another country and be the product of a mating between a sire and dam both of which were registered prior to the 1st January 1980 in a Stud Book approved by the International Stud Book Committee, or trace in all lines of its pedigree to horses so registered;

(c) horses which are recorded in the New Zealand Register of Non-Stud Book Mares and have been promoted to Stud Book status by NZTR supported by the unanimous agreement of the International Stud Book Committee; and

(d) horses, other than those referred to in the last three preceding paragraphs, which are approved for entry by NZTR and the entry of which is supported by unanimous agreement of the International Stud Book Committee, provided that any such horse is the product of a natural service, which is the physical mounting of a mare by a stallion with intromission of the penis and ejaculation of semen into the reproductive tract. A natural gestation must have taken place and delivery must have been from the body of the same mare in which the foal was conceived. For the avoidance of doubt:

(e) such natural service may include the immediate reinforcement of the stallion’s service by using residual semen ejaculated by the stallion whilst it penetrated the mare during that service of the same mare, provided that the reinforcement of service must be immediately after the natural covering and the semen must not be processed or altered in any way before it is used for reinforcement; and

(f) semen obtained from a stallion by any artificial means may not be used to reinforce a service, and any other form of artificial breeding (including artificial insemination, embryo transfer or transplant, cloning, or any other form of genetic manipulation) may not be used to produce the horse.

Popular stallions in New Zealand may serve more than 220 individual mares each season (Rogers et al 2007). The combination of an imposed start date to the season, which occurs prior to the onset of the mare's natural breeding season, and the need for natural service means that very intensive reproductive management is required to maximise the number of mares conceiving each season.

Given the importance of the Thoroughbred industry to New Zealand's culture and economy, it is surprising that there have been no previous studies investigating the factors that influence the reproductive performance of Thoroughbred stud farms in this country. This thesis reports the findings from a series of studies conducted to describe the reproductive performance of New Zealand Thoroughbred mares, to determine those factors that significantly affect reproductive performance, and to investigate strategies aimed at improving reproductive outcomes. This thesis is presented as a literature review (Chapter 2) and a series of chapters each representing a manuscript submitted to a peer-reviewed journal.

Chapters 3 and 4 describe the findings of an epidemiological study investigating the reproductive performance of five Thoroughbred stud farms over three consecutive breeding seasons in the Waikato region of New Zealand. Chapter 3 presents descriptive statistics and univariate associations whilst Chapter 4 outlines a multivariable approach to the analysis of the data, identifies the most important mare-level factors affecting reproductive performance, and determines the relative contribution of the mare, stallion and stud farm to reproductive performance.

Chapter 5 presents the results of a study that investigates the endocrine profiles and follicular dynamics of transitional mares treated with a progesterone-releasing intra-vaginal device. Chapter 6 presents the results of a controlled study that investigates the reproductive performance of transitional mares treated with a progesterone-releasing intra-vaginal device at the start of the breeding season.

Chapter 7 presents the findings of a study that aims to create a potential model for ovulation failure in the mare.

Chapter 8 presents general conclusions for consideration by relevant industry sectors, and outlines potential areas for future research.

Chapter 2: Literature review

Equine reproductive seasonality

The mare is seasonally polyoestrous with the onset of normal, regular oestrous cycles occurring during the spring (Ginther 1992). Because regular oestrous cycles commence during a period of increasing day length, mares are classified as “long-day breeders”; in contrast to “short-day breeders” such as the ewe, where oestrous cycles commence during a period of decreasing day length during autumn. Seasonally breeding females conceive at a time of the year that ensures that their offspring are born when nutritional and environmental conditions are optimal. Given the relatively long gestation length (335 ± 6 days (Ginther 1992)) of the mare, conception needs to occur during late spring or summer to enable this to occur. Throughout the year, the annual cycle of reproductive activity of the mare is best described as:

1. Spring/Summer – normal, regular ovulatory oestrous cycles

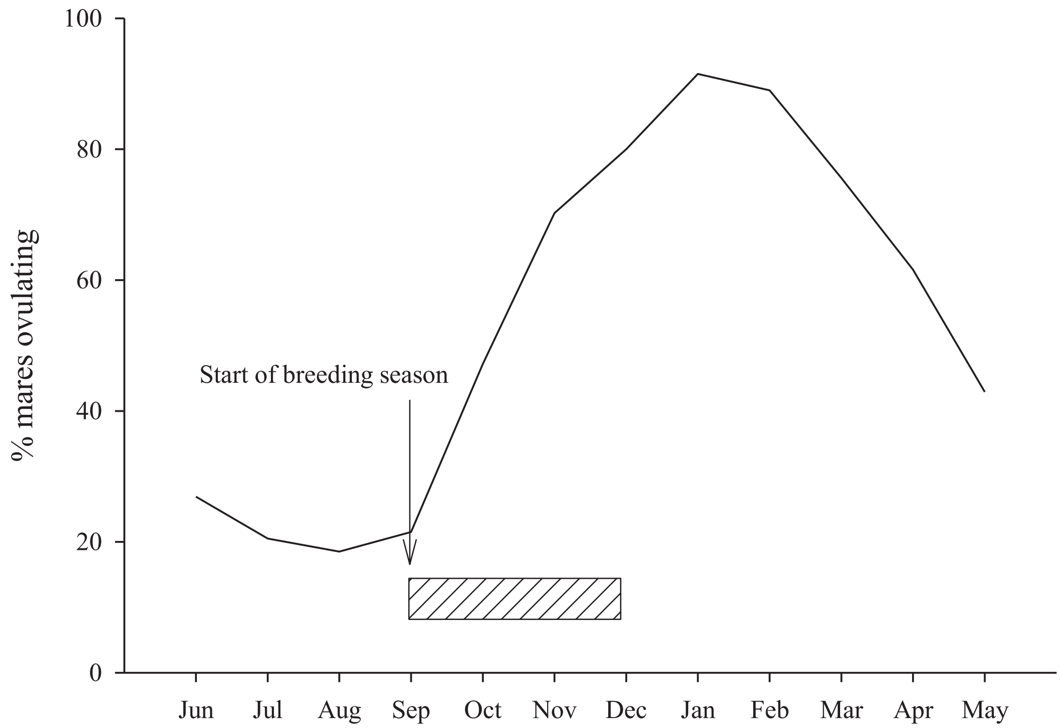
2. Autumn Transition – transition from regular oestrous cycles into winter anovulatory anoestrus

3. Winter anovulatory anoestrus – the reproductive axis is “dormant”

4. Spring Transition – transition from winter anovulatory anoestrus to normal, regular ovulatory oestrous cycles.

As outlined above, the spring transition period is of particular interest and consequence because the artificially imposed uniform birth date occurs at a time when mares are in the transition period. In an extensive abattoir survey of over 5000 mares in Australia, Osborne (1966) showed that the proportion of mares ovulating each month varied considerably over the course of the year. Of major significance is the finding that only around 20% of mares were ovulating at the commencement of the “official” breeding season on 1st September (see Fig 2.1). Hence most research on mare reproductive seasonality has focussed on what drives the transition period and ways to either overcome or advance the transition period.

Figure 2.1: Proportion of 5198 mares ovulating each month over a 12 month period in South Eastern Australia. Data obtained from a Sydney horse abattoir. Hatched area represents "imposed" breeding season as determined by Australian and New Zealand Stud Book regulations (adapted from (Osborne 1966)).



Characteristics of the transition period

The transition period is characterised by a resurgence of follicular activity, irregular oestrous behaviour, and increased secretion of gonadotrophins and ovarian steroids (Donadeu and Watson 2007). The seasonal reproductive pattern of the mare appears to represent an endogenous rhythm that is entrained by external environmental factors such as photoperiod, temperature, nutrition and body condition (Nagy et al 2000). The evidence to support an endogenous rhythm of reproductive function comes from studies that show that when mares are exposed to either constant periods of light or darkness they will resume (in the case of constant dark-exposed mares) or cease (in the case of constant light-exposed mares) cyclic ovarian activity at varying time periods after the start of treatment (Sharp et al 1975; Palmer et al 1982).

Photoperiod

Burkhardt (1947) was the first to show that artificial light exposure could influence the seasonal onset of normal ovulatory cycles in the mare and it is now well established that day length is the major factor controlling the seasonal nature of equine reproduction (Donadeu and Watson 2007; Sharp 2011b). Despite a tremendous amount of research in this area it appears that the exact mechanism by which light exerts its effect in the mare is unclear (Nagy et al 2000; Sharp 2011b). In "short-day breeders" like the ewe, melatonin appears to play a major role in the onset of normal ovulatory cycles. Melatonin is produced by the pineal gland and is increased during darkness. The administration of exogenous melatonin to ewes prior to the onset of the normal breeding season causes them to start cycling earlier than untreated controls (Goodman et al 2010). The evidence to support the fact that melatonin also plays a role in the seasonal onset of ovulatory cycles in the mare comes from studies involving pinealectomised mares. The onset of ovarian activity in these mares was not affected by artificial light exposure, and pinealectomy during winter prolonged the anoestrus period (Grubaugh et al 1982). In addition, feeding melatonin to mares has been shown to delay the onset of the first ovulation of the breeding season (Peltier et al 1998). In sheep, the effect of melatonin is modulated through the stimulation of GnRH production by the hypothalamus (Goodman et al 2010). In contrast, mares commence normal ovulatory cycles during periods of increasing day length (ie. a time of reduced melatonin concentrations). If GnRH concentrations in the mare are influenced by melatonin, then it appears to have the opposite effect to that in short-day breeders. It is postulated by some authors that reduced melatonin concentrations in the mare are somehow stimulatory to GnRH production and that this is modulated through a different pathway to that found in the ewe (Nagy et al 2000; Sharp 2011a). Alternatively, melatonin may have an inhibitory effect on GnRH release in the mare and the decline in melatonin levels that is associated with increasing day length may allow the hypothalamus to "escape" from this negative effect (Sharp 2011a). Whatever the mechanism, the existence of melatonin receptors in the hypothalamus of the mare tends to suggest that GnRH production is influenced by melatonin to some extent (Nonno et al 1995).

Recent research in other mammals (mice, humans) suggests that other mechanisms may be just as important, or even more important, than melatonin in regulating seasonal biological functioning (Kalsbeek et al 2006; Sharp 2011b). The discovery of "clock genes" in the suprachiasmatic nucleus (SCN) of the hypothalamus, has provided new insights into how photoperiod potentially regulates reproductive function (Reppert and Weaver 2002). It may be that the alteration of clock gene expression in the SCN (in direct response to photoperiod rather than via melatonin pathways) modulates the expression of genes that encode for the production of GnRH, as there are seasonal differences in the amount of GnRH in the equine hypothalamus (Hart et al 1984).

The recent discovery of a group of neuropeptides known as "kisspeptins" has also shed new light on how seasonal breeding in many species may be regulated by external cues such as light, temperature and nutrition (Greives et al 2007). Kisspeptins are produced by specific neurons (KiSS-1 neurons) in the hypothalamus and have been found in all mammals studied to date, including horses (Magee et al 2009; de Tassigny and Colledge 2010). The evidence suggests that kisspeptins act as the "master controller" of GnRH production in the hypothalamus (de Tassigny and Colledge 2010). GnRH neurons are directly innervated by KiSS-1 neurons and the negative/positive feedback effect of sex steroids (oestrogen, progesterone) on GnRH production is directly mediated via the up- or down-regulation of kisspeptin gene expression in the KiSS-1 neurons (Smith et al 2007). In addition, the expression of the gene that encodes KiSS-1 is seasonally up- or down-regulated in seasonally breeding animals such as the ewe (Smith et al 2007) and hamster (Wagner et al 2008), and is mediated by photoperiod.

The effect of photoperiod on the seasonal onset of normal ovulatory cycles in the mare is therefore likely to be co-ordinated via a complex interrelationship between melatonin release and the differential (seasonally dependent) expression of clock and KiSS-1 genes in the hypothalamus.

Nutrition and body condition

Body condition has been shown to be an important factor in determining the length of the transition period. Mares with a body condition score of ≥ 5 (on a 1 to 9 scale) were found to ovulate earlier in the year compared to mares with a condition score of < 5 (Kubiak et al 1987). Mares on a rising plane of nutrition also ovulated earlier than mares maintained on a stable energy intake (Ginther 1974), and mares fed a high quality protein source ovulated sooner than mares maintained on a low quality protein source (van Niekerk and van Niekerk 1997). The available data suggests that body condition during winter and spring is probably more important than diet per se as a determinant of ovulation date, as feeding a high energy diet shortened the interval to first ovulation in mares with a low body condition score, but did not affect the ovulation date of mares that were already in good body condition (Kubiak et al 1987). A positive effect of pasture has also been reported, with mares grazing green grass during winter and early spring ovulating about a week earlier than mares maintained in a dry lot and fed hay (Carnevale et al 1997).

Kisspeptins may also be involved in mediating the effect of nutritional status on the transition period. KiSS-1 neurons express the leptin receptor (de Tassigny and Colledge 2010) and it is proposed that these neurons monitor peripheral fat reserves via leptin to modulate reproductive function under conditions of negative energy balance (Backholer et al 2010). Leptin is a protein synthesised by adipose cells (Barb 1999) and in mares there is a direct, positive relationship between body condition and plasma leptin concentrations (Ferreira-Dias et al 2005) therefore supporting the concept that leptin/KiSS-1 interaction is involved in the nutritional control of the transitional period.

Environmental temperature

The effect of environmental temperature on the transition period has not been studied extensively. Only one report exists (from Australia) where a substantial number of individual mares were observed over a 10 year period (Guerin and Wang 1994). The authors found that the mean minimum daily temperature during the 5 week period immediately prior to ovulation was negatively correlated to the number of days to the first ovulation of the year, ie. the colder the weather the longer the interval to first ovulation. At the latitude of the study (37^{o}S), a 1^{o} C decrease in temperature was associated with a 9 day delay in the mean date of ovulation.

Endocrinology of the transition period

The production and release of GnRH from the hypothalamus is the main hormone responsible for controlling reproductive seasonality in the mare (Donadeu and Watson 2007). The seasonal period of anoestrus is associated with low levels of GnRH in the hypothalamus and decreased levels of LH in the anterior pituitary (Hart et al 1984; Sharp 2011c). The reduced pituitary LH content is a direct result of down-regulation of the gene that encodes the β-subunit of equine LH (Sharp et al 2001). Pituitary FSH content remains unchanged during anoestrus as evidenced by the ability to induce an FSH surge in response to exogenous GnRH (Evans and Irvine 1976; Silvia et al 1987).

As the transition period progresses, GnRH production is re-established in the hypothalamus as described above. In association with a return of GnRH production there is a re-establishment of co-ordinated FSH surges (Irvine et al 2000) each of which precedes the emergence of a follicular wave (Donadeu and Ginther 2002). FSH concentrations tend to decline during the last 10 to 20 days of the transition period as maturing follicles produce inhibin (Hines et al 1991; Watson et al 2002; Donadeu and Ginther 2003) which has a negative feedback effect on the release of FSH. Pituitary LH content (Silvia et al 1987) and circulating LH concentrations (Donadeu and Ginther 2002) remain low until just a few days before the first ovulation of the year.

Oestradiol concentrations remain low throughout most of the transition period and rise just before the first ovulation of the year (Seamans and Sharp 1982; Donadeu and Ginther 2002). It is likely that the pre-ovulatory rise in oestradiol concentrations is responsible for inducing the LH surge that causes ovulation; identical to the sequence of events that occurs in normally cycling mares (Donadeu and Ginther 2002, 2003; Sharp 2011c).

Changes in prolactin (PRL) concentrations are also worthy of note because pharmacological manipulation of PRL is often used in an attempt to advance the transition period (Besognet et al 1997). PRL is produced by the pituitary gland and in mares levels vary throughout the year with concentrations highest in summer and lowest during winter (Evans et al 1991). PRL release from the pituitary is under the control of dopamine which is a catecholamine neurotransmitter produced in several areas of the brain including the hypothalamus (Goodman et al 2010). The finding that PRL levels in the mare are highest during the breeding season has led many researchers to believe that PRL has a direct effect on reproductive seasonality in the mare. This assumption most likely comes from studies in sheep where GnRH secretion is inhibited by dopamine during seasonal anoestrus (Goodman et al 2010). In the mare, dopamine concentrations in the CSF are higher during the anovulatory period than during the breeding season (Melrose et al 1990). The link between PRL and dopamine is clear and direct, ie. dopamine exerts a negative feedback effect on PRL release, however the link between PRL concentrations and the onset of normal ovulatory cycles is tenuous at best. The treatment of transitional mares with dopamine antagonists such as sulpiride (Besognet et al 1997; Panzani et al 2011) or domperidone (Mari et al 2009) has been shown to increase PRL concentrations and advance the transition period in some mares, however this effect is most likely mediated through changes in dopamine concentrations rather than an effect of PRL per se. Although there are seasonal variations in PRL concentrations, there appears to be no conclusive evidence to suggest that PRL has a direct effect on reproductive seasonality in the mare.

Ovarian dynamics during the transition period

During the normal ovulatory season, ovarian follicles develop in waves every 10 - 12 days and each wave is preceded by an FSH surge occurring 3 days prior to wave emergence (Ginther et al 2001). Follicle waves are usually associated with the development of large (> 30 mm) dominant follicles during an ovulatory cycle (Ginther 1992).

During winter anovulatory anoestrus, follicular development is curtailed and the ovaries become small and inactive with the largest follicles reaching about 16mm in size (Donadeu and Ginther 2002). The end of the winter anovulatory period and the start of the transition period is marked by a significant increase in the size of the largest follicle (≥ 21mm) and an abrupt increase in the number of follicles > 15mm (Donadeu and Ginther 2002; Donadeu and Watson 2007). The co-ordinated surges of FSH that occur in response to the re-instatement of GnRH release from the hypothalamus results in the development of follicle waves throughout the transition period (Donadeu and Ginther 2002). These waves are associated with the development of large (> 35mm) follicles which fail to ovulate (Watson et al 2002). On average, mares develop 3.7 anovulatory follicle waves during the transition period (Ginther 1990; Watson et al 2002), but there is substantial variation between different mares (Ginther 1990; Donadeu and Ginther 2002). The development of anovulatory follicular waves means that the transition period lasts between 30 and 90 days in individual mares (Ginther 1990; Nagy et al 2000; Donadeu and Watson 2007).

The reason why follicles grow to a relatively large size but fail to ovulate during the transition period is likely to be multifactorial. The lack of LH production and release from the pituitary is one reason why follicles fail to ovulate, as an LH surge is required for ovulation to occur (Ginther 1992). It is likely that increasing LH production which occurs during progression of transition supports the growth of large dominant follicles that emerge in response to FSH surges, but there is insufficient LH to induce ovulation until the end of the transition period (Donadeu and Ginther 2002). Transition period follicles also express low levels of mRNA for the LH receptor and are therefore less likely to ovulate in response to either an endogenous LH surge (Watson et al 2004), or an hCG-induced LH surge (Morel and Newcombe 2008).

Transition period anovulatory follicles also produce less steroid hormones (oestradiol, progesterone) than ovulatory follicles (Davis and Sharp 1991; Bogh et al 2000; Acosta et al 2004) and as a result they are often referred-to as being “steroidogenically incompetent” (Sharp 2011c). Oestradiol is responsible for up-regulating the expression of LH receptors in the follicle wall and is therefore a vital component of follicular growth, maturation and ovulation (Beg et al 2003). Large transitional phase follicles exhibit reduced expression of several enzymes (StAR, P450scc, P540c17 and P450arom) involved in the steroid pathway (Watson et al 2004). The expression of these enzymes is up-regulated by LH (Kerban et al 1999; Ginther et al 2003b), therefore the lack of circulating LH during the transition period is likely to be responsible for the steroidogenic incompetence of transition period follicles (Donadeu and Watson 2007). The increase in LH production towards the end of the transition period is therefore likely to up-regulate the expression of the steroid pathway enzymes resulting in increased production of oestradiol from the dominant follicle (Gastal et al 2007). The subsequent positive feedback effect of oestradiol on the pituitary results in an LH surge, and the up-regulation of LH receptors (by oestradiol) in the follicle wall finally leads to ovulation of the dominant follicle and the end of the transition period (Donadeu and Ginther 2003; Gastal et al 2007).

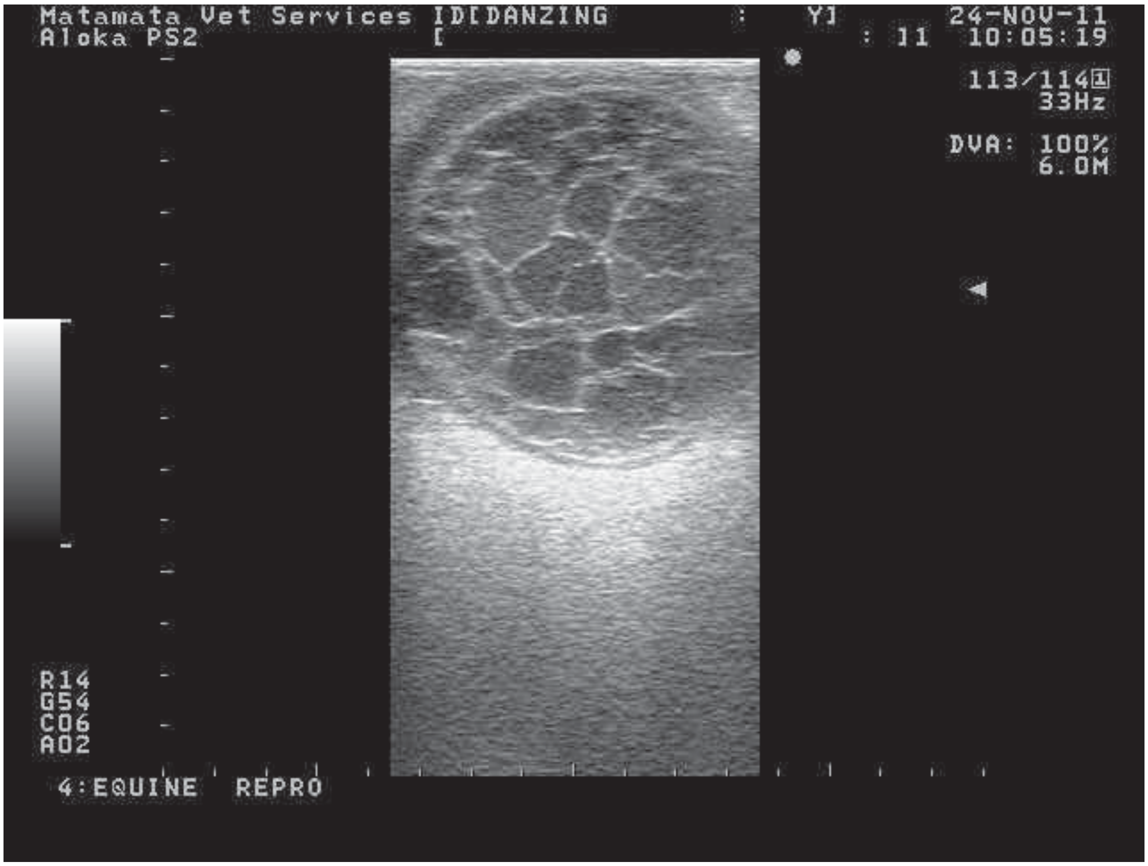
Haemorrhagic anovulatory follicles

Unlike the normal anovulatory follicles that grow and regress during the transition period, haemorrhagic anovulatory follicles (HAFs) are a distinct morphological entity where the follicle fails to rupture and its cavity becomes filled with blood and fibrin strands (Ginther et al 2007)(Fig. 2.2). HAFs attain sizes of around 50 – 60 mm and usually become luteinised, thereby producing progesterone and a prolonged period of anoestrus (McCue and Squires 2002; Ginther et al 2007; Ellenberger et al 2008). HAFs are of significance because they occur in up to 20% of follicular waves and are most common during the transition period (McCue and Squires 2002; Ginther et al 2007).

The predisposing factors for HAF development in the mare are unclear. Several mechanisms have been proposed which include: a) inappropriate exposure of small follicles (< 20mm) to elevated LH concentrations (Ginther et al 2008b), b) insufficient gonadotrophic stimulation to induce ovulation (McKinnon 1993), c) reduced oestrogen secretion by the dominant follicle (McKinnon 1993), d) physical blockage of the ovulation fossa due to ovarian cysts (Kolling and Allen 2006), e) repeated use of ovulation-inducing hormones (Lefranc and Allen 2003), and f) the use of prostaglandins to shorten the dioestrus period (Cuervo-Arango and Newcombe 2008).

HAF formation in mares has significant economic impacts because it is associated with infertility due to failure of the follicles to evacuate the oocytes (Cuervo-Arango and Newcombe 2008). The ability to predict which dominant follicles will become HAFs is difficult because during ultrasound examination they appear to be morphologically similar or identical to normal pre-ovulatory follicles (Ginther et al 2007). Therefore much time and expense can be wasted on breeding mares during infertile oestrus cycles due to HAF formation. HAFs can also persist for several weeks resulting in a prolonged anoestrus period (McCue and Squires 2002).

Figure 2.2: Typical ultrasonographic appearance of a haemorrhagic anovulatory follicle (HAF) in the ovary of a Thoroughbred mare. The antrum is filled with blood and fibrin strands are obvious.



Oestrous behaviour during the transition period

One of the unusual characteristics of the transition period is the irregular, erratic oestrous behaviour exhibited by most mares (Ginther 1992). The reason why mares exhibit oestrous behaviour during a period of reduced oestradiol production is not fully understood (Ginther 1974), although even ovariectomized mares can display overt oestrous behaviour (Asa et al 1980). One proposed mechanism is that conjugated oestrogens (not oestradiol) are produced by steroidogenically incompetent transitional follicles and these are responsible for inducing oestrous behaviour (Sharp 2011c). The evolutionary reason for the display of oestrus at a time when matings are infertile is hard to explain, but may be related to the bonding of feral herds of horses and ensuring that mares remain close to the stallion in such herds (Asa et al 1979). In modern management systems the expression of oestrous behaviour by anovulatory mares is frustrating because large amounts of time and effort can be wasted on the reproductive management of mares that are not capable of conceiving.

Methods to advance the transition period

A variety of methods have been used in an attempt to advance the onset of normal ovulatory cycles in transitional mares.

Artificial light exposure

The most effective method for advancing the transition period is through the use of controlled light exposure commencing several weeks prior to the start of the breeding season (Burkhardt 1947; Nagy et al 2000). The effect of artificial light exposure is not to shorten the transition period, but to cause it to start earlier (Sharp and Ginther 1975; Sharp 2011b). At least 35 days of light exposure is required to advance the transition period (Palmer et al 1982) but it is generally accepted that light exposure should commence soon after the winter solstice to reliably advance the first ovulation of the year (Ginther 1992; Nagy et al 2000). In the southern hemisphere this means artificial light exposure should start towards the end of June. Artificial day length needs to be at least 14.5 hours in duration to be effective (Palmer et al 1982; Ginther 1992), however a “pulse” of light, 1 hour in duration, 9.5 hours after the onset of darkness is also effective (Palmer et al 1982). The “pulse lighting” effect is probably mediated through the up-regulation of clock genes in the hypothalamus (Sharp 2011a). The intensity of light required to be effective in artificial lighting programmes appears to be relatively low, with a single 100 Watt bulb in a 4m x 4m stable being sufficient to induce a positive response (Burkhardt 1947). In the southern hemisphere, mares are maintained at pasture all year round, as opposed to the northern hemisphere where most mares are stabled indoors during the winter. The exposure of mares to an artificial lighting programme is therefore logistically more difficult in the southern hemisphere. As a result only 40% of Thoroughbred stud farms in a New Zealand study utilised artificial light exposure to advance the transition period (Rogers et al 2007), compared to 100% of farms in a study from the USA (Bosh et al 2009b).

Pharmacological methods

Hormonal treatments are often used as an alternative to artificial light exposure to advance the onset of the transition phase. These treatments include GnRH, FSH, progestagens and dopamine antagonists. From a practical point of view, only progestagens and dopamine antagonists are used commercially (McCue et al 2007b).

GnRH

Evans and Irvine were the first to show that GnRH given twice daily, in combination with progesterone, induced follicular development and ovulation in acyclic (seasonally anovulatory) mares (Evans and Irvine 1977). Since then, various regimens using different formulations of GnRH (buserelin, deslorelin, goserelin), different routes of administration (subcutaneous implants, mini-pumps, IV) and different treatment durations have been described (Hyland and Jeffcott 1988; Ginther and Bergfelt 1990; Turner and Irvine 1991; McCue et al 1992; Fitzgerald et al 1993; McKinnon et al 1997; Williams et al 2007). Whilst GnRH treatment can induce follicular development and ovulation in a high proportion of treated mares, the response to treatment is more reliable when mares possess follicles > 25mm in size, ie. mares that are in late transition (Hyland et al 1987; Ginther and Bergfelt 1990; McKinnon et al 1997). In addition, although GnRH can induce ovulation in transitional mares, the corpus luteum (CL) resulting from such ovulations may be defective and not produce sufficient progesterone to maintain pregnancy (Evans and Irvine 1977; McKinnon et al 1997). As a result, induction of ovulation in transitional mares with GnRH has been often associated with a higher than normal incidence of early embryonic death (Bergfelt and Ginther 1992b). Given the variable response to GnRH treatment, the need to give multiple doses, and the substantial cost of commercially available preparations, GnRH therapy is not routinely used to advance the transition period (McCue et al 2007b).

FSH

Equine FSH (eFSH) is commercially available in some countries (USA, Brazil), and has been shown to induce follicular development and ovulation in around 80% of treated mares after a 7 – 12 day treatment programme (Niswender et al 2004; McCue et al 2007a; Raz et al 2009). eFSH needs to be given twice daily by IM injection (McCue et al 2007a) and as with GnRH therapy, eFSH is more effective when given in late transition (Niswender et al 2004; Raz et al 2009). Because of the need to give multiple doses, and the cost of commercially available preparations, eFSH therapy is not routinely used to advance the transition period (McCue et al 2007a).

Progestagens

Progestagens include natural progesterone and synthetic progestins such as altrenogest and norgestomet (Squires 2011). Progestagens can be delivered orally (Squires et al 1979b; Wiepz et al 1988), by IM injection (Evans and Irvine 1979; Alexander and Irvine 1991), or intravaginally (Newcombe 2002; Newcombe et al 2002; Handler et al 2006). Intravaginal progesterone is usually delivered by using devices designed for cattle (PRID, CIDR) (Newcombe et al 2002), although a device specifically designed for mares is now commercially available (Cue-Mare) (Grimmett et al 2002). There have been conflicting reports on the efficacy of progestagens in advancing the first ovulation of the year. Some studies have shown a positive effect (Squires et al 1979b; Newcombe et al 2002) and others have not (Allen et al 1980; Alexander and Irvine 1991). The variation in reports regarding the efficacy of progestagen treatment is most likely due to the stage of transition at which treatment commenced. Progestagen therapy is much more effective in inducing follicle growth and ovulation when given in late transition compared to early transition (Squires et al 1979b; Allen et al 1980; Turner et al 1981; Newcombe 2002; Newcombe et al 2002; Handler et al 2006, 2007). Some authors propose that progestagen therapy simply serves to synchronise the first ovulation of the year in mares that were destined to ovulate naturally (McCue et al 2007b). This assumption comes from the fact that most studies have not included untreated controls (Newcombe 2002; Handler et al 2006, 2007; Squires 2011).

Dopamine antagonists

The finding that PRL levels vary throughout the year and are highest during the breeding season (Evans et al 1991) has led to studies on the use of dopamine antagonists as a potential method for advancing the transition period (Besognet et al 1997). There are conflicting reports on the efficacy of treatment of transitional mares with dopamine antagonists such as sulpiride (Besognet et al 1997; Panzani et al 2011) or domperidone (McCue et al 1999; Mari et al 2009). Some studies have shown a beneficial effect

(Besognet et al 1997; Mari et al 2009; Panzani et al 2011) whereas others have shown no effect of treatment (McCue et al 1999; Donadeu and Thompson 2002). In studies where a positive effect of treatment has been reported, a prolonged treatment period (between 20 – 50 days of twice daily administration) and starting treatment when mares have follicles > 25mm is required. In addition, treatment appears to be more effective when combined with artificial light exposure (Duchamp and Daels 2002). Given the long treatment period, the need for twice daily administration and a more predictable response in mares under artificial photoperiod, dopamine antagonists appear to be limited in their efficacy for managing the transition period on commercial stud farms (McCue et al 2007b).

Assessment of equine reproductive performance

Surprisingly, unlike other livestock production systems, there are no standard measures of reproductive performance in the equine industry (Ginther 1992; Amann 2006). Amann (2006) describes the situation by stating "…for horses there is no standard format for calculation, presentation of data, or minimum numbers of records considered reliable" and "The horse industry should, at least informally within breed, define a minimum acceptable practice for presenting 'fertility data' in oral communications, handouts, websites etc. Apparent fertility should be viewed with far greater scepticism than displayed by many people in the horse industry."

Reproductive success has been defined as the ability of a mare to produce a live foal and this outcome is usually defined as the foaling rate (Ginther 1992; Schulman et al 2003). Other outcome measures commonly used to describe reproductive performance are the pregnancy rate per cycle, and the end-of-season pregnancy rate (Ginther 1992; Love and Samper 2009). Ginther (1992) was the first to define several potential measures of reproductive performance (Table 2.1), however since then, there has been a lack of consistency amongst investigators in the outcome measures used to describe reproductive performance (Amann 2005).

It should also be noted that in epidemiological terms the use of various “rates” as measures of fertility is erroneous. Strictly speaking, a rate is a ratio in which the denominator is the number of animal-time units at risk (Dohoo et al 2009). Therefore the description of various fertility “rates” without regard to a time period in the denominator is technically incorrect, although the terminology is entrenched in the literature.

The pregnancy rate per cycle is the most commonly used measure to describe reproductive performance in the Thoroughbred industry (Amann 2006). This is defined as the number of mares diagnosed pregnant at a specific time point after mating, divided by the total number of mares mated, multiplied by 100 (Ginther 1992). On commercial stud farms, pregnancy diagnosis is usually first performed 14 – 16 days after mating (Perkins and Grimmett 2001). The per cycle pregnancy rate is often calculated on a first cycle basis because it reduces the influence of repeated cycles being contributed by subfertile mares or stallions (Amann 2006; Blanchard et al 2010b; Nath et al 2010).

The end-of-season pregnancy rate is defined as the total number of mares diagnosed pregnant at a specific time point after mating at the conclusion of the breeding season, divided by the total number of mares mated during the season, x 100 (Ginther 1992). On most New Zealand Thoroughbred stud farms, a stallion service fee is payable on the diagnosis of a 42 day positive pregnancy test. Therefore as an example, if a stud farm breeds a total of 450 mares during the season and of those mares 360 have a positive 42 day pregnancy test, the end-of-season pregnancy rate for that farm is 80%. Whilst the end-of-season pregnancy rate is a useful measure of overall reproductive performance, it does not allow for an assessment of the efficiency in obtaining the outcome (Schulman et al 2003). For example, two stud farms may have a similar end-of-season pregnancy rate, but if one farm has a pregnancy rate per cycle of 60% and the other farm has a pregnancy rate per cycle of 40%, after only 2 cycles per mare, the first farm will have 84% of its mares pregnant whilst the second farm will require 3 cycles per mare to obtain a 78% end-of-season pregnancy rate.

Terminology	Definition
Pregnancy rate per cycle	No. mares diagnosed pregnant on a specified day after mating (eg. day 16) divided by No. mated per cycle x 100
End-of-season pregnancy rate	No. mares diagnosed pregnant on a specified day (eg. day 42) divided by total No. Mares mated at least once during the season x 100
Foaling rate	No. mares producing a live foal divided by the total No. Mated x 100
Pregnancy-loss rate	No. mares not foaling divided by the No. pregnant on a specified day (eg. day 16) x 100
Embryo-loss rate	No. mares not pregnant at end of embryo stage (day 40) divided by No. pregnant on a specified day (eg. day 16) x 100
Twinning rate	No. mares diagnosed with two embryos on a specified day (eg. day 16) divided by the No. mares diagnosed pregnant on the same day x 100

Table 2.1: Measures of equine reproductive performance commonly used in the literature (adapted from Ginther 1992).

Factors affecting equine reproductive performance

In comparison to other domestic species, the reproductive performance of horses is relatively low. In the Thoroughbred industry, breeding animals are primarily selected for desirable traits other than fertility, such as the athletic performance of the individual or their closely-related family members, pedigree and/or conformation. Unlike other livestock industries, horses are rarely culled for poor reproductive performance because of the amount invested in any one individual animal and large differences in the cull value compared to replacement costs (Bosh et al 2009a).

Factors that influence the reproductive performance of horses can be broadly categorised into intrinsic (related to the mare and stallion) and extrinsic (environmental effects, nutritional status, imposed breeding season etc) factors. The extrinsic effects of season, nutrition and seasonality and how they affect reproductive performance have already been discussed.

Mare-related factors

Age

There is clear evidence within the literature that mare age has a profound effect on reproductive performance. Without exception, all studies that have investigated the effect of mare age on reproductive outcomes have come to the same conclusion, that advancing age has a negative effect on reproductive performance (Carnevale and Ginther 1992; Bruck et al 1993; Hearn et al 1993; Hemberg et al 2004; Langlois and Blouin 2004; Allen et al 2007; Bosh et al 2009b; Benhajali et al 2010; Blanchard et al 2010b; Nath et al 2010; Sharma et al 2010a). Less clear is whether or not there is an “absolute” age after which reproductive performance declines or a gradual decline in age-related reproductive performance. Most studies grouped mare age into 3 or 4 arbitrary categories, and therefore it is difficult to determine at what point reproductive performance declines. From the available data it appears that the decline in reproductive performance occurs somewhere between 11 and 14 years of age.

Older mares have a lower pregnancy rate per cycle and a higher incidence of embryonic loss than younger mares (Carnevale and Ginther 1992; Carnevale 2008). The decreasing fertility associated with increasing mare age appears to be multi-factorial. Older mares have an increased incidence of endometrial changes such as fibrosis and inflammation (Kenney 1978; Held and Rohrbach 1991; Ricketts and Alonso 1991) and decreased uterine contractility and clearance of intra-uterine fluid (Carnevale and Ginther 1992; LeBlanc et al 1998; Cadario et al 1999) which predisposes them to post-mating endometritis. Older mares are also more likely to have poor vulval conformation which predisposes them to bacterial contamination of the reproductive tract (Pascoe 1979). Pathological changes of the oviducts are also observed at greater frequency in older mares, which may interfere with fertilization and/or embryo transport (Liu et al 1991). Aging is also associated with a decrease in oocyte quality (Ball et al 1989; Carnevale 2008) which is proposed to be due to mutations in mitochondrial DNA (Keefe et al 1995). Carnevale (2008) showed that the transfer of oocytes from old mares (≥ 20 years) into young recipient mares (6 – 10 years) resulted in a reduced pregnancy rate compared to the transfer of oocytes obtained from young mares (Carnevale 2008). Older mares also have decreased follicular activity secondary to depleted reserves of primordial follicles (Carnevale et al 1994; Carnevale 2008).

Reproductive status

The reproductive status of mares is generally divided into three categories (Ginther 1992):

- Maiden – a mare that has never had a foal
- Barren (or “Dry”) – a mare that has had foals previously but is currently not pregnant or lactating

- Foaling (or "Wet") – a lactating mare with a foal at foot

There are conflicting reports in the literature regarding the effect of reproductive status on the fertility of mares. Some studies have shown no effect of reproductive status on per cycle pregnancy rates (Hemberg et al 2004; Bosh et al 2009b; Benhajali et al 2010; Sharma et al 2010a), whilst others have shown that maiden mares have the highest per cycle pregnancy rate (Nath et al 2010). Others have shown similar pregnancy rates for maiden and foaling mares with both being higher than those of barren mares (Hearn et al 1993; Morris and Allen 2002; Allen et al 2007). Yet another study showed that foaling mares had the highest per cycle pregnancy rate with barren and maiden mares being similar (Blanchard et al 2010b). Most studies have shown no effect of reproductive status on the live foal rate (Morris and Allen 2002; Hemberg et al 2004; Allen et al 2007), however in a large survey of French mares of 24 different breeds, foaling mares were shown to have the highest foaling rate and maiden mares the lowest (Langlois and Blouin 2004) which is contrary to other studies where maiden mares produced more live foals than foaling mares (Bosh et al 2009b; Sharma et al 2010a). The effect of mare status on reproductive performance is largely confounded by the age of the mare, ie. maiden mares are generally the youngest and therefore the most fertile. Most studies have not utilised statistical procedures to account for this fact (Bruck et al 1993; Morris and Allen 2002; Hemberg et al 2004; Allen et al 2007; Bosh et al 2009b; Nath et al 2010; Sharma et al 2010a). In those that have, barren mares had reduced odds of pregnancy (Hearn et al 1993; Benhajali et al 2010) and foaling mares a higher odds of pregnancy (Blanchard et al 2010b). It is generally accepted that the barren mare category comprises mares that are subfertile or "problem breeders" and this is the reason for their reduced performance (Morris and Allen 2002; Bosh et al 2009b; Nath et al 2010), however, in some production systems, the barren mare category is largely composed of mares that have been voluntarily unmated for various reasons and as such their fertility is equal to that of maiden or foaling mares (Nath et al 2010).

The effect of reproductive status on pregnancy loss is unclear. Some studies have shown that maiden mares have the lowest incidence of pregnancy loss compared to barren or foaling mares (Bruck et al 1993; Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b; Sharma et al 2010a) whereas others have shown no effect of reproductive status on pregnancy loss rates (Hearn et al 1993). As mentioned above, mare age probably confounds the effect of reproductive status on pregnancy losses as maidens are usually the youngest mares.

Reproductive history

The effect of a mare's previous reproductive history (ie. whether she has previously aborted or failed to conceive) on her future fertility is not well established. Most studies have shown no difference in the reproductive performance of mares that had aborted or failed to conceive in the previous season compared to those that had voluntarily been left non-pregnant (Morris and Allen 2002; Hemberg et al 2004; Nath et al 2010; Sharma et al 2010a).

Post-partum breeding

The first post-partum oestrus ("foal-heat") usually begins 5-12 days after foaling with the average interval from foaling to first ovulation being around 10 days (Ginther 1992). This interval is seasonally affected, with mares foaling earlier in the season having a longer interval to the first post-partum ovulation than mares foaling later in the season (Palmer and Driancourt 1983). The effect of season is light dependent.

In order for a mare to continue to produce a foal each year, she must conceive within 30 days of foaling. Therefore, breeding mares on foal-heat has the potential to increase reproductive efficiency. There have been conflicting reports on the efficacy and benefits of breeding mares on foal-heat, with some studies reporting a reduced pregnancy rate (Lowis and Hyland 1991; Ginther 1992) and a higher incidence of embryonic loss (Morris and Allen 2002; Allen et al 2007), while other studies have shown that the pregnancy rates of mares served on foal-heat are equal to those of mares served on subsequent cycles after foaling (Blanchard et al 2004; Sharma et al 2010b). In addition, mares served on foal-heat have been shown to conceive earlier in the breeding season (Lowis and Hyland 1991).

One of the main factors in determining the success of foal-heat breeding appears to be the appropriate selection of suitable mares for foal-heat service (Blanchard et al 2004; MacPherson and Blanchard 2005). In particular the interval from foaling to ovulation affects the pregnancy rate to foal-heat service. Mares ovulating less than 10 days after foaling have a reduced pregnancy rate and a higher rate of embryonic loss compared to mares ovulating after 10 days (McKinnon et al 1988; Lowis and Hyland 1991). The reason for this is that the embryo remains within the oviduct for 5 days after fertilisation before entering the uterus, and normal uterine involution takes approximately 14-15 days (Ginther 1992). Therefore, embryos that enter the uterus prior to day 14 (day of ovulation = day 9) are likely to enter a uterine environment that is still not fully capable of maintaining and nurturing an embryo (McKinnon et al 1988; Ginther 1992). Other periparturient problems such as dystocia, retained foetal membranes and endometritis have also been associated with a reduction in fertility of mares served on foal-heat (McKinnon et al 1988; Lowis and Hyland 1991; MacPherson and Blanchard 2005).

Breed

It is difficult to determine from the literature if there is an effect of breed on mare fertility per se. Very few studies have compared the reproductive performance of mares of different breeds under the same management conditions. One large French study showed differences amongst breeds in the live foal rate, but the authors concluded that different management practices (eg. the use of AI and intensive veterinary intervention) most likely explained the differences (Langlois and Blouin 2004). In a recent Australian study, the reproductive performance of Thoroughbred mares was similar to that of Standardbred mares managed by the same veterinary practice (Nath et al 2010).

However, the Standardbred mares required more inseminations per cycle than the Thoroughbred mares to achieve the same level of reproductive performance. It is likely that the convenience and availability of semen through the use of AI on the Standardbred farms was the reason for this difference rather than any difference in the actual fertility of the two breeds.

Stallion-related factors

Just as there are differences in the inherent fertility of individual mares, there is variation in the inherent fertility of individual stallions (Morris and Allen 2002; Bosh et al 2009b; Blanchard et al 2010b). Whilst there are hundreds of studies published on the effects of sperm numbers, motility, morphology and various semen extenders (in those breeds that allow for the use of AI), surprisingly there is very little published data available on specific factors that affect Thoroughbred stallion fertility. It appears that stallion fertility is mainly affected by age, breeding frequency, testicular size, semen characteristics and season.

Age

Sperm production in stallions commences at puberty (around 12-24 months of age) and gradually increases until sexual maturity is reached at around 6 years of age (Johnson and Thompson 1983). Thoroughbred stallions usually commence stud duties at around 4 or 5 years of age at the cessation of an athletic career, an age when they are not yet sexually mature. Breeding a young stallion to too many mares in his first few seasons at stud may result in poor reproductive performance (Varner et al 1991). An age-related decline in fertility is observed in some, but not all stallions from around 15 to 18 years of age (Varner et al 1991; Roser 1997; Blanchard et al 2010a) and this is most likely due to testicular degeneration (Roser 1997). Within the age range of actively breeding stallions (from 4-18 years), it appears that stallion age has only minor effects on fertility (Davies Morel and Gunnarsson 2000; Blanchard et al 2010b).

Breeding frequency and "Book size"

The total number of sperm in each ejaculate decreases with increasing frequency of ejaculation (Amann et al 1979; Squires et al 1979a). Breeding frequency is largely determined by the number of mares "booked" to a particular stallion (Blanchard et al 2010b). For stallions with low sperm production, frequent ejaculation may result in potentially suboptimal sperm delivery per mare (Varner et al 1991). Stallions that breed more than four mares per day may have a lower pregnancy rate per cycle than stallions breeding less than three mares per day (Blanchard et al 2010b). Despite this, some studies have shown no effect of stallion book size on fertility (McDowell et al 1992; Bosh et al 2009b; Blanchard et al 2010a). The finding that book size does not generally affect stallion fertility is likely due to more intensive veterinary management of mares visiting busy stallions, such that the number of matings per oestrous cycle is minimised (McCarthy and Umphenour 1992).

Testes size

An individual stallion's testicular volume is an accurate predictor of his daily sperm production (Thompson et al 2004) and there is an age-related linear increase in testicular size up until about 16 years of age (Amann et al 1979). Whilst reduced sperm production does not equate to reduced fertility per se, stallions with low testicular volume are unable to breed as many mares (Pickett and Voss 1998). Minimising the number of mares bred to a stallion with small testes may result in normal fertility. It is generally accepted however, that stallions with small testicular volume should not be classified as satisfactory breeding animals during a Breeding Soundness Examination (BSE) (Parlevliet et al 1994; Love 2011).

Semen characteristics

Analysis of stallion semen characteristics largely focuses on the assessment of sperm motility and morphology (Jasko 1992). It is generally accepted that sperm motility is an unreliable predictor of stallion fertility (Jasko et al 1988; Jasko et al 1992a; Jasko et al 1992b; Love 2011). Sperm morphology seems to be better correlated to fertility with head and mid-piece defects associated with the greatest decrease in fertility (Parlevliet and Colenbrander 1999; Love et al 2000). Genetic sperm abnormalities such as acrosomal defects, head defects, mid-piece defects and tail defects are rare in stallions but may be the cause of inherent subfertility (Bosard et al 2005; Chenoweth 2005).

Season

Reproductive function in stallions is subject to the same seasonal variation as mares. Testicular size, sperm production and libido are all increased during the physiological breeding season (Clay et al 1987; Johnson and Thompson 1987). Serum concentrations of LH and testosterone are also increased during the breeding season compared to the non-breeding season (Harris et al 1983; Johnson and Thompson 1983; Thompson et al 1986). Although sperm production is decreased during the non-breeding season, it does not cease (Clay et al 1987).

Previous studies of Thoroughbred reproductive performance

Published studies that have described the reproductive performance of Thoroughbred mares are listed in Table 2.2. Three main points are clear from this: 1. some studies have relied on breed registry information to gather data, 2. only one peer-reviewed study used a multivariable approach to analyse the data, and 3. there are no studies from New Zealand. The use of breed registry data relies on accurate and timely information provided by breeders. Retrospective information from the USA Stud Book shows that up to 20% of annual mare returns are not submitted by breeders and therefore relying on registry data to calculate reproductive performance is associated with a high degree of inaccuracy (Bosh et al 2009b). In addition, registry data is simply a record of whether a mare produces a registerable foal each year. It does not include detailed individual mare reproductive information such as the number of cycles required for conception, pregnancy loss information etc.

Live foal rates

Per season live foal rates have increased over the years. Breed registry data from the 1950s to 1970s generally reported a live foal rate of around 50 – 60% (Osborne 1975; Schulman et al 2003), but within the last decade, a live foal rate of around 80% has been reported (Hemberg et al 2004; Allen et al 2007; Bosh et al 2009b). The improvement in live foal rate is most likely due to improvements in technology, such as the routine use of ultrasonography to monitor reproductive cycles and pregnancy, and the development of veterinary treatments to manage the reproductive cycle (Schulman et al 2003; Allen et al 2007; Nath et al 2010).

Per cycle pregnancy rates

It is difficult to determine whether per cycle pregnancy rates have increased over the years. This is because there are differences in how (ultrasound versus manual palpation), and at what stage (early or late gestation) pregnancy was diagnosed in different studies. The use of ultrasonographic pregnancy diagnosis did not become commonplace on commercial stud farms until the late 1980s (Squires et al 1988; Ginther 1992). Studies in which pregnancy diagnosis is performed earlier after service (eg. 14 – 16 days) are likely to report a higher pregnancy rate per cycle than studies where pregnancy diagnosis is performed at a later stage because embryonic death may have occurred prior to pregnancy diagnosis. In the UK, Sanderson and Allen (1987) reported a 53.3% pregnancy rate per cycle, and Morris and Allen (2002) reported a 59.9% pregnancy rate per cycle. These two studies were conducted in a very similar population of mares using similar methods to diagnose pregnancy, and tend to suggest that per cycle pregnancy rates have improved. Most studies generally report a per cycle pregnancy rate of around

50 – 60% per cycle (Bruck et al 1993; Hemberg et al 2004; Allen et al 2007; Bosh et al 2009b; Blanchard et al 2010b; Nath et al 2010) when pregnancy diagnosis is performed 14 – 16 days after service.

Table 2.2: Studies of the reproductive performance of Thoroughbred mares published in the literature ranked according to publication date.

Authors	**Year published**	**Year(s) data collected**	**No. mares**	**Country**	**Data source**[#]	**Method of analysis**[‡]
Nath et al	2011	1990-2001	4369	Australia	Farm	D
Blanchard et al	2010b	2005-2007	3788	USA	Farm	M
Sharma et al	2010	1998-2005	1181	India	Farm	D
Bosh et al	2009	2004	1011	USA	Farm	D
Allen et al	2007	2002	3373	UK	Farm	D
Hemberg et al	2004	1997-2001	430	Sweden	Farm	D
Schulman et al	2003	1975-1999	162 768	S. Africa	Registry	D
Morris and Allen	2002	1998	1393	UK	Farm	D
Morley and Townsend	1997	1988	1201	Canada	Registry	D
Bruck et al	1993	1981-1986	1630	Australia	Farm	D
Hearn et al[*]	1993	1988-1991	704	Canada	Farm	M
Sanderson and Allen[*]	1987	1982-1983	2673	UK	Farm	D
Osborne[*]	1975	1950-1972	98 020	Australia	Registry	D

[*] Non-refereed publication

[#] Data obtained from either farm records or breed registry data.

[‡] Statistical method of data analysis. D = descriptive study, M = multivariable analyses.

Pregnancy loss rates

There are differences in the literature in terms of how pregnancy loss rates are reported. Some studies report the percentage of all pregnancies lost between first diagnosis (usually 14 – 16 days after service) and foaling, whereas other studies further define the stage of gestation at which losses occur, eg. between days 14 and 42 of gestation, and from day 42 to foaling. When pregnancy loss rates between first diagnosis and foaling are examined, around 15 – 20% of all pregnancies are lost (Bruck et al 1993; Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b; Nath et al 2010; Sharma et al 2010a). Pregnancy losses are higher earlier in gestation; the loss rate between first pregnancy diagnosis and 42 days of gestation was around twice that of the loss rate between 42 days and term (Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b).

Multiple pregnancy rate

Of all pregnancies first diagnosed at 14 -16 days after service, around 10 – 15% are twins and there appears to be little variation between studies in the twinning rate (Bruck et al 1993; Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b; Nath et al 2010; Sharma et al 2010a). Twinning rate is affected by mare age and reproductive status as previously discussed. In addition, Thoroughbreds appear to have a higher multiple pregnancy rate than other breeds (Ginther 1992).

"Interval length"

Only one study has attempted to investigate how long it takes for mares to conceive after the start of the breeding season (Bosh et al 2009b). These authors used the term "interval length" and defined this as the mean number of days from the start of the breeding season (for dry mares) or from foaling date (for wet mares) to the last service date for each mare. Interval length is therefore a description of how long the breeding season is, rather than an assessment of the start of mating to conception interval.

Statistical methods appropriate for the analysis of equine reproductive performance

Reproductive outcomes are measured in two main ways:

1. Binary (yes, no) outcomes - for example pregnancy, delivery of a live foal,
2. Time-to-event outcomes - for example days to conception after the start of the breeding season.

Analysis of these outcomes is achieved by investigating single (univariate) or multiple (multivariable) effects of exposure variables on the outcome of interest.

Univariate analyses

Univariate analysis is the simplest form of data analysis and examines the relationship between a single exposure variable and the outcome of interest. Univariate analysis is a useful tool for screening potential predictors of an outcome and should always be used as the first step in analysing a data set (Dohoo et al 2009). Simple descriptive statistics are used to report means, standard deviations etc. for continuously distributed data and frequency tabulations, proportions and counts are used to describe categorical variables. Simple analysis of the data is then performed using the appropriate tests, eg. *t*-tests for continuous variables and χ^2 tests for comparisons of proportions. Virtually all previously published studies on Thoroughbred reproductive performance have utilised a univariate approach to analyse the effects of independent variables on the outcomes of interest (see Table 2.2).

Multivariable analyses

Whilst univariate analyses can identify factors that appear to influence the outcome it does not take into account the fact that some predictors may be related to one another, nor does it determine which predictors are the most important. Multivariable analysis involves the analysis of several predictors at the same time and involves building a statistical model which can predict the outcome of interest. When building a model there needs to be a balance between the desire to get the model that best "fits" the data (ie. one that can perfectly predict the response given a range of values of the predictors) with the desire for parsimony (simplicity in the model) (Dohoo et al 2009). For example, a model that perfectly fits the data would be one that contains a value for each variable in the data set, ie. not very practical. In biological systems we are usually more interested in determining what predictors are the most important in explaining the outcome rather than the exact ability of the model to predict a certain outcome. This is because there are large inherent variations in biological systems. Thus obtaining precise estimates of the coefficients in a model is less important than determining whether a predictor has a positive or negative effect on the outcome of interest.

In studies of reproductive performance, the outcome of interest (pregnancy) has a binary (yes, no) outcome. The most appropriate technique for examining the relationship between a binary outcome and several exposure variables is multiple logistic regression. The multiple logistic regression model is represented by:

$$\operatorname{logit}(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_{1,i} + \cdots + \beta_k x_{k,i}.$$

Where p_i is the probability of the outcome occurring, β_0 is the intercept, and β_1, βk...... are the regression coefficients for each variable χ_1, χ_k.........

Each β coefficient relates to the influence of an individual exposure variable on the outcome of interest, while adjusting for the effect of all other exposure variables in the model. The β coefficient represents the amount the logit of the probability of the outcome changes with a unit increase in the predictor. Intuitively, this is difficult to interpret so the results are generally expressed as odds ratios (OR) by the exponentiation of each β coefficient (OR = e^{β}). If the OR > 1 then the predictor increases the odds of developing the outcome, and if the OR < 1 it decreases the odds of developing the outcome (Dohoo et al 2009).

Methods that deal with clustered data

One of the key assumptions of multiple logistic regression analysis is that the observations are independent (McDermott et al 1994). If animals are kept together in herds then this assumption has been violated because the variation between animals results not only from the usual variation amongst animals, but also the variation that is due to differences between herds (Dohoo et al 2009). The differences between herds are a product of farm and animal management practices and possibly even differences in location, topography, and climate etc. The herd therefore imposes a clustered structure to the data. Given that on a stud farm, the reproductive performance of mares is likely to be correlated, statistical techniques that account for the clustered nature of the data should be used (McDermott and Schukken 1994; Zyzanski et al 2004). None of the previously published studies that have investigated the reproductive performance of mares have accounted for the clustered structure of the data.

Ignoring the effects of clustering in studies that investigate the reproductive performance of mares has two potential negative effects (McDermott and Schukken 1994; Dohoo et al 2009):

1. The standard errors (SE) of parameter estimates may be incorrect and the variance is likely to be underestimated, and

2. If the number of mares on different stud farms is highly variable then an unadjusted analysis gives unreasonably large weight to farms with a greater number of mares.

There are no standard tests to determine whether clustering is present within a given dataset (McDermott and Schukken 1994; Dohoo et al 2009). Determining whether clustering is present within the data (and whether its effect is worthy of statistical consideration) is usually based on the investigator's awareness of the data structure (McDermott et al 1994). However, a useful concept that applies to the investigation of clustered data is the intra-class correlation (ICC) which is a measure of the relatedness of observations within a cluster (Otte and Gumm 1997; Killip et al 2004). It is calculated using the formula:

$$\text{ICC } (\rho) = \sigma_b^2/(\sigma_b^2 + \sigma_w^2)$$

Where σ_b^2 is the variance between clusters, and σ_w^2 is the variance within clusters.

Values of ρ range from 0 to 1. A very small value for ρ implies that the within-cluster variance is much greater than the between-cluster variance (ie. clustering has a minimal effect). Small, medium and large values of the ICC are defined as < 0.05, 0.05-0.10, and > 0.10 respectively (Zyzanski et al 2004). If ρ is small, the investigator may decide that statistical techniques for clustered data are unnecessary.

Fixed effects models

The simplest (and most common) method used to deal with clustering is to include the cluster variable (eg. stud farm) as a fixed effect in the regression model (Dohoo et al 2009). In fixed effects models, dummy (indicator) variables representing the group (stud farm) are included in the model. The fixed effects analysis then effectively estimates a separate parameter for each stud farm. The main disadvantage of this approach is that the model only contains the within-farm variance as the between-farm variance is removed by the fixed effects (Dohoo et al 2009). Therefore any conclusions from the data are specific to the actual farms. This is of value if it is the differences between farms that are the object of the analysis. However, if the farms are deemed to be representative of the population as a whole, and the outcome is measured at the individual mare level, then a fixed effects model is less informative (McDermott et al 1994).

Mixed effects models

An appropriate statistical method for dealing with clustered data is the use of mixed effects models (also known as “random effects models” or “hierarchical models” (Greenland 2000; Dohoo et al 2001). Mixed effect models take two main forms depending on the scale of the outcome variable. Linear mixed models (LMMs) are used when the outcome is a continuous variable (such as age, for example). Generalised linear mixed models (GLMMs) are used when the outcome follows a discrete (eg. binary) scale. These models have become used more commonly over the last decade as computing power has increased and more sophisticated software has been developed to deal with the complexity involved in fitting such models (McDermott et al 1994; Dohoo et al 2001; Bolker et al 2009; Bates 2010). Mixed effects models are so-named because they include both fixed effects terms and random effects terms. Fixed effects terms are defined as those in which the possible values of the variable are fixed and repeatable, and it is assumed that observations are independent of one another, for example age would be classified as a fixed effect variable. Random effects terms are defined as those in which the possible values of the variable can change, and are therefore not repeatable (Hedeker et al 1994). In addition, random effects terms are factors whose levels are sampled from a larger population, or whose interest lies in the variation amongst them rather than the specific effects of each level (Bolker et al 2009). To give an example, we may fit a mixed model to relate the effect of mare age (a fixed effect) by selecting a sample of stud farms (a random effect), on the pregnancy rate to first service (the outcome variable).

Mixed models have two main statistical applications (Hedeker et al 1994; Greenland 2000; Dohoo et al 2001):

1. To determine the effects of the independent variables in the model on the outcome of interest whilst accounting for the clustered nature of the data and,

2. To determine the contribution of the different random effects terms in the model and how they contribute to the total variance of the parameter of interest.

In a LMM model the total variance is the summation of the variance components that are included in the model. For example, in a model of a continuous outcome that included mare-related variables (fixed effects) and stallion and farm (as random effects), the total variance would be calculated using the following equation:

$$var_{(total)} = var_{(mare)} + var_{(stallion)} + var_{(farm)}$$

In a GLMM the total variance is calculated slightly differently because the error terms follow a logistic distribution rather than a normal distribution (Dohoo et al 2009). In GLMMs the error variance is fixed at $\pi^2/3$ such that the equation for the calculation of total variance in this situation is:

$$var_{(total)} = var_{(mare)} + var_{(stallion)} + var_{(farm)} + \pi^2/3$$

Using mixed models to identify important sources of variation means that intervention strategies can be targeted at those factors that contribute most to the overall outcome (Hedeker and Gibbons 1994; Greenland 2000; Dohoo et al 2001).

There appears to be some degree of confusion in the literature regarding the terminology used to describe mixed effects models (Bates 2010). In most circumstances mixed models are referred to in the context of a hierarchical data structure (Baayen et al 2008), for example, cows within herds, within regions, within countries. Each level represents a distinct random effect and hence such models are often called “nested” or “multi-level” models (Bolker et al 2009). These models allow for the calculation of the total variance component at each level of the model. However, mixed models are also used to include random effects that do not represent levels in a hierarchy (Hedeker et al 1994; Baayen et al 2008). For example, mares on a stud farm that are served by the same stallion are exposed to two random effects, the stud farm and the serving stallion. In this situation mares would be considered clustered at both the stud farm level and the stallion level. This type of data structure is referred to as “crossed random effects” as mares from different stud farms can be served by different stallions (Baayen et al 2008; Bolker et al 2009). Applying a mixed model in this situation separates the total variance attributable to each of the random effects.

Methods to analyse time-to-event data

Survival analysis

Certain reproductive performance outcomes are measured in terms of how long it takes for an event to occur. For example, how long does it take for mares to conceive after the start of the breeding season? In the equine literature, Bosh (2009b) is the only author to have attempted to describe this by performing a study involving Kentucky broodmares (Bosh et al 2009b). In that study however, the mean ± SD number of days to the last service date was used as the end-point. Unfortunately, the last service date may or may not have resulted in pregnancy and therefore it was not possible to calculate the start of mating to conception interval. In addition, using the mean ± SD to describe a time-to-event outcome only allows for the inclusion of mares that actually experienced the event of interest (ie. conception).

Survival analysis is the term used to describe a technique of analysing time-to-event data that allows for the inclusion of missing “events” (Dohoo et al 2009). The term survival analysis comes from the fact that these analytical techniques were first developed to analyse the “survival time” of patients after certain medical procedures, eg. surgery (Kaplan and Meier 1958) and the “event” (failure) of interest was death of the patient. The term “failure” seems an inappropriate term to describe the event of interest because often the outcome of interest is a success, eg.cure, day of conception (Meadows et al 2006), however the terminology is accepted and used regardless.

The inclusion of missing events in survival analysis is referred to as censoring (Dohoo et al 2009). Right censoring is the most common form of censoring and a subject is right censored if it is known that failure occurs some time after the recorded follow-up period (Kaplan and Meier 1958). In the case of broodmare reproductive performance the end of the breeding season is effectively the end of the follow-up period because no mares are served after that date. Therefore survival analysis allows for the calculation of the time taken for mares to conceive and for the inclusion of data from those mares that don’t conceive by the end of the season.

In the veterinary literature, the description of time-to-event data is usually performed graphically using a survival curve (Dohoo et al 2009). The Kaplan-Meier survival curve plots the cumulative proportion of animals that have “survived” (not experienced the event of interest) as a function of time (Kaplan and Meier 1958). In studies investigating the reproductive performance of dairy cattle, survival analysis is used frequently (Morton 2010). Examination of the survival curve allows for inferences to be made from the data, eg. how long does it take for 50% of cows to conceive after the planned start of mating? (Meadows et al 2006). The risk of an animal experiencing the outcome event during the interval of interest, divided by the length of the interval of interest is known as the hazard (Dohoo et al 2009). There are virtually no studies in the equine literature that have used survival analysis to analyse reproductive outcomes.

Comparison of survival curves is often of interest, for example, do old mares take longer to conceive than young mares? The univariate comparison of survival curves is also an effective screening procedure to identify potential variables that should be included in multivariable model fitting (Dohoo et al 2009). The log-rank test is the most common method of comparing survival functions between groups and is based on a series of contingency tables of observed and expected events for each group at each time point at which an event occurred. The number of events at each time point is compared to the expected number and a χ^2 test performed (Dohoo et al 2009).

Multivariable survival models are used to quantify the effect of multiple explanatory variables on failure time. The Cox proportional hazards model is the most commonly used method to perform multivariable survival analyses (Cox 1972). The Cox proportional hazards model is based on the assumption that the hazard for an individual is a product of a baseline hazard (h_0) and an exponential function of a series of explanatory variables (Dohoo et al 2009):

$$h(\mathrm{t}) = h_0(\mathrm{t})\mathrm{e}^{\beta X}$$

and, $$\mathrm{HR} = h(\mathrm{t})/h_0(\mathrm{t}) = \mathrm{e}^{\beta X}$$

where HR is the hazard ratio. The hazard ratio is interpreted in a similar way to an odds ratio, ie. if the HR > 1 then the predictor increases the hazard of the outcome occurring, and if the HR < 1 it decreases the hazard of the outcome occurring.

Random effects terms can be included in the Cox proportional hazard model in much the same way as GLMMs (Therneau 2011). In survival analysis, random effects terms are referred to as “frailty” terms. This nomenclature comes from the fact that some observations are more prone to experiencing the event in ways that cannot be accounted for by a simple Cox model and hence are more “frail” (Dohoo et al 2009). To account for this unobserved frailty, a random effect parameter is introduced into the model (Therneau 2011). Such models are referred to as mixed effects Cox models. If one was to analyse the time taken for mares to conceive after the start of the breeding season, the clustering of mares on stud farms can only be accounted for by including a random effect (frailty) term for stud farm in a mixed effects Cox model.

Conclusion

This review describes the New Zealand Thoroughbred breeding industry in general terms, the physiology of equine reproductive seasonality, and in particular, details the characteristics of the transition period. It also reviews previous studies of reproductive performance in Thoroughbred mares and statistical techniques appropriate for the analysis of reproductive outcomes.

It is clear from the literature review that the mismatch between the natural breeding season of the mare and the imposed breeding season, as stipulated by regulatory authorities, are at odds with each other. This creates a situation where the normal physiological characteristics of the transition period become problematic for the Thoroughbred industry. Strategies to hasten the transition period and to manage pathological conditions such as the development of haemorrhagic anovulatory follicles (HAFs) are therefore essential.

It is clear from the available literature that poor reproductive performance is a significant lost opportunity for the Thoroughbred industry worldwide; no other livestock industry would accept a situation where 20% of its breeding herd failed to reproduce each year. It is therefore essential to understand what limits equine reproductive performance so that strategies can be developed to increase performance. However, a review of previous studies describing the reproductive performance of Thoroughbred mares clearly shows deficiencies in the literature. Statistical techniques that account for the fact that mare reproductive outcomes are multivariable in nature and that mares are clustered at the stud farm level are limited. It is therefore difficult to determine what factors are the most important drivers of reproductive performance in the Thoroughbred industry.

An indisputable conclusion reached on completion of the literature review is that there is virtually no information regarding the reproductive performance of Thoroughbred mares in New Zealand. Management and veterinary intervention strategies in New Zealand must therefore be extrapolated from studies performed elsewhere, ie. under different environmental, nutritional and managerial systems. This thesis attempts to rectify this situation by investigating aspects of reproductive performance in New Zealand Thoroughbred mares.

Chapter 3: Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses.

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Abstract

Aim: The aim of this study was to describe the reproductive performance of a population of Thoroughbred mares on stud farms in the Waikato region of New Zealand.

Methods: A prospective cohort study was performed involving five stud farms in the Waikato region of New Zealand during three consecutive breeding seasons (2006-2008). A total of 1482 individual mares contributed 2007 mare years and 3402 oestrous cycles over the three breeding seasons. Mares were served by 87 individual stallions.

Results: The mean first-cycle pregnancy rate (FCPR) was 53.6%, the end-of-season pregnancy rate (SPR) was 85.3% and the foaling rate was 80.2%. There were significant differences amongst farms for FCPR (range 47.3 – 68.2%; $P < 0.01$), SPR (range 81.4 – 92.6%; $P < 0.001$) and foaling rate (range 77.8 – 90.1%; $P < 0.001$). There were significant differences amongst stallions in their FCPR (range 38 – 74%; $P < 0.001$) and SPR (range 72 – 97%; $P < 0.001$). The proportion of pregnancies lost between day 14-16 and day 42 of gestation was 5.5% and the proportion of pregnancies lost between day 42 and term was 3.0%. Mares older than 14 years of age and barren mares had the highest pregnancy loss rates. Of all pregnancies diagnosed at 14-16 days, 12.9% were multiple pregnancies. The length of the breeding season was relatively short with 87.3% of services occurring in the 91 day period between 1September and 30 November.

Conclusions: The reproductive performance of Thoroughbred mares in the Waikato region of New Zealand was similar to that reported from other major Thoroughbred breeding countries. Although the per cycle pregnancy rates in this study were lower than those reported in some studies, the foaling rate was similar to previous reports and this was achieved during a relatively short breeding season.

Abbreviations: FCPR (first cycle pregnancy rate), SPR (end-of-season pregnancy rate).

Key words: mare, stallion, reproductive performance

Introduction

Descriptive studies of Thoroughbred reproductive performance have been published from the UK (Morris and Allen 2002; Allen et al 2007), USA (Bosh et al 2009b), Sweden (Hemberg et al 2004), South Africa (Schulman et al 2003), Australia (Nath et al 2010) and India (Sharma et al 2010a) over the past decade. Surprisingly, there are no standard measures of reproductive performance in the equine industry (Ginther 1992; Amann 2005). One measure of reproductive success is the ability of a mare to produce a live foal, and this outcome is usually referred to as the foaling rate which is defined as the proportion of mares served in a particular breeding season that produce a live foal (Ginther 1992; Schulman et al 2003). In most circumstances the foaling rate is obtained from stud book returns which rely on accurate and timely information provided by breeders. Retrospective information from the USA Stud Book shows that up to 20% of annual mare returns are not submitted by breeders and for this reason the use of registry data to determine reproductive success is associated with a high degree of inaccuracy (Bosh et al 2009b). Other outcome measures commonly used to analyse reproductive performance are the pregnancy rate per cycle, and the end-of-season pregnancy rate (Ginther 1992; Love and Samper 2009). These parameters can be calculated from data collected from farm records and provide a more accurate and timely assessment of reproductive performance.

The New Zealand Thoroughbred breeding industry is the sixth largest in the world, however there are no published reports detailing the reproductive performance of Thoroughbred mares from this country. Therefore, the objective of this study was to investigate the reproductive performance of a population of Thoroughbred mares on stud farms in the Waikato region of New Zealand. This paper describes reproductive performance outcomes in such a way as to allow comparisons to previous studies from other countries, utilising similar methodology. In a companion paper, we describe multivariable statistical methods appropriate for clustered data to identify mare-related exposure variables that significantly contribute to the reproductive performance of Thoroughbred mares.

Materials and methods

Study design

A prospective cohort study was conducted on mares from five stud farms in the Waikato region of New Zealand (latitude 37°49'S) during three consecutive breeding seasons (2006-2008). The Thoroughbred breeding season commences on 1 September in New Zealand. The five stud farms were selected from the client database of a single veterinary practice and were all under the routine care of the same veterinarian (DH). Farms were enrolled in the study based on their willingness to be involved and a known history of good, accurate record-keeping.

Farm description

Farm size ranged from 50 to 200 ha (mean 109 ha). All mares were maintained on pasture and were supplemented with grass conserved as hay or haylage, and fed commercially available concentrate feed as required. Grazing management of mares was similar to that outlined previously (Rogers et al 2007) and was typical of the relatively homogeneous nature of stud farm management in the Waikato region. Mares were not stabled unless there were health issues with their foals or for the management of foal limb deformities. Foaling occurred outside, under supervision, on all farms as previously reported (Rogers et al 2007). Three of the stud farms stood resident stallions. On farms with resident stallions, most mares were owned by outside clients. The two stud farms without resident stallions were private facilities where the mares were owned by the owner of the stud farm and no outside mares were present. Characteristics of each stud farm are presented in Table 1.

Breeding management

On farms that stood resident stallions, most mares visited the stud for breeding, remained on the farm and then left the farm after a 42 day positive pregnancy test or when the owner decided to cease breeding attempts in non-pregnant mares, whichever came first. Oestrous behaviour was monitored daily by teasing with a pony stallion. Each farm was visited by the same veterinarian (DH) at least 6 days per week throughout the breeding season. Transrectal ultrasonographic examination (Aloka SSD-500V; Hitachi Aloka Medical Ltd., Tokyo, Japan) was performed daily or every other day during oestrus to monitor follicular development and normal/abnormal changes in characteristics of the reproductive tract. Intra-uterine antibiotics (ceftiofur, neomycin, procaine penicillin) were administered for the treatment of endometritis and/or post-breeding intra-uterine fluid accumulation when indicated as previously described (Nath et al 2010). Systemic treatment with oxytocin, antibiotics (trimethoprim sulphonamide) and anti-inflammatory medication (prednisolone) was also administered when appropriate. On all farms, a proportion of the dry mares were exposed to an artificial lighting programme, commencing on 1 July with the duration of light exposure calculated to ensure that the artificial day length was 14 hours; the other mares had no exposure to artificial light at all. Selection of mares for light exposure was entirely the choice of the farm manager or mare owner. Foal heat breeding was practiced on four farms. Mares were only served on foal heat if they met the following selection criteria: ovulation occurring 10 or more days post-partum, less than 1cm of intra-uterine fluid present on ultrasonography, and no history of dystocia or retained foetal membranes. Mares were served by stallions that were either resident on the same farm as the mare or by stallions that were located on other stud farms ("walk-out" services). Mares that were "walked-out" to stallions for service returned to their stud farm of origin immediately after service. All mares were given human chorionic gonadotropin (hCG) intravenously (1667 iu, Chorulon, Intervet, Auckland, New Zealand) the day before service. Pregnancy determination by ultrasonographic examination was first performed 14-16 days after service in all mares. In mares diagnosed pregnant at 14-16 days, further examinations to confirm pregnancy (or detect pregnancy loss) were performed at 21, 28 and 42 days of gestation. It is standard industry practice in New Zealand for stallion service fees to be payable on a positive 42 day pregnancy test.

The data collected at each veterinary visit were entered into a spreadsheet by the same veterinary technician present at each visit for later analysis. The foaling outcome was obtained the following season for resident mares from farm records; for non-resident mares the foaling outcome was obtained via the New Zealand Thoroughbred Studbook (http://www.nzracing.co.nz/Breeding/Studbook.aspx).

Reproductive performance measures

The main measures of reproductive performance (dependent variables) of interest were the pregnancy rate per cycle, and the end-of-season pregnancy rate. The pregnancy rate per cycle was defined as the number of mares diagnosed pregnant (at 14-16 days after mating) divided by the number mated per cycle x 100 (Ginther 1992). For example if 100 mares are mated on one cycle and 55 of them are diagnosed pregnant 14-16 days after mating, the per cycle pregnancy rate is 55%. For the description of main effects the first cycle pregnancy rate (FCPR) was used. The FCPR was used because it is accepted as a reliable indicator of fertility in the equine industry as it removes the influence of repeated cycles being contributed by subfertile mares or stallions (Amann 2006; Blanchard et al 2010b; Nath et al 2010). The end-of-season pregnancy rate (SPR) was defined as the total number of mares diagnosed pregnant at 42 days of gestation by the end of the season divided by the total number of mares mated during the season x 100 (Ginther 1992). For example if a stud farm breeds a total of 450 mares during the season and of those mares 360 have a positive 42 day pregnancy test, the SPR for that farm is 80%. Other measures of reproductive outcome were (i) the foaling rate, (ii) the incidence of twin pregnancies, and (iii) the incidence of pregnancy losses (and the stage of gestation at which they occurred). The foaling rate was defined as the number of mares producing a live foal of those that were mated in the preceding season x 100 (Ginther 1992).

Mares that left the farm or died before their first mating were excluded from the study. Barren mares were defined as those that had previously produced a foal but had not conceived (or were not mated) in the preceding season. Maiden mares were defined as those that had never been mated. Both barren and maiden mares were defined collectively as “dry” mares.

Statistical analyses

Univariate associations between each of the two main dependent variables of interest (FCPR and SPR) and the independent variables (stud farm, year of study and serving stallion) were performed. For the binomial outcome variable of pregnancy, chi-squared tests were used to determine the association between each independent variable and the dependent variable by comparison of proportions. The correlation between SPR and foaling rate was calculated using the Spearman Rank Correlation method. Data are presented as mean (SD) and percentages with 95% CI where appropriate. Significance was determined at $P < 0.05$. Analyses were performed using R Version 2.13.0 (R Development Core Team, 2011; R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). Some mares remained in the study in consecutive years and contributed more than one breeding season, for the purposes of analysis each mare was considered to contribute a separate “mare-year” for each breeding season, therefore the terms mares and mare-years are used synonymously (Bruck et al 1993).

Results

A total of 1482 individual mares contributed 2007 mare years and 3402 oestrous cycles over the three breeding seasons. Information from the New Zealand Stud Book indicated that this represented 10% (2007/19729) of the total number of mares served in New Zealand during the same period. Mares were served by 87 different stallions, representing 48% (87/182) of the total number of active stallions present during the three year period with twenty-four of the stallions resident on the study farms. Three of the five farms were represented in all years, whereas one (Farm 3) was present for one breeding season and another farm (Farm 1) for two seasons; these farms left the study after they were sold and used for other farming enterprises. The population characteristics of each farm are outlined in Table 3.1. The mean age of mares was 9.58 (SD 4.1) years and the median age was 9 years (min 3, max 24). Mare reproductive status was 60% (1201/2007) foaling, 27% (533/2007) barren, and 13% (273/2007) maiden. Of foaling mares, 32% (384/1201) were served on foal heat. There were 283 dry mares (35% of all dry mares) exposed to an artificial lighting programme.

No mares were served on any farm after 31December although for the majority of non-pregnant mares (79% (233/295)) this cut off was much earlier, being 15 December. Only 13% (456/3505) of all services occurred in December. Of the stallions used, 26 stallions served more than 10 study mares (range 1 – 273; mean 23.1 mares per stallion).

Pregnancy rate per cycle

The overall mean FCPR was 53.6% (1075/2007) and varied amongst farms (Table 3.2). The first and second cycle pregnancy rates were similar (53.6 and 53.0% respectively, $P = 0.76$). The pregnancy rate per cycle for cycles 3 to 6 combined (no mares were served on more than six cycles) was 45.3% (229/505) which was lower than the pregnancy rates to first and second cycles ($P = 0.002$; Table 3.3). The FCPR was not significantly different between years. There were significant differences amongst stallions in their FCPR (range 38 – 74% for stallions that served ≥ 10 mares; $P<0.001$). Of the 26 stallions that served more than 10 study mares, those that served more than 100 study mares had a lower FCPR than those that served less than 100 mares (51% (637/1258) vs 59% (345/584); $P < 0.001$). There was no significant difference in the FCPR between years. The FCPR of mares served on foal heat was not different from those served on later cycles (49.5% (190/384) vs 52.4% (428/817) respectively; $P = 0.35$).

End-of-season pregnancy rate (SPR)

The mean SPR was 85.3% (1712/2007) and varied amongst farms (range 81.4 – 92.6%; $P<0.001$; Table 3.2). There were significant differences amongst stallions in their SPR (range 72 – 97%; $P<0.001$), however there was no correlation between the number of mares served and the SPR for each stallion. The SPR was not significantly different between years. The SPR was higher in mares mated on foal heat compared with those that were not mated on foal heat (87.6% (336/384) vs 79.9% (653/817) respectively; $P < 0.001$).

Foaling rate

The foaling outcome was available for 1592 mares (93% of all pregnant mares) that foaled in the study. Information on foaling outcome was obtained from farm records for 82% (1305/1592) of the mares with the remainder being obtained from the New Zealand stud book. The overall foaling rate (live foals born) in this study was 80.2% (1277/1592) with a range amongst farms of 77.8 – 90.1% ($P < 0.001$; Table 3.2). The correlation between SPR and foaling rate was 0.82 ($P < 0.001$). The foaling rate was not significantly different between years. The foaling rate was higher in mares mated on foal heat compared with those that were not mated on foal heat (81.3% (312/384) vs 75.2% (614/817) respectively; $P = 0.001$).

Pregnancy losses

The mean proportion of pregnancies lost between first diagnosis (14-16 days) and 42 days of gestation was 5.5% (98/1775). There were no differences amongst farms in the pregnancy loss rate. The pregnancy loss rate was greater in older mares, with mares 14 years and older having a mean loss rate of 9.0% (28/302) compared with 4.7% (70/1473) in mares younger than 14 years ($P = 0.002$). Barren mares had the highest pregnancy loss rate between first diagnosis and 42 days (7.9% (39/494)) which was higher than both maiden (3.7% (10/260); $P = 0.03$) and foaling (4.8% (49/1021); $P = 0.02$) mares (Table 3.4). There were no differences amongst stallions in the proportion of pregnancies lost. There was no difference in the pregnancy loss rate between mares conceiving on a foal heat cycle compared with mares conceiving on later cycles (5.3% (10/190) vs 4.6% (39/831) respectively; P = 0.82). From 42 days of gestation to term the pregnancy loss rate was 3% (51/1704) and there was no effect of stud farm, mare age, mare status, foal heat breeding or stallion on pregnancy losses after 42 days of gestation.

Multiple-pregnancy rate

The overall rate of multiple pregnancies diagnosed at 14-16 days was 12.9% (229/1775). Manual reduction to a single embryo was performed in all cases of multiple pregnancies on the day they were first detected. Eight (3.5%) of the multiple pregnancies diagnosed were triplets. There was no effect of farm, mare age or stallion on the incidence of multiple pregnancies. Mare status affected the multiple pregnancy rate, with barren and maiden mares having a higher rate than foaling mares (Table 3.4). The pregnancy loss rate following manual reduction to a single embryo was not different from the loss rate in mares diagnosed with a singleton pregnancy (5.8% (13/229) vs 5.5% (85/1546) respectively; P = 0.96).

Services per oestrous cycle

The mean number of services per oestrous cycle was 1.03 (stud farm range 1.0 – 1.1) and this did not vary with any of the independent variables.

Discussion

This is the first study to describe the reproductive performance of Thoroughbred stud farms in New Zealand. The New Zealand Thoroughbred breeding industry differs from that in the northern hemisphere in that horses are maintained outside, all-year-round on a predominantly pasture-only diet, with relatively little supplementary feed (Rogers et al 2007). This system allows a large number of horses to be managed by relatively few farm staff. The stud farms involved in this study were characteristic of the New Zealand industry as outlined previously (Rogers et al 2007). However, it is important to realise that these farms were all selected from the same geographical region and were under the care of the same veterinarian for the duration of the study. Therefore conclusions from the findings of this study need to be interpreted with this in mind.

The median age of mares in our study (9 years) was higher than that reported in studies from the UK (Allen et al 2007) and USA (Bosh et al 2009b) where the median age in both studies was 8 years. The proportion of foaling mares was lower in our study (60%) compared with previous studies from the northern hemisphere where 65 – 70% of mares were foaling mares (Morris and Allen 2002; Bosh et al 2009b) but was similar to that described in a recent Australian study where 59.5% of mares were foaling mares (Nath et al 2010). The proportion of barren mares in our study (27%) was also similar to that of the Australian study (26.4%) (Nath et al 2010) and probably reflects similarities between the Australian and New Zealand breeding industries. The relative cost of keeping a non-pregnant mare is substantially higher in the northern hemisphere (Bosh et al 2009a). In Australasia, the relatively low-cost, pasture-based production system means that mare owners are more inclined to leave a mare non-pregnant for a season in order to produce an early foal the following year.

The length of the breeding season in this study was around 105 days which is significantly shorter than in the northern hemisphere where the season runs from 15 February to early July and is therefore around 150 days in duration (Hemberg et al 2004; Allen et al 2007; Bosh et al 2009b). The most likely reason for the short Thoroughbred breeding season in New Zealand is the fact that early foals are desirable because they are generally larger than their later-born peers by the time they are sold at yearling sales at around 14-17 months of age. The main Thoroughbred yearling sale in New Zealand is held in the last week of January; therefore foals born after 1 December are only 12-13 months of age at sale time and are at a distinct growth disadvantage compared with foals born earlier in the season.

The overall first-cycle pregnancy rate reported here of 53.6% was slightly lower than other studies where a per-cycle pregnancy rate of around 60% has been reported (Allen et al 2007; Bosh et al 2009b), but the foaling rate of 80.2% we report is very similar to that reported in the USA (78.3% (Bosh et al 2009b)) and the UK (79.7% (Allen et al 2007)). In our study there were significant differences amongst stud farms in the FCPR and SPR. Variation in stud farm fertility could be due to differences in mare age and reproductive status, stallion fertility and other management practices such as foal heat breeding and artificial light exposure. The effect of stud farm and any confounding exposure variables can only be determined by multivariable analyses which are described in a companion paper.

In agreement with the findings of others (Morris and Allen 2002; Bosh et al 2009b; Blanchard et al 2010b) there were significant differences amongst stallions in their FCPR and SPR. Inherent stallion fertility varies considerably, but in addition, stallion breeding management practices such as the number of mares served per day and per season, and breeding frequency all affect stallion fertility (Blanchard et al 2010b). The overall fertility of stallions in this study was lower than that previously reported (Bosh et al 2009b; Blanchard et al 2010b; Nath et al 2010). It is unlikely that there are large differences in the inherent fertility of stallions in New Zealand compared with other countries, especially given the fact that most stallions at stud in New Zealand were either bred-in or are sired-by stallions from the northern hemisphere. Stallion management practices are more likely to explain the differences between stallion fertility in different countries. With the longer breeding season in the northern hemisphere, rarely do stallions need to breed more than three times per day, even with book sizes of around 150 mares (Blanchard et al 2010b). Serving more than three times a day has been associated with a reduction in fertility (Blanchard et al 2010b). We found that stallions that served more than 100 study mares per year over the 3 year period had a significantly lower FCPR than those that served less than 100 mares (50.6 vs 59.1%). It is important to realise that these stallions were also serving outside mares and therefore the total number of mares served by these stallions was even greater. The short breeding season in New Zealand means that it is common for most popular stallions to serve at least four times a day. The majority of services (87%) occurred in the 90 day period from 1 September to 30 November. The average number of services per oestrus cycle in this study was only 1.03, compared with 1.1 services per cycle reported from the UK (Allen et al 2007). This indicates that stallion managers are reluctant to re-serve mares on the same oestrous cycle and supports anecdotal evidence that mares are served once per cycle in order to maximize the number of individual mares served by any given stallion. Further studies are required to investigate stallion breeding management practices in New Zealand and their potential effect on fertility.

In this study 32% of foaling mares were served on foal heat, which is significantly higher than that reported in other studies where only 12 - 14% of mares were served on foal heat (Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b). There was no difference in the FCPR of mares served on foal heat compared with mares served on later cycles, and foal heat breeding significantly improved the SPR. The higher rate of foal heat breeding reported here is probably a reflection of the relatively short breeding season and underlines the necessity to get mares back in foal as soon as possible after foaling. Some studies have reported a lower pregnancy rate and a higher pregnancy loss rate in mares served on foal heat (Morris and Allen 2002; Allen et al 2007) whereas other studies have found no detrimental effect of foal heat breeding (Blanchard et al 2004; MacPherson and Blanchard 2005). The overall positive effect of foal heat breeding in this study is most likely due to careful selection criteria, since of the mares examined at foal heat only 45% were actually deemed suitable for breeding on foal heat. There was no difference in pregnancy loss rates between mares conceiving on a foal heat cycle compared with mares conceiving on later cycles. The similar rate of pregnancy loss indicates that uterine involution had occurred normally and the uterus was physiologically capable of maintaining pregnancy in the selected mares. In the context of the New Zealand breeding system, foal heat breeding appears to offer significant benefits.

In our study 35% of all dry mares were exposed to an artificial lighting programme prior to the start of the breeding season which is much lower than that reported in a recent study from the USA where 100% of dry mares were exposed to artificial lights (Bosh et al 2009b). This difference most likely reflects the fact that mares in the USA are housed indoors and therefore the exposure to artificial lighting is relatively easy to achieve. In New Zealand, artificial light exposure is generally performed by bringing mares into holding yards each night and is therefore relatively time-consuming and labour intensive (Rogers et al 2007).

Pregnancy loss rates in the present study were significantly lower than that reported in other studies where around 20% of all pregnancies first diagnosed at 14 -16 days after service were subsequently lost (Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b). This may be due to differences in the incidence of clinical and/or subclinical endometritis which may cause early embryonic death. Whilst we did not specifically investigate this, anecdotal evidence suggests that mares housed indoors have a higher incidence of intra-uterine fluid accumulation post-breeding and are therefore more susceptible to mating-induced endometritis (Riddle et al 2007). Racing has also been shown to have a beneficial effect on fertility (Sairanen et al 2011). Under the management system used in New Zealand, all mares are routinely at pasture and have ample opportunity to exercise. In a recent study from Australia (Nath et al 2010) where management systems are similar to those in New Zealand, the pregnancy loss rates were similar to our study, thus supporting the theory that spontaneous exercise at pasture may have a positive effect on fertility. In our study, pregnancy loss rates were greatest in mares older than 14 years of age, and barren mares, which is in agreement with previous studies (Allen et al 2007; Bosh et al 2009b; Nath et al 2010).

The multiple pregnancy rate in this study was similar to that reported elsewhere and was highest in barren mares which is consistent with other reports (Perkins and Grimmett 2001; Allen et al 2007; Nath et al 2010).

In the equine industry there are no standard or universal measures of reproductive performance (Amann 2006). Whilst the foaling rate is commonly used to compare the reproductive performance of different populations of mares, the data required to measure this parameter are often difficult to obtain for the following reasons. Firstly, the foaling rate is usually obtained from stud book records which depend on accurate foaling returns. In this study we found that the foaling rate of the study mares was 80.2% which was significantly higher than the 65.3% reported in the stud book for all mares in New Zealand in the same period. This is similar to the findings of other studies where the reported foaling rate is significantly higher than the registry data from their respective countries (Morris and Allen 2002; Hemberg et al 2004) and emphasizes the unreliability of using stud book records to determine reproductive performance. Secondly, the foaling rate is a retrospective measure and is based on events that occurred in the previous breeding season (i.e. 12 months ago). The main goal of any reproductive herd-health management program is to monitor performance within the breeding season so that any deviations from targets can be detected early and potentially rectified before the season ends. As a measure of reproductive performance within the breeding season the foaling rate is therefore of little use. We found that the end-of-season pregnancy rate (SPR) was highly correlated to the foaling rate in our study. Given that the SPR can be determined relatively soon after the end of the season, we propose that this parameter is a more useful index of reproductive performance than the foaling rate.

In conclusion, our findings show that the reproductive performance of Thoroughbred mares in the Waikato region of New Zealand was similar to that reported from other major Thoroughbred breeding countries. Although the per cycle pregnancy rates in this study were lower than those reported in some studies, the foaling rate was similar to previous reports and this was achieved during a relatively short breeding season.

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Table 3.1: Characteristics of five Thoroughbred stud farms from the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008).

Stud farm	Total mares	Age (mean ± SD)	Total No. dry mares	No. maiden mares	No. barren mares	No. foaling mares	No. stallions
1	367	8.6 ± 3.4	152	56	96	215	9
2	148	9.1 ± 4.1	63	24	39	85	-
3	44	9.2 ± 3.8	12	4	8	32	-
4	463	10.1 ± 4.1	186	59	127	277	8
5	985	9.8 ± 4.2	393	130	263	592	7
Total	2007	9.6 ± 4.1	806	273	533	1201	24

Table 3.2: First-cycle pregnancy rate (FCPR), end-of-season pregnancy rate (SPR) and foaling rate of five Thoroughbred stud farms (n = 2007 mares) in the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008).

Variable	ID	FCPR (%)	SPR (%)	Foaling rate (%)
Stud	1	58.6	86.1	78.1
	2	54.7	92.6	82.3
	3	68.2	90.9	90.1
	4	60.9	90.1	81.2
	5	47.3	81.4	77.8
Mean		53.6	85.3	80.2

Table 3.3: Pregnancy rate per cycle of 2007 Thoroughbred mares from the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008).

Cycle number	No. cycles	No. pos	Pregnancy rate (%)	95% CI
1	2007	1075	53.6[a]	51.4-55.8
2	890	471	53.0[a]	49.6-56.2
3-6	505	229	45.3[b]	40.9-49.8

[a,b] Values within the same column with different superscripts are significantly different ($P < 0.05$)

Table 3.4: Pregnancy loss rates and multiple pregnancy rate by reproductive status for 2007 Thoroughbred mares from the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008).

	Maiden			Barren			Foaling		
	%	No.	95% CI	%	No.	95% CI	%	No.	95% CI
Pregnancy losses									
14 - 42 d	3.7^{a}	10/260	1.9-7.0	7.9^{b}	39/494	5.7-10.6	4.8^{a}	49/1021	3.6-6.3
42 d - term	2.8	7/253	1.1-5.6	3.0	14/468	1.6-5.0	3.1	30/983	2.1-4.3
Multiple preg. at 15d	15.8^{a}	41/260	11.6-20.8	19.0^{a}	94/494	15.7-22.8	9.2^{b}	94/1021	7.5-11.1

^{a,b} Values within the same row with different superscripts are significantly different ($P < 0.05$)

Chapter 4: Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 2. Multivariable analyses and sources of variation at the mare, stallion and stud farm level.

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Abstract

Aim: The objective of this study was to utilise multivariable statistical methods appropriate for clustered data to identify mare-related exposure variables that significantly affected the reproductive performance of Thoroughbred mares in the Waikato region of New Zealand. In addition, we aimed to determine the relative contribution of the mare, stallion and stud farm to reproductive performance.

Methods: A prospective cohort study was performed involving five stud farms in the Waikato region of New Zealand during three consecutive breeding seasons (2006-2008). A total of 1482 individual mares contributed 2007 mare years and 3402 oestrous cycles over the three breeding seasons. Reproductive performance was measured using three parameters; a) first cycle pregnancy rate (FCPR), b) end-of-season pregnancy rate (SPR), and c) the start of mating to conception interval.

Results: When controlled for the effects of serving stallion, stud farm and year of study the only significant mare-related variables included in the final models of FCPR, SPR and conception interval were the age of the mare and her reproductive status (classified as dry or foaling). Advancing mare age significantly reduced reproductive performance regardless of reproductive status and foaling mares had significantly poorer reproductive outcomes compared to dry mares when controlled for age. For each additional increase in year of age the FCPR was reduced by a factor of 0.94 (95% CI: 0.92 - 0.96) and the SPR was reduced by a factor of 0.91 (95% CI: 0.88 – 0.93). Mares older than 14 years of age took longer to conceive after the start of mating compared to younger mares. The daily hazard of conception for mares 14 years and older was 0.64 (95% CI: 0.47-0.83) times less than mares younger than 9 years of age. Determining the relative contribution of the mare, stallion and stud farm to the FCPR indicated that 95.9% of the variation was at the mare level, 4.1% was at the stallion level and 0% was at the stud farm level. For the SPR the variance components indicated that 92.5% of the variation was at the mare level, 6.7% was at the stallion level and 0.8% was at the stud farm level.

Conclusions: The reproductive performance of Thoroughbred mares in the Waikato region of New Zealand is influenced by two main mare-related factors; the age of the mare and her reproductive status (dry or foaling). The majority of variation in reproductive performance was associated with mare-level factors and the contribution of the stallion and stud farm was relatively minor.

Abbreviations: FCPR (first cycle pregnancy rate), SPR (end-of-season pregnancy rate)

Key words: mare, stallion, reproductive performance

Introduction

Factors that influence the reproductive performance of Thoroughbred mares can be broadly categorised into mare-related, stallion-related and management-related exposure variables. The most important mare-related exposure variables that appear to influence reproductive outcomes are age and reproductive status (Allen et al 2007; Bosh et al 2009b). Despite these findings there is no clear understanding of the relative importance and the interrelationships between the various mare-level variables on reproductive performance. Factors that affect Thoroughbred stallion fertility are also poorly defined, and stallion breeding management and inherent variation in Thoroughbred stallion fertility have not been thoroughly investigated (Blanchard et al 2010b). Studies of farm-level management practices that influence reproductive performance are also limited (Bosh and Gluck 2006). To the best of the authors' knowledge there are no reported studies quantifying the relative contribution of the mare, stallion and stud farm on reproductive performance.

Most studies describing the reproductive performance of Thoroughbreds have evaluated factors which vary at a single level (univariate analysis) (Morris and Allen 2002; Allen et al 2007; Nath et al 2010). Univariate analysis involves examining the effect of a single exposure variable, for example mare age, on the outcome of interest assuming conditions of independence (McDermott et al 1994). Univariate analysis does not take into account the fact that some variables influencing reproductive performance are related to each other, for example maiden mares are most likely to be the youngest, nor does it determine which of the exposure variables are the most important. Given that many independent effects can contribute to reproductive performance, multivariable analyses are required to determine the relative contribution of each on the outcome of interest (Dohoo et al 2009).

Although reproductive performance in Thoroughbreds is usually analysed at the mare level, mare-level outcomes cannot be considered independent events. Mares are grouped within stud farms and are therefore exposed to the same nutritional, environmental and farm-management practices. In addition, groups of mares are served by the same stallion (defined as a stallion's "book" of mares) and hence there is likely to be an influence of the stallion's inherent fertility on the reproductive performance of the mares that he is bred to. Mare-level reproductive outcomes are likely to be correlated within groups (stud farm and covering stallion), a phenomenon referred to as "clustering" in the observational epidemiological literature (McDermott and Schukken 1994). When data are clustered there are two sources of variation, between clusters and within clusters: the first arises from the effect of the cluster (the stud farm or covering stallion) and the second from the individual mare. These two sources of variation cause the variance of effect measures to inflate, meaning that there is greater uncertainty in their true value compared with a study that is completely randomised at the individual mare level, in other words, when clustering is present the effective sample size (and consequently the study power) is reduced. Failure to account for clustering when it is present results in confidence intervals that are too narrow, increasing the chance of making a type I error (rejecting the null hypothesis when, in reality, it is true) (McDermott and Schukken 1994). Statistical techniques that account for the clustered nature of the data should therefore be used to investigate factors that influence mare reproductive performance (McDermott et al 1994).

There are no published reports detailing the reproductive performance of Thoroughbred mares in New Zealand. To address this knowledge gap, the objective of this study was to describe the reproductive performance of a population of Thoroughbred mares on stud farms in the Waikato region of New Zealand. In a companion paper we outlined descriptive statistics and explanatory data in such a way as to allow comparisons to previous studies from other countries, utilising similar methodology. In this paper, the objective was to utilise multivariable statistical methods appropriate for clustered data to examine the effects of mare-related exposure variables and their interrelationships on the reproductive performance of Thoroughbred mares. In addition, we aimed to determine the relative contribution of the mare, stallion and stud farm to reproductive performance.

Materials and methods

Study design

A more detailed description of the study design has been published in a companion paper (Hanlon et al 2012). Briefly, a prospective cohort study was performed involving five stud farms in the Waikato region of New Zealand during three consecutive breeding seasons (2006-2008). The Thoroughbred breeding season commences on 1 September in New Zealand. A total of 1482 individual mares contributed 2007 mare years and 3402 oestrous cycles over the three breeding seasons. Mares were served by 87 individual stallions, with 24 of the stallions residing on the study farms.

Each farm was visited by the same veterinarian (DH) at least 6 days per week throughout the breeding season. Ultrasonographic examination was performed daily or every other day during oestrus to monitor follicular development and normal/abnormal changes in characteristics of the reproductive tract. Appropriate veterinary treatments were administered when required. On all farms, a proportion of the dry mares were exposed to an artificial lighting programme, commencing on 1 July with the duration of light exposure calculated to ensure that the artificial day length was 14 hours; the other mares had no exposure to artificial light at all. Foal heat breeding was practiced on four farms. Mares were served only on foal heat if they met the following selection criteria: ovulation occurring 10 or more days post-partum, less than 1cm of intra-uterine fluid present on ultrasonography, and no history of dystocia or retained foetal membranes. Mares were served by stallions that were either resident on the same farm as the mare or by stallions that were located on other stud farms (“walk-out” services). Pregnancy determination by ultrasonographic examination was first performed 14-16 days after service in all mares. In mares diagnosed pregnant at 14-16 days, further examinations to confirm pregnancy (or detect pregnancy loss) were performed at 21, 28 and 42 days of gestation.

Reproductive performance measures

The three measures of reproductive performance (dependent variables) of interest were (i) the first cycle pregnancy rate, (ii) the end-of-season pregnancy rate, and (iii) the start of mating to conception interval. The first cycle pregnancy rate (FCPR) was defined as the number of mares diagnosed pregnant (at 14-16 days after mating) divided by the number mated on their first oestrous cycle x 100 (Ginther 1992). The end-of-season pregnancy rate (SPR) was defined as the total number of mares diagnosed pregnant at 42 days of gestation by the end of the season divided by the total number of mares mated during the season x 100 (Ginther 1992). The start of mating to conception interval was defined as the number of days from 1 September to the last service date for mares diagnosed pregnant at 42 days of gestation. If a mare lost her pregnancy between first pregnancy diagnosis (at 14-16 days) and 42 days of pregnancy then the initial date of conception was excluded from the calculation of conception interval. If such a mare conceived again and had a positive 42 day pregnancy test then the last service date was taken as the date of conception for that mare. The denominator for the start of mating to conception interval was the total number of mares mated, so that mares that did not conceive during the season were still included in this calculation.

Mares that left the farm or died before their first mating were excluded from the study. Barren mares were defined as those that had previously produced a foal but had not conceived (or were not mated) in the preceding season. Maiden mares were defined as those that had never been mated. Both barren and maiden mares were collectively defined as “dry” mares.

Statistical analyses

Table 4.1 outlines the mare-level exposure variables included in model building of FCPR, SPR and conception interval. Generalised linear mixed models (GLMMs) were used to examine the effects of mare-level variables on the binomial outcome variables FCPR and SPR. Mare-level variables were included in each model as fixed effects, and the variables stud farm and stallion were included in each model as random effects terms to account for the clustered structure of the data (McDermott et al 1994; Bolker et al 2009). Model building proceeded as follows: Firstly, restricted maximum likelihood estimates (REML) for each dependent variable were obtained for a simple random intercept (null) model containing only a constant and the random effects terms, stud farm and stallion, using the Laplace approximation method in the “lme4” package in R (Bates 2010). Because one of the aims of this study was to determine the relative contribution of the stud farm and stallion on each dependent variable, these terms were retained in all models even if the variance estimate was 0 (Bolker et al 2009). Initially GLMMs were fitted that included the effect of mare reproductive status classified as either foaling or dry, on both FCPR and SPR (these models were referred to as being for “all mares”). Further models were then built to describe the reproductive performance of foaling and dry mares separately. This was done because some of the exposure variables were mutually exclusive, for example only foaling mares can be served on foal heat, and only dry mares can be maidens. All independent mare-level variables (including interactions) were then added to the null model as fixed effects (full model). A backward, step-wise model building process was then used where non-significant (Wald’s test, $P > 0.05$) interactions were removed sequentially, followed by non-significant main effects. After each variable was dropped from the model a likelihood ratio test was performed to compare the new, reduced model to the previous model, to determine significance of the reduced model (Bolker et al 2009). To test the significance of each of the two random effects (stallion and stud farm) the log-likelihoods of the final models were compared with and without each of the random effects included.

The latent variable approach was used to determine the relative contribution of the variance components at the mare, stud farm and stallion level (Dohoo et al 2001).

Survival analysis was used to analyse the time-to-event outcome of time to conception after the start of the breeding season. All independent mare-level variables (including interactions) were entered into a Cox proportional hazards regression model using the “coxme” package in R (Therneau 2011) which allows for the inclusion of frailty (random effects) terms. Frailty terms for stud farm and stallion were included in the model and both were retained in all models to account for the clustered nature of the data. A backward, step-wise model building process was then used where non-significant (Wald's test, $P > 0.05$) interactions were removed sequentially, followed by non-significant main effects. After each variable was dropped from the model, a likelihood ratio test was performed to compare the new, reduced model to the previous model, to determine significance of the reduced model. Kaplan-Meier survival curves were used to describe the main effects of age, reproductive status, artificial light exposure and foal heat service on the conception interval. For the purposes of survival analysis, age was categorised into three groups: ≤ 8 years, 9 to 13 years and ≥ 14 years. Foaling date was categorised into quartiles; Q1 - foaling on or prior to 11th September, Q2 - foaling between 12th September and 2nd October, Q3 - foaling between 3rd October and 21st October, and Q4 – foaling on or after 22nd October.

All analyses were performed using R Version 2.13.0 (R Development Core Team, 2011; R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). Some mares remained in the study in consecutive years and contributed more than one breeding season, for the purposes of analysis each mare was considered to contribute a separate "mare-year" for each breeding season, therefore the terms mares and mare-years are used synonymously (Bruck et al 1993).

Results

The mean age of mares in the study was 9.58 ± 4.1 years and the median age was 9 years (IQR 6-12 years). Mare reproductive status was; 60% (1201/2007) foaling, 27% (533/2007) barren, and 13% (273/2007) maiden. Of the foaling mares, 32% (384/1201) were served on foal heat. There were 283 dry mares (35% of all dry mares) exposed to an artificial lighting programme.

First cycle pregnancy rate (FCPR)

All mares

The mean FCPR for all mares in the study, regardless of reproductive status was 53.6% (1075/2007). When controlled for serving stallion, stud farm and year of study the only significant fixed effects included in the final model of FCPR for all mares were the age of the mare and the reproductive status categorised as either foaling or dry (Table 4.2). Increasing age was negatively associated with the FCPR and foaling mares had a lower OR for FCPR than dry mares. Using the latent variable approach to determine the relative contribution of the mare, stallion and stud farm on the FCPR, the variance components indicated that 95.9% of the variation in FCPR was at the mare level, 4.1% was at the stallion level and 0% was at the stud farm level. The likelihood ratio test comparing final GLMMs with and without the two random effects (stallion and stud farm) indicated that stallion was a significant random effect in the model for FCPR (change in -2LL=29.6, df=1, $P < 0.0001$) whereas stud farm was not.

Dry mares

The mean FCPR for dry mares was 56.8% (458/806). The only significant fixed effects included in the final model of FCPR for dry mares were the age of the mare, whether the mare had been exposed to a lighting programme, and the number of days to first service after 1st September (Table 4.2). Increasing age was negatively associated with the FCPR when controlled for serving stallion, stud farm, light exposure and days to first service. Light exposure increased the OR for FCPR when controlled for serving stallion, stud farm, age and days to first service. The FCPR was positively associated with increasing days to first service after 1st September after controlling for serving stallion, stud farm, age, and light exposure.

Foaling mares

The mean FCPR of foaling mares was 51.3% (616/1201). When controlled for serving stallion, stud farm, foal heat service and foaling date the only significant fixed effect included in the final model of FCPR for foaling mares was the age of the mare, with increasing age being negatively associated with the FCPR in foaling mares.

End of season pregnancy rate (SPR)

All mares

The mean SPR for all mares in the study was 85.3% (1712/2007). When controlled for serving stallion, stud farm and year of study the only significant fixed effects included in the final model of SPR for all mares were the age of the mare and reproductive status categorised as either foaling or dry (Table 4.2). Increasing age was negatively associated with SPR and foaling mares had a lower OR for SPR than dry mares. Using the latent variable approach, the variance components indicated that 92.5% of the variation was at the mare level, 6.7% was at the stallion level and 0.8% was at the stud farm level. The likelihood ratio test comparing final GLMMs with and without the two random effects (stallion and stud farm) indicated that stallion was a significant random effect in the model for SPR (change in -2LL=20.8, df=1, $P < 0.0001$) whereas stud farm was not.

Dry mares

The mean SPR of dry mares was 89.8% (724/806). The only significant fixed effects included in the final model of SPR for dry mares were the age of the mare, whether the mare had been exposed to a lighting programme, and the number of days to first service after 1st September (Table 4.2). Increasing age was negatively associated with the SPR when controlled for serving stallion, stud farm, light exposure and days to first service. Light exposure increased the OR for SPR when controlled for serving stallion, stud farm, age and days to first service. Increasing days to first service decreased the OR for SPR when controlled for serving stallion, stud farm, age and light exposure.

Foaling mares

The mean SPR of foaling mares was 82.4% (990/1201). The only significant fixed effects included in the final model of SPR for foaling mares were the age of the mare, whether the mare was served on a foal heat, and the foaling date (Table 4.2). Increasing age was negatively associated with the SPR when controlled for serving stallion, stud farm, foal heat service and foaling date. Breeding mares on foal heat was positively associated with the SPR when controlled for serving stallion, stud farm, age and foaling date. A later foaling date was negatively associated with the SPR when controlled for serving stallion, stud farm, age and foal heat service.

Start of mating to conception interval

All mares

The only significant fixed effects included in the final model for the start of mating to conception interval for all mares was the age category of the mare and the reproductive status categorised as either foaling or dry (Table 4.3). When controlled for serving stallion, stud farm and reproductive status the interval to conception was significantly longer in mares older than 14 years of age compared to mares younger than 14 years. Kaplan-Meier survival curves of the cumulative proportion of mares conceiving after the start of the breeding season, stratified by age are shown in Figure 4.1. Foaling mares took longer to conceive than dry mares when controlled for age, serving stallion and stud farm. Kaplan-Meier survival curves of the cumulative proportion of mares conceiving after the start of the breeding season, stratified by reproductive status are shown in Figure 4.2.

Dry mares

The only significant fixed effects included in the final model of conception interval for dry mares were the age of the mare, whether the mare had been exposed to a lighting programme and the number of days to first service after 1^{st} September (Table 4.3). When controlled for serving stallion, stud farm, artificial light exposure and days to first service, dry mares older than 14 years of age took longer to conceive after the start of the breeding season than mares younger than 9 years of age. Artificial light exposure significantly reduced the conception interval when controlled for serving stallion, stud farm, age and days to first service. Kaplan-Meier survival curves of the cumulative proportion of mares conceiving after the start of the breeding season, stratified by artificial light exposure are shown in Figure 4.3. The start of mating to conception interval also increased with advancing days to first service after the 1^{st} September.

Foaling mares

The only significant fixed effects included in the final model of conception interval for foaling mares were the age of the mare, whether the mare was served on a foal heat, and the foaling date (Table 4.3). When controlled for serving stallion, stud farm, age and foaling date, mares served on foal heat conceived sooner than those not served on foal heat. Kaplan-Meier survival curves of the cumulative proportion of mares conceiving after the start of the breeding season, stratified by foal heat service are shown in Figure 4.4. There was also an effect of foaling date, such that, when controlled for serving stallion, stud farm, age, and foal heat service mares foaling after the 22nd October had a longer start of mating to conception interval than mares foaling prior to the 11th September.

Discussion

This is the first study to describe a multivariable approach appropriate for clustered data to investigate factors that influence the reproductive performance of Thoroughbred mares. The use of multivariable analyses in the context of equine reproductive performance is important because many exposure variables can contribute to the outcomes of interest and these analyses can identify those that are the most significant.

The results of this study indicate that the most important mare-level factors associated with the overall reproductive performance of mares in the Waikato region of New Zealand were mare age and reproductive status (ie. foaling or dry). When controlled for the effects of serving stallion, stud farm, year of study, date of service and reproductive status, increasing mare age significantly reduced the FCPR and SPR. For each unit increase in year of age the FCPR was reduced by a factor of 0.94 (95% CI: 0.92 - 0.96) and the SPR was reduced by a factor of 0.91 (95% CI: 0.88 – 0.93). In practical terms the OR can be used to estimate the change in FCPR and SPR for mares of different ages. For example, given the findings of this study, a 5 year increase in mare age is associated with a 0.73 fold decrease in the FCPR. Mares older than 14 years of age took longer to conceive after the start of mating compared to younger mares. The daily hazard of conception for mares 14 years and older was 0.64 (95% CI: 0.47-0.83) times less than mares younger than 8 years of age. The decreasing fertility of individual mares as they age is well recognised (Ball et al 1989; Ricketts and Alonso 1991).

In terms of reproductive status, dry mares had a significantly higher FCPR and SPR compared to foaling mares. The dry mare group of mares is made up of two sub-groups, maiden mares (those that have never had a foal) and “barren” mares (those that have previously produced foals). It has been generally accepted that barren mares represent a population of mares that are inherently sub-fertile (Morris and Allen 2002; Allen et al 2007; Bosh et al 2009b). However, in the New Zealand breeding industry it appears that most mares in the dry mare category are a result of a voluntary “rest” rather than inherent subfertility. New Zealand breeders would rather leave a mare non-pregnant for a season than produce a “late” foal (Hanlon et al 2012). This finding is also supported by the fact that there was no effect of maiden or barren reproductive status on dry mare reproductive performance in the present study.

The start of mating to conception interval of dry mares was significantly shorter than for foaling mares. This is most likely because dry mares are eligible for breeding right from the start of the breeding season whereas foaling mares have to foal and therefore have an imposed “wait period” before they enter the pool of mares eligible for breeding. As such, the two broad categories of dry and foaling mares represent two different physiological populations of mares.

When controlled for the effects of stud farm, serving stallion, status (maiden or barren sub-category) and year of study, the only mare-level variables that significantly affected the reproductive performance of dry mares were age, artificial light exposure and days to first service after the 1st September. The exposure to an artificial lighting program prior to the start of the breeding season significantly improved the reproductive performance of dry mares in the present study. Light exposure increased the FCPR by a factor of 2.98 (95% CI: 2.02 – 4.40). The finding that the FCPR was improved in mares exposed to lights has not been previously reported. Some studies have reported that the first ovulation of the season is associated with similar pregnancy rates to subsequent ovulations (Cuervo-Arango and Clark 2010). However, to the best of our knowledge no studies have compared the first cycle pregnancy rates of mares exposed to lights versus those under natural light at the start of the breeding season. In this study, it is likely that mares exposed to artificial light had ovulated at least once prior to their first breeding. It is possible that subsequent ovulations are more fertile than first ovulations due to progesterone priming and/or enhanced acceleration of the LH/FSH profile during the transition period (Donadeu and Watson 2007; Cuervo-Arango and Clark 2010). Light exposure also increased the SPR of dry mares by a factor of 2.45 (95% CI: 1.14 – 5.27) which probably resulted from a combination of both an increased FCPR and the fact that these mares were cycling earlier and therefore had more breeding opportunities than mares not exposed to lights. This is supported by the fact that the start of mating to conception interval was also shorter in mares exposed to lights with the daily hazard of conception 1.60 (95% CI: 1.29 – 1.89) times higher in mares exposed to lights compared to those that were not. Increasing days to first service (after 1st September) positively affected the FCPR and negatively affected the SPR and conception interval of dry mares. No seasonal effect on FCPR has been reported previously but it is possible that nutrition, body condition and environmental temperature had a positive effect on the FCPR. Anecdotally, in New Zealand's pasture-based management system, pasture growth and body condition increase with increasing day length from late winter through spring. Pasture has been shown to have a positive effect on follicular development in mares (Carnevale et al 1997). In addition, mares served earlier in the season are more likely to still be in the transition period and therefore the pregnancy rate to services occurring at this time is likely to be reduced. The negative effect of advancing days to first service on the SPR and the start of mating to conception interval is understandable because the later a mare starts the breeding season the fewer the breeding attempts available for conception.

When controlled for the effects of stud farm, serving stallion and year of study, the only mare-level variables that significantly affected the reproductive performance of foaling mares in this study were age, foal heat breeding and foaling date. The only significant variable influencing FCPR was the age of the mare; foal heat breeding and foaling date had no significant effect on the FCPR. Foal heat breeding increased the SPR of foaling mares by a factor of 2.04 (95% CI: 1.39 – 2.99) compared to mares not bred at foal heat. The start of mating to conception interval was also shorter in mares bred at foal heat with the daily hazard of conception 1.78 (95% CI: 1.29 – 1.89) times higher in mares bred on foal heat compared to those that were not. The findings of our study indicate that the selection of suitable mares for foal heat breeding has major positive benefits in terms of reproductive performance. Foaling date significantly affected the SPR and start of mating to conception interval. For each additional day that a mare foaled later in the season the SPR was reduced by a factor of 0.97 (95% CI: 0.97 – 0.98). The daily hazard of conception for mares foaling after the 22nd October was 0.76 (95% CI: 0.64 – 0.92) times less than mares foaling before the 11th September. Foaling date has not been included in previous studies that have assessed the reproductive performance of mares even though the results of our study show that it has a major influence on reproductive performance. Clearly, increasing the number of mares that foal prior to the 22nd October improves reproductive performance.

One of the aims of this study was to determine the relative contribution of the mare, stallion and stud farm on reproductive performance. To the best of our knowledge there have been no previous studies in which the sources of variation in reproductive performance have been quantified. We found that the variation in reproductive outcome was virtually all associated with the mare. The proportion of variance contributed by the mare, stallion and stud farm was 95.9, 4.1 and 0% respectively for FCPR and 92.5, 6.7 and 0.8% respectively for SPR. Mare-related exposure variables are clearly the most important determinants of reproductive performance in this population and management should therefore concentrate on mare-level practices. Strategies that maximise the performance of dry mares should aim to have mares cycling as early as possible in the breeding season to maximise the number of cycles available for conception. This is likely to have a beneficial effect in the following breeding season as well because early foaling mares have superior reproductive performance. The results of this study clearly demonstrate a major improvement in dry mare reproductive performance as a result of artificial light exposure. Hormonal treatments that attempt to induce the early onset of normal cycles in transitional mares such as the use of exogenous progestagens (Wiepz et al 1988; Hanlon and Firth 2012), eFSH administration (Niswender et al 2004; Raz et al 2009), GnRH or its analogues (Evans and Irvine 1977; Hyland and Jeffcott 1988; McKinnon et al 1997), and dopamine antagonists such as domperidone or sulpiride (Besognet et al 1997; Panzani et al 2011) should also be considered, but their efficacy needs to be compared to artificial light exposure. Foaling mares should be managed appropriately to ensure rapid uterine involution after foaling, therefore making them suitable candidates for foal heat breeding.

Whilst the results of this study indicate that mare-level factors are the most important determinant of reproductive success, care needs to be taken in the interpretation and extrapolation of these findings. We identified virtually no variation at the stud farm level in reproductive performance. Assessment of the significance of the random effect term for stud farm included in the multivariable models also revealed that stud farm was not a significant variable. This is probably due to two main factors; a) the stud farms were all visited by the same veterinarian and followed similar reproductive management protocols, therefore there were no differences amongst farms in terms of their reproductive management procedures, and b) all farms followed similar farm management procedures. The homogeneous nature of stud farm management in the Waikato region of New Zealand has been described previously (Rogers et al 2007). The Waikato region contains the majority of New Zealand's Thoroughbred stud farms. The Waikato area is geographically condensed and therefore it is not surprising that the management protocols are similar amongst farms within the region and anecdotally, at least, there is free exchange of ideas amongst farm managers. Further studies to include farms from different regions and/or those under the care of different veterinary practices would be required to assess the effects (if any) of different farm management practices.

Whilst the variance estimates contributed by the stallion were small, there were significant differences amongst stallions in their reproductive performance (Hanlon et al 2012). Assessment of the significance of the random effect term for stallion included in the multivariable models also revealed that stallion was a significant variable. Stallions with known inherent subfertility need to be managed appropriately to maximise their reproductive performance. Stallion breeding management has been shown to affect reproductive success in the Northern Hemisphere (Blanchard et al 2010b), however there have been no studies that have investigated the breeding management of stallions in New Zealand.

In conclusion, the reproductive performance of Thoroughbred mares in the Waikato region of New Zealand is influenced by two main mare-related factors; the age of the mare and her reproductive status (dry or foaling). Reproductive performance declines with advancing age regardless of reproductive status. Dry mares served early in the season have the highest reproductive performance. Strategies that ensure dry mares are cycling at the start of the season, such as artificial light exposure, maximise reproductive performance. Foaling mares served on foal heat and mares that foal early in the breeding season have the highest reproductive performance. The majority of the variation in reproductive performance is associated with mare-level factors and the contribution of the stallion and stud farm is relatively minor. Management strategies to maximise reproductive performance on New Zealand stud farms should focus on mare-level practices.

Acknowledgements

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Table 4.1: Description of mare-level exposure variables considered as potential "risk" factors for first cycle pregnancy rate, end-of-season pregnancy rate and the start of mating to conception interval for 2007 Thoroughbred mares in the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008).

Variable	**Description**	**Levels or unit**
All mares	Age	years
	Status	dry, foaling
	Serving stallion	n = 87 stallions
	Stud farm of origin	n = 5 stud farms
	Year of study	2006, 2007, 2008
	Service date	calendar date of each service
Dry mares	Status	barren, maiden
	Artificial light exposure	yes, no
	Days to 1st Service	days from 1st Sept. to 1st service
Foaling mares	Foaling date	calendar date mare foaled
	Foal heat service	yes, no

Table 4.2: Significant fixed-effects variables retained in generalized linear mixed models (with random effects included for stallion and stud farm) of the two dependent variables, first cycle pregnancy rate and end-of-season pregnancy rate based on 2007 Thoroughbred mares from the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008). The three models relate to “All mares”, “Dry mares” and “Foaling mares” separately.

Variables	**First cycle pregnancy rate**			**End-of-season pregnancy rate**		
	Coefficient (SE)	OR (95% CI)	P-value	Coefficient (SE)	OR (95% CI)	P-value
All mares:						
Age (years)	-0.057 (0.012)	0.94 (0.92-0.96)	< 0.001	-0.099 (0.015)	0.91 (0.88-0.93)	< 0.001
Status = dry	Ref	1.00		Ref	1.00	
Status = foaling	-0.205 (0.095)	0.81[a] (0.67-0.98)	0.03	-0.691 (0.144)	0.50 (0.38-0.66)	< 0.001
Dry mares:						
Age (years)	-0.067 (0.016)	0.93 (0.91-0.97)	< 0.001	-0.088 (0.023)	0.92 (0.87-0.96)	< 0.001
Light exposure = no	Ref	1.00		Ref	1.00	
Light exposure = yes	1.093 (0.199)	2.98 (2.02-4.40)	< 0.001	0.897 (0.389)	2.45 (1.14-5.27)	0.02
Days to first service	0.018 (0.004)	1.02 (1.01-1.03)	< 0.001	-0.012 (0.005)	0.97 (0.98-0.99)	0.02
Foaling mares:						
Age (years)	-0.049 (0.017)	0.95 (0.92-0.98)	0.003	-0.069 (0.022)	0.93 (0.89-0.97)	< 0.01
Foal heat service = no				Ref	1.00	
Foal heat service = yes	ns			0.714 (0.195)	2.04 (1.39-2.99)	< 0.001
Foaling date	ns			-0.024 (0.003)	0.97 (0.97-0.98)	< 0.001

ns = not significant in the model. Note - Variance components for random effects not shown

[a] Interpretation: Foaling mares were 0.81 times less likely to conceive to their first service than dry mares.

Table 4.3: Significant risk factors retained in Cox proportional hazards models for the start of mating to conception interval (with frailty terms for stud and stallion included in each model to account for clustering) based on 2007 Thoroughbred mares in the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008). The three models relate to "All mares", "Dry mares" and "Foaling mares" respectively.

Variable	Category	Hazard	95% CI	P-value
All mares:				
Age (years)	≤ 8	Ref		
	9 to 13	0.95	0.87 – 1.08	0.39
	≥ 14	0.67[a]	0.60 – 0.80	< 0.0001
Status	Dry	Ref		
	Foaling	0.89	0.78 – 0.95	0.02
Dry mares:				
Age (years)	≤ 8	Ref		
	9 to 13	1.01	0.84 – 1.17	0.89
	≥ 14	0.59	0.48 – 0.72	< 0.0001
Artificial light exposure	No	Ref		
	Yes	1.60	1.29 – 1.89	< 0.0001
Days to first service[b]		0.98	0.98 – 0.99	< 0.0001
Foaling mares				
Age (years)	≤ 8	Ref		
	9 to 13	0.91	0.80 – 1.05	0.23
	≥ 14	0.68	0.55 – 0.83	< 0.0001
Foal heat service	No	Ref		
	Yes	1.78	1.54 – 2.04	< 0.0001
Foaling date	≤ 11th Sept.	Ref		
	12th Sept. to 2nd Oct.	0.88	0.74 – 1.05	0.65
	3rd Oct. to 21st Oct.	0.92	0.77 – 1.09	0.42
	≥ 22nd Oct.	0.76	0.64 – 0.92	0.002

[a] Interpretation: Compared to the reference category (mares ≤ 8 years of age), mares ≥ 14 years of age had 0.67 (95% CI: 0.60 – 0.80) times the daily hazard of conception over the breeding season.

[b] Days from 1st September to first service

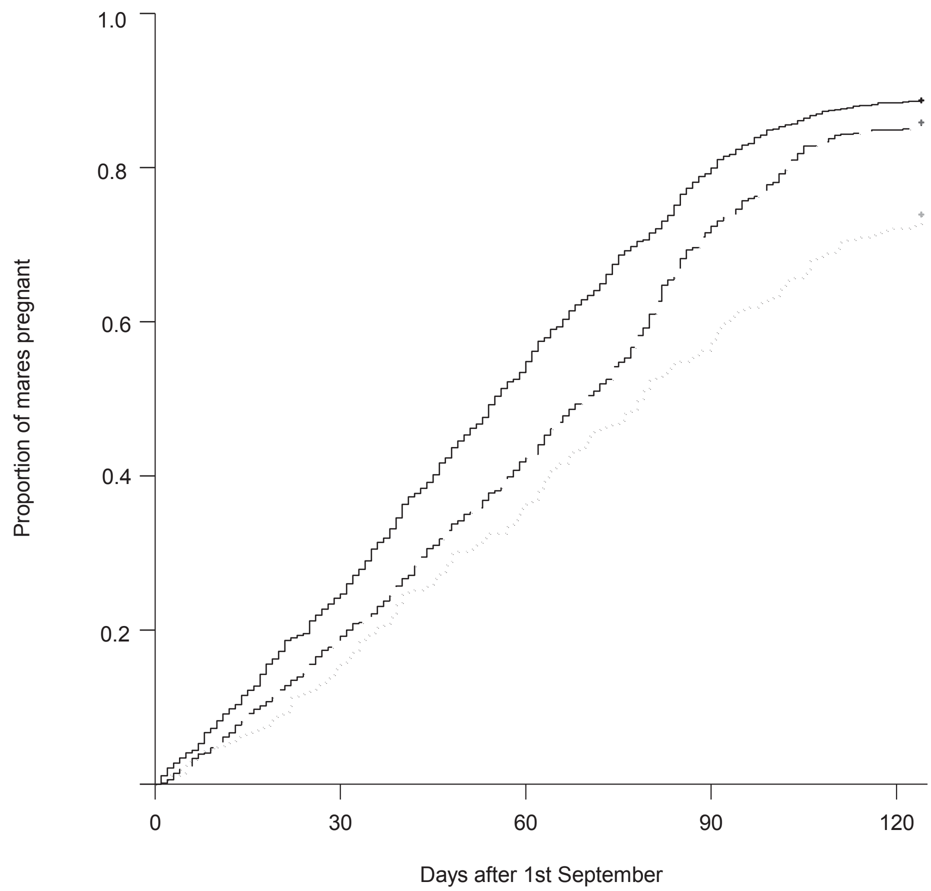

Figure 4.1: Kaplan-Meier survival curves, stratified by age, for the cumulative proportion of mares conceiving after the 1st September. Data represents 2007 Thoroughbred mares from five stud farms in the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008). The three different age categories are; 3 – 8 years of age (solid line), 9 – 13 years of age (dashed line) and ≥ 14 years of age (dotted line).

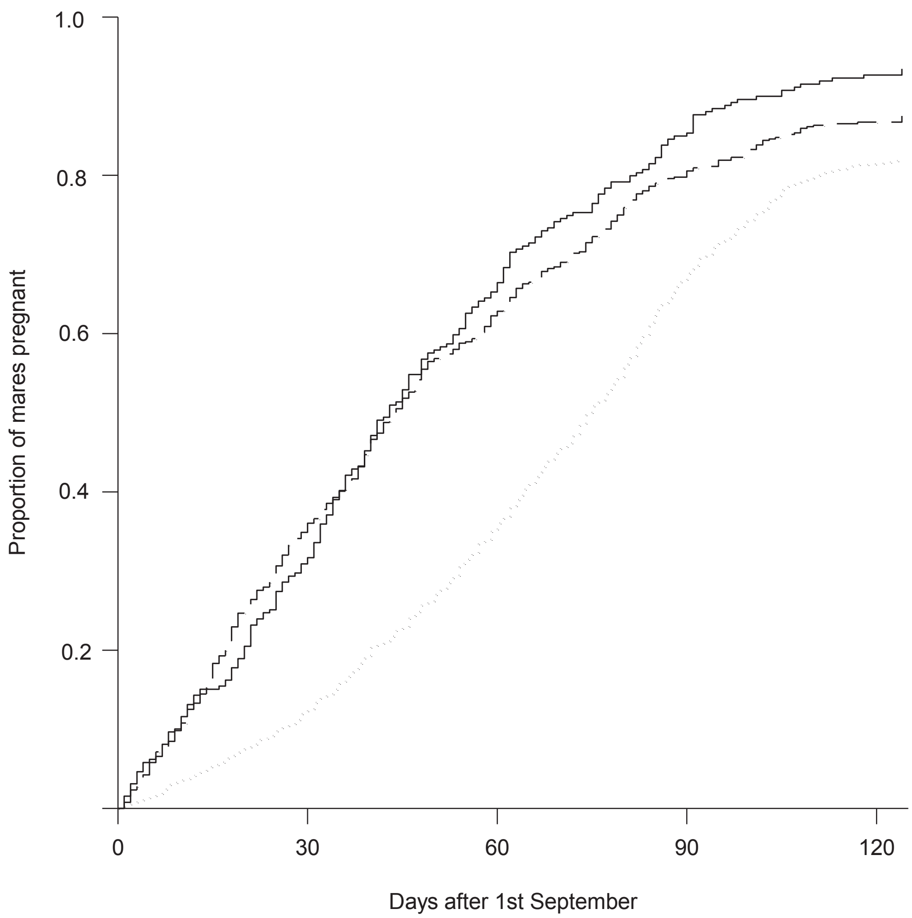

Figure 4.2: Kaplan-Meier survival curves, stratified by reproductive status, for the cumulative proportion of mares conceiving after the 1st September. Data represents 2007 Thoroughbred mares from five stud farms in the Waikato region of New Zealand over three consecutive breeding seasons (2006-2008). Reproductive status categorized as; maiden (solid line), barren (dashed line) and foaling (dotted line).

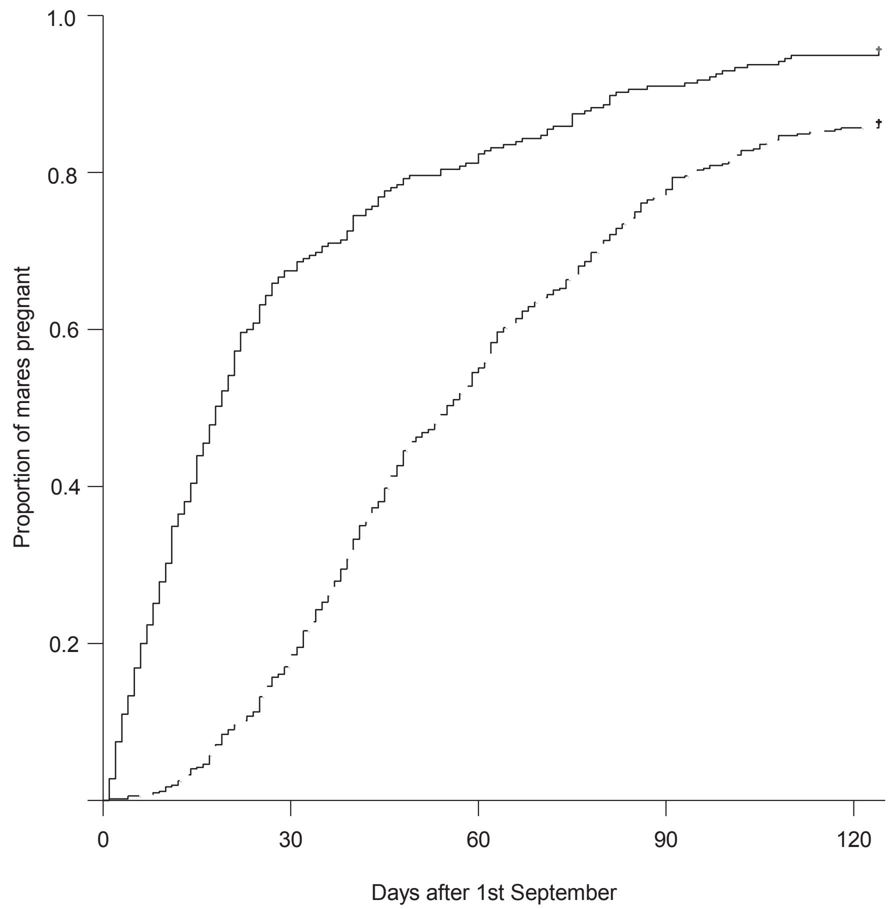

Figure 4.3: Kaplan-Meier survival curves for the cumulative proportion of mares conceiving after the 1st September for 806 dry Thoroughbred mares from five stud farms in the Waikato region of New Zealand either exposed to an artificial lighting programme prior to the start of the breeding season (n = 283, solid line) or not exposed to artificial light (n = 523, dashed line) over three consecutive breeding seasons (2006-2008).

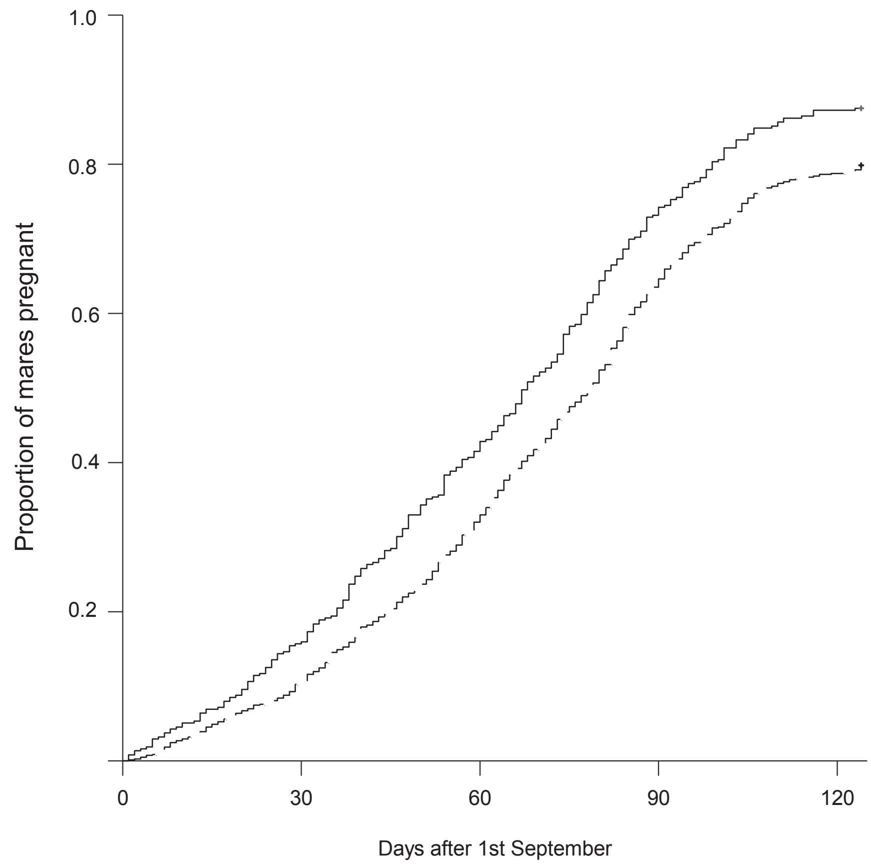

Figure 4.4: Kaplan-Meier survival curves for the cumulative proportion of mares conceiving after the 1st September for 1201 Thoroughbred mares from five stud farms in the Waikato region of New Zealand either served on foal-heat (n = 384, solid line) or not served on foal-heat (n = 817, dashed line) over three consecutive breeding seasons (2006-2008).

Chapter 5: The effects of intra-vaginal progesterone on follicular dynamics and FSH, LH and progesterone concentrations in transitional mares.

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Abstract:

The aims of this study were to investigate the effects of intra-vaginal progesterone administration on follicular development and FSH, LH and progesterone profiles in transitional mares. Ten non-pregnant Thoroughbred mares were treated with an intra-vaginal progesterone-releasing device (Cue-Mare®) for 10 days. Daily ultrasound examinations and blood sampling was performed from day -1 to day 14 (day 0 = day of device insertion). The mean diameter of the largest follicle on the day of insertion was 21.3 ± 0.3 mm. The diameter of the largest follicle increased significantly on day 6 and follicle growth continued thereafter so that at the time of device removal the mean diameter of the largest follicle was 37.2 ± 1.1 mm. All mares ovulated within 48 hours of administration of 1667 iu hCG given when a follicle > 35mm was detected after device removal. The mean time from removal to ovulation was 3.3 ± 0.2 days. Progesterone concentrations during treatment peaked at 3.3 ± 0.7 ng/ml the day after device insertion and remained above 1 ng/ml for the entire treatment period. FSH concentrations peaked (9.4 ± 0.8 ng/ml) 3 days before the first significant increase in follicle diameter, before declining during the period from day 5 to 9. LH concentrations were significantly elevated on days 6 and 7 corresponding to the period of increased growth of the dominant follicle. Follicle development in transitional mares resulting from intra-vaginal progesterone treatment appears to be mediated through a close temporal association between progesterone, FSH and LH during treatment.

Key words: mare, progesterone, transition, Cue-Mare, ovulation

1. Introduction

The imposed start of the Thoroughbred breeding season in both the northern and southern hemispheres commences at a time when the majority of non-pregnant mares are still in the transition phase from winter anestrus to normal, regular estrus cycles

(Ginther 1992). The transition period is associated with erratic estrous behavior, the growth and regression of ovarian follicles which fail to ovulate, and lasts between 60 and 80 days (Nagy et al 2000; Donadeu and Watson 2007). A variety of methods have been utilized in an attempt to advance the onset of normal estrous cycles in transitional mares. The most effective method for shortening the transition period is through the use of artificial light exposure commencing several weeks prior to the start of the breeding season (Sharp and Ginther 1975; Palmer et al 1982; Nagy et al 2000). Unfortunately, controlled light exposure is labour intensive, and as a result, only 40% of Thoroughbred stud farms in a recent New Zealand survey used artificial lighting in an attempt to advance the transition period (Rogers et al 2007). Pharmacological methods used to reduce the duration of the transition period include; a) GnRH or its analogues (Evans and Irvine 1977; Hyland and Jeffcott 1988; McKinnon et al 1997), b) oral progestagens (Webel and Squires 1982; Wiepz et al 1988), c) progesterone administered parenterally (Alexander and Irvine 1991), d) eFSH administration (Niswender et al 2004; Raz et al 2009), and f) dopamine antagonists such as domperidone (Besognet et al 1997; McCue et al 1999). None of these methods have been shown to reliably advance the transition phase compared to untreated control mares, and many of them have been shown to only be effective when used in combination with artificial light exposure (Nagy et al 2000). In addition, all of these methods involve the daily administration of the treatment.

The off-label use in mares of progesterone-containing intravaginal devices designed for cattle (CIDR, PRID) has been described (Foglia et al 1999; Klug and Jochle 2001; Newcombe 2002; Newcombe et al 2002). The intravaginal route offers a relatively economic and convenient method of administering progesterone to mares that avoids the need for daily treatment. Generally, these devices have not gained widespread acceptance because clinically they are associated with discomfort and marked vaginitis (Grimmett 1992; Newcombe 2002). However, it has been shown that these devices are effective in stimulating follicle growth in transitional mares (Foglia et al 1999; Klug and Jochle 2001; Newcombe 2002; Newcombe et al 2002). Similarly, we recently showed that treatment with Cue-Mare®, an intravaginal progesterone-releasing device specifically designed for use in mares, for 10 days was effective in advancing the transition period compared to untreated controls (Hanlon 2007). The mechanisms whereby intravaginal progesterone stimulates follicular growth in transitional mares are not fully understood but may be mediated through alterations in steroid hormone concentrations during the treatment period (Heijltjes et al 2006).

The aims of this study were to investigate the effects of intra-vaginal progesterone on follicular development and FSH, LH and progesterone profiles in transitional mares.

2. Materials and methods

2.1. Animals

Ten non-lactating Thoroughbred mares aged between 5 and 13 years were used for this study. All mares remained outside on pasture (consisting of predominantly perennial ryegrass) under natural light for the duration of the study and had ad lib access to water and hay. All mares were based on the same stud farm and remained together in the same group for the duration of the study. The study location was the Waikato region of New Zealand (latitude 37°49'S). Mares were enrolled for treatment on the 9th September. The official Thoroughbred breeding season commences on the 1st September in the Southern Hemisphere. Animal ethics approval for this study was granted by the Massey University Animal Ethics Committee (Protocol 07/57).

2.2. Mare selection

The ten mares used for this study were selected from a group of 27 non-pregnant mares that were resident on the study farm. Mares were selected for the study if they had no abnormalities of the reproductive tract as determined by palpation and ultrasound examination, had been exhibiting estrous behavior continuously for at least 10 days prior to treatment and the diameter of the largest follicle at any examination during this period was between 20 and 25 mm. Selection of mares based on follicle size and estrous behavior was performed to ensure that they were in the transitional phase of the anovulatory period (Donadeu and Watson 2007).

2.3. Intra-vaginal progesterone treatment

Each mare was treated with an intra-vaginal progesterone releasing device (Cue-Mare®, Bioniche Animal Health Australasia, Victoria, Australia) for 10 days. The device consists of two main components: i) the carrier body or "wishbone" which is used to hold the treatments inside the vagina, and ii) two "pods" which are attached to the arms of the wishbone and contain progesterone (1.72g, 10% w/w) which is formulated into a soft, silicone matrix (Grimmett et al 2002).

2.4. Ultrasound examination

Mares were examined by transrectal ultrasound using a 5MHz probe (Aloka SSD 500V) every second day from day -10 until day 0 (Day 0 = day of device insertion). They were then examined daily from day 0 to day 14 (Day 10 = day of device removal). All examinations were performed at the same time each day (0800 hours). Each ultrasound examination session was recorded using a portable digital media player (Archos 404) for later analysis. The diameter of all follicles greater than 15mm in diameter was measured at each examination and follicle diameter was determined from the average of the height and width of the maximum cross-sectional area of a single frozen image on the ultrasound screen (Gastal et al 1997). Day-to-day identity of individual follicles was not performed. This technique has been shown to be a reliable method for tracking follicular development in mares (Ginther and Bergfelt 1992a; Donadeu and Ginther 2002; Ginther et al 2003a).

2.5. Estrous behavior

Estrous behavior was determined by exposing each mare to a pony stallion on a daily basis starting from day -10 and continuing until day 14.

2.6. Breeding

After device removal, daily ultrasound examinations continued and each mare was exposed to a pony stallion to determine estrous behavior. Human chorionic gonadotrophin (hCG, 1667 iu IV, Chorulon®, Intervet Australia Pty Ltd, Victoria, Australia) was administered when a follicle > 35 mm was detected in conjunction with estrous behavior. Each mare was served by natural service 24-36 hours after hCG administration to one of eight different Thoroughbred stallions. All stallions were of proven fertility. Pregnancy was determined 14 days after service by transrectal ultrasound examination.

2.7. Blood sampling and hormone assays

A jugular blood sample was collected daily into EDTA tubes and centrifuged at 1600 rpm for 10 minutes. Plasma was stored at –20 degrees Celsius. LH was measured by radioimmunoassay using anti-equine LH antiserum (AFP-240580), equine LH as standards (AFP-5130A), and radioactively-labelled equine LH (AFP-5130A) prepared using ^{125}I and iodogen coated vials with recovery of the ^{125}I-eLH by elution from a PD 10 column (Sephadex G25, Sigma Chemical Co, Mo, USA). Separation used a mix of anti-rabbit gamma globulin (in house, prepared in sheep) and 9% poly ethylene glycol solution. Inter-assay coefficients of variation (CV) were 8.1%, 8.1% and 10.1% at 7.34, 3.59 and 1.37ng/ml LH, respectively. Intra-assay CVs were 5.6%, 5.4% and 7.9% near 14, 4 and 1 ng/ml, respectively, and the mean ± SEM detection limit (DL) was 0.37 ± 0.05 ng/ml. Equine FSH was measured by radioimmunoassay as previously described (Evans et al., 2002), using radioactively-labelled equine FSH (Papkoff e265B), anti-equine FSH antiserum (AFP1391675), and equine standards (Papkoff e265B). The inter-assay CVs were 7.1% and 10.3% at 9.45 and 5.41ng/ml, respectively. The intra-assay CVs were 7.9%, 5.4% and 11.7% near 10, 4 and 1.5ng/ml, respectively and the DL was 0.74 ± 0.08ng/ml. Progesterone was measured by commercial double antibody radioimmunoassay (DSL-3400, DSL Australia Pty Ltd, NSW, Australia). The inter-assay CV was 6.8 and 4.7% at 1.1 and 10ng/ml, respectively, and the DL was 0.12ng/ml.

2.8. Statistical analyses

Data were analyzed by repeated measures ANOVA to account for the within-mare and between-mare effects of day on hormone profiles and follicle growth. For nonparametric data, analysis was performed by Friedman repeated measures analysis on ranks. All post-hoc pair-wise comparisons were performed using Tukey's test. Significance was considered at $P<0.05$. Data are presented as mean ± SE. Analyses were performed using SigmaPlot Version 11.0 (2008, SPSS Software Inc, Chicago, IL, USA) and R Version 2.9.0 (2009, http://www.r-project.org).

3. Results

3.1. Follicle development

The mean diameter of the largest follicle on the day of device insertion was 21.3 ± 0.3 mm. During the 10 day period prior to device insertion the mean diameter of the largest follicle was 20.7 ± 0.3 mm (range 19 to 23 mm). The first significant increase in the diameter of the largest follicle when compared to the previous day occurred on day 6 (26.2 ± 0.4 mm vs 22.5 ± 0.5 mm for day 6 vs day 5 respectively, $P<0.05$; Fig. 5.1). The average growth rate of the largest follicle from day 6 to device removal was 2.8 mm/day. At device removal the mean diameter of the largest follicle was 37.2 ± 1.1 mm. Three mares developed two dominant follicles each; all remaining mares developed a single dominant follicle. On the day after device removal (day 11), 7 mares had a follicle > 35mm in diameter and were administered hCG. The 3 remaining mares had follicles > 35mm on day 12 and were then given hCG. All dominant follicles ovulated within 48 hours of hCG administration, therefore 7 mares had ovulated by day 13 and the remaining 3 mares had ovulated by day 14. The mean time to ovulation after device removal was 3.3 ± 0.2 days.

3.2. Estrus behavior and breeding

Prior to treatment, all mares had been exhibiting estrous behavior for at least 10 continuous days. In all mares, estrous behavior ceased the day after device insertion and resumed the day after device removal. All ten mares were served naturally 24-36 hours after hCG administration. Five of the 10 mares conceived to this service.

3.3. Progesterone

None of the intra-vaginal devices were lost during the treatment period. Progesterone concentrations peaked at 3.3 ± 0.7 ng/ml the day after device insertion and remained there for 24 hours (Fig. 5.2). There was no significant difference in mean progesterone concentrations between days 1 and 2. Concentrations declined from day 2, with the first significant reduction in progesterone levels below peak concentrations occurring on day 7 (3.3 ± 0.7 ng/ml vs 1.7 ± 0.1 ng/ml for day 1 vs day 7 respectively; $P<0.05$; Fig. 5.2). After device removal, concentrations significantly declined so that they were similar to pre-treatment levels on day 11. Progesterone concentrations began to rise from day 12 onwards, corresponding to ovulation of dominant follicles.

3.4. FSH

Plasma FSH concentrations declined significantly the day after device insertion (8.6 ± 1.1 ng/ml vs 7.2 ± 1.3 ng/ml for day 0 and day 1 respectively; $P<0.05$; Fig. 5.2). They then increased to plateau on days 3, 4 and 5 (peak concentration of 9.4 ± 0.8 ng/ml on day 3) before declining during the period from day 5 to 9. There was a gradual rise in FSH concentrations between day 9 and 14.

3.5. LH

LH concentrations tended to decrease the day after device insertion (1.1 ± 0.2 ng/ml vs 0.8 ± 0.1 ng/ml for day 0 and day 1 respectively; $P=0.07$; Fig. 5.2). During the 10 day period of device insertion, LH concentrations were significantly elevated compared to day 1 levels on days 6 and 7. After device removal, concentrations were significantly increased to above pre-treatment levels on all days.

4. Discussion

In this study, treatment of transitional Thoroughbred mares with an intra-vaginal progesterone-releasing device for 10 days resulted in follicle growth and ovulation within 4 days of device removal in all mares. The efficacy of progesterone delivered intra-vaginally in stimulating follicle growth has been reported previously (Foglia et al 1999; Klug and Jochle 2001; Newcombe 2002; Newcombe et al 2002; Handler et al 2006; Hanlon 2007). However, in comparison to previous studies using devices designed for cattle (CIDR, PRID (Foglia et al 1999; Newcombe 2002; Newcombe et al 2002; Handler et al 2006)) we found that the follicle growth rate was higher when using a device specifically designed for mares. In this study, follicles developed from 21.3 ± 0.3 mm at the time of device insertion to 37.2 ± 1.1 mm at the time of removal, a growth rate of 1.6 mm/day. Using CIDRs, Foglia et al (Foglia et al 1999) reported a growth rate of 1.04 mm/day and Newcombe et al (Newcombe et al 2002) reported a growth rate of 0.52 mm/day. Handler et al (Handler et al 2007) reported a growth rate of 0.4 mm/day when PRIDs were used in transitional mares. The slower growth rate in mares treated with cattle devices explains why treatment periods of 11-12 days are necessary to produce a pre-ovulatory sized follicle by the end of treatment (Newcombe et al 2002). Direct comparisons between this study and previous studies using cattle devices cannot be made however as differences could also be due to mare selection, breed, seasonality, location and nutrition. The growth rate from the first significant increase in follicle diameter (day 6) until removal was 2.8 mm/day, which is similar to the growth rate of dominant follicles in normally cycling mares (Ginther et al 2008a).

The time from removal of devices to ovulation was 3.3 ± 0.2 days which is similar to some studies (Foglia et al 1999; Grimmett et al 2002) but was shorter than that reported in other studies (Newcombe 2002; Handler et al 2006). The time taken from device removal to ovulation is dependent on the size of the dominant follicle at the time of device removal (Newcombe 2002; Handler et al 2007), the larger the follicle at device removal, the shorter the interval to ovulation. In addition, the use of hCG or GnRH analogues to induce ovulation shortens the interval to ovulation (Newcombe et al 2002). Follicle size at device removal is also dependent on the size of the largest follicle at the time of insertion (Newcombe et al 2002), which is mainly influenced by season, ie. mares in deep anestrus are less likely to develop pre-ovulatory sized follicles in response to treatment than mares in the late transition phase (Newcombe et al 2002; Handler et al 2006). The mares used in our study were selected based on follicle size and estrous behavior to ensure they were in the transitional phase of the anovulatory period (Ginther 1992; Donadeu and Ginther 2002; Donadeu and Watson 2007).

The pregnancy rate to the induced ovulation in this study was 50% which is similar to the 52.9% previously reported by Grimmett et al following Cue-Mare treatment in transitional mares (Grimmett et al 2002).

Progesterone concentrations during treatment in the present study were similar to those previously reported (Grimmett et al 2002). Peak concentrations occurred 1 day after device insertion, remained there for 24 hours and then steadily declined for the rest of the treatment period. Concentrations remained above 1 ng/ml for the entire treatment period, the minimum level during normal diestrus (Nett et al 1976). Progesterone concentrations during Cue-Mare insertion appear to be lower than those reported during PRID (Handler et al 2006) or CIDR (Klug and Jochle 2001) treatment, despite Cue-Mare containing more progesterone than PRID (1.72g vs 1.55g w/w for Cue-Mare and PRID respectively) but not CIDR (1.9g w/w). Obviously there are differences in progesterone dissolution and absorption from these different devices. The higher progesterone concentrations during treatment with CIDR and PRID may explain the slower follicle growth rate when these devices are used compared to Cue-Mare. Follicle growth rates have been shown to be reduced by daily intra-muscular injection of progesterone in a dose-dependent manner (Alexander and Irvine 1991; Gastal et al 1999; Gastal et al 2000). Likewise, in field observations involving large numbers of mares, Newcombe et al (Newcombe 2002; Newcombe et al 2002) reported that during PRID treatment follicles regressed whereas during CIDR treatment follicle growth occurred. This would be expected given that PRID produces higher progesterone concentrations during treatment than CIDR.

Concentrations of FSH decreased within 1 day of device insertion, coinciding with peak progesterone concentrations. Peak FSH levels were observed on day 3 and remained at plateau levels for 3 days. These findings are in agreement with Heijltjes et al (Heijltjes et al 2006) who reported an elevation in FSH concentrations in transitional pony mares during the first 5 days of PRID treatment. The same findings have been reported when progesterone is administered by daily intra-muscular injection (Alexander and Irvine 1991; Gastal et al 1999; Bergfelt et al 2001). The relationship between the FSH profile and follicle growth in our study was similar to that previously reported in both transitional and cycling mares. The first significant increase in follicle diameter occurred on day 6, three days after the initial peak in FSH concentrations. This relationship is in agreement with the findings of Ginther et al (Ginther et al 2003a) who showed that deviation (defined as a distinctive change in growth rates among follicles of a wave (Gastal et al 1997; Ginther et al 2001) occurs 3 days after the FSH peak. It has also been shown that FSH levels decrease over a period of 3-5 days after deviation (Bergfelt and Ginther 1992a, 1993; Donadeu and Ginther 2002), which is what we observed in this study. In addition, follicle deviation has been shown to occur when the largest follicle is between 21-23 mm in diameter in normally cycling mares (Bergfelt et al 2001; Ginther et al 2001; Ginther et al 2003a). In our study, the mean diameter of the largest follicle on day 5 (the day before a significant increase in follicle diameter) was 22.5 ± 0.5 mm.

The temporal relationships between FSH concentrations and follicle growth that we observed during the first 6 days of progesterone treatment indicate that transitional mares have a pool of follicles capable of developing in response to elevations in FSH, a finding supported by the fact that administration of eFSH also results in follicle growth in transitional mares (Niswender et al 2004; Raz et al 2009). The decline in FSH concentrations observed from day 6 to 9 presumably occurred as a result of inhibin production by the dominant follicles (Bergfelt and Ginther 1993; Donadeu and Ginther 2001, 2002, 2004).

LH concentrations were significantly increased on days 6 and 7 during treatment. As outlined above, this period corresponded to the time of the first detectable increase in follicle diameter. It has been shown that a close temporal relationship exists between FSH, LH and follicle growth whereby once FSH levels decline, LH concentrations increase and are vital for continued growth of the dominant follicle (Ginther 1992; Gastal et al 1999; Bergfelt et al 2001). Absolute circulating progesterone concentrations have also been shown to affect LH concentrations with progesterone levels > 2ng/ml depressing LH concentrations (Ginther et al 2006b). In our study progesterone concentrations fell below 2 ng/ml on day 5 and therefore the “permissive” effect of decreasing progesterone may have also contributed to the significant rise in LH on days 6 and 7 supporting growth of the dominant follicle at this time. The short peak and rapid decline in progesterone concentrations following treatment with Cue-Mare may explain why follicle development is faster than with CIDR, PRID or injectable progesterone which all elevate progesterone for a longer period and always above 2 ng/ml. After device removal LH concentrations significantly increased which is in agreement with the findings of others after the cessation of progesterone treatment (Driancourt and Palmer 1982; Klug and Jochle 2001). However, in our study this rise was also attributable to the administration of hCG to induce ovulation which has been shown to elevate endogenous LH concentrations significantly (Evans et al 2006; Ginther et al 2009).

In conclusion, we have shown that intra-vaginal delivery of progesterone results in follicle growth in transitional mares. Follicle development occurs in close association with rising FSH concentrations during the initial treatment period resulting in a significant increase in the diameter of the largest follicle on day 6 of treatment. The combination of declining progesterone levels, falling FSH concentrations and growth of the dominant follicle results in an increase in LH concentrations on days 6 and 7 supporting continued growth of the dominant follicle. Within two days of device removal mares are in estrus and dominant follicles respond to hCG-induced ovulation.

5. Acknowledgements

Cue-Mare devices were kindly provided by Bioniche Animal Health Australasia Pty. Ltd.

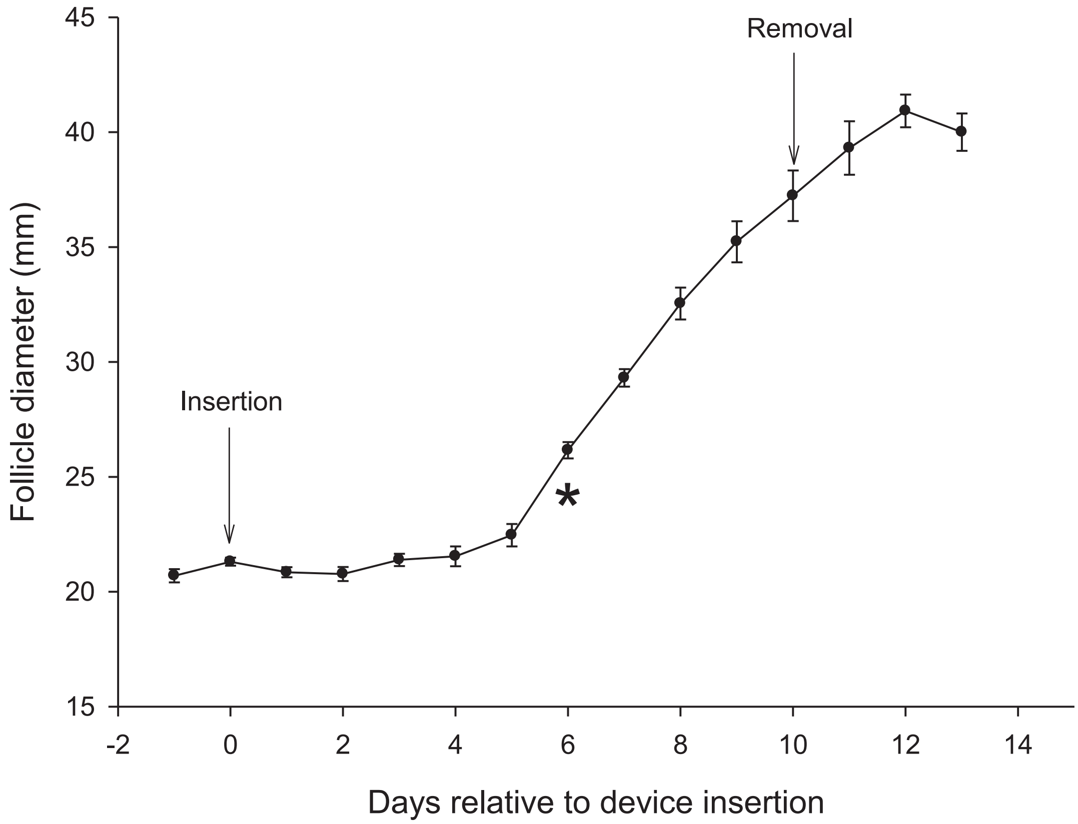

Figure 5.1: Follicle development (mm ± SE) in transitional mares (n=10) treated with an intra-vaginal progesterone-releasing device for 10 days and hCG after removal when a follicle > 35 mm was detected. * indicates the first significant (P<0.05) increase in follicle diameter when compared to the previous day.

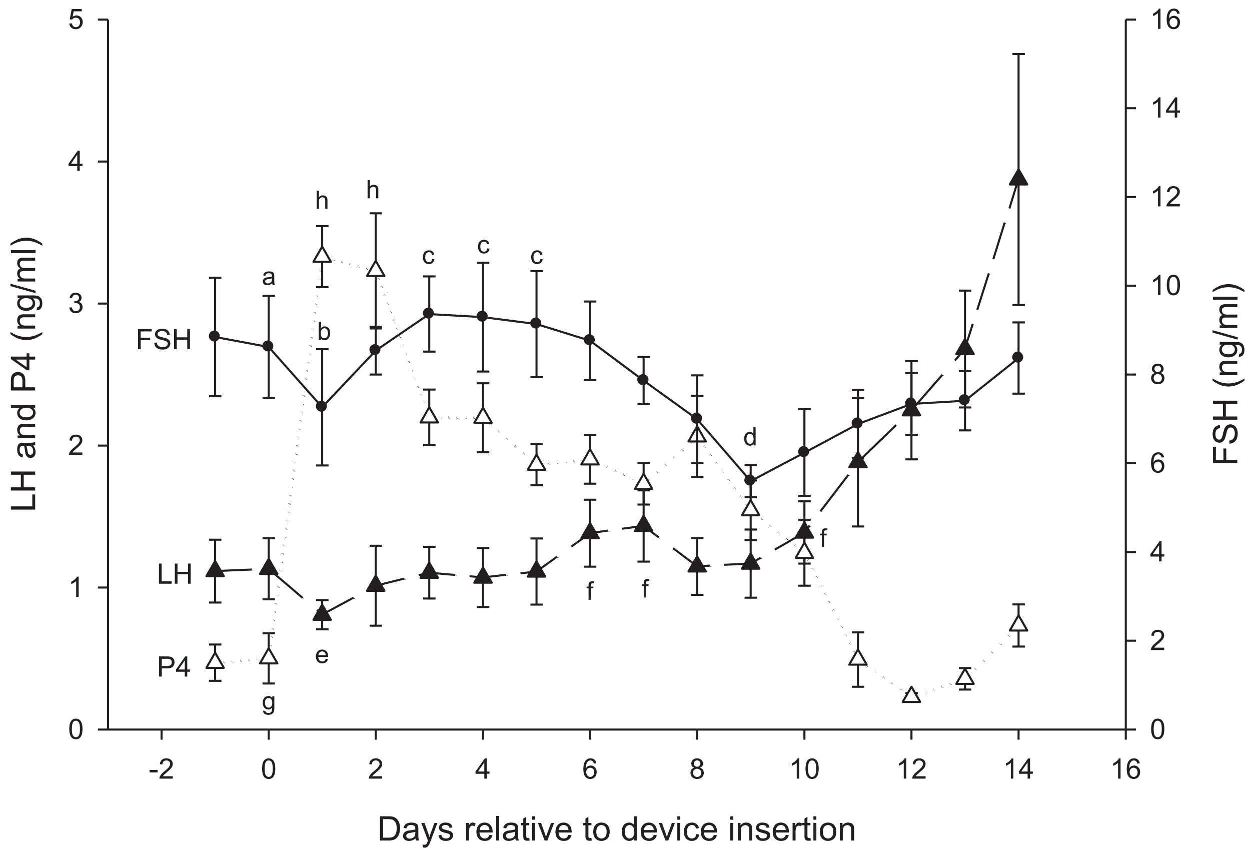

Figure 5.2: LH, FSH and progesterone profiles (mean ± SE) in transitional mares (n=10) treated with an intra-vaginal progesterone-releasing device for 10 days and hCG after removal when a follicle > 35 mm was detected. Means with different letters indicate differences (P<0.05) within FSH (abcd), LH (ef) and progesterone (gh) between days.

Chapter 6: The reproductive performance of Thoroughbred mares treated with intra-vaginal progesterone at the start of the breeding season.

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Abstract

The aim of this study was to investigate the effects of intra-vaginal progesterone on the reproductive performance of transitional Thoroughbred mares on commercial stud farms.

Two hundred and twenty seven (227) non-lactating transitional Thoroughbred mares aged between 4 and 18 y (mean 9.4 ± 3.2 y) located on three stud farms in the Waikato region of New Zealand were used in the study performed during four consecutive breeding seasons (2007-2010). Mares were age-matched in pairs and either treated with an intra-vaginal progesterone releasing device (Cue-Mare®, 1.72g progesterone, 10% w/w) for up to 10 d (Treated; n = 126) or left untreated (Control; n = 101). In both groups, human chorionic gonadotrophin (hCG, 1667 iu IV) was administered when a follicle ≥ 35 mm was detected in conjunction with estrous behavior and each mare was served by natural service.

Treated mares were served earlier in the breeding season (mean number of days to first service 13.9 ± 3.0 d vs 26.7 ± 13.2 d for Treated and Control groups respectively; $P < 0.001$). In the Treated group, 95.2% of mares were served within the first 21 d of the season compared to 42.6% of Control mares ($P < 0.001$). Treated mares conceived earlier in the breeding season (mean number of days to conception 37.5 ± 14.2 d vs 50.8 ± 21.3 d for Treated and Control groups respectively; $P = 0.01$). There was no difference between groups in the first service pregnancy rates (53.9% and 50.5% for Treated and Control mares respectively; $P = 0.89$).

Treatment with an intravaginal device increased the number of mares conceiving by the end of the breeding season (91.3 vs 82.3% for Treated and Control groups respectively; $P = 0.04$). This treatment protocol appears to offer a convenient, economical and reliable method for managing transitional mares on commercial Thoroughbred stud farms.

Key words: mare, progesterone, transition, Cue-Mare, ovulation

1. Introduction

The mare is seasonally polyestrous with regular ovulatory cycles occurring in response to increasing day length (Donadeu and Watson 2007). The Thoroughbred breeding season officially commences on 1st September in the southern hemisphere and the desire to breed mares as early as possible in the season means that most non-pregnant mares are still in the transition phase from winter anestrus to normal ovulatory estrous cycles (Ginther 1992). The transition period lasts between 60 and 80 days and is characterized by erratic estrous behavior and the growth and regression of ovarian follicles which fail to ovulate (Nagy et al 2000; Donadeu and Watson 2007).

A variety of methods have been utilized in an attempt to advance the onset of normal ovulatory estrous cycles in transitional mares. These methods include the use of artificial light exposure commencing several weeks prior to the start of the breeding season (Sharp and Ginther 1975; Palmer et al 1982; Nagy et al 2000) and pharmacological methods such as, a) GnRH or its analogues (Evans and Irvine 1977; Hyland and Jeffcott 1988; McKinnon et al 1997), b) oral progestagens (Webel and Squires 1982; Wiepz et al 1988), c) progesterone administered parenterally (Alexander and Irvine 1991), d) eFSH administration (Niswender et al 2004; Raz et al 2009), and f) dopamine antagonists such as domperidone or sulpiride (Besognet et al 1997; McCue et al 1999; Panzani et al 2011). The effectiveness of these methods varies considerably, and in addition, all involve at least once daily treatment administration.

The intravaginal route offers a relatively cheap and convenient method of administering progesterone to mares that avoids the need for daily treatment. Intravaginal devices designed for cattle (CIDR, PRID) have been used off-label in mares and are effective in stimulating follicle growth in transitional mares (Foglia et al 1999; Klug and Jochle 2001; Newcombe 2002; Newcombe et al 2002). Generally, cattle devices have not gained widespread acceptance because clinically they are associated with discomfort and marked vaginitis (Grimmett 1992; Newcombe 2002).We recently showed that treatment of transitional mares with an intravaginal progesterone-containing device specifically designed for mares (Cue-Mare®, Bioniche Animal Health Australasia, Victoria, Australia) for 10 days, resulted in follicle growth and ovulation within 4 days of device removal (Hanlon et al 2010), and that the devices were associated with minimal discomfort and vaginitis (Grimmett et al 2002). Such a treatment therefore offers a potential method of managing the transition period on commercial stud farms.

The aim of this study was to investigate the effects of intra-vaginal progesterone on the reproductive performance of transitional Thoroughbred mares on commercial stud farms.

2. Materials and methods

2.1. Animals

Two hundred and twenty seven (227) non-lactating Thoroughbred mares between 4 and 18 y of age (mean 9.4 ± 3.2 y) located on three stud farms were used. The study was performed during four consecutive breeding seasons (2007-2010). All mares remained outside on pasture (consisting of perennial ryegrass) under natural light for the duration of the study and had *ad lib* access to water and hay. The study location was the Waikato region of New Zealand (latitude 37°49'S). Mares were enrolled for treatment on or before the 6th September in each breeding season. The official Thoroughbred breeding season commences on the 1st September in the Southern Hemisphere. Approval for the study was granted by the Massey University Animal Ethics Committee.

2.2. Mare selection

On each stud farm, the reproductive tracts of all non-pregnant resident mares were examined by transrectal ultrasonography every second day between the 18th and 31st of August in each breeding season. Mares with a corpus luteum were deemed to be cycling and were excluded from the study. The remaining mares were included in the study if they had no abnormalities of the reproductive tract as determined by palpation and ultrasound examination, had been exhibiting estrous behavior continuously for at least 10 d prior to treatment and the diameter of the largest follicle at any examination during this period was 20 - 25mm. Selection of mares based on follicle size and estrous behavior was performed to ensure that they were in the transitional phase of the anovulatory period (Donadeu and Watson 2007). Treatment commenced between 1st and 6th September in each season. After each examination session mares were age-matched in pairs and assigned randomly to the treatment and control groups by tossing a coin. All mares remained with their original paddock-mates and were managed identically. In 2009, two of the participating stud farms did not allow an equal number of control mares to be left untreated. This increased the number of mares in the treatment group.

2.3. Treatments and examinations

In the Treatment group each mare was treated with an intra-vaginal progesterone releasing device (Cue-Mare®, Bioniche Animal Health Australasia, Victoria, Australia) for 7 or 10 d. The device consists of two main components: i) the carrier body or "wishbone" which is used to hold the treatments inside the vagina, and ii) two "pods" which are attached to the arms of the wishbone and contain progesterone (1.72g, 10% w/w) which is formulated into a soft, silicone matrix (Grimmett et al 2002).

All mares were examined by transrectal ultrasonography using a 5MHz probe (Aloka SSD 500V) every second day from day -10 until day 0 (day 0 = day of device insertion). Mares in the treatment group were examined again on day 7 and the intravaginal device removed if a follicle ≥ 35mm in diameter was detected. Mares with a follicle < 35mm on day 7 were re-examined on day 10. Mares in the control group were managed as per the usual routine on the stud farm, ie. mares were teased every second day and presented for ultrasound examination on a bi-weekly basis.

2.4. Breeding

After device removal in the treatment group, daily ultrasound examinations continued and each mare was exposed to a pony stallion to determine estrous behavior. Human chorionic gonadotrophin (hCG, 1667 iu IV, Chorulon®, Intervet Australia Pty

Ltd, Victoria, Australia) was administered when a follicle ≥ 35 mm was detected in conjunction with estrous behavior. Each mare was served by natural service 24-36 h after hCG administration to one of 9 different Thoroughbred stallions all of proven fertility. Each mare was examined by ultrasonography at 24 h intervals after service to determine the day of ovulation and at 14-16 d after service to determine pregnancy status. Pregnancy was re-confirmed at 28 and 42 d after service.

In control mares, ultrasound examinations were performed bi-weekly (every 3-4 d) commencing on day 0. Breeding management and pregnancy diagnosis was identical to the Treatment group. Control mares were served by the same stallions as the Treatment group mares.

Regardless of group allocation, all mares that failed to conceive to their first service were managed the same way for the remainder of the season. This involved repeated teasing, ultrasound examinations and breeding by natural service until the mare conceived or until the end of the breeding season. On all three stud farms, the breeding season ended on or before 24th December.

2.8. Statistical analyses

The specific measures of reproductive performance (dependent variables) of interest were: (i) interval from 1st September to first service, (ii) interval from 1st September to conception, (iii) first service pregnancy rate and, (iv) end-of-season pregnancy rate. The end-of-season pregnancy rate was defined as the proportion of mares in each group pregnant at ≥ 42 d of gestation at the end of the breeding season (Ginther 1992). Multiple logistic regression was used to determine the effects of the independent variables, namely, treatment, mare age, covering stallion, stud farm, and year on the first service and end-of-season pregnancy rates. Multiple linear regression was used to determine the effects of the independent variables; treatment, mare age, covering stallion, stud farm and year on days to first service and days to conception after 1st September. The cumulative proportion of mares in each group conceiving after the 1st September was calculated using the Kaplan-Meier method of survival analysis. Data are presented as mean ± SD. Analyses were performed using SigmaPlot Version 11.0 (2008, SPSS Software Inc, Chicago, IL, USA) and R Version 2.9.0 (2009, http://www.r-project.org). Significance was determined at $P < 0.05$.

3. Results

During the treatment period none of the intravaginal devices was expelled. Thirteen mares (10.3%) had the device removed on day 7 when a follicle ≥ 35mm in diameter was detected. The remaining mares (89.7%) had the device removed on day 10. Reproductive outcomes for mare age, farm and year of the study are presented in Table 6.1.

3.1. Time to first service and to conception

Mare age, covering stallion, stud farm and year of the study had no significant effects on the time to first service (Table 6.1). The only independent variable significantly associated with time to first service was treatment group. Mares treated with an intravaginal device were served significantly earlier in the breeding season (mean number of days to first service 13.9 ± 3.0 d vs 26.7 ± 13.2 d for treated and control groups respectively; $P < 0.001$; Table 6.2). In the treatment group, 95.2% of mares were served within the first 21 d of the season compared to 42.6% of control mares ($P < 0.001$; Table 6.2). Treatment duration reduced the time to first service with mares treated for 7 d being served 2.5 d earlier than mares treated for 10 d ($P=0.002$; Table 6.3).

Mare age, covering stallion, stud farm and year of the study had no significant effects on the time to conception (Table 6.1). However, mares treated with an intravaginal device conceived earlier in the breeding season (mean number of days to conception 37.5 ± 14.2 d vs 50.8 ± 21.3 d for treated and control groups respectively; $P = 0.01$; Table 6.2, Fig. 6.1).

3.2. First service pregnancy rate

None of the independent variables had a significant effect on the first service pregnancy rate. The first service pregnancy rates were 53.9% and 50.5% for treated and control mares respectively (P=0.89).

3.3. End-of-season pregnancy rate

Mare age, covering stallion, stud farm and year of the study had no significant effects on the end of season pregnancy rate (Table 6.1). However, treatment with an intravaginal device was associated with a higher number of mares conceiving by the end of the breeding season (91.3 vs 82.3% for treated and control groups respectively; P = 0.04; Table 6.2).

4. Discussion

In both the Northern and Southern hemispheres the Thoroughbred breeding season commences at a time when most non-pregnant mares are in the transition phase from winter anestrus to normal, regular estrus cycles. Early foals are desired because they are generally larger than their later-born peers by the time they are sold at yearling sales at around 14-17 months of age (Morel et al 2007). In addition, earlier born foals are more physically advanced than their counterparts when they start racing as two-year-olds (Smith et al 2006). For these reasons there is considerable pressure on stud managers and veterinarians to ensure that mares conceive as early as possible.

Progesterone and synthetic progestagens are often used to manage the transition period in mares (McCue et al 2007b). Delivery is either by daily injection, daily oral administration or from intravaginal devices. The intravaginal route offers a relatively economical and convenient method of administering progesterone to mares that avoids the need for daily treatment and/or injections (Newcombe 2002; Newcombe et al 2002; Hanlon 2007). We recently showed that treatment of transitional mares with an intravaginal progesterone-containing device for 10 d resulted in follicle growth and ovulation within 4 d of device removal (Hanlon et al 2010). Follicle development occurred in close association with rising FSH concentrations during the initial treatment period, with an increase in LH concentrations on days 6 and 7 supporting continued growth of the dominant follicle. Within two days of device removal mares were in estrus and dominant follicles responded to hCG by induced ovulation.

Treated mares were served significantly earlier in the breeding season compared to untreated control mares. On average, treated mares were served 12.8 d earlier than control mares. Treated mares also conceived 13.3 d earlier than control mares. Breeding mares as early as possible in the season has been shown to have a positive effect on the end of season pregnancy rate (Hearn 2000; Love 2003). This is because the earlier in the season a mare commences normal estrous cycles, the more breeding opportunities she has during that season. The duration of the breeding season in New Zealand and Australia is around 105 d (3.7 months), which is significantly shorter than that of the Thoroughbred breeding season in the Northern Hemisphere (around 5 months) (Bosh 2006). Therefore, it is of even greater importance that in the Southern Hemisphere mares are cycling as early as possible in the season.

The efficacy of progesterone delivered intra-vaginally in stimulating follicle growth has been reported previously (Foglia et al 1999; Klug and Jochle 2001; Newcombe 2002; Newcombe et al 2002; Handler et al 2006; Hanlon 2007; Hanlon et al 2010) although there have been conflicting reports on the efficacy of progesterone or progestagens in advancing the first ovulation of the year. Some studies have shown a positive effect (Squires et al 1979b) and others have not (Allen et al 1980; Alexander and Irvine 1991). The results of our study clearly show that the intravaginal device used (Cue-Mare) advanced the first ovulation of the season in treated mares. We have previously shown that follicle growth during Cue-Mare treatment is mediated through changes in FSH and LH concentrations during the treatment period (Hanlon et al 2010). The reported variation in efficacy of progesterone delivered intravaginally is most likely due to the stage of transition during which treatment commenced: Progesterone and progestagen therapy does not reliably advance the first ovulation of the year when treatment commences in anestrus or early transition (Squires et al 1979b; Allen et al 1980; Turner et al 1981; Newcombe 2002; Newcombe et al 2002; Handler et al 2006, 2007), but in late transition, treatment is more effective (Squires et al 1979b; Allen et al 1980; Turner et al 1981; Alexander and Irvine 1991; Newcombe 2002; Newcombe et al 2002; Handler et al 2006, 2007). In the present study, mares were selected only if they had follicles 20-25mm diameter at the start of treatment, to ensure that the enrolled mares were truly transitional and not in anestrus (Ginther 1992; Donadeu and Watson 2007). There was no significant difference between the first service pregnancy rates of treated and control mares. This agrees with the findings of Cuervo-Arango and Clark (2010) who found that transitional mares treated with an intravaginal device designed for cattle (CIDR-B) had similar pregnancy rates to untreated mares served at the first ovulation of the year (Cuervo-Arango and Clark 2010).

Treatment with intravaginal progesterone significantly increased the number of mares that were confirmed as being pregnant by the end of the breeding season. The advancement of the first service date for treated mares effectively extended the breeding season of treated mares compared to control mares by 12.8 d. In the first 21 d of the season, 95.2% of treated mares were served compared to 42.6% of control mares. An improvement in the overall number of mares conceiving obviously has significant economic advantages to mare owners.

Treatment commenced on or before the 6th September each year. Given the study design, this meant that mares were not served until the 9th September at the earliest. Examining mares and starting treatment even earlier, so that intravaginal devices are removed in the days just prior to 1st September is likely to produce an even greater improvement in the reproductive performance of treated mares. Further studies are required to confirm this.

In conclusion, intravaginal treatment with a progesterone-releasing device was effective in inducing estrus and ovulation in transitional Thoroughbred mares. Compared to control mares, treated mares were served earlier and conceived sooner after the start of the breeding season. By the end of the breeding season, significantly more treated mares were in foal compared to untreated mares. This treatment protocol appears to offer a convenient, economical and reliable method for managing transitional mares on commercial stud farms.

5. Acknowledgements

Cue-Mare devices were kindly provided by Bioniche Animal Health Australasia Pty. Ltd. The participation of the mare owners and stud managers and their staff is gratefully acknowledged. Funding support for this project was provided by the New Zealand Racing Board through the Equine Partnership for Excellence.

Variable	n	Days to first service (mean)	Days to conception (mean)	First service pregnancy rate (%)	End of season pregnancy rate (%)
Stud					
1	30	20.5	40.4	60.0	86.7
2	94	19.0	41.9	52.1	88.0
3	103	19.5	44.9	47.6	84.5
Year					
2007	38	21.2	49.9	50.0	86.8
2008	69	19.6	45.3	52.2	85.5
2009	68	17.5	41.7	50.0	86.7
2010	52	20.5	37.8	51.9	86.5
Mare Age (years)					
4-8	86	19.4	40.4	57.0	86.0
9-13	126	19.9	43.4	48.4	84.9
14-18	15	15.2	53.2	40.0	100
Stallion (N=9)					
range	12 - 55	19.1 - 21.6	39.9 - 41.4	42.0 - 73.7	81.2 - 100

Table 6.1: Descriptive end-points for the independent variables; stud farm, year of study, age of mare and serving stallion, included in multiple logistic regression models (first service pregnancy rate and end of season pregnancy rate) and linear regression models (days to first service and days to conception). None of the independent variables were significant when included in multiple regression models that included the variable “Treatment” as an effect.

Treatment group	n	Age	Days to first service	Mares served in first 21d (%)	Days to conception	First service pregnancy rate (%)	End of season pregnancy rate (%)
Treated	126	9.4 ± 3.1	13.9 ± 3.0	95.2	37.5 ± 14.2	53.9	91.3
Control	101	9.4 ± 3.2	26.7 ± 13.2	42.6	50.8 ± 21.3	50.5	82.3
P value*			<0.001	<0.001	0.01	0.89	0.04

Table 6.2: Reproductive performance of transitional Thoroughbred mares either treated with an intravaginal progesterone-releasing device (Treated) or left untreated (Control) at the start of the breeding season. *P value of dependent variable.

Treatment duration	n	Days to first service	Days to conception	First service pregnancy rate (%)	End of season pregnancy rate (%)
7 d	13	11.4 ± 2.9	39.2 ± 12.5	53.8	84.6
10 d	113	13.9 ± 3.1	37.3 ± 11.9	53.9	92.0
P value*		0.002	0.87	1.0	0.37

Table 6.3: Reproductive performance of transitional Thoroughbred mares treated with an intravaginal progesterone-releasing device for 7 or 10 days at the start of the breeding season. *P value of dependent variable.

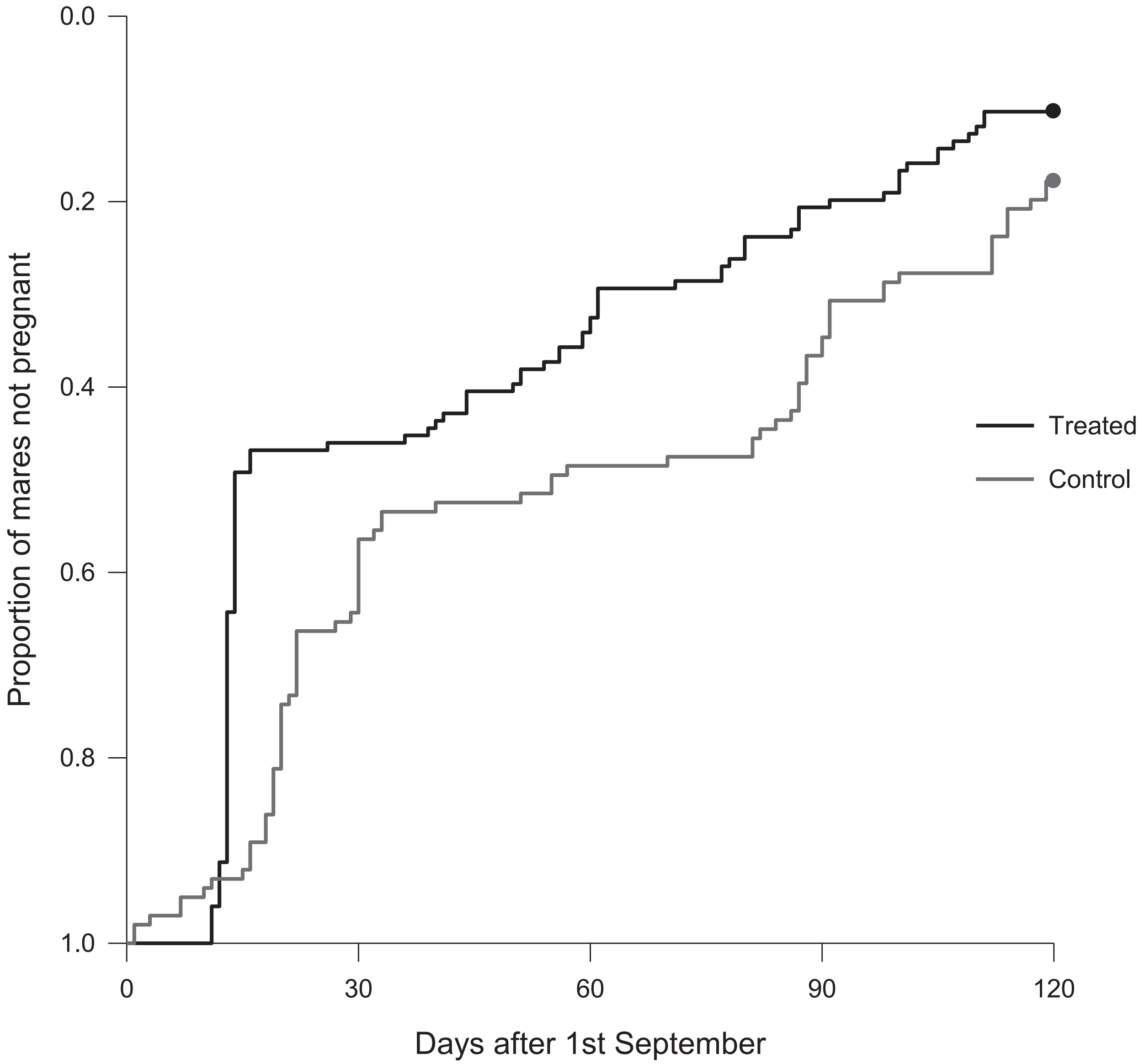

Figure 6.1: Kaplan-Meier survival curves for the cumulative proportion of mares conceiving after the 1st September for mares either treated with an intravaginal progesterone-releasing device (Treated) or left untreated (Control) at the start of the breeding season.

Chapter 7: A model to induce hemorrhagic anovulatory follicle (HAF) formation in non-pregnant mares using long term exposure to eCG

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Abstract

A common cause of infertility in several species is the development of anovulatory follicles. In the mare, the most commonly described anovulatory condition is the development of hemorrhagic anovulatory follicles (HAF). Progress in understanding the mechanisms leading to ovulation failure has been hampered by the lack of a suitable model. The aim of this study was to determine whether transplantation of equine chorionic girdle into non-pregnant recipient mares and the subsequent secretion of eCG by the trophoblast cells would modulate the ovarian dynamics of the mares. Segments of chorionic girdle from day 34 conceptuses were transplanted into the vulval submucosa of four non-pregnant recipient mares. Three control mares were transplanted with allantochorion and one control mare was transplanted with serum-free media only. Mares were examined by transrectal ultrasonography bi-weekly for up to 105 days after transplant and serum samples for eCG and P4 assay were collected at the same time. We observed the development of 18 HAFs during the 105 day observation in recipient mares. No HAF formation occurred in the control mares. The diameter of follicles that became HAFs was smaller (30.9 ± 1.3 mm) than the diameter of follicles that ovulated normally in control mares (41.2 ± 1.9 mm; $P<0.001$). eCG concentrations were elevated above pre-treatment levels in all recipient mares. In conjunction with HAF development, serum P4 levels were also elevated. We propose that HAF formation in this study was a result of the exposure of follicles to eCG produced by the trophoblast cells of the transplanted chorionic girdle. This method appears to offer a model for studying the HAF syndrome in mares and possibly luteinized unruptured follicle (LUF) syndrome, a similar condition, in women.

1. Introduction

Ovulation failure has been reported in horses (Ginther 1992), cattle (Peter 2004), dogs, (Meyers-Wallen 2007), camelids (Adams et al 1991) and humans (Qublan et al 2006). In the mare, the most commonly described anovulatory condition is the development of hemorrhagic anovulatory follicles (HAF), where the follicle fails to rupture and its cavity becomes filled with blood and fibrin strands (Ellenberger et al 2008). This condition has a reported incidence of 5-20% of all estrus cycles and is more common during the spring and autumn transition periods (Ginther et al 2007). Other risk factors reported include increasing mare age (McCue and Squires 2002; Ginther et al 2007; Cuervo-Arango and Newcombe 2008) and the use of exogenous prostaglandin treatment to induce luteolysis (Cuervo-Arango and Newcombe 2008; Ginther et al 2008b). Certain individuals apparently are predisposed to HAF development in repeated oestrous cycles (Cuervo-Arango and Newcombe 2008).

HAF formation in mares has significant economic impacts because it is associated with infertility due to failure of the follicles to evacuate the oocytes (Cuervo-Arango and Newcombe 2008). The ability to predict which dominant follicles will become HAFs is difficult because during ultrasound examination they appear to be morphologically similar or identical to normal pre-ovulatory follicles (Ginther et al 2007). Therefore much time and expense can be wasted on breeding mares during infertile oestrus cycles due to HAF formation. HAFs can also persist for several weeks resulting in a prolonged anoestrus period (McCue and Squires 2002).

The predisposing factors for HAF development in the mare are unclear. Several mechanisms have been proposed which include: a) insufficient gonadotrophic stimulation to induce ovulation (McKinnon 1993), b) reduced oestrogen secretion by the dominant follicle (McKinnon 1993), c) physical blockage of the ovulation fossa due to ovarian cysts (Kolling and Allen 2006), d) repeated use of ovulation-inducing hormones (Lefranc and Allen 2003), e) the use of prostaglandins to shorten the dioetrus period (Cuervo-Arango and Newcombe 2008) and f) changes in the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Riley et al 2001).

Due to their size and the ease with which their reproductive tract can be imaged ultrasonographically, mares may offer a suitable comparative model for the study of a similar anovulatory condition in women known as luteinized unruptured follicle syndrome (LUF) (Ginther et al 2008b; Mihm and Evans 2008). This syndrome is defined as a failure of ovulation with intra-follicular hemorrhage with luteinization (Qublan et al 2006). The morphology and functional properties of LUFs are very similar to HAFs in the mare (Liukkonen et al 1984; Zaidi et al 1995). A model which reliably induces HAF formation in the mare would enable study of this condition in mares, and also possibly serve as a model for LUF in women. Previous attempts to create a "HAF model" in mares have focused on exposing immature follicles to supraphysiological concentrations of LH through a combination of follicle ablation and prostaglandin treatment (Ginther et al 2008b). However, this model resulted in HAF formation in only 20% of follicular waves.

We have previously shown that chorionic girdle tissue, in either intact strips or as trophoblast vesicles formed after in vitro culture in serum-free medium can be successfully transplanted to ectopic sites such as the vulval submucosa in non-pregnant mares where the trophoblast cells differentiate (Adams and Antczak 2001; de Mestre et al 2006). Recently we demonstrated that these transplanted trophoblast cells not only survived in the submucosa for a similar length of time as the endometrial cups in the uterus during pregnancy, but they produced sufficient quantities of eCG to be detected in the serum of the recipient mares (de Mestre et al 2008). Equine CG has LH-like activity in the mare (Sherman et al 1992; Saint-Dizier et al 2004b).The aim of this study was to determine whether transplantation of equine chorionic girdle into non-pregnant recipient mares and the subsequent secretion of eCG by the trophoblast cells would modulate the ovarian dynamics of the mares.

2. Materials and methods

2.1. Animals

Eight non-lactating mares of various breeds aged 3-7 years were used as recipients for this study, and were maintained at the Baker Institute for Animal Health, Cornell University, USA. Pregnancies were established as previously described (Adams and

Antczak 2001). Animal care was performed in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of Cornell University. The results of the eCG and P4 assays for the recipient mares used in this study have also been reported as part of a related manuscript (de Mestre et al 2011) but not the results of the ovarian characteristics of the mares.

2.2. Transplantation of Chorionic girdle

Transplantation of chorionic girdle was performed during estrus in three recipient mares and during diestrus in one mare. Chorionic girdles of day 34 conceptuses were obtained non-surgically by established methods (de Mestre et al 2011). Girdles were dissected free of allantochorion and chorion and placed into serum-free medium containing Dulbecco's-Modified Eagle Medium (DMEM) enriched with 2 nM L-glutamine (Gibco, Carlsbad, CA), 0.5 µg/ml vitamin C, 0.4 µg/ml insulin (Sigma, St Louis, MO) and 100 U/ml Penicillin-streptomycin. Following dissection, the chorionic girdle was sectioned into three to four pieces of equal size, each approximately 3-4 cm in length. Recipient mares were sedated with Xylazine hydrochloride (Lloyd Laboratories, IO) 0.4 mg/kg and butorphanol tartrate (Fort Dodge, IO) 0.001 mg/kg given intravenously and the transplant site (vulva) was prepared aseptically. Sectioned chorionic girdle strips were suspended in 0.4 ml of serum-free medium and injected superficially into the vulval submucosa of the four recipient mares. Preparation and injection of an equivalent volume of allantochorion was performed in the same way. Three control mares were transplanted with allantochorion and one control mare was transplanted with media only.

2.3. Ovarian and uterine ultrasonography

Mares were examined bi-weekly by transrectal ultrasound using a 5MHz probe (Aloka SSD 500; Aloka America, Wallingford, CT, USA) commencing 7 days prior to chorionic girdle transplantation. In chorionic girdle transplant recipient mares, ultrasound examination continued until 105 days after transplant. In control mares, ultrasound examination continued until 35 days after transplantation. Each ultrasound examination session was recorded using a portable digital media player (Archos 404, www.archos.com) for later analysis. The relative location of follicles, corpora lutea, and HAFs were used as references for identifying and tracking follicles (Gastal et al 2000). Follicle, corpus luteum and HAF diameter was determined from the average of the height and width of the maximum cross-sectional area of a single frozen image on the ultrasound screen (Ginther 1995). The beginning of the formation of an HAF was considered equivalent to the day of ovulation and both ovulation and the beginning of an HAF were designated as Day 0 (Ginther et al 2006a). HAF regression was deemed to have occurred when the diameter of the HAF was less than 15mm.

2.4. Hormone assay

Blood samples were collected into serum tubes and centrifuged at 1600 rpm for 10 minutes and the separated serum stored at –20 degrees Celsius. The concentration of eCG in serum was determined using an enzyme linked immunoassay as previously described (Meadows 1995). The concentration of progesterone in serum samples was determined using a solid-phase radioimmunoassay (Reimers et al 1991).

2.5. Statistical analyses

Data for follicle end-points were compared using unpaired *t*-tests. Data are expressed as mean ± SEM. A P value < 0.05 was considered significant.

3. Results

In four mares transplanted with chorionic girdle, the development of 18 HAFs was recorded during the 105 day observation period. Descriptive data for each mare are presented in Table 7.1. HAF development was characterized by the appearance of echogenic “flecks” or strands within the follicle that increasingly filled the antrum, combined with hyperechoic thickening of the follicle wall (Fig. 7.1A and 7.1B). Seventeen of the 18 HAFs formed in a period between 10 and 60 days after chorionic girdle transplant (Fig. 7.2). One transplant mare (3883) did not produce any HAFs during the observation period but ovulated 4 small follicles (mean diameter 27.0 ± 2.1 mm) during the first 54 days of the observation period (Fig. 7.2). During the 35 day period in which they were examined by ultrasound, none of the 4 control mares produced a HAF but ovulated 9 individual dominant follicles.

In all mares transplanted with chorionic girdle, eCG concentrations remained above pre-transplant levels for the duration of the study period. Peak eCG levels were obtained, on average, 27.3 ± 2.3 days after chorionic girdle transplant with a mean peak eCG concentration of 15.9 ± 3.4 iu/ml (Fig. 7.2). In association with the increase in eCG, serum progesterone concentrations remained elevated in transplant mares for at least 74 days (Fig. 7.3).

The diameter of follicles that became HAFs was smaller (30.9 ± 1.3 mm) than the diameter of follicles that ovulated normally in control mares (41.2 ± 1.9 mm; $P<0.001$, Fig. 7.4). Three days prior to ovulation or HAF formation the diameter of HAF follicles was 24.2 ± 1.0 mm, which was smaller ($P<0.001$) than the diameter of normally ovulating follicles (35.9 ± 1.5 mm; Fig. 7.4) The mean diameter of HAFs after formation was 49.1 ± 3.6 mm. The mean lifespan of HAFs (from first detection to regression) was 27.4 ± 2.9 days. The accumulation of HAFs occurred over time such that at any one point in time a single mare had up to 4 HAFs present.

4. Discussion

Transplantation of equine chorionic girdle into the submucosa of the vulva in non-pregnant mares resulted in the development of 18 HAFs in four individual mares during the 105 day observation period. The process of HAF development that we observed was typical of that previously described (McCue and Squires 2002; Ginther et al 2007; Cuervo-Arango and Newcombe 2008; Ginther et al 2008b; Ellenberger et al 2009).

Previous attempts to induce HAFs in mares have utilized $PgF_{2\alpha}$ treatment, with or without the ablation of all follicles ≥ 6mm in diameter 10 days after $PgF_{2\alpha}$ treatment (Ginther et al 2008b; Ginther et al 2008c). The combined effects of $PgF_{2\alpha}$ treatment and follicle ablation resulted in the exposure of immature follicles of the post-ablation wave to elevated concentrations of LH, which resulted in HAF formation in 20% of the induced waves (Ginther et al 2008b). The concentration of LH was significantly higher in those follicular waves that resulted in HAF formation compared to those that ovulated normally.

Equine CG has LH-like activity in the mare and the protein structure of eCG is identical to that of eLH with a single gene encoding the β-subunits of both hormones (Sherman et al 1992; Saint-Dizier et al 2004b). In addition, both hormones bind to the same receptor in equine tissue (eLH/CG-R receptor) (Saint-Dizier et al 2004b), although eCG binds with only 3-4% of the affinity shown by equine LH (Stewart and Allen 1981). The eLH/CG-R is expressed in thecal, granulosa and luteal cells in the ovary (Saint-Dizier et al 2003; Saint-Dizier et al 2004a; Saint-Dizier et al 2004b). During pregnancy, eCG concentrations increase rapidly from day 35 to reach peak values between days 50 and 75 (Allen 2001). Concentrations of eCG decline at around 100 to 120 days of pregnancy as the endometrial cups regress (Allen and Stewart 2001). During the lifespan of the endometrial cups, eCG luteinizes or ovulates the dominant follicle of successive waves of follicles that are stimulated to develop under the endogenous rhythm of FSH production every 10-12 days (Evans and Irvine 1975; Ginther and Bergfelt 1992b). Thus the ovaries accumulate supplementary CLs which produce progesterone to maintain pregnancy until the feto-placental production of progestagens takes over. Equine CG also binds to eLH/CG-R on the primary CL to induce resurgence of progesterone production.

The results of our study supports the theory proposed by Ginther et al (Ginther et al 2008b) that LH plays a role in the formation of HAFs. Transplantation of chorionic girdle resulted in elevated eCG concentrations for the duration of the observation period. The immature follicles of each successive wave were therefore constantly exposed to an environment of elevated eCG concentrations much in the same way that immature follicles were exposed to elevated LH concentrations in the model used by Ginther et al (2008b). It therefore appears that the exposure of a newly emerging follicular wave to eCG or LH predisposes the follicle to the later development of an HAF. One of the benefits of our model is that the production of eCG occurs over a prolonged time period resulting in repeated HAF formation in subsequent follicular waves. We found that 17 of the 18 HAFs occurred within the first 60 days following chorionic girdle transplant. Although the binding affinity of eCG to eLH/CG-R is lower than the affinity shown by LH, the half-life of eCG is relatively long (6 days) (Cole et al 1967). This, in combination with the continued production of eCG by the transplanted trophoblast cells results in a sustained exposure of follicles to eCG for a prolonged period and explains the successive development of HAFs in the transplanted mares over a long time period.

In our study, three of the four mares transplanted with chorionic girdle produced HAFs during the period of elevated eCG production, whereas in the remaining mare ovulation of four small follicles (27.0 ± 2.1 mm) occurred over a 56 day period. In pregnant mares, Ginther (1992) reported that approximately two-thirds of supplementary CLs form from luteinization of follicles without ovulation. Variation in eCG levels in normal pregnant mares may explain the wide degree of individual variation in ovarian responses during pregnancy as described previously (Wesson and Ginther 1980). Further studies are required to determine whether HAF formation as a result of long-term exposure to eCG is dependent on the concentration of eCG.

One proposed mechanism for HAF formation is insufficient gonadotrophin production (McKinnon 1993). The results of our study and that of Ginther et al (2008b) appear to dispel this theory. In the study by Ginther et al (2008b) HAF formation was more likely to occur in those follicular waves associated with elevated LH concentrations. Likewise in our study, HAF formation occurred in the face of elevated eCG concentrations. Rather than an elevation of eCG/LH per se, it is also possible that the timing of exposure of follicles is important in determining whether an HAF will form. In our study successive follicular waves were continuously exposed to eCG. The diameter of follicles that became HAFs was significantly smaller than the diameter of follicles that ovulated normally. This agrees with the findings of Ginther et al (2008b) and Allen et al (2007b) and is most likely a result of the exposure of immature follicles to eCG resulting in the cascade of events that lead to HAF formation. Previous reports of administering exogenous eCG to mares have shown no significant ovarian effects (Dinger et al 1982; Daels et al 1998). However, in those studies the dose of eCG used (1000-4000 IU per mare as a single dose) was probably insufficient to obtain concentrations similar to those reported in our study or in pregnant mares, and therefore was unlikely to have a physiological effect. Further studies are required to determine whether the timing of eCG administration relative to the stage of follicle growth influences HAF development.

Whilst HAF is the term that has been used in broad-terms to describe the failure of ovulation associated with hemorrhage into the follicle, further classification into lueinized or non-luteinized anovulatory follicles has been described (McCue and Squires 2002). Luteinized anovulatory follicles are characterized by an elevation in progesterone concentrations throughout their life-span. In a study performed by McCue and Squires (2002) 85.7% of mares that developed HAFs spontaneously had elevated progesterone levels indicating luteinization of the anovulatory follicle. In association with the formation of HAFs in our study, we observed a sustained elevation in serum progesterone concentrations in all mares indicating luteinization of the anovulatory follicles.

Luteinized unruptured follicle syndrome (LUF) occurs in women, and is defined as a failure of ovulation in which the unruptured follicle undergoes luteinization under the influence of LH (Zaidi et al 1995; Qublan et al 2006). Qublan et al (2006) reported a 0% pregnancy rate in 42 women undergoing intra-uterine insemination during a LUF cycle. This is similar to the findings of McCue and Squires (2002) who reported a pregnancy rate of 0% in 71 mares inseminated during a HAF cycle. During the follicular growth phase, no morphological differences between normally ovulating follicles and LUF follicles can be detected by ultrasonography (Hamilton et al 1985; Zaidi et al 1995; Merce et al 2001). A “LUF cycle” can only be diagnosed once luteinization has occurred (Coulam et al 1982) similar to HAF development in the mare. Ultrasonographically, LUFs appear morphologically identical to HAFs (Merce et al 2001). It is therefore possible that the HAF syndrome in mares offers a suitable model for studying the LUF syndrome in women. In mares, perhaps the term "LUF" should be adopted to replace "HAF", as it seems to better reflect the morphological and endocrinological description of the syndrome.

Other risk factors proposed for HAF development in mares include advancing age (McCue and Squires 2002; Ginther et al 2007; Cuervo-Arango and Newcombe 2008), the use of exogenous prostaglandin to induce luteolysis (Ginther et al 2007; Cuervo-Arango and Newcombe 2008), repeated use of ovulation-inducing hormones (Cuervo-Arango and Newcombe 2008) and physical blockage of the ovulation fossa due to ovarian cysts (Kolling and Allen 2006). Whilst we did not specifically aim to investigate these factors in our study, our mares were all less than 8 years of age thus reducing any age-related effects on HAF development. In addition, prostaglandin was not administered to any of the mares after chorionic girdle transplant, nor did we administer ovulation-inducing hormones (such as GnRH or hCG). None of our mares were observed to have cysts within the ovulation fossa.

In conclusion, we have shown that HAF formation occurs at high frequency and multiple times in individual mares after ectopic transplantation of chorionic girdle into the vulvar submucosa. The mechanism of action is presumed to be via the exposure of follicles to eCG produced by the trophoblast cells of the chorionic girdle. This model appears to offer a potential method of studying the HAF syndrome in mares and possibly the LUF syndrome, a similar condition, in women.

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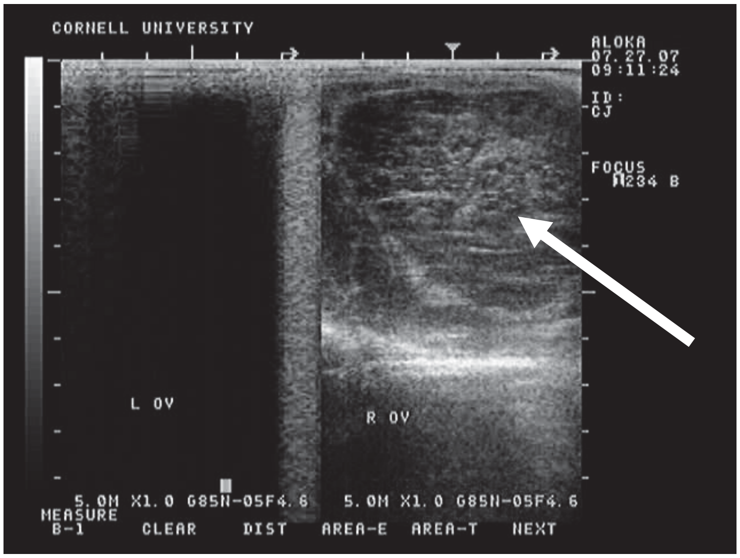
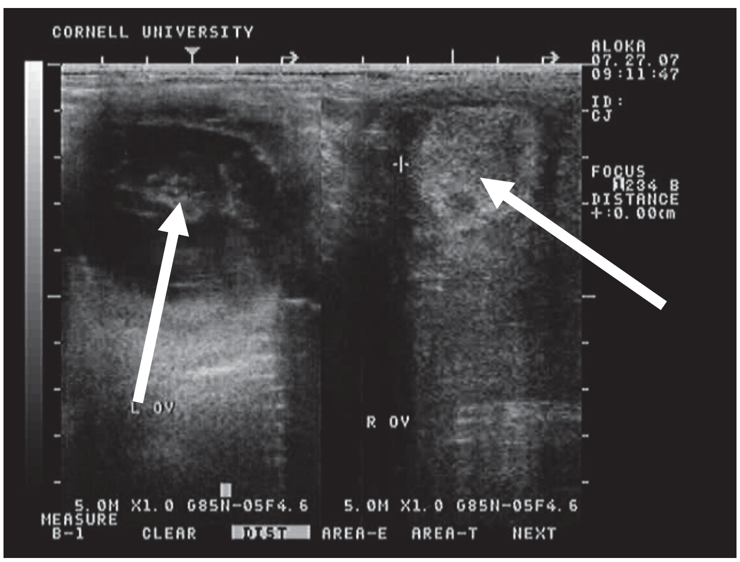
Table 7.1: Descriptive data for mares receiving chorionic girdle (CG) trophoblast transplants or control treatments.

Mares[a]	# of HAFs[b]	# of Ovulations[c]	Peak eCG (iu/ml)	Day of peak eCG[d]	Pre HAF follicle diameter[e] (mean ± SE, mm)	Post HAF diameter[f] (mean ± SE, mm)	Mean follicle diameter at ovulation (mm)	Mean HAF lifespan (days)
CG Transplant								
3836	4	0	9.7	32	34.0 ± 3.9	52.5 ± 6.7	NA[g]	29.8 ± 9.2
3883	0	4	25.2	28	-[h]	-	27.0 ± 2.1	-
3901	9	2	12.0	21	28.3 ± 1.9	49.4 ± 5.9	38 ± 0	20.6 ± 2.9
3902	5	1	16.7	28	34.8 ± 1.5	57.4 ± 1.9	52	31.4 ± 2.4
Total	18	7						
Mean			15.9 ± 3.4	27.3 ± 2.3	30.9 ± 1.3	49.1 ± 3.6		27.4 ± 2.9
Control								
3601	0	2	ND[i]	NA	-	-	45.5 ± 4.5	-
3820	0	3	ND	NA	-	-	37.3 ± 1.9	-
3837	0	2	ND	NA	-	-	48.5 ± 1.5	-
3916	0	2	ND	NA	-	-	42.0 ± 3.0	-
Total		9						
Mean							41.2 ± 1.9	

[a] Mare identification numbers. Four mares received transplants of chorionic girdle (CG) trophoblast; three of the controls received an equivalent amount of allantochorion membrane tissue, while the fourth control mare received medium alone.
[b] Total number of HAFs detected during the examination period
[c] Total number of ovulations detected during the examination period
[d] Relative to day of CG transplant.
[e] Follicle diameter at the time that HAF development was first noted.
[f] HAF diameter at the first examination after HAF development was first noted (second examination).
[g] NA – not applicable. This mare did not ovulate during the observation period.
[h] – This mare did not develop HAFs.
[i] ND – Not detected. eCG was never detected in control mares.



A



B

Figure 7.1: A. Ultrasound image of a typical HAF observed during the study period after transplantation of chorionic girdle into non-pregnant recipient mares. This image was recorded 31 days after CG transplant. B. Ultrasound image of a pre-HAF follicle (on the left) taken 31 days after CG transplant with blood and fibrin strands in the antrum. The image on the right shows an HAF 14 days after formation, characterized by hyperechoic, dense luteal tissue.

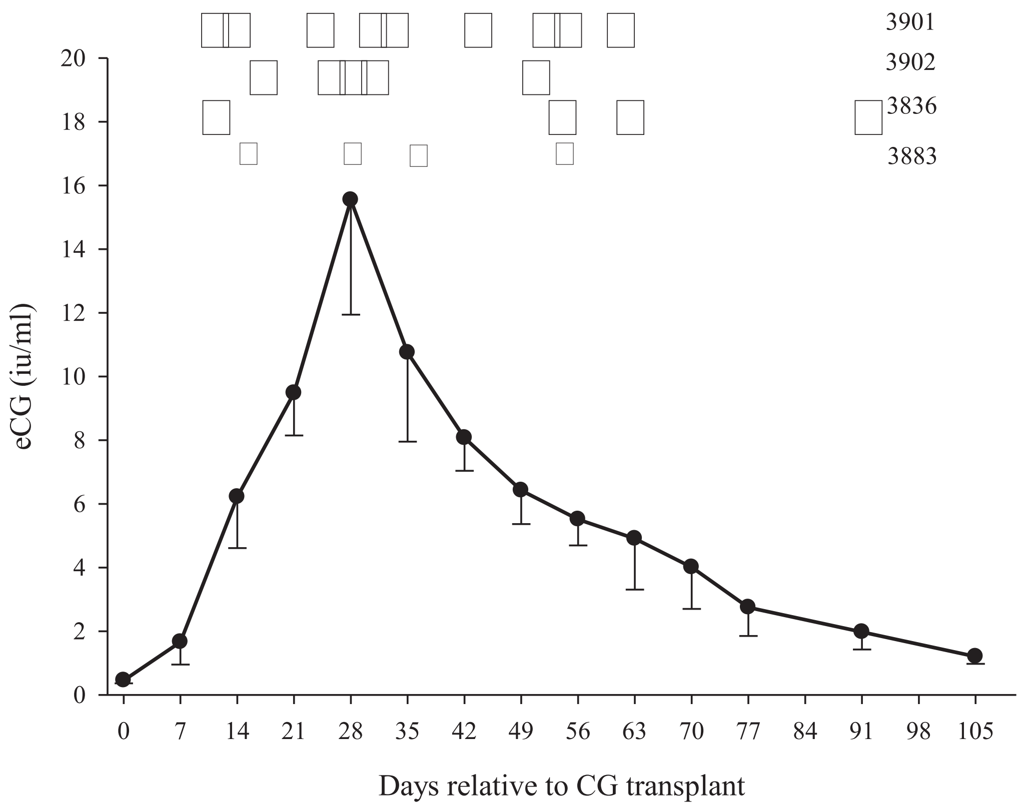


Figure 7.2: Concentrations of eCG (mean ± SE) and relative ovarian changes (* = HAF formation, + = ovulation) in non-pregnant mares (n=4) transplanted with chorionic girdle. Accession number of each mare is given in the top right corner.

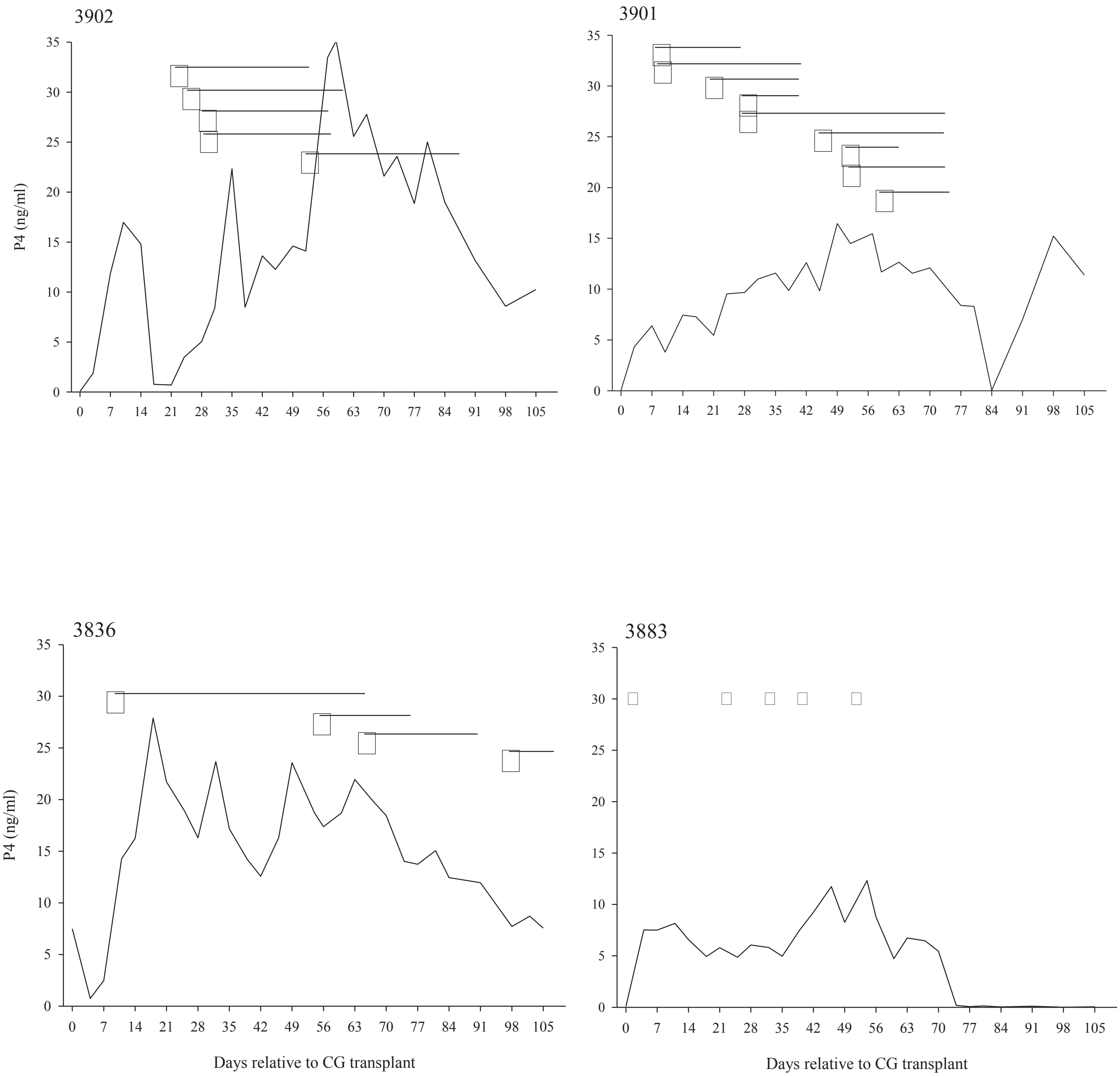


Figure 7.3: Progesterone profiles and relative ovarian changes (* = HAF formation, + = ovulation) in non-pregnant mares (n=4) transplanted with chorionic girdle. Horizontal lines indicate HAF lifespan.

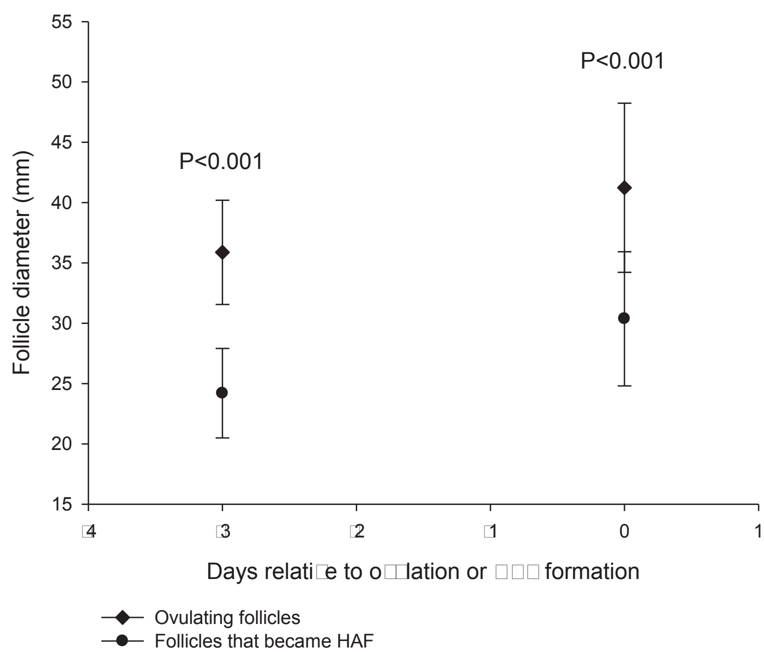


Figure 7.4: Follicle diameter at 3 days prior to and at the time of ovulation or HAF formation (Day 0) in non-pregnant mares (n=4) transplanted with chorionic girdle or control mares (n=4).

Chapter 8: General Discussion

The New Zealand Thoroughbred breeding industry is one of the largest in the world and makes a significant contribution to the nation’s economy. Historically, the racing and breeding of Thoroughbreds has played a major part in the country’s heritage and culture. However, in recent times, there has been a steady decline in the number of foals produced each season. In 1994 a total of 5264 foals were born and in 2009 only 4207 foals were born, a reduction of 20%. New Zealand is not alone in this trend with a declining foal crop occurring in recent times in all of the major Thoroughbred breeding countries.

Anecdotal evidence suggests that the decline in foal production is due to a gradual reduction in the size of the broodmare population because the profitability of breeding Thoroughbreds is considered to be marginal by many breeders. A reduction in foal numbers has a flow on effect - reducing export earnings and the number of horses available for domestic racing. Declining field numbers in New Zealand’s domestic racing scene reduces betting turnover and directly decreases the amount of stakes money injected back into the industry, further reducing the profitability of racing and/or breeding Thoroughbreds. One potential way to increase the profitability of the New Zealand Thoroughbred breeding industry is to produce more foals from the existing broodmare population.

In any livestock production system, strategies to improve production can only be investigated once industry standards are defined and the main factors that influence performance are identified. In the Thoroughbred breeding industry, published standards of reproductive performance are relatively scarce and the methodology in collecting, analyzing and describing reproductive outcomes is inconsistent. This is in contrast to other livestock industries, such as the New Zealand dairy industry for example, where a centralised database is used for the storage of production and reproductive information on an individual cow basis. Such a database allows for the analysis and reporting of key industry data and the comparison of farm performance to industry targets. Although each individual country keeps its own Thoroughbred stud book, the information recorded in the stud book is simply a record of whether or not individual mares produce a foal each year. Calculating any industry targets from stud book records is unreliable because around 20% of breeders do not submit their annual mare returns, and the information is not of sufficient detail to determine even the most basic reproductive performance parameters.

Descriptive studies of Thoroughbred reproductive performance have been published from the UK, USA, Sweden, South Africa, Australia and India over the past decade. All of these studies have analysed reproductive performance by evaluating mare-level factors which vary at a single level such as mare age (univariate analysis). Univariate analysis does not take into account the fact that some variables influencing reproductive performance are related to each other, nor does it determine which of the exposure variables are the most important. The limitations of univariate analyses were discussed in Chapter 2 of this thesis.

Whilst reproductive performance in Thoroughbreds is usually analysed at the mare level, mare-level outcomes cannot be considered independent events. Mares are clustered within stud farms and are therefore exposed to the same nutritional, environmental and farm-management practices. In addition, groups of mares are served by the same stallion and hence there is likely to be an influence of the stallion's inherent fertility on the reproductive performance of the mares to which he is bred. None of the previous studies of Thoroughbred reproductive performance have utilised statistical techniques appropriate for clustered data to analyse reproductive performance.

Despite the importance of the Thoroughbred industry to New Zealand, there are no published reports detailing the reproductive performance of Thoroughbred mares in this country. Therefore one of the primary objectives of this thesis was to describe the reproductive performance of a population of Thoroughbred mares on stud farms in the Waikato region of New Zealand. Of critical importance was the desire to use multivariable statistical techniques appropriate for the analysis of clustered data to examine the effects of mare-related exposure variables and their interrelationships on the reproductive performance of Thoroughbred mares. The major strengths of this study were the prospective nature of data collection, the use of farm data rather than breed registry data, the large sample size and the relatively short time-span during which data collection occurred in comparison to some previous studies.

Chapter 3 outlined descriptive statistics and explanatory data in such a way as to allow comparisons to previous studies from other countries, utilising similar methodology. The results of this study show clear differences between the New Zealand breeding industry and that in the northern hemisphere. Mares in this New Zealand study were older and the proportion of barren mares was higher in this study compared with previous studies from the northern hemisphere. The older age and higher proportion of barren mares is likely a reflection of the fact that New Zealand breeders are more inclined to leave mares "dry" (non-pregnant) for a season in order to produce an early foal the following year. The low-cost, pasture-based grazing system in New Zealand means that carrying a non-pregnant mare is relatively cheaper than in the northern hemisphere. The breeding season in this study was only 105 days long which is significantly shorter than in the northern hemisphere where the season is around 150 days duration. This difference is likely due to the different timing of the annual yearling sales in each country. The main Thoroughbred yearling sale in New Zealand is held in the last week of January; therefore foals born after 1st December are only 12-13 months of age at sale time and are at a growth disadvantage compared with foals born earlier in the season. In the northern hemisphere the main "yearling" sales are held in September (USA) and October (UK) when horses are actually between 17 – 21 months of age, therefore later born foals have more time to "catch-up" to their earlier born peers compared with the situation in New Zealand where yearlings are sold at a younger age.

There were other differences in the reproductive management of mares in New Zealand compared with those from other countries. In this study, 32% of foaling mares were served on foal heat, which is significantly higher than that reported in previous northern hemisphere studies where only 12 - 14% of mares were served on foal heat. The higher rate of foal heat breeding in New Zealand is probably a reflection of the short breeding season and the necessity to get mares back in foal as soon as possible after foaling. In this study, 35% of all dry mares were exposed to an artificial lighting programme prior to the start of the breeding season which is much lower than that reported in the northern hemisphere, where 80 – 100% of dry mares were exposed to artificial lights. This difference most likely reflects the fact that mares in the northern hemisphere are housed indoors and therefore the exposure to artificial lighting is relatively easy to achieve. In New Zealand, artificial light exposure is generally performed by bringing mares into holding yards each night and is therefore perceived as being relatively time-consuming and labour intensive.

The first-cycle pregnancy rate of mares in the present study was 54%, which is lower than that previously reported from the northern hemisphere where a per-cycle pregnancy rate of around 60% has generally been reported. The lower per-cycle pregnancy rate could be due to several factors. Firstly, the median age of mares was older in this than previous studies, and increasing mare age was found to significantly reduce reproductive performance. Secondly, the shorter breeding season means that stallion breeding frequency is very high in New Zealand with 87% of services occurring in the 91 day period between 1st September and 30th November. Stallions that served more than 100 study mares per year had a significantly lower first cycle pregnancy rate than those that served less than 100 mares. Other management-related differences such as the proportion of dry mares exposed to lights and the proportion of foaling mares served on foal heat could also contribute to the difference in the per-cycle pregnancy rate between different studies.

The foaling rate of 80% in this study was equal to or higher than previous reports from several countries. The relatively high foaling rate, achieved over a short breeding season, was the product of a high end-of-season pregnancy rate (85%) and a relatively low pregnancy loss rate between 42 days of gestation and foaling (3%). Previous studies from the northern hemisphere have reported pregnancy loss rates of around 15 – 20%. The difference is most likely due to the fact that mares in New Zealand are outdoors all year round and therefore have ample opportunity to exercise. Moderate exercise appears to have a beneficial effect on the clearance of intra-uterine fluid and may reduce the incidence of post-breeding endometritis and subsequent pregnancy loss.

In Chapter 4, multivariable statistical methods appropriate for clustered data were used to analyse the results of the prospective cohort study. In addition, the relative contribution of the mare, stallion and stud farm towards reproductive performance was determined. Another aspect of this study was the introduction of the start-of-mating to conception interval as a measure of equine reproductive performance. This indicator is commonly used in seasonally calving dairy herds and is a measure of how quickly cows conceive after the start of mating. It is a product of the submission rate (number of cows mated) multiplied by the pregnancy rate per cycle and is commonly used to compare the reproductive performance of dairy herds with standard industry targets. The results of the study clearly support the concept of using the start of mating to conception interval (SMCI) as a useful within-season reproductive monitoring tool on horse studs.

This study described novel techniques for the analysis of equine reproductive performance that are reported in this thesis for the very first time. These findings are of direct relevance to the New Zealand breeding industry and are also of value to the rest of the world, and provide a clear description of how Thoroughbred reproductive performance should be measured and analysed.

The results of the multivariable analyses indicated that the most important mare-level factors associated with the overall reproductive performance of mares in the Waikato region of New Zealand were mare age and reproductive status (categorised as foaling or dry). When controlled for the effects of serving stallion, stud farm, year of study, date of service and reproductive status, increasing mare age significantly reduced the first-cycle pregnancy rate, reduced the end-of-season pregnancy rate, and increased the interval from the start of the breeding season to conception. In terms of reproductive status, dry mares had a significantly higher first-cycle pregnancy rate and end-of-season pregnancy rate compared with foaling mares. There was no effect of reproductive status sub-group for dry mares (maiden, barren) thus reinforcing the notion that barren mares in New Zealand are largely a result of management decisions (intentional non-breeding) rather than infertility.

When controlled for the effects of stud farm, serving stallion, status (maiden or barren sub-category) and year of study, the only mare-level variables that significantly affected the reproductive performance of dry mares were mare age, artificial light exposure and days to first service after the 1st September. The exposure to an artificial lighting program prior to the start of the breeding season significantly improved the first-cycle pregnancy rate, the end-of-season pregnancy rate and reduced the start of mating to conception interval. Increasing days to first service (after 1st September) also positively affected the first-cycle pregnancy rate when controlled for artificial light exposure. Mares served earlier in the season that have not been exposed to artificial lights are more likely to still be in the transition period and therefore the pregnancy rate to services occurring at this time is lower.

When controlled for the effects of stud farm, serving stallion and year of study, the only mare-level variables that significantly affected the reproductive performance of foaling mares were mare age, foal heat breeding and foaling date. Foal heat breeding increased the end-of-season pregnancy rate, and the start of mating to conception interval was also shorter in mares bred on foal heat. Interestingly, the first-cycle pregnancy rate was not significantly different between mares bred on foal heat and those bred on subsequent cycles. This indicates that the criteria used for determining the suitability of mares for foal heat breeding was effective in selecting those mares that had undergone normal uterine involution. Foaling date significantly affected the end-of-season pregnancy rate and the start of mating to conception interval. Mares that foaled before the 22nd October had a higher end-of-season pregnancy rate and a shorter conception interval compared with mares foaling after the 22nd October.

One of the aims of the cohort study was to determine the relative contribution of the mare, stallion and stud farm on reproductive performance. Previous studies have attempted to determine the effects of stud farm and stallion on mare-level reproductive outcomes, but have simply relied upon a univariate approach; none have utilised the appropriate multivariable analytical techniques. When stud farm and stallion were included in multivariable models as random effects terms, and the latent variable approach was used to determine the variance components, it was clear that virtually all of the variation in reproductive outcome was associated with the mare. The proportion of variance contributed by the mare, stallion and stud farm was 95.9, 4.1 and 0% respectively for first-cycle pregnancy rate and 92.5, 6.7 and 0.8% respectively for the end-of-season pregnancy rate. The results therefore suggest that reproductive management should concentrate on mare-level practices. There was virtually no variation at the stud farm level, and the inclusion of stud farm as a random effect term did not significantly improve the fit of any of the multivariable models. The most likely reason for this finding was the fact that all farms were under the care of the same veterinarian and therefore followed very similar reproductive management practices. In addition, all farms were from the Waikato region where farm and animal management practices are relatively homogeneous.

Whilst the variance estimates contributed by the stallion were small, there were significant differences amongst stallions in their reproductive performance (Chapter 3). Assessment of the significance of the random effect term for stallion included in the multivariable models also revealed that stallion was a significant variable. Stallion breeding management has been shown to affect reproductive performance in the northern hemisphere; however there have been no studies that have investigated the breeding management of stallions in New Zealand.

The fact that the Thoroughbred industry has imposed an artificial breeding season on itself that is not compatible with the natural seasonal cycle of the mare was discussed in Chapter 2. The results of the cohort study confirmed the “problem” of commencing the breeding season during the transitional period. Strategies to advance the transition period and to manage peculiar characteristics such as ovulation failure therefore become of relevance to the industry. Dry mares that were cycling early in the season had the highest reproductive performance, therefore strategies that ensure dry mares are cycling at the start of the season, such as artificial light exposure, help to maximise reproductive performance. However, in the cohort study only 35% of all dry mares were exposed to an artificial lighting programme which is most likely due to the fact that it is relatively expensive and time-consuming to expose mares to lights in a pasture-based management system. Alternative methods are required in New Zealand that are cheap, effective and not labour intensive. A variety of hormonal treatment methods as alternatives to light exposure to advance the transition period were reviewed in Chapter 2. None of these methods has been shown to reliably advance the transition phase compared to untreated control mares, and many of them have been shown to be effective only when used in combination with artificial light exposure. In addition, all of these methods involve daily administration which is time-consuming and costly.

In Chapters 5 and 6 the use of an intravaginal progesterone-releasing device to manage transitional mares was investigated. The intravaginal route offers a relatively cheap and convenient method of administering progesterone to mares that avoids the need for daily treatment. The aim of the first study (Chapter 5) was to investigate the effects of intravaginal progesterone on follicular development and FSH, LH and progesterone profiles in transitional mares. The aim of the second study (Chapter 6) was to investigate the effects of intravaginal progesterone on the reproductive performance of transitional Thoroughbred mares on commercial stud farms.

The results from the study in Chapter 5 showed that the treatment of transitional Thoroughbred mares with an intravaginal progesterone-releasing device for 10 days resulted in follicle growth and ovulation within 4 days of device removal. Follicle development occurred in close association with rising FSH concentrations during the initial treatment period resulting in a significant increase in the diameter of the largest follicle on day 6 of treatment. The combination of declining progesterone levels, falling FSH concentrations and growth of the dominant follicle resulted in an increase in LH concentrations on days 6 and 7 supporting continued growth of the dominant follicle. Within two days of device removal mares were in oestrus and dominant follicles responded to hCG-induced ovulation.

The results of the field trial (Chapter 6) showed that mares treated with intravaginal progesterone at the start of the season were served about 2 weeks earlier than untreated control mares. In the first 21 days of the season, 95% of treated mares were served compared with 43% of control mares. Treated mares also conceived earlier and had a higher end-of-season pregnancy rate than control mares. This treatment protocol appears to offer a convenient and economical method of managing transitional mares on commercial stud farms and may be a suitable alternative to artificial light exposure.

In Chapter 7 the development of a potential model for ovulation failure in the mare was investigated. As discussed in Chapter 2, the most common anovulatory condition in mares is the development of haemorrhagic anovulatory follicles (HAFs) and the syndrome is most frequently observed during the transition period. HAF formation in mares is associated with infertility, and the ability to predict which dominant follicles will become HAFs is difficult because during ultrasound examination they appear to be morphologically similar to normal pre-ovulatory follicles.

Whilst the primary objective of this study was to create a model for the study of HAF development in the mare, a review of the literature revealed a potential comparative application in the field of human reproduction. Due to their size and the ease with which their reproductive tract can be imaged ultrasonographically, mares may offer a suitable model for the study of a similar anovulatory condition in women known as luteinized unruptured follicle syndrome (LUF). The morphology and functional properties of LUFs are very similar to HAFs in the mare. Previous attempts to create a HAF model in mares have focused on exposing immature follicles to supraphysiological concentrations of LH through a combination of follicle ablation and prostaglandin treatment. However, such models have resulted in HAF formation in only around 20% of follicular waves. The aim of this study was to deliver eCG (which has LH-like activity in the mare) to non-pregnant mares through the transplantation of chorionic girdle and to determine whether this could induce HAF development in the treated mares.

Transplantation of chorionic girdle tissue resulted in elevated eCG concentrations in all mares for the duration of the observation period resulting in the development of multiple

HAFs in the treated mares over a prolonged time-span. The immature follicles of each successive wave were constantly exposed to an environment of elevated eCG concentrations in much the same way that immature follicles were exposed to elevated LH concentrations in previous HAF models.

These findings support the theory proposed by some previous investigators that LH plays a role in the formation of HAFs. The increased incidence of HAF development that occurs during the transition period may be due to several factors such as the inappropriate timing of exposure of transitional follicles to LH, or a mismatch between follicle development, the expression of LH receptors, and the release of LH from the pituitary gland during the transition period. The fact that the chorionic girdle transplantation model reliably induces HAF formation will now enable further studies to be undertaken to investigate the HAF syndrome.

Limitations

One of the limitations of the cohort study was the fact that all stud farms were under the routine care of the same veterinarian and all farms were from the same geographical region. Therefore the conclusions from this study should be interpreted with this in mind and extrapolation of the findings from the cohort study to other regions and/or countries may not be possible. However, the study has clearly defined the methodology for analysing and describing equine reproductive performance and this can most certainly be applied to any equine breeding industry.

Another limitation of the cohort study was the fact that only one breed (Thoroughbred) was represented. Initially, the intention of this study was to include data from Standardbred stud farms. In the early stages of the study, three large Standardbred farms (with a combined total of around 2500 mares) from the south Auckland and Canterbury regions were enrolled. Harness Racing New Zealand (HRNZ) maintains a centralised database at its head office in Christchurch. The intention of the database is to record horse details (race performance, produce records, breeding details etc) in a computerised version of the stud book. Interrogation of the database to extract reproductive performance should be possible. However, after several visits to HRNZ and discussions with the programmers responsible for maintaining the database it became clear that extracting reproductive data in a format that would allow for appropriate analysis was not possible.

Future research

This thesis has identified several areas for further research.

Further studies to include farms from different regions and/or those under the care of different veterinary practices are required to assess the effects of different veterinary and farm management practices.

The effect of stallion breeding management practices deserves further study. The short duration of the New Zealand breeding season imposes considerable pressure on stallion managers to breed as many mares as possible in a limited time period. The effect of factors such as different breeding intervals, number of mares served per day and per season, time of breeding, and month of breeding could all potentially affect stallion fertility. Determining which factors significantly contribute to stallion fertility would be of immense value to the industry and would provide stallion owners and managers with information on which to base their breeding management decisions.

Further studies on the use of in-season performance parameters such as the start-of-mating to conception interval (SCMI) are indicated. In other seasonally-based breeding systems such as the New Zealand dairy industry, the “in-calf” rate is a useful indicator of dairy herd reproductive performance that can be calculated as soon as the herd has been pregnancy tested. The 6-week in calf rate is used as a comparative measure amongst different herds and is defined as the proportion of cows pregnant at 6 weeks after the start of mating, divided by the total number of cows in the herd. A 6-week “in-foal” rate could similarly be calculated for stud farms and would be a useful comparative measure of reproductive performance amongst different stud farms. Using the in-foal rate during the season would enable any deviations from target to be detected early and (hopefully) rectified before the end of the breeding season.

Whilst the use of intravaginal progesterone-releasing devices appeared to be beneficial, any hormonal treatment to advance the transition period should be compared with artificial light exposure as the “gold standard”. Therefore, further studies are required using mares from the same farms to compare the reproductive performance of mares treated with either intravaginal progesterone or exposed to artificial light treatment. In addition, starting treatment with intravaginal progesterone several days before the start of the breeding season may improve the response to treatment. Timing the removal of devices so that all mares are cycling on the first day of the season could effectively increase the number of mares bred as early as possible. This is analogous to the dairy industry where anovulatory dairy cows are treated with intravaginal progesterone so that they are inseminated on the first day of mating.

Further studies on the HAF syndrome and its relevance to the New Zealand Thoroughbred industry are required. Whilst it is accepted that the syndrome is of clinical and economic significance in other countries, the incidence of HAFs and the risk factors for HAF formation in New Zealand broodmares needs to be determined.

Conclusions

This thesis is the first to describe the reproductive performance of Thoroughbreds in New Zealand and is the first study anywhere in the world to use appropriate techniques for the analysis of clustered data to analyse equine reproductive performance.

The reproductive performance of Thoroughbred mares in New Zealand was similar to that reported from other major Thoroughbred breeding countries. There were obvious differences however in reproductive performance between the New Zealand industry and those from other countries. In the study population, the breeding season was shorter, mares were older and there were a higher proportion of barren mares compared with previous studies from the northern hemisphere. Mare-level variables contributed the most to reproductive performance and the effect of the stud farm and stallion was relatively minor. Dry mares that were served early in the season and foaling mares that foaled before 22nd October had superior reproductive performance. There appears to be several opportunities for New Zealand breeders to increase production. Increasing the proportion of dry mares exposed to an artificial lighting programme prior to the start of the season is likely to significantly increase the number of dry mares conceiving each season. Also, increasing the number of mares conceiving each season could be achieved by simply prolonging the breeding season. Such a strategy most likely relies on the industry recognising that the current date of the annual yearling sales is potentially limiting foal production by adding pressure to produce early foals. Changing the date of the yearling sales to later in the year would alleviate this pressure. Additionally, the imposed start of the breeding season of 1st September limits foal production because dry mares are still in their transition period when the breeding season starts. Changing the date of the start of the breeding season to 1st October could potentially increase the number of mares conceiving each season and reduce the reliance on hormonal treatments or artificial light exposure to advance the transition period.

This study has clearly identified a need for the New Zealand Thoroughbred industry to establish a centralised database to record individual mare data. Such a database would allow the industry to determine key performance indicators and enable stud farm managers to make informed decisions regarding reproductive management in much the same way as the New Zealand dairy industry. It is inevitable that such a resource would improve the reproductive efficiency of the industry and thereby increase profitability.

In conclusion, it is hoped that this thesis will serve as a valuable resource for the Thoroughbred industry in New Zealand and for the rest of the world.

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