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Interovulatory intervals in mares receiving deslorelin implants in Ireland (2009-2010)

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Abstract

Deslorelin acetate implants, recently licensed in Ireland and the UK for ovulation induction in mares, have been associated with prolonged interovulatory intervals in USA studies, leading to the practice of removing implants post-ovulation. Trial data in Australia indicates a less pronounced effect on interovulatory intervals, suggesting possible geographical variation. Objectives of the current study were to assess the effect of deslorelin implants, with and without removal on oestrous cycle length in Irish and UK-based Thoroughbred broodmares. Data was collected retrospectively from 88 oestrus cycles. A statistically significant difference was found between interovulatory intervals in mares in which the deslorelin implant was not removed, compared to administration and removal of the implant or the use of human chorionic gonadotrophin, with lactating mares being significantly affected. These results suggest that implant removal when possible is advisable. The delay in subsequent ovulations was, however, less marked than that reported in some USA studies. This information is useful in deciding when to schedule subsequent breeding for mares which received a deslorelin implant during the previous oestrous period and provides evidence to counter concerns that mares treated with deslorelin implants may experience a long delay in return to oestrus if the implant is not removed.

Introduction

Increases in oestrous cycle length in mares following the administration of subcutaneous implants containing deslorelin, a synthetic gonadotrophin releasing hormone (GnRH)

analogue, for induction of ovulation have been reported (Johnson and others 2000, Morehead and Blanchard 2000, Blanchard and others 2002, Farquhar and others 2002, McCue and others 2002). Anecdotal reports of prolonged interovulatory intervals in mares in the USA followed the initial use of deslorelin implants. Subsequent studies found an increase in the interovulatory period in mares receiving deslorelin implants in relation to control or human chorionic gonadotrophin (hCG) treated mares in a clinical setting (Morehead and Blanchard 2000, Blanchard and others 2002) and in deslorelin treated mares administered prostaglandin F2 alpha (PGF2 α) at day 5-10 post-ovulation (Johnston and others 2000, Farquhar and others 2002, McCue and others 2002). Removal of the deslorelin implants post-ovulation has resulted in normal ovulation intervals (Farquhar and others 2002, McCue and others 2002); however, removal of the implant may not always be practical in mares that are not resident at the stud. It is common practice in Australia not to remove the deslorelin implant post-ovulation and trial data (Peptech Animal Health trial data, 2010) indicates an incidence of oestrous cycles greater than 26 days of 15% in Australia. In Kentucky, USA, an incidence of 32% of oestrus cycles greater than 26 days was found (Peptech Animal Health trial data, 2010), suggesting that there may be geographical variations in the response to the implant, as mares are of the same breed and veterinary management is similar. A deslorelin implant for ovulation induction in mares (Ovuplant[®]) became licensed for use in the UK in 2005 and Ireland in 2009. The objectives of the current study were to compare the effect of using Ovuplant[®], with and without removal of the implant and the traditionally used ovulation induction agent hCG (Chorulon[®]), on interovulatory intervals in mares in the

Irish stud farm setting and to compare the results to those published for mares in the USA and Australia.

Materials and methods

Animals and treatments

Records from a total of 284 Thoroughbred broodmares, resident on a one large stud in Ireland during the 2009 or 2010 breeding seasons were analysed retrospectively. Sixty-seven mares did not conceive on the first cycle and data on subsequent, interovulatory intervals was available for inclusion in the study. Implants containing 2.1mg deslorelin acetate (Ovuplant[®] Dechra Ltd Sansaw Business Park, Hadnall, Shrewsbury, Shropshire, UK) were used in a proportion of mares, the remainder of the mares receiving the traditionally used ovulation induction agent hCG (Chorulon[®] Intervet Schering Plough Ireland Ltd, Boghall Road, Bray, Co Wicklow, Ireland). Mares visiting a stallion with a service fee at the higher end of the range received Ovuplant[®]. This selection was considered to be random for the purposes of the parameters measured in the study because age, breeding status and breeding history were not factors in determining nominations to a particular stallion. Post-mating treatment of mares also did not differ between mares visiting different stallions. Mares were covered when a dominant preovulatory follicle reached 35- 40 mm. Cross-covering of mares which failed to ovulate within 48 hours was assessed on an individual basis according to the stallion used and these mares were excluded from the study. In 2010, all mares received Ovuplant[®]. Implants were administered at the time of covering, either subcutaneously in the neck or in the vulval mucosa. Mares receiving hCG were also injected at the time of covering.

Vulval implants were removed where possible once ovulation was confirmed. Lignocaine hydrochloride (Norocaine solution for injection, Norbrook laboratories Ltd, Rossmore Industrial Estate, Monaghan, Ireland) was used to provide local anaesthesia to facilitate insertion and removal. The site of the implant and whether it was removed post-ovulation was recorded. The factors investigated (age and breeding status) were not used to decide whether or not implants were removed. Implants were not removed if they had been administered subcutaneously in the neck or in too deep a subcutaneous site in the vulval mucosa and therefore were not easily accessible. The choice of site of administration was at the discretion of the stud. Mares were routinely examined to confirm ovulation at 24 and 48 hours post-covering. Pregnancy diagnosis was performed by transrectal ultrasonography at 14-15 days post-covering. Mares which were not confirmed to be in foal at this examination were re-examined at approximately 18 days post covering and subsequently as required depending on the rate of follicular development, until covering was scheduled and ovulation was confirmed. PGF2 α was not routinely administered to non-pregnant mares to hasten the return to oestrus. PGF2 α was administered in some cases where there was ultrasonographic evidence of a persistent corpus luteum (CL) or failure to show signs of returning to oestrus (negative teasing behaviour and/or no ultrasonographic evidence of uterine oedema and a dominant follicle or follicles) by day 20 post-ovulation. Measurement of progesterone to confirm the presence of luteal tissue was not performed and mares receiving PGF2 α were not excluded from the analysis. Mares showing evidence of premature luteal regression due to endometritis (defined as uterine oedema, with or without luminal fluid and the absence of a visible CL) at the 14-15 day scan were excluded from the study during the affected oestrous cycle. Inter-

ovulatory intervals were compared for three groups of mares: those receiving hCG, which served as a control group (CON), those receiving a deslorelin implant which was not removed (ONR) and those receiving a deslorelin implant which was removed post-ovulation (REM). Mares with an interovulatory interval greater than 22 days (Witherspoon 1971) were defined as exhibiting a prolonged interovulatory interval for the purposes of the study.

Statistical analysis

Differences in interovulatory intervals in the different treatment groups, effect of year and mare age on interovulatory intervals between the three treatment groups and effect of mare status (maiden or barren cycling, maiden or barren mares covered at the first ovulation of the year (end-transitional), foal heat, second or subsequent natural oestrus post-foaling or PGF2 α induced oestrus post foal heat) on interovulatory interval between the three treatment groups were then statistically analysed with a multiple variance ANOVA with Bonferroni post hoc testing using PASW 18.0 for Windows. A significance level of $p < 0.05$ was set. Results are expressed as the mean \pm s.e.m. The correlation between increasing age and interovulatory interval was calculated using Microsoft Excel (2007 for Windows).

Results

Twenty-two mares were covered on one or subsequent cycles during 2009 and 45 mares were covered on one or more cycles during 2010. The total number of oestrous cycles available for analysis was 32 in 2009 and 56 in 2010. Deslorelin implants were

administered and not removed in 8 of these cycles in 2009 and 15 cycles in 2010 (ONR n=23). Implants were administered and subsequently removed in one cycle in 2009 and 41 cycles in 2010 (REM n=42). Twenty-three mares received hCG (control group) in 2009 (CON n=23). There were no control mares in 2010. Experience throughout the duration of the study suggests that even if efforts are made to remove the implant in most cases, circumstances will arise in which it is not feasible, such as mares which are not resident on the stud, the implant being placed too deeply, difficulty in palpating the implant in mares with significant fibrous scarring of the vulva following multiple Caslicks procedures and mare temperament. In 3 mares which failed to ovulate within 48 hours, the implant appeared to have dissolved by the time of ovulation, making removal impossible. The mean interovulatory interval was 23.86 days in the ONR group, 21.95 days in the REM group and 21.86 days in the CON group. There was a significant difference ($P=0.02$) in interovulatory intervals between mares in the ONR group and the 2 other treatment groups (Table 1). There was no significant difference in interovulatory intervals between CON mares in 2009 and the REM group in 2010. There was no significant difference between the group ONR in 2009 and ONR in 2010 (Table 2), suggesting that there was no effect of year on interovulatory intervals. There was no significant difference in interovulatory interval between mares aged up to and over 10 years of age in any of the three treatment groups (Table 3) although a significant positive correlation ($P<0.05$) between increasing age and interovulatory interval was found in the group of control mares only ($r=0.35$).

Double ovulations (synchronous to within 48 hours) not resulting in pregnancy were recorded in 12 mares, of which six had received hCG, three had the deslorelin implant removed and three did not have the implant removed. For cycles in which a double ovulation was recorded, the mean cycle length was 21.9 days, range 21-25 days. For mares with single ovulations, the mean cycle length was 22.5 days, range 19-38 days.

Interovulatory intervals were significantly longer ($P=0.02$) in ONR foaling mares covered at the second or subsequent natural oestrous period when compared to CON or REM foaling mares (Table 4). No significant differences in interovulatory intervals were identified between treatment groups when non-lactating mares were analysed collectively (maiden and barren cycling and end-transitional mares). Statistical analysis of non-lactating end-transitional mares, non-lactating cycling mares, foal heat mares and lactating mares covered at PGF2 α -induced oestrus (Table 4) was not possible due to the small numbers in each treatment group.

Discussion

The results show a small but statistically significant difference in the interovulatory interval in mares in which deslorelin implants were not removed in comparison to control mares (2 days) and mares in which the implant was removed (1.94 days). These results are similar to those reported in Colorado by McCue and others (2002) who found a mean increase in interovulatory intervals in mares in which the deslorelin implant was not removed of 1.8 days when compared to mares receiving hCG and 1.9 days when no ovulation induction agent was used. The increase in interovulatory interval in the current study is, however, less than that of 3.5 days reported by Morehead and Blanchard (2000),

the 9.6 days reported by Vanderwall and others (2001) or the 19.7 days reported in foal-heat mares (Blanchard and others 2002) in North America. The interval was greater than the difference of 0.6 days, (interovulatory interval of 21.2 ± 3.4 days in untreated mares versus 21.8 ± 4.6 days in mares receiving a 2.2mg deslorelin implant, which was not statistically significant) in another USA study by Mumford and others (1995) and the mean increase in cycle length of 1.2 days in Australian field studies (Peptech Animal Health trial data, 2010). The findings of the current study are of clinical significance for practitioners using deslorelin implants and broodmare managers and provide reassurance that, should it be impossible or impractical to remove the implant, the effect on the subsequent oestrous cycle length is likely to be small if the mare fails to become pregnant. Follicle size at the time of administration of the implant is similar between the different studies (35-40 mm in the current study, 40-42mm in the study by Morehead and Blanchard [2000] 38.9 ± 0.5 mm in the study by Vanderwall and others [2001] , 35 mm in the study by Blanchard and others [2002], 35mm in the study by McCue and others [2002]). Mumford and others (1995) inserted the implant at a follicle size of 30mm and reported shorter interovulatory intervals than the current study. Although Vanderwall et al (2001) found a tendency towards an increased incidence of prolonged (>30 days) interovulatory intervals in mares receiving the implant at a smaller follicle size of 37.1 ± 1.1 , rather than 40.1 ± 0.6 mm this was not statistically significant ($P < 0.1$) and the follicle sizes at which this effect was noticed were larger than those in the study by Mumford and others (1995) and similar to those of the other studies (Morehead and Blanchard (2000), Blanchard and others (2002), McCue and others (2002) and the current study, suggesting that follicle size at the time of administration of the deslorelin implant

was unlikely to have contributed to differences in the results between the current and other published studies.

It is interesting that failure to ovulate within 48 hours occurred in only three of 65 cycles in which deslorelin implants were used and all three mares were aged 19 or over. Farquhar and others (2000) found that mares aged 15-19 years and 20 years or over had a reduced ovulation rate in response to a deslorelin implant (87.9% and 83.8% respectively) when compared to mares aged 2-4 years (90.2% ovulation rate) or 5-9 years (91.0% ovulation rate). Age-related ovulation dysfunction is suggested to occur in some mares from approximately 20 years of age onwards (McCue 1998). Carnevale and others (1993) identified longer interovulatory intervals in mares of 20 years of age or older (mean 26.5 days) when compared to mares 5-7 years of age (23.9 days) or 15-19 years (23.0 days). This longer interovulatory interval appeared to be due to a longer follicular phase (11.7 days versus 9.4 days in the 5-7 year age group and 8.0 days in the 15-19 year group). A subsequent study by the same authors (Carnevale and others 1994) showed elevated FSH and LH concentrations in older mares, suggesting a decreased ovarian sensitivity to these hormones, which may explain the failure of some of the older mares in the current study to respond to exogenous GnRH preparations such as deslorelin. Due to these published effects of aging on cycle length, mares in the current study were divided into categories of up to and over 10 years of age within the different treatment groups to examine whether there was an age effect. In the current study, although a significant positive correlation was noted between increasing age and cycle length in the control group, differences in cycle length between mares up to and over 10 years of age in the different treatment groups were not statistically significant suggesting that,

although there were proportionally more mares over 10 years of age in the ONR group, this was unlikely to have contributed to the overall finding of longer interovulatory intervals in this group. The small number of mares in their later teens and twenties limits further analysis of the effect of age in the current study, however, this also reflects the commercial Thoroughbred breeding population in which older mares, unless proven producers, are often retired. It would be interesting to re-examine the use of deslorelin implants in a larger population of older mares as this data may be of particular relevance for Sport Horse mares, many of which may not begin a stud career until their teens and which may require precise timing of ovulation to facilitate shipments of chilled semen. Longer oestrous cycles have also been reported in mares after double ovulations (24.0 days following a double ovulation versus 20.8 days for single ovulations) (Urwin and Allan 1983), however, in the current study, the mean interovulatory interval was shorter in mares following a double ovulation. Only three mares in which the deslorelin implant was not removed were recorded as having a double ovulation, indicating that multiple ovulations were unlikely to be a contributory factor to longer interovulatory intervals in mares where the implant was not removed. The overall incidence of multiple pregnancies was not evaluated for the different treatment groups; however, triplets were confirmed in three mares, all of which had received Ovuplant[®].

The effect of year was evaluated to examine whether this could have had an influence on cycle length, possibly due to different numbers of early foaling mares, or more adverse weather conditions, which may be factors contributing to lactational anoestrus or mares being mated later in May or June, when oestrous cycle length has been found to be shorter (Daels and Hughes 1993). It would have been preferable to have a control group

of mares receiving hCG in 2010 also, however, due to farm management decisions this was not possible. As the mean difference in interovulatory intervals was not significantly different between years in the comparable groups (ONR) in 2009 and 2010, or between the control and REM groups, this limitation in the study design is unlikely to have significantly affected the results. Further studies matching mares between treatment groups according to age, status, month of foaling where applicable and month in which the mare received the ovulation induction agent would be desirable before drawing final conclusions about this matter. It should be noted that the second ovulation recorded to define the interovulatory interval was subsequent to the administration of the same ovulation induction agent used previously and was not a spontaneous ovulation. The effectiveness of the deslorelin implant as an ovulation induction agent (ovulation occurring within 48 hours in all but 3 of 65 cycles (4.6%) of mares in the current study, could be a reason for a less prolonged interovulatory interval than that reported in some earlier studies, however, as an ovulation induction agent will be used on almost all stud farms, this feature of the study reflects the normal situation in practice and therefore does not detract from the clinical usefulness of the information obtained.

Prolonged interovulatory periods in non-lactating mares administered PGF2 α post-ovulation (Johnson 2000, McCue 2002) and in foal heat mares following a deslorelin implant-induced ovulation have been reported, (Blanchard 2002) however, there are no reports in the literature of the effect of deslorelin implants in lactating mares short-cycled with PGF2 α or at the second and subsequent natural ovulations post-foaling. The increase in interovulatory interval in the non-lactating ONR mares in the current study was not statistically significant. Non-lactating mares did not routinely receive PGF2 α ,

which was only administered in some cases which were exhibiting a delay in returning to oestrus. A persistent corpus luteum was not confirmed by progesterone measurement in any mares and cannot be excluded, but the clinical impression was that there was minimal response (such as development of uterine oedema and a change in teasing behaviour) to PGF2 α administration. Results of the current study suggest that the down regulatory effects of deslorelin implants on GnRH secretion described by Farquhar and others (2002) and Johnson (2002) may be more significant in lactating mares with a tendency towards a greater increase in interovulatory interval at a PGF2 α induced oestrus and significant increase in interovulatory interval at the second or subsequent natural oestrus. It is possible that increasing milk production to meet the needs of the foal places additional nutritional demands on the mare at this time a decrease in body condition may affect pituitary function and sensitivity to GnRH agonists, however, the pathophysiology of lactational anoestrus in mares, which may aid our understanding of the response to deslorelin implants in the current and other studies, is not fully elucidated. In contrast to earlier studies (Blanchard 2002), the mean interovulatory interval in the foal heat mares in the current study did not appear to differ between mares which did or did not have the implant removed, however statistical analysis was not possible due to the small number of foal heat mares in the current study.

Conclusions

Results from the current field study show that failing to remove the deslorelin implant post-ovulation can result in a statistically significant increase in the interovulatory interval, which can be avoided if the implant is removed. This effect has a tendency to be

more pronounced in lactating mares covered at the PGF2 α - induced oestrus or second or subsequent natural oestrous period and in mares over 10 years of age, but in most cases, the delay in the subsequent ovulation is short (1-3 days) and unlikely to be of serious concern. This information may help to schedule repeat breeding or insemination more accurately in relation to the subsequent ovulation.

Table 1: Interovulatory intervals in the different treatment groups

Treatment Group	Number of cycles	Mean interovulatory interval (days)	Range (days)	No of cycles >22 days	Percent >22 days
Ovuplant [®] not removed	23	23.86*	19-37	14	60.8%
Ovuplant [®] removed	42	21.95	19-38	9	21.4%
Control (Chorulon [®])	23	21.86	19-27	7	30.4%

* Indicates significantly different to other groups.

Table 2 Effect of year on interovulatory intervals

Year	Number of cycles	Mean cycle length \pm s.e in days in control (2009) or REM (2010) mares	Mean cycle length \pm s.e in d mares
2009	32	21.86 \pm 0.44	23.25 \pm 0.68
2010	56	22.04 \pm 0.49	24.2 \pm 1.00

There were no significant differences between 2009 and 2010 for any of the treatment groups

Table 3 Effect of age of mare on cycle length between the three treatments

Age of mare (Years)	Control		REM		ONR
	No of cycles	Mean cycle length \pm s.e in days	No of cycles	Mean cycle length \pm s.e in days	No of cycles
Up to 10	16	21.05 \pm 0.67	28	22.28 \pm 0.47	14
Over 10	7	20.71 \pm 0.64	14	21.33 \pm 0.97	9

There was a significant positive correlation ($P<0.05$) between increasing age and interovulatory interval in the group of mares aged 10 years and over ($r=0.35$).

Table 4 Effect of mare status on cycle length within the three treatment groups

Mare status	Control		Ovuplant® removed		Ovuplant®
	n	Mean cycle length	n	Mean cycle length ±	
		± s.e in days		s.e in days	
Maiden/barren cycling	6	22.16±1.18	19	22.10±0.44	8
Maiden/barren end- transition	2	21.5±0.71	1	21±0	3
Foal heat	1	21±0	3	26.33±7.15	1
Foaling PG induced oestrus	5	23±0.79	3	20.67±1.47	2
Foaling 2 nd /subsequent natural oestrus	8	21.25±0.85	15	21.26±0.40	7

* Indicates statistically significant difference between treatment groups for mares (P=0.02).

The status was unknown for four mares which were covered shortly after arriving on the stud and these mares were excluded from the analysis.

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