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

I. S. F. Henderson, P. Brama, M. Osborne, M. E. Beltman

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 postovulation. Trial data in Australia indicate 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 were collected retrospectively from 88 oestrous cycles. A statistically significant difference (P=0.02) was found between interovulatory intervals in mares in which the deslorelin implant was not removed, compared with administration and removal of the implant or the use of human chorionic gonadotrophin. The results suggest that implant removal when possible is advisable. The delay in subsequent ovulations was less marked than that reported in some studies from the USA. This information is useful in deciding when to schedule subsequent breeding for mares which received a deslorelin implant during the previous oestrus 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.

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, 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). Longer interovulatory intervals were also reported in deslorelin-treated mares administered prostaglandin F2 alpha (PGF2α) at day 5 to 10 postovulation (Johnson and others 2000, Farquhar and others 2002, McCue and others 2002). Removal of the deslorelin implants postovulation 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 postovulation. Experimental data from Australia (Peptech Animal Health trial data 2010) indicated a 10.7 per cent incidence of oestrous cycles 26 to 30 days long and 4.5 per cent incidence of cycles exceeding 30 days when the implant was not removed. For mares receiving hCG, 7 per cent of cycles were 26 to 30 days in length while 4.5 per cent were greater than 30 days; for mares receiving no ovulation induction agent, these figures were 9.2 per cent and 3.9 per cent, respectively. Data from the same study in Kentucky, USA, showed that mares in which the implant was not removed experienced an incidence of 20.5 per cent of oestrous cycles 26 to 30 days long with 13.6 per cent in control mares and 11.5 per cent of cycles greater than 30 days with 15.6 per cent in control mares (Peptech Animal Health trial data 2010). These studies suggest 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 formulation of the implant used is identical to the one available in Australia, the USA and Europe. 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 with 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 one large stud in Ireland during the 2009 or 2010 breeding seasons, were analysed retrospectively. Seventy-six per cent of mares (217 mares) conceived during the first oestrous cycle in which they were covered. Sixty-seven mares did not conceive on the first cycle and data on subsequent, interovulatory intervals were available for inclusion in the study. Fifty-two mares conceived during the second cycle, 10 mares conceived on a third cycle, four mares conceived on the fourth cycle and one mare was covered on five cycles and failed to conceive. Data from...
evidence of premature luteal regression due to endometritis (defined as clinical signs of endometritis, as defined below). Implants containing 2.1 mg deslorelin acetate (Ovuplant Decha Sansaw Business Park) 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). 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. Postmating treatment of mares did not differ between mares visiting different stallions. Mares were examined using an Aloka SSD-500 V ultrasound scanner with a 5 MHz probe (Keymed (Ireland), Keymed House, C calmount Park). Mares were covered when a dominant preovulatory follicle reached 35 to 40 mm. Repeat 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 2009, mares that received hCG were treated at the time of covering, while in 2010 all mares received Ovuplant at the time of covering. Implants were administered either subcutaneously in the neck or in the vulval mucosa. Vulval implants were removed where possible once ovulation was confirmed. Lignocaine hydrochloride (Norocaine solution) was used to provide local anaesthesia to facilitate insertion and removal for injection, Norbrook laboratories, Rossmore Industrial Estate) was used to provide local anesthesia to facilitate insertion and removal of the implant which was removed postovulation, making removal impossible. The implant being placed too deeply, difficulty in palpating the implant in mares with significant fibrous scarring of the vulva following multiple Caslick’s procedures and mare temperament. In the case of three mares, which failed to ovulate within 48 hours, the implant appeared to have dis- solved by the time of ovulation, making removal impossible. The study suggests that even if efforts are made to remove the implant in most cases, circumstances would arise in which it was not feasible, as such 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 Caslick’s procedures and mare temperament. In the case of three 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. 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 would arise in which it was 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 Caslick’s procedures and mare temperament. In the case of three 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 two other treatment groups (Table 1). There was no significant difference in interovulatory intervals between mares in 2009 and 45 mares were covered on one or more cycles during 2010. The total number of oestrous cycles available for analysis was 52 in 2009 and 56 in 2010. Deslorelin implants were administered and not removed in eight of these cycles in 2009 and 15 cycles in 2010 (ONR, n=25). 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. 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There was no significant difference in interovulatory intervals between mares in the three treatment groups were analysed. The effect of mare status (mated or barren cycling, mated or barren mares covered at the first ovulation of the year (end-transitional), foal heat, second or subsequent natural oestrus postfoaling or PGF2α-induced oestrus postfoal heat) on interovulatory interval between the three treatment groups was also considered. Statistical analysis was performed via 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±SEM. The correlation between increasing age and interovulatory interval was calculated using Microsoft Excel (2007 for Windows).

Table 1: Interovulatory intervals in the different treatment groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of cycles</th>
<th>Mean interovulatory interval (days)</th>
<th>Range (days)</th>
<th>Number of cycles &gt;22 days</th>
<th>% of cycles &gt;22 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovuplant not removed</td>
<td>23</td>
<td>23.86±0.44</td>
<td>19-37</td>
<td>14</td>
<td>60.8%</td>
</tr>
<tr>
<td>Ovuplant removed</td>
<td>23</td>
<td>21.95±0.44</td>
<td>19-38</td>
<td>9</td>
<td>42.9%</td>
</tr>
<tr>
<td>Control (Chorulon)</td>
<td>23</td>
<td>21.86±0.42</td>
<td>19-27</td>
<td>7</td>
<td>30.4%</td>
</tr>
</tbody>
</table>

* Indicates significantly different from other groups

Table 2: Effect of year on interovulatory intervals

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cycles</th>
<th>Mean cycle length (days) in control (2009) or REM (2010) mares</th>
<th>Mean cycle length (days) in ONR mares</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>32</td>
<td>21.86±0.44</td>
<td>23.25±0.68</td>
</tr>
<tr>
<td>2010</td>
<td>56</td>
<td>22.04±0.49</td>
<td>24.21±0.00</td>
</tr>
</tbody>
</table>

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

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 52 in 2009 and 56 in 2010. Deslorelin implants were administered and not removed in eight of these cycles in 2009 and 15 cycles in 2010 (ONR, n=25). 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 would arise in which it was 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 Caslick’s procedures and mare temperament. In the case of three 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 two other treatment groups (Table 1). There was no significant difference in interovulatory intervals between mares in 2009 and 2010 for any of the treatment groups (Table 2). There was no significant difference in interovulatory intervals between mares in 2009 and 2010. There was no significant difference between the group ONR in 2009 and ORN 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 ovolations (synchronous to within 48 hours) not resulting in pregnancy were recorded in 12 mares, six in the CON group, three in the REM group and three in the ONR group. For cycles in which a double ovulation was recorded, the mean cycle length was 21.9 days, range 21 to 25 days. For mares with single ovulations, the mean cycle length was 22.5 days, range 19 to 38 days.

Interovulatory intervals were significantly longer (P=0.02) in ONR foaling mares covered at the second or subsequent natural
The results show a small but statistically significant difference in the interovulatory interval in ONR mares compared with CON mares (two days) and REM mares (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 with 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 foil-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 vs 21.8 ± 4.6 days in mares receiving a 2.2 mg 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 the 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 broodmares and managers to 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 to 40 mm in the current study; 40 to 42 mm 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, 35 mm in the study by McCue and others 2002). Mumford and others (1995) inserted the implant at a follicle size of 30 mm and reported shorter interovulatory intervals than the current study. Although Vanderwall and others (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 57.1 ± 1.1, rather than 40.1 ± 0.6 mm, this was not statistically significant (P<0.1). 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 55 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 to 19 years and 20 years or over had a reduced ovulation rate in response to a deslorelin implant (57.9 per cent and 55.8 per cent, respectively) when compared with mares aged two to four years (90.2 per cent ovulation rate) or five to nine years (91.0 per cent 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 with mares five to seven years of age (23.9 days) or 15 to 19 years (23.0 days). This longer interovulatory interval appeared to be due to a longer follicular phase (11.7 days vs 9.4 days in the five to seven year age group and 8.0 days in the 15 to 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, a significant positive correlation was noted between increasing age and cycle length in CON mares. Barbacini and others (2000) reported that failure to ovulate within 48 hours of administration of hCG was more common in barren mares and mares over 16 years old. There are conflicting reports in the literature regarding a decrease in the response to repeated doses of hCG. Sullivan and others (1975) reported a reduction in the efficacy of hCG when it was used more than twice during a breeding season and Wilson and others (1990) observed an increase in the mean interval from hCG administration to ovulation when hCG was used during five consecutive oestrous cycles. Blanchard and others (2003) found no decrease in response rates when hCG was used in up to four oestrous cycles during a season and the presence of antibodies to hCG was not associated with a decrease in efficacy (Roser and others 1979). It is possible that the control mares experienced a reduced response to the second administration of hCG, leading to a longer interovulatory interval. 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, age was unlikely to have contributed to the overall finding of longer interovulatory intervals in this group.

### TABLE 3: Effect of age of mare on cycle length between the three treatments

<table>
<thead>
<tr>
<th>Age of mare (years)</th>
<th>Number of cycles</th>
<th>Control Mean cycle length±se in days</th>
<th>Number of cycles</th>
<th>REM Mean cycle length±se in days</th>
<th>Number of cycles</th>
<th>ONR (Ovuplant Not Removed) Mean cycle length±se in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 10</td>
<td>16</td>
<td>21.05±0.67</td>
<td>28</td>
<td>22.28±0.47</td>
<td>14</td>
<td>22.63±0.58</td>
</tr>
<tr>
<td>Over 10</td>
<td>7</td>
<td>20.71±0.64</td>
<td>14</td>
<td>21.33±0.97</td>
<td>9</td>
<td>23.44±1.12</td>
</tr>
</tbody>
</table>

There was a significant positive correlation (P<0.05) between increasing age and interovulatory interval in the group of control mares only (r=0.35) REM Removed postovulation.

### TABLE 4: Effect of mare status on cycle length within the three treatment groups

<table>
<thead>
<tr>
<th>Mare status</th>
<th>n</th>
<th>Control Mean cycle length±se in days</th>
<th>n</th>
<th>Ovuplant removed Mean cycle length±se in days</th>
<th>n</th>
<th>Ovuplant not removed Mean cycle length±se in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maiden/barren cycling</td>
<td>6</td>
<td>22.16±1.18</td>
<td>19</td>
<td>22.10±0.44</td>
<td>8</td>
<td>23.37±0.92</td>
</tr>
<tr>
<td>Maiden/barren end transition</td>
<td>2</td>
<td>21.5±0.71</td>
<td>1</td>
<td>21±0</td>
<td>3</td>
<td>21.33±0.41</td>
</tr>
<tr>
<td>Foal heat</td>
<td>2</td>
<td>21±0</td>
<td>3</td>
<td>26.33±7.15</td>
<td>2</td>
<td>24±0</td>
</tr>
<tr>
<td>Foaling PG-induced oestrus</td>
<td>5</td>
<td>23±0.79</td>
<td>3</td>
<td>20.67±1.47</td>
<td>2</td>
<td>24±0</td>
</tr>
<tr>
<td>Foiling second/subsequent natural oestrus</td>
<td>8</td>
<td>21.25±0.85</td>
<td>15</td>
<td>21.26±0.47</td>
<td>7</td>
<td>24.92±2.24</td>
</tr>
</tbody>
</table>

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

* Indicates statistically significant difference between treatment groups for mares (P<0.02)
The small number of mares in their later teens and 20s 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 these data may be of particular relevance for gestation. This may be from any of which may begin a second cycle prior to the administration of deslorelin implants. Longer oestrus cycles have also been reported in mares after double ovulations (24.0 days following a double ovulation vs 20.8 days for single ovulations) (Urwin and Allen 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. It is possible that the use of the deslorelin implants may stimulate smaller follicles which may otherwise have regressed, to ovulate; however, a study published by Louvis and others (1997) which examined the effect of deslorelin implants on twinning rates found no significant difference between the incidence of twins in treated and control mares. Stallion factors (particularly longevity of spermatozoa) may contribute to the incidence of multiple pregnancies when asynchronous or dioestrous oovulations occur; however, the size of the multiple concepts did not suggest marked asynchrony in the timing of the oovulations.

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 have contributed to the prolonged anovulatory status of mares being mated later in May or June, when oestrus cycle length has been found to be shorter (Daels and Hughes 1995). It would have been preferable to have a CON group 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 CON 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 time required to determine the length of 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 45 hours in all but three of 65 cycles) (4.6 per cent) 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 PGF2α postfoaling (Johnson 2000, McCue 2002) and 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 PGF2α or at the second and subsequent natural ovulations postfoaling. 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 PGF2α, which was only administered in some cases which were exhibiting a delay in returning to oestrus. A persistent CL 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 PGF2α administration. Results of the current study suggest that the downregulatory 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 PGF2α-induced oovulation and significant increase in interovulatory interval at the second or subsequent natural oovulation. It is possible that increasing milk production to meet the needs of the foal places additional nutritional demands on the mare at this time, or prolactin secretion may have a negative effect on pituitary gonadotrophin production, resulting in an increased sensitivity to downregulatory effects of GnRH analogues; 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.

In conclusion, results from the current field study show that failing to remove the deslorelin implant postovulation 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 mares over 10 years of age and lactating mares covered at the PGF2α-induced oovulation and was statistically significant in lactating mares covered at the second or subsequent normal oovulatory period, but in most cases the delay in the subsequent oovulation is short (one to three 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 oovulation.

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