The Influence of Progesterone During Early Pregnancy in Cattle

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Early pregnancy in the cow

In dairy cattle, failure to conceive to a particular mating represents a major constraint to fertility. Current estimates suggest that in the UK only around 50% of dairy cows calve to a particular insemination indicating that in 50% of cows pregnancy fails. The timing and extent of these losses has been reviewed extensively by Sreenan and Diskin (1986) and more recently by Peters (1996). These reviews report a fertilisation rate of around 90%. Pregnancy rate then falls to around 80% by day 10-13 due to failed embryo development. By day 19 this figure has fallen to around 60-65% due to failure of the embryo to prevent luteolysis and maintain the secretion of progesterone necessary for its continued development. Thus around 25-30% of pregnancies fail due to early embryo mortality. Further late embryo losses of around 5-10% and an abortion rate of around 5% lead to a final calving rate of around 50%. From these figures, based on a number of studies, it can clearly be seen that the most important factor contributing to early pregnancy failure is embryo mortality. This mortality results partly from the direct failure of the embryo to develop, but more commonly from the failure of the embryo to prevent luteolysis and maintain the pregnancy.

The establishment of pregnancy in the cow

In the cow the establishment of pregnancy depends on the effective functioning of an endocrine communication system between the mother and the embryo. This system underpins the decision, by the cow, to either maintain the corpus luteum and thus the pregnancy or to undergo luteolysis and reovulate, generating a new opportunity to become pregnant. In cyclic cows, luteolysis results from the release of luteolytic episodes of uterine PGF2α, produced predominantly by the luminal epithelial cells of the uterine endometrium (Fortier et al. 1988). An important component of the luteolytic mechanism is the development of oxytocin receptors on the luminal epithelium of the endometrium as this allows oxytocin to exert a stimulatory action on PGF2α secretion. In the absence of luteal oxytocin it has been demonstrated that the release episodes of PGF2α exhibit a lower amplitude and longer duration in both sheep (Mann and Lamming 1995a) and cows (Mann and Peters, unpublished observation). During early pregnancy in the cow, the embryo inhibits oxytocin stimulated PGF2α release by inhibiting the development of oxytocin receptors on the luminal epithelium (Mann et al. 1995a) and through the induction of a prostaglandin synthesis inhibitor (Thatcher et al. 1995). The embryo achieves this by producing a protein, interferon-τ, which acts locally within the uterus to inhibit PGF2α secretion. In the cow, mRNA for interferon-τ is first detected in the trophectoderm, the principle site of production, on around day 12 and is maximal on days 15-16 (Farin et al., 1990). Interferon-τ protein is first detected in significant quantities in uterine flushes between days 14-16, when embryos have begun elongation (Mann et al. 1998a). To prevent luteolysis the embryo must be sufficiently well developed to allow the secretion of sufficient interferon-τ to prevent luteolytic PGF2α secretion. Poor embryo development is associated with low interferon-τ production, failed inhibition of luteolysis and embryo loss (Mann et al. 1996, Mann et al. 1998b). A full understanding of the control of embryo development and interferon-τ production is therefore, of paramount importance in determining strategies to reduce the high level of early embryo mortality experienced in dairy cattle. The principle hormone controlling this process is progesterone.
Progesterone levels and the outcome of early pregnancy
It has been established for many years that the concentration of progesterone during early pregnancy has a marked effect on the outcome of insemination. A number of studies have revealed lower concentrations of progesterone in both milk (Lamming et al. 1989, Mann et al. 1995) and plasma (Lukaszewska and Hansel 1980, Mann et al. 1995) in inseminated cows in which pregnancy fails than in cows in which pregnancy is successfully established. These studies have demonstrated lower progesterone from around day 10-12 following insemination. It should be noted, however, that the success of early pregnancy cannot be defined by the attainment of a particular progesterone level. For example, in the study of Mann et al. (1995), mean plasma progesterone concentration from day 12-17 following insemination was 8.2±0.2ng/ml in those cows in which insemination was successful and 6.6±0.2ng/ml in those cows in which pregnancy failed. However the ranges of progesterone concentrations in the individual cows were 5.7-12.3ng/ml and 4.8-10.5ng/ml in the two groups respectively. Thus maternal progesterone concentration should not be regarded as an absolute determinant of early pregnancy, but rather as a factor which influences the probability of success or failure.

As well as lower progesterone from day 10-12 onwards, low progesterone much earlier in the cycle has been implicated in pregnancy failure. In 1971 Henricks et al. reported lower concentrations of progesterone 6 days post mating in cows with a failed pregnancy. A delay in the post ovulatory rise in progesterone results in impaired embryo development (Mann et al. 1998a). A delay in the post ovulatory rise in progesterone has also been demonstrated to result in a marked reduction in pregnancy rate in mated animals (Lamming and Darwash 1995). Darwash and Lamming (1998) demonstrated that in inseminated cows, progesterone rises (to >3ng/ml milk) on day 4 following insemination in cows becoming pregnant compared with day 5 in cows in which insemination did not result in successful pregnancy. Thus a delay of only one day in the post ovulatory rise in progesterone can have a dramatic negative effect on pregnancy rate.

The influence of progesterone on embryo development
It is well established that progesterone plays a major role in stimulating the production of a variety of endometrial secretions necessary for the successful development of the embryo (see Geisert et al. 1992 for review). The importance of maternal progesterone in this process is further emphasised by numerous asynchronous embryo transfer studies where appropriate treatment of donor animals with progesterone can render their uteri receptive to embryos transferred from asynchronous donors (Lawson and Cahill 1983). We have recently demonstrated the importance of the pattern of maternal progesterone secretion on the development of the embryo in the cow (Mann et al. 1998a). On day 16 following insemination poorly developed embryos producing little or no interferon-τ were found in cows that had exhibited a late post ovulatory rise in progesterone to low luteal phase concentrations. Conversely cows with an earlier rise in progesterone to high luteal phase concentrations possessed embryos that were well elongated and which produced large quantities of interferon-τ. The importance of the post ovulatory progesterone rise in the control of embryo development has been demonstrated by a number of other studies. In ewes, Nephew et al., (1991) demonstrated that a one day delay in the post ovulatory progesterone rise resulted in retarded embryo development on day 13 with an associated 3 fold decrease in production of interferon-τ. In cattle, Garrett et al. (1988) found that by increasing progesterone from days 2 - 5, embryo development on day 14 was advanced significantly (10 fold increase in conceptus length). In a recent study, Kerbler et al. (1997) demonstrated only a slight increase in interferon-τ production on day 18 by bovine embryos collected from cows in which progesterone had been artificially increased from around day 8 by induction of accessory corpora lutea. Thus it would appear that it is the post ovulatory progesterone rise and not the level of progesterone secretion finally achieved that is most important in the control of embryo development in the cow. This is supported by a recent study in
which administration of supplementary progesterone from day 5-9, but not from day 12-16 resulted in a significant increase in interferon-\(\tau\) production on day 16 (Mann et al. 1998b). While administration of progesterone has been shown to increase endometrial protein secretion (Garrett et al. 1988), the specific nutrients and growth factors involved in the control of early embryo development and the mechanisms by which their secretion is controlled are poorly understood. Recent work in the cow has, however, identified the uterine IGF system as an important component in the mechanism controlling the development of the early embryo (Wathes et al. 1998a, Wathes et al. 1998b).

**The influence of progesterone on the luteolytic mechanism**

As well as its effects on embryo development, progesterone is also responsible for controlling the development of the luteolytic mechanism in the mother. In cattle, a low level of luteal phase progesterone leads to a stronger release of luteolytic PGF\(_{2\alpha}\) from the uterus (Mann and Lamming 1995b), thus presenting the developing embryo with a larger inhibitory task. In cyclic cows, administration of progesterone early in the luteal phase to advance the post ovulatory progesterone rise shortens the luteal phase (Burke et al. 1994), demonstrating the ability of the early progesterone rise to control the timing of subsequent luteolysis. It would follow that delaying the progesterone rise might be expected to delay luteolysis and increase cycle length. However, this theory would be difficult to test in intact cows as it is hard to delay the rise in progesterone in cyclic animals without affecting other aspects of the cycle. We have previously shown that treatment of ovariectomized cows with progesterone and oestradiol to recreate normal oestrous cycle plasma concentrations, results in the development of an active population of endometrial oxytocin receptors and PGF\(_{2\alpha}\) release to exogenous oxytocin challenge, analogous to the luteolytic signal in intact animals (Lamming and Mann 1995). Using this model we have shown that while advancing the rise in progesterone advances luteolysis, delaying the progesterone rise does not delay luteolysis (Mann et al. 1994, Mann et al. 1998c). Lamming and Darwash (1995) have studied this finding in intact cows. These authors found that for every one day delay in the time of the progesterone rise (after day 5) there was a 0.7 day shortening of the subsequent luteal phase. Thus a late progesterone rise was associated with luteolysis at the normal time resulting in a reduced length of the period of progesterone secretion. This progressive shortening of the luteal phase was accompanied by a progressive reduction in conception rate, demonstrating the detrimental effect of a reduced period of progesterone secretion on embryo survival.

**The effect of progesterone supplementation on the outcome of pregnancy**

The knowledge that progesterone is a vital hormone during early pregnancy and associated with the control of early embryo development has prompted a plethora of studies in which progesterone supplementation has been utilise in an attempt to improve pregnancy rate in cattle (Table 1). While many studies have show benefit of this approach others have not and in many studies numbers of cows are too small to allow worthwhile statistical analysis to be employed. An overall analysis of these studies (Table 2) shows a moderate, though highly significant, 5\% enhancement of pregnancy rate following progesterone supplementation. However, by breaking down the figures some very interesting trends become apparent. If the time of treatment is examined it can be seen that if progesterone treatment is commenced after day 6 no benefit results while if treatment is started before day 6 a 10\% improvement in pregnancy rate results. Furthermore, if initial pregnancy rate was low (<50\%) a large improvement is seen following progesterone treatment (19\%) whereas if initial conception rate is high (>50\%) no benefit is seen. Thus early supplementation or supplementation of cows with low fertility results in significant benefits in terms of pregnancy rate while later supplementation or supplementation of cows with good initial conception rates, while not detrimental to conception rate, confers no benefit.
Table 1. Summary results of a number of studies carried out in a wide variety of cattle populations in a number of different environments to investigate the effects of progesterone supplementation on pregnancy rate. The studies involved a variety of supplementation methods carried out over various time periods following insemination (column 1 indicates the day on which supplementation commenced). In many studies too few cows were included to allow a meaningful statistical analysis of the results to be performed.

<table>
<thead>
<tr>
<th>Start day</th>
<th>Control</th>
<th>Treated</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0% (1/20)</td>
<td>35.0% (7/20)</td>
<td>+ 30.0%</td>
<td>Herrick (1953)</td>
</tr>
<tr>
<td>4</td>
<td>16.7% (3/18)</td>
<td>46.8% (22/47)</td>
<td>+ 30.1%</td>
<td>Dawson (1954)</td>
</tr>
<tr>
<td>3</td>
<td>29.9% (20/67)</td>
<td>41.8% (28/67)</td>
<td>+ 11.9%</td>
<td>Wiltbank et al. (1956)</td>
</tr>
<tr>
<td>2 - 9</td>
<td>37.7% (26/69)</td>
<td>70.0% (49/70)</td>
<td>+ 32.3%</td>
<td>Johnson et al. (1958)</td>
</tr>
<tr>
<td>5</td>
<td>45.0% (9/20)</td>
<td>73.7% (14/19)</td>
<td>+ 18.7%</td>
<td>Sreenan and Diskin (1983)</td>
</tr>
<tr>
<td>10</td>
<td>61.1% (102/167)</td>
<td>65.4% (102/156)</td>
<td>+ 4.3%</td>
<td>Sreenan and Diskin (1983)</td>
</tr>
<tr>
<td>5</td>
<td>40.0% (26/65)</td>
<td>47.5% (29/61)</td>
<td>+ 7.5%</td>
<td>Diskin and Sreenan (1986)</td>
</tr>
<tr>
<td>5</td>
<td>30.0% (9/30)</td>
<td>60.7% (17/28)</td>
<td>+ 30.7%</td>
<td>Robinson et al. (1989)</td>
</tr>
<tr>
<td>10</td>
<td>30.0% (9/30)</td>
<td>59.3% (16/27)</td>
<td>+ 29.3%</td>
<td>Robinson et al. (1989)</td>
</tr>
<tr>
<td>5</td>
<td>57.1% (8/14)</td>
<td>68% (17/25)</td>
<td>+ 10.9%</td>
<td>Walton et al. (1990)</td>
</tr>
<tr>
<td>10 - 16</td>
<td>67.0% (421/628)</td>
<td>64.3% (317/493)</td>
<td>- 2.7%</td>
<td>Macmillan et al. (1991)</td>
</tr>
<tr>
<td>14</td>
<td>63.6% (300/472)</td>
<td>64.0% (329/514)</td>
<td>+ 0.4%</td>
<td>Macmillan et al. (1991)</td>
</tr>
<tr>
<td>4</td>
<td>66.3% (309/466)</td>
<td>74.6% (344/461)</td>
<td>+ 8.3%</td>
<td>Macmillan et al. (1991)</td>
</tr>
<tr>
<td>13</td>
<td>42.4% (39/92)</td>
<td>50.0% (18/36)</td>
<td>+ 7.6%</td>
<td>Stevenson &amp; Mee (1991)</td>
</tr>
<tr>
<td>7</td>
<td>53.6% (83/155)</td>
<td>57.9% (92/159)</td>
<td>+ 4.3%</td>
<td>Van Cleef et al. (1991)</td>
</tr>
<tr>
<td>3</td>
<td>34.9% (22/63)</td>
<td>47.8% (32/67)</td>
<td>+ 12.9%</td>
<td>Larson and Butler (1995)</td>
</tr>
<tr>
<td>10</td>
<td>53.3% (72/135)</td>
<td>56.0% (75/134)</td>
<td>+ 2.7%</td>
<td>Mann et al. (1998d)</td>
</tr>
</tbody>
</table>

Table 2. An overall analysis of the progesterone studies presented in Table 1. An overall analysis of progesterone supplementation was carried out as well as an analysis based on day of commencement of supplementation (before or after day 6) and an analysis based on the initial conception rate of the control cows (less than or greater than 50% conception). In all cases statistical comparison of the treatment effect was carried out by Chi-Square analysis (ns = p>0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Treated</th>
<th>Effect</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>58.1% (1459/2511)</td>
<td>63.3% (1508/2384)</td>
<td>+ 5.2%</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Start &lt; day 6</td>
<td>54.6% (406/743)</td>
<td>64.9% (503/775)</td>
<td>+10.3%</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Start &gt; day 6</td>
<td>61.1% (1026/1679)</td>
<td>62.5% (949/1519)</td>
<td>+1.4%</td>
<td>ns</td>
</tr>
<tr>
<td>Control &lt; 50%</td>
<td>34.3% (124/362)</td>
<td>53.6% (207/386)</td>
<td>+19.3%</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Control &gt; 50%</td>
<td>62.7% (1334/2129)</td>
<td>65.4% (1294/1978)</td>
<td>+ 2.7%</td>
<td>ns</td>
</tr>
</tbody>
</table>

Conclusions
It can clearly be seen that progesterone has a major influence on the outcome of pregnancy in the cow, controlling the development of both the embryo and the luteolytic mechanism. During early pregnancy the synchrony between these two events is critical to the outcome of pregnancy. The development of a luteolytic signal before the embryo has produced sufficient interferon - τ to prevent this will result in luteolysis and the loss of the pregnancy. Through its controlling influence on these two processes, progesterone acts to maintain...
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The necessary synchrony between embryo development and the inhibition of the luteolytic mechanism. The benefits of early progesterone on both embryo development and pregnancy rate suggest that while progesterone concentrations during the luteal phase are an important determinant of the outcome of pregnancy, they do not appear to be as important as the timing of the post ovulatory progesterone rise.

Acknowledgements
Many of the studies included in this review were funded by the Ministry of Agriculture Fisheries and Food and the Milk Development Council under the Link Sustainable Livestock Production Programme. This support is gratefully acknowledged.

References
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