INTRODUCTION

Retention of fetal membranes after parturition has been a problem that has been described in the earliest texts on bovine reproduction. For example, Strebel, 1890, reviewed the occurrence and treatment of retained placenta in domestic animals. Nonetheless, it continues to an important problem on many cattle operations. Published studies on the incidence of retained placenta report from 3% to 39% of calvings (Joosten et al., 1987; Laven and Peters, 1996) but this rate depends on the definition of retained placenta. A more complete analysis of the timing of placental retention is provided in Table 1.

As can be seen there were a total of 1010 cows evaluated in this study and 34% had not lost their placenta by 6 h after calving. In other words, about 2/3 of the cows had lost their placenta by 6 h. There were about 15% of cows that retained the placenta for more than 1 d (>23 h). If we examine the effect of parity it seems clear that as cows get older they have a greater tendency to retain their placenta using any of the times. For example, there are about 28% of older cows (more than 3 lactations) that retain their placenta >12h. However, there are only 10% of first lactation heifers that retain their placenta more than 12 h.

The conclusion from this study is that retained placenta may be any cow that does not lose the placenta by 6 h after parturition. About 1/3 of cows fall into this category. If a

Table 1. Incidence of retained placenta (vanWerven et al., 1992.)

<table>
<thead>
<tr>
<th>Duration of Parity Retained Placenta</th>
<th>1</th>
<th>2&amp;3</th>
<th>&gt;3</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours</td>
<td>n=277</td>
<td>n=394</td>
<td>n=339</td>
<td>n=1010</td>
</tr>
<tr>
<td>&gt;6</td>
<td>27%</td>
<td>33%</td>
<td>41%</td>
<td>34%</td>
</tr>
<tr>
<td>&gt;8</td>
<td>16%</td>
<td>26%</td>
<td>30%</td>
<td>25%</td>
</tr>
<tr>
<td>&gt;12</td>
<td>10%</td>
<td>20%</td>
<td>28%</td>
<td>18%</td>
</tr>
<tr>
<td>&gt;23</td>
<td>8%</td>
<td>17%</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td>&gt;47</td>
<td>7%</td>
<td>14%</td>
<td>17%</td>
<td>13%</td>
</tr>
<tr>
<td>&gt;71</td>
<td>7%</td>
<td>14%</td>
<td>14%</td>
<td>12%</td>
</tr>
</tbody>
</table>
definition of lack of placental loss by 24 h is used as the definition of retained placenta then about 15% of cows will have this problem and the incidence is about twice as frequent in older cows.

The average economic costs of retained placenta have been estimated at $285 per event. (Kimura et al., 2002). However, this cost can vary dramatically for individual cows particularly related to the parity of the cow. In the vanWerven et al., 1992 study, the reproductive performance of first parity heifers and of second and third parity cows was not significantly affected by the duration of placental retention. For first parity heifers there was no cut-off point at which delayed expulsion of the placenta became detrimental to any production or reproduction variable that was measured in the study. However, fourth parity and higher cows showed the best reproductive performance when they expelled their placenta within 6 hours. Retention of the placenta for longer than 6 hours resulted in an increase of 17 days to the first service and 26 additional days open. Older cows also showed decreased milk production with an increase in the duration of retention. Second and third parity cows as well as fourth parity and higher cows demonstrated the highest overall performance in all the parameters tested when they expelled their placentas within 6 hours after parturition. Table 2 shows an example of the type of data generated in this study. The only statistically significant problem in first parity heifers is a slightly higher incidence of mastitis in cows that had retained placenta more than 6 h (4% vs. 9% during the first week). In older cows there was a reduction in milk production in cows with retained placenta. The decrease in reproductive efficiency in the older cows that had retained placenta is also illustrated. Values that are significantly different are shown in bold. Most studies show large variation between farms in the effect of retained placenta on mastitis and reproduction; however, most studies show that older cows have the most problems after having retained placenta.

There is also a great deal of variability in incidence of RP between farms. Some farms have as low as 1% incidence of RP and some have as high as 50%. Some studies have found a high incidence of RP with dramatic effect of treatments on reducing the incidence; whereas,

**Table 2.** Association of various parameters with retained placenta (retained >6h).

<table>
<thead>
<tr>
<th>Age</th>
<th>RP</th>
<th>n</th>
<th>Milk Production (100 d)</th>
<th>Culling Rates Overall</th>
<th>Repro</th>
<th>Mastitis First Week</th>
<th>First AI</th>
<th>Preg Rate Per AI</th>
<th>Days Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Parity</td>
<td>≤6</td>
<td>202</td>
<td>2400 kg</td>
<td>23%</td>
<td>20%</td>
<td>4%</td>
<td>79d</td>
<td>47%</td>
<td>109d</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>75</td>
<td>2309 kg</td>
<td>28%</td>
<td>10%</td>
<td>9%*</td>
<td>76d</td>
<td>48%</td>
<td>113d</td>
</tr>
<tr>
<td>2nd or 3rd Parity</td>
<td>≤6</td>
<td>265</td>
<td>3256 kg</td>
<td>18%</td>
<td>30%</td>
<td>4%</td>
<td>75d</td>
<td>44%</td>
<td>106d</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>130</td>
<td>3112 kg*</td>
<td>23%</td>
<td>37%</td>
<td>6%</td>
<td>77d</td>
<td>41%</td>
<td>115d</td>
</tr>
<tr>
<td>4+ Parity</td>
<td>≤6</td>
<td>199</td>
<td>3479 kg</td>
<td>38%</td>
<td>24%</td>
<td>6%</td>
<td>71d</td>
<td>47%</td>
<td>95d</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>140</td>
<td>3347 kg*</td>
<td>36%</td>
<td>35%</td>
<td>9%</td>
<td>88d*</td>
<td>41%</td>
<td>121d*</td>
</tr>
</tbody>
</table>
other studies have found a low incidence and many times no effect of treatments on the incidence of retained placenta. Thus, the incidence and impact of retained placenta is highly variable. Age of the cow and farm-specific factors are obviously important in producing the condition and in causing an impact of this condition on production and reproduction.

CAUSES AND PREVENTION OF RETAINED PLACENTA:

The placenta must remain tightly attached to the maternal uterine lining to maintain the nutrient and oxygen exchange that is required for normal pregnancy. However, the membranes must be very quickly broken down at the time of parturition in order to have a normal expulsion of the fetal membranes. Obviously this process of placental breakdown must be precisely controlled to allow continued function until the correct time for loss of the placenta.

There are a number of risk factors that have been identified for retained placenta. For example: abortion, stillbirth, twin births, dystocia, induction of parturition, metabolic disorders, and short gestation length have all been associated with a high incidence of retained placenta (Laven and Peters, 1996). Nevertheless, only about a third of retained placentas are associated with these risk factors. Normal pregnancy and delivery is still associated with a surprisingly high incidence of retained placenta. For example in a study with an overall incidence of 6.6% in retained placenta, the normally calving cows that had no other health problem that could be identified still had a 4.1% incidence (Joosten et al., 1987; 1991). Thus, there are clear risk factors for RP but much of the problem of RP is not accounted for by these risk factors.

Some authors have suggested that lack of uterine contractility may be the primary cause of retained placenta. However, most recent work has indicated that uterine contractility, while somewhat reduced in cows with retained placenta, appears to be more than sufficient to produce fetal membrane expulsion. Most recent data has suggested that the main cause of retained placenta is due to a lack of breakdown of the caruncle-cotelydon attachments and not to a lack of contractility of the uterus (Lavern and Peters, 1996; Davies et al., 2004; Martins et al., 2004).

In order to understand the underlying causes of retained placenta, 5 well-characterized causes of retained placenta will be introduced at this time. A model will then presented that may help in understanding these causes of retained placenta based on the current body of evidence that is available. Finally, the use of this model to explain the reason for retained placenta with these conditions and to formulate prevention programs will be discussed.

**Five conditions closely associated with retained placenta:**

1. Inbreeding or genetic similarity between dam and calf: When a dam and her calf have MHC class I compatibility or identity there is a high likelihood of RP (Joosten et al., 1991).

2. Induction of parturition with prostaglandin F2α: When cows were treated with
PGF2α on day 276 of pregnancy, parturition occurred much earlier (In 44±2 h after treatment compared to 163±30 h in controls). However, there was a high incidence of retained placenta (86%) with an average time of placental retention of 153±25 h (Rasmussen et al., 1996). This high retained placenta was not reduced by increasing E2 concentrations (50 mg estradiol-benzoate).

3. High stress prior to calving: Cows that are exposed to overcrowding, transportation, or other forms of stress are much more likely to have an increased incidence of retained placenta. Indeed, Peters and Bosu, 1987 showed that the one hormone most associated with RP was elevated cortisol for the 5 days prior to calving. In Figure 1 is shown the results of a study from Brazil on circulating hormones and RP (Wischral et al., 2001).

**Figure 1.** Changes in circulating hormones in cows with retained placenta (RP; triangles) or without retained placenta (NRP; squares).
Clearly, circulating progesterone was not different between RP and NRP indicating that changes in progesterone concentrations are not the underlying cause of RP. Circulating estradiol was also not different prior to calving indicating that it is unlikely to be a primary cause of retained placenta. It was different after calving probably because the placenta remained functional and intact in these cows. In contrast, cortisol was more than twice the concentration in NRP than RP prior to calving suggesting the high circulating cortisol may be an important component of the RP process.

4. Induction of parturition with dexamethasone or other long-acting glucocorticoids: Induction of parturition with dexamethasone results in a predictable time of calving (30-48 h later) but also an unacceptably high rate of retained placenta (50-90%).

5. Low selenium or vitamin E: In cows with low circulating selenium or vitamin E concentrations there is a marked increase in the rate of retained placenta ((LeBlanc et al., 2004; Allison and Laven, 2000).

A Model for Normal and Delayed Placental Expulsion

Figure 2 shows the initial events in the parturition process. Cortisol on the fetal side is the first critical initiator of this process. This increase in fetal cortisol comes from the fetal adrenal gland. The fetal adrenal gland is stimulated by ACTH due to maturation of the fetal hypothalamus and stress axis near the time of expected parturition. This process causes fetal cortisol to reach a critical level by about 30 h prior to normal parturition. It should be noted that this increase in circulating cortisol concentration is only on the fetal side of the circulation. Any increase in circulating cortisol in the maternal circulation is due to production of cortisol by the maternal adrenal gland.

There is a population of large granulated binucleate cells in the trophectoderm (fetal side) of the placenta that play a critical role in the parturition process. These cells produce placental lactogen and pregnancy-associated glycoproteins. They account for about 15-20% of the cells in the ovine and bovine trophectoderm during most of pregnancy but they dramatically decrease near the time of parturition at the same time as the increase in circulating cortisol in the fetus (Ward et al., 2002). Fetal adrenalectomy prevented the normal migration and decline in binucleate cells. Conversely, treating the fetus with exogenous cortisol prior to the expected time of parturition caused a premature migration of binucleate cells and decline in their number (Wooding et al., 1986; Ward et al., 2002). Thus, the increase in cortisol causes migration of binucleate cells from the fetal side of the placenta to the maternal side. As these cells migrate there is a dramatic spike in the concentrations of placental lactogen and other pregnancy-specific proteins as these cells degranulate. These cells fuse with the endometrial epithelium and express MHC class I on their surface. Prior to this time there is almost no MHC class I expression on bovine placentomal, endometrial epithelial cells. The villous/crypt portion of the placentome is the area of tight attachment and nutrient exchange. Very little, if any, MHC class I expression is occurring prior to binucleate cell migration in these areas (Davies et al., 2000). There is, however, MHC class I expression
in other regions of the placenta such as between placentomes. The mechanisms causing lack of MHC class I expression in the placental crypts until the time of parturition are not yet known but are probably critical for proper timing of placental release.

Gunnink, 1984 was the first scientist to suggest that an immune inflammatory response was responsible for normal placental separation. These elegant studies demonstrated that the bovine placentomes secrete a chemotactic factor that they felt attracted leukocytes (potentially this was actually monocytes from the buffy coat; Kimura et al., 2002). In addition, they found that cows with retained placenta had less of this chemoattractant and that leukocytes from cows with retained placenta were much less responsive to the placental chemotactic factor. More recent studies (see Kimura et al., 2002), have shown that neutrophils seem to be the more likely factor involved in the immune rejection of the placenta and that cows with RP had decreased neutrophil function. Thus, cows with RP have reduced maternal immune function and this may prevent the immunological rejection of the placenta and may be the underlying cause for the RP.

How does this model help explain the five conditions that cause retained placenta:

1. Inbreeding: This is fairly obvious. If the MHC Class I molecules are identical
between the dam and calf then the maternal immune system will not recognize the fetal cells as foreign. Even in cases without complete identity, the greater the similarity between the MHC Class I molecules in the dam and calf, the greater the likelihood of retained placenta. This is obviously because the immune system would not attack the placenta to cause a breakdown of placental tissue at the time of placental expulsion.

2. Induction of parturition with PGF2a: This also seems fairly straightforward. Treatment with PGF2a prior to the normal time of parturition will cause regression of the corpus luteum. Regression of the corpus luteum will decrease progesterone concentrations and initiate the calving process. However, the calving process will be initiated without any rise in cortisol concentrations in the fetal circulation. Therefore, there will not be migration of binucleate cells to the maternal circulation. This lack of migration will prevent the rise in MHC Class I expression in the placental crypts and so the maternal immune system will not reject the fetal placenta at the time of parturition. In other words, the final stages of placental maturation have not occurred because PGF2a induced parturition with binucleate cell migration.

3. Stress: This appears to be a major cause of retained placenta in many dairy herds. Nevertheless, it may not be easily clear why the increase in cortisol will increase retained placenta. Obviously, increased stress will increase circulating cortisol concentrations. An increase in circulating cortisol is the classical hormonal marker of stress. High cortisol will also dramatically inhibit immune system function. This fact is used to help prevent rejection by the immune system of tissue and bone marrow transplants. Unfortunately, in this situation, the high circulating cortisol concentrations on the maternal side of the circulation serve to inhibit the rejection of the placenta by inhibiting the activity of the maternal immune system. Thus, stress by dramatically elevating cortisol during the week prior to parturition, will inhibit immune rejection of the placenta and therefore increase the rate of retained placenta.

The mechanism described above is based on a good deal of physiological logic but has not been experimentally tested at this time. Nevertheless, it also matches much of my experience in observing retained placental rates on farms. Farms that expose their cows to a high degree of stress during the period prior to calving seem very likely to have high retained placenta rates. Clinicians that are attempting to lower retained placenta rates on farms need to look carefully at the situation for the cows in the transition pens prior to calving. In other words, they need to examine every change or stress that the cows will be exposed to during the week prior to calving. The clinician needs to look at these areas and situations with an eye to whether some of the cows might be encountering stress during this time. The general instinct of cows is to hide from other people and animals during the calving process. Will the cow be feeling stress as parturition approaches? Can you envision low or high circulating cortisol concentrations in the cows? The same situation may be stressful to one cow (increase cortisol) but not be perceived as stressful in another cow.

4. Induction of parturition with dexamethasone: It may appear that the reason for retained placenta in this situation would be the same as when cows are induced to calve early with prostaglandin F2a. However, this is not the case. Treatment of the cow with an injection
of dexamethasone will also cause the dexamethasone concentration to rise in the fetal circulation. This is because this hormone can readily cross to the fetal side of the circulation. Thus, this is somewhat similar to the experiments mentioned above in which the fetus was given a treatment with cortisol and this caused premature parturition. However, as mentioned above, treatment of the fetus with cortisol caused migration of the binucleate cells from the maternal to the fetal circulation (Wooding et al., 1986; Ward et al., 2002). Therefore this should allow the maternal immune system to be exposed to the foreign antigens that should cause immune rejection and normal expulsion of the placenta. Unfortunately, dexamethasone also is present in very high concentrations in the maternal circulation after intramuscular dexamethasone treatment. These high concentrations are well-known to inhibit the maternal immune system. Therefore, the reason for retained placenta varies for cows induced with dexamethasone as compared to PGF2a. In PGF2a, the early induction of parturition without migration of the binucleate cells prevents maternal immune rejection; whereas, with dexamethasone, the binucleate cells migrate but the maternal immune system is inhibited due to the high circulating glucocorticoid concentrations.

Based on this very clear model, it seems likely that we should be able to develop a method to induce a consistent time of parturition without the problem of retained placenta (CalfSynch). Unfortunately, this has not yet been possible. Inducing binucleate cell migration without inhibition of the maternal immune system may seem straightforward but has been surprisingly difficult to attain.

5. Low selenium or vitamin E: This nutritional problem can clearly cause retained placenta. It is well-known that selenium and vitamin E are essential for proper immune system function. Thus, any nutritional deficiency that lowers immune function would be expected to increase the rates of retained placenta by the mechanisms discussed above.

Recent studies have indicated that supplementation of vitamin E to high levels (at least 1000 iu per day) during the dry period and early lactation can reduce the incidence of mastitis (Alison and Laven, 2000). This is possibly due to an increase in immune system activity and function. A recent study analyzed the relationship of peripartum serum vitamin E concentrations with retained placenta and early lactation clinical mastitis in over 1000 lactating dairy cows in Canada (LeBlanc et al., 2004). This study found that for every 1 µg/ml increase in circulating alpha-tocopherol, there was a reduction in retained placenta of 20%. Similarly, cows with low alpha-tocopherol prepartum were much more likely to have clinical mastitis during the first 30 days post-partum.

LeBlanc et al., 2002 also did a extensive study of the effect of vitamin E supplementation prior to calving on the incidence of retained placenta. Cows (n = 1142) were randomized to receive 3000 IU of vitamin E or placebo at 1 week prior to expected calving. This treatment increased circulating alpha-tocopherol concentrations at 7 and 14 days post-partum. Overall there was not significant effect of this treatment on rate of retained placenta or clinical mastitis. However, cows with marginal vitamin E status prior to calving (serum alpha-tocopherol to cholesterol mass ratio < 2.5 X 10-3) had a reduction in retained placenta if they received vitamin E treatment. In cows with adequate vitamin E there was no improve-
ment. They also reported that the primiparous cows were most likely to benefit from the vitamin E treatment.

In areas with soil that is lacking in selenium there can be very high rates of retained placenta. Selenium is primarily utilized in selenite amino acids. Therefore supplementation of inorganic selenium in the diet can sometimes not provide a great deal of elevation in “physiological” selenium concentrations before it begins to reach toxic concentrations. Thus, supplementation of cows with organic selenium or fertilization of pastures with selenium have appeared to provide improved selenium delivery to lactating dairy cows.

Thus, this relatively simple physiological model is able to explain a large percentage of reasons for retained placenta. It is also able to provide a rational basis for how to prevent and treat retained placenta. Figure 3 shows the extended model including the effect of cortisol to inhibit the immune system or vitamin E or selenium to enhance the immune system if these factors are limiting.

**TREATMENTS AFTER RETAINED PLACENTA OCCURS:**

A number of studies have outlined a variety of different methods to treat retained placenta. Nevertheless, it seems clear that prevention is a much better pathway to deal with this problem. Many farms have extremely low incidences of retained placenta and, based on the model above, many of the underlying causes of retained placenta can be effectively prevented.

One of the most common treatments is prostaglandin F2a. There are at least 14 different published scientific studies that have evaluated the effect of various PGF2a products on retained placenta.

**Figure 3.** An extension of the model from Figure 2. If vitamin E or selenium is low then this will cause the immune response to be weak. If cortisol is high in the maternal circulation this will block the immune response. If immune rejection does not occur a retained placenta may result.
retained placenta. About half of these studies have shown some improvement, particularly when PGF2a was given very close to the time of parturition (see Peters and Laven, 1996 and references in Drillich et al., 2006).

Oxytocin and oxytocin analogs have been also evaluated in many studies on retained placenta. Most of these studies have used 50-100 iu of oxytocin. Again about half of these studies have shown some improvement, particularly if longer acting oxytocin analogs or multiple treatments with oxytocin were utilized (see Peters and Laven, 1996 and references in Drillich et al., 2006).

A number of recent studies have focused on use of intrauterine antibiotics and manual removal of the placenta. Manual removal and intrauterine antibiotic do not appear to be effective treatments for retained placenta based on these recent studies. For example, a recent study randomized a total of 501 cows with retained placenta into 4 treatment groups: Reference (REF = Control group; n = 131), treatment with an intrauterine antibiotic pill (AP; n = 119), treatment by manually removing the placenta (MR; n = 121), or antibiotic pill plus manual removal (PR; n = 130). All cows in this study were monitored for temperature and if temperature increased to > 39.5 degrees C (79.8% of cows) then they were treated for ceftiofur (1 mg/kg body weight) for 3-5 consecutive days. The cows were evaluated for many variables related to reproduction (days to first service, first service conception rate, total conception rate, days to pregnancy, % of cows pregnant at 200 days in milk, and % of cows culled). None of these variables were significantly affected by treatment.

Based on a number of recent and older publications, the current recommendation for treatment of RP is to not use intrauterine antibiotic or manual removal of the placenta. However, all cows should be monitored and treated with systemic antibiotic. There may also be a case made for treating all cows having retained placenta with antibiotics because almost all (~80%) will show this clinical sign. The cows that had a fever and were treated with systemic antibiotic had an improvement in reproductive outcome in the above study (Drillich et al., 2006) as compared to cows with RP in which a fever was not detected and therefore they were not treated with antibiotics. There were 2 other significant results in this experiment. First there was a highly significant effect of herd on the reproductive outcomes.

**Table 3.** Some reproductive variable after different treatments in cows with RP [Drillich et al., 2006].

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Antibiotic Pill</th>
<th>Manual Removal</th>
<th>Pill+Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to first AI</td>
<td>77.0</td>
<td>74.0</td>
<td>75.5</td>
<td>75.0</td>
</tr>
<tr>
<td>First-service CR</td>
<td>32.7%</td>
<td>32.7%</td>
<td>36.1%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Pregnant by 200DIM</td>
<td>61.1%</td>
<td>64.7%</td>
<td>58.7%</td>
<td>52.3%</td>
</tr>
<tr>
<td>Cows culled</td>
<td>16.0%</td>
<td>21.0%</td>
<td>19.0%</td>
<td>20.8%</td>
</tr>
</tbody>
</table>
This was obviously much more important than the effect of treatment (which was not significant). In addition, there was a significant effect of parity with primiparous cows with RP having much better reproductive outcomes (regardless of treatment group) than older cows with RP. This is consistent with the discussion in the first section of this manuscript, that older cows are more likely to have RP and are more likely to have a production and reproduction effect due to RP.

REFERENCES


