1   The Effect of Manual Removal of Placenta Immediately after

2   Foaling on Subsequent Fertility Parameters in the Mare

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12   **Abstract**

13   Retained placenta is considered to be a common problem in post-partum mares. The

14   incidence varies from 6 to 54 % depending on the breed, with higher incidence in heavy

15   draught mares than in lightweight mares. Retained placenta has been linked to lower

16   post-partum oxytocin concentration, impaired uterine involution and dystocia. The

17   objective of this study was to assess the effect of early manual removal of placenta

18   immediately post-partum on subsequent fertility parameters (development of free intra-

19   uterine fluid, inflammatory status of endometrium and on pregnancy rates) and to

20   compare them with mares with spontaneous expulsion of placenta. A total of 29 mares

21   mainly Irish Draught were closely monitored during foaling by CCTV and allocated to

22   two groups: 1) mares that expelled the placenta spontaneously within 3 h of foaling; and

23   2) mares that were cleansed manually immediately after foal delivery. All mares were

24   examined and scanned 5 and 9 days post-partum and free-intrauterine fluid (IUF)

25   recorded; endometrial swabs were taken 9 days post-partum for endometrial cytology

26   and culture. None of the fertility parameters analysed showed statistical difference

27   between groups 1 and 2. Therefore it can be concluded that early manual removal of

placenta has no detrimental effects of subsequent mare's fertility and therefore can be recommended when a veterinarian attends a foaling.

Introduction

Retention of fetal membranes (RFM) in the mare is considered to be a clinical emergency in field situations and during the breeding season is reported to be the most common problem of postpartum mares requiring veterinary assistance.[1] Major causes of concern of RFM for veterinarians are increased likelihood of endometritis, with subsequent reduction in fertility, endotoxaemia, laminitis and even death.[2,3] Nowadays, however, with improvement of treatment protocols the relevance of these sequelae has been reduced.[4] Prevalence of RFM in the mare population varies significantly between breeds, with lower prevalence (6 to 10 %) in lightweight breeds[5], higher in draught breeds and values as high as 54 % in Friesians.[6] Cut off point to consider a placenta to be retained varies but is usually set at 3 h post-partum. Urgency of removal is greatest in the larger, heavier and older mares and following dystocia.

Recent studies have linked increased likelihood of RFM to post-partum reduced serum concentration of calcium, magnesium[7] and oxytocin[8] as well as to previous history of RFM and dystocia.[5]

Treatment options vary from conservative with administration of ecbolic drugs, infusion of fluids into the chorioallantoic space, and injection of collagenases into the umbilical arteries[9] to more aggressive treatment consisting in traction of placenta while the allantochorion is manually separated from the endometrium. There has always been controversy as to which treatment is more effective and less detrimental to the mare's

53 subsequent fertility and well being. Some of the negative consequences attributed to

54 manual removal are excessive haemorrhage which favours bacterial growth, increase of

55 free intra-uterine fluid, delayed uterine involution, increased likelihood of endometritis,

56 longer remaining of an open cervix and permanent damage to the endometrium resultant

57 in decrease in fertility.[1,3,10] On the other hand, a recent study did not find any difference

58 in reproductive performance (foal heat pregnancy rates and foaling rates) between

59 mares with RFM with or without manual removal and mares with placentas expelled

60 normally.[4]

61 Whether manual removal or conservative treatments are chosen, RFM is still a reason

62 for concern with the associated veterinary costs involved for the horse owner. The

63 objectives of this study were to define the technique of early placental removal

64 immediately after foaling and to assess its effect on subsequent fertility parameters.

65

66 **Materials and methods**

67

68 *Animals*

69 29 pregnant mares (Irish Draught (18) and some Irish Draught crossed with

70 Thoroughbred (6) as well as 5 Standardbred) were used in the study. Once estimated to

71 be close to foaling (according to udder development and secretion appearance) they

72 were stabled and monitored with closed circuits television (CCTV).

73

74 *Foaling and cleansing protocol*

75 All mares were allocated to one of two groups: 1) mares that expelled the placenta

76 spontaneously within 3 h of foaling (n = 12); and 2) mares that were cleansed manually

77 immediately after foaling (n = 12) as follows: while the mare was still laying down the

delivered foal was placed in front of the mare after cutting and disinfecting the

umbilical cord so that the mare did not attempt to stand. Immediately after delivery, the

foal was pulled over the mares back legs sufficient for her to make nose to nose contact.

Then she was encouraged to remain lying during placental removal. If she stood up,

then spontaneous removal was allowed. Then the allantochorion membrane was grasped

manually from the distal (hanging) part. In some cases it was necessary to insert a hand

to wrist depth in order to grasp the cervical part of the placenta. Pulling firmly and

steadely (avoiding traction on the umbilical vessels), the entire placenta was

exteriorised. Following removal of the placenta, it was extended on a clear surface and

checked for completeness, especially loss of the non-pregnant horn.

An attempt to include in each group mares paired by age and parity was made. However

in some occasions (n = 2) the mare stood up before the placenta could be removed in

which no further attempt of manual removal was performed. These mares were included

in group 1 if the placenta was expelled within 3 h.

Mares not cleansed manually at foaling which retained the placenta for longer than 3 h

(n = 4) or those needing assistance at foaling (dystocia; n = 1) were excluded from the

study.

Fertility parameters

- **Free intra-uterine fluid**: all mares were ultrasonographically examined daily

    from day 5 post-partum until detection of ovulation. Depth (mm) of free intra-

    uterine fluid was recorded at the first examination and on day 9 post-partum or

    on the day before ovulation had been detected (whichever happened first). In

    addition, echogenicity of uterine fluid was noted and subjectively graded into

    four categories according to the echogenicity of floating particles: anechoic (0),

    slightly echoic (1), moderately echoic (2) and echoic fluid (3).

103   -   **Endometrial cytology**: double guarded endometrial swabs were taken from all

104   mares on day 9 post-partum or on the day ovulation had been detected. Swabs

105   were smeared on sterile slides, Diff-quick stained and assessed under light

106   microscope for number of neutrophils, debris and bacteria. Cytology smears

107   were classified into negative, mild and acute inflammation according to Card

108   (2005). [11]

109   -   **Endometrial culture**: swabs used for cytology smears were immediately plated

110   into blood agar Petri dishes and incubated for 48 h at 38º Celsius before final

111   interpretation. Cultures were read and classified into negative (no micro-

112   organism growth or scanty mixed growth of non-pathogens) and positive (pure

113   heavy growth of pathogens: *E. coli, S. zooepidemicus, Pseudomonas spp.,*

114   *Klebsiela spp., or Candida spp.*).

115   -   **Pregnancy rate**: the outcome after first service was recorded as well as whether

116   the mare became pregnant or remained barren at the end of the season. No mare

117   was mated on the foal heat.

118

119   *Statistical analysis*

120   Two-sample t-test was used to test statistical difference in free IUF 5 days post-partum

121   between groups 1 and 2 (all mares had some IUF). Wilcoxon non-parametric test was

122   used to test any difference in echogenicity of IUF. Fisher's exact test was used to test

123   the difference in endometrial inflammation (endometrial cytology and culture),

124   percentage of mares with free IUF 9 days post-partum (some mares did not have any

125   IUF at 9 days post-partum) and pregnancy rates between groups 1 and 2. All data was

126   computed in the statistical software Minitab 15®.

127

128 **Results**

129 All manual cleansings (MC) were easily performed within 5 minutes of foaling and in

130 no case was part of fetal membranes left in the uterus. None of the fertility parameters

131 investigated was significantly (P > 0.05) different between MC at foaling and those that

132 cleansed spontaneously (SC). Mean age and parity for groups SC and MC was 7.1 ± 1.2

133 years and 3.7 ± 0.7 foalings; 8.4 ± 1.5 years and 2.9 ± 0.6 foalings respectively.

134 All mares from group SC and MC had some free IUF 5 days post-partum whereas only

135 31 and 40 % of mares from groups SC and MC respectively still remained with free IUF

136 9 days post-partum. Fluid echogenicity in both groups was highest (score of 2) five days

137 post-partum and decreased four days later to anechoic values. Assessment of

138 inflammatory status of endometrium nine days post-partum as evidenced by

139 endometrial cytology showed that only one mare (8 %) had an acute endometritis (from

140 group MC) while two mares in each group (17 %) were considered to have mild

141 endometritis. Pregnancy rate after first service was 55 and 63 % for SC and MC mares

142 respectively; whereas the percentage of pregnant mares (> 40 days) by the end of the

143 season was 78 and 82 % respectively. Four mares (MC = 1 mare; SC = 3 mares) were

144 left barren for several reasons: prolonged anoestrus (n = 1), anovulatory haemorrhagic

145 follicles (n = 1) and diseases unrelated to the reproductive tract (n = 2). Bacterial

146 positive cultures (pure heavy growth) (n = 2, one each from SC and MC mares) were

147 identified (as per colony morphology) as ß-haemolytic *S. zooepidemicus*.

148 Four mares allocated to SC group had RFM (4/16) for longer than 3 h needing further

149 assistance. These mares were excluded from the study. A detailed record of fertility

150 parameters values for both groups are shown in Table 1.

151

152 **Discussion**

6

153 The aim of this report was to characterise and define the technique for early manual

154 removal of the fetal membranes immediately after foaling as well as to assess the effect

155 of this procedure on subsequent mare's fertility by comparing it with untreated mares

156 which passed the placenta within the time considered physiological. Results of this

157 report indicated no detrimental effect of early manual removal of placenta on any of the

158 parameters analysed.

159 Unlike in other reports assessing the effect of different treatments for retained placenta

160 on the mare's fertility, a great deal of fertility factors was taken into account as well as a

161 total control (thanks to 24 h CCTV) of times taken for the 3rd stage of parturition. The

162 reason for using the fertility parameters chosen was the fact that reports against manual

163 removal of placenta[1,2,12] claimed that the most harmful effect of manual detachment of

164 the chorioallantoic membrane was the possibility of retention of numerous microvilli in

165 large areas where they have broken free from their attachments remaining embedded in

166 the maternal crypts of the endometrium. As a result, the presence of attached microvilli

167 would favour the growth of bacteria, development of free intra-uterine fluid and

168 decrease rate of uterine involution. In this respect, mares cleansed manually did not

169 develop more free fluid 5 days post-partum than mares cleansed spontaneously.

170 Similarly, echogenicity score of uterine fluid was not different between groups.

171 Development of free intra-uterine fluid and increase in echogenicity score has been

172 linked to endometritis[13,14] and although they are typically regarded as indicators of post-

173 breeding inflammation, they can be also good indicators of the inflammatory status of

174 the endometrium.[11] Mares were first examined 5 days post-partum at which all of them

175 had some free IUF. In practice, during this early post-partum period, some amount of

176 free IUF is considered normal. In fact, all mares with negative endometrial cytology and

177 culture had some fluid (< 20 mm depth). On the other hand, mares with pure heavy

178 bacterial growth 9 days post-partum presented 4 days previously > 20 mm of fluid with

179 high echogenicity scores.

180 The overall incidence of RFM in the present study was 25 %. This value is not

181 surprisingly high since many of the mares were heavy draught types. Whether the

182 incidence of RFM is low or high in a given population of mares, the results of this study

183 suggest that manual removal of fetal membranes at the time of foaling can be performed

184 easily and without apparent detrimental effects on subsequent reproductive

185 performance. By doing this the incidence of RFM could be significantly reduced. Care

186 must be taken however, when removing the placenta after foaling and not entering the

187 uterus. In addition, pulling from the umbilical cord alone should be avoided as this is

188 contraindicated and could provoke uterine prolapse. In the author's field experience, it

189 was noted that placentae of mares standing up immediately after foaling have usually

190 higher resistance to subsequent manual removal. If this is the case, excessive

191 manipulation should be avoided and the mare left to cleanse spontaneously. However

192 this technique has been invariably possible in well over 100 mares.

193 In conclusion, early removal of placenta did not appear to affect negatively any of the

194 fertility parameters analysed in this study and therefore it would be advisable for the

195 veterinarian to remove the placenta if present at foaling.

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