# The effect of dietary protein on reproduction in the mare. VII. Embryonic development, early embryonic death, foetal losses and their relationship with serum progestagen

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## ABSTRACT

Sixty-four Thoroughbred and Anglo-Arab mares aged 6–12 years were randomly allocated to 4 dietary groups and fed diets that differed in the total protein content and quality (essential amino-acids). Forty mares were non-lactating and 24 lactating. Eight mares were withdrawn from the investigation owing to injuries or gynaecological pathology. An overall conception rate of 94.6 % and a foaling rate of 80 % was achieved. Five of 14 (35.7 %) mares (Group 1) fed a low-quality protein diet suffered from early embryonic loss before 90 days of pregnancy compared to 3 of 41 (7.3 %) mares in the remaining groups that received the higher-quality protein in their diets. Serum progestagen concentrations of mares in Group 1 that suffered foetal loss were indicative of luteal function insufficiency during the 1st 40 days post-ovulation. Non-lactating mares in all 4 groups gained on average approximately 30 kg in mass during the 90 days before the breeding period. Lactating mares in Group 1 (low-quality protein) lost on average 25 kg in mass during lactation, with no weight loss observed among the lactating mares in the other 3 groups. No difference in the diameter of the embryonic vesicle was found between dietary groups until Day 35 of pregnancy.


**Key words**: equine, pregnancy loss, protein nutrition, serum progestagen.

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## INTRODUCTION

Horses, particularly Thoroughbreds, have a history of low reproductive efficiency compared to other domestic animals, with earlier reports indicating a 45–50 % live foal rate[23]. Subsequently, foaling rates of 76–84 % for Thoroughbred, Standardbred and Quarter-horse farms have been reported[4,5,8,16,22,23,24]. Embryonic losses of 5 % from Days 23–43[7], 5 % from Days 20–45[25], 16 % from Days 15–40[13], 17 % from Days 15–50[33], 10 % from Days 14–48[36] and 8.9 % from Days 22–44[6] of pregnancy have been reported previously. Marlow[17] reported a total foetal loss of 12.6 % in Thoroughbred mares in South Africa. Losses that can occur throughout pregnancy could be either of infectious or non-infectious aetiology. Factors include age of mares[35], maiden mares served when very young[20], endometrial cysts and inflammation[1,28,36], twin pregnancies[10,21,24], environmental factors causing stress such as high or very low ambient temperatures, transport of pregnant mares resulting in a decrease in serum progestagen concentrations to levels where embryonic or foetal loss is experienced[28], mares with a history of embryonic loss[36], immunological rejection, chromosomal abnormalities, endometrial fibrosis[2] and mating at foal heat[7,15,18,35]. A mathematical approach by Ginther[11], based on the fact that the vesicle is not detectable during the early intra-uterine life of the conceptus and that embryonic loss is therefore extremely difficult to determine[12], indicate embryonic loss at 8.5 % before Day 10 post-ovulation.

Adequate progesterone is important for uterine and endometrial function and maintaining pregnancy. Luteal production of progesterone is therefore of the utmost importance during early pregnancy. Hypofunction of a *corpus luteum* (CL) could be the result of either underdevelopment or lack of functional maintenance. Inadequate stimulation of the granulosa cells at ovulation by LH could result in improper CL formation[11].

Studies concerning the relationship between nutrition and embryonic or foetal loss in mares are limited. Mares that conceive at foal heat have an increased incidence of pregnancy loss that is most probably due to high milk production and consequent negative energy balance of the mare[18]. Other studies indicate an increase in embryonic loss from Days 25–31 in poorly fed mares[3], while dietary restriction of mares carrying twins normally results in the loss of one embryo[19]. Embryonic losses observed were not related to energy metabolism. The extent to which dietary protein influences these findings has not been determined.

The purpose of this study was to determine the effect of dietary protein quality and quantity on embryonic development and foetal loss during early pregnancy.

## MATERIALS AND METHODS

Sixty-four Thoroughbred and Anglo-Arab mares aged 6–12 years were used. Of these mares, 40 were non-lactating (NL) and 24 lactating (L). The mares were randomly assigned according to age and body weight to 1 of 4 dietary groups as previously described[32]. All foals were weaned at the age of 6 months. Mares were teased and covered as previously described[32]. Mares were examined rectally and the diameter of the embryos measured ultrasonographically from Days 14–16, 18–22, 28–30 and 33–36 post-ovulation and monthly thereafter by rectal palpation until 8 months pregnancy duration. All pregnancy losses were recorded. Blood samples of pregnant mares were collected, processed and the serum stored for progestagen determinations as previously described[31]. Blood samples were collected daily for the 1st 3 days after ovulation and then every 3rd day until Day 33 of pregnancy. From Days 35 to 50, blood samples were collected daily. Thereafter blood samples were collected weekly until foaling. All blood samples were collected between 06:00 and 07:30.

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Initially, mares were kept as 8 different groups, of which 4 were NL and 4 L, in paddocks of 50 × 30 m. After weaning, the mares were placed together and kept as 4 dietary groups in paddocks of 80 × 30 m as previously described[29]. At this stage all mares that were not pregnant were removed from the trial. The composition of the diets, and the total daily crude protein and amino-acid intake of the mares in the different dietary groups have been described by Van Niekerk *et al.*[29]. All aborted foetuses were submitted to the Provincial Veterinary Laboratory, Middelburg, Eastern Cape Province, for examination. All mares were weighed weekly from parturition until the time of weaning.

### *Statistical analysis*

Results were compared using the mixed model LSML 76 described by Harvey[14].

## RESULTS

Pregnancy rates, foetal losses before 90 days of pregnancy and abortions in mares in the 4 dietary groups are shown in Table 1. Mares withdrawn from the experiment owing to injuries or gynaecological pathology were: Group 1 (NL): 1 mare did not show signs of oestrus; Group 2 (NL): 1 mare had a cyst in a uterine horn; Group 3 (NL): 1 mare had a torn cervix and 1 mare was intractable and was not covered; Group 4 (NL): 1 mare had a physical injury; Group 3 (L): 2 mares had physical injuries; Group 4 (L): 1 mare had endometritis and was not covered. Therefore only 56 mares are included in the results. In 1 mare (No. 32; Group 4 NL) implantation occurred in the body of the uterus immediately anterior to the cervix (body pregnancy) and was expelled before Day 60.

Serum progestagen concentrations of the 3 mares in Group 1 (NL) that lost their embryos before 90 days are shown in Fig. 1, from the time of foaling until the time of embryonic loss. No embryonic vesicle was detectable in Mare 1 (NL) by Day 43. In Mare 63 (NL) foetal loss occurred between Days 65 and 75. In Mare 6 (NL) embryonic loss occurred between Days 30 and 33. The 3 mares did not show signs of oestrus after foetal loss and were not covered again.

The serum progestagen concentrations of lactating mares 1 (L) and 6 (L) in Group 1 that lost their foetuses are shown in Fig. 2. Mare 1 (L) lost her foetus between Days 85 and 95 and Mare 6 (L) between Days 60 and 70. Neither mare showed further signs of oestrus and consequently they were not covered again.

The serum progestagen concentrations of the non-lactating mares that lost their foetuses before 90 days of pregnancy (Mares 31, 32 and 36) in Group 4 (NL) are shown in Fig. 3. Mare 31 lost her embryo between Days 40 and 46 and ovulated again on *c.* Day 48, had a normal oestrous cycle and was mated during the following oestrus, conceived and carried to term. Mare 32 had a body pregnancy and serum progestagen concentrations increased to reach maximum values of 10–16 ng/mℓ from Days 9 to 17. Values then declined to 8–10 ng/mℓ reaching a minimum of 4.4 ng/mℓ by Day 33. After Day 34, values increased to normal (8–15 ng/mℓ) from Days 37–50 and then declined rapidly to baseline values by Day 60. This mare ovulated again on *c.* Day 63, which was followed by a normal oestrous cycle, was mated during the next oestrous period, conceived and carried to term. Mare 36 showed a normal increase in serum progestagen concentrations after ovulation that remained high (10–12 ng/mℓ) between Days 6 and 21 and then declined to a minimum value of 6.4 ng/mℓ by Day 39, followed by an increase to a value of 26 ng/mℓ by Day 47. These values remained high until Day 66 and then declined to 4 ng/mℓ by Day 73, when foetal loss occurred. This mare did not show any further signs of oestrus and was not covered again.

Serum progestagen concentrations of Mare 11 in Group 2 (NL) that aborted on Day 245 of pregnancy and Mare 5 in Group 1 (L) that aborted on Day 170 of pregnancy are shown in Fig. 4. The foetus of Mare 11 showed advanced autolysis. A haemolytic *Streptococcus* sp. was isolated from the foetal specimens (Provincial

Table 1: **Pregnancy rates, foetal losses before 90 days of pregnancy, and abortions in mares in 4 dietary groups.**

| Group[a] | Number of mares | | | | |
|---|---|---|---|---|---|
| | Total | Withdrawn | Pregnant | Foetal loss <90 days | Abortion |
| 1 NL | 10 | 1 | 9 | 3 | |
| 2 NL | 10 | 1 | 9 | | 1 |
| 3 NL | 10 | 2 | 8 | | |
| 4 NL | 10 | 1 | 9 | 3 | |
| 1 L | 6 | 5 | 2 | | 1 |
| 2 L | 6 | 4 | | | |
| 3 L | 6 | 2 | 4 | | |
| 4 L | 6 | 1 | 5 | | |
| Total | 64 | 8 | 49 | 8 | 2 |

[a]NL = non-lactating; L = lactating.
Group 1 = tef hay and cubes.
Group 2 = lucerne hay and cubes.
Group 3 = tef hay, cubes and fishmeal.
Group 4 = lucerne hay, cubes and fishmeal.

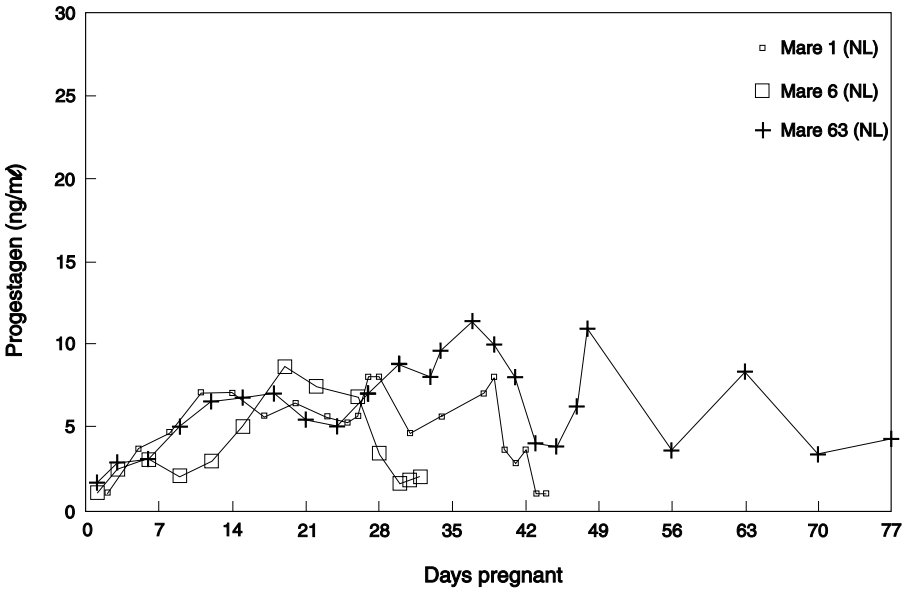


Fig. 1: **Serum progestagen concentrations of non-lactating mares in Group 1 (NL) that lost their foetuses before 90 days of pregnancy.**

Veterinary Laboratory, Middelburg, Eastern Cape Province). In this mare, serum progestagen concentrations were high until the day of abortion. In Mare No. 5, serum progestagen concentrations remained at high normal values until Day 138, when they declined to reach very low values of 3.2 ng/mℓ before abortion on Day 170. The decline in progestagen concentrations occurred gradually during the 30 days preceding abortion. The foetus was normal and showed no signs of autolysis.

The mean body mass of the lactating mares in the 4 dietary groups is shown in Fig. 5. Mares in dietary Groups 2 (L), 3 (L) and 4 (L) maintained their body mass throughout lactation. Mares in dietary Group 1 (L) lost on average 25 kg during lactation.

The mean diameter of the embryonic vesicles of the lactating and non-lactating mares are shown in Table 2. Although no significant differences in the embryonic vesicle diameter (mm) between dietary groups were found at 14–15 d, 29–30 d and 34–35 d post-ovulation, a significant difference of 29.6 ± 1.5 mm (NL) compared to 35.3 ± 1.7 mm (L) ($P \leq 0.05$) was found from Days 19–20.

Table 2: **Mean diameter** (mm) **of embryonic vesicles of non-lactating and lactating mares on Days 14–15, 19–20, 29–30 and 34–35 of pregnancy.**

| Days after ovulation | Diameter of embryonic vesicle (mm) | |
|---|---|---|
| | Non-lactating ($n$ = 35) | Lactating ($n$ = 16) |
| 14–15 | 21.3 ± 1.3 | 21.4 ± 1.7 |
| 19–20 | 29.6[a] ± 1.5 | 35.3[b] ± 1.7 |
| 20–30 | 40.2 ± 1.5 | 42.6 ± 2.3 |
| 43–35 | 48.9 ± 1.5 | 50.1 ± 1.9 |

[a,b]Values in the same row with different superscripts differ significantly ($P \leq 0.05$) (SE ± mean).

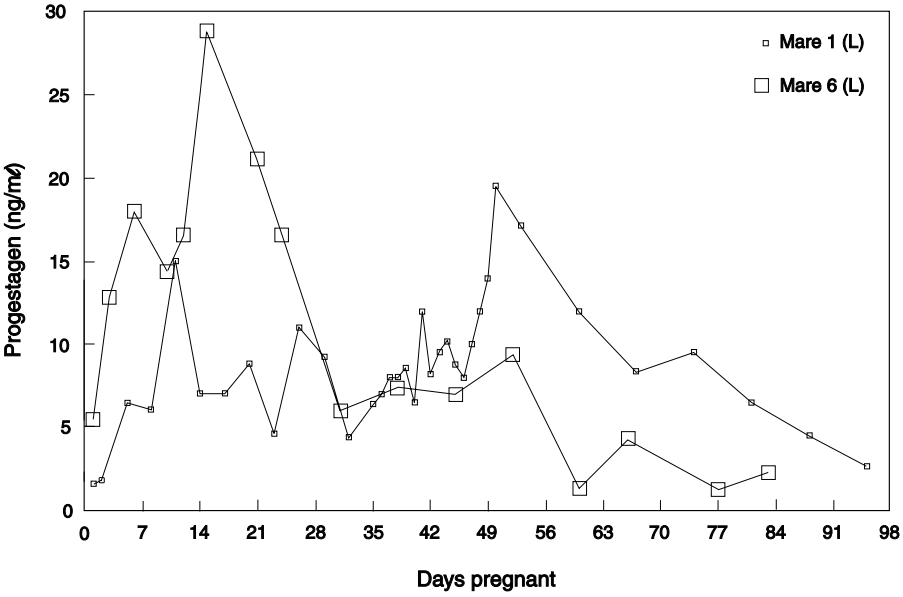


Fig. 2: **Serum progestagen concentrations of 2 lactating mares in Group 1 (L) that lost their foetuses before 90 days of pregnancy.**

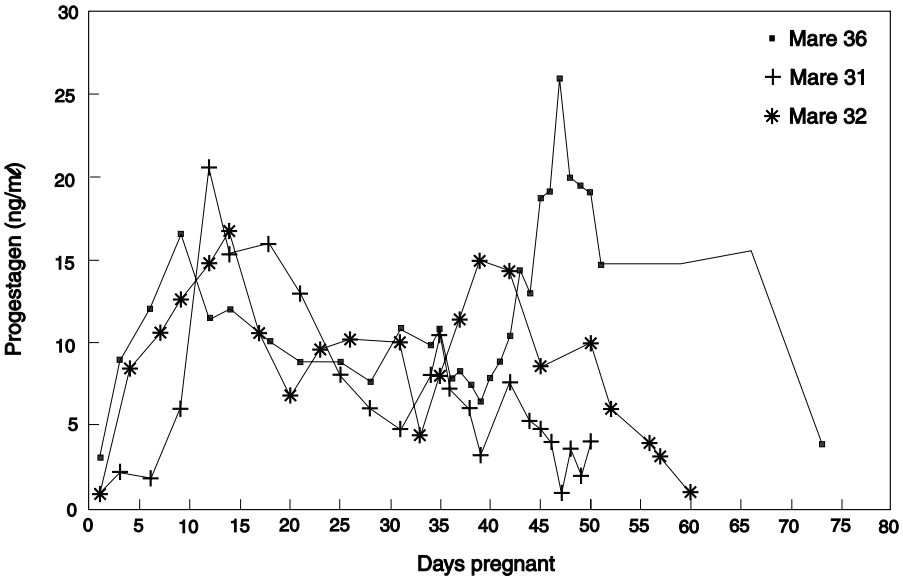


Fig. 3: **Serum progestagen concentrations of non-lactating mares in Group 4 (NL) that lost their foetuses before 90 days of pregnancy. Mare 32 suffered from a uterine body pregnancy.**

## DISCUSSION

An overall conception rate of 94.6 % of mares covered was achieved. The 3 mares that did not conceive were all lactating mares. Eight mares in the 4 dietary groups suffered foetal losses before 90 days of pregnancy. Two of these mares were remated, conceived and carried to term. Two abortions occurred after Day 90 of pregnancy. This effectively resulted in an 80 % foaling percentage and 14 % pregnancy loss rate. These results are in accordance with those reported in previous studies[17]. Ginther[12] attempted to unify the interpretation of reports on pregnancy loss in the literature and pointed out that results generally originated from studies with divergent examination programmes and reference points in pregnancy. He concluded that an 18 % pregnancy loss could be expected when the initial diagnoses were performed on *c.* Day 20 and at 12 % when performed on Day 40[12].

Possible causes of embryonic and foetal loss as cited in literature, namely the effect of age, abnormalities and inflammation of the endometrium, twin pregnancy, conception at foal heat, infectious diseases, and mares with a history of embryonic or foetal loss were excluded in this study. Immunological rejection and chromosomal abnormalities usually result in very early embryonic death and are generally undetected. In the event of early embryonic death before Day 12 post-ovulation, luteolysis occurs as in a normal cycle and the mare will usually come into oestrus before Day 21. As cited in literature, environmental factors, particularly those that cause stress in pregnant mares, are important causes of embryonic and foetal loss[28]. All mares in this study were kept under the same

environmental conditions, the only exception being the differences in composition of the 4 diets, specifically the total protein content and the quality of the essential amino-acids. The total daily protein intakes of the mares in all 4 dietary groups were met as described by Van Niekerk *et al.*[29,32] and only the essential amino-acid content that was shown to be inadequate in the mares allocated to dietary Group 1 varied[32].

The question remains about the extent to which nutrition, and specifically the quality of dietary protein, contributes to the prevention and/or reduced incidence of embryonic loss. The results obtained from mares in dietary Group 1 (low-quality protein), including both lactating and non-lactating mares, clearly show that 5 of 14 mares (35.7 %) lost their embryos before Day 90. Of the 41 L and NL mares in Groups 2, 3 and 4 only 3 (7.3 %) lost their embryos and 2 of these came into oestrus, were covered again, conceived and carried to term.

Mares in Group 1 (L) lost on average 25 kg (Fig. 5) in body mass during lactation, and although mares in Group 1 (NL) gained *c.* 30 kg on average during the same period, an embryonic loss rate of 33 % was recorded. This indicates that both the negative energy balance during lactation and the total dietary protein intake and amino-acid composition, despite the fact that the mares in Group 1 (NL) gained weight, are important factors contributing to embryonic loss.

It is well-known that progesterone is essential for maintenance of pregnancy in most species[12]. Progestagens desensitise the myometrium and in doing so block the stimulating effect of oestrogens, which remain fairly high throughout pregnancy[12]. Under the influence of progesterone the cervix remains contracted and tightly closed throughout pregnancy, and should serum progesterone concentrations fall below critical levels for that specific stage of pregnancy, the myometrium will contract under the influence of oestrogens, the cervix will relax and embryonic or foetal loss may occur.

The pattern of progesterone secretion during pregnancy in the mare differs from other species. In the pregnant mare there are certain stages during pregnancy when serum progestagen concentrations normally decline. These stages are at 30–40 d when the primary CL produces less progesterone and eventually degenerates. At the same time large follicles develop and ovulate to form the secondary CL[32]. Some mares may even show symptoms of oestrus and allow copulation at this stage of pregnancy[27].

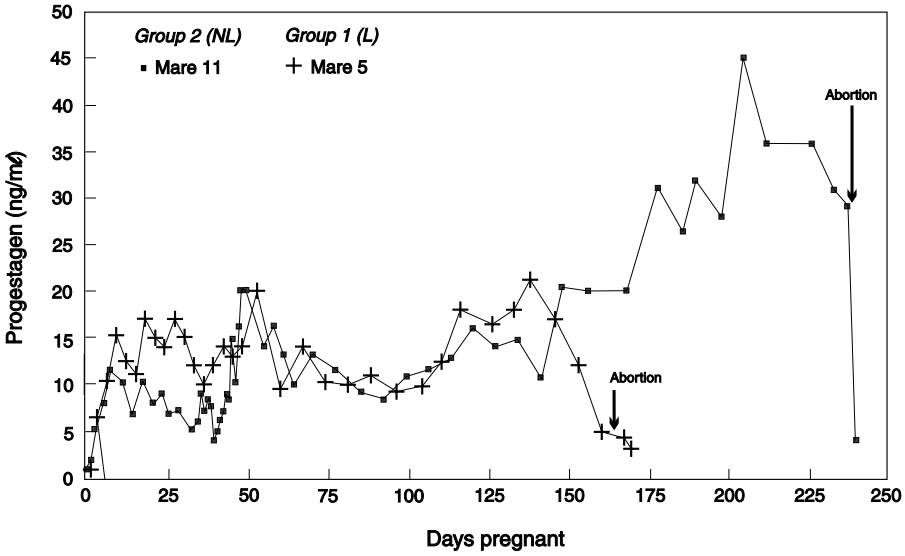


Fig. 4: **Serum progestagen concentrations of Mare 11 in Group 2 (NL) and Mare 39 in Group 1 (L) that aborted after 90 days of pregnancy.**

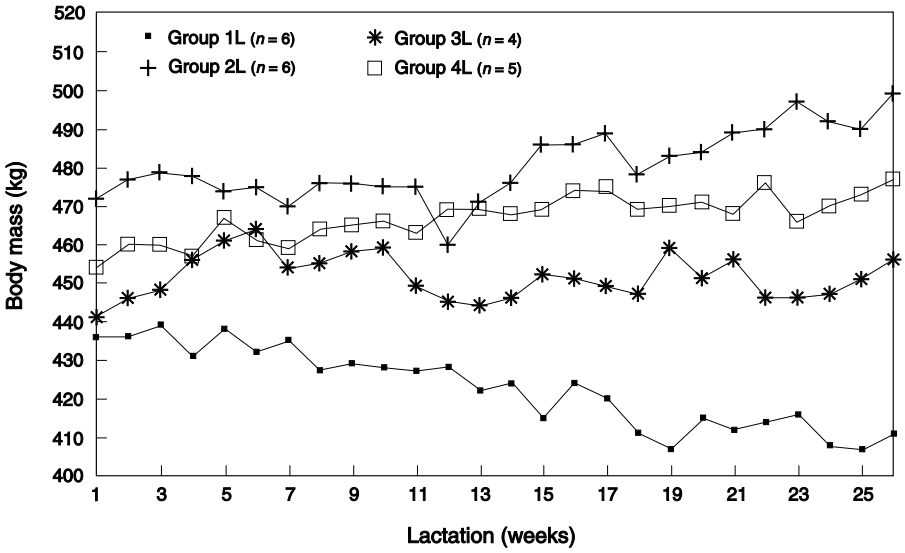


Fig. 5: **Mean body mass of lactating mares in 4 dietary groups during lactation.**

The 2nd critical stage is between 60 and 90 d when the secondary CL may produce less progesterone and even degenerate before the placenta takes over the function of progesterone production. During this stage, serum progestagen concentrations usually decline in most mares and often reach critically low concentrations. Embryonic or foetal loss can easily occur during these stages, particularly when any form of stress, nutritional stress included[18,28], results in a further reduction in serum progesterone values.

Normally, progestagen concentrations increase within 24 h post-ovulation to reach maximum values of 8–20 ng/mℓ from Days 12–20 and then decline, reaching the lowest concentrations from Days 27–40[32]. The mares ($n$ = 3) in dietary Group 1 (NL) (Fig. 1) all showed abnormally low progestagen concentrations during the 1st 3 days of pregnancy, and this indicates inadequate secretory function of the primary CL. Luteal insufficiency could be caused by a primary defect in the CL that occurs during development (full function not attained) or after development (full function attained but not maintained), or could be the result of premature luteal regression (luteolysis). In considering these 3 possibilities it appears that stimulation and therefore formation and maintenance of the primary CL by LH was inadequate. No similar low progestagen secretion patterns were observed among mares in the other 3 groups of NL mares. In the mare the LH surge only attains

maximum concentrations approximately 1 day after ovulation[9,34] and the presence of LH during this time of early luteal development is important for functional development. This prominent LH surge in mares, with the highest concentration occurring after ovulation, may account for the early post-ovulatory rise in progesterone concentrations in this species.

Although the LH concentrations were not determined at this stage of pregnancy in this study, it was shown earlier[30,31] that mares in dietary Group 1 (NL) (low-quality protein) commenced ovulatory cycles 3–4 weeks later than the mares receiving high-quality protein diets[30,31]. Follicular development of these mares proceeded to the LH-dependent stage and was then delayed[30]. All early embryonic losses in Group 1 (NL) occurred during the late transitional and early ovulatory season[30]. It therefore appears that the concentration and bioactivity of gonadotrophin, specifically LH, was inadequate for optimum luteal stimulation, resulting in a hypofunction of the CL probably caused by the low-quality protein (essential amino-acid) intake of the mares in this group.

The inverse relationship that exists between nutrition and progesterone in mares on a low-quality protein diet that carry their foetuses to term was described in an earlier article and has also been reported for sheep and goats[32]. It is also clear that a compensatory mechanism for progesterone production is activated in the mare in situations of nutritional stress[32]. It is obvious that in the case of the 5 mares in dietary Groups 1 (NL and L) that received the low-quality protein diet, this compensatory mechanism did not function and resulted in embryonic loss. This may be an inherited defect and/or that these mares are more susceptible to deficiencies of essential amino-acids in their diets. Consequently a lowered production of LH at and immediately after ovulation could occur that would lead to inadequate luteal stimulation and resultant subnormal and even subcritical levels of progesterone production. When the progesterone values decrease under normal circumstances from Days 30–40 and again from Days 60–90, these mares cannot maintain their progesterone levels above the critical threshold and embryonic or foetal loss results, probably because the compensatory mechanism that is activated under these circumstances[32] remains inactive. In another study[26], it was also shown that the post-ovulatory LH concentrations are higher early in the breeding season compared to those in the latter half of the ovulatory season. The nutritional stress imposed by the low-quality protein in the diet in these genetically low-progesterone-producing mares is possibly reflected by lower LH secretion at and after ovulation, which could be aggravated by the possibly higher requirement for LH early in the breeding season.

In the case of Mare 1 (L) in Group 1, the progestagen pattern was similar to the 3 non-lactating mares (1, 6, 63) until Day 45, when progestagen concentrations increased dramatically to Day 50 due to the secondary CL. Levels then declined. A similar explanation of an inadequate LH surge in this case validates this progestagen concentration pattern. In the case of Mare 6 (Group 1L), no increase in serum progestagen was found at the time of expected secondary ovulations.

The 3 mares in dietary Group 4 (NL) all showed a rapid increase of progesterone after ovulation and followed a normal secretion pattern that indicated a normally-functioning primary CL. The serum progestagen concentrations of Mare 31 show that secondary ovulations did not occur. This resulted in pregnancy failure, but the oestrous cycle was resumed, the mare was mated and pregnancy to full term ensued. Mare 32, after having a uterine (body) pregnancy and losing her foetus, was also mated, conceived and carried to term and embryonic loss is therefore not considered to be nutritionally related. Mare 36 lost her embryo after Day 65 after showing a normal progestagen secretion pattern that indicates some other cause of foetal loss.

Mares 11, Group 2 (NL) and 39, Group 1 (L), that aborted after 150 days of pregnancy both showed normal progestagen secretion patterns during the 1st 120 days of pregnancy. The cause of the abortions was not considered to be related to diet.

The mean diameter of the embryonic vesicles did not differ between dietary groups, but when pooled the lactating mares revealed a larger embryonic vesicle diameter from Days 19–35 compared to non-lactating mares. In both the L and NL mares, embryonic development was rapid until Day 15, followed by the plateau phase. Although the reason for measuring the embryonic vesicles was purely to confirm pregnancy, no nutritional effect on early foetal development was established. The mean values were in accordance with published results[10,22]. Although the differences in vesicle diameter were not significant, this might have been the result of increased myometrial activity and improved endometrial secretion resulting from repeated oxytocin release during suckling.

## CONCLUSION

The high incidence of early embryonic loss (35.7 %) in mares receiving a diet containing low-quality protein (low in the essential amino-acids) was most probably caused by inadequate production of progesterone from the primary CL. This luteal insufficiency could be the result of low levels of circulating gonadotrophic hormones, especially LH, at the time of ovulation, which could lead to understimulation of luteal tissue. The metabolic pathways in the production of progesterone differ between the CL, placenta and foetus on account of different enzymes present in the different structures. Whether the metabolic pathway and its associated enzymes in the CL are sensitive to dietary protein intake is unknown. Embryonic vesicle growth was unaffected by dietary protein intake. The early embryonic losses in the non-lactating mares were not associated with a loss of body mass, in contrast to the lactating mares, which lost on average 25 kg during lactation. We therefore conclude that a dietary intake of low-quality protein lacking in essential amino-acids could result in low LH concentrations and consequently luteal insufficiency, inadequate progesterone production and early embryonic loss.

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