Early embryonic death in mares: clinical and hormonal aspects*

Morte embrionária precoce em éguas: aspectos clínicos e hormonais

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SUMMARY

The present study aimed to diagnose early embryonic death in 128 mares. Blood samples were collected at the 4th, 7th, 10th, 13th, 16th, 19th, 21st and 30th day after ovulation and the diameter of the corpus luteum was measured. The diameter and characteristics of the embryonic vesicle were evaluated by ultrasonography. Microbiology, cytology and histopathology were carried out in mares with embryonic death. Plasma oestrogen and progesterone concentration were measured by radioimmunoassay. From 128 mares, 17 (13.28%) showed early embryonic death. The embryonic losses took place at 19 days of pregnancy in 47.05% and at 21 days of pregnancy in 29.4% of the mares. The diameter of the corpus luteum in the mares that maintained pregnancy was similar to those with embryonic loss. Otherwise, the diameter of the embryonic vesicles was bigger at the 16th, 19th, 21st and 30th day of pregnancy when compared to the mares with embryonic loss. The mean plasma progesterone concentration was similar in both groups and the median plasma oestrogen concentration was higher in the pregnant mares. The cytological, microbiological and histopathological exams revealed that most of the mares had endometritis. Ultrasonography provided an early diagnosis of pregnancy (from the 12th day post-ovulation) and important information about the development of embryo and embryonic death. Endometritis was considered the main cause of embryonic losses in this study.

UNITERMS: Embryo mortality; Progesterone; Estrogen; Histopathology; Equine.

INTRODUCTION

Estimation of embryonic death in mares has been published, but little is known about the aetiology of this problem. Ginther[4], using rectal palpation, reported that the incidence of embryonic and/or foetal losses in mares between 20 and 90 days post-ovulation ranged from 7 to 16%. More recent ultrasonography studies show a rate of embryonic death ranging from 5 to 24% between 11 and 50 days post-ovulation[6].

Endometritis is considered an important cause of embryonic loss in mares[7]. Ginther *et al.*[6] related the process of endometritis with high evidence of embryonic loss (18.2%) in pony mares between 11 and 15 days post-ovulation. The authors concluded that these losses were probably due to premature luteolysis secondary to endometritis. They also proposed that the primary luteal insufficiency and failure of the maternal recognition mechanism of gestation could lead to low progesterone concentration and embryonic death.

The equine embryo produces detectable oestrogen concentration between 6 and 12 days post-ovulation[10]. It is not known whether the low concentration of oestrone sulphate associated with embryonic death is the cause or effect of embryonic death, but it is suggested that measurement of oestrogen concentration may help to monitor the foetal viability after 40 days of gestation.

Ginther *et al.*[6] and Adams *et al.*[1] reported that the diameter of the embryonic vesicles measured by ultrasonography was smaller in mares with embryonic losses compared to the pregnant ones.

This study aimed to diagnose and study the early embryonic death in mares using ultrasonography, plasma oestrogen and progesterone concentrations and microbiology, cytology and histopathology of the endometrium.

MATERIAL AND METHOD

One hundred and twenty eight thoroughbred, Quarter horses and Mangalarga Paulista adult mares were used. They were in normal reproductive activity and were teased daily, for identification of the oestrus. The mares underwent rectal palpation

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and ultrasonography**, for control of follicular development, ovulation and gestation up to 30 days post-ovulation. The mares were artificially inseminated when follicles with pre-ovulation characteristics were detected.

Blood samples were collected at 4, 7, 10, 13, 16, 19, 21 and 30 days post-ovulation for measurement of oestrogen and progesterone plasma concentrations. The diameter of the corpus luteum was measured at 4, 10, 13, 16, 19, 21 and 30 days post-ovulation and the diameter and characteristics of the embryonic vesicles were studied by ultrasonography from the 10th post-ovulation day. Microbiology, cytology and histopathology were performed in those mares with embryonic death for correlation between the endometrium change and the embryonic death.

Oestrogen and progesterone concentrations were measured by solid phase radioimunnoassay, using 125I and gamma counter***. Oestrogen and progesterone concentrations were expressed in ng/ml and pg/ml in the plasma, respectively.

Samples for microbiology were collected using protected swabs introduced inside the uterus. Samples were placed in 10 ml sterile phosphate buffer solution and maintained for two hours at 4ºC, sowed in blood agar and Levine plates and incubated at 37ºC for 24, 48 and 72 hours in aerobic condition.

A mechanical collector containing gynaecological brushes**** was used to collect material for uterine cytology. Laminae were stained by Leishman. Interpretation was based on the relative presence of polimorphonuclear leukocytes[2].

The uterine fragment obtained by biopsy***** was fixed in Bouin for 24 hours, placed many times in alcohol 70%, dehydrated, diafanized, included in paraffin according to standard technique and coloured by hematoxylin-eosin (HE).

Statistical analysis: Statistics were based on Student's unpaired "t" test to compare the differences between the groups at each time point. Differences were considered significant when $p<0.05$.

RESULTS

From the 128 mares studied, 17 (13.28%) showed early embryonic loss. Most of the death occurred at the 19th day (47.05%) and at the 21st day post-ovulation (29.4%). Plasma hormonal concentrations and measurements performed by ultrasonography are show in Tab. 1, 2, 3 and 4 and Fig. 1, 2 and 3.

Table 1

Mean (standard deviation) diameter (mm) of the corpus luteum in the mares with normal pregnancy (G1) and in mares with embryonic loss (G2), Botucatu, 1996.

		4th	10th	13th	16th	19th	21st	30th
G1	Mean	32.46	26.63	25.30[a]	24.37	24.36	24.30	24.01
	SD	6.53	5.40	4.82	5.69	4.70	5.19	4.87
G2	Mean	32.47	25.94	22.58[b]	21.94	22.46	23.18	23.28
	SD	8.38	3.99	3.64	5.60	4.17	5.45	3.45

[a,b]: $p<0.05$; difference between groups.

Table 2

Mean (standard deviation) diameter (mm) of embryonic vesicles in mares with normal pregnancy (G1) and in mares with embryonic loss (G2), Botucatu, 1996.

		10th	13th	16th	19th	21st	30th
G1	Mean	5.42	14.00	26.03[a]	31.95[a]	33.19[a]	39.91[c]
	SD	2.14	3.88	5.70	4.48	5.19	7.12
G2	Mean	6.25	13.57	22.80[b]	27.23[b]	29.00[b]	25.20[b]
	SD	1.50	4.86	7.18	7.03	5.97	8.89

[a,b]: $p<0.05$; difference between groups;
[c,d]: $p<0.001$; difference between groups.

Table 3

Mean (standard deviation) plasma progesterone concentrations (ng/ml) in mares with normal pregnancy (G1) and in mares with embryonic loss (G2), Botucatu, 1996.

		4th	7th	10th	13th	16th	19th	21st	30th
G1	Mean	5.88	7.12	5.45	5.61	4.29	3.97	4.76	4.13
	SD	2.46	2.61	2.30	2.49	2.16	1.78	1.98	1.66
G2	Mean	5.98	8.83	7.17	6.66	4.72	4.61	5.05	5.59
	SD	2.19	3.04	3.15	3.33	2.63	2.33	2.14	3.47

no difference between groups.

Table 4

Median (Md) plasma oestrogen concentrations (pg/ml) in mares with normal pregnancy (G1) and in mares with embryonic loss (G2), Botucatu, 1996.

		4th	7th	10th	13th	16th	19th	21st	30th
G1	Mean	30.73	62.49[a]	38.51	37.85[a]	45.29	96.61[a]	47.11[a]	38.36[a]
	P25	7.92	8.82	10.89	10.17	9.41	17.09	14.2	16.4
	P75	179.48	174.06	118.16	135.19	107.22	160.52	124.81	117.56
G2	Mean	15.21	11.19[b]	34.48	7.64[b]	7.64	9.06[b]	6.26[b]	19.04[b]
	P25	5.25	4.92	3.88	3.00	4.69	5.69	2.48	1.14
	P75	69.05	54.60	139.98	14.99	64.49	52.37	49.23	37.27

a, b: $p<0.05$; difference between groups.

** Scanner 450 vet with transducer of 5 Mhz – Pie Medical – Netherlands.
*** Gamma Counter mod. 5500 – Beckman.
**** Cytobrush - Laborlex - S.P. – Brazil.
*****Biopsy forceps - model Krause.

Median and percents were used for oestrogen data due the great variability between samples.

The microbiological exam was negative in 58.8% of the mares with embryonic death and the cytological exam was negative in 52.8%. Otherwise 100% of these mares showed chronic infiltrative and/or degenerative endometritis in the histopathological exam (Tab. 5).

Table 5
Histology, cytology and microbiology in mares ambryonic loss (G2).

Mares	Histological Classification (Category)	Cytological Interpretation (% PMN*s)	Microbiology
1	II A	(-)	Negative
2	II A	(-)	*E. coli* (mild)
3	II B	(-)	*Corynebacterium equi*
4	II B	26%	Negative
5	II B	(-)	*Streptococcus* ß *haemolytic*
6	II B	39%	Corynebacterium sp
7	II B	30%	E. coli (exuberant)
8	II B	32%	Negative
9	II B	68%	*Streptococcus* ß *haemolytic*
10	II B	38%	Corynebacterium sp (mild)
11	II B	35%	Negative
12	II B	(-)	*Streptococcus* ß *haemolytic* (exuberant)
13	II B	(-)	Negative
14	II B	10%	Negative
15	II B	(-)	Negative
16	II B	(-)	Negative
17	III	(-)	Negative

* PMNs: polymorphonuclear neutrophils.

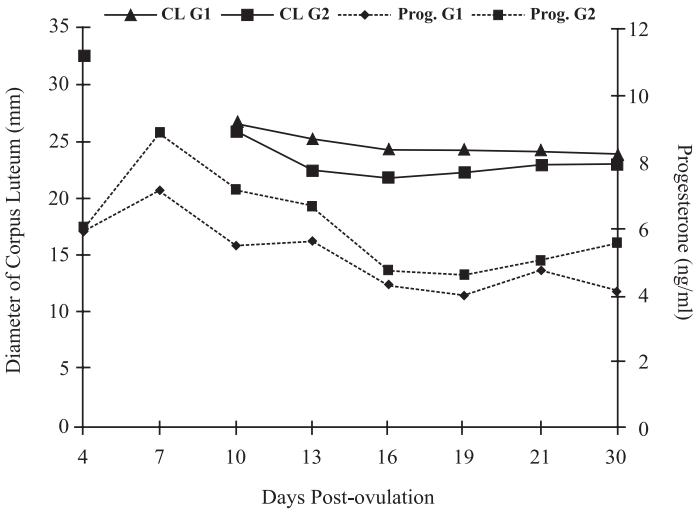

Figure 1
Plasma progesterone concentrations and mean diameter of the corpus luteum in mares with embryonic loss (G2). There was no difference between groups.

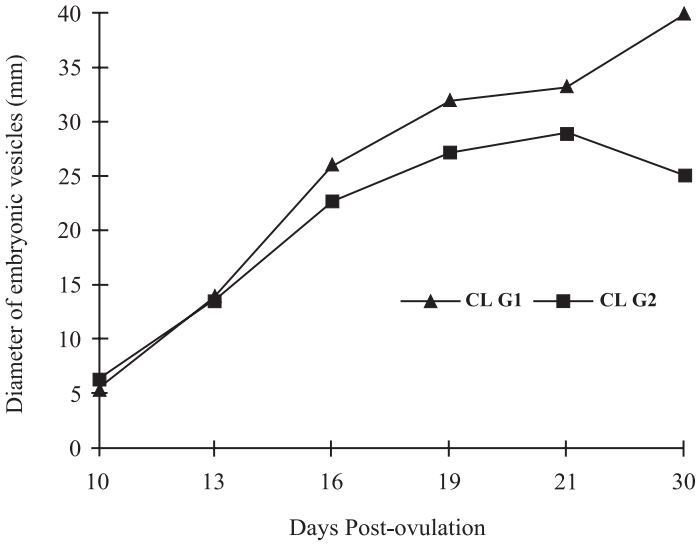

Figure 2
Mean diameter of the embryonic vesicles in mares normal pregnancy (G1) and in mares with embryonic loss (G2). G1>G2 at 16, 19, 21 and 30 days ($p<0.05$).

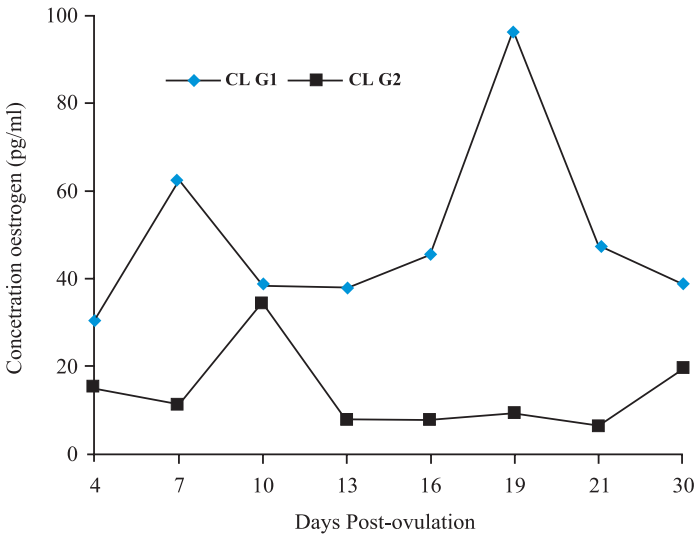

Figure 3
Median (Md) plasma oestrogen concentration in mares with normal pregnancy (G1) and in mares with loss (G2). G1>G2 at 7, 13, 16, 19, 21 and 30 days ($p<0.05$).

DISCUSSION

The incidence of embryonic losses in our study between the 10th and 30th post-ovulation day was 13.28%. Most of these losses occurred up to 21 days post-ovulation, what is in agreement with previous studies[5,6,9].

Primary dysfunction of the corpus luteum did not appear to be the cause of the embryonic death, because plasma progesterone concentrations were similar ($p>0.05$) in pregnant mares and in mares with embryonic loss. These results were similar to those reported by

Darenius *et al.*[6], but different from Ginther *et al.*[6], who found a significant decrease in progesterone concentration between 7 and 11 days post-ovulation in mares with embryonic loss. Besides that, the plasma progesterone concentration was reduced after the embryonic loss, showing that the progesterone decrease is the effect and not the cause of embryonic death.

There was no correlation between the diameter of the corpus luteum and maintenance of gestation. Similar results were obtained by Ginther[5], who also reported a reduction of progesterone concentration after embryonic loss. Despite of that, these authors observed a reduction in the diameter of the corpus luteum and plasma progesterone concentration after the 33rd day of pregnancy.

In relation to the cytopathological, microbiological and histopathological studies, most of the mares with embryonic death showed a compromised endometrium. From the seventeen mares which showed early embryonic death, nine showed acute and/or chronic inflammation, two periglandular fibrosis and six a combination of both, suggesting that the inflammatory processes involving the endometrium is the main cause of embryonic death in the equine species.

Ginther[5] reported a relation between uterine inflammation with secondary luteolysis and low progesterone concentration leading to embryonic death. Woods *et al.*[9] diagnosed endometrium inflammation in 60% of the mares with early embryonic death.

The ultrasonographic study showed that the diameter of the embryonic vesicles after 16 days of ovulation were smaller in the mares with embryonic loss. Similar results were reported by Ginther[5].

In relation to oestrogen concentration the difference between the two groups is clear. The plasma oestrogen concentrations between the 13th and 21st post-ovulation days in the mares with embryonic losses, were lower than in the pregnant mares. This could be an indication of embryonic viability, but more detailed studies should be done to confirm this fact.

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RESUMO

O presente estudo objetivou diagnosticar as causas da morte embrionária precoce em 128 éguas. Amostras de soro foram coletadas nos dias 4, 7, 10, 13, 16, 19, 21 e 30 após ovulação, para dosagens hormonais e mensurações do corpo lúteo. O diâmetro e as características da vesícula embrionária foram avaliados, através da ultra-sonografia, a partir do 12º dia pós-ovulação. Das 128 éguas estudadas, 17 (13.28%) apresentaram morte embrionária. O diâmetro do corpo lúteo, bem como as concentrações plasmáticas de progesterona, foi semelhante nos grupos que apresentaram morte embrionária e nos que mantiveram a gestação. Os níveis de estrogeno plasmático foram mais elevados no grupo das fêmeas que mantiveram a gestação. Os exames citológicos, microbiológicos e histopatológicos revelaram que a maioria das éguas com diagnóstico de morte embrionária eram portadoras de endometrites.

UNITERMOS: Mortalidade embrionária; Progesterona; Estrógeno; Histopatologia; Éguas.

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