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Multivariable analysis to determine risk factors associated with early pregnancy loss in thoroughbred broodmares

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Abstract

Early pregnancy loss (EPL) between days 15 to 65 after breeding has been shown to occur in 7.9% of equine pregnancies with substantial economical, welfare and safety implications. Whilst maternal age has been recognised as an important risk factor in relation to the incidence of EPL, few other risk factors have been conclusively identified. Further, multivariable data analysis of risk factors for EPL is sparse. A prospective cohort investigation of thoroughbred broodmares in the United Kingdom was conducted over the 2013 and 2014 breeding seasons. Information relating to 28 factors including mare, stallion, pregnancy and therapeutic interventions was collected using questionnaires and entered into a custom-designed Microsoft Access database. Mixed effects logistic regression was used to determine risk factors for EPL, including 'mare' as a random effect to account for repeat pregnancies in the same mare. Stallion, stud and veterinarian were also evaluated as random effects. Variables with a p-value of <0.25 in univariable analysis were taken forward for consideration in the multivariable model which was built using a forward stepwise approach. Data were collected on 2245 pregnancies in 1753 mares. Increasing mare age (OR=1.11, 95% confidence interval (CI)=1.04, 1.18, p=0.001), having had one previous foal (OR=3.52, 95% CI=1.56, 7.95, p=0.002) and presence of uterine cysts (OR=1.76, 95% CI=1.07, 2.91, p=0.03) were all associated with increased odds of EPL following multivariable analysis. Increasing day 15/16 scan vesicle size (OR=0.24, 95% CI=0.16, 0.38, p<0.001) and the use of ovulatory induction agents (OR=0.31, 95% CI=0.17, 0.55, p<0.001) were negatively associated with EPL. Stallion, stud and veterinarian were not significantly associated with EPL. Analysis of a subpopulation of 344 multiple (twin and triplet) pregnancies found that the use of flunixin meglumine at the time of manual reduction of a multiple pregnancy resulted in reduced odds of EPL (OR=0.34, 95% CI=0.14, 0.84, p=0.02). Results from this study can be used by stud farm personnel when assessing their broodmare population and by clinicians when deciding upon therapeutic strategies. Additional work can be focused around these risk factors to further our understanding of the pathophysiology of EPL.

Keywords

Pregnancy loss, equine, broodmare, flunixin meglumine, ovulation induction, altrenogest

1. Introduction

Numerous studies have shown that the majority of losses of thoroughbred pregnancies occur in the embryonic and early fetal period [1-3]. Pregnancy losses have significant implications in terms of economics, time and mare and stallion welfare [4, 5]. Despite this, few studies have looked at risk factors specifically relating to early pregnancy loss (EPL). Furthermore, most studies investigating overall reproductive efficiency report risk factors for EPL identified using univariable rather than multivariable analysis [3, 6-8] and thus do not take account of potential confounding due to associations between proposed risk factors, which may lead to erroneous conclusions.

Univariable analyses repeatedly show mare age to be the most significant factor impacting on the incidence of EPL, with increasing age linked to increases in losses [3, 6, 7]. The start of season reproductive status of the mare has also repeatedly been found to relate to incidence of loss, with maiden mares consistently reported to have the lowest incidences of EPL [3, 6, 7]. There is, however, a clear association between mare age and status with maiden mares principally being the youngest members of the cohort. Conflicting results have been found in relation to foaling to covering period and uterine cysts [2, 9, 10]. Whilst the contribution of a range of reproductive therapies on various aspects of reproductive efficiency have been measured [7, 11, 12] there is little work investigating their impact on EPL.

Manual reduction of twins is common practice in broodmares [13] and various medications are used at the time of reduction in attempts to minimise its impact on the remaining conceptus [14, 15]. The effect of the individual operator and the use of the prostaglandin synthetase inhibitor flunixin are the most common factors investigated in relation to pregnancy loss in twin pregnancies [14-16]. Again, investigations into their effect on EPL have only been conducted with univariable analyses and results of studies have been contradictory [14, 16, 17].

Multivariable analyses investigating reproductive efficiency in thoroughbreds more broadly were conducted in a 2012 study of 1482 New Zealand mares [9] and a 2016 study of 2385 mares in Ireland [18]. These studies looked at pregnancy rates, mating to conception times and pregnancy loss overall (day 15 to term) but did not investigate factors specifically influencing EPL. A 2011 study of 1476 Japanese thoroughbreds did use multivariable analysis to investigate factors associated with losses up to day 35 of pregnancy [2]. They found foal heat mating and the presence of uterine cysts to contribute to the incidence of EPL (defined as loss from gestational day 17-35). This study did not, however, assess the impact of therapeutic agents, stallions or twin pregnancies on pregnancy outcome.

With few diagnostic and therapeutic options available to minimise the incidence of EPL, investigations to understand risk factors associated with the condition are paramount. A comprehensive multivariable study to address risk factors associated with EPL in this population of thoroughbred mares was, therefore, warranted. We hypothesised that a combination of mare, stallion, conceptus and therapeutic factors would all independently contribute to the risk of EPL with the greatest risk being mare age. We also proposed that of the factors associated with mare age, uterine cysts but not post mating uterine fluid or mare status would independently increase the risk of EPL. Further, based on the observation by Kohne et al, 2014 that hCG administered to induce ovulation results in increased levels of progesterone 5-15 days post ovulation, we proposed that use of ovulatory agents would reduce the risk of EPL. The objectives of this study were to investigate the effects of (i) mare, stallion, pregnancy and therapeutic level variables on EPL and (ii) therapies/factors associated with twin or triplet reduction on EPL, using multivariable analysis.

2. Materials and methods

2.1. Study design and period

A prospective cohort study was conducted recording details on the reproductive management and occurrence of EPL in thoroughbred broodmares located on 29 stud farms under the care of two large equine veterinary practices based in Newmarket, UK. Data were collected over the 2013 and 2014 breeding seasons and the results of a descriptive analysis of the data have been reported [1]. The definition of EPL was pregnancy loss from day of detection to gestation day 65. This was to account for losses occurring in the embryonic period as well as during early implantation and microcotyledon development.

2.2. Sample size calculation

Sample size calculations were conducted using the online software Epitools Epidemiological Calculators (AusVet Pty Ltd). Calculations for the risk factor analysis assuming 80% power and a 30-40% exposure frequency in controls indicated around 140 cases of EPL (and the same number of controls) would be required to detect an odds ratio of 2 as statistically significant at a 5% level.

2.3 Data collection and processing

Data were collected from a combination of sources. An initial questionnaire was completed by stud secretaries, attending veterinarians or one of the investigators at the time of the initial positive pregnancy scan 11 to 17 days post covering. Mare, stallion and veterinary reproductive data were recorded. A further questionnaire was completed at each subsequent reproductive scan until gestation day 65. Additional information was collected from two veterinary hospital databases, Weatherbys (Wellingborough, UK) and Racing Post online (https://www.racingpost.com/bloodstock/). The inclusion criterion for a mare to be entered into the study was a positive day 14 to 17 routine pregnancy scan. Data were only collected for mares with clinical findings recorded throughout the entire period of early pregnancy (days 15-65). Therefore only pregnancies with a known pregnancy status at 65 days of gestation as ascertained by ultrasonography or birth of a live foal of pregnancy were included in the analysis. Data collected were entered into a custom designed Microsoft Access database and stud, veterinarian, stallion and mare names were coded to ensure anonymity. Data were transferred to Microsoft Excel and subsequently to Stata software package for statistical analysis. The total number of cases included for each variable is listed in Supplementary Tables 1 and 2).

2.4 Statistical analysis

2.4.1 Risk factors for EPL

Incidence of EPL was derived for different levels of each exposure variable, together with 95% confidence intervals (CI). Mixed effects logistic regression was conducted to identify factors associated with EPL, and odds ratio (OR) and its 95% CI reported. Mare was included as a random effect in all analyses, to account for repeat pregnancies in the same mare within the dataset. A total of 22 exposure variables (listed in Supplementary Table 1) were evaluated as fixed effects in univariable analyses. Continuous variables were initially categorised to evaluate the linearity of their association with the outcome. Where no departure from linear trend was identified using a likelihood ratio test (LRT), the variable was subsequently modelled as continuous predictor in the analysis. Variables with a univariable LRT p-value of <0.25 were considered for inclusion in a multivariable model, which was built using a forward stepwise approach. Inclusion of a variable in the final model was based on a LRT p-value of <0.05. Biologically plausible interactions between variables in the final model were assessed using a LRT comparing models with and without the interaction terms. Given the number of levels, stud farm, stallion and veterinarian were assessed as random effects both on their own (i.e. with no other variables included in the model) and in the final model, regardless of their univariable p-value. As an *a priori* variable of interest, stallion was also evaluated as a fixed effect using a subset of the data including only stallions with >30 pregnancies in the dataset [7]. In this analysis, stallion was added to a multivariable model containing the variables found to be significantly associated with EPL in the main analysis, using the stallion with most observations as the reference category.

2.4.2. Risk factor analysis for EPL in multiple pregnancies

A separate analysis was also conducted to investigate risk factors associated with EPL specifically relating to multiple (twin or triplet) pregnancies. With previous studies having indicated that veterinary operator may have an effect on pregnancy outcome following manual reduction [16], ‘vet’ was included in this analysis as a fixed effect. Stud and stallion were evaluated for inclusion as random effects. This dataset included nine mares with repeated pregnancies. For these mares, one pregnancy was randomly selected for inclusion in the analysis, using a random number generator. The other pregnancies from these mares were removed, resulting in a dataset with only one pregnancy per mare. The fixed effect of day 15 or 16 vesicle size used the size of the conceptus which was not subject to manual reduction. Variables with a univariable LRT p-value of <0.25 were considered for inclusion in a multivariable model. Additionally, all factors found to be significant in the final multivariable analysis of the ‘all pregnancies’ dataset were considered for inclusion in the final model, regardless of their significance in the univariable analysis, as was the ‘vet’ variable. A forward stepwise building process was used with a p value of <0.05 considered significant in the final model.

3. Results

3.1. Descriptive results

Information was collected on 2245 pregnancies in 1753 mares, 492 of which had repeat pregnancies in the dataset either due to a pregnancy in both 2013 and 2014 or because they lost a pregnancy and had a second or third pregnancy within the same season. Mares were located on 29 different stud farms and were covered by a total of 86 stallions. Veterinary care was provided by 13 veterinarians. The overall incidence of EPL in this population was 7.9% (178/2245; 95% CI=6.8, 9.1). A description of the risk factors investigated, the levels for each factor and the incidence of EPL for each level can be seen in Supplementary Table 1A-D.

3.2 Factors associated with EPL

Univariable analysis was performed on 22 potential risk factors. A total of five mare factors (age, status, EPL previous season, number previous live foals, uterine cysts), two therapeutic factors (ovulatory induction agents, oxytocin at cover) and one pregnancy factor (size of day 15/16 vesicle) had an overall LRT p-value <0.25 and went forward for assessment in a multivariable model.

The final multivariable model is presented in Table 1. Mare age, the number of previous live foals and uterine cysts were associated with increased odds of EPL whereas the use of an ovulatory induction agent (3000 iu Chorulon (MSD Animal Health, Milton Keynes, UK) intravenously, 2.1 mg Ovuplant® (Dechra Veterinary Products, Shrewsbury, UK) or 0.04 mg Receptal® (MSD Animal Health, Milton Keynes, UK)) and increasing size of the embryonic vesicle at gestation day 15 or 16 reduced EPL occurrence. Stud, vet or stallion were not significant when included in this model as random effects and no significant interactions were identified.

3.3. Effect of stallion on EPL

A total of 22 stallions were represented in the data set 30 or more times with the incidence of EPL amongst these stallions ranging from 1.5% (95% CI=0.0, 8.2) to 16.2% (95% CI=6.2, 32.0). The overall effect of stallion was not statistically significant (p=0.07) after accounting for other factors associated with EPL. Just one stallion had significantly higher odds of EPL compared to the reference stallion (OR=8.5, 95% CI=2.1, 35.0, p=0.003).

3.4. Multiple pregnancy dataset

A sub-population of 344 mares with multiple pregnancies was analysed representing 26 stud farms and 57 covering stallions. Incidence of EPL in this sub-population was 7.6% (26/344; 95% CI=5.0, 10.9). Univariable analysis was performed on 28 factors described in Supplementary Table 2A-D. Of these, five mare factors (age, status, EPL previous season, number of previous live foals, uterine cysts), three therapeutic factors (ovulatory induction agents, dexamethasone at cover and uterine lavage) and four twinning factors (flunixin meglumine, sedation, same location at reduction, multiple ovulations in the same versus bilateral ovaries) had a univariable LRT p-value of <0.25 and were, therefore, considered for inclusion in the multivariable model. The final model found mare age, the use of ovulatory induction agents, the use of dexamethasone at cover and the use of flunixin at twin pregnancy reduction to be significantly associated with EPL (Table 2). Veterinarian was not significantly associated with EPL, either in univariable analysis (p=0.27) or when forced into the final multivariable model (p=0.42), and neither were stallion or stud farm.

4. Discussion

This is the largest study to date to describe a multivariable approach for the investigation of factors influencing EPL in thoroughbred mares. Key findings were the increased occurrence of EPL in the presence of uterine cysts and the reduction in EPL associated with the use of ovulatory induction agents (chorulon and deslorelin) and flunixin to treat twin pregnancies at the time of reduction. In agreement with previous studies using univariable approaches, results show the important contribution of maternal age on pregnancy loss and an increased risk of EPL associated with small vesicle sizes. Whilst mare status has been widely thought to contribute to EPL [3, 6, 7], multivariable findings show status per se to be unrelated to EPL although animals which had had a single previous foal were at increased risk of EPL when compared to maiden mares. These findings open avenues for ongoing investigations to allow underlying causes of EPL to be more comprehensively understood whilst also assisting clinicians wishing for evidence based rationales in management of broodmares.

Studies using univariable analysis have repeatedly shown mare age to be a well-recognised factor associated with an increased risk of EPL [3, 5, 7, 19]. Although an odds ratio of 1.11 per year may seem low, this would lead to a 15-year-old mare having an OR for EPL of 2.84 in comparison with a 5 year-old-mare. The transfer of oocytes from aged mares to young recipient mares and vice versa indicates that the main contributor to reduced fertility in early pregnancy is an age-related decline in oocyte quality rather than uterine factors [20, 21]. Cytogenetic studies in humans have shown 45-70% of all first trimester losses to be attributed to chromosomal anomalies and that this proportion increased substantially with maternal age [22, 23]. The contribution of chromosomal abnormalities to EPL in the mare is not currently known, although two chromosomal abnormalities have been demonstrated experimentally in equine embryos [24] and new methods to study conceptual material from clinically failed pregnancies are now available [25]. It is also plausible that EPL derived from oocyte defects could arise by mitochondrial defects or chromosome misalignment on the metaphase II spindle which have been shown to increase in aged mares [26, 27]. These oocyte abnormalities are likely to also contribute to earlier pregnancy losses (prior to day 15) not assessed as part of this study.

Early pregnancy vesicle size, as determined at routine ultrasound examinations, has previously been shown to be associated with EPL [28-30], however, the magnitude of this effect has not previously been quantified. The current study shows that for each cm increase in vesicle size at gestational day 15 or 16, the odds of EPL were reduced by a factor of 0.28. It is possible that intrinsic flaws within the oocyte or early conceptus result in a smaller vesicle size and, hence, this is a visual sign representative of an underlying pathology. Conversely, it may be that a small yet viable vesicle is more likely to result in failure due to an inability to adequately progress within the uterus. For example, we know that contact between the conceptus and maternal endometrium is essential for maternal recognition of pregnancy [31].

The relationship between parity and EPL has not previously been described. The findings of a foaled uterus (one live foal) being at higher risk of losing a pregnancy than a maiden uterus seem relatively intuitive. Increasing parity is known to coincide with a lengthening of the vulva and an increased angle of declination [32]. Further, fixation in a previously gravid horn in consecutive pregnancies can results in a higher incidence of early pregnancy loss [33], a possibility after one live foal. Loss of structural support of the caudal reproductive tract and the broad ligament resulting from repeat pregnancies may result in the uterus tilting ventrocaudally and scintigrams taken after intrauterine infusion of radiocolloid revealed the position of the uterus may affect uterine clearance [34]. Therefore, it was of note that we did not identify delayed uterine clearance as a risk factor for EPL in this study. Additionally, links between parity and endometrosis have been found [35], as have links relating elastosis of the myometrial vessels to parity [36]. These studies also reported the presence of elastosis in the myometrial vessels to be related to chronic uterine infection and delayed uterine clearance with the number of previous foals being found to have the strongest association with uterine vascular degeneration. Therefore one would have expected the risk to extend beyond one live foal to 2-5 and 6+ foals, although statistically this was not the case. This specific risk of one live foal over 2-5 is currently unexplained.

There has been a substantial increase in the use of ovulatory induction agents in the UK from 59.1% in 2002 to 91.8% in 2013/14 [1, 7]. Although primarily used to reduce the number of covers per oestrous cycle, here we show ovulatory agents are associated with a reduced odds of EPL. The exact mechanisms remain unknown. Timing of ovulation in respect to cover is known to impact on EPL and it is speculated that an oocyte ageing in the oviduct post ovulation and prior to fertilisation may reduce oocyte quality [37] and increase the likelihood of the pregnancy being lost [38]. None of the oocytes in our study had ovulated at the last reproductive evaluation prior to cover (typically 12 to 24 hours prior to mating). Furthermore, post hoc analysis revealed no differences in the last detected follicle size prior to cover in mares receiving or not receiving treatment. It seems, therefore, that this explanation is unsatisfactory. Studies quantifying progesterone levels following hCG administration in the mare have yielded contrasting results [39, 40]. Mares treated with 1000 iu of hCG on days 3 to 5 post ovulation had enhanced day 7 to 14 post oestrus progesterone concentrations [41, 42]. More recently, Köhne et al. showed that the use of 1500 iu of hCG to induce ovulation significantly increased progestin concentrations between days 5 to 15 post ovulation compared with untreated controls [39]. It is plausible that surges in progesterone levels associated with gonadotrophic preparations may bring similar benefits in horses to those seen in cattle [15, 16] although this remains speculative at this point. A further mechanism worthy of consideration is the finding of increased ovarian vascular perfusion and luteal blood flow in GnRH or hCG treated mares when compared to saline treated controls [40]. Improved ovarian blood flow may result in a higher quality corpus luteum better able to support the developing vesicle.

Contradictory results have previously been found when ascertaining the relationship between uterine cysts and EPL. A study from 1995 solely investigating the effects of uterine cysts on loss rates found no overall effect of cysts once mare age had been taken into account [10]. However, Miyakoshi et al. found uterine cysts greater than 10 mm in diameter to be positively associated with EPL in their multivariable analysis [2]. With mare age being accounted for but the size and number of cysts not being quantified in our current study, the presence of cystic structures was still found to increase the odds of EPL. It has been suggested that reduced uterine vascular perfusion may occur in the vicinity of cysts and impedance on embryo dynamics and implantation may result [43]. The correlation between uterine cysts and endometrial fibrosis has also been recognised [42]. Therefore, it may be that cysts are representative of underlying uterine insufficiencies rather than being directly responsible for losses. Future analyses giving consideration to the location, size and number of uterine cysts would be of benefit to allow improved management and prognostic evaluations.

Whilst primary luteal insufficiency is not a well-defined cause of EPL in mares [45], experimental studies have clearly shown exogenous progesterone use in the face of an absence of luteal support to be capable of maintaining a pregnancy [46]. With a scarcity of treatment options available to clinicians in light of a pregnancy recognised of being at risk, altrenogest is often resorted to [45]. In this study we identified 128/2245 pregnancies that were treated with altrenogest starting at the first pregnancy examination. We did not include this data in the risk factor analysis as these treatments were initiated due to signs of impeding loss such as uterine fluid accumulation, endometrial oedema, abnormal vesicle shape and a slowed heart beat [29] and as such case selection was bias. In the majority of EPL cases either progesterone inadequacy is not the cause of loss [46] or recognition of the problem is too late. A controlled study specifically attempting to accurately determine any benefits of altrenogest in these situations and or when initiated 5 days post ovulation is required but remains challenging due to the large population size that would be required.

No other factor was found to have an association with EPL following multivariable analysis in the 'all pregnancies' dataset, despite some having a significant association in the univariable analysis. The effect of foal heat breeding on EPL has been inconsistent between studies with no effect seen in a New Zealand study [19] and a significant increase in rates of loss seen when mares were bred on foal heat in two Asian studies [2, 49]. Blanchard et al. (2012) found increased odds ratios (OR) for pregnancy loss associated with the length of breeding post-partum with the OR for pregnancy loss not decreasing to 1.00 or below until day 78 post-partum. In our study, a numerically higher incidence of EPL was detected in the group of mares mated between 8 to 19 days postpartum than in any other group. However, this represented only 33/2246 pregnancies and analysis found it did not modify risk of EPL. Again these discrepancies may relate to stringent case selection and relatively few mares mated at foal heat in the current investigation. When looking at the effects of twin/triplet pregnancies on EPL, an Australian study found significantly fewer embryonic losses in mares diagnosed with a multiple pregnancy compared with a singleton pregnancy [3]. This was not repeated elsewhere [2] and a South Korean study found the reverse [49]. No association between the incidence of EPL and vesicle number was detected in the present study. Given the rise in occurrence of multiple pregnancies seen when comparing to the previous data from 2002 (16.1% compared to 10.5%) [7], it is reassuring that no negative impacts on early pregnancy outcome were found.

Pascoe et al. showed that the release of endometrial PGF2α secondary to manual twin reduction was directly related to the pressure required [17] and flunixin was found to inhibit PGF2α. This small study followed ten mares receiving no treatment with manual reduction and 40 mares which received progestagens and flunixin. A recent Kentucky study found the use of flunixin and progesterone reduce the incidence of pregnancy loss [16] but it is unclear if it was flunixin, progesterone or the combination of treatments which had this effect. Equally, as the results were from univariable analysis the effects could have been related to differences in operators using or not using flunixin as a significant difference in incidence of EPL was found between operators. The results of the current multivariable study show flunixin to be beneficial in regards to pregnancy maintenance when used at the time of manual reduction, reducing the occurrence of EPL by a factor of 0.34. No effect of operator was elucidated. A negative association of dexamethasone on pregnancy outcome was found only in the multiple pregnancy sub population. Dexamethasone usage has been advocated in particularly problematic mares [50]. With only 21 mares treated in this sub population, and a wide resulting 95% confidence interval (1.94, 22.1), it is likely that this effect is due to low numbers and case selection rather than being a real sequela of dexamethasone usage.

Conclusion

Multivariable analysis has found the mare level factors of increasing age, one previous live foal and the presence of uterine cysts to be positively associated with EPL. Evidence suggests it is oocyte deficits rather than endometrial dysfunction which are the main contributors relating mare age to EPL [20, 21, 26, 27]. Cytogenetic analysis to establish chromosomal aberrations would be beneficial to explore the pathophysiology behind oocyte-related failures. However, evidence linking uterine dysfunction to parity [34, 36] and the influence of foal number and uterine cysts seen in the present study suggest the uterus does indeed play a role in EPL. Further studies quantifying endometrial changes, for example, assessing the size and location of cysts in relation to EPL are also warranted. Therapeutically, the use of ovulatory agents were shown to result in reduced odds of EPL and flunixin given at the time of manual reduction of twin pregnancies was similarly beneficial. Despite its increasing use, intrauterine covering therapies were not found to reduce the occurrence of EPL, although it is possible their use brings other benefits such as improved conception. By taking into account the risk factors identified in this study, clinicians will be more fully equipped to make evidence-based decisions regarding therapeutic use and treatment options whilst providing stud managers with more accurate prognostic information.

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Conflicts of Interest: None

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Table 1. Final multivariable model of risk factors associated with early pregnancy loss (Days 15 to 65) in a cohort of 1753 thoroughbred mares based in the Newmarket area of the UK over the 2013 and 2014 breeding seasons (n=2245 pregnancies). Mare included as a random effect (p=0.07).

Variable	**Level**	**Odds ratio**	**95% Confidence interval**	**Wald test p-value**	**LRT* p-value**
Mare age (years)		1.11	1.04, 1.18	0.001	0.001
Number of previous live foals	0	*Reference*			0.006
	1	3.52	1.56, 7.95	0.002	
	≥2	2.00	0.90, 4.42	0.09	
Use of ovulatory induction agent[#]	No	*Reference*			<0.001
	Yes	0.31	0.17, 0.55	<0.001	
Presence of uterine cysts	No	*Reference*			0.03
	Yes	1.76	1.07, 2.91	0.03	
Day 15/16 vesicle size (mm)		0.24	0.16, 0.38	<0.001	<0.001

*Likelihood ratio test

[#]3000 iu Chorulon intravenously (n=621), 2.1 mg Ovuplant® (n=1434) or 0.04 mg Receptal® (n=11)

Table 2. Final multivariable model of risk factors associated with early pregnancy loss in 344 thoroughbred mares with initial multiple pregnancies (twin or triplet) based in the Newmarket area of the UK over the 2013 and 2014 breeding seasons.

Variable	**Level**	**Odds ratio**	**95% Confidence interval**	**Wald test p-value**	**LRT* p-value**
Mare age (years)		1.13	1.03, 1.25	0.009	0.009
Use of ovulatory induction agent					0.02
	No	*Reference*			
	Yes	0.23	0.07, 0.72	0.01	
Use of dexamethasone at cover					0.006
	No	*Reference*			
	Yes	6.51	1.93, 22.0	0.003	
Use of flunixin# at reduction					0.02
	No	*Reference*			
	Yes	0.34	0.14, 0.84	0.02	

*Likelihood ratio test

#Flunixin meglumine 50 mg/45kg IV

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Supplementary Table 1. Descriptive and univariable analysis results of potential **A.** mare **B.** stallion **C.** therapeutic and **D.** pregnancy risk factors for early pregnancy loss (EPL) in a cohort of 1753 thoroughbred mares (2245 pregnancies) in the Newmarket region of the UK in the 2013 and 2014 breeding seasons. 'Mare' was included in the models as a random effect to account for repeat pregnancies in the same mare.

Supplementary Table 1A.

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Mare age (n=2239)	2	1	0	0.0	0.0, 95.0	1.14*	1.09, 1.18	<0.001
	3	38	0	0.0	0.0, 7.6			
	4	269	7	2.6	1.1, 5.3			
	5	266	16	6.0	3.5, 9.6			
	6	261	12	4.6	2.4, 7.9			
	7	218	10	4.6	2.2, 8.3			
	8	184	15	8.2	4.6, 13.1			
	9	165	10	6.1	2.9, 10.9			
	10	148	10	6.8	3.3, 12.1			
	11	133	17	12.8	7.6, 19.7			
	12	111	16	14.4	8.5, 22.4			
	13	97	16	16.5	9.7, 25.4			
	14	88	12	13.6	7.2, 22.6			
	15	68	10	14.7	7.3, 25.4			
	16	59	6	10.2	3.8, 20.8			
	17	51	6	11.8	4.4, 23.9			
	18	34	5	14.7	5.0, 31.1			
	19	21	3	14.3	3.0, 36.3			
	20	17	5	29.4	10.3, 56.0			
	21	6	0	0.0	0.0, 39.3			
	22	3	1	33.3	0.8, 90.6			
	24	1	0	0.0	0.0, 95.0			
Mare status (n=2245)	maiden	416	13	3.1	1.7, 5.3	*Reference*		<0.001
	barren	308	34	11.0	7.8, 15.1	3.70	1.86, 7.38	
	foaled	1407	122	8.7	7.3, 10.3	3.06	1.67, 5.61	
	rested	114	9	7.9	3.7, 14.5	2.73	1.08, 6.88	
EPL previous season (n=1542)	no	1423	91	6.4	5.2, 7.8	*Reference*		0.05
	yes	119	15	12.6	7.2, 19.9	2.01	1.07, 3.77	
Previous live foals	0	469	14	3.0	1.6, 5.0	*Reference*		<0.001

(n=2206								
	1	347	30	8.6	5.9, 12.1	3.20	1.61, 6.33	
	2-5	840	63	7.5	5.8, 9.5	2.69	1.45, 5.00	
	6+	550	69	12.5	9.9, 15.6	4.98	2.67, 9.30	
Number of cycles covered (n=2234)	1	1655	125	7.6	6.3, 8.9	*Reference*		0.48
	2	455	42	9.2	6.7, 12.3	1.28	0.86, 1.92	
	≥3	124	10	8.1	3.9, 14.3	1.04	0.50, 2.17	
Uterine cysts (n=2245)	no	1844	122	6.6	5.5, 7.8	*Reference*		<0.001
	yes	401	56	14.0	10.7, 17.7	2.40	1.64, 3.50	
Days from foaling to cover (n=1346)	8-19	33	4	12.1	3.4, 28.2	*Reference*		0.80
	20-89	1284	110	8.6	7.1, 10.2	0.67	0.20, 2.22	
	90+	29	3	10.3	2.2, 27.4	0.57	0.09, 3.58	
Month of cover (n=2243)	Feb	304	17	5.6	3.3, 8.8	*Reference*		0.43
	March	677	48	7.1	5.3, 9.3	1.32	0.72, 2.43	
	April	673	58	8.6	6.6, 11.0	1.64	0.90, 2.98	
	May	514	47	9.1	6.8, 12.0	1.67	0.91, 3.10	
	June/July	75	7	9.3	3.8, 18.3	1.51	0.55, 4.15	
Uterine fluid (cm) (n=2201)	0	1449	116	8.0	6.7, 9.5	*Reference*		0.37
	1	525	37	7.0	5.0, 9.6	0.84	0.55, 1.29	
	2	147	13	8.8	4.8, 14.6	1.10	0.56, 2.16	
	≥3	80	3	3.8	0.8, 10.6	0.41	0.12, 1.42	

*Mare age modelled as a continuous variable

Supplementary Table 1B

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Stallion age (n=2165)	3-5	310	26	8.4	5.6, 12.0	*Reference*		0.40
	6-9	748	54	7.2	5.5, 9.3	0.83	0.49, 1.41	
	10-13	518	51	9.8	7.4, 12.7	1.19	0.69, 2.07	
	14-18	294	19	6.5	3.9, 9.9	0.74	0.38, 1.45	
	19+	295	21	7.1	4.5, 10.7	0.80	0.42, 1.55	

Supplementary Table 1C

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Pre-oestrus PG (n=2245)	no	1584	120	7.6	6.3, 9.0	*Reference*		0.56
	yes	661	58	8.8	6.7, 11.2	1.11	0.78, 1.59	
Pre-oestrus altrenogest (n=2245)	no	2106	167	7.9	6.8, 9.2	*Reference*		0.98
	yes	139	11	7.9	4.0, 13.7	1.01	0.50, 2.01	
Pre-oestrus domperidone (n=2245)	no	2195	175	8.0	6.9, 9.2	*Reference*		0.59
	yes	50	3	6.0	1.3, 16.5	0.72	0.20, 2.54	
Pre-oestrus sulpiride (n=2245)	no	2187	172	7.9	6.8, 9.1	*Reference*		0.45
	yes	58	6	10.3	3.9, 21.2	1.46	0.56, 3.76	
Pre-oestrus P and E (n=2245)	no	2195	172	7.8	6.7, 9.0	*Reference*		0.40
	yes	50	6	12.0	4.5, 24.3	1.54	0.58, 4.09	
Ovulatory induction agent (n=2245)	no	183	36	19.7	14.2, 26.2	*Reference*		<0.001
	yes	2062	142	6.9	5.8, 8.1	0.26	0.16, 0.42	
Intrauterine antibiotics at cover (n=2245)	no	1132	86	7.6	6.1, 9.3	*Reference*		0.78
	yes	1113	92	8.3	6.7, 10.0	1.05	0.75, 1.47	
Dexamethasone at cover (n=2245)	no	2081	163	7.8	6.7, 9.1	*Reference*		0.63
	yes	164	15	9.1	5.2, 14.6	1.16	0.63, 2.14	

Oxytocin at cover (n-2245)	no	1090	95	8.7	7.1, 10.5	*Reference*		0.15
	yes	1155	83	7.2	5.8, 8.8	0.78	0.55, 1.10	
Intrauterine lavage at cover (n=2245)	no	1718	135	7.9	6.6, 9.2	*Reference*		0.92
	yes	527	43	8.2	6.0, 10.8	0.98	0.66, 1.46	

Supplementary Table 1D

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Number of vesicles (n=2241)	1	1881	148	7.9	6.7, 9.2	*Reference*		0.93
	2	344	27	7.8	5.2, 11.2	0.98	0.61, 1.56	
	3	16	1	6.3	0.2, 30.2	0.66	0.07, 5.96	
Size of vesicle at day 15/16 (mm) (n=1881)	0-10	90	21	23.3	15.1, 33.4	0.26*	0.17, 0.39	<0.001
	11-20	1111	98	8.8	7.2, 10.6			
	21-30	658	25	3.8	2.5, 5.6			
	31-40	22	0	0.0	0.0, 12.7			

*Vesicle size modelled as a continuous variable; size grouped into 10mm intervals to illustrate EPL incidence in each category

Supplementary Table 2. Descriptive and univariable analysis results of potential **A.** mare **B.** stallion **C.** therapeutic **D.** pregnancy risk factors for early pregnancy loss in a cohort of 344 thoroughbred mares with multiple (twin/triplet) pregnancies in the Newmarket region of the UK in the 2013 and 2014 breeding seasons.

Supplementary Table 2A

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Mare age (n=341)	3	9	0	0.0	0.0, 28.3	1.13*	1.04, 1.23	0.006
	4	54	1	1.9	0.0, 9.9			
	5	39	3	7.7	1.6, 20.9			
	6	27	2	7.4	0.9, 24.3			
	7	28	2	7.1	0.9, 23.5			
	8	20	0	0.0	0.0, 13.9			
	9	26	0	0.0	0.0, 10.9			
	10	25	0	0.0	0.0, 11.3			
	11	22	2	9.1	1.1, 29.2			
	12	15	2	13.3	1.7, 40.5			
	13	16	2	12.5	1.6, 38.3			
	14	9	4	44.4	13.7, 78.8			
	15	11	2	18.2	2.3, 51.8			
	16	12	3	25.0	5.5, 57.2			
	17	15	2	13.3	1.7, 40.5			
	18	5	0	0.0	0.0, 45.1			
	19	2	0	0.0	0.0, 77.6			
	20	5	0	0.0	0.0, 45.1			
	21	1	0	0.0	0.0, 95.0			
Mare status (n=344)	maiden	89	4	4.5	1.2, 11.1	*Reference*		0.13
	barren	67	9	13.4	6.3, 24.0	3.30	0.97, 11.22	
	foaled	166	13	7.8	4.2, 13.0	1.81	0.57, 5.71	
	rested	22	0	0.0	0.0, 12.7	0	-	
EPL previous season (n=240)	no	213	10	4.7	2.3, 8.5	*Reference*		0.004
	yes	27	6	22.2	8.6, 42.3	5.80	1.92, 17.55	
Live foals (n=337)	0	98	4	4.1	1.1, 10.1	*Reference*		0.09
	1-5	142	10	7.0	3.4, 12.6	1.78	0.54, 5.85	
	6+	97	12	12.4	6.6, 20.6	3.32	1.03, 10.68	

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Number of cycles covered (n=344)	1	275	20	7.3	4.5, 11.0	*Reference*		0.92
	2	56	5	8.9	3.0, 19.6	1.25	0.45, 3.48	
	≥3	13	1	7.7	0.0, 36.0	1.06	0.13, 8.59	
Uterine cysts (n=343)	no	287	17	5.9	3.5, 9.3	*Reference*		
	yes	56	9	16.1	7.6, 28.3	3.04	1.28, 7.23	0.02
Days from foaling to cover (n=153)	10-19	5	1	20.0	0.5, 71.6	*Reference*		0.38
	20+	148	11	7.4	3.8, 12.9	0.32	0.03, 3.13	
Month of cover (n=344)	Feb	62	4	6.5	1.8, 15.7	*Reference*		0.73
	March	111	9	8.1	3.8, 14.8	1.28	0.38, 4.34	
	April	95	5	5.3	1.7, 11.9	0.81	0.21, 3.12	
	May	70	7	10.0	4.1, 19.5	1.61	0.45, 5.79	
	June	6	1	16.7	0.4, 64.1	2.90	0.27, 31.15	
Uterine fluid (cm) (n=343)	0	222	17	7.7	4.5, 12.0	*Reference*		0.81
	1-2	107	9	8.4	3.9, 15.4	1.11	0.48, 2.57	
	≥3	14	0	0.0	0.0, 19.3	0	-	

*Mare age modelled as a continuous variable

Supplementary Table 2B

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Stallion age (n=339)	3-5	76	6	7.9	3.0, 16.4	*Reference*		0.86
	6-9	103	6	5.8	2.2, 12.2	0.72	0.22, 2.33	
	10-13	85	8	9.4	4.2, 17.7	1.21	0.40, 3.67	
	14-18	35	3	8.6	1.8, 23.1	1.09	0.26, 4.65	
	19+	40	2	5.0	0.6, 16.9	0.61	0.12, 3.19	

Supplementary Table 2C

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Pre-oestrus PG (n=344)	no	219	18	8.2	4.9, 12.7	*Reference*		0.53
	yes	125	8	6.4	2.8, 12.2	0.76	0.32, 1.81	
Pre-oestrus altrenogest (n=344)	no	322	24	7.5	4.8, 10.9	*Reference*		0.78
	yes	22	2	9.1	1.1, 29.2	1.24	0.27, 5.63	
Pre-oestrus domperidone (n=344)	no	336	25	7.4	5.1, 10.8	*Reference*		0.62
	yes	8	1	12.5	0.1, 49.2	1.78	0.21, 15.02	
Pre-oestrus sulpiride (n=344)	no	337	25	7.4	4.9, 10.8	*Reference*		0.54
	yes	7	1	14.3	0.4, 57.9	2.08	0.24, 17.96	
Pre-oestrus P and E (n=344)	no	334	25	7.5	4.9, 10.9	*Reference*		0.78
	yes	10	1	10.0	0.3, 44.5	1.37	0.17, 11.28	
Ovulatory induction agent (n=344)	no	23	5	21.7	7.5, 43.7	*Reference*		0.02
	yes	321	21	6.5	4.1, 9.8	0.25	0.09, 0.75	
Intrauterine antibiotics at cover	no	170	11	6.5	3.3, 11.3	*Reference*		0.45

(n=344)								
	yes	174	15	8.6	4.9, 13.8	1.36	0.61, 3.06	
Dexamethasone at cover (n=334)	no	323	21	6.5	4.1, 9.8	*Reference*		0.02
	yes	21	5	23.8	8.2, 47.2	4.49	1.50, 13.46	
Oxytocin at cover (n=344)	no	174	13	7.5	4.0, 12.4	*Reference*		0.95
	yes	170	13	7.6	4.1, 12.7	1.03	0.46, 2.28	
Intrauterine lavage at cover (n=344)	no	259	17	6.6	3.9, 10.3	*Reference*		0.24
	yes	85	9	10.6	5.0, 19.2	1.69	0.72, 3.94	

Supplementary Table 2D

Variable	Level	n	EPL n	% EPL	95% confidence interval (EPL incidence)	OR	95% confidence interval (OR)	Likelihood ratio test p-value
Number of vesicles	2	327	25	7.6	5.0, 11.1	*Reference*		0.78
	3	17	1	5.9	0.1, 28.7	0.76	0.10, 5.93	
Ovulation (n=240)	multiple same ovary	141	7	5.0	2.0, 10.0	*Reference*		0.21
	bilateral	99	9	9.1	4.2, 16.6	1.91	0.69, 5.33	
Size of vesicle at day 15/16 (mm) (n=262)	0-10	16	0	0	0.0, 17.1	*0.0*	-	0.48
	11-20	173	14	8.1	4.5, 13.2	*Reference*		
	21-30	72	4	5.6	1.5, 13.6	0.67	0.21, 2.10	
	31-40	1	0	0.0	0.0, 95.0	0.0	-	
Flunixin (n=344)	no	136	15	11.0	6.3, 17.5	*Reference*		0.05
	yes	208	11	5.3	2.7, 9.3	0.45	0.20, 1.01	
Buscopan (n=344)	no	336	26	7.7	5.1, 11.1	*Reference*		
	yes	8	0	0.0	0.0, 31.2	0.00	-	
Sedation (n=344)	no	262	23	8.8	5.6, 12.9	*Reference*		0.10

	yes	82	3	3.7	0.8, 10.3	0.39	0.12, 1.35	
Same location at reduction (n=249)	no	157	8	5.1	2.2, 9.8	*Reference*		0.10
	yes	92	10	10.9	5.3, 19.1	2.27	0.86, 5.98	
Vet (n-344)	V01	16	0	0.0	0.0, 17.1	0.00	-	0.27
	V02	50	4	8.0	2.2, 19.2	0.62	0.18, 2.19	
	V03	5	0	0.0	0.0, 45.1	0.00	-	
	V04	89	4	4.5	1.2, 11.1	0.34	0.10, 1.17	
	V05	65	8	12.3	5.5, 22.8	*Reference*		
	V06	2	1	50.0	12.6, 98.7	7.13	0.40, 125.52	
	V07	34	2	5.9	0.7, 19.7	0.45	0.09, 2.23	
	V10	2	0	0.0	0.0, 77.6	0.00	-	
	V11	54	3	5.6	1.2, 15.4	0.42	0.11, 1.67	
	V12	14	3	21.4	4.7, 50.8	1.94	0.44, 8.50	
	V13	13	1	7.7	0.2, 36.0	0.59	0.07, 5.20	