Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12739

Likelihood of pregnancy after embryo transfer is reduced in recipient mares with a short preceding oestrus

J. CUERVO-ARANGO*, A. N. CLAES, M. RUIJTER-VILLANI and T. A. STOUT

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.

*Correspondence email: j.cuervo-arangolecina@uu.nl; Received: 27.02.17; Accepted: 05.08.17

Summary

Background: Previous surveys reported a positive association between the length of the follicular phase and subsequent fertility in embryo transfer donor and Thoroughbred mares. However, it is unclear whether a longer oestrus positively influences fertilisation and oviductal development (oocyte quality, oviductal environment), or uterine receptivity and survival of the embryo in the uterus.
Objectives: To determine the effect of length of oestrus (characterised by duration of endometrial oedema) on likelihood of pregnancy and early embryo loss (EEL) in recipient mares after embryo transfer (ET).
Study design: Retrospective clinical study.
Methods: A total of 350 embryos recovered from 161 donor mares were transferred into 231 recipient mares during three consecutive breeding seasons. The following variables were analysed via two binary logistic regression models to determine their effect on pregnancy and EEL: 1) year of transfer, 2) season of transfer, 3) age of the recipient mare, 4) age of the donor mare, 5) operator performing the transfer, 6) singleton or twin embryo, 7) embryo size, 8) number of transfers to a given recipient in any one season, the use of 9) d-cloprostenol and 10) hCG in the recipient mare, 11) day of ovulation of the recipient mare at ET, 12) number of corpora lutea (CLs) at ET, and 13) duration of oestrus in the recipient mare.
Results: Age of the donor mare ($P = 0.01$), operator ($P = 0.008$), number of CLs at ET ($P = 0.05$) and the number of days of endometrial oedema during the oestrus preceding ET to the recipient mare ($P = 0.004$) influenced the likelihood of pregnancy. Early embryonic loss was influenced only by the year of transfer ($P = 0.014$).
Main limitations: Retrospective design of the study. The involvement of several veterinary surgeons over the 3-year period could have affected data recording.
Conclusions: The likelihood of pregnancy in recipient mares is positively correlated with the duration of endometrial oedema during the oestrus preceding ET. This suggests a role for an adequate duration of oestrogenic priming during oestrus on uterine receptivity and embryo survival.

Keywords: horse; recipient mare; embryo transfer, endometrial oedema; oestrus; fertility

Introduction

The interovulatory interval (IOI) in mares is highly variable, ranging from 16 to 28 days [1,2], but with a mean of 21 days [1]. While dioestrus (luteal phase) has a relatively constant duration (with a reported mean of 14.9 days, and a range of 12–16 days [3]), the duration of oestrus (follicular phase), i.e. the period between luteolysis and ovulation, can vary tremendously between mares and cycles (reported mean duration of 7.3 days, with a range of 3–13 days [2]). This variation can be exacerbated by inducing luteolysis with $PGF_{2\alpha}$ or one of its synthetic analogues (PGF), with a reported interval range from PGF treatment to ovulation of 2–16 days [4].

In a field situation, oestrus and dioestrus can be differentiated on the basis of uterine and cervical tone, uterine echotexture and the ultrasonographic detection of a CL [1,5]. Dioestrus is characterised by the absence of endometrial oedema, the presence of a visible CL and high levels of progesterone (>1 ng/mL) [5]. The development of endometrial oedema is a phenomenon observed during oestrus, and involves engorgement of the endometrial folds with fluid in response to follicular oestrogens in the absence of progesterone [5,6]. As for oestrous duration, there is significant variability in the duration of the period of endometrial oedema during oestrus, with individual mares showing endometrial oedema for none, one, a few, or several days prior to ovulation [1,7]. The primary factor regulating both the development and subsequent dissipation of endometrial oedema is the balance between circulating concentrations of oestradiol and progesterone [6]. It has been suggested that the threshold circulating progesterone concentration necessary to inhibit endometrial oedema ranges from 1 to 2 ng/mL [6]. However, the progesterone concentration does not appear to be the only factor regulating the presence of endometrial oedema since a small percentage of mares [1,7] show little or no oedema during the follicular phase despite basal concentrations of progesterone (1 ng/mL) during a so called ‘silent oestrus’ [1].

The variation in the duration of oestrus accompanied by endometrial oedema appears to be relevant to fertility. Several studies have shown a positive correlation between the length of the follicular phase and likelihood of establishing pregnancy in mares following PGF-induced [4,7,8] or spontaneous luteolysis [7]. Moreover, since the odds of embryo recovery in embryo transfer (ET) donors and the likelihood of pregnancy in Thoroughbred mares increased with the length of the preceding follicular phase, a longer duration of oestrus seems to be beneficial for the process of fertilisation, presumably by increasing either oocyte quality and/or improving the oviductal environment. It has also been shown that the switch in dominance of ovarian steroids (oestradiol to progesterone) during the transition from oestrus to dioestrus [9] affects endometrial gene expression in a manner that should help support the survival of an embryo. It is unclear, however, whether the length of oestrus also influences uterine receptivity and endometrial support of embryonic growth and development.

Comparing the pregnancy outcome after ET in recipient mares that had a preceding oestrus of different lengths represents an excellent model to test the hypothesis that the duration of oestrus critically affects uterine receptivity for pregnancy. Therefore, the objectives of this study were to determine the effect of the duration of the period of endometrial oedema in the oestrus prior to ET on the likelihood of pregnancy and EEL in recipient mares. It was hypothesised that a longer duration of oestrus/endometrial oedema would be associated with a more favourable uterine environment, and consequently, a higher likelihood of pregnancy.

Materials and methods

The data used in this study were obtained retrospectively from reproductive records of donor and recipient mares presented to or owned by the Department of Equine Sciences of the Utrecht University during three consecutive breeding seasons (2014–2016). More than 90% of the recipient and donor mares were Warmbloods. Overall, data for 350 day-8 embryos recovered from 161 different donor mares during 297 embryo recovery attempts, and subsequently transferred to 231 different recipient mares, were investigated. All recipients were maiden or barren at the time of transfer. The ages of the recipient and donor mares ranged from 3 to 17 and 3 to 23 years old, with means of 7.2 ± 2.9 and 11.3 ± 5.6 years, respectively.

Embryo flushing and recovery

The uterus of donor mares was flushed on day 8 after ovulation using 3–6 L of lactated Ringer's solution (3000 mL Ringer Lactate)[a] supplemented with 15 mL of fetal calf serum. In flushes requiring two bags, the 15 mL of fetal calf serum was added only to the first bag of 3 L lactated Ringer's, and therefore, the second bag contained plain lactated Ringer's solution only. Recovered embryos were loaded into 0.5 mL straws and transferred by one of the five veterinary surgeons transcervically into a recipient mare that had ovulated 4–9 days previously. All recipient mares were sedated with 4 mg of detomidine hydrochloride (10 mg/mL detomidine, Domosedan)[b] immediately prior to ET.

Routine recipient mare management

Most recipient mares (90.4%) belonged to the Department of Equine Sciences of Utrecht University. Mares were kept in small groups (5–20 mares) in grass paddocks (ad libitum feeding) or in individual boxes with daily exercise in a horse-walker or sand paddock, and fed hay and concentrate with ad libitum access to fresh water. During the first examination of the breeding season, the reproductive tract of recipient mares was palpated and evaluated via transrectal ultrasonography, and the cervix was palpated per vaginam to diagnose cervical pathology and aid in the determination of the stage of oestrous cycle.

Mares in dioestrus (the presence of one or more CL, the absence of endometrial oedema and a tight cervix) were treated with 75 μg of d-cloprostenol (75 μg/mL d-cloprostenol)[c] and evaluated ultrasonographically 1–4 days later (depending on the diameter of the largest dioestrous follicle at the time of treatment) to ensure luteolysis had occurred. Similarly, recipient mares that did not receive an embryo during a given dioestrus (there were more recipients available than embryos) were either administered d-cloprostenol 8–10 days' post-ovulation or allowed to return to oestrus spontaneously (examined 15 days after last ovulation). A total of 210/350 (60%) oestrous periods were PGF-induced.

Once in oestrus, mares were scanned every 24 h until ovulation was detected. The presence of endometrial oedema was scored subjectively on a scale of 0–3 depending on the intensity of the endometrial fold pattern (0 = no oedema, 1 = some oedema, 2 = obvious oedema and 3 = a lot of oedema). The 'duration of oestrus with endometrial oedema' was recorded as the interval from the first day on which the mare showed obvious endometrial oedema (score of 2–3) until the day of ovulation (D0). This period will from here on be referred to as the 'duration of oestrus', even though it may not necessarily exactly match the total length of physiological oestrus (interval between luteolysis and ovulation). All follicles larger than 25 mm were followed until the first ovulation was detected by the disappearance of a previously recorded preovulatory follicle and replacement with a corpus haemorrhagicum. When a recipient mare had two or more preovulatory follicles, additional follicles were not monitored to determine the time of the asynchronous ovulation. However, all recipient mares were examined on the day of ET and the number of CLs was noted.

Oestrous cycles from mares that were in oestrus with endometrial oedema and a follicle >35 mm at the first examination were excluded from the analysis of the effect of duration of oedema on likelihood of pregnancy (n = 40 oestrous cycles), since the duration of oestrus with endometrial oedema could not be determined accurately.

Mares without a CL and with the largest follicle being <20 mm for at least 10 days at the beginning of the breeding season (February–April) were considered to be in anoestrus. Anoestrous mares were examined once a week until they entered the transitional phase (follicles between 20 and 30 mm with varying amounts of endometrial oedema), from which time they were examined 2–3 times a week as they progressed towards the first ovulation of the season (n = 18 cycles).

Ovulation was induced with 1500 IU of hCG i.v.[d] in 229/350 (65.4%) cycles when the mare was in oestrus with a follicle ≥35 mm in diameter. The decision to induce ovulation was dependent on veterinary preference and to help ensure adequate donor-recipient synchrony. Donor-recipient synchrony was always between −1 and +4 days (only mares that ovulated between 1 day before and 4 days after the donor mare were used as embryo recipients).

Pregnancy diagnosis was performed 4–6 days after ET and then confirmed at 2 weeks intervals until the pregnancy was approximately 45 days old. Nonpregnant mares were reexamined 2 days after a negative pregnancy examination to confirm the negative diagnosis, and then allowed to enter oestrus spontaneously.

Data analysis

The experimental unit was considered to be the oestrous cycle of the recipient mare prior to receiving an embryo.

The following variables were taken into account when analysing factors that could affect likelihood of pregnancy and EEL: 1) year of transfer: 2014 (n = 129), 2015 (n = 110) and 2016 (n = 111); 2) season: early season (February–April, n = 68), peak season (May–July, n = 195) and late season (August–November, n = 87); 3) age of the recipient mare in years; 4) age of donor mare; 5) operator, with five different veterinary surgeons that performed 49, 94, 137, 57 and 13 transfers, respectively; 6) type of embryo: singleton (n = 244) or twin embryos recovered in a single flush (n = 106); 7) embryo size: small (<400 μm; n = 65), medium (400–800 μm; n = 101) and large (>800 μm; n = 35); 8) number of transfers for each recipient in a single season: first transfer of the year (n = 281), second transfer after a previous failure (n = 58) and third or more transfer after two or more consecutive failures (n = 11); 9) induction of oestrus with d-cloprostenol: yes (n = 210) or no (n = 140); 10) induction of ovulation with hCG: yes (n = 229) or no (n = 131); 11) day of ovulation of the recipient mare at ET, from day 4 to day 9 after ovulation in single days (n = 26, 42, 71, 70, 90 and 51, respectively); 12) number of CLs at the time of ET: 1 CL (n = 249) or ≥2 CL (n = 101); and 13) length of oestrus accompanied by endometrial oedema in the recipient mare, in days (n = 310).

Binary logistic regression (SPSS statistical software) was used to analyse the data with separate analyses for each of the two outcomes. The first outcome was pregnancy status at 4–6 days post-ET (0 = not pregnant, 1 = pregnant). The second outcome was pregnancy status at 45 days' post-ovulation of the donor mare, conditional upon recipient mares having been diagnosed as pregnant at 4–6 days post-ET (0 = not pregnant meaning EEL, 1 = pregnant). All independent variables defined above were considered for inclusion in both analyses. No stepwise regression was used in the model, and model checking was performed following the guide for data assumptions for binomial logistic regression included in the SPSS software; the Box-Tidwell test was used to confirm the absence of multicollinearity of the independent variables. Checking for outliers, leverage and influential points was performed using Stata (Graphical user interface) in SPSS. The goodness of fit test indicated that the model fitted the data well ($P>0.1$). The statistical significance was set at $P\leq0.05$ to identify variables that significantly influenced the likelihood of pregnancy or EEL. For each variable that showed a significant effect, a Chi-square test was used to examine the difference between conditions on likelihood of pregnancy and EEL. A Spearman's rank correlation was used to determine the strength of the association between the duration of oestrus with oedema and the interval from PGF treatment to ovulation (ITO) in mares treated with d-cloprostenol to induce the oestrus preceding ET. Finally, a Chi-squared test was used to examine the effect of ITO, and to compare the difference between the first vs. subsequent ovulations of the year on likelihood of pregnancy.

Results

The overall percentage of recipients that became pregnant after embryo transfer was 76.3% (267/350) and the incidence of EEL by day 45 was 14.2% (38/267). The effects (statistical significance) of the different variables examined on likelihood of pregnancy and EEL are shown in Table 1. Age of the donor mare (P = 0.01), operator (P = 0.008), the number of CLs at the time of ET (P = 0.05) and the duration of endometrial oedema in the oestrus preceding ET (P = 0.004) influenced the likelihood of pregnancy. The intensity score of endometrial oedema was not associated with the likelihood of pregnancy. The year of the study was the only variable that influenced EEL (P = 0.01).

The likelihood of pregnancy decreased as the age of the donor mare increased, an age effect was not apparent for the recipient mares included in this study (Fig 1). The veterinary surgeon that performed the ET influenced the outcome, with a significant difference between the operator with the lowest (63.8%, n = 94) and highest (94.3%, n = 57) likelihood of establishing pregnancy.

The likelihood of pregnancy increased with increasing length of the recipient's preceding oestrus (Fig 2). The median duration of oestrus in all recorded cycles (n = 310) in recipient mares was 3 days. The likelihood of pregnancy in recipient mares with 3 or more days of oedema in the oestrus preceding ET was significantly higher (83.1%; 157/189) than that in recipients with less than 3 days of oedema (63.6%; 77/121). The likelihood of pregnancy of recipients with zero days of oedema during the follicular phase was the lowest (50%; 11/22).

The likelihood of pregnancy and EEL of recipient mares classified according to oestrous cycle characteristics is shown in Table 2. The

TABLE 1: Factors affecting likelihood of pregnancy in recipient mares after ET using logistic regression

Variable	P-value	Odds ratio	95% confidence interval Lower	95% confidence interval Upper
1. Year	0.5	–	–	–
2. Season	0.1	–	–	–
3. Age of recipient	0.4	–	–	–
4. Age of donor	0.01	0.94	0.89	0.99
5. Operator	0.008	3.6	0.8	17.4
6. Twin/singleton	0.06	–	–	–
7. Embryo size	0.1	–	–	–
8. Number of transfer	0.5	–	–	–
9. $PGF_{2\alpha}$	>0.9	–	–	–
10. hCG	>0.9	–	–	–
11. Day post-Ov	0.08	–	–	–
12. Number of CL	0.05	1.8	0.8	3.9
13. Length of oestrus	0.004	1.3	1.1	1.5

Odds ratio and 95% CI are shown for variables that influenced the pregnancy status significantly. For categorical variables with more than two levels ('operator'), the P-value and odds ratio represent the comparison between the levels with the lowest and highest likelihood of pregnancy after ET.

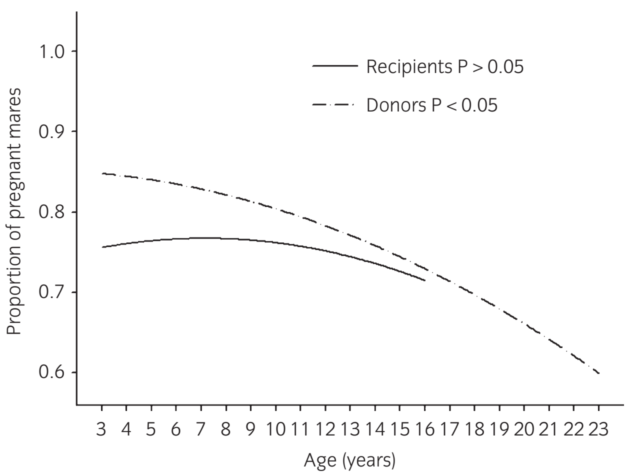

Fig 1: Effect of donor and recipient age on likelihood of pregnancy after embryo transfer. The regression model indicated a significant effect of age of donor mare (P = 0.011) but not recipient mare (P>0.05) on likelihood of pregnancy. The association between age and the proportion of pregnant recipients are depicted by regression curves that best fits the data. The likelihood of pregnancy was defined as the number of pregnant recipient mares 4–6 days after ET divided by the total number of transfers.

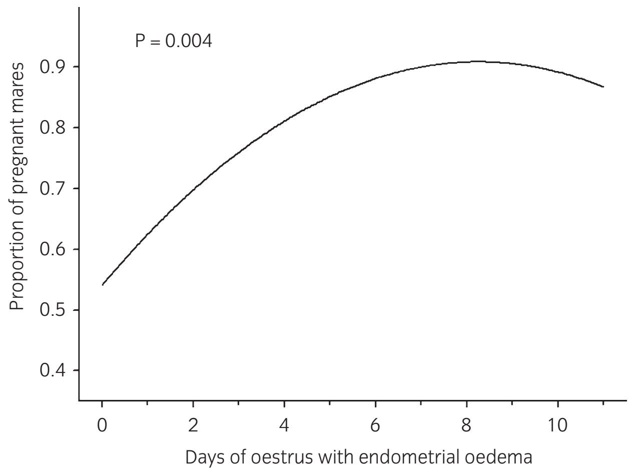

Fig 2: Effect of length of oestrus (days with endometrial oedema score of 2 or 3 prior to ovulation) in recipient mares during the oestrus preceding embryo transfer on likelihood of pregnancy. The association between the proportion of pregnant recipients and the length of oestrus is depicted by the regression curve that best fits the data (P = 0.004). The likelihood of pregnancy was defined as the number of pregnant recipient mares 4–6 days after ET divided by the total number of transfers.

TABLE 2: Oestrous cycle characteristics of recipient mares and their association with likelihood of pregnancy and EEL after ET

Type of cycle	ITO	n	Median length of oedema (days)	MO (%)	P (%)	EEL (%)
PGF-induced	3–6 days	81	2	25.9	65.4[a]	20.8
	7–8 days	46	3	30.4	82.6[b]	10.5
	≥9 days	83	4	39.8	84.3[b]	17.1
Spontaneous	–	122	3	26.2	74.6[b]	14.3
1st Ov of year	–	18	6	16.7	88.9[b]	6.3

Oestrous cycles were classified according to the use of d-cloprostenol to induce oestrus (PGF-induced or spontaneous) and whether mares were already cyclic or emerging from the winter anoestrus (first ovulation of the year). ITO: Interval from d-cloprostenol (PGF) treatment to ovulation, expressed in days. Median length of oedema: median duration of the follicular phase with endometrial oedema until ovulation, expressed in days. MO: multiple ovulation rate determined by the number of corpora lutea (two or more) at the time of embryo transfer (ET). P: Proportion of pregnant recipient mares (number of pregnant mares divided by the number of embryo transfers performed × 100) 4–6 days after ET. EEL: early embryonic loss between the first pregnancy diagnosis and day 45 after ovulation in the donor mare. Different superscripts (a,b) indicate a significant difference (P<0.05) in the proportion of pregnant mares.

duration of endometrial oedema was significantly correlated (r = 0.475; P<0.01) with the ITO. The likelihood of pregnancy in recipient mares with an ITO of 6 days or less (65.4%; 53/81) was lower (P = 0.004) than that for mares with an ITO more than 6 days (83.9%; 108/129). The likelihood of pregnancy in recipient mares with 2 or more CLs at the time of transfer (88.1%; 89/101) was higher (P≤0.05) than that of recipients with 1 CL (71.5%; 178/249). However, the number of CLs did not affect EEL, with incidences of 15.7% vs. 11.2% for recipients with 1 and 2 CLs, respectively. The only factor that influenced EEL was the year of transfer (P = 0.01), with the highest EEL observed in 2014 (24.5%; 24/98), followed by 2015 (9.8%; 7/71) and 2016 (7.1%; 7/98).

Discussion

The hypothesis that a longer duration of oestrus accompanied by endometrial oedema would positively influence the likelihood of pregnancy was substantiated by the results of this study. Furthermore, the ITO was also positively correlated with the likelihood of pregnancy, which agrees with previous observations in Thoroughbred mares [7,8]. Not surprisingly, the ITO was significantly correlated with the duration of oestrus. Mares with a short ITO (i.e. <6 days) are expected to have a short oestrus because the period with basal progesterone (between induced luteolysis and the next ovulation) is short. A basal peripheral progesterone concentration and a high concentration of circulating oestrogens, are necessary for the uterus to show endometrial oedema [5,6].

The oestrous cycles with the longest duration of endometrial oedema corresponded to the ones preceding the first ovulation of the year (median of 6 days of endometrial oedema). Embryo transfer to recipient mares following the first ovulation of the year has been reported to be more successful than transfer after subsequent ovulations (74% vs. 62%, respectively) [10]. Although our results showed no significant difference between the first and subsequent ovulations, the number of mares that received an embryo following the first ovulation of the year was probably too small (n = 18) to make meaningful comparisons.

It is well known that exposure to oestrogens prior to a rise in progesterone is critical for inducing uterine receptivity. This is confirmed by the successful use in practice of oestrogen administration followed by progesterone treatment to prepare anoestrous mares to receive embryos [11–13]. The current study has demonstrated that the length of this exposure may also be crucial for optimising the likelihood of pregnancy.

The variation in likelihood of pregnancy as a factor of the ITO was high in recipients subjected to PGF-induced luteolysis (65–84%). Following PGF-induced luteolysis, mares had significant differences in the duration of oestrus, and consequently the likelihood of pregnancy, with a marked difference between mares that ovulated 6 or fewer days after treatment with PGF compared to mares that ovulated >6 days after PGF. These results parallel previous studies in Polo, Standardbred and Thoroughbred mares [4,7,8], in which there was a clear association between the ITO and the likelihood of pregnancy, with the lowest fertility in mares that ovulated soon after PGF. As a result of those studies, it was hypothesised that the reduction in fertility was most likely related to reduced oocyte quality since mares with a short ITO usually ovulated from a large dioestrous follicle that may have been present for a prolonged period prior to ovulation, and in which the oocyte may already have started to 'age' [4]. However, the similar effects of the different ITO and oestrous length groups on the likelihood of pregnancy in the ET recipients in the current study and mated or inseminated mares in the previous studies, suggests a significant effect of length of the preceding oestrus with endometrial oedema on uterine receptivity and embryo survival. Further studies are required to examine how the length of oestrus and circulating oestrogens affect the endometrial environment and receptivity to embryo development 1–2 weeks later.

The likelihood of pregnancy has been affected not only by physiological variation in oestrous length, but also by artificial manipulation of the cycle with exogenous progesterone [7,14,15]. The interval from removal of a progesterone intravaginal device (PRID) to ovulation was significantly correlated with fertility in Thoroughbred mares [7,14]. Mateu-Sánchez *et al.* [7] reported likelihoods of pregnancy in mares that ovulated within 4, between 5 and 7, and beyond 7 days after PRID removal as 42.9, 63.4 and 81%, respectively. The correlation between the length of oestrus and the interval between PRID removal and ovulation was high (r = 0.83). Furthermore, while the administration of progestogens for 2 days to oestrous mares failed to induce the desired delay in ovulation, it did reduce the fertility of treated mares compared with controls [15]. In the latter study, mares were treated with altrenogest when they were in oestrus with a follicle >35 mm for 2 days. Altrenogest treatment led to the disappearance of endometrial oedema and teasing behaviour within 2 days, but both were reestablished immediately after cessation of treatment. Despite having no effect on follicular growth, preovulatory diameter and ovulation time, the treatment protocol was associated with a reduction in likelihood of pregnancy compared with controls (36% vs. 61%, respectively). Apparently, the split and shortened oestrus induced by altrenogest had a negative effect on fertility [15]. These results further support a positive association between the length of oestrus accompanied by endometrial oedema and ability to establish pregnancy.

The greatest limitation of the current study is its retrospective design. The involvement of several veterinary surgeons over the 3-year period could have affected data recording. However, the endometrial oedema scoring system was unchanged throughout the entire study period. In addition, the statistical regression model took the effect of season and year into account, and should therefore have addressed any potential bias of data associated with the retrospective study design. In this regard, there was no obvious effect of year or season on likelihood of pregnancy. On the other hand, there were marked differences in the likelihood of pregnancy for the different veterinary surgeons performing the transfers, even though all were specialists in equine reproduction and had training and experience in performing the transfers. Clearly, it is possible that different vets had different competence in performing the ET procedure. It is, however, also possible that the variation in likelihood of pregnancy is explained by the use of different criteria to choose a recipient mare for a given transfer (e.g. a preference to use a recipient mare with a spontaneous cycle or one with a longer oestrus). It is more difficult to explain the difference in EEL between years. Perhaps, environmental conditions, or other variables not accounted for, had a confounding effect.

An unexpected observation was the association between the number of CL at the time of ET and likelihood of pregnancy. Interestingly, a similar finding was observed in a recent study which investigated the outcome of 181 embryo transfers and found a superior likelihood of pregnancy (86%) in recipients with 2 CLs compared to mares with 1 CL (70%) at the time of transfer [16]. The authors of that study suggested a possible beneficial effect of higher progesterone concentrations on embryo survival in mares with 2 CLs, although progesterone concentrations were not determined. However, this seems unlikely since there is no clear evidence of a positive role of progesterone supplementation on fertility in embryo recipients [17,18], despite widespread use [19]. On the basis of the results of the present study, it seems more plausible that the higher likelihood of pregnancy observed in recipient mares with 2 CLs reflects the higher multiple ovulation rate (MO) in mares with a longer follicular phase, interval from PGF treatment to ovulation and duration of oestrus [8]. In the present study, the MO rates for recipient mares with an oestrus of ≤2 and ≥3 days of oedema were 20.1 and 33.2%, respectively. The association between a longer follicular phase and a higher MO rate has been shown previously [8,20].

The results of this study confirm a positive association between the duration of oestrus and the subsequent likelihood of pregnancy in ET recipient mares. However, the underlying mechanisms behind this phenomenon are unknown. A previous publication [9] has demonstrated marked changes in the global endometrial gene expression pattern during the oestrous cycle as a result of length of exposure to the different ovarian steroids (oestrogens and progesterone) during the follicular and luteal phases; the change was considered critical to the preparation to support embryo survival. It is logical to conclude that the variation in the duration of the follicular phase period characterised by low progesterone and high oestrogen concentrations might affect the pattern of endometrial transcription and translation of genes required to support uterine receptivity and conceptus development. In mice, it has similarly been proposed that the timing and intensity of exposure of the uterus to oestrogens is a key determinant in the development of uterine receptivity for embryo implantation [21,22]. Long-term treatment with oestrogens (i.e.

11–40 days) is also often performed to enhance endometrial preparation for pregnancy before embryo transfer in women [23]. Furthermore, a shorter duration of oestrogen treatment (<11 days) was associated with reduced birth rates in women after embryo transfer compared with those receiving longer treatments [23].

In conclusion, the duration of the period of oestrus accompanied by endometrial oedema influenced the likelihood of establishing pregnancy in recipient mares after both PGF-induced and spontaneous luteolysis. This finding has potential relevance for practitioners, who could use the number days of endometrial oedema during the preceding oestrus as an additional criterion to select a recipient mare for embryo transfer.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight not required by this journal: retrospective analysis of clinical data. Explicit owner informed consent for inclusion of animals in this study was not stated.

Source of funding

None.

Authorship

J. Cuervo-Arango contributed to study design, data production, analysis and interpretation, preparation of the manuscript, figure creation, editing and final approval of the manuscript. T. Stout and A.N. Claes contributed to study design, data production, data analysis and interpretation, editing and final approval of the manuscript. M. Ruijter-Villani contributed to data production, editing and final approval of the manuscript.

Manufacturers' addresses

[a]Baxter Nederland B.V., Utrecht, the Netherlands.
[b]Vetoquinol B.V., 's Hertogenbosch, the Netherlands.
[c]Eurovet Animal Health B.V., Bladel, the Netherlands.
[d]Intervet Nederland B.V., Boxmeer, the Netherlands.

References

1. Ginther, O.J. and Pierson, R.A. (1989) Regular and irregular characteristics of ovulation and the interovulatory interval in mares. *J. Equine. Vet. Sci.* **9**, 4-12.
2. Santos, V.G., Bettencourt, E.M.V. and Ginther, O.J. (2015) Long-term characteristics of idiopathic persistent corpus luteum in the mare. *Theriogenology* **84**, 242-251.
3. Ginther, O.J. (Ed.) (1992) Characteristics of the ovulatory season. In: *Reproductive Biology of the Mare: Basic and Applied Aspects*, 2nd edn. Equiservices Publishing, Cross Plains, Wisconsin, USA.
4. Cuervo-Arango, J., Mateu-Sánchez, S., Aguilar, J.J., Nielsen, J.M., Etcharren, V., Vettorazzi, M.L. and Newcombe, J.R. (2015) The effect of the interval from PGF treatment to ovulation on embryo recovery and pregnancy rate in the mare. *Theriogenology* **83**, 1272-1278.
5. Ginther, O.J. (Ed.) (1995) Uterus (Chapter 6). In: *Ultrasonic Imaging and Animal Reproduction: Book 2 (horses)*. Equiservices Publishing, Cross Plains, Wisconsin, USA.
6. Pelehach, L.M., Greaves, H.E., Porter, M.B., Desvousges, A. and Sharp, D.C. (2002) The role of estrogens and progesterone in the induction and dissipation of uterine edema in mares. *Theriogenology* **54**, 441-444.
7. Mateu-Sánchez, S., Newcombe, J.R., Garcés-Narro, C. and Cuervo-Arango, J. (2016) The period of the follicular phase during which the uterus of mares shows estrus-like echotexture influences the subsequent pregnancy rate. *Theriogenology* **86**, 1506-1515.
8. Cuervo-Arango, J. and Newcombe, J.R. (2009) Cloprostenol in equine reproductive practice: something more than a luteolytic drug. *Reprod. Domest. Anim.* **45**, e8-e11.
9. Gebhardt, S., Merkl, M., Herbach, N., Wanke, R., Handler, J. and Bauersachs, S. (2012) Exploration of global gene expression changes during the estrous cycle in equine endometrium. *Biol. Reprod.* **87**, 1-13.
10. Carnevale, E.M., Ramirez, R.J., Squires, E.L., Alvarenga, M.A., Vaderwall, D.K. and McCue, P.M. (2000) Factors affecting pregnancy rate and early embryonic death after equine embryo transfer. *Theriogenology* **54**, 965-979.
11. Kaerch, F., Kozicki, L.E., Camargo, C.E., Weiss, R.R., Santos, I.W., Muradas, P.R., Bertol, M.A.F. and de Abreu, R.A. (2013) Embryo transfer in anovulatory recipient mares treated with estradiol benzoate and long-acting progesterone. *J. Equine. Vet. Sci.* **33**, 205-209.
12. Botelho, J.H.V., Pessoa, G.O., Rocha, L.G.P. and Yeste, M. (2015) Hormone supplementation protocol using estradiol benzoate and long-action progesterone is efficient in maintaining pregnancy of anovulatory recipient mares during autumn transitional phase. *Anim. Reprod. Sci.* **152**, 39-43.
13. Rocha Filho, A.N., Pessôa, M.A., Gioso, M.M. and Alvarenga, M.A. (2004) Transfer of equine embryos into anovulatory recipients supplemented with short or long acting progesterone. *Anim. Reprod.* **1**, 91-95.
14. Newcombe, J.R. (2002) Field observations on the use of a progesterone-releasing intravaginal device to induce estrus and ovulation in seasonally anestrous mares. *J. Equine. Vet. Sci.* **22**, 378-382.
15. Canisso, I.F., Gallacher, K., Gilbert, M.A., Korn, A., Schweizer, C.M., Bedford-Guaus, S.J. and Gilbert, R.O. (2013) Preovulatory progestagen treatment in mares fails to delay ovulation. *Vet. J.* **197**, 324-328.
16. Pedersen, H.G., Niklasson, M., Vullers, A. and Christoffersen, M. (2016) Embryo quality grade and recipient ovulation rate affect pregnancy rates in embryo transfer recipient mares. *J. Equine. Vet. Sci.* **41**, 61-62.
17. Pinto, M.R., Miragaya, M.H., Burns, P., Douglas, R. and Neild, D.M. (2017) Strategies for increasing reproductive efficiency in a commercial embryo transfer program with high performance donor mares under training. *J. Equine. Vet. Sci.* **54**, 93-97.
18. Iuliano, M.F. and Squires, E.L. (1986) Effect of exogenous progesterone on pregnancy rates after surgical embryo transfer in mares. *Theriogenology* **26**, 291-298.
19. Allen, W.R. (2001) Luteal deficiency and embryo mortality in the mare. *Reprod. Domest. Anim.* **36**, 121-131.
20. Ginther, O.J., Jacob, J.C., Gastal, M.O., Gastal, E.L. and Beg, M.A. (2008) Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. *Anim. Reprod. Sci.* **106**, 181-187.
21. Simon, C., Dominguez, F., Valbuena, D. and Pellicer, A. (2003) The role of estrogen in uterine receptivity and blastocyst implantation. *Trends Endocrinol. Metab.* **14**, 197-199.
22. Ma, W.G., Song, H., Das, S.K., Paria, B.C. and Dey, S.K. (2003) Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc. Natl Acad. Sci. USA* **100**, 2963-2968.
23. Borini, A., Dal Prato, L., Bianchi, L., Violini, F., Cattoli, M. and Flamigni, C. (2001) Effect of duration of estradiol replacement on the outcome of oocyte donation. *J. Assist. Reprod. Genet.* **18**, 185-190.