# ORIGINAL ARTICLE





# Reproductive performance of a cohort of Standardbred mares under a commercial breeding system

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# Abstract

**Background:** Despite being a large commercial breeding industry, there is little published data on the reproductive success of Standardbred mares.

Objectives: To quantify the reproductive performance of Standardbred mares under artificial breeding systems in a commercial setting and determine the incidence of early embryonic and other pre-partum losses.

Study design: Retrospective cohort study.

Methods: Data from four commercial farms were collected across four breeding years, and all mares were bred via artificial insemination. A total of 3995 mares contributed 7229 mare years. First-cycle pregnancy rate (FCPR) and end of season pregnancy rate (SPR) were analysed in mixed-effects logistic regression models. Time-to-conception interval was analysed in a Cox regression model.

Results: The mean FCPR was 61.4% (confidence interval [CI] 60.3%-62.6%), the mean end of SPR was 84.7% (CI 83.8-85.5%), the mean live foal rate (FR) was 73.1% (CI 72.1%-74.2%). Mares located on-farm were more probable to be pregnant in terms of both FCPR (odds ratio [OR] 1.168, CI 1.018–1.340, p = 0.026) and SPR (OR 2.026, CI 1.673-2.454, p < 0.001), mares inseminated with thawed-frozen semen were less probable to be pregnant in terms of FCPR (OR 0.598, CI 0.457-0.783, p < 0.001) and SPR (OR 0.479, CI 0.354-0.647, p < 0.001) compared with insemination with fresh-extended semen. Older mares (14 years and older) were less probable to be pregnant in terms of FCPR (OR 0.795, CI 0.688–0.919, p = 0.002) and SPR (0.435, CI 0.352-0.538, p < 0.001) compared with young mares (3- to 8-year old). Main limitations: Retrospective data relied on accurate record keeping of stud farms and no mare-treatment or ovulation induction records were available. Live FRs relied mostly on annual foaling returns so fetal/foal deaths may be underrepresented.

Conclusion: This study provides substantial baseline data on reproductive performance for Standardbred mares managed under a commercial artificial breeding system.

### KEYWORDS

artificial breeding, fertility, horse, mare, reproduction, Standardbred

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# 1 | INTRODUCTION

The breeding of Standardbred horses for harness racing is a significant activity in many countries. However, despite the existence of a large commercial industry, there is little published literature on the reproductive efficiency of Standardbred horses in any of the harness racing countries. One study has reported an overall 13-21 day pregnancy rate per mating cycle of 68.3% for Standardbred mares in North-East Victoria, Australia,<sup>1</sup> and a Finnish study reported a foaling rate of 71% for Standardbreds.<sup>2</sup> Most published studies about the reproductive success of racehorses have pertained to Thoroughbreds and these have been described for mares in the United Kingdom,<sup>3,4</sup> Ireland,<sup>5</sup> United States.<sup>6,7</sup> Australia.<sup>1,8</sup> New Zealand<sup>9-12</sup> and Sweden<sup>13</sup> with overall success rates of about 53%-66% pregnancies per mating cycle. The Standardbred breeding industry differs markedly from that of Thoroughbreds as the former allows the use of artificial breeding techniques, namely artificial insemination (AI) with fresh cooled or thawed-frozen semen, and more recently embryo transfer (ET) technology, whereas the Thoroughbred industry allows natural mating only. The use of artificial breeding techniques in Standardbreds means that reproductive efficiency in Standardbreds may differ from that arising from the natural mating processes and from systems where a restrictive breeding season is applied.<sup>14</sup>

A measure of reproductive performance used in horse racing industries is the 42-day positive pregnancy and this provides the basis on which stallion service fees are generally paid<sup>9</sup> and can be defined as an end of season pregnancy rate (SPR). However, there are no industry-wide standard measures of reproductive performance for horses.<sup>9,15</sup> Pregnancy rate per mating cycle is a commonly used performance measure which provides a helpful indicator of one aspect of reproductive efficiency.<sup>9</sup> First-cycle pregnancy rate (FCPR) may be the most accurate measure of mare fertility as it removes the effect of sub-fertile mares in the breeding population. The foaling rate is another measure, which is effectively the ultimate outcome since the whole objective of a breeding programme is to produce a live foal. Nevertheless, the foaling rate is calculated from the annual breeding returns sent by breeders to their officiating Stud Book, so it does not provide the full picture of individual mating/insemination success.<sup>6</sup>

The New Zealand Thoroughbred breeding industry places emphasis on early-born foals, particularly for the annual yearling sales and for the strong export market to Australia and Asia,<sup>16</sup> with a breeding season (start of mating in New Zealand is officially from 1 September each year) of approximately 105 days,<sup>9</sup> much shorter than the 150 days reported for Northern Hemisphere breeding farms.<sup>4</sup> The New Zealand racing season begins on 1 August and runs until 31 July of the following year. Given the long gestation length of mares and the relatively short commercial breeding season, a mare must become pregnant within the first two oestrous cycles post-foaling to maintain commercial viability. Mares potentially foaling later in the breeding season are often left non-pregnant to miss a season so they can conceive early in the following year.<sup>9</sup> The Standardbred breeding industry in New Zealand does not appear to have pressure to produce early foals, indeed foaling for northern hemisphere time (born 1 January onwards) was a minor industry trend for the United States export market. However, there is anecdotal evidence that owners and trainers prefer early-born foals that will reach maturity earlier for the lucrative 2- and 3-year-old racing seasons.

As there is expected to be increasing scrutiny on the well-being of animals in the racing industries, it will be important to have sound information about wastage, and reliable data on reproductive success are required for this, especially for Standardbreds for which there is little in the way of existing information. The current study was carried out to quantify the reproductive performance of horses in the Standardbred industry, which is where artificial breeding systems are commonly used. It was expected that critical steps in the breeding programmes that are instrumental in reproductive wastage would be identified, by determining the incidence of early embryonic and prepartum pregnancy losses, time-to-conception and gestation lengths in a population of Standardbred mares.

# 2 | MATERIALS AND METHODS

# 2.1 | Study

A retrospective study was conducted on mares that were served by stallions attached to four commercial Standardbred stud farms in New Zealand. Breeding data were collected from the Infohorse Database at Harness Racing New Zealand (HRNZ) for Farm 2 and Farm 3 that used the Infohorse system and breeding data from stud masters' spreadsheets were collected for Farm 1 and Farm 4. The foaling outcome for each mare service (i.e., mating event) was collated from the Infohorse Database. Data were obtained from Farm 1, Farm 2 and Farm 3 for the 2012 season, Farm 1, Farm 2, Farm 3 and Farm 4 for the 2013 and 2014 seasons, Farm 2 and Farm 4 for the 2015 season and Farm 4 for the 2016 season. The data for 2016 was subsequently dropped from analysis due to small numbers.

# 2.2 | Farms

Two farms were located in the South Island (Farm 1 and Farm 4) and two in the North Island (Farm 2 and Farm 3). Each farm had resident stallions from which semen was collected plus frozen semen straws obtained from other non-resident stallions attached to that farm, so in effect the farms are semen bases for particular stallions often under common ownership. Some of the resident stallions were 'shuttle stallions', meaning that they move between Northern Hemisphere (North America) and Southern Hemisphere (New Zealand or Australia) breeding seasons and others were resident stallions that remained onfarm. Stallions whose germplasm was available by frozen semen only were either locally deceased or were based in the United States, Canada or Europe with their semen straws being air freighted to New Zealand. Each farm had brood mare herds living on-farm, either owned by the farm or were client-owned mares based there permanently, or client mares which arrived to reside on-farm for the breeding season. In addition, Farm 1, Farm 2 and Farm 3 distributed fresh,

chilled-transported or frozen semen to clients' mares in different parts of the country. These mares were either at different stud farms or broodmare bases or lived on the clients' properties and were referred to as 'off-farm' mares.

# 2.3 | Mare and stallion management

On-farm and off-farm mares were maintained on pasture and foaling occurred outdoors under supervision, as is standard practice in New Zealand.<sup>9,12</sup> All mares in this study were bred using AI with either fresh semen or thawed frozen semen. Fresh semen was collected every second day from resident stallions and was prepared for insemination by splitting into doses with added semen extender. The optimal dosage is  $25-50 \times 10^6$  sperm/mL for an insemination dose of 10-20 mL containing a minimum of 500 million progressively motile spermatozoa.<sup>17,18</sup> If mares were not inseminated within a few hours or semen was required to be transported to mares in other regions, it was cooled rapidly from 37°C to 20°C and then placed in refrigerated shipping containers to further cool (cooling rate 0.05°C/min) to 5°C. Within 24 h the semen was removed from the shipping container and warmed immediately before insemination.<sup>18</sup> Frozen semen is prepared in 0.5 mL plastic tubes, commonly referred to as 'straws', and involves rapid cooling of freshly collected semen with the addition of semen extender and 5% glycerol from 37°C to 20°C and then further cooling at a steady rate below 20°C to prevent damage using a computer-controlled gradient freezing unit.<sup>18</sup> Standard farm practice involves rectal scanning every 6-10 h so that insemination with thawedfrozen semen was within a maximum of 10 h post ovulation. Under the breeding regulations set out by HRNZ<sup>19</sup> AI of these mares may be conducted only by a registered veterinary surgeon or an approved AI technician. ETs in mares may be conducted only by a registered veterinary surgeon.

Mares were diagnosed as pregnant (or not) via a transrectal ultrasound scan with the first scan performed at 14–16 days postinsemination. Subsequent scans to confirm pregnancy occurred at around 21 and 28 days with the final pregnancy confirmation scan at 42 days.

### 2.4 | Reproductive performance measures

The main measures of reproductive performance used were the outcome variables: FCPR, end of SPR, live foal rate (FR) and time to conception interval. The FCPR is calculated as the number of mares diagnosed pregnant (14–16 day ultrasound scan) divided by the number of mares inseminated (×100). The SPR is the number of mares diagnosed pregnant (42-day ultrasound scan) divided by the number of mares inseminated (×100). The FR is the number of live foals (alive for 7 days in accordance with breeding definitions of payment on the presence of a live foal) produced divided by the number of mares inseminated (×100). The time to conception interval is the number of days from 1 September until the last date of service (LDOS) for mares

that were diagnosed pregnant at 42 days. The denominator for the time to conception interval was the total number of mares mated. Information pertaining to the mares inseminated included mare name, mare age, serving stallion, semen status (fresh-extended, chilled-transported or thawed-frozen), mare status (foaling/non-foaling/maiden), mare location when served, insemination dates, conception date (LDOS), pregnancy scan outcomes and outcome and date of foaling. Gestation length was reported as number of days from the conception date (Day 1) to the foaling date.

# 2.5 | Statistical analysis

Data from the various sources were collated and imported to MS Excel (Microsoft Corporation) for formatting and data analysis. Univariate logistic regression was used to compare associations between the outcome variables (FCPR and SPR) and the independent variables; mare age (categorised as ≤8 years, 9–13 years or ≥ 14 years), reproductive status (foaling, non-foaling or maiden), semen status (fresh-extended, chilled-transported or thawed-frozen), and mare location (on-farm or off-farm) as shown in Tables S1 and S2. Variables showing some univariable association (p < 0.2) with the outcomes were evaluated in a mixed-effects logistic regression model with stallion and mare added as random effects to account for clustering. Variables were removed sequentially from the model manually backwards stepwise if non-significant (Wald's test, p > 0.05). Variables that were significant ( $p \le 0.05$ ) or that improved model fit (likelihood ratio test statistic [LRST]  $p \le 0.05$ ) were retained in the model. The goodness-of-fit of the model was assessed using the Hosmer-Lemeshow test statistic.<sup>20</sup> To increase the strength of the full model results, additional models for FCPR and SPR were run to compare the results with data restricted to Farms 1 and Farm 3 only as they were balanced for year and on-farm vs. off-farm mares (shown in Table S3).

Survival analysis was used to analyse the time-to-conception outcome from the start of the breeding season (1 September) in each year. Cox regression analysis with the Breslow method of handling ties was performed to examine the mare-level independent variables with time-to-conception, measured as days from 1 September to conception, and a frailty term for a stallion was included in the model. Variables were removed sequentially from the model manually backwards stepwise if non-significant (Wald's test, p > 0.05). Variables that were significant ( $p \le 0.05$ ) or that improved model fit (LRST  $p \le 0.05$ ) were retained in the model. The goodness-of-fit of the model was tested using a Nelson-Aalen cumulative hazard function plotted with Cox-Snell residuals.<sup>21</sup>

All data analyses were performed in STATA 15 (StataCorp).

# 3 | RESULTS

A total of 3955 individual mares contributed 7229 mare years over four breeding seasons. The mares were served by 97 different stallions where the stallions served a median of 19 (interquartile range [IQR] 4–101) study mares and 46 stallions served 20 or more mares over the study period. Table 1 shows the number of mares and stallions and farms participating in the study population for each year. The overall mean mare age was 10.2 years (±4.4 SD), and the median age was 9 years (IQR 7–13). Mare status was classified as either foaling (wet mares), non-foaling or maiden mares and made up 51.1% (3693), 32.0% (2310) and 17.0% (1226) of the study population, respectively. Table S4 shows the average mare age on each farm and the overall number of foaling and non-foaling mares on each farm.

Of the 6123 occasions that mares that had a positive pregnancy diagnosis at 42 days after their last date of insemination, 68.2% (4176/6123) pregnancies occurred on the first cycle, 23.7% (1449/6123) on the second cycle, 7.0% (430/6123) on the third cycle, 1.8% (112) on the fourth cycle and 0.2% (14) on the fifth cycle. There were 15.3% (1106/7229) negative end of season pregnancy scans overall.

The overall incidence of early embryonic or fetal death before the 42-day scan was 424 mares diagnosed pregnant at 14- to 16-day scan that subsequently scanned negative before or at the 42-day scan, this was at the cycle level as some mares had more than one positive 14- to 16-day scan that was negative at the 42-day scan in 1 year. The incidence of conceptus loss after the 42-day positive pregnancy diagnosis was 7.8% (563/7229). Table 2 shows the percentage of live foals, foal deaths, pregnancy loss after 42 days, and early embryonic or fetal loss (before 42 days) for each year on each farm. The number of twin pregnancies was available from mares located on-farm at Farms 1, 2 and 3 and is shown in Table S5. The number of mares in the study population inseminated with fresh-extended, chilled-transported and thawed-frozen semen and the associated FCPR and SPR percentages are presented in Table S6.

The overall mean FCPR was 61.4% (4442/7229, 95% confidence interval [CI] 60.3–62.6) and the range of means across farms is shown

**TABLE 1** The number of mares and stallions in the study population (and their percentage of the total population bred) in each year and the comparison with the number of mares and stallions in the New Zealand Standardbred breeding population for each study year.

Year	No. of study mares (%)	No. of mares served NZ	No. of stallions used study (%)	No. of stallions used NZ	No. of study farms
2012	2177 (68.0%)	3201	57 (72.2%)	79	3
2013	2273 (73.9%)	3077	70 (79.6%)	88	4
2014	2136 (74.0%)	2887	61 (72.6%)	84	4
2015	643 (23.8%)	2698	38 (44.2%)	86	2

	Mares served	Early embryonic death	Aborted after 42 day positive	Foal dead/died	Live foals
Farm 1					
2012	557	19 (3.4%)	55 (9.9%)	13 (2.3%)	387 (69.5%)
2013	713	23 (3.2%)	50 (7.0%)	19 (2.7%)	542 (76.0%)
2014	606	26 (4.3%)	55 (9.1%)	19 (3.1%)	446 (74.8%)
Total	1867	68 (3.6%)	160 (8.6%)	51 (2.7%)	1375 (73.6%)
Farm 2					
2012	505	16 (3.2%)	26 (5.2%)	23 (4.6%)	363 (71.9%)
2013	517	13 (2.5%)	34 (6.6%)	25 (4.8%)	377 (72.9%)
2014	383	29 (7.6%)	20 (5.2%)	14 (4%)	306 (79.9%)
2015	474	20 (4.2%)	29 (6.1%)	11 (2%)	347 (73.2%)
Total	1879	78 (4.2%)	109 (5.8%)	73 (3.9%)	1393 (74.1%)
Farm 3					
2012	1115	60 (5%)	95 (8.4%)	56 (5.0%)	800 (71.8%)
2013	769	47 (6.1%)	64 (8.3%)	28 (3.6%)	542 (70.5%)
2014	960	64 (6.7%)	81 (8.4%)	32 (3.3%)	720 (75.0%)
Total	2844	171 (6.0%)	240 (8.4%)	116 (4.1%)	2062 (72.5%)
Farm 4					
2013	274	20 (7.3%)	23 (8.4%)	11 (4.0%)	204 (74.5%)
2014	188	5 (2.7%)	16 (8.5%)	8 (4.3%)	139 (73.4%)
2015	169	5 (3.0%)	15 (8.9%)	4 (2.4%)	114 (67.5%)
Total	631	30 (4.8%)	54 (8.6%)	23 (3.6%)	457 (72.4%)

TABLE 2	The number and percentage of	early embryonic/fetal o	deaths (before 42	days pregnancy)	, aborted foetuses	after 42 day <sub>l</sub>	positive
pregnancy sc	an, foals born dead or died and l	ive foals (foaling rate) o	on each stud farm	in each study yea	ar.		

in Table 3. In the final model when controlled for serving stallion and mare (LRST p < 0.001), and adjusting for the fixed effect of year, the significant fixed effects associated with FCPR were mare age, on-farm or off-farm mare location, semen type and year (Table 4). Mares aged 14 years and older were significantly less probable to become pregnant on the first cycle (odds ratio [OR] 0.795 [CI 0.688-0.919], p = 0.002) compared with the reference age mares (3- to 8-year olds), however, there was no significant difference from the reference age mares for those aged 9–13 years. Mares located on-farm were more probable to become pregnant on the first cycle (OR 1.168 [CI 1.018-1.340], p = 0.026) compared with mares located offfarm. Mares inseminated with chilled-transported (OR 0.864 [CI 0.752-0.992], p = 0.038) or thawed-frozen (OR 0.598 [0.457-0.783], p < 0.001) semen were significantly less probable to be pregnant on their first cycle compared with the reference fresh-extended semen. In the comparison model with Farms 1 and 3 data only (Table S3), the on-farm versus off-farm mares became non-significant but all other variables had a similar association to FCPR as with the full model.

The overall mean SPR was 84.7% (6123/7229, 95% CI 83.8–85.5) and the range of means across farms and a comparison of on-farm and off-farm SPR rates is shown in Table 3. In the final model when controlled for serving stallion and mare (LRST p = 0.014), and adjusting for the fixed effect of year, increasing mare age was negatively associated with SPR (aged 9–13 years OR 0.721

[CI 0.597-0.873], p = 0.001, aged 14 years and older OR 0.435 [CI 0.352-0.538], p < 0.001). Mares located on-farm were more probable to be pregnant at 42 days (OR 2.026 [CI 1.673-2.454], p < 0.001). Mares inseminated with thawed-frozen semen (OR 0.479 [CI 0.354-0.647], p < 0.001) were less probable to be pregnant at 42 days than those inseminated with fresh-extended semen. However, there was no significant difference between freshextended and chilled-transported semen on SPR. Non-foaling mares (OR 1.433 [Cl 1.207-1.701], p < 0.001) were more probable to be pregnant at 42 days but maiden mares were not significantly different to the reference category of foaling mares. An increasing number of cycles mated had a negative effect on SPR (p < 0.001) as shown in Table 4. The comparison model for SPR (Table S3) restricted to Farms 1 and 3 data had the same association for all variables as the full model except mares aged 9-13 years were nonsignificant.

The median time-to-conception from the start of the breeding season (number of days from 1 September until conception) was 82 (IQR 59–107) days and ranged from 79 (IQR 56–105) to 86 (IQR 63–110) days across farms. In the final Cox proportional hazards model (Table 5), adjusting for the fixed effects of farm and year, when compared with mares aged ≤8 years, mares aged 14 years and older were at 0.805 hazard ratio (HR) (CI 0.747–0.869) the daily hazard for conception over the breeding season (p < 0.001).

**TABLE 3** A comparison of FCPR (first-cycle pregnancy rate) and SPR (end of season pregnancy rate) between mares served on-farm and off-farm for each farm for each study year.

	On-farm			Off-farm	Off-farm		
	Mares served	FCPR	SPR	Mares served	FCPR	SPR	
Farm 1							
2012	291	170 (58.4%)	261 (89.7%)	266	141 (53.0%)	201 (75.6%)	
2013	419	262 (62.5%)	375 (89.5%)	294	196 (66.7%)	238 (81.0%)	
2014	410	240 (58.5%)	351 (85.6%)	196	132 (67.3%)	169 (86.2%)	
Total	1120	672 (60.0%)	987 (88.1%)	756	469 (62.0%)	608 (80.4%)	
Farm 2							
2012	102	61 (59.8%)	88 (86.3%)	403	229 (56.8%)	325 (80.6%)	
2013	133	87 (65.4%)	116 (87.2%)	384	221 (57.6%)	320 (83.3%)	
2014	118	82 (69.5%)	111 (94.1%)	265	165 (62.3%)	229 (86.4%)	
2015	140	88 (62.9%)	123 (87.9%)	334	166 (49.7%)	264 (79.0%)	
Total	493	318 (64.5%)	438 (88.8%)	1386	781 (56.3%)	1138 (82.1%)	
Farm 3							
2012	201	144 (71.6%)	179 (89.0%)	914	569 (62.2%)	760 (83.2%)	
2013	171	124 (72.5%)	151 (88.3%)	598	371 (62.0%)	493 (82.4%)	
2014	157	104 (66.2%)	148 (94.3%)	803	516 (64.5%)	686 (85.4%)	
Total	529	372 (70.3%)	478 (90.4%)	2315	1456 (62.9%)	1939 (83.8%)	
Farm 4							
2013	274	167 (61.0%)	239 (87.2%)				
2014	188	117 (62.2%)	164 (80.3%)				
2015	169	89 (52.7%)	132 (78.1%)				
Total	631	373 (59.1%)	535 (82.7%)				

	OR	95% (1	Wald test	I RST n value
Outcome: ECPR		7570 CI	Wald test	EROT p value
Mare age				
<8 vers	Reference			
9–13 years	0.963	0 851-1 090	n = 0.553	
>14 years	0.795	0.688-0.919	p = 0.002	
On-farm	0.775	0.000 0.717	p = 0.002	
No	Reference			
Ves	1 168	1 018-1 340	n - 0.026	
Semen type	1.100	1.010 1.040	p = 0.020	
Fresh-extended	Reference			n < 0.001
Chilled-transported	0.864	0 752-0 992	n = 0.038	p • 0.001
Frozen	0.598	0.457-0.783	p = 0.000	
Vear	0.370	0.457 0.700	p • 0.001	
2012	Pafaranca			
2012	1 179	1 0 2 1 2 5 0	n = 0.024	
2013	1.170	1.022-1.339	p = 0.024	
2014	0.7/0	1.049-1.427	p = 0.010	
2015	0.768	0.018-0.953	p = 0.017	D . 0.001
Stallion				P < 0.001
Outcome: SPR				
Mare Age	Deferrer			
≤8 years	Reference	0.507.0.070	0.004	
9–13 years	0.721	0.597-0.873	p = 0.001	
≥14 years	0.435	0.352-0.538	p < 0.001	
On-farm	D (			
No	Reference	4 (70, 0.454	0.004	
Yes	2.026	1.673-2.454	p < 0.001	
Semen type	D (			
Fresh-extended	Reference	0.77/ 4.40/	0.400	
Chilled-transported	0.927	0.//6-1.106	p = 0.400	
Frozen	0.479	0.354-0.647	p < 0.001	
Mare reproductive status	D (			0.004
Foaling	Reference			p < 0.001
Non-foaling	1.433	1.207-1.701	p < 0.001	
Maiden	1.126	0.892-1.423	p = 0.316	
No. of cycles mated				
Cycle 1	Reference			
Cycle 2	0.399	0.336-0.474	p < 0.001	
Cycle 3	0.176	0.139-0.223	p < 0.001	
Cycle 4	0.109	0.076-0.156	p < 0.001	
Cycle 5	0.049	0.024-0.099	p < 0.001	
Year				
2012	Reference			
2013	1.101	0.916-1.323	<i>p</i> = 0.302	
2014	1.264	1.038-1.540	<i>p</i> = 0.020	
2015	0.779	0.595-1.018	p = 0.067	
Stallion				<i>p</i> = 0.013
Mare				

**TABLE 4** Results of multivariable mixed-effects logistic regression models of the variables significantly associated with the pregnancy success outcomes: first-cycle pregnancy (FCPR), and end of season pregnancy (SPR) for Standardbred mares in the study population.

Abbreviations: CI, confidence interval; OR, odds ratio; LRST, likelihood ratio test statistic.

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**TABLE 5**Results of a multivariableCox regression model of the associationbetween variables significantlyassociated with start of mating toconception interval (with frailty term forstallion added to account for clustering)for Standardbred mares in the studypopulation.

Outcome: Time to conception from 1 September						
	Coefficient	SE	HR	95% CI	p Value	
Mare age						
≤8 years			Reference			
9–13 years	-0.061	0.032	0.941	0.883-1.002	<i>p</i> = 0.06	
≥14 years	-0.216	0.039	0.806	0.747-0.869	p < 0.001	
On-farm						
No			Reference			
Yes	0.089	0.037	1.093	1.017-1.175	<i>p</i> = 0.02	
Mare reproductive status						
Foaling			Reference			
Non-foaling	0.476	0.031	1.61	1.517-1.709	p < 0.001	
Maiden	0.352	0.039	1.421	1.315-1.536	p < 0.001	
Semen type						
Fresh-extended			Reference			
Chilled-transported	-0.268	0.035	0.765	0.714-0.819	p < 0.001	
Frozen	-0.216	0.076	0.727	0.627-0.843	p < 0.001	
Farm						
1			Reference			
2	0.147	0.071	1.158	1.008-1.330	<i>p</i> = 0.04	
3	0.113	0.058	1.12	1.000-1.254	<i>p</i> = 0.05	
4	0.023	0.064	1.023	0.902-1.161	p = 0.82	
Year						
2012			Reference			
2013	0.079	0.035	1.083	1.011-1.159	p = 0.023	
2014	0.088	0.037	1.092	1.016-1.174	p = 0.02	
2015	-0.008	0.057	0.992	0.887-1.109	p = 0.9	

Abbreviations: CI, confidence interval; HR, hazard ratio; SE, standard error.

Other significant fixed effects were non-foaling mares (p < 0.001) and maiden mares (p < 0.001), mares located on-farm (p < 0.001). When compared with mares inseminated with fresh-extended semen, mares inseminated with chilled-transported were at 0.765 HR ([CI 0.715–0.819], p < 0.001) the daily hazard for conception and mares inseminated with thawed-frozen semen were at 0.727 HR ([CI 0.627–0.844], p < 0.001) the daily hazard for conception over the breeding season. Stallion was a significant frailty term (LRST p < 0.001) in the model.

The overall mean live FR was 73.1% (5287/7229, CI 72.1–74.2) with no difference detected between farms (range of means 72.4%–74.1%), Table S7 shows the FCPR, SPR and FR in each study year. The correlation between SPR and the foaling rate was 0.70 (p < 0.001). The median overall gestation length was 350 (IQR 344–357) days and was the same across all four farms.

# 4 | DISCUSSION

This study provides a substantial data set for the reproductive performance of Standardbred mares bred under commercial artificial breeding conditions. The overall foaling rate recorded here (73.1%) accords well with values for Standardbreds provided in the literature  $(71\%)^2$  so it is probably indicative of an acceptable level of fertility in these horses and is suggestive of considerable competence of personnel involved in the management and care of these animals throughout the whole reproductive process. In addition, these findings provide a benchmark against which the reproductive performance of mares in the Standardbred industries can be compared and the competence of persons working in this industry can be judged.

The overall first-cycle pregnancy rate of 61.4% recorded in this study was much higher than the 53.6% reported for Thoroughbred mares mated in Waikato, New Zealand.<sup>10</sup> A study on the fertility of Norwegian Coldblood trotters under AI conditions reported an FCPR of 51.2%.<sup>22</sup> A study incorporating 11 farms in Victoria, Australia reported a FCPR of 65.3%–75% in Thoroughbreds (7 farms) and 57.1%–74.0% in Standardbreds (4 farms).<sup>1</sup> Our current study showed considerably greater variability in FCPR for mares inseminated both on-farm (range 52.7%–72.5%) and off-farm (range 49.7%–67.3%) and we contend that this may reflect the differences in mare age

and semen type. The end of SPR recorded here (84.7%) is higher than those reported from other studies (range 58.2%–82.5%).<sup>4,10</sup>

In the case of Standardbred mares in the current study, pregnancy loss between early pregnancy diagnosis (Days 14–16) and final pregnancy scan (Day 42) was 5.9%, which is comparable to the 5.5% reported by Hanlon et al.<sup>9</sup> for Thoroughbreds in New Zealand but is lower than the 7.2%–8.0% reported by Allen et al.<sup>4</sup> for Thoroughbred flat racing and national hunt mares in Newmarket, United Kingdom and is much lower than the 17.3% loss in pregnancies diagnosed after 15 days, also for Thoroughbred mares in the United Kingdom.<sup>3</sup> However, in the current study the pregnancy loss between Day 42 of gestation to term was 7.8% which is higher than the 3% recorded for this period in Thoroughbred mares based in Waikato, New Zealand.<sup>9</sup> We suspect that this apparently inferior fetal survival rate in the Standardbreds may have resulted from some underreporting of live foal births in the stud book registry, whereas the Thoroughbred data were derived directly from stud records which may be more accurate.

The results of the current study indicate that mare age, mare location (i.e., located on-farm or not), and semen status (i.e., fresh-extended, chilled-transported or thawed-frozen) were all factors associated with reproductive performance in the Standardbred mares. Increasing mare age had a significant negative effect on FCPR and SPR and mares aged 14 years and older were much less probable to be pregnant at both outcomes. This is in agreement with other studies reporting increasing age as being negatively associated with a pregnancy outcome.<sup>4,5,10,22-24</sup> Reasons for subfertility in older mares include degenerative changes to the external and internal reproductive tract,<sup>24</sup> reduced oocyte viability<sup>24</sup> and being more susceptible to endometritis.<sup>25</sup>

Mares located on-farm were more probable to be pregnant than mares located at other farms or at owners' properties. Under an artificial breeding system, a mare may stay on the owner's property for breeding purposes thus there is no requirement to travel to a stud farm for service by a stallion. This reduces the risk of injury to the mare (and/or foal) during transport and the social stress accompanying exposure to a herd of unfamiliar mares. However, this may provide a different set of issues, such as the mare not receiving the benefits and reproductive management of highly experienced commercial stud operators. The higher pregnancy rates for mares based on-farm on one of the four farms under study here is a strong indication of the potential benefit to be obtained from the intensive management that can be provided on commercial stud farms.

Mares inseminated with fresh-extended semen were more probable to be pregnant for both FCPR and SPR outcomes than those inseminated with thawed-frozen semen, insemination with chilledtransported semen had a negative effect on FCPR but there was no significant effect on SPR. There appear to be few comparable data for fresh versus frozen semen pregnancy rates in horses. One study used data across the United States and Europe to compare pregnancy rates of mares inseminated with fresh semen, cooled semen and thawedfrozen semen.<sup>26</sup> Those mares were inseminated with semen from a variety of sources so there may have been variation in the quality of semen 'straws', additionally there was no mention of breed type

of mares inseminated. That study reported pregnancy rates of 60% with fresh semen, 44% with cooled semen and 46% with thawedfrozen semen.<sup>26</sup> Heckenbichler et al.<sup>17</sup> reported FCPR of 40% in mares inseminated with cooled-shipped semen and reported variation in processing and storing methods with most semen doses arriving at less than optimal storage temperatures. Norwegian Coldblood trotting mares inseminated with fresh-extended semen had FCPR of 55.1% compared with FCPR of 42.2% for cooled-shipped semen<sup>22</sup> which is lower than the 64.9% and 60.7% in our study. Higher pregnancy rates in horses were reported using AI with fresh semen (OR 1.1) compared with natural/in-hand mating (OR 1.0), which had similar results to AI with cooled-shipped semen (OR 1.0) or frozen semen (0.9),<sup>27</sup> the differences in fresh, cooled-shipped and frozen semen were similar to this current study. The lack of available data for semen dosage or the number of straws used per insemination for frozen inseminations is a limitation of the current study as Vidament et al.<sup>28</sup> reported increased pregnancy rates with a dose of  $300 \times 10^6$  spermatozoa. In addition, two or more inseminations per cycle increased the pregnancy rates when compared with mares inseminated only once.

Commercial breeding of horses, especially for racing, requires mares to become pregnant again soon after foaling, and in order to maintain a yearly foaling pattern (given the length of gestation), mares must become pregnant within 25 days of foaling.<sup>29</sup> There is also a requirement for mares to become pregnant early in the breeding season, as there is anecdotal evidence for a preference of buyers for early-born Standardbred foals, particularly at the annual yearling sales. In our study the time from the beginning of the breeding season until conception (time to conception interval) was shorter for non-foaling mares, mares younger than 14 years of age, mares located on a study farm and mares inseminated with fresh semen. Mare status (foaling, non-foaling or maiden) had no effect on the FCPR but non-foaling mares were more probable to be pregnant at SPR, so, although lactating mares conceived later in the breeding season compared with their non-foaling counterparts, their status had no effect on their ability to conceive initially. Thus, if lactating mares do not conceive or hold their pregnancy on the first or second cycle post-partum or foal later in the season owners may elect to miss breeding from them that year to ensure they can rebreed early in the following year.

The main limitations of this study were the unavailability of mare-treatment records for induction of oestrus and ovulation, or treatment of reproductive disease which may affect pregnancy rates. Also, the number of inseminations per cycle were not available for all mares and there was no information available for the number of straws used per insemination. In addition, the foaling rate recorded here (in Standardbreds) was lower than that reported for Thoroughbreds by Hanlon et al.<sup>9</sup> and this may have been due to the reliance on stud book registry records of foaling for many of the foaling outcomes we recorded, rather than farm records due to the differing mare locations at foaling. Other studies have found higher foaling rates reported on farm compared with official stud book register figures.<sup>1,4</sup> Thus, farm records seem to provide a more accurate description of foaling outcomes than official registry records which rely on breeder/ owner submission of a foaling return, so foals that are born and

subsequently die some weeks later may be classified as a 'no return'. The mean gestation length recorded here (350 days) is similar to the gestation lengths reported as 349 days in two studies on Standardbreds in New Zealand<sup>30,31</sup> and slightly longer compared with 344.1  $\pm$  0.49 days reported in Thoroughbreds.<sup>32</sup> The variation in gestation lengths can cause issues for rebreeding for the following season in terms of producing later-born foals.

In conclusion, it is submitted that this study provides benchmark information against which the Standardbred industry can monitor its level of performance in the management of mares at insemination and during gestation, and of them and their newborn foals postnatally.

# AUTHOR CONTRIBUTIONS

Jasmine C. Tanner contributed to the study design, had full access to the data and takes responsibility for the accuracy and integrity of the data, contributed to data analysis and interpretation and preparation of the manuscript. Graham K. Barrell contributed to the study design, supervision, writing and review of the manuscript. Both authors approved the final version of the manuscript.

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# CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https://www. webofscience.com/api/gateway/wos/peer-review/10.1111/evj.13989.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Open sharing exemption granted by editor for this retrospective clinical report.

### ETHICAL ANIMAL RESEARCH

Research ethics committee oversight not currently required by this journal: retrospective analysis of breeding data.

### INFORMED CONSENT

Representatives of the farm owners gave consent for horses' inclusion.

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### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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