Reproductive outcomes in sport horse commercial French breeding farms: clinical relevance of mares' age, parity and lactating status

Emilie Derisoud[a,b,c,+]*, Bénédicte Grimard[b,c,+], Clothilde Gourtay[d], Juliette Auclair-Ronzaud[e], Pascale Chavatte-Palmer[b,c]*

[a]Department of Physiology and Pharmacology, Karolinska Institutet, Solna, Stockholm, Sweden; [b]Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France; [c]Ecole Nationale [c]Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France

[d]IFCE, Plateau technique du Pin, 61310, Exmes, France

[e]IFCE, Plateau technique de Chamberet, 19370, Chamberet, France

[+] authors contributed equally to this work

*Corresponding author.

Emails:

emilie.derisoud@gmail.com

benedicte.grimard@vet-alfort.fr

clothilde.gourtay@ifce.fr

pascale.chavatte-palmer@inrae.fr

juliette.auclair-ronzaud@ifce.fr

Mail address:

Pascale Chavatte-Palmer

BREED (UMR1198) – Bâtiment 230

Biology de la reproduction, Environnement, Epigénétique et Développement

Domaine de Vilvert

78352 Jouy-en-Josas, FRANCE

Abstract

Background: For a long time, important and progressive fertility decline is observed with mare age. Parity and yearly breeding, however, are controversial factors impacting fertility. Most of these studies were performed on race horses and before 2010, on data accumulated before. The cumulative effects of age and nulliparity/nursing with improved reproductive technologies and practices, often observed in sport horses, has not been investigated yet.

Objectives: To investigate the effect of age, parity and nursing, as well as reproductive management, on post-insemination inflammation and fertility in sports mares.

Study design: Age, parity, nursing status, follicular size before ovulation, estrus and/or ovulation induction, artificial insemination (AI) protocol, post-breeding inflammation and treatment, Day 14 pregnancies rates (PR) and number of embryos, as well as subsequent foaling the next year were recorded for 277 mares (506 cycles) over one breeding season in two French commercial studs.

Methods: Multivariate logistic regression models were used.

Results: PR was 41.9% per cycle and 76.5% at the end of the season. Post-insemination fluid accumulation risk was increased in >10-year-old, barren or July or August inseminated mares ($p<0.0001$, OR=3.29, 5.389 and 3.329, respectively). PR were reduced in mares >10-year-old vs younger or inseminated with frozen vs fresh or refrigerated semen ($p<0.05$, OR=0.622 and 0.582, respectively). More pregnancy on Day 14 were observed in mares with multiple ovulations compared to mono-ovulation ($p<0.05$, OR=1.791). Regardless the age, PR only tended to be improved in multiparous ($p=0.07$, OR=1.434 in parous vs nulliparous) but in >10-year-old mares, multiparity greatly increased PR (44.09% in parous vs 30.89% in nulliparous, $p<0.05$).

Main limitations: Limited number of mares.

Conclusion: In sport horse, maternal age was more important than parity and lactating status for reproductive success but cumulative effects of nulliparity and aging was deleterious on PR, demonstrating their importance in the management of sport mares.

Keywords

Horse, equine, reproduction, insemination, lactation, fertility

1. Introduction

Effectiveness of mare reproduction is an important economical factor in the horse industry. Once mares begin their reproductive career, breeders generally aim to produce one foal per year per mare to remain profitable. This goal can only be achieved if mares remain fertile throughout their life span.

The decline in mare fertility associated with age is well described [1–18] but mainly on Thoroughbreds where the career is short and mares are bred from and culled at younger age compared to sport horses. It is common in sport horses to breed older mares: in Canada, 37% of the standardbred mares [19] and in Finland, 53% of Standardbred and 63% of Finnhorse mares exceed 11 years old at insemination [3]. Furthermore, mares can be bred shortly after foaling and are often nursing at the time of breeding. Lactation affects mares' metabolism [20] but there is no consensus on whether it may induce effects on fertility and embryo/fetal loss [21–24]. As for lactation, the putative effects of mare's parity on fertility and embryo loss are controversial [2–5,9,10,12–16,18]. Plus, in sport horses, mares are often both nulliparous and old at the same time and in Finland, 20.5% of Finnhorse and 15.5% of Standardbred broodmares are both older than 10 and nulliparous [3].

Transient uterine inflammation is a normal physiological reaction in mares after breeding. For some mares, however, a persistent infection can develop and interfere with fertility outcomes (for review [25]). Indeed, 45% (N=22) of the 8-16 years old mares and 88% of the older mares (≥17 years old, N=26) had uterine fluid retention 48h after insemination with frozen semen, whereas none of the 9 younger mares was affected [26]. Parity and/or lactation on the post-breeding inflammation have not been explored yet.

The Thoroughbred studbook only allows hand breeding whereas the French Trotter studbook allows only insemination with fresh semen. Such limitations do not exist in sport horse and no recent study considered at the same time the method of reproduction and the effects of age, parity and lactation on mare fertility.

90 This study aimed to identify effects of mare age, parity and nursing on post-breeding fluid
91 accumulation, fertility and embryonic/fetal death in commercial stud conditions for sport mares.

92

93 2. Material and methods

94 *2.1. Animals*

95 Data were recorded in 2 French commercial stud farms, one located about 60km south west of Paris,
96 the other 150 km south of Paris, both managed by one artificial insemination cooperative during one
97 breeding season (2019).

98 From beginning of February to late August 2019, the study enrolled 277 sport mares over 506 estrus
99 periods. For each mare, date of birth, breed, number of previous breedings and foalings were extracted
100 from the national database (Infochevaux: https://infochevaux.ifce.fr/fr/info-chevaux). Mares were
101 clustered according to age in two (≤ 10 years old *vs* > 10 years old) or four classes (≤ 5 years old,]5,
102 10],]10, 15], > to 15 years old). They were also clustered in 2 classes according to previous fertility
103 (number of previous foaling/number of previous breedings <0.7 or >0.7) and in 2 classes according to
104 parity: nulliparous (never foaled before) vs parous (foaled at least once before) at the beginning of the
105 breeding season.

106

107 *2.2. Breeding management*

108 *2.2.1. Estrus monitoring*

109 First, luteolysis was induced in mares that had a persistent corpus luteum or that were sent for
110 breeding late in the season, using prostaglandin F2α analogs: Alfaprostol (Alfabedyl©) or Luprostol
111 (Prosolvin®, 1 to 1.5 mL IM according to the mare size).

112 Estrus detection was performed using uterine horn firmness estimated by rectal palpation together
113 with ultrasound examination where the uterine oedema score (0-5) [27] and the diameter of the
114 largest follicle were determined. Mares were monitored once or twice daily (according to breeding
115 method, see below) from the time when the diameter of the largest follicle reached 35 mm and until
116 ovulation was detected.

117 As ovulation approached (follicle diameter ≥35 mm and reduction of the uterine oedema score),
118 ovulation was induced with hCG (Chorulon®, 1500 to 5000 IU IM related to the mare weight) or with a
119 GnRH analog (Decapeptyl®, 0.1 mg triptorelin in 1 mL SC). The GnRH analog option was chosen when
120 mares had a known resistance to hCG or when hCG had already been used before during the breeding
121 season and was not effective to induce ovulation.

122 ***2.2.2. Insemination management***

123 All mares were bred using artificial insemination (AI). Mares were inseminated with fresh (FAI), cooled
124 transported (CTAI) or frozen semen (FZAI). As this study has been performed in a commercial breeding
125 farm, insemination dose was dependent on the number of straws bought by the broodmare's owner
126 and the country where straws have been produced. Mare management differed according to the type
127 of semen used.

128 Mares entitled to be inseminated with fresh semen were examined once daily during estrus. A first
129 insemination was performed maximum 24h before ovulation (follicle diameter ≥ 35 mm and decrease
130 in the uterine oedema score) and mares that had not ovulated 48h later were inseminated a second
131 time. This procedure was repeated until ovulation was observed.

132 Mares entitled to be inseminated with cooled transported semen were also examined once daily and
133 inseminated before ovulation, but subsequent inseminations were performed every 24h until
134 ovulation.

135 Mares bred with frozen semen were examined every 12h if the number of available straws was >4
136 while examinations were performed every 6 h when the number of available straws was ≤4. In this
137 latter case, deep horn insemination was performed. The aim was to inseminate as close as possible of
138 ovulation (*i.e.* maximum 6 hours before or after ovulation), as determined by ultrasound observation
139 of preovulatory follicular wall thickening and follicle deformation.

140

141 ### *2.2.3. Post-breeding fluid accumulation and pregnancy diagnosis*

142 All mares were examined by ultrasound the day after insemination. If uterine oedema and/or fluid
143 accumulation were observed, mares were treated either with a single dose of oxytocin (Ocytocine S©,
144 10-20 UI in 1 to 1.5 mL IM or IV), or oxytocin in association with uterine lavage (one or two litters of
145 warm, sterile saline solution), or in association with antibiotics (in accordance with the
146 recommendation of the use of antibiotics in veterinary medicine) or by uterine lavage alone.
147 Treatment was decided by the veterinary surgeon and depended on the volume of fluid accumulated.

148 Pregnancy was assessed 14 days (D14) after AI by ultrasonography. In the case of twin pregnancy,
149 squeezing was recommended to the breeder but not always performed. Pregnancy was confirmed on
150 Day 30 if the mare was brought back to the stud. In addition, some mares came back again in autumn
151 for late pregnancy diagnosis.

152

153 ### *2.2.4. Data recording*

154 All data were recorded in the same dedicated software (Gynebase©, Equidéclic, France) by the
155 veterinarians and technicians of the 2 studs.

156 Age, parity, lactational status at breeding (nursing *vs* non nursing), number of monitored cycles for
157 each mare, estrus induction (yes/no), heat duration (number of days of observation by

158 ultrasonography), size of the preovulatory follicle, induction of ovulation (yes/no), hormone used to
159 induce ovulation (hCG or GnRH analog), ovulation observed (yes/no), number of follicles ovulated, AI
160 mode (fresh semen, cooled transported or frozen semen), number of AI during estrus, date of
161 insemination (last AI if more than one AI were performed during estrus), stallion identity, number of
162 straws used (for frozen semen), uterine fluid accumulation after AI (yes/no), treatment used (oxytocin
163 alone vs other), presence of single or twin embryos, squeezing when twins were diagnosed (yes/no),
164 pregnancy diagnosis on D14 as well as on D30 or later, when possible, were recorded. The number of
165 the monitored cycles needed to obtain a gestation was classified as 1 *vs* >1 when pregnancy was
166 achieved after more than one cycle. The number of straws used for frozen insemination was classified
167 as either <8 *vs* ≥ 8 or ≤ 4 vs > 4. Cutoff values of 4 and 8 were chosen according to previous (8 straws
168 for 400 million mobile spermatozoa) and the current (4 straws of 50 x 10^6 mobile spermatozoa, 4 mL
169 each) recommendation of the French Institute for Horses and Horse-riding (IFCE) for frozen semen.

170 Pregnancy rate was calculated as the ratio between the number of pregnant mares at the end of the
171 breeding season and total number of mares bred (overall pregnancy rate at the end of the season) and
172 as the ratio of number of positive diagnoses on D14 / total number of used cycles (pregnancy rate per
173 cycle). Embryonic loss was estimated by the number of mares not pregnant on D30 after a positive
174 pregnancy diagnosis on D14. Late embryonic loss was calculated by the number of mares not pregnant
175 during the autumn after a positive pregnancy diagnosis on D14. Foal birth was checked the next year
176 using the national database. Total embryonic/fetal loss was defined as the number of mares that did
177 not foal after a positive pregnancy diagnosis on D14. Mares were only considered as non-foaling when
178 abortion/stillbirth or new breeding without foaling were recorded in the national database in 2020.

179

180 *2.3. Statistical analysis*

181 Data were analyzed using SAS® Studio 3.8 (SAS® University Edition).

182

183 ### *2.3.1. Univariate & multivariate analysis*

184 Univariate analysis was used to evaluate the effects of qualitative variables on (1) the incidence of
185 post-breeding fluid accumulation, on (2) pregnancy rate and (3) embryonic/fetal loss. For (1) and (2),
186 all recorded cycles were used while for (3), only pregnant mares on D14 were considered. The
187 statistical unit was, therefore, the cycle for (1) and (2) while it was the mare for (3).

188 For each criterion analyzed, results were compared between the different classes using a Chi-Squared
189 test. Variables associated at $p < 0.20$ were included in the second step of the analysis.

190 As a second step, multivariate analysis was conducted using logistic regression (GLIMMIX procedure
191 of SAS® Studio). Individual effects were considered by including mare identity in each model as a
192 random effect. A backward stepwise elimination of non-associated ($p > 0.10$) variables was performed
193 to develop multivariate models. Models presenting the lowest Akaike's Information Criterion were
194 retained.

195 For these analyses, breeds and stallions could not be used as their numbers were too high without
196 enough repeats to be considered in the statistical analysis.

197 For quantitative data, results are presented as mean ± standard error.

198

199 ### *2.3.2. Analysis of age, parity and nursing status*

200 The analyses were performed using the estrous cycle as the reference unit. Young (≤ 10 years) *vs* old
201 (>10 years), nulliparous *vs* parous, nursing *vs* non nursing mares were compared using T test for
202 quantitative variables and Xi Square for qualitative ones. For further characterization of the suckling

status and mares' parity, these factors were analyzed using Xi Square analysis in each population of young or old mares.

3. Results

3.1. Descriptive analysis

3.1.1. Population of study

The 277 mares belonged to 32 breeds approved by the French Institute of the Horse (IFCE), with French saddlebred (Selle français) being the most represented breed (56% of mares included in this study). No other breed reached more than 15 individuals. Eight mares did not belong to any French approved breed. In addition, 13 mares belonged to racehorse breeds (3 English Thoroughbreds, 10 French Trotters). There were also 22 ponies and one draft horse. All mares were used for sport and/or leisure.

Characteristics of the 277 mares are detailed in Table 1. The average age was 12.7 years old (min 2, max 23, σ = 4.8). Altogether, 196 mares had been bred at least in one previous breeding season and the overall average of previous breeding seasons was 2.5 (min 0, max 17, σ = 3.0). For previously bred mares, mean fertility (number of foalings/number of breeding) was 0.71 (min 0, max 1, σ = 0.31). The interval between previous breeding and the 2019 reproductive season ranged from 1 to 13 years. Altogether, 66 (21.9%) mares were nulliparous and aged more than 10 years and 44 (15.8%) were suckling and older than 10 years.

Table 1: Characteristics of the 277 mares bred in 2019.

Variable	n	Percent
Age in 2 classes (Years)		
≤ 10	96	34.7
> 10	181	65.3

Age in 4 classes (Years)		
≤5	24	8.7
5-9	72	26.0
10-14	94	33.9
> 15	87	31.4
Parity		
Nulliparous	98	35.4
Parous	179	64.6
Lactation status in 2019		
Suckling	76	27.4
Non suckling	201	72.6
Fertility at previous breeding (n=196)		
< 0.7	82	41.8
≥ 0.7	114	58.2
Month of first AI		
March	36	13.0
April	70	25.3
May	86	31.0
June	67	24.2
July - August	18	6.5

222

223 **3.1.2. Pregnancy rate per mare**

224 Mares were bred with 154 different stallions. Altogether, 212 mares were diagnosed pregnant on D14
225 at the end of the season (76.5% of D14 pregnancy). These performances were obtained within an
226 average of 1.8 cycle per mare (min 1, max 6). From the 154 mares that came back for pregnancy
227 confirmation, 130 were still pregnant at Day 30, which represents 9.1% of pregnancy loss. Only forty-
228 six mares came back later in the autumn, of which 44 mares were confirmed still pregnant (4.3% of
229 pregnancy loss during this period).

230 Foaling information in 2020 were obtained for 206 pregnant mares on Day 14. Among them, 168
231 foalings were recorded indicating a total embryonic/fetal loss of 18.4%.

232

233 ### 3.1.3. Breeding management

234 Data recorded per estrus period are presented on Table 2.

235 Among the 506 estrus recorded periods, 92 were induced by luteolysis of a previous corpus luteum
236 using prostaglandins (18.2%). Data were recorded in average during 5.1 days per estrus period (min 1,
237 max 16, σ = 2.1).

238 Of the 506 estrous periods, fresh semen was used for 90 cycles (FAI, 17.8%), cooled transported semen
239 for 142 cycles (CTAI, 28.1%) and frozen semen for 274 (FZAI, 54.1%). Ovulation was induced in 360
240 estrous periods (71.2%). The induction of ovulation was performed in 40.0% of the FAI, 74.0% of the
241 CTAI and 80.0% of the FZAI. Mostly hCG was used (n=322, 89.4%) with GnRH analog used in the other
242 cases (n=38, 10.6%). Preovulatory dominant follicle mean diameter was 42.4 mm (min 28, max 60, σ =
243 4.8). Mares were inseminated in average 1.2 times per estrus (min 1, max 5, σ = 0.6). For FZAI, the
244 mean number of straws used was 5.1 (min 1, max 12, σ = 2.4, recorded in 251 AI by 274).

245 Ovulation was observed by ultrasonography in 501 of the 506 estrous periods (99%). In most cases,
246 only one ovulation was observed (n=425, 84.8%). Double or triple ovulations were observed in 75
247 (15.0%) and 1 estrus, respectively.

248 After insemination, uterine fluid accumulation was observed in 180 estrous periods (35.6%). Oxytocin
249 was used as treatment for 173/180 cases, mostly alone (139/180), sometimes associated with uterine
250 lavage (27 cases). Antibiotics were used for local treatment (10/180) associated to oxytocin (n=3), to
251 uterine lavage (n=4) or alone (n=3).

252 After AI, 212 cycles among the 506 led to a pregnancy on D14 (pregnancy rate per cycle: 41.9%). Twins
253 were detected in 27 cases (5% of breeding and 12.7% of pregnancies). Squeezing was performed for
254 25/27 pregnancies. After squeezing, pregnancy was checked on Day 30 and 21/24 mares were still
255 pregnant (87.5%).

256

257 **Table 2: Characteristics of the 506 monitored estrus**

Variable	n	Percent
Cycle number		
1	277	54.7
2	144	28.5
3	65	12.8
4	18	3.7
5	1	0.2
6	1	0.2
Luteolysis before AI		
No	414	81.8
Yes	92	18.2
Induction of ovulation		
No	146	28.8
Yes	360	71.2

Number of observed ovulations (n=501)		
1	425	84.8
2	75	15.0
3	1	0.2
Method of semen preservation		
Fresh semen (FAI)	90	17.8
Cooled transported (CTAI)	142	28.1
Frozen (FZAI)	274	54.1
Number of straws (n= 251)		
< 8	177	70.5
≥ 8	74	29.5
Number of straws (n= 251)		
≤ 4	126	50.2
> 4	125	49.8
Month of AI		
March	36	7.1
April	104	20.6
May	150	29.6
June	155	30.6
July-August	61	12.1
Number of AI by estrus period		
1	431	85.2
2	57	11.2
3	11	2.2
4	5	1.0
5	2	0.4

Observed ovulation		
Yes	501	99.0
No	5	1.0
Uterine fluid accumulation after AI		
Yes	180	35.6
No	327	64.4
Uterine treatment (n=180)		
Ocytocin alone	139	77.2
Other	41	22.8

258

259 ## 3.2. Univariate & multivariate analysis

260 ### 3.2.1. Factors associated with post-breeding inflammation

261 After univariate analysis, variables associated with post-breeding fluid accumulation were: mare's age
262 (using 10 years old as cutoff value), parity, lactation (suckling *vs* non suckling), induction of estrus by
263 luteolysis, cycle number and month of AI (Figure 1). Post-breeding inflammation was not associated
264 with semen conditioning (33.6, 40.2 and 37.9% respectively for FAI, CTAI and FZAI, p=0.45), number of
265 AI per estrus (35.2% for 1 AI *vs* 40.3% for more than one AI per estrus, p=0.40), nor number of straws
266 used (33.9 vs 33.8% for number of straws < 8 vs ≥ 8, p=0.98 and 37.3 vs 30.4% for number of straws ≤
267 4 vs > 4, p=0.25).

268 After multivariate analysis, only 3 variables were significantly associated (p<0.05) with post-breeding
269 inflammation: mare age, lactation and month of AI (Supplementary table 1). Inflammation was more
270 frequent in mares older than 10 years than in younger mares (Figure 1). Non suckling mares at the time
271 of insemination were also more affected in comparison with suckling ones (Figure 1). There was no
272 interaction between age and nursing status (p = 0.38) but increased age amplified the number of post-
273 breeding inflammations in both non-suckling and suckling mares. Respectively, 22.9% and 8.2% of the

274 non-suckling and suckling young mares were affected by post-breeding inflammation while 50.7% and
275 18.3% of mares older than 10 years old were affected.

276 The risk of inflammation was also increased in July and August compared to previous months (Figure
277 1).

278

279 ### 3.2.2. Factors associated with pregnancy rate per cycle

280 After univariate analysis, the following factors were associated with pregnancy rate at the 20%
281 threshold: age in 2 classes, parity, cycle number, AI modality, month of insemination, number of
282 observed ovulations and post-breeding inflammation. All the associated variables were introduced in
283 multivariate models. As a significant effect of number of ovulations was observed after univariate
284 analysis, only estrous cycles with ovulations that were observed by ultrasonography were considered
285 (*i.e.*, 501 estruses among the 506 recorded).

286 Stepwise regression and backward elimination led to a model containing only 3 significant variables
287 ($p<0.05$): mare age, number of ovulations and semen conditioning (Supplementary Table 2). Data that
288 significantly influenced pregnancy rates are summarized in Figure 2. Pregnancy rate was higher in
289 mares younger *vs* older than 10 years old. Pregnancy rate was increased when multiple ovulations
290 were observed. FAI and CTAI resulted in more pregnancies on Day 14 than FZAI. Trends were observed
291 ($p < 0.10$) for the effects of month of AI and parity. Pregnancy rate tended to be higher in April, May,
292 July and August vs March and June. Parous mares tended also to have better pregnancy rates than
293 nulliparous mares ($p=0.07$, OR=1.434 in parous mares).

294 The study of the interaction between maternal age and parity showed that in mares aged of 10 years
295 or less, being nulliparous or parous did not alter pregnancy rates (49.25 and 46.15% of pregnancy rate
296 per cycle for young nulliparous and parous respectively, $n = 158$, $p = 0.70$). In mares older than 10
297 years old, however, nulliparity accentuated the decrease in pregnancy rates. Indeed, the pregnancy

298 rates per cycle was only 30.89% for old nulliparous mares *vs* 44.09% for old multiparous mares (OR =
299 1.96 in parous mares, p = 0.016).

300 After multivariate analysis, pregnancy rate was not significantly affected by post-breeding
301 inflammation (37.8% of pregnancy rate after inflammation vs 44.2% in healthy mares, p = 0.16). In
302 treated mares, treatment after post-breeding treatment did not affect pregnancy rate (37.4% of
303 pregnancy for treatment with oxytocin alone, n = 139 *vs* 39.0% for other treatment, n = 41, p = 0.85).

304

305 **3.2.3. Factors associated with embryonic/fetal loss between Day 14 and foaling**

306 After the univariate analysis, age in four classes, induction of ovulation and month of insemination
307 were associated with embryonic/fetal loss at the threshold of 20%. However, after multivariate
308 analysis, none of these factors affected embryonic/fetal loss.

309

310 3.3. Focus on maternal age, parity and suckling status

311 **3.3.1. Effect of maternal age**

312 Mares 10 years old or younger were 7.2 ± 0.2 years old while mares older than 10 years were 15.6 ±
313 0.2 years old.

314 Neither parity, induction of the estrus cycle or of ovulation, month of AI, number of cycles required for
315 a gestation at Day 14, number of straws in case of FZAI or the number of twin embryos at Day 14 were
316 significantly related to maternal age (Supplementary Table 3).

317 Nevertheless, more young mares were pregnant at Day 14 and less had a post-breeding inflammation
318 compared to old mares (Table 3). In addition, 31% of young mares were nursing while it represented
319 only 20.7% of old mares ($p < 0.05$). Multiple ovulations occurred more frequently in old mares

320 compared to young mares. Young mares tended to be more inseminated using fresh and frozen and
321 less with refrigerated semen compared to older mares.

322 Estrus tended to be longer in old *vs* young mares (respectively, in average, 5.2 ± 0.1 *vs* 4.8 ± 0.2 days,
323 p = 0.086). The preovulatory follicle diameter tended to be smaller in old compared to young mares
324 (respectively, in average, 42.2 ± 0.2 *vs* 43.0 ± 0.4 mm, p = 0.086).

325 **Table 3: Characteristics in young (≤10 years old) vs old mares (>10 years old)**

Variable	**n**	**Young**	**Old**	**p**
		≤ 10 years old	**> 10 years old**	
		n=158	**n=343**	
Pregnancy rates on Day 14	501	47.5%	39.4%	0.021**
Post-breeding inflammation	501	18.3%	44.0%	< 0.0001**
Lactation				
Non-nursing	381	69.0%	79.3%	0.012
Nursing	120	31.0%	20.7%	
Method of the semen preparation *				
FAI				
CTAI	87	19.0%	16.6%	0.093
FZAI	140	21.5%	30.9%	
	274	59.5%	52.5%	
Number of ovulations per cycle				
1				
> 1	425	90.5%	82.2%	0.016
	76	9.5%	17.8%	

326 * AI: Artificial insemination, FAI: insemination with fresh semen, CTAI: insemination with cooled
327 transported semen, FZAI: insemination with frozen semen

328 ** Results from multivariate analysis

329

330 ### 3.3.2. Effect of maternal parity

331 Neither age, induction of the estrus cycle, semen preparation, month of AI, number of cycles or AI
332 required for a gestation at Day 14, number of straws in case of FZAI nor the number of ovulation or
333 twin embryos at Day 14 were significantly affected by maternal age (Supplementary Table 4).

334 Nulliparous mares tended to be less pregnant on Day 14 (Figure 2). Parity tended to be related to post-
335 breeding inflammation. Maternal parity was different according to maternal age. More nulliparous
336 mares were present in 5 or less and 10-15 years old group while less nulliparous were observed in 5-
337 10- and 16 or more-years old groups. Nulliparous mares were mostly older than 10 years (64.7%,
338 Supplementary Table 4) but nulliparous were in average younger than parous mares (Table 4) with a
339 mean 2-year difference between nulliparous and parous mares (in average, respectively, 11.6 ± 0.3 *vs*
340 13.7 ± 0.3 years old, $p < 0.0001$). Moreover, suckling status was obviously related to parity and 38.6%
341 of parous mares were suckling at insemination. Ovulation was induced more frequently in parous than
342 in nulliparous mares. Parity did not modify estrus duration (5.1 ± 0.2 days for nulliparous *vs* 5.1 ± 0.1
343 days for parous mares, $p = 0.11$) but the size of the preovulatory follicle at ovulation was reduced in
344 nulliparous compared to parous mares (respectively, 41.9 ± 0.3 and 42.8 ± 0.2mm in diameter, $p=0.03$).

345 **Table 4: Characteristics in nulliparous vs parous mares**

Variable	n	Nulliparous n=190	Parous n=311	p

Pregnancy rates on D14	501	37.4%	44.7%	0.066**
Age				
≤ 5	40	17.4%	2.3%	< 0.0001
5 < Age ≤ 10	118	17.9%	27.0%	
10 < Age ≤ 15	185	43.2%	33.1%	
Age > 15	158	21.6%	37.6%	
Lactation				
Non nursing	381	100.0%	61.4%	< 0.0001
Nursing	120		38.6%	
Induction of ovulation				
No	145	35.8%	24.8%	0.008
Yes	356	64.2%	75.2%	

AI: Artificial insemination, FAI: insemination with fresh semen, CTAI: insemination with cooled transported semen, FZAI: insemination with frozen semen

** Results from multivariate analysis

3.3.3. Effect of nursing at insemination

Neither induction of the estrus cycle nor ovulation induction, semen preparation, number of AI per estrus required for a gestation at Day 14, number of straws in case of FZAI nor the number of ovulations or twin embryos at Day 14 were significantly affected by maternal suckling status (Supplementary Table 5).

355 Significantly less suckling mares were affected by post-breeding infection than non-suckling ones
356 (Table 5) but pregnancy rates were not affecting by lactational status (45.8% and 40.7% of pregnancy
357 rate per cycle for, respectively suckling and non-sucking mares, p = 0.32).
358 There were more suckling mares younger than 10 years of age and more non-suckling mares aged
359 more than 10 years. Mares younger than 5 and mares aged between 10 to 15 years were less
360 frequently nursing than mares aged 5-10. In the group of 5-10 years old, more suckling mares were
361 present in comparison to non-suckling ones while the lactational status was similarly represented in
362 16 years old and older mares. In average, however, the age of suckling *vs* non-suckling mares was not
363 different (respectively 13.0 ± 0.2 *vs* 12.6 ± 0.2 years old, p = 0.39). Less nursing mares were bred in
364 March and April but more were bred in June than non-nursing ones (Table 5). Moreover, more nursing
365 mares were pregnant at Day 14 within the first exploited cycle than non-nursing ones.
366 Estrus was shorter (respectively, 4.7 ± 0.16 *vs* 5.2 ± 0.1 days, p = 0.03) and preovulatory follicle
367 diameter was larger (respectively 43.8 ± 0.4 *vs* 42.0 ± 0.2, p < 0.0001) in nursing vs non-nursing mares.
368 **Table 5: Characteristics in non-suckling *vs* suckling mares**

Variable	**n**	**Non suckling**	**Suckling**	**p**
		n=381	**n=120**	
Post-breeding inflammation	501	42.8%	14.2%	< 0.0001
Age				
≤ 10 years old	158	28.6%	40.8%	0.012
> 10 years old	343	71.4%	59.2%	
≤ 5	40	9.7%	2.5%	< 0.0001
5 < Age ≤ 10	118	18.9%	38.3%	
10 < Age ≤ 15	185	39.6%	28.3%	

Age > 15	158	31.8%	30.8%	
Month of AI				
March	35	8.1%	3.3%	0.005
April	103	22.8%	13.3%	
May	149	29.7%	30.0%	
June	153	26.7%	42.5%	
July - August	61	12.6%	10.8%	
Number of cycles for pregnancy				
1	276	52.5%	63.3%	0.037
> 1	225	47.5%	36.7%	

* AI: Artificial insemination, FAI: insemination with fresh semen, CTAI: insemination with cooled transported semen, FZAI: insemination with frozen semen

4. Discussion

Data presented here are summarized in Figure 3.

4.1. Post-breeding fluid accumulation

4.1.1. Prevalence

In this study, more than a third of the monitored cycles were followed by post-breeding uterine fluid accumulation. In a normal population of Thoroughbred mares, around 15% of mares are susceptible to post-breeding inflammation that persists for several days [28]. In a more recent study in Thoroughbreds in UK, however, post-breading fluid accumulation occurred in 47.7% of analyzed pregnancies [17]. The observed rate in the present study is therefore in the range of the literature. In

381 comparison to other studies, however, the post-breeding inflammation rate might have been
382 overestimated. Indeed, as mares could be kept on the breeding stud for several cycles, the same mares
383 could have had several inflammations over the entire breeding period, as this condition is generally
384 persistent [29].

385

386 #### 4.1.2. Effect of maternal age, parity and lactation status

387 In the studied population, there was twice more post-breeding fluid accumulation in mares older than
388 10 years than in younger mares. These results agreed with literature as one recent study in
389 Thoroughbred stud farms showed that the percentage of mares with post-breeding intrauterine fluid
390 accumulation [17], increase with mare age. Frequency of endometritis has been demonstrated to
391 increase with mare age as well as [26,30–34].

392 In addition, suckling at insemination appeared to have a protective effect against post-breeding fluid
393 accumulation. To the authors' knowledge, there is no study on the effect of nursing at insemination
394 on post breeding fluid accumulation. Since lactating mares are producing endogenous oxytocin, the
395 well-known and widespread treatment against post-breeding inflammation [35], it could explain the
396 observed improvement in nursing mares. Indeed, nursing induces oxytocin release in the blood
397 circulation, reaching a peak of around 10mIU/L in nursing pony and broodmares [36,37]. Plasma
398 oxytocin concentrations of 10-7 mIU/L were reported to be present 20min after the beginning of the
399 suckling period [36]. Since foals nurse until 72times a day in the first weeks of their life, the release of
400 oxytocin happens in a regular manner throughout the day [38].

401

402 #### 4.1.3. Effect of the method of semen preservation and semen quantity

403 In this study, neither semen preservation method nor the volume determined by number of frozen
404 straws, nor the number of inseminations were related to post-breeding fluid accumulation. One study
405 showed a similar volume of uterine fluid accumulated in the uterus after insemination with cooled or
406 frozen semen which is consistent with the results obtained here [39]. One recent study, however,
407 showed that the uterine inflammatory response was positively corelated to the number of
408 spermatozoids used for the insemination with frozen semen [40]. This high inflammatory response was
409 also faster to resume than the response for low doses of semen [40].

410

411 #### 4.1.4. Effect of month of insemination

412 Data showed that more post-breeding fluid accumulation occurred when insemination was performed
413 late in the season, i.e., during July and August. Several hypotheses could explain this result. The first is
414 that mares with good quality uteri needed less cycles to become pregnant and therefore, more mares
415 with reproductive troubles were inseminated late in the season as they often need to be inseminated
416 for several cycles to start a pregnancy. Another hypothesis regarding the increase of post-breeding
417 inflammation late in the season would be the increase of temperatures occurring from June/July . So
418 far, there is no study on the effects of outdoor temperature on the incidence of endometritis in mares
419 but in dairy cows, environmental heat, as evaluated by comparing the possibility of sheltering or not
420 from the sun, increased rectal temperature by almost one degree [41] and reduced uterine blood flow
421 [42]. The temperature increase, out of the thermoneutrality zone (5-25°C for horses), could directly
422 affect uterine temperature and/or on uterine blood flow and therefore promote inflammation in the
423 mares' uterus.

424

425 #### 4.1.5. Treatment and pregnancy rates

426 In the present study, when a post-breeding fluid accumulation was detected, treatment was
427 systematically applied. Most of the time, this treatment was limited to an injection of oxytocin. In a
428 recent study about therapeutic practices in intensively managed Thoroughbred mares, almost half of
429 the pregnant mares were treated with intrauterine antibiotics and the same proportion was treated
430 with oxytocin. Oxytocin combined with intrauterine antibiotics are used prophylactically in
431 Thoroughbreds in the UK to avoid uterine infections as breeders believe it to be a cause of conception
432 failure and embryo loss [17]. In Thoroughbreds, however, less than 10% of early pregnancy failures
433 were associated with uterine inflammation [17,31]. Another study on 99 Thoroughbred mares showed
434 no association between post-breeding uterine fluid accumulation and pregnancy rates nor embryonic
435 death [43], as observed here. In all studies, the systematic management to prevent/cure anormal
436 inflammation could explain this lack of association. Therefore, nowadays, the post-breeding
437 inflammation seems to be well handled by stud farms.

438 Contrarily, in different breeds, the combination of antibiotics with oxytocin to reduce post-breeding
439 fluid accumulation was shown to be more efficient than antibiotics or oxytocin alone to prevent
440 decreased pregnancy rates after mating [44]. In another study on warmblood mares artificially
441 inseminated, however, oxytocin alone was sufficient to increase pregnancy rates in comparison to no
442 treatment [35]. In this study, oxytocin alone was mostly applied and was sufficient to avoid altered
443 pregnancy rate. Therefore, it suggests that this treatment alone is efficient to avoid decreased
444 pregnancy rates in artificially inseminated mares.

445

446 4.2. Pregnancy rates

447 4.2.1. Prevalence

448 In studies on different breeds conducted in several countries using artificial insemination, pregnancy
449 rates were between 40-80%, according to the semen preservation method [15,45–47]. In a comparable

450 recent study on 328 sport mares artificially inseminated in the Netherlands, 46.6% of gestations were
451 obtained on Day 12 – 18 [48]. Here, pregnancy rates per cycle were similar with 41.9% of mares being
452 pregnant by cycle regardless of the method used for semen preservation.

453

454 #### 4.2.2. Effect of maternal age, parity and lactation status

455 Pregnancy rates were reduced when mares were older than 10 years old at the time of insemination.
456 Several studies already showed a reduction of pregnancy rates with increased maternal age
457 [1,7,10,30,49–51]. Most studies, however, observed differences in mares older than 14 years old and
458 not as early as 10 years old. Here, the clustering in 4 classes (≤ 5, 5-9, 10-14 and ≥ 15 years old) was
459 not associated with alteration of pregnancy rates. Our inability to demonstrate such changes might be
460 due to the only slight reduction of pregnancy rates induced by aging and to the limited number of
461 mares studied.

462 Maternal parity tended to not reduce pregnancy rates in the overall population. The effects of
463 maternal parity on pregnancy rates are contrasted as being closely related to mares' age. Indeed, when
464 nulliparous mares were mostly older than 7 years, a detrimental effect of nulliparity was observed
465 [2,15] but when nulliparous mares were younger, no deleterious or a favorable effect of parity was
466 observed [2,14]. Here, more than 60% of nulliparous were older than 10 years which could explain the
467 observed tendency.

468 When considering only mares older than 10 years, D14 pregnancy rates were reduced in nulliparous
469 mares while in the youngest group, the parity did not affect the incidence of pregnancy. Thus, the
470 present data indicate a deleterious cumulative effect of nulliparity and aging on fertility in mares. This
471 was previously suggested but never demonstrated in any broodmares population.

472 One other important finding is that lactation at the time of insemination did not influence pregnancy
473 rates, 14 days post ovulation. Foaling mares, however, needed less cycles to become pregnant. In the

474 literature, the effect of lactation on fertility is controversial as some studies observed that nursing
475 mares are more fertile than non-nursing ones [2,8,30,50,51] while others do not observe any
476 difference [4,7,10,14]. Insemination at foal heat was previously shown to reduce pregnancy rates
477 [23,52] and to be associated with increased embryonic death [21,22,52]. Here, most foaling mares
478 were bred on foal heat without adverse effect on pregnancy rates.

479

480 #### 4.2.3. Effect of the number of ovulations

481 The more ovulations were observed, the higher the likelihood that the mare was to be pregnant at 14
482 days post ovulation. The result obtained here seems obvious as double ovulations increase the number
483 of oocytes that could be fertilized.

484

485 #### 4.2.4. Effect of the method of semen preservation and semen quantity

486 As previously observed [45,46,48,53], the modalities of insemination influenced the probability for a
487 mare to be pregnant on Day 14. Indeed, pregnancy rate was higher after FAI or CTAI than after FZAI. It
488 is well known that stallion semen quality decreases with cryopreservation, thus a higher critical
489 number of mobile spermatozoa per dose is required to reach the same pregnancy rates than with fresh
490 semen [53]. In this study, the number of straws was not related to pregnancy rate, as also shown by
491 others [45]. In France, the concentration of spermatozoa/straw is standardized to 100 millions of
492 spermatozoa per ml by the National French Institute. Thus, stallion coming from abroad had a different
493 spermatozoa concentration, usually lower, that could also impact the results. Despite these
494 considerations, the protocol involving ultrasonography every 6h and deep intra-uterine AI after
495 observed ovulation seemed to be effective to reach acceptable pregnancy rates with 4 straws or less.

496

497 #### 4.2.5. Effect of month of insemination

498 The month of insemination tended to influence the pregnancy rate, with reduced incidence of
499 pregnancies in March and June. In another French study, March was one of the more prolific month in
500 terms of foal productivity per mare, all breed considered (Thoroughbred, Standardbred and sport
501 bred) [2]. In racehorse breeding, it is common to use light to advance the breeding season [54] since
502 foals born early in the year have an advantage when they are sold as yearling. In sport horse breeding
503 under European latitudes, however, March is often the beginning of the breeding season. At this time,
504 mares enter the spring transitional period and less mares are bred, which could explain the reduced
505 pregnancy rates on this month. The other French study also reported a fertility decrease when mares
506 were bred in June and after [2]. The June effect, here, is more complicated to explain as July and August
507 did not affect pregnancy rates. During their stay, all mares were housed in individual stable with no
508 access to fresh grass. They were fed with hay, thus a change in food quality in June could not explain
509 the present results. Nevertheless, increasing ambient temperature could explain the differences in
510 pregnancy rates as temperatures reached a maximum of 35.8°C in one stud farm in June 2019. In July
511 and August, temperature were also high with more than 33°C as maximal temperature in the 2 studied
512 stud farms. The lack of difference in July and August could be explained by the fact that only few mares
513 were bred during this period and that more attention could have been given to these last mares.

514

515 ### 4.3. Effects of age on other reproductive outcomes

516 A survey sent to breeders participating in competing events, showed that mare's age was the least
517 important consideration for the selection of mares for reproduction [55].

518 #### 4.3.1. Choice of semen preservation method

519 Maternal age did not influence the breeders' choice concerning ovulation management but influenced
520 the modality of AI. Indeed, less frozen and fresh semen were chosen for the insemination of older
521 mares. On one side, frozen semen is less efficient for producing a foal, as confirmed by literature [53]
522 and this study. Moreover, reduced fertility has been observed in old mares, both in literature [for
523 review see 47] and in the present study. The combination of both factors could explain that older
524 mares tended to be inseminated less with frozen semen as the financial risk might have been too costly
525 for breeders. In addition, currently, equine breeders do not use more straws for old than for young
526 mares, even if it has been shown that increasing straw number could improve fertility [53].

527

528 #### 4.3.2. Length of estrus and ovulation outcomes

529 This retrospective study shows that the length of estrus tended to increase with mare age (only 0.4
530 day more in old mares), with a smaller preovulatory follicle. This was already observed as a prolonged
531 interovulatory interval was associated with a prolonged follicular growth [57–64] and to a reduction in
532 follicular growth and preovulatory follicular size in older mares [65–68].

533 In the studied population, more multiple ovulations were also observed in older mares. Several studies
534 reported that the incidence of multiple ovulations continuously increases until the mare reaches 20
535 years of age [63,68–70]. More multiple ovulations in older mares, however, is not associated with more
536 twinning at 14 days post ovulation. Studies have shown that even if fertilization appears to be equal
537 between young and old mares, early embryo mortality is increased in older mares [49,71] which could
538 explain the absence in increase of twin embryos observed at 14 days in older mares compared to
539 younger ones.

540

541 ### 4.4. Effects of parity on other reproductive outcomes

542 Nulliparous mares had smaller preovulatory follicles. As explained above, the monitoring of mares was
543 not performed more than twice a day: since follicles continuously grow until breakdown, this observed
544 difference should be considered with caution.

545

546 ### 4.5. Effects of suckling at insemination on other reproductive outcomes

547 In sport horses, mares are often bred and subsequently foal during the spring. Foaling mares are most
548 of the time pregnant at the beginning of the spring. It is, therefore, not surprising to observe a peak of
549 breeding in May and June in foaling mares while barren mare breedings were more spread throughout
550 the reproductive season. Moreover, as it appears that less estrus cycles were required to obtain a
551 pregnancy in lactating mares, it is not surprising either to not observed many lactating mares in August.

552 Finally, the heat period was shortened and the preovulatory follicle was larger when mares were in
553 lactation without changing the number of ovulations. To the author knowledge, there is no study on
554 the effect of lactation at insemination time on estrus and follicle parameters.

555

556 ## 5. Conclusion

557 In conclusion, maternal age appeared to be the most important factor affecting both post-breeding
558 inflammation and pregnancy rates. Both could be explained by the degenerative changes that are
559 observed in older mares. Breeders, should, therefore, be encouraged to pay more attention to the age
560 of their broodmares and either, breed earlier in their life or culling them earlier to avoid excessive
561 costs. Older mares are also of less interest concerning genetics point of view [72] that should also be
562 considered by breeders. Moreover, as frozen semen was associated with decreased pregnancy rates,
563 the use of fresh or cooled semen for the insemination of old mares should be advised.

564 In this study, it has also been shown that nulliparity as the same time to aging affected pregnancy
565 rates. For their first gestation, mares should not be more than 10 years old to increase chances of
566 pregnancy. One advice to owners could be to breed the mares before starting their sportive career to
567 produce their first foal as it will help for the later inseminations.

568 Suckling at insemination prevented post-breeding inflammation. The most probable hypothesis could
569 be that sucking provokes oxytocin release that is acting as a natural treatment of uterine fluid
570 accumulation.

571 Here, pregnancy rates were not affected by post-breeding inflammation, although uterine fluid
572 accumulation were more frequent than in other studies, showing that the excessive fluid accumulation
573 related to insemination is well handled and that oxytocin only as a treatment is efficient.

574

References

[1] Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. Equine Veterinary Journal 2007;39:438–45. https://doi.org/10.2746/042516407X1737581.

[2] Langlois B, Blouin C. Statistical analysis of some factors affecting the number of horse births in France. Reprod Nutr Dev 2004;44:583–95. https://doi.org/10.1051/rnd:2004055.

[3] Katila T, Reilas T, Nivola K, Peltonen T, Virtala A-M. A 15-year survey of reproductive efficiency of Standardbred and Finnhorse trotters in Finland - descriptive results. Acta Veterinaria Scandinavica 2010:11.

[4] Nath L, Anderson G, McKinnon A. Reproductive efficiency of Thoroughbred and Standardbred horses in north-east Victoria. Australian Veterinary Journal 2010;88:169–75. https://doi.org/10.1111/j.1751-0813.2010.00565.x.

[5] Hanlon D, Stevenson M, Evans M, Firth E. Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses. New Zealand Veterinary Journal 2012;60:329–34. https://doi.org/10.1080/00480169.2012.693039.

[6] Haadem CS, Nødtvedt A, Farstad W, Thomassen R. A retrospective cohort study on fertility in the Norwegian Coldblooded trotter after artificial insemination with cooled, shipped versus fresh extended semen. Acta Vet Scand 2015;57:77. https://doi.org/10.1186/s13028-015-0161-8.

[7] Bosh KA, Powell D, Neibergs JS, Shelton B, Zent W. Impact of reproductive efficiency over time and mare financial value on economic returns among Thoroughbred mares in central Kentucky. Equine Veterinary Journal 2009;41:889–94. https://doi.org/10.2746/042516409X456059.

[8] Lane E, Bijnen M, Osborne M, More S, Henderson I, Duffy P, et al. Key Factors Affecting Reproductive Success of Thoroughbred Mares and Stallions on a Commercial Stud Farm. Reprod Dom Anim 2016;51:181–7. https://doi.org/10.1111/rda.12655.

[9] Platt H. Aetiological aspects of abortion in the thoroughbred mare. J Comp Pathol 1973;83:199–205.

[10] Morris LHA, Allen WR. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Veterinary Journal 2002;34:51–60. https://doi.org/10.2746/042516402776181222.

[11] Gibbs PG, Davison KE. A field study on reproductive efficiency of mares maintained predominately on native pasture. Journal of Equine Veterinary Science 1992;12:219–22. https://doi.org/10.1016/S0737-0806(06)81449-8.

[12] Baker CB, Little TV, McDOWELL KJ. The live foaling rate per cycle in mares. Equine Veterinary Journal 1993;25:28–30. https://doi.org/10.1111/j.2042-3306.1993.tb04819.x.

[13] Hemberg E, Lundeheim N, Einarsson S. Reproductive Performance of Thoroughbred Mares in Sweden. Reprod Domest Anim 2004;39:81–5. https://doi.org/10.1111/j.1439-0531.2004.00482.x.

[14] Brück I, Anderson G, Hyland J. Reproductive performance of Thoroughbred mares on six commercial stud farms. Australian Vet J 1993;70:299–303. https://doi.org/10.1111/j.1751-0813.1993.tb07979.x.

[15] Squires E, Barbacini S, Matthews P, Byers W, Schwenzer K, Steiner J, et al. Retrospective study of factors affecting fertility of fresh, cooled and frozen semen. Equine Veterinary Education 2006;18:96–9. https://doi.org/10.1111/j.2042-3292.2006.tb00425.x.

[16] Samper JC, Vidament M, Katila T, Newcombe JR, Estrada A, Sargeant J. Analysis of some factors associated with pregnancy rates of frozen semen: a multi-center study. Theriogenology 2002;58:647–50. https://doi.org/10.1016/S0093-691X(02)00754-9.

[17] Rose BV, Firth M, Morris B, Roach JM, Wathes DC, Verheyen KLP, et al. Descriptive study of current therapeutic practices, clinical reproductive findings and incidence of pregnancy loss in intensively managed thoroughbred mares. Animal Reproduction Science 2018;188:74–84. https://doi.org/10.1016/j.anireprosci.2017.11.011.

[18] Chevalier-Clément F. Pregnancy loss in the mare. Animal Reproduction Science 1989;20:231–44. https://doi.org/10.1016/0378-4320(89)90088-2.
[19] Physick-Sheard PW. Demographic analysis of the Canadian Standardbred broodmare herd. Preventive Veterinary Medicine 1995;24:285–99. https://doi.org/10.1016/0167-5877(95)00492-F.
[20] Heidler B, Aurich JE, Pohl W, Aurich Chr. Body weight of mares and foals, estrous cycles and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. Theriogenology 2004;61:883–93. https://doi.org/10.1016/S0093-691X(03)00279-6.
[21] Merkt H, Günzel A-R. A survey of Early Pregnancy Losses in West German Thoroughbred Mares. Equine Veterinary Journal 1979;11:256–8. https://doi.org/10.1111/j.2042-3306.1979.tb01359.x.
[22] Blanchard TL, Thompson JA, Love CC, Brinsko SP, Ramsey J, O'Meara A, et al. Influence of day of postpartum breeding on pregnancy rate, pregnancy loss rate, and foaling rate in Thoroughbred mares. Theriogenology 2012;77:1290–6. https://doi.org/10.1016/j.theriogenology.2011.10.034.
[23] Camillo F, Marmorini P, Romagnoli S, Vannozzi I, Bagliacca M. Fertility at the first post partum estrous compared with fertility at the following estrous cycles in foaling mares and with fertility in nonfoaling mares. Journal of Equine Veterinary Science 1997;17:612–6. https://doi.org/10.1016/S0737-0806(97)80189-X.
[24] Camargo CE, Kozicki LE, Ruda PC, Breno Pedrosa V, Talini R, Weiss RR, et al. Reproductive efficiency in lactating mares inseminated early in the puerperium (<10 days post partum) versus non-lactating mares inseminated 180 days post partum: PHK 2017;33:458–64. https://doi.org/10.21836/PEM20170506.
[25] Christoffersen M, Troedsson M. Inflammation and fertility in the mare. Reproduction in Domestic Animals 2017;52:14–20. https://doi.org/10.1111/rda.13013.
[26] Woodward EM, Christoffersen M, Campos J, Squires EL, Troedsson MHT. Susceptibility to persistent breeding-induced endometritis in the mare: Relationship to endometrial biopsy score and age, and variations between seasons. Theriogenology 2012;78:495–501. https://doi.org/10.1016/j.theriogenology.2012.02.028.
[27] Samper JC. Ultrasonographic Appearance and the Pattern of Uterine Edema to Time Ovulation in Mares. Proceedings of the 43rd Annual Convention of the American Association of Equine Practitioners, vol. 43, 1997, p. 189–91.
[28] Zent WW, Troedsson MHT, Xue J-L. Postbreeding Uterine Fluid Accumulation in a Normal Population of Thoroughbred Mares: A Field Study. Proc Am Assoc Equine Pract, vol. 44, 1998, p. 64–5.
[29] Canisso IF, Segabinazzi LGTM, Fedorka CE. Persistent Breeding-Induced Endometritis in Mares—A Multifaceted Challenge: From Clinical Aspects to Immunopathogenesis and Pathobiology. IJMS 2020;21:1432. https://doi.org/10.3390/ijms21041432.
[30] Barbacini S, Marchi V, Zavaglia G. Equine frozen semen: results obtained in Italy during the 1994-1997 period. Equine Veterinary Education 1999;11:109–12. https://doi.org/10.1111/j.2042-3292.1999.tb00930.x.
[31] Ricketts SW, Alonso S. The effect of age and parity on the development of equine chronic endometrial disease. Equine Veterinary Journal 1991;23:189–92. https://doi.org/10.1111/j.2042-3306.1991.tb02752.x.
[32] Brinsko SP, Ball BA, Miller PG, Thomas PGA, Ellington JE. In vitro development of day 2 embryos obtained from young, fertile mares and aged, subfertile mares. Reproduction 1994;102:371–8. https://doi.org/10.1530/jrf.0.1020371.
[33] Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. Theriogenology 1992;37:1101–15. https://doi.org/10.1016/0093-691X(92)90108-4.
[34] Adams GP, Kastelic JP, Bergfelt DR, Ginther OJ. Effect of uterine inflammation and ultrasonically-detected uterine pathology on fertility in the mare. J Reprod Fertil Suppl 1987;35:445–54.
[35] Rasch K, Schoon HA, Sieme H, Klug E. Histomorphological endometrial status and influence of oxytocin on the uterine drainage and pregnancy rate in mares. Equine Veterinary Journal 1996;28:455–60. https://doi.org/10.1111/j.2042-3306.1996.tb01617.x.

[36] Sharma OP. RELEASE OF OXYTOCIN ELICITED BY SUCKLING STIMULUS IN MARES. Reproduction 1974;37:421–3. https://doi.org/10.1530/jrf.0.0370421.
[37] Ellendorff F, Schams D. Characteristics of milk ejection, associated intramammary pressure changes and oxytocin release in the mare. Journal of Endocrinology 1988;119:219–27. https://doi.org/10.1677/joe.0.1190219.
[38] Martin-Rosset W, DOREAU M, CLOIX J. Etude des activités d'un troupeau de poulinières de trait et de leurs poulains au pâturage. Annales de Zootechnie 1978;27:33–45.
[39] Samper JC, Stanford MS, French HM, Chapwanya A. Post-breeding inflammation in mares after insemination with large and low doses of fresh or frozen semen: PHK 2016;32:24–6. https://doi.org/10.21836/PEM20160103.
[40] Cazales N, Estradé MJ, Pereyra F, Fiala-Rechsteiner SM, Mattos RC. Sperm transport and endometrial inflammatory response in mares after artificial insemination with cryopreserved spermatozoa. Theriogenology 2020;158:180–7. https://doi.org/10.1016/j.theriogenology.2020.09.021.
[41] Ealy AD, Drost M, Hansen PJ. Developmental Changes in Embryonic Resistance to Adverse Effects of Maternal Heat Stress in Cows1. Journal of Dairy Science 1993;76:2899–905. https://doi.org/10.3168/jds.S0022-0302(93)77629-8.
[42] Roman-Ponce H, Thatcher WW, Caton D, Barron DH, Wilcox CJ. Thermal Stress Effects on Uterine Blood Flow in Dairy Cows. Journal of Animal Science 1978;46:175–80. https://doi.org/10.2527/jas1978.461175x.
[43] Malschitzky E, Schilela A, Mattos ALG, Garbade P, Gregory RM, Mattos RC. Intrauterine fluid accumulation during foal heat increases embryonic death. Pferdeheilkunde 2003;19:1–4.
[44] Pycock JF, Newcombe JR. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. Veterinary Record 1996;138:320–3. https://doi.org/10.1136/vr.138.14.320.
[45] Sieme H, Bonk A, Hamann H, Klug E, Katila T. Effects of different artificial insemination techniques and sperm doses on fertility of normal mares and mares with abnormal reproductive history. Theriogenology 2004;62:915–28. https://doi.org/10.1016/j.theriogenology.2003.12.011.
[46] Sieme H, Schäfer T, Stout TAE, Klug E, Waberski D. The effects of different insemination regimes on fertility in mares. Theriogenology 2003;60:1153–64. https://doi.org/10.1016/S0093-691X(03)00113-4.
[47] Watson ED, Barbacini S, Berrocal B, Sheerin O, Marchi V, Zavaglia G, et al. Effect of insemination time of frozen semen on incidence of uterine fluid in mares. Theriogenology 2001;56:123–31. https://doi.org/10.1016/S0093-691X(01)00548-9.
[48] Cuervo-Arango J, Claes AN, Stout TA. A retrospective comparison of the efficiency of different assisted reproductive techniques in the horse, emphasizing the impact of maternal age. Theriogenology 2019;132:36–44. https://doi.org/10.1016/j.theriogenology.2019.04.010.
[49] Ball BA, Little TV, Hillman RB, Woods GL. Pregnancy rates at Days 2 and 14 and estimated embryonic loss rates prior to day 14 in normal and subfertile mares. Theriogenology 1986;26:611–9. https://doi.org/10.1016/0093-691X(86)90168-8.
[50] Woods GL, Baker CB, Baldwin JL, Ball BA, Bilinski J, Cooper WL, et al. Early pregnancy loss in brood mares. Journal of Reproduction and Fertility Supplement 1987;35:455–9.
[51] Sharma S, Dhaliwal GS, Dadarwal D. Reproductive efficiency of Thoroughbred mares under Indian subtropical conditions: A retrospective survey over 7 years. Animal Reproduction Science 2010;117:241–8. https://doi.org/10.1016/j.anireprosci.2009.05.011.
[52] Malheiros de Souza JR, Dias Gonçalves PB, Bertolin K, Ferreira R, Sardinha Ribeiro AS, Ribeiro DB, et al. Age-dependent effect of foal heat breeding on pregnancy and embryo mortality rates in Thoroughbred mares. Journal of Equine Veterinary Science 2020:102982. https://doi.org/10.1016/j.jevs.2020.102982.
[53] Vidament M, Dupere AM, Julienne P, Evain A, Noue P, Palmer E. Equine frozen semen: freezability and fertility field results. THERIOGENOLOGY 1997;48:907–17. https://doi.org/10.1016/S0093-691X(97)00319-1.

[54] Davies Morel MCG, Newcombe JR, Holland SJ. Factors affecting gestation length in the Thoroughbred mare. Animal Reproduction Science 2002;74:175–85. https://doi.org/10.1016/S0378-4320(02)00171-9.
[55] Whitaker TC, Brace C. Mare age as a selection consideration for sport horse broodmares; a breeder survey. Proc BrSoc Anim Sci 2007;2007:30–30. https://doi.org/10.1017/S1752756200019335.
[56] Derisoud E, Auclair-Ronzaud J, Palmer E, Robles M, Chavatte-Palmer P. Female age and parity in horses: how and why does it matter? Reproduction, Fertility and Development In press.
[57] Ginther OJ, Carnevale EM, Bergfelt DR. DELAY IN EMERGENCE OF THE OVULATORY FOLLICULAR WAVE IN OLD MARES. JOURNAL OF EQUINE VETERINARY SCIENCE 1993;13:5.
[58] Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Siddiqui MAR, Beg MA. Effects of age on follicle and hormone dynamics during the oestrous cycle in mares. Reprod Fertil Dev 2008;20:955. https://doi.org/10.1071/RD08121.
[59] Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA. Age-related dynamics of follicles and hormones during an induced ovulatory follicular wave in mares. Theriogenology 2009;71:780–8. https://doi.org/10.1016/j.theriogenology.2008.09.051.
[60] Carnevale EM, Bergfelt DR, Ginther OJ. Aging effects on follicular activity and concentrations of FSH, LH, and progesterone in mares. Animal Reproduction Science 1993;31:287–99. https://doi.org/10.1016/0378-4320(93)90013-H.
[61] Vanderwall DK, Woods GL, Freeman DA, Weber JA, Rock RW, Tester DF. Ovarian follicles, ovulations and progesterone concentrations in aged versus young mares. Theriogenology 1993;40:21–32. https://doi.org/10.1016/0093-691X(93)90338-6.
[62] Carnevale EM, Bergfelt DR, Ginther OJ. Follicular activity and concentrations of FSH and LH associated with senescence in mares. Animal Reproduction Science 1994;35:231–46. https://doi.org/10.1016/0378-4320(94)90039-6.
[63] Marinone AI, Losinno L, Fumuso E, Rodríguez EM, Redolatti C, Cantatore S, et al. The effect of mare's age on multiple ovulation rate, embryo recovery, post-transfer pregnancy rate, and interovulatory interval in a commercial embryo transfer program in Argentina. Animal Reproduction Science 2015;158:53–9. https://doi.org/10.1016/j.anireprosci.2015.04.007.
[64] Claes A, Ball BA, Scoggin KE, Roser JF, Woodward EM, Davolli GM, et al. The influence of age, antral follicle count and diestrous ovulations on estrous cycle characteristics of mares. Theriogenology 2017;97:34–40. https://doi.org/10.1016/j.theriogenology.2017.04.019.
[65] Claes A, Ball BA, Scoggin KE, Esteller-Vico A, Kalmar JJ, Conley AJ, et al. The interrelationship between anti-Müllerian hormone, ovarian follicular populations and age in mares: Anti-Müllerian hormone and ovarian follicular populations. Equine Vet J 2015;47:537–41. https://doi.org/10.1111/evj.12328.
[66] Uliani RC, Conley AJ, Corbin CJ, Friso AM, Maciel LFS, Alvarenga MA. Anti-Müllerian hormone and ovarian aging in mares. Journal of Endocrinology 2019:147–56. https://doi.org/10.1530/JOE-18-0391.
[67] Korkmaz Ö, Emre B, Polat İM, Zonturlu AK, PiR Yağci İ, Pekcan M, et al. The Correlation Between Anti-Müllerian Hormone Concentrations and Reproductive Parameters in Different Age Groups in Purebred Arabian Mares. Kafkas Univ Vet Fak Derg 2020;26:53–7. https://doi.org/10.9775/kvfd.2019.22227.
[68] Davies Morel MCG, Newcombe JR, Hayward K. Factors affecting pre-ovulatory follicle diameter in the mare: the effect of mare age, season and presence of other ovulatory follicles (multiple ovulation). Theriogenology 2010;74:1241–7. https://doi.org/10.1016/j.theriogenology.2010.05.027.
[69] Morel MCGD, Newcombe JR, Swindlehurst JC. The effect of age on multiple ovulation rates, multiple pregnancy rates and embryonic vesicle diameter in the mare. Theriogenology 2005;63:2482–93. https://doi.org/10.1016/j.theriogenology.2004.09.058.
[70] Panzani D, Rota A, Marmorini P, Vannozzi I, Camillo F. Retrospective study of factors affecting multiple ovulations, embryo recovery, quality, and diameter in a commercial equine embryo

transfer program. Theriogenology 2014;82:807–14. https://doi.org/10.1016/j.theriogenology.2014.06.020.

[71] Ball BA, Little TV, Weber JA, Woods GL. Survival of Day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. Reproduction 1989;85:187–94. https://doi.org/10.1530/jrf.0.0850187.

[72] Palmer E, Chavatte-Palmer P. Contribution of reproduction management and technologies to genetic progress in horse breeding. Journal of Equine Veterinary Science 2020:103016. https://doi.org/10.1016/j.jevs.2020.103016.

Declaration of competing interest

The authors have declared no conflicting interests.

Funding information

This work was supported by the PHASE (Physiologie animale et Systèmes d'Elevage) department of INRAE, the French national institute for horses and horse-riding (Institut Français du cheval et de l'Equitation) and the Karolinska Institute.

Acknowledgements

The authors tanks V. Lehuraux, A Jugland, B. Brisset and P. Drevillon from the Stud of "Charmoy" (89) and Stud of "Les Bréviaires" (78) for the availability of the data and for their hospitality during the visits of their organizations. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Many thanks to Juliette Auclair-Ronzaud for the reviewing of this article.

Author contribution

CG generated data. BG formatted datasets and performed the analyses. ED, JAR and PCP wrote the draft. All authors read, revised, and approved the submitted manuscript.

810 # Figure legends

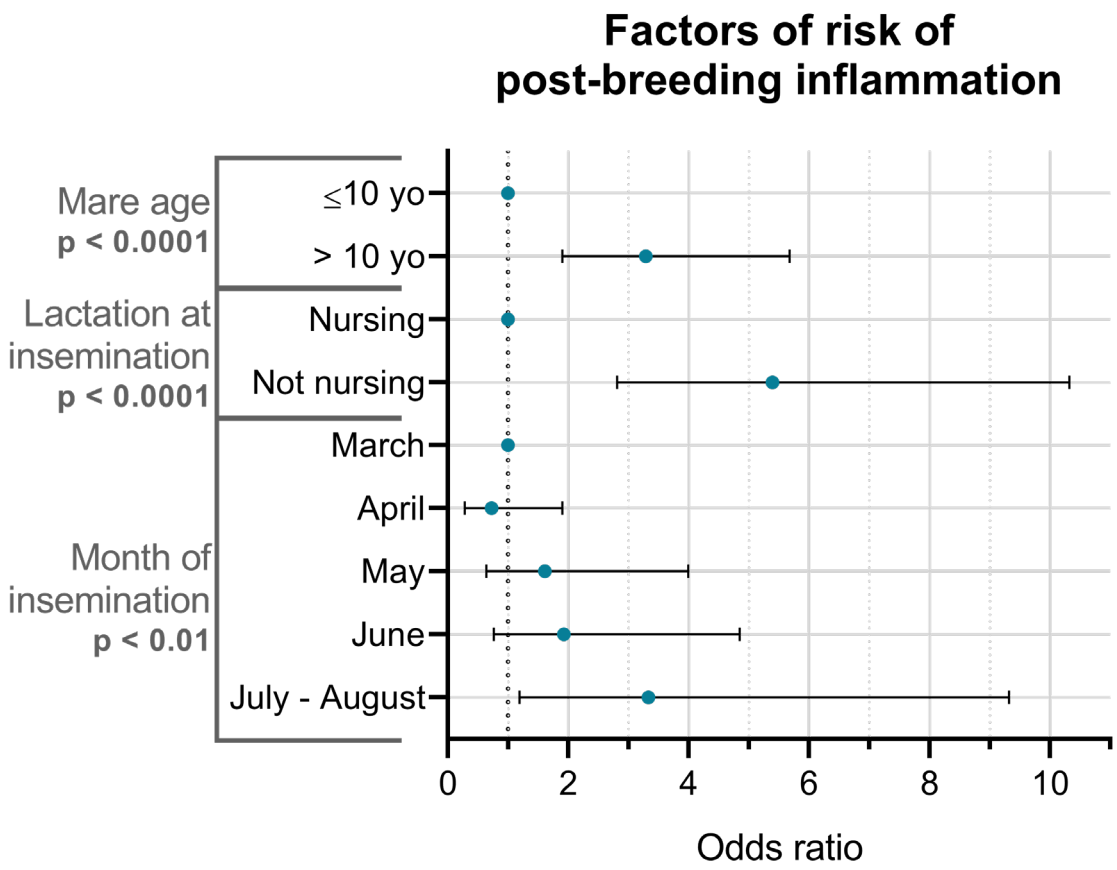
811 Figure 1: Odds ratio for factors influencing the likelihood of post-breeding endometritis in sport mares

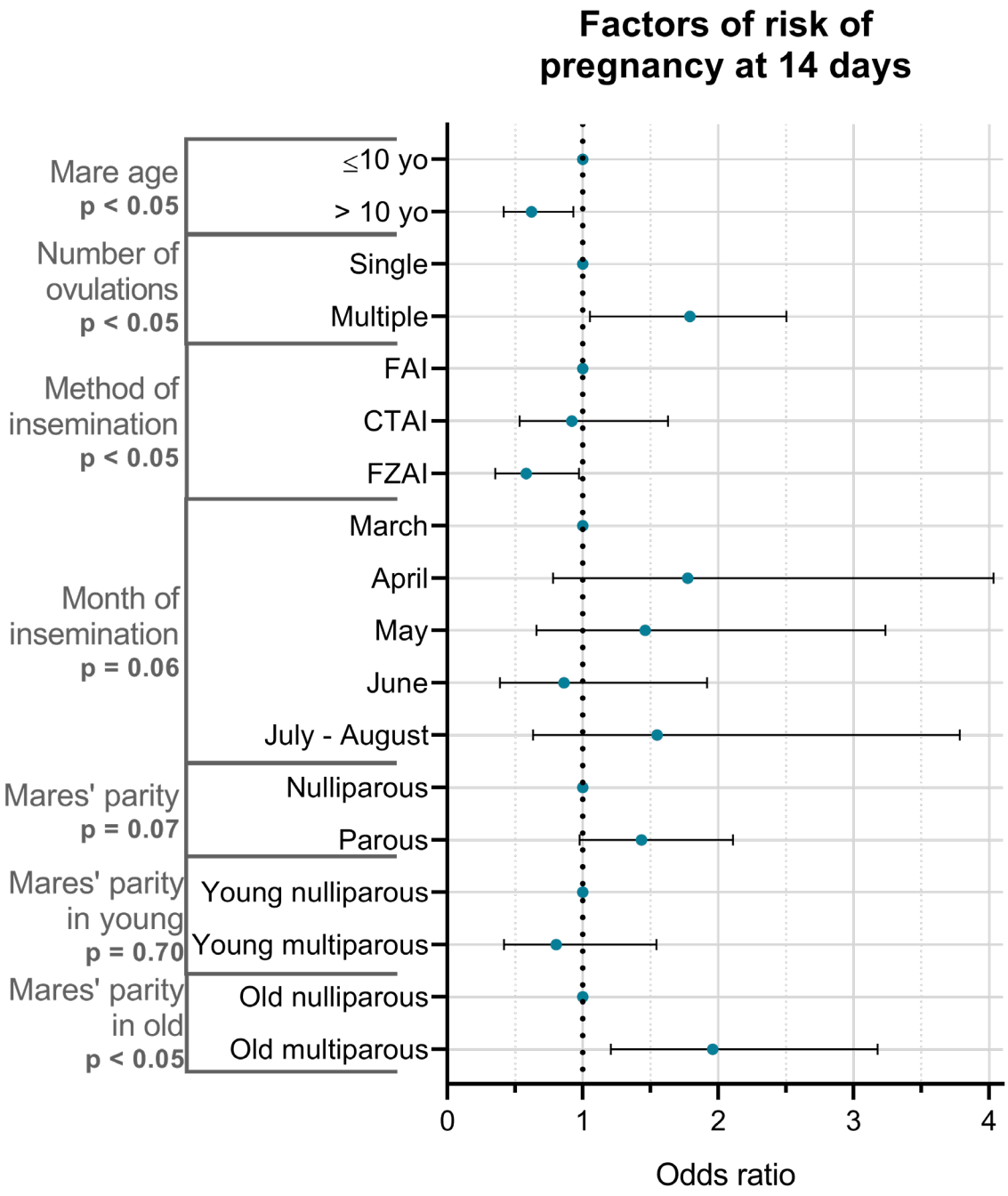
812 Figure 2: Odds ratio for factors influencing the likelihood of pregnancy in sport mares

813 Figure 3: Factors affecting post-breeding endometritis and pregnancy rates in the studied population
814 of sport mares

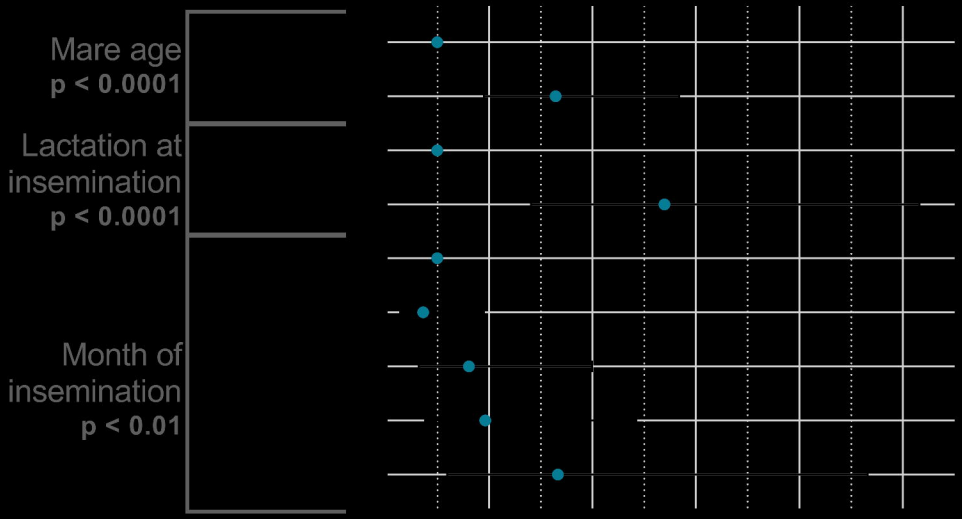
815 Red arrows significates that the factor increased the likelihood while blue arrows indicated that the
816 factor decreased the likelihood.

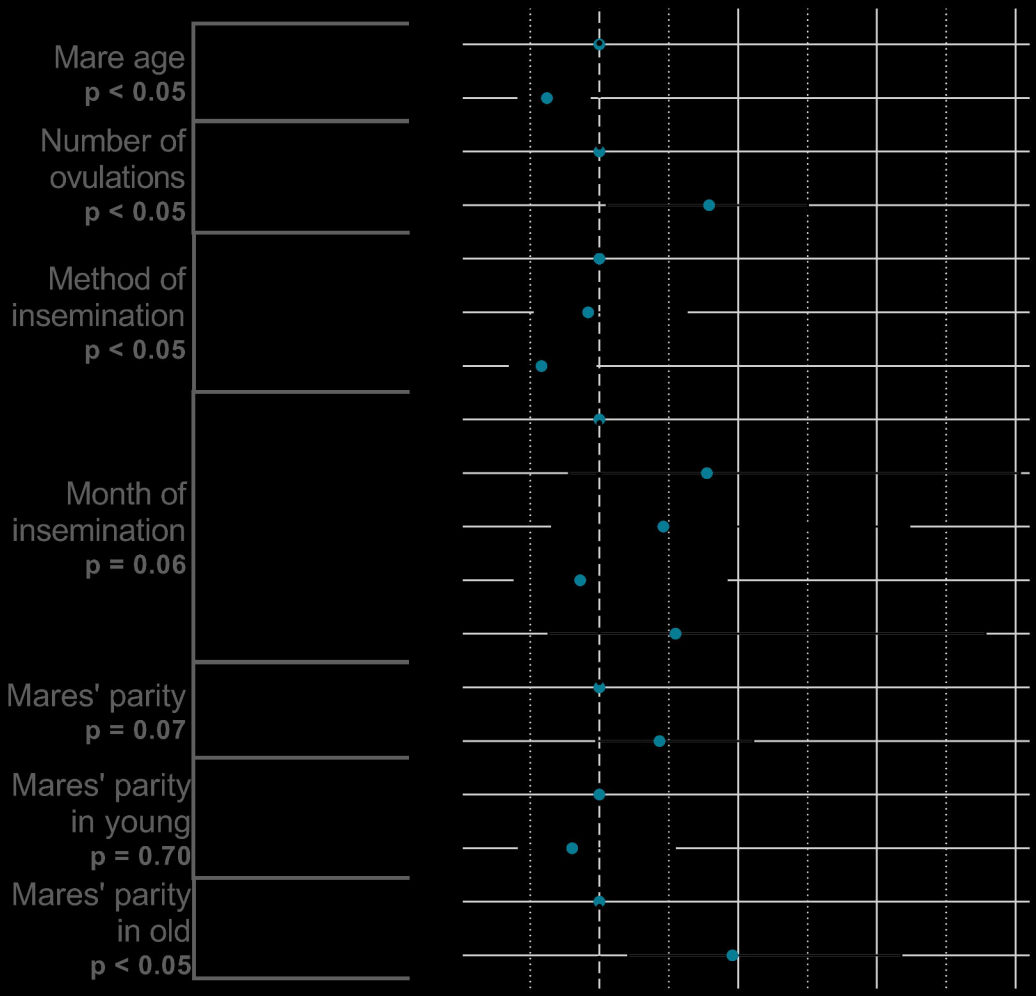
817

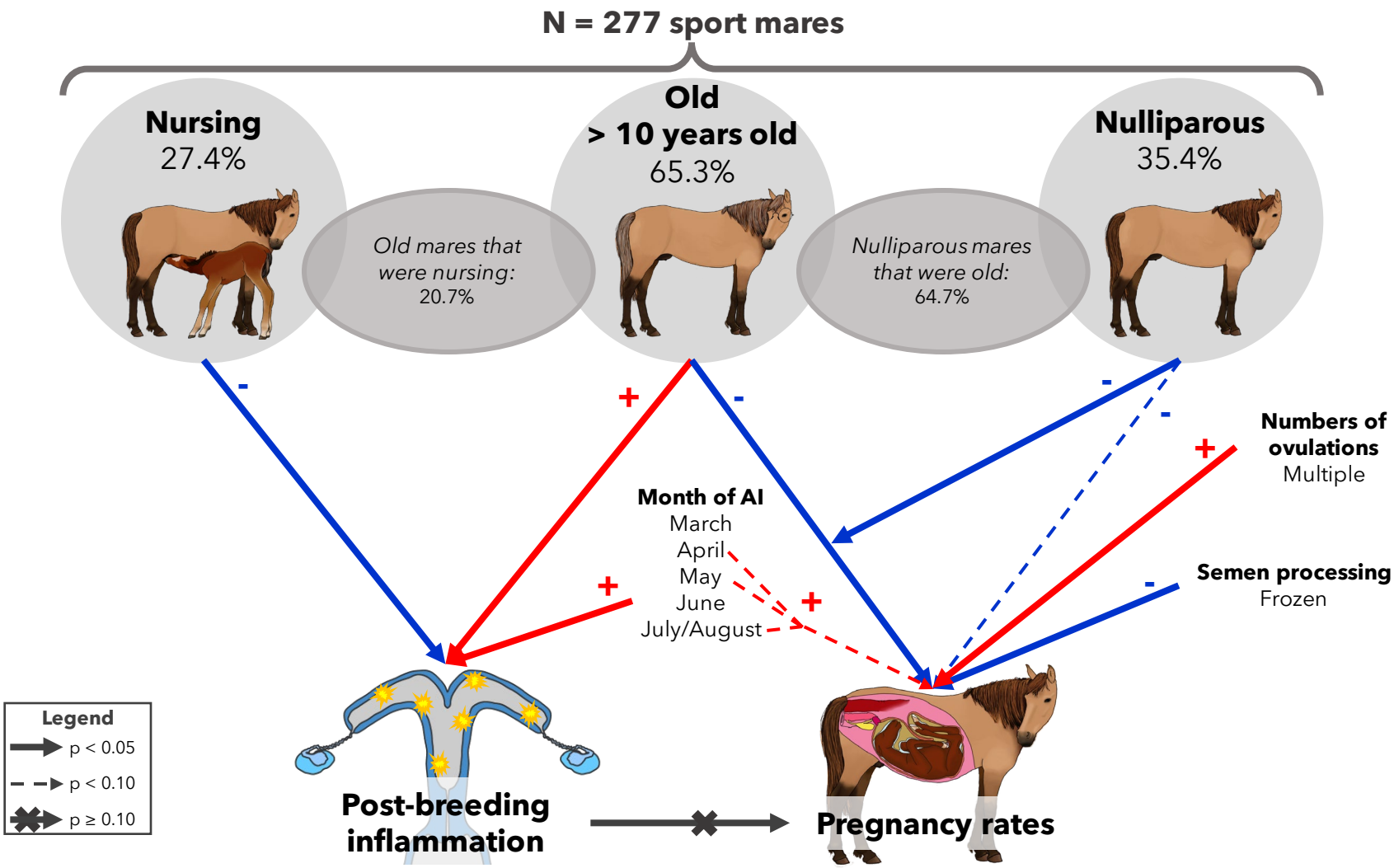

Factors of risk of
post-breeding inflammation
Mare age
p < 0.0001
≤10 yo
> 10 yo
Lactation at
insemination
p < 0.0001
Nursing
Not nursing
Month of
insemination
p < 0.01
March
April
May
June
July - August
0
2
4
6
8
10
Odds ratio


Factors of risk of pregnancy at 14 days
Mare age
p < 0.05
≤10 yo
> 10 yo
Number of ovulations
p < 0.05
Single
Multiple
Method of insemination
p < 0.05
FAI
CTAI
FZAI
Month of insemination
p = 0.06
March
April
May
June
July - August
Mares' parity
p = 0.07
Nulliparous
Parous
Mares' parity in young
p = 0.70
Young nulliparous
Young multiparous
Mares' parity in old
p < 0.05
Old nulliparous
Old multiparous
0
1
2
3
4
Odds ratio

823


Mare age
p < 0.0001
Lactation at insemination
p < 0.0001
Month of insemination
p < 0.01


Mare age
p < 0.05
Number of ovulations
p < 0.05
Method of insemination
p < 0.05
Month of insemination
p = 0.06
Mares' parity
p = 0.07
Mares' parity in young
p = 0.70
Mares' parity in old
p < 0.05


N = 277 sport mares
Nursing
27.4%
Old
> 10 years old
65.3%
Nulliparous
35.4%
Old mares that were nursing:
20.7%
Nulliparous mares that were old:
64.7%
Numbers of ovulations
Multiple
Month of AI
March
April
May
June
July/August
Semen processing
Frozen
Legend
p < 0.05
p < 0.10
p ≥ 0.10
Post-breeding inflammation
Pregnancy rates