



## FERTILITY AT THE FIRST POST PARTUM ESTROUS COMPARED WITH FERTILITY AT THE FOLLOWING ESTROUS CYCLES IN FOALING MARES AND WITH FERTILITY IN NONFOALING MARES

F. Camillo,<sup>1</sup> P. Marmorini,<sup>2</sup> S. Romagnoli,<sup>1</sup> I. Vannozi,<sup>1</sup> M. Bagliacca<sup>3</sup>

### SUMMARY

A retrospective study on the reproductive performance of 401 artificially inseminated trotter mares during six breeding seasons is presented. Mares, 279 post partum (PP) and 122 maiden and barren, or nonlactating (NL), were inseminated with fresh semen obtained from four fertile stallions of the same breed. Pregnancy rate (PR) of mares inseminated at the foal heat (182/253, 71.9%) was lower, but not significantly different, than the PR (22/26, 84.6%) of mares inseminated for the first time at the second post partum cycle and similar to the PR at the first and second cycle of NL mares (95/112, 77.8% and 25/33, 75.7%, respectively). PR of mares inseminated at the foal heat was higher, but nonsignificantly different, from PR of the post partum mares not pregnant after artificial insemination (AI) at foal heat and inseminated again at the following estrous cycle. The PR after AI at the foal heat was significantly higher than the PR when the AI was performed at the third or later cycle in NL mares (71.9% vs 22.2%,  $P < 0.01$ ). The estrus cycle/pregnancy ratio for the PP mares inseminated for the first time at the foal heat or at the second heat and for NL mares was, respectively, 1.4, 1.2, 1.3 at first cycle and 1.4, 1.3 and 1.4 at the end of the season or when mares left the stud. The proportion of open mares at the end of the season or when leaving the stud was 7.1% (18/253), 3.8% (1/26) and 4.1% (5/122) for PP mares first inseminated at the first or second post partum cycle and for NL mares, respectively; the total rate of open mares was 6% (24/401). The foaling rate (FR) following concep-

tion at the foal heat (72.1%) was not statistically different from the FR following conception at any other cycle (50-100%). Based on the absence of significant differences in fertility at the first post partum estrus cycle versus any other estrus cycle, we conclude that breeding at the foal heat should be advisable.

### INTRODUCTION

Foaling mares are the higher proportion of the mares bred each year. As mean pregnancy length in equine is 335-340 days, the goal to produce one foal/mare/year is achievable only if foaling mares conceive by 25-30 days post partum.

Pregnancy rate when breeding at the foal heat is generally reported to be lower than when breeding at successive estrous cycles.<sup>1,2,3,4</sup> To improve uterine involution and/or pregnancy rates at the foal heat the following techniques have been proposed: uterine lavage<sup>5</sup>; injection of ecboic drugs<sup>6,7</sup>; delaying the first ovulation post partum using ovarian steroids<sup>8</sup> or progestins.<sup>9</sup> Breeding at the second cycle post partum after shortening the first post partum luteal phase by the use of prostaglandin  $F_{2\alpha}$  was proposed by others.<sup>10</sup>

Most studies on equine post partum reproductive performance reported pregnancy rates, but not foaling rates, of mares conceiving at the foal heat or at following post partum cycles without a comparison with the reproductive performances of nonlactating mares bred in the same stud and managed in the same way.

Therefore, the aim of this study was to compare pregnancy rates and foaling rates of mares inseminated at the foal heat with those of mares inseminated at the following post partum estrous cycle and with those of nonlactating mares.

**Authors' addresses:** <sup>1</sup>Istituto di Patologia Speciale e Clinica Chirurgica Veterinaria dell'Università, <sup>2</sup>Equine Practitioner, <sup>3</sup>Dipartimento di Produzioni Animali dell'Università - Pisa - Italia.

**Acknowledgements:** Dr. Alessandra Rota for her contribution in reviewing this paper. This research was supported in part by the Fondo di Ateneo of University of Pisa.

## MATERIALS AND METHODS

Data from six consecutive breeding seasons (1990-1995) were studied retrospectively.

### Animals

A total of 401 (279 post partum = PP, and 122 maiden and barren or nonlactating = NL) trotter mares were inseminated with fresh semen of four trotter fertile stallions.

Management of the mares - PP and NL mares were palpated and scanned with ultrasounds for uterine status and ovarian activity at their arrival at the stud, or at day 7-8 post partum when foaling at the stud. Mares showing uterine folds typical of estrous and an ovarian follicle  $\geq 30$  mm were considered in estrous; teaser stallions were not employed. All estrous mares were inseminated every second day from the detection of a growing follicle/s  $\geq 35$  mm until the ovulation. Inseminations were performed 4 times a week (Monday, Wednesday, Friday, Saturday). Pregnancy was diagnosed by ultrasound at day 14 post ovulation and confirmed at days 16 and 25, when most pregnant mares left the stud. Mares remaining at the stud were examined again at day 35, 60, 90, and then in July and in October. The occurrence of parturition was verified consulting the official Italian Stud Book of Trotters.\* In twenty mares the occurrence of foaling was not known.

PP mares with a history of foaling complications as well as PP and NL mares showing clinically evident reproductive tract pathology were not included in the study. Normal foaling mares were inseminated at the first post partum cycle ( $n=253$ ) with the exception of those mares arriving at the stud right after the foal heat ovulation or foaling very early in the year ( $n=26$ ) which were inseminated at the second cycle post partum. NL mares ( $n=122$ ) were inseminated as soon as possible after their arrival at the stud.

PP and NL mares were generally not treated during the first two insemination cycles with the exception of some cases when Caslik vulvoplasty (both PP and NL mares) or induction of ovulation by hCG (only NL mares) were performed. PP and NL mares not pregnant after two estrous cycles were examined for the presence of endometritis by uterine cytology and bacterial cultures and, if positive, treated before or after a new insemination cycle. Reproductive performance of PP and NL mares still open after two insemination cycles were included in this study.

Semen and artificial inseminations - Stallions were collected 4 times a week using an artificial vagina; semen was evaluated for volume, color, pre- and post-dilution sperm motility (subjectively evaluated using a light microscope at 400x) and sperm concentration (using in a Thomas chamber). Semen was extended in partially skimmed milk added with 50,000 IU/L sodium penicillin and 50 mg/L

gentamicin.<sup>11</sup> An insemination dose of extended semen contained a minimum of 500 million progressively motile spermatozoa in a total volume of 20 ml. Mares were inseminated within one hour from semen collection.

### Definitions -

- Day 0 = day of ovulation.
- Pregnancy rate (PR) = pregnant mares at day 14/ inseminated mares (%).
- Foaling rate (FR) = foaling mares/pregnant mares at day 14 (%).
- Estrus cycles/pregnancy (EC/P) ratio = number of estrus cycles in which mares were inseminated/pregnant mares at day 14.
- Open mares rate = OMR = nonpregnant mares at the end of the breeding seasons (July) or when leaving the stud/ inseminated mares (%).

### Statistical analysis

Data were analyzed with the chi-square test.<sup>12</sup> Differences were considered statistically significant when  $P < 0.05$ .

## RESULTS

Mares inseminated at their foal heat had a PR (71.9%) lower but not significantly different from the PR of the mares inseminated for the first time at their second PP cycle (84.6%). NL mares inseminated at their first and second cycle after arrival at the stud had a PR (77.8% and 75.7%, respectively) similar to that of mares inseminated at foal heat. Foal heat PR was higher but nonstatistically different from PR of PP mares open after the insemination at foal heat and inseminated again at the following estrous cycles. PR at foal heat was higher than PR at cycles later than the third in NL mares (71.9% vs 22.2%,  $P < 0.01$ ). PR for the 3 groups of mares in different cycles are reported in Table 1.

Table 1. Pregnancy rate per cycle in post partum mares first inseminated at the foal heat, post partum mares first inseminated at the second estrous cycle and nonlactating mares. Values within a column that differ significantly have different letters (A, B =  $P < 0.01$ ; a, b =  $P < 0.05$ ). Differences were analyzed per row and per column.

Cycle	Pregnancy rate			Total
	Post partum (1st AI at foal heat)	Post partum (1st AI at 2nd heat)	Non lactating	
1st n°	182/253	22/26	95/122	299/401
%	71.9	84.6	77.8 A	74.5 A
2nd n°	61/92	2/5	25/33	88/130
%	66.3	40	75.8 A	87.7 a
3rd n°	24/38	2/3	4/8	30/49
%	60.5	66.7	50	61.2
>3rd n°	8/14	/	2/9	10/23
%	57.1	/	22.2 B	43.5 B b
Total	275/397	26/34	128/172	427/803
%	69.3	76.5	73.2	70.8

\*Registro del Cavallo Trotatore Italiano.

**Table 2.** Foaling rate per cycle in post partum mares first inseminated at the foal heat, post partum mares first inseminated at following estrous cycles and nonlactating mares. Differences, analyzed per row and per column, are not significant.

Cycle	Foaling rate			Total
	Post partum (1st AI at foal heat)	Post partum (1st AI at 2nd heat)	non-lactating	
1st n°	127/176	16/22	68/88	211/286
%	72.1	72.7	77.3	73.8
2nd n°	39/56	1/2	17/24	57/82
%	69.5	50	70.8	69.5
3rd n°	20/23	2/2	2/4	24/29
%	86.9	100	50	82.7
>3rd n°	8/8	/	2/2	10/10
%	100		100	100
Total	194/263	19/26	89/118	302/407
%	73.8	73.1	75.4	74.2

**Table 3.** Pregnancy rate per cycle in trotter mares inseminated during six different breeding seasons. Differences, analyzed per row and per column, are not significant.

Cycle	Year and Pregnancy rate					
	90	91	92	93	94	95
1st n°	31/45	56/83	49/68	18/25	80/87	65/83
%	68.7	67.5	72	72	82.5	78.3
2nd n°	14/16	20/30	9/19	8/10	17/28	20/27
%	87.5	66.7	47.4	80	60.7	74.1
3rd n°	1/2	10/14	3/8	4/4	5/12	7/9
%	50	64.3	37.5	100	41.7	77.8
>3rd n°	/	2/6	2/6	1/1	2/4	3/6
%		33.3	33.3	100	50	50
Total	46/63	88/133	63/101	31/40	104/141	95/125
%	73	66.2	62.4	77.5	73.8	76

The EC/P ratio at first insemination and the overall EC/P ratio were 1.4, 1.2, 1.3 and 1.4, 1.3 and 1.4 for PP mares inseminated at foal heat, PP mares inseminated at the second cycle and NL mares, respectively.

OMR was 7.1% (18/253), 3.8% (1/26) and 4.1% (5/122) for PP mares first inseminated at the first or second post partum cycle and for NL mares, respectively; average OMR was 6% (24/401).

The FR following conception at the foal heat was similar to the FR following conception at any other cycle (Table 2).

Considering all mares together, per cycle PR and total PR were not affected by year (Table 3) or stallion (Table 4), but were affected by age of the mares: older mares showed a lower total PR than younger mares did 65/117 (55.5%) vs 67/81 (82.7%), 159/214 (74.3%), 136/191 (71.2%) for  $\geq 15$  years,  $\leq 5$  years, 6-9 year and 10-14 year old mares, respectively ( $P < 0.01$ ) (Table 5).

Mares equal to or higher than 15 years of age showed also a lower total FR than 10-14 year old mares: 42/66 (63.6%) vs 104/132 (78.8%), respectively ( $P < 0.05$ ) (Table 6).

**Table 4.** Pregnancy rate per cycle of the four different stallions used for AI in the mares included in this study. Differences, analyzed per row and per column, are not significant.

Cycle	Stallion and pregnancy rate				Total
	1	2	3	4	
1st n°	68/88	93/125	121/172	11/16	299/401
%	77.3	79.2	70.3	68.7	74.5
2nd n°	21/31	26/38	39/56	2/5	88/130
%	67.7	68.4	69.1	40	67.7
3rd n°	9/13	6/14	12/19	3/3	30/49
%	69.2	42.8	63.1	100	61.2
>3rd n°	3/6	4/9	3/8	/	10/23
%	50	44.4	40		43.5
Total	101/138	135/186	175/255	16/24	427/603
%	73.2	72.6	68.6	66.7	70.8

**Table 5.** Pregnancy rate per cycle in mares of different age groups. Values that differ significantly have different letters (A, B =  $P < 0.01$ ; a, b =  $P < 0.05$ ). Differences were analyzed per row and per column.

Cycle	Age and pregnancy rate				Total
	$\leq 5$	6-9	10-14	$\geq 15$	
1st n°	53/63	116/152	90/126	40/60	299/401
%	84.1 a	76.3	71.4	66.7 b	74.5
2nd n°	10/14	36/47	31/43	11/26	88/130
%	71.4	76.6 A	72.1 a	42.3 B b	67.7
3rd n°	4/4	5/11	12/16	9/18	30/49
%	100	45.4	75	50	61.2
>3rd n°	0/0	2/4	3/6	5/13	10/23
%		0	50	38.5	43.5
Total	67/81	159/214	136/191	65/117	427/603
%	82.7A	74.3 A	71.2 A	55.5 B	70.8

## DISCUSSION

PR of the mares inseminated at the foal heat (71.9%) was about 13% lower (but not statistically different) than the PR (84.6%) of the mares inseminated for the first time at the second cycle post partum and similar to the PR at the first and second cycles of nonlactating mares (77.8% and 75.7%, respectively). The EC/P ratio and the OMR in each group of mares were similar.

This observation partially agrees with previous reports where a 9% to 20% lower PR was described following breeding at the first rather than following PP estrous cycles<sup>1,2,4</sup> and a 23% lower embryo recovery rate was observed following insemination at the foal heat compared to insemination of nonlactating control mares.<sup>13</sup>

In these previous reports PR or embryo recovery rate at foal heat was quite low, 39%,<sup>2</sup> 50%,<sup>1</sup> and 48%<sup>13</sup> compared to the PR of 71.9% observed in our study; we cannot explain this difference but this is certainly not due to a selection of mares to be bred at foal heat (only mares with severe foaling complications or evident reproductive tract pathology were not inseminated) or to pre or post ovulation treatments, as they were not performed in our

**Table 6.** Foaling rate per cycle and in total for trotter mares of different ages. Values that differ significantly have different letters (A,B = P<0.01; a,b = P<0.05). Differences were analyzed per row and per column.

Cycle	Age and Foaling rate				Total
	<5	5-10	10-15	>15	
1st n°	32/44	83/118	71/88	25/41	211/286
%	72.7	73.4	80.7	61	73.8
2nd n°	8/9	25/32	19/29	5/12	57/82
%	88.9	78.1	65.5	41.7	69.5
3rd n°	2/4	4/5	11/12	7/8	24/29
%	50	80	91.7	87.5	82.7
>3rd n°	1	2/2	3/3	5/5	10/10
%		100	100	100	100
Total	42/57	114/152	104/132	42/66	302/407
	73.7	75	78.8 a	63.6 b	74.2

study. Arrott et al.<sup>14</sup> observed the higher pregnancy rate (82%, 9 of 11) following mating at foal heat.

Ginther<sup>4</sup> reported a 17% lower PR in mares bred at the first post partum estrus compared with mares open following breeding at foal heat and bred again at the second estrous cycle. We observed a PR of 71.9% and of 66.3% for the same groups of mares, respectively.

Mating at the foal heat increased overall pregnancy loss rate in some reports but not in others.<sup>4</sup> In our study, FR, and thus overall pregnancy loss rate, was similar following conception at the foal heat or at any other cycle of PP and NL mares and averaged 74% and 26%, respectively. Chevalier<sup>15</sup> reported a significantly higher fetal loss in mares conceiving at the foal heat, compared with mares conceiving at the subsequent cycles, and an overall pregnancy loss (day 22 to 310) of 20% slightly lower than the pregnancy loss that we observed.

Uterine lavage on days 2 and 4 or day 3 or days 3, 4 and 5 post partum with 5-10 l of saline did not improve the degree of uterine involution or the pregnancy rate at first post partum estrus.<sup>6,16</sup> Although in a small number of mares treatment twice daily with PGF<sub>2α</sub> (1 mg of prostalene) from the day of parturition for 10 days or until breeding, tended to improve PR at foal heat and improved significantly PR at the second post partum heat.<sup>6,7</sup> observed no improvement in uterine involution following treatment of 5 post partum mares with PGF<sub>2α</sub> (500 mg of fluprostenol twice a day for 10 days) or oxytocin (20 iu of oxytocin twice a day for ten days; n = 4 mares). Recently post breeding treatment with oxytocin and intrauterine broad-spectrum antibiotics was reported as increasing PR at foal heat.<sup>17</sup>

Some authors hypothesized that delaying post partum breeding improves conception rate. This delay has been obtained either administering PGF<sub>2α</sub> 6 days after the ovulation at foal heat and then breeding at the second estrus cycle,<sup>10</sup> or delaying the onset of the foal heat and the ovulation by means of ovarian steroids<sup>8,9,10</sup> or Altrenogest found a higher PR in mares treated with PGF<sub>2α</sub> 7-9 days after post partum ovulation and bred at the induced estrus. Burns et al.,<sup>18</sup> and Almeida et al.,<sup>19</sup> observed no improve-

ment in fertility using the same protocol.

Loy et al.,<sup>6</sup> and Almeida et al.,<sup>19</sup> found no improvement of reproductive performance when treating mares with progesterone and oestradiol or with progesterone only for 6-20 days from the day of foaling and breeding at the delayed estrus. McKinnon et al.,<sup>9</sup> but not Almeida et al.,<sup>19</sup> reported a significant improvement in PR following the treatment of foaling mares with altrenogest for 8 or 20 days, respectively, and then breeding mares at the delayed estrus.

## CONCLUSIONS

Foaling rate, open mare rate and the cycle/pregnancy ratio for mares inseminated for the first time at their first or second PP estrus and for nonlactating mares was similar. Pregnancy rates at foal heat tended to be lower but this difference was not statistically significant. This observation, together with the negative or controversial results and costs of attempts to improve conception rate at the foal heat, suggest that probably the best reproductive management for PP mares is to breed at the foal heat without any treatment. Exceptions may be represented by mares with a history of foaling complications or with evidence of reproductive tract pathology, and when semen quality and/or availability is poor, such as for surmenaged or low fertile stallions or when using frozen semen.

## BIBLIOGRAPHY

- Loy RG: Characteristics of post-partum reproduction in mares. *Vet Clinics of North America: Large Animal Practice* 1980;2:345-359.
- Lieux P: Comparative results of breeding on first and second post-foaling heat periods. *Proc AAEP* 1980;129-132.
- Koskinen E, Katila T: Uterine involution, ovarian activity, and fertility in the post-partum mare. *J Reprod Fertil* (1987);S 35: 733-734.
- Ginther OJ: *Reproductive Biology of the Mare. Basic and Applied Aspects*. 2nd Edition. Cross Plains, Wisconsin, Equiservices USA (1992).
- Bianchard TL, Varner DD, Brinsko SP, Meyers SA, Johnson L: Effects of postparturient uterine lavage on uterine involution in the mare. *Theriogenology* 1989;32(4):527-533.
- Lay WB, Purkswell BJ, Bowen JM: Effect of PGF<sub>2α</sub> analogue administered during the postpartum period on pregnancy rate. *J Eq Vet Sci* 1988;8(2):141-143.
- Bianchard TL, Varner DD, Brinsko SP, et al.: Effects of ecobolic agents on measurements of uterine involution in the mare. *Theriogenology* 1991;36(4):559-572.
- Loy RG, Evans MJ, Pemstein R, Taylor TB: Effects of injected ovarian steroids on reproductive patterns in post-partum mares. *J Reprod Fert* (1982);(S32):199-204.
- McKinnon AO, Squires EL, Harrison LA, Blach EL, Shideler RK: Ultrasonographic studies on the reproductive tract of mares after parturition: Effect of involution and uterine fluid on pregnancy rates in mares with normal and delayed first postpartum ovulatory cycles. *JAVMA* 1988;192(3):350-353.
- Kreider JL, Murrell VV, Longwell LC, Godke RA: Control of estrus in the lactating postpartum mare with fluprostenol (ICI-

81,008) *Theriogenology* 1978;10:(5)371-380

11. Palmer E: L'insemination Artificielle des juments: Bilan de 5 années de recherches et d'utilisation pratique. CEREOPA. 9eme Journee d'Etudes, 1983;90-109.

12. SAS (1995) JMP. *Statistics and Graphics Guide, Version 3.1*. SAS Institute Inc. Cary NC, USA.

13. Huhtinen M, Reilas T, Katila T: Recovery rate and quality of embryos from mares inseminated at the first post partum oestrus. *Acta Veterinaria Scandinavica* 1996;37(3):343-350.

14. Arrott CA, Blanchard TL, Varner DD, et al.: Biodegradable estradiol microspheres do not affect uterine involution or characteristics of postpartum oestrus in mares. *Theriogenology* 1994;42:(2)371-384.

15. Chevalier-Clement F: Pregnancy loss in the mare. *Anim Reprod Sci* 1988;20:231-244.

16. McCue PM, Hughes JP: The effect of postpartum uterine lavage on foal heat pregnancy rate. *Theriogenology* 1990;33:(5) 1125-1129.

17. Pycock JF: Assessment of oxytocin and intrauterine antibiotics on pregnancy rate in the mare. *Proc AAEP* 1994:129-132.

18. Burns SJ, Irvine CHG, Amoss MS: Fertility of prostaglandin-induced oestrus compared to normal post-partum oestrus. *J Reprod Fertil* 1979;S27:245-250.

19. Almeida FO, Fonseca FA, Espechit CJB et al.: Effects of PGF<sub>2a</sub> and progesterone on the reproductive efficiency of crossbred mares during the postpartum period. *Rev Soc Brasil Zootec* 1995;24:(4)652-659.

Original work published in the *Journal of Equine Veterinary Science* is peer-reviewed by two or more referees familiar with the content of the paper. Referees' comments are incorporated into a revised manuscript and reviewed for a second time before publication. Authors are encouraged to obtain a complete copy of "Author's Guidelines." FAX: 909-678-1885.

Printed by M&M Productions, 357 E. Arrow Highway, #203, San Dimas, CA 91773. Phn: 909-592-4419.

*Journal of Equine Veterinary Science* (ISSN No. 0737-0806) is published monthly (12 times a year) by William E. Jones, DVM, PhD, 20225 Grand Ave., Wildomar, CA 92595. Periodical postage paid at Wildomar, CA 92595. **POSTMASTER:** Send address changes to: *Journal of Equine Veterinary Science*, P.O. Box 1209, Wildomar, CA 92595.

Copyright © 1997 by William E. Jones. All rights reserved. No part of this publication may be reprinted or copied either wholly or in part without permission of the publisher. Subscriptions: P.O. Box 1209, Wildomar, CA 92595; phone (909) 678-1889. U.S. individual subscription rate \$155; Canada and Mexico \$160; overseas \$185; libraries \$210, \$220 and \$240 respectively. Payment must accompany order. Members of IAEP receive a subscription as part of their dues.