

## **The impact of uterine therapies on reproductive efficiency in Thoroughbred mares; the possible effect of mare age and reproductive status**

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### **ABSTRACT**

To optimise the reproductive efficiency of Thoroughbred mares, several uterine therapies have been administered during the limited period of the breeding season. Reproductive and treatment records of 253 Thoroughbred mares kept at nine commercial stud farms during the period from 1998 to 2005 were used to assess the incidence of various uterine therapies and their curative effect on the overall reproductive efficiency of mares. Furthermore, the effects of mare age on the efficacy of uterine therapy in mares were ascertained. On the basis of type and protocol of uterine therapy application, the mares were grouped as follows: saline/antiseptic lavage, intrauterine infusion of antibiotics and/or parenteral administration of oxytocin and their combinations. The overall incidence of uterine therapy was 18.07% (357) of the 1976 oestrous cycles covered and all the uterine therapies used were equally efficient regarding reproductive parameters. Out of the total treated cases, 25.21% of the mares received a combination therapy of intrauterine antibiotics, along with oxytocin injections. Further, the incidence of uterine treatment was least ( $P < 0.0001$ ) in mares aged 3-7 years (9.35%) and maiden mares (5.92%). The pregnancy rates at Day 39 post ovulation and foaling rates were higher ( $P < 0.05$ ) in untreated mares than treated mares (46.39 vs. 40.62% and 41.75 vs. 35.85%, respectively) whereas no difference was recorded in terms of overall pregnancy losses (21.95 vs. 18.55%). In treated mares, significantly higher day 39 pregnancy rates and foaling rates were observed in mares aged 3-7 years (47.27 and 41.82%) and maiden mares (60.00 and 60.00%) than in  $\geq 18$  years old (22.58 and 12.90%) mares and barren (31.94 and 27.78%) mares, respectively. In conclusion, despite the higher incidence of administration in older and barren mares,

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uterine treatment did not consistently improve the reproductive efficiency of reproductively incompetent mares and the outcome of uterine therapies was entirely dependent on mare age and reproductive status.

**Key words:** mares, thoroughbred, reproductive efficiency, uterine therapy, age

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### **Introduction**

Reproductive competence, manifested embryo survival rate and production of viable foals, is of major importance in equine stud management and is, therefore, of significant interest for breeders and veterinarians. As such it has been the subject of extensive research and scientific investigation.

The horse is considered to have relatively low reproductive efficiency, and among horses the Thoroughbred is considered to have the lowest reproductive efficiency (SULLIVAN et al., 1975; ENGELKEN, 1999; MORRIS and ALLEN, 2002). Both physiological and managerial factors have been implicated as contributors to the failure of Thoroughbred mares to conceive and carry a pregnancy to term (SHARMA et al., 2010a and 2010b). In Thoroughbreds of the northern hemisphere, a four month breeding period (Feb-June) is arbitrarily imposed for the purpose of age related racing. During this limited period of time, in order to maximise the reproductive efficiency of Thoroughbred mares, a variety of uterine treatments are applied (ALLEN et al., 2007). To date, it is also unclear whether and how these various uterine therapies affect the mare's reproductive ability, particularly in older mares with compromised fertility.

Therefore, the present study was undertaken in Thoroughbred mares to:

- i) assess the incidence of various uterine therapies and their effect on the overall reproductive performance of mares
- ii) ascertain whether the response to such uterine treatments varied with mare age and reproductive status.

It is believed that this study will help to identify the mares with the greatest risk of pregnancy loss and to offer advice about the most appropriate uterine therapy management in order to obtain optimal fertility.

### **Materials and methods**

*Animals and management.* Reproductive and treatment parameters were analysed for 253 mares over seven consecutive (1998 to 2005) breeding seasons (February-June) on nine organised Thoroughbred stud farms situated in the north-western part of India (between latitude 28°28' and 30°30'N, longitude 74°31' and 77°02'E). Fertility data for the mares and any associated uterine treatment applied was recorded on the prepared survey forms. Since the majority of mares remained on the same stud farm for several years and bred each year, many mares appear in the data more than once.

Oestrus was monitored daily by teasing and by ultrasound scanning of the ovaries. All mares were first mated when the follicle diameter exceeded 35mm and/or follicle consistency was deemed by an experienced veterinarian to indicate imminent ovulation. The mating was repeated at 36-48h intervals until ovulation was confirmed. In a small proportion of mares in which either the ovulation was not confirmed after covering, or the record was unavailable, the day after the last mating was assumed to be the day of ovulation (Day 0). Pregnancy diagnosis was performed on Day 16 post-ovulation, by ultrasonography and/or transrectal palpation and subsequently the pregnant mares were re-examined on Days 21, 32, 39, 50, 65, 90, 135 and 198 post-ovulation. Day 16 and Day 39 pregnancy rates were calculated, along with pregnancy loss at various intervals from Day 16 until term. All reproductive performance parameters after Day 16 were expressed as the percentage of mares confirmed to be pregnant at Day 16. Overall pregnancy losses analysed included embryonic losses between Day 16 and 39 and further foetal losses from Day 40 until live foaling. Still-born foals were also included as pregnancy loss. All pregnancy rates were calculated per oestrus.

The reproductive management decisions about the specific protocol for administration of uterine therapy for all mares, such as: criterion for disease diagnosis and identification of problem mares, type of uterine therapy, dosage, frequency etc. were all made by and based on the experience of stud managers and their veterinarians, with the ultimate aim of maximising conception rates.

*Database and groups.* The data recorded on the survey forms were analysed retrospectively after incomplete records had been omitted. Finally, fertility analysis was carried out on 1976 covered oestruses. The mares were divided into groups according to their age (3-7, 8-12, 13-17,  $\geq 18$  years) and reproductive status viz., maiden (never mated), barren (mated but never diagnosed pregnant during the last breeding season), lactating (foaled in the current season and lactating throughout the season), rested (parous mare deliberately not mated during the last breeding season) and aborted (pregnant at the end of last breeding season but lost pregnancy later on). In addition, the mares were grouped according to the usage and type of uterine treatment received (saline/antiseptic lavage, intrauterine infusion of antibiotics and/or parenteral administration of oxytocin as deemed appropriate by veterinarian).

*Statistical analysis.* Chi-squared and Fisher's exact tests were used to test for significance throughout, using statistical software Minitab 14 version (Minitab Inc., State college, PA, USA). Differences were considered significant when  $P < 0.05$  (DYTHAM, 1999).

## Results

The incidence of various uterine therapies and their effect on the overall reproductive efficiency of Thoroughbred mares. Uterine treatments were applied in 357 (18.07%) of 1976 covered oestrous cycles (Table 1). Significantly higher ( $P<0.05$ ) Day 39 pregnancy rates and foaling rates were observed in untreated mares than treated mares (Table 1). However, Day 16 pregnancy rates and pregnancy losses were similar ( $P>0.05$ ) in both treated and untreated groups. Further, none of the treatment protocols resulted in a significant difference ( $P>0.05$ ) in any of the analysed reproductive parameters (Table 1).

Table 1. The influence of different uterine therapies on reproductive efficiency parameters in treated and untreated cycles of Thoroughbred mares

Type of oestrus (% of total)	Reproductive efficiency parameters					
	Day 16 pregnancy rate/ oestrus (%)	Day 39 pregnancy rate/ oestrus (%)	Foaling rate/ oestrus (%)	*Embryonic losses (%) (Day 16-39)	*Foetal losses (%) (Day 39- term)	*Overall pregnancy losses (%) (Day16- term)
Treated oestrus (18.07)	45.94	40.62 <sup>a</sup>	35.85 <sup>a</sup>	11.59	10.37	21.95
Untreated oestrus (81.93)	51.27	46.39 <sup>b</sup>	41.75 <sup>b</sup>	9.52	9.04	18.55
Treatment protocol (% of total)						
Intrauterine antibiotics (I. ut. anti) (15.97)	38.60	33.33	29.82	13.64	9.09	22.73
Uterine lavage (U.L.) (8.40)	43.33	40.00	40.00	7.69	0.00	7.69
Oxytocin (15.41)	54.55	47.27	43.63	13.33	6.66	20.00
I. ut. anti. plus oxytocin (25.21)	45.55	41.11	35.55	9.76	12.20	21.95
U. L. plus oxytocin (14.01)	52.00	46.00	40.00	11.53	11.53	23.07
I. ut. anti. plus U.L. (10.92)	41.00	35.90	30.77	12.50	12.50	25.00
I. ut. anti. plus U.L. plus oxytocin (10.08)	44.44	38.89	30.55	12.50	18.75	31.25

<sup>a,b</sup> Within a column percentages with different superscript alphabets differ significantly ( $P<0.05$ ). \* All expressed as a percentage of mares pregnant at Day 16.

Table 2. Incidence of uterine therapies and reproductive efficiency parameters in regard to mare age in treated Thoroughbred mares

Reproductive efficiency parameters	Mare age (years)			
	3-7	8-12	13-17	≥18
Number of oestrous cycles	588	830	480	78
Incidence of uterine therapies (%)	9.35 <sup>a</sup>	16.51 <sup>b</sup>	27.92 <sup>c</sup>	39.74 <sup>d</sup>
Day16 pregnancy rate/oestrus (%)	52.72 <sup>a</sup>	49.64 <sup>a</sup>	43.28	29.03 <sup>b</sup>
Day 39 pregnancy rate/oestrus (%)	47.27 <sup>a</sup>	44.53 <sup>a</sup>	38.06	22.58 <sup>b</sup>
Foaling rate/oestrus (%)	41.82 <sup>a</sup>	40.88 <sup>a</sup>	33.58 <sup>a</sup>	12.90 <sup>b</sup>
*Embryonic losses (%) (Day 16-39)	10.34	10.29	12.07	22.22
*Foetal losses (%) (Day 39-term)	10.34	7.35 <sup>a</sup>	10.34	33.33 <sup>b</sup>
*Overall pregnancy losses (%) (Day16-term)	20.69 <sup>a</sup>	17.65 <sup>a</sup>	22.41 <sup>a</sup>	55.55 <sup>b</sup>

<sup>a,b,c,d</sup> Within a row percentages with different superscript alphabets differ significantly (P<0.05). \* All expressed as a percentage of mares pregnant at Day 16

Table 3. Incidence of uterine therapies and reproductive efficiency parameters in regard to the mare reproductive status of treated Thoroughbred mares

Reproductive efficiency parameters	Reproductive status				
	Lactating	Barren	Maiden	Aborted	Rested
Number of oestrous cycles	1156	287	169	249	115
Incidence of uterine therapies (%)	17.30 <sup>a</sup>	25.09 <sup>b</sup>	5.92 <sup>c</sup>	21.29 <sup>ab</sup>	19.13 <sup>ab</sup>
Day16 pregnancy rate/oestrus (%)	49.50	38.89	60.00	41.51	40.91
Day 39 pregnancy rate/oestrus (%)	45.50 <sup>a</sup>	31.94 <sup>b</sup>	60.00	33.96	31.81
Foaling rate/oestrus (%)	40.50	27.78 <sup>a</sup>	60.00 <sup>b</sup>	28.30	27.27
*Embryonic losses (%) (Day 16-39)	8.08	17.86	0.00	18.18	22.22
*Foetal losses (%) (Day 39-term)	10.10	10.71	0.00	13.64	11.11
*Overall pregnancy losses (%) (Day16-term)	18.18	28.57	0.00	31.82	33.33

<sup>a,b</sup> Within a row percentages with different superscript alphabets differ significantly (P<0.05). \*All expressed as a percentage of mares pregnant at Day 16

*Effect of mare age and reproductive status on response to uterine therapy.* The incidence of uterine treatment increased significantly ( $P < 0.0001$ ) with increased mare age (Table 2). Significantly ( $P < 0.0001$ ) fewer maiden mares received uterine treatments than other reproductive status groups (Table 3). Further, in treated mares, the age of the mares markedly affected the reproductive efficiency as mares at ages 3-7 and 8-12 years had significantly higher pregnancy rates ( $P < 0.05$ ) and foaling rates ( $P < 0.01$ ) than  $\geq 18$  years old mares. Similarly, a difference ( $P < 0.05$ ) in overall percent pregnancy losses existed between  $\geq 18$  years old mares and the rest of the other age groups. Day 39 pregnancy rates were comparatively lower ( $P < 0.05$ ) in barren than in lactating mares (31.94% vs. 45.50%, respectively). Finally, foaling rates were significantly ( $P < 0.05$ ) higher in maiden mares than barren mares (60.00% vs. 27.78%, respectively). However, neither between different mare age groups, nor in reproductive statuses was any difference ( $P > 0.05$ ) observed in reproductive efficiency parameters between treated and untreated oestrus.

### Discussion

*The incidence of various uterine therapies and their effect on the overall reproductive efficiency of Thoroughbred mares.* To the best of our knowledge, this is the first extensive report to determine the effects of uterine therapies on the reproductive efficiency of Thoroughbred mares reared in commercial stud farms. Similar to the results of our study, MORRIS and ALLEN (2002) recorded a similar value of treated oestrus (12.24%) whereas ALLEN et al. (2007) had more than double (36.3%) the treatment rate in English Thoroughbreds. ZENT et al. (1998) also recorded an overall incidence of 15.6% intrauterine fluid (IUF) accumulation for all examined oestruses, which strengthens our findings. Furthermore, in our study it is evident that the reproductive efficiency of treated Thoroughbred mares was comparatively lower than of those that remained untreated. Interestingly, MALSCHITZKY et al. (2002) also reported that in mares with IUF post-breeding uterine lavage treatment combined with oxytocin administration did not restore pregnancy rates to that achieved in mares without IUF. As IUF accumulation (ZENT et al., 1998) and susceptibility to uterine infections (VAN ITTERSUM and VAN BUITEN, 1999) have been reported to be a consequence of advanced age and a cause of subfertility (BARBACINI et al., 2003; HURTGEM, 2006) in older mares, it might be speculated that the mares which require uterine treatment have a compromised endometrium defence mechanism (RICKETTS and ALONSO, 1991; MORRIS and ALLEN, 2002) and a lower fertility rate than mares which do not need uterine treatment.

Although the majority of the mares received intrauterine antibiotics, this therapy was not applied after bacterial endometritis had been proven. Several veterinarians were involved in the study and no uniform criteria were used to determine which protocol for fluid accumulation/uterine infection should be applied. Also it was not possible

to determine whether the adverse effect on pregnancy rates was related to IUF and/or infection, the amount of IUF, or the treatment. Trials performed in mares have failed to show an adverse effect of either the oxytocin injection (NIE et al., 2003) or uterine lavage (VANDERWALL and WOODS, 2003) on the establishment of pregnancy. However, it is possible that the presence of IUF (MALSCHITZKY et al., 2002) and/or uterine infections (RIDDLE et al., 2007) were responsible for lower fertility. It seems that uterine treatment does not necessarily improve foaling rates, but might improve day 16 pregnancy rates. Nonetheless a recent survey (SHARMA et al., 2010c) also indicated improvement in live foaling rate with the application of uterine treatments at foal heat in Thoroughbred mares. The probable reason for improved reproductive efficiency in infertile mares achieved by uterine treatments may be attributed to reduced inflammation and expulsion of accumulated IUF (MAISCHBERGER et al., 2008) which has spermicidal and embryocidal effects (WATSON, 2000). Earlier studies (PYCOCK and NEWCOMBE, 1996; BARBACINI et al., 2003; AZWAI, 2008) also demonstrated improved pregnancy rates in problem mares treated with oxytocin and uterine lavage, which supports our findings. Similar to the results of our study, ZENT et al. (1998) also reported no effect of different treatments on conception rates. Perhaps this is a reflection of sound clinical judgment and judicious use of uterine therapies by experienced stud veterinarian.

*Effect of mare age and reproductive status on uterine therapy response.* In the current study, the response to the uterine treatment was significantly affected by mare age. Previous studies (GHAESMZADEH-NAVA et al., 2004; SHARMA and DHALIWAL, 2010) also found similar negative response to uterine therapies in mares of advanced age. Similarly, in Thoroughbred mares numerous authors (MORRIS and ALLEN, 2002; ALLEN et al., 2007; SHARMA et al., 2010a; b; c) have also shown that increasing age in general has an adverse impact on reproductive performance. This decline in fertility with age has been attributed to many ovarian /uterine factors, such as chronic progressive degenerative changes in the endometrium (endometriosis) (RICKETTS and ALONSO, 1991); increased susceptibility to post coital endometritis (ZENT et al., 1998) and general uterine infection caused by age related degeneration in perineal conformation and uterine competence (KALIRAJAN and RAJASUNDARAM, 2008); multiple uterine cysts, causing a reduction in the uterine surface and vascular perfusion (FERREIRA et al., 2008); frequent delayed/irregular ovulation (CARNEVALE et al., 1994) that results in morphologically/genetically abnormal oocytes (CARNEVALE et al., 1999; RAMBAGS et al., 2005); and intrauterine fluid accumulation (ZENT et al., 1998). All these factors have an impact on reproductive efficiency and are responsible for the reduced uterine treatment success observed in older mares.

In our case, the response to treatment varied with reproductive status and might reflect the population of subfertile problem mares in the barren and aborted groups with

compromised fertility, that remained non pregnant in the early season or because uterine intrinsic defects have lower fertility rates. It is worth noting that the greater proportion of older (>12 years) mares fell into the barren (40.65%) category, whereas all maiden mares and 3/4th of the foaling mares were younger than 13 years of age. This might explain the differences in reproductive efficiency in response to uterine treatment. Therefore, despite the potential difficulty in differentiating the effect of age and reproductive status, it is likely that both may have an effect. Furthermore, we have not observed any improvement in any of the reproductive efficiency parameters with the age and reproductive status of the mare, regardless of the administration of uterine therapy. The effects of mare age and reproductive status on the reproductive efficiency prevailed. Thus, for better consideration of economical stud farming and to achieve optimum therapeutic results in older mares as well as in suspected cases of uterine incompetence, a biopsy sample of the endometrium should be assessed concurrently to detect its functionality and ability to clear infection. Breeding of competent mares only will have economic consequences, with the loss of foal income but may well be more than offset by reduced travelling, keep and veterinary costs and by the increased value of earlier and possibly stronger foals.

### **Conclusion**

Uterine treatment was administered in 18.07% of oestrous cycles and its incidence increased with mare age. Furthermore, uterine treatment may compensate for Day 16 pregnancy rates and pregnancy losses to some extent in reproductively incompetent mares. However this trend was not demonstrated for overall foaling rates. Irrespective of the administration of uterine therapy, the effects of mare age and reproductive status on the reproductive efficiency were of major importance in our study. Response to uterine therapy in Thoroughbred broodmares is attributable, directly or indirectly, to a wide range of factors. Therefore to maximise stud farm economic return, veterinarians need to ensure that mares are in optimum condition for breeding by evaluating mares thoroughly, especially their uterine health condition.

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**SAŽETAK**

Radi poboljšanja reprodukcijske sposobnosti punokrvnih kobila provedeno je nekoliko načina liječenja u sezoni njihove spolne aktivnosti. Protokoli o reprodukciji i liječenju 253 punokrvne kobile držane na devet komercijalnih ergela u razdoblju od 1998. do 2005. bili su uzeti za procjenu incidencije različitih načina

liječenja maternice i njihova učinka na prosječnu reprodukcijску sposobnost kobila. Određen je i utjecaj dobi na učinkovitost liječenja maternice. Na osnovi načina i protokola liječenja kobile su bile podijeljene u skupine. Jednoj skupini maternica je bila ispirana fiziološkom otopinom odnosno antiseptikom, a drugoj skupini bili su primijenjeni antibiotici u maternicu i/ili parenteralno u kombinaciji s oksitocinom. Incidencija liječenja maternice iznosila je 18,07% (357) od 1976 promatranih estrusnih ciklusa. Svi načini liječenja bili su jednako učinkoviti s obzirom na reprodukcijске pokazatelje. Od ukupno promatranih, 25,21% kobila bilo je liječeno kombiniranom intrauterinom primjenom antibiotika i oksitocina. Incidencija liječenja maternice bila je najmanja ( $P < 0,0001$ ) u kobila u dobi od tri do sedam godina (9,35%) i ždrjebica (5,92%). Stopa bređosti 39. dana nakon ovulacije i stopa ždrjebljenja bile su veće ( $P < 0,05$ ) u neliječenih kobila. Stopa ovulacije u neliječenih je kobila iznosila 46,39%, a u liječenih 40,62% dok je stopa ždrjebljenja u neliječenih iznosila 41,75%, a u liječenih 35,85%. Nije bila ustanovljena značajna razlika u ukupnim gubitcima tijekom bređosti (21,95% u neliječenih u odnosu na 18,55% u liječenih kobila). U liječenih kobila, stopa bređosti 39. dana nakon ovulacije i stopa ždrjebljenja bile su značajno veće za skupinu u dobi od tri do sedam godina (47,27% i 41,82%) te u ždrjebica (60,0% i 60,0%) nego u onih u dobi  $\geq 18$  godina (22,58% i 12,90%) i jalovih (31,94% i 27,78%) kobila. Zaključno se može iznijeti da usprkos većoj incidenciji liječenja starijih i jalovih kobila, samo liječenje nije proporcionalno poboljšalo reprodukcijску učinkovitost u kobila s poremećenom reprodukcijom te da ishod liječenja maternice u cijelosti ovisi o dobi kobile i njezinu reprodukcijskom statusu.

**Ključne riječi:** kobile, punokrvnjaci, reprodukcijška sposobnost, liječenje maternice, dob

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