

Relationships between stallion age, book size, number of matings (covers), breeding soundness examination findings, and fertility parameters in 15 Thoroughbred stallions (34 stallion years)

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Abstract

Pre-season breeding soundness examinations were performed on 15 stallions at one breeding farm in central Kentucky during the years 2005–2007 (34 stallion years). Pregnancy rates per cycle, seasonal pregnancy rates, and pregnancy loss rates were determined from breeding and foaling reports. Spearman rank order correlations or Pearson's product moment correlations between semen quality in second ejaculates and fertility parameters were calculated, as well as between fertility parameters and stallion age, stud fee, testicular size, mare age, book size, and number of covers required to service the mare book. Stallion age was negatively correlated to both pregnancy rate per cycle ($r = -0.462$; $P < 0.01$) and seasonal pregnancy rate ($r = -0.412$; $P < 0.02$). Seasonal pregnancy rate was positively correlated to both book size ($r = 0.584$; $P < 0.001$) and number of covers required to service the mare book ($r = 0.552$; $P < 0.001$). Pregnancy rate per cycle was linearly correlated to percent ($r = 0.495$; $P < 0.01$) and total number ($r = 0.396$; $P = 0.02$) of progressively motile sperm, and percent ($r = 0.484$; $P < 0.01$) and total number ($r = 0.437$; $P = 0.01$) of morphologically normal sperm, in second ejaculates. Pregnancy rate per cycle and mean- α_t determined in the sperm chromatin structure assay of second ejaculates were negatively correlated ($r = -0.398$; $P < 0.02$). These data support breeding soundness examination findings as beneficial for assessing potential fertility of Thoroughbred stallions prior to the onset of the breeding season.

Keywords: Equine, stallion, Thoroughbred, breeding soundness examination, fertility

Introduction

Performance of breeding soundness examinations prior to onset of the breeding season has been recommended for most domestic large animal species, including the horse.¹⁻³ Breeding soundness examinations of the stallion typically include physical examination of the genitalia (including measurement of testicular size), assessment of mating ability, and evaluation of at least two ejaculates collected an hour or more apart.^{1,2} Aerobic culture of urethral swabbings procured after washing, and prior to and after ejaculation, is also recommended. In the last 15 years, performance of the sperm chromatin structure assay has become an integral component of the semen evaluation, as it may detect aberrations in DNA integrity that are related to subfertility/infertility, even when sperm quality is sometimes within normal limits.⁴

When assessing fertility of a given stallion, it should be remembered that a number of factors besides inherent fertility of the stallion contribute to pregnancy outcome, such as number of mares bred in a season,^{5,6} daily mating frequency,⁷ and inherent fertility of mares bred (affected by age and/or beginning status [maiden, foaling, barren, etc.]).⁸⁻¹⁵ It has also been postulated that higher fertility is achieved by stallions with higher stud fees,* perhaps due to more fertile mares comprising their book (i.e., younger, fewer problem breeding mares, etc.) or a higher level of management being involved due to the increased financial risk.⁶ These factors could preclude finding significant relationships between semen quality and fertility. The purpose of this study was to examine the relationships between stallion age, stud fee, mare age, number of mares bred, number of matings (covers) required to service the mare book, and breeding soundness examination findings to fertility of Thoroughbred stallions.

Materials and methods

Fifteen stallions at one farm in central Kentucky had breeding soundness examinations performed prior to the onset of the 2005-2007 breeding seasons (34 stallion years). Breeding soundness examinations were performed according to the guidelines of the Society for Theriogenology,¹ with total testicular volumes predicted from individual caliper measurements of the testes according to Love et al.¹⁶ A minimum of two ejaculates were collected in an artificial vagina using a mare in estrus as the mount source. Swabbings were procured from the urethra after washing, and prior to and after ejaculation, as well as a swabbing of the raw filtered semen, for aerobic bacterial culture on blood and MacConkey's agar. Volume of gel-free semen was measured in a graduated cylinder. Sperm concentration in gel-free semen was determined spectrophotometrically (590B Equine Densimeter, Animal Reproduction Systems, Chino, CA). An aliquot of gel-free semen was diluted in warm semen extender (INRA 96 semen extender, IMV Technologies, Maple Grove, MN) to a concentration of 25 million sperm/ml. A 5 μ l drop of extended semen was placed on a warm microscope slide and cover slipped, and sperm motility (total/progressive) was assessed by one examiner using a phase contrast microscope (250X) with warming stage. An aliquot of raw semen was mixed in 2% buffered formal saline, and a 5 μ l drop was placed on a microscope slide and cover slipped, and examined under oil immersion (1250X) using differential contrast microscopy to assess sperm morphologic defects in 100 sperm. The examiner (TLB) of sperm motility and morphology was not blinded to the stallion. An aliquot of raw gel-free semen was pipetted into a cryovial and snap frozen in liquid nitrogen until assessed by sperm chromatin structure assay. After staining with acridine orange, a minimum of 5000 sperm cells were evaluated for DNA fluorescent patterns by flow cytometry according to the method of Kenney et al.⁴

Data retrieved from records included results of the pre-season breeding soundness examinations (total testicular volume, total number of sperm in second ejaculate, percentage and total number of progressively motile sperm in second ejaculate, percentage and total number of morphologically normal sperm in second ejaculate, total number of progressively motile, morphologically normal sperm (product of total number of sperm, percent progressively motile sperm, and percent morphologically normal sperm) in second ejaculate, and results of the sperm chromatin structure assay from sperm in second ejaculate (mean- α_t , standard deviation- α_t , and COMP- α_t).

Breeding records from this farm were examined to retrieve data on pregnancy rate per cycle, seasonal pregnancy rate, and pregnancy loss (at any time after establishment of pregnancy) rate for the years 2005 to 2007. Additional data retrieved for comparisons included age of the stallion, book size (number of mares bred) for each given year, number of matings (covers) required to service the book of mares for each given year, mean mare age, and stud fee for each given year.

The Shapiro-Wilk statistic was used to test normality of the data. Data for fertility parameters (pregnancy rate per cycle, seasonal pregnancy rate, and pregnancy loss rate) were normally distributed ($P > 0.01$). Pearson product moment correlations were performed to determine linear relationships among normally distributed data (book size, number of covers, mean mare age, total testicular volume, percentage and number of progressively motile sperm in second ejaculates, percentage of morphologically normal sperm in second ejaculates, number of progressively motile, morphologically normal sperm in second ejaculates, and mean- α_t and standard deviation- α_t in second ejaculates, and fertility parameters. Spearman rank order correlations were determined to examine linear relationships among fertility parameters and non-normally distributed data (stallion stud fee, stallion age, total sperm number in second ejaculates, total number of morphologically normal sperm in second ejaculates, and COMP- α_t of sperm in second ejaculates; $P < 0.01$).

Results

Stallion ages during the study ranged from 4 to 25 years. Stud fees for stallions ranged from \$5,000 to \$60,000. The range in book size and number of covers per year for stallions were 23 to 199 mares, and 27 to 305 covers per year, respectively.

Overall pregnancy rates per cycle achieved by stallions during this study ranged from 50 to 73%. Yearly pregnancy rates per cycle for individual stallions varied from none (0%) to as much as 15%

between years (Table 1). Stallion age was negatively correlated to both pregnancy rate per cycle ($r = -0.462$; $P < 0.01$) and seasonal pregnancy rate ($r = -0.412$; $P < 0.02$). No relationship ($P > 0.05$) existed between pregnancy rate per cycle or pregnancy loss rate and stud fee, book size, or number of covers required to service the mare book. While stud fee was not correlated with seasonal pregnancy rate ($P > 0.05$), book size ($r = 0.584$; $P < 0.001$) and number of covers ($r = 0.552$; $P < 0.001$) were correlated to seasonal pregnancy rate.

Mean mare age for the mares within a stallion's yearly book was not correlated ($P > 0.05$) with pregnancy rate per cycle or per season, or with pregnancy loss rate. However, stallion stud fee was positively correlated to book size ($r = 0.409$; $P < 0.02$), and was negatively correlated with mean mare age ($r = -0.520$; $P < 0.01$).

No stallion had *Pseudomonas aeruginosa* or *Klebsiella pneumoniae* (considered potential venereal pathogens) isolated from urethral swabbings procured before or after ejaculation, nor from swabbings of the raw semen. No stallions had neutrophils present in semen, suggesting internal genital infections did not exist in these stallions.

Using guidelines proposed by Kenney et al.,¹ all stallions had normal total testicular size (except for two stallions, stallions 10 and 11, each having only one scrotal testis of normal size). Total testicular volumes in stallions with two scrotal testes ranged from 271 cc to 536 cc. No correlations between total testicular volume and fertility parameters were detected ($P > 0.05$).

Per cycle pregnancy rate was positively correlated to percentage of progressively motile sperm ($r = 0.495$; $P < 0.01$), total number of progressively motile sperm ($r = 0.396$; $P = 0.02$), percent morphologically normal sperm ($r = 0.484$; $P < 0.01$), total number of morphologically normal sperm ($r = 0.437$; $P < 0.01$), and total number of progressively motile, morphologically normal sperm ($r = 0.402$; $P < 0.02$) in second ejaculates. Pregnancy rate per cycle was negatively correlated with mean- α_t of second ejaculates determined in the sperm chromatin structure assay ($r = -0.398$; $P < 0.02$), but no correlation ($P > 0.05$) was detected among any fertility parameters and standard deviation- α_t or COMP- α_t . No correlations ($P > 0.05$) were detected between semen quality parameters and seasonal pregnancy rate. Pregnancy loss rate was not correlated with any of the parameters recorded/measured ($P > 0.05$).

Discussion

It has been postulated that higher fertility is achieved by those stallions with higher stud fees. Reasoning provided for a relationship between stud fee and fertility is that more fertile, better managed mares will comprise the mare book when stud fees are high because of the increased financial risk. However, within the range of stud fees for stallions standing at this farm, we found no significant linear relationship between stud fee and fertility parameters. Stud fees for Thoroughbred stallions standing in central Kentucky can exceed \$500,000. Perhaps if more stallions with very high stud fees were included in the data, significant correlations between stud fee and fertility might have existed. Alternatively, the management level of mares bred at Thoroughbred farms in central Kentucky may be optimal (e.g., negative cultures were required from all but first cycle foaling mares at this farm; use of ovulation-inducing drugs to promote ovulation within two days of breeding and examining dismount samples to ensure the stallion ejaculated during mating are common practices at most Thoroughbred farms in central Kentucky), regardless of the stud fee for the stallion used for mating, thereby negating influence of management on overall fertility.

The range in pregnancy rates per cycle included in this study was similar to that described by others when surveying fertility of large groups of Thoroughbreds, and were within ranges reported as normal.^{10,15,17} Ages for stallions during the study ranged from four to 25 years. The negative correlation between stallion age and pregnancy rate per cycle could be explained, in part, by some age-related decline in testicular function that typically occurs in older stallions. Others have described age-related decline in fertility in stallions,^{7,12,18} as well as individual stallion variation in fertility.^{7,10,12,19,20}

No attempt was made to determine sources of variation in fertility among years within stallions, as the beginning status and age of a stallion's book of mares can vary significantly from year to year based on his popularity as a sire. In one report of a Thoroughbred stallion experiencing an 11% decline in

pregnancy rate per cycle and per season between two successive years of breeding, the percentage of barren mares comprising the mare book more than tripled to 25%, and the associated low pregnancy rate in this group of mares accounted for most of the decline in fertility achieved between years.³ However, how popularity of a stallion influences the age and beginning status of mares within the mare book has not been well described.

The finding of positive correlations between seasonal pregnancy rate and book size, and between seasonal pregnancy rate and number of covers required to service a stallion's book of mares, is interesting. Baker et al.⁵ reviewed Jockey Club foaling records for stallions standing in Kentucky during 1987, and found reproductive success increased as book size increased, with foaling rates being 47% for stallions with the smallest book sizes and 70% for stallions with the largest book sizes. Turner and McDonnell⁶ confirmed similar findings in a retrospective study of Jockey Club records for Thoroughbred stallions of various sized mare books during 2003–2005.⁶ Baker et al.⁵ postulated that mares on large farms were managed better so that they had increased opportunity to become pregnant. They theorized the primary reason mares on larger farms had a better chance of becoming pregnant in a season was that a higher level of management resulted in earlier detection of failure to become pregnant, and thus more chances for rebreeding. Our finding that pregnancy rates per cycle were higher in stallions with larger books would suggest that either more fertile mares were presented to these stallions, or the mares were managed better to increase their initial opportunity to become pregnant. Stallion stud fee was positively correlated to book size, indicating that at this farm the stallions with higher stud fees were more popular. Also of interest was our finding that stallion stud fee was significantly negatively correlated with mean mare age. It is well known that younger mares are more fertile than older mares,^{8–15} so perhaps more young, fertile mares are presented to stallions with higher stud fees because more investment is at risk. This could explain some of the increase in fertility of the more popular stallions.

We were not surprised that no correlations were detected between total testicular volume and fertility parameters. Poor semen quality due to testicular dysfunction is commonly associated with small testes, and none of the stallions in this study had smaller than normal testicular volumes.^{20,21}

Semen parameters often reported to be correlated with fertility in stallions include percent progressively motile sperm and percent morphologically normal sperm.^{4,22–25} Our finding that percentage and number of progressively motile sperm, or percentage and number of morphologically normal sperm, in second ejaculates were significantly correlated with pregnancy rate per cycle supports earlier findings. So, preseason breeding soundness examination findings were associated with fertility parameters in this group of fertile stallions. Perhaps if some stallions at this farm were subfertile, more variation in semen quality parameters would have existed, thereby resulting in greater correlations among semen characteristics and fertility parameters. However, the fact that correlations could be found in this small subset of fertile stallions suggests that the breeding soundness examination could be even more useful to separate fertile from subfertile/infertile stallions.

Of additional interest is the finding of significant correlations of semen quality parameters with pregnancy rate per cycle, but not with seasonal pregnancy rate. Pregnancy rate per cycle is probably a more representative indicator of stallion fertility than seasonal pregnancy rate. Stallions with low pregnancy rates per cycle can achieve satisfactory seasonal pregnancy rates if mares that do not become pregnant on the first estrous cycle of breeding have sufficient opportunity to be rebred promptly on subsequent estrous cycles.⁵

We expected COMP- α_t to be significantly associated with fertility parameters, as it has been cited as the sperm chromatin assay statistic of semen quality either better correlated with infertility in the horse,^{4,26} or showing a greater percentage of change in cooled semen quality between fertile and subfertile stallions.²⁷ Perhaps the reason it was not associated with fertility parameters in this study was that only three stallions (stallion 9 in 2006 [18%], stallion 12 in 2006 [18%], and stallion 7 in 2005 [27%]) had values elevated over 16% COMP- α_t , the maximum value reported as normal.⁴ Kenney et al.⁴ reported all SCSA statistics were elevated in subfertile stallions achieving a 38% seasonal pregnancy rate when compared to normal stallions achieving a 70% seasonal pregnancy rate. The finding of a negative association between mean- α_t and pregnancy rate per cycle in this group of normally fertile stallions could

suggest that it is a more sensitive indicator of unstable sperm chromatin. A shift in mean- α_t represents a shift in the chromatin stability of the entire population of sperm in the ejaculate.

We were also interested in investigating the relationship between sperm chromatin integrity and pregnancy loss rate. If sperm with lowered chromatin integrity resulted in fertilization, it is possible the developing conceptus would be compromised and thus more prone to loss. However, SCSA statistics were not significantly related to pregnancy loss rates in this study.

Summary

Our findings suggest seasonal pregnancy rate is improved in Thoroughbred stallions breeding large books of mares, requiring more covers to service the mare book, than in those stallions breeding smaller books of mares. Whether this relationship is due to increased level of management could not be determined from this study. Our finding of linear relationships between pregnancy rate per cycle and progressive sperm motility, morphologically normal sperm, and mean- α_t of sperm confirms the value of these semen parameters for evaluating stallions for potential breeding soundness. Since pregnancy rate per cycle was significantly correlated with several semen quality parameters while seasonal pregnancy rate was not, it may be a more sensitive indicator of fertility of a stallion.

*Pickett BW, personal communication, 1982.

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Table 1. Semen quality findings in second ejaculates obtained during breeding soundness examinations and pregnancy rate per cycle (PR/cycle), seasonal pregnancy rate (SPR) and pregnancy loss rate (PLR), achieved by 15 Thoroughbred stallions on one farm in central Kentucky during 2005 – 2007 (34 stallion years).

Stallion	Year	Age	Book	AMAge	No. sperm	% PMS	%MNS	No. PMMNS	Mean- α	SD- α	% COMP- α	PR/cycle	SPR	PLR
1	2005	6	142	9.31	5.921	65%	77%	2.963	238	54	5%	64.8%	90.1%	18.8%
1	2006	7	172	9.33	8.720	55%	69%	3.309	198	40	3%	69.9%	90.1%	18.2%
1	2007	8	33	9.62	11.418	65%	71%	3.197	247	45	7%	76.9%	90.9%	3.3%
2	2005	6	170	10.56	10.816	50%	56%	3.028	240	23	5%	68.7%	93.5%	24.3%
2	2006	7	146	10.02	7.056	40%	46%	1.298	229	70	13%	66.5%	85.6%	25.2%
2	2007	8	140	10.16	8.352	50%	55%	2.297	238	51	13%	78.4%	92.9%	22.1%
3	2005	9	64	10.07	15.120	65%	56%	5.504	233	34	5%	64.2%	84.4%	13.1%
4	2005	8	121	9.51	12.250	55%	72%	4.891	240	44	7%	67.7%	90.9%	14.2%
4	2006	9	195	9.26	31.680	35%	51%	5.655	233	69	14%	69.8%	93.9%	17.9%
4	2007	10	115	9.63	7.220	60%	63%	2.729	215	45	8%	67.1%	85.2%	11.0%
5	226	5	187	9.99	6.816	45%	42%	1.288	244	42	5%	55.4%	86.1%	16.0%
5	2007	6	101	10.57	8.200	30%	25%	0.656	226	35	7%	69.7%	91.1%	16.2%
6	2005	21	27	11.91	5.539	45%	48%	1.196	248	56	12%	59.1%	92.6%	19.2%
6	2006	22	28	10.68	6.685	30%	32%	0.642	259	53	13%	59.5%	78.6%	13.6%
7	2005	18	88	11.08	8.588	50%	52%	1.475	272	49	27%	53.2%	81.2%	15.7%
8	2005	6	184	9.89	8.588	65%	67%	3.740	255	30	8%	67.9%	94.6%	11.1%
9	2005	14	113	9.58	11.088	60%	70%	4.657	258	38	7%	58.9%	89.4%	25.7%
9	2006	15	65	10.02	14.188	60%	56%	4.767	259	88	18%	70.7%	93.9%	24.6%
9	2007	16	114	9.69	12.780	70%	55%	4.920	250	42	8%	58.0%	85.1%	17.1%
10	2006	4	197	9.68	6.031	55%	51%	1.692	231	49	7%	70.1%	93.4%	17.4%
10	2007	5	112	10.25	11.661	60%	68%	4.758	230	44	4%	78.1%	90.2%	21.1%
11	2005	6	120	9.74	3.225	45%	38%	0.551	240	55	11%	54.8%	90.0%	11.4%
12	2005	11	159	8.96	12.208	60%	61%	4.505	253	60	13%	63.9%	89.9%	22.7%
12	2006	12	191	9.36	8.720	35%	50%	1.526	238	67	18%	61.2%	98.5%	16.9%
12	2007	13	199	9.70	6.836	50%	48%	1.641	254	50	10%	68.1%	91.5%	23.4%
13	2005	23	42	8.44	16.440	35%	47%	2.704	258	38	7%	58.3%	88.1%	14.3%
13	2006	24	50	10.35	11.330	25%	35%	0.991	244	39	9%	52.3%	76.0%	15.6%
13	2007	25	36	9.88	10.212	20%	35%	0.715	257	40	10%	35.7%	77.8%	25.0%
14	2005	5	136	9.14	3.276	65%	58%	1.235	246	51	10%	66.0%	92.7%	20.1%
14	2006	6	136	9.76	7.710	60%	52%	2.406	241	68	16%	64.2%	87.5%	16.3%
14	2007	7	127	9.71	8.154	55%	57%	2.556	255	59	13%	72.2%	94.5%	16.9%
15	2005	9	40	10.37	10.948	60%	64%	4.204	238	54	8%	61.4%	77.5%	14.3%
15	2006	10	35	9.98	11.269	55%	55%	3.409	232	66	10%	67.3%	88.6%	21.2%
15	2007	11	23	11.59	13.113	65%	64%	5.455	241	39	6%	70.4%	82.6%	15.8%

Key: Age = years, Book = number of mares bred in season; No. sperm = total number of sperm in billions; AMAge = average age in years of mares in book; 9.74%PMS = percentage of progressively motile sperm; %MNS = percentage of morphologically normal sperm; No. PMMNS = total number of progressively motile, morphologically normal sperm in billions; Mean- α = mean channel of entire distribution of sperm cells stained with acridine orange that fluoresce green or red depending on extent of DNA denaturation as determined by flow cytometer in Sperm Chromatin Structure Assay; SD- α = standard deviation of sperm cells stained with acridine orange that fluoresce green or red depending on extent of DNA denaturation as determined by flow cytometer in Sperm Chromatin Structure Assay; % COMP- α = percentage of sperm cells stained with acridine orange outside main population depending on extent of DNA denaturation as determined by flow cytometer in Sperm Chromatin Structure Assay; PR/cycle = pregnancy rate per cycle; SPR = seasonal pregnancy rate; PLR = pregnancy loss rate (includes pregnancies lost during any stage of gestation).

