Estimation of genetic parameters and effects of cytoplasmic line on scrotal circumference and semen quality traits in Angus bulls

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ABSTRACT: The purpose of this study was to estimate the heritability of scrotal circumference (SC) and semen traits, genetic correlations between SC and semen quality traits, and the effect of cytoplasmic line on SC and semen traits. Breeding soundness exam (BSE) data were collected on registered Angus bulls at 4 ranches over 7 yr. The American Angus Association provided historical pedigree information to estimate the effect of cytoplasmic line on SC and semen quality traits. After editing, the evaluated data set contained 1,281 bulls with breeding soundness exam data that traced back to 100 founder dams. Data were analyzed using a 2-trait animal model to obtain heritability, genetic correlation between SC and semen quality traits, as well as the effect of cytoplasmic line as a random effect for SC, percent motility (MOT), percent primary abnormalities (PRIM), percent secondary abnormalities (SEC), and percent total abnormalities (TOT) using multiple-trait derivative-free REML. Fixed effects included source ranch and collection year, and test age was used as a covariate. Estimates of heritability for SC, MOT, PRIM, SEC, and TOT were 0.46, 0.05, 0.27, 0.23, and 0.25, respectively. Genetic correlations between SC and MOT, PRIM, SEC, and TOT were 0.36, −0.19, −0.11, and −0.23, respectively. The proportions of phenotypic variance accounted for by cytoplasmic line for SC, MOT, PRIM, SEC, and TOT were <0.001, 0.013, 0.023, 0.002, and <0.001, respectively. Genetic correlations between SC and semen quality traits were low to moderate and favorable. Cytoplasmic line may have a marginal effect on MOT and PRIM, but is likely not a significant source of variation for SC, SEC, or TOT.

Key words: bull, cytoplasmic line, semen

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INTRODUCTION

Mitochondrial DNA (mtDNA) is believed to be inherited solely from the mother (Dawid and Blackler, 1972; Hutchison et al., 1974; Shitara et al., 1998). Sutovsky et al. (2000) determined that ubiquitination is the mechanism that regulates the destruction of sperm mtDNA, ensuring maternal inheritance. Establishing maternal inheritance of mtDNA opens up the possibility of these genes serving as an additional source of genetic variation. Wagner (1972) was the first to propose cytoplasmic inheritance in animals as an avenue for introducing additional genetic material.

Numerous studies evaluated the effect of cytoplasmic line in milk and preweaning performance traits using the sire or sire-maternal grandsire model and found cytoplasmic effect accounted for a significant source of phenotypic variance (Bell et al., 1985; Tess et al., 1987; Schutz et al., 1992). Technological advances allowed others to analyze the effect of cytoplasmic line using the animal model to account for all nuclear additive genetic variance. Upon reevaluation of previous data, Tess and Robison (1990) failed to find significant differences in milk performance traits based on maternal line. Northcutt et al. (1991) found maternal lineage accounted for 0 to 5% of the phenotypic variance for birth weight and had virtually no effect on weaning weight. Tess and MacNeil (1994) also reported cytoplasmic line was not a significant source of variation for birth weight or ADG when cytoplasm was treated as a fixed or random effect. More recently, Keenan (2003) found cytoplasmic line did not contribute a significant source of variation to growth traits, but did appear to affect fat deposition.

Currently, no studies have investigated the effect of cytoplasmic line on bull fertility. Several factors contribute to the potential effects of mtDNA and, therefore, cytoplasmic line on bull fertility. Numerous studies have linked male infertility in humans with mitochondrial dysfunction or mutations of the mitochondrial genome (Kao et al., 1995, 1998; Holyoake et al., 2001; Kasai et
al., 2002). Furthermore, due to the role of mitochondria in energy production and their location in the sperm midpiece, mtDNA may have an especially important role in semen quality traits, particularly sperm motility.

The purpose of this study was to estimate the heritability of scrotal circumference (SC) and semen traits, genetic correlations between SC and semen quality traits, and the effect of cytoplasmic line on SC and semen traits.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because the data were obtained from an existing database at the College of Veterinary Medicine Teaching Hospital at Kansas State University.

Data

Breeding soundness exam (BSE) data (procedure outlined by Chenoweth et al., 1993), were collected on 1,281 registered Angus bulls. The pedigree file included 4 generations of ancestors for every measured animal. Traits were recorded on bulls ranging in age from 288 to 549 d (mean age = 387 d). Scrotal circumference was measured to the nearest 0.5 cm. Semen was collected by electroejaculation and evaluated for motility and morphology. Semen from bulls with SC less than 30 cm was not collected. The BSE data were collected on bulls from 4 private producers in the Flint Hills region of Kansas from 1994 to 2000. Bulls were born and subsequently evaluated in the spring (January to April) or fall (August to November) seasons. Calving year and season were components of contemporary group. Exams were performed by clinical veterinarians from the Kansas State University College of Veterinary Medicine. Although not all exams were performed by the same veterinarian, the same veterinarian performed all exams within each contemporary group. Table 1 outlines the number of bulls tested at each source for each year of collection.

Immediately after collection, a sample of ejaculate was placed on a warm slide and evaluated under a light microscope at a magnification of 100× to assess gross motility. Percentage of motile sperm (MOT) was recorded as the percentage of spermatozoa that exhibited rapid linear movement as evidenced by the presence of swirls and the wave action of the sample. A second slide was prepared by mixing equal-sized drops of eosin-nigrosin stain with the ejaculate, spreading the resulting mixture across the slide, and allowing it to air dry. One hundred randomly sampled sperm were evaluated per slide for morphologic abnormalities at 400× light microscopy. The classification system used by Chenoweth et al. (1993) was used for distinguishing between primary and secondary abnormalities. Percent primary (PRIM) and secondary abnormalities (SEC) were recorded as a percentage of the total sperm counted. Percent total abnormalities (TOT) were obtained by adding PRIM and SEC. If the ejaculate had motility <30% or was <70% morphologically normal, a sample from the bull was collected at least 2 additional times on the day of examination. The results from the best sample, as determined by MOT and percent morphologically normal spermatozoa, were recorded.

The American Angus Association provided pedigree information for the bulls that extended 6 generations. In addition, historical pedigree information was obtained to determine the cytoplasmic dam for each bull. The cytoplasmic dam was defined by tracing the pedigree file of the association to determine the female contributing the mtDNA in the maternal lineage of the animal. The final data set contained BSE data on 1,281 bulls tracing to 100 cytoplasmic dams. Descriptive statistics of the data set are presented in Table 2.

Model

The effect of cytoplasmic line was evaluated on the following traits: SC, MOT, PRIM, SEC, and TOT. Data were analyzed using a 2-trait animal model to

| Table 1. Number of bulls collected from each source from 1994 through 2000 |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| Source | 1 | 2 | 3 | 4 | Total |
| Year 1 | 10 | 3 | 23 | 0 | 36 |
| Year 2 | 44 | 3 | 29 | 0 | 76 |
| Year 3 | 58 | 82 | 31 | 35 | 206 |
| Year 4 | 67 | 79 | 24 | 46 | 216 |
| Year 5 | 111 | 10 | 29 | 48 | 198 |
| Year 6 | 128 | 132 | 29 | 42 | 331 |
| Year 7 | 0 | 138 | 31 | 49 | 218 |
| Total | 418 | 447 | 196 | 220 | 1,281 |

| Table 2. Descriptive statistics for scrotal circumference (SC) and semen traits |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| Trait | Records | Mean | SD | Minimum | Maximum | Cytoplasmic lines |
| SC, cm | 1,280 | 34.70 | 2.82 | 20.00 | 49.00 | 100 |
| Motility, % | 1,245 | 44.47 | 12.34 | 0.00 | 85.00 | 100 |
| Primary abnormalities, % | 1,238 | 13.77 | 16.88 | 2.00 | 100.00 | 100 |
| Secondary abnormalities, % | 1,238 | 12.24 | 10.34 | 0.00 | 90.00 | 100 |
| Total abnormalities, % | 1,238 | 26.01 | 19.99 | 6.00 | 100.00 | 100 |
obtain heritability, genetic correlation between SC and semen quality traits, and the effect of cytoplasmic line as a random effect for SC, MOT, PRIM, SEC, and TOT. The mixed model equations used were described by Quaas and Pollak (1980). Test age was used as a covariate, and fixed effects included source ranch and collection year. The multiple-trait derivative-free REML software package described by Boldman et al. (1995) was used to calculate variance and covariance components for estimation of heritability and genetic correlations. Modifications were made to the data set to account for missing values to calculate SE (Nephawe et al., 2003). The mathematical model used for each trait is represented by the following:

$$y = X\beta + Z_1a + Z_2c + e,$$

where $y$ is the vector of observations; $X$ is the incidence matrix relating fixed effects ($\beta$) to $y$; $Z_1$ and $Z_2$ are incidence matrices relating random direct additive genetic ($a$) and cytoplasmic line ($c$) effects to $y$, respectively; and $e$ is the vector of residual error effects. The variance structure for the analyses can be described by the following: $\text{Var}(a) = \sigma_a^2 A$, $\text{Var}(c) = \sigma_c^2 I$, $\text{Var}(e) = \sigma_e^2 I$, where $I$ is an identity matrix and $A$ is the relationship matrix. Direct additive genetic and cytoplasmic line effects were considered independent for all analyses. Convergence criterion was assumed to have been met when the variance of the logarithmic value of the likelihood function was equal to or less than $10^{-9}$. Each trait was restarted at least twice to ensure convergence was not falsely obtained only at local maximum sites.

## RESULTS AND DISCUSSION

### Heritabilities

Estimates of heritability for SC, MOT, PRIM, SEC, and TOT were 0.46, 0.05, 0.27, 0.23, and 0.25, respectively (Table 3). Heritability estimates of SC have been reported in numerous studies and range from 0.16 to 0.71. A sample of literature estimates of SC and sperm motility heritabilities are reported in Table 4. The heritability estimate of SC was comparable with previous studies (Bourdon and Brinks, 1986; Gipson et al., 1987; Smith et al., 1989; Meyer et al., 1990; Keeton et al., 1996; Martínez-Velázquez et al., 2003). These studies primarily examined yearling British breed-type bulls. However, greater estimates of 0.71, 0.65, and 0.57 were reported by Evans et al. (1999), Mwansa et al. (2000), and Kealey et al. (2006), respectively. The Hereford bulls tested by Kealey et al. (2006) were considerably older (37 mo) than the bulls used in the current study, which may help explain the variation in results. Conversely, Kriese et al. (1991) reported a SC heritability estimate of 0.16 in Brangus bulls, whereas Morris et al. (1992) and Vargas et al. (1998) also reported lower estimates than the current study in crossbred and Brahman bulls, respectively. Biological type appears to affect SC heritability as Bos indicus bulls (or B. indicus-influenced bulls) have lower estimates than all other breeds reviewed in this paper. The increased degree of variation among heritability estimates in previous studies was likely due to the different breeds and ages of the bulls tested, as well as the models used for analysis. Although the heritability estimate for MOT was slightly less than previously reported values, prior work supports the low heritability of MOT (Knights et al., 1984; Gipson et al., 1987; Smith et al., 1989). However, Kealey et al. (2006) reported MOT was moderately heritable (0.22), whereas others have shown MOT was highly heritable (Druet et al., 2009). Smith et al. (1989) and Kealey et al. (2006) reported heritability of PRIM was moderately heritable (0.31 and 0.30, respectively), which aligns with the current results. The estimate for SEC was considerably greater than the value of 0.02 reported by Smith et al. (1989), but somewhat less than the estimate of 0.33 by Kealey et al. (2006). Druet et al. (2009) reported heritability of TOT was moderately heritable (0.23) in Holstein bulls, which is consistent with the results of the current study.

### Genetic Correlations

Genetic correlations between SC and MOT, PRIM, SEC, and TOT were 0.36, −0.19, −0.11, and −0.23, respectively (Table 3). Genetic correlations between SC and semen quality traits were low to moderate and favorable. Results would indicate selection for SC in Angus bulls will positively influence semen quality; however, results must be interpreted cautiously due to large SE. Genetic correlations between SC and semen

<table>
<thead>
<tr>
<th>Trait</th>
<th>SC</th>
<th>MOT</th>
<th>PRIM</th>
<th>SEC</th>
<th>TOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>0.46 ± 0.08</td>
<td>0.11</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.11</td>
</tr>
<tr>
<td>MOT</td>
<td>0.36 ± 0.29</td>
<td>0.05 ± 0.03</td>
<td>0.27 ± 0.07</td>
<td>0.23 ± 0.08</td>
<td>0.25 ± 0.07</td>
</tr>
<tr>
<td>PRIM</td>
<td>-0.19 ± 0.17</td>
<td>0.27 ± 0.07</td>
<td>0.23 ± 0.08</td>
<td>0.25 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>SEC</td>
<td>-0.11 ± 0.19</td>
<td>0.23 ± 0.08</td>
<td>0.25 ± 0.07</td>
<td></td>
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</tr>
<tr>
<td>TOT</td>
<td>-0.23 ± 0.18</td>
<td>0.25 ± 0.07</td>
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<td></td>
</tr>
</tbody>
</table>

1Heritabilities ± SE on diagonal; genetic correlations ± SE below the diagonal; and phenotypic correlations above the diagonal.

2MOT = sperm motility, PRIM = primary abnormalities, SEC = secondary abnormalities, TOT = total abnormalities.
traits could actually be positive or near zero due to SE. The sample size should be increased to obtain more reliable estimates of genetic parameters. Even so, estimates were not in agreement with those reported by Smith et al. (1989). Their estimates of −0.04, 0.14, and 1.22 for genetic correlations of SC with MOT, PRIM, and SEC, respectively, were low to high and unfavorable. In addition, they were opposite in sign from their phenotypic correlations, as well as opposite in sign from the results of the current study. Gipson et al. (1987) reported a somewhat greater value (0.65) for the genetic correlation between SC and MOT. The genetic correlation between SC and MOT was in agreement with the value (0.34) reported by Kealey et al. (2006); however, their values of −0.36 and −0.45 for PRIM and SEC, respectively, were somewhat less than the values reported in the current study.

**Phenotypic Correlations**

Phenotypic correlations between SC and MOT, PRIM, SEC, and TOT were 0.11, −0.10, −0.05, and −0.11, respectively (Table 3). Phenotypic correlations between SC and semen traits were low and favorable. This is in agreement with the values of 0.13, −0.09, and −0.10 for phenotypic correlations between SC and MOT, PRIM, and SEC, respectively, reported by Smith et al. (1989). Palasz et al. (1994) reported bulls with a greater SC tended to have a reduced percentage of sperm abnormalities, but did not detect any significant correlations between SC and different types of abnormal sperm. However, Palasz et al. (1994) did find a significant correlation (r = 0.10) between SC and the percentage of normal spermatozoa. The phenotypic correlation for motility in the present study is not in agreement with the significantly lower value of −0.52 reported by Knights et al. (1984). Conversely, Gipson et al. (1987) reported a moderately high value of 0.57 for the phenotypic correlation between SC and MOT, which is similar to the relationship (r = 0.45) between SC and MOT in pubertal Holstein bulls (Devkota et al., 2008).

**Cytoplasmic Line**

The proportions of phenotypic variance accounted for by cytoplasmic line for SC, MOT, PRIM, SEC, and TOT were <0.001, 0.013, 0.023, 0.002, and <0.001, respectively. Estimates are reported in Table 5. Cytoplasmic line may have a marginal effect on MOT and PRIM, but is likely not a significant source of variation for SC, SEC, or TOT. However, due to relatively small sample size, SE were large for estimates and results must be interpreted with caution.

Sperm motility is a major determinant for male fertility, particularly in males being used for AI. Sperm use a large amount of ATP for propulsion, and this energy is produced in the mitochondria surrounding the
sperm midpiece. Problems with mitochondrial respiratory chain function can adversely affect sperm motility and, consequently, male fertility. Ruiz-Pesini et al. (1998) conducted a study in human males to determine the relationships between sperm motility and mitochondrial chain enzyme activities. Results indicated semen samples of males with normal sperm motility had significantly greater activities of complexes I, II, and IV compared with asthnozoospermic subjects. Results also showed motility is largely dependent on the amount of mitochondria surrounding the midpiece (Ruiz-Pesini et al., 1998). Although cytoplasmic line accounted for only 1.3% of the phenotypic variance for motility in the current study, this correlation between sperm motility and mitochondrial enzymatic activities could explain why cytoplasmic line accounted for a greater amount of variance for motility than other semen traits.

Variation in mtDNA between cytoplasmic lines has been documented and associated with phenotypic variation of reproductive traits. Sutarno et al. (2002) identified polymorphisms in bovine mtDNA that were associated with female fertility in 2 beef breeds, indicating variation of mtDNA between cytoplasmic line. Moreover, Lei et al. (2004) sequenced the complete mitochondrial D-loop and found significant mitochondrial genetic diversity in 8 Chinese cattle breeds.

### Implications

The heritability estimate for SC was high indicating SC should respond to selection. Heritability estimates for semen quality traits were low to moderate, thus increasing the difficulty of using those traits as predictors of bull fertility. Genetic correlations between SC and semen quality traits were low to moderate and favorable. Phenotypic correlations between SC and semen traits were less than genetic correlations, but were still favorable. Therefore, the selection for increased SC should also improve semen quality traits in Angus bulls; however, results should be interpreted cautiously due to the large SE of genetic parameters. The results of this study indicate cytoplasmic line may have a marginal effect on MOT and PRIM, but is likely not a significant source of variation for SC, SEC, or TOT.

Due to the strict maternal inheritance of mtDNA, significant cytoplasmic effects could increase the value of certain maternal lines. With the increased popularity of cloning to perpetuate superior genetics, it is important to evaluate the contribution of mtDNA found in recipient eggs because the cloned animal will carry alternative mtDNA if the recipient egg did not originate from the same maternal line. Significant cytoplasmic effects for certain traits may warrant alternative genetic analyses for cloned animals to account for this variation. Variation in mtDNA between cytoplasmic lines has been documented and associated with phenotypic variation. However, beef cattle breed associations will not likely improve accuracy of predicting bull fertility by including cytoplasmic line in their genetic evaluations.

### LITERATURE CITED


