

# **The relationship between end-of-test measures of reproductive soundness, behavior, and hair testosterone concentrations in performance-tested bulls**

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## **Introduction**

Beef cattle production is vital to both the state economy of Tennessee and its role in the national agricultural sector. Serving as the top agricultural product in the state with regards to the amount of revenue generated, beef cattle and calves dominate Tennessee agriculture, with 53 percent of its farms dedicated to producing them. This role continues to grow presently with the decline in the state dairy industry. Tennessee is also among top beef-producing states in the nation, ranking as the ninth state nationwide and as the second state among those east of the Mississippi River in terms of numbers of beef cattle. Cow-calf operations make up 88% of the state's beef-related business (Neel, 2014), and, as a result, success in the industry relies heavily on the ability to employ knowledge of reproductive characteristics in order to breed cattle efficiently. Moreover, applied understanding of carcass traits is always vital to the beef industry in any state, since the quantity and quality alike of meat harvested depends on this. With the beef industry playing such a significant economic part, within the state especially, continuing to better comprehend and predict both reproductive and carcass traits, along with their indicators, is critical to Tennessee agriculture.

Similarly, understanding of cattle behavior and temperament is of supreme significance for Tennessee in particular. Temperament, or the behavioral fear response of cattle to human interactions (Cooke, 2014), has a clear impact on the safety of handlers. Recent trends have shown the ages of Tennessee farmers to be relatively high and continuing to rise, with the average age recorded in the 2012 Census of Agriculture at 59.2 years, after a 2.4% increase from

the 2011 figure (Fewell, 2014). This exceeds both the national average age and the magnitude of its growth. These statistics highlight the state's need to focus on docility in beef and on ideal practices for handling and testing cattle. Therefore, in order to maximize the safety and well-being of Tennessee cattlemen, it is imperative that we increase emphasis on docility to match the rise in senility among handlers.

### **Literature Cited**

Testosterone is the primary androgen in the male body. In addition to being essential for male sexual development and behavior as well as reproductive function, it plays a heavy role in aggressive behavior across many species, including birds, fish, dogs, deer, and rodents (Brain, 1983) as well as monkeys (Rada et al., 1976). This has been shown through a variety of studies involving both testosterone injections and castration as techniques to control hormone levels. Examination of testosterone concentrations in hair has yielded similar results. An experiment focusing on wild male hyrax observed in the field demonstrated that hair testosterone levels had significant positive correlation with dominant ranking in the social hierarchy (Koren et al., 2001). These collectively support the existence of a weak positive correlation between testosterone levels and aggressive social behavior, with testosterone increasing alongside inter-animal aggression.

However, this link does not seem to carry over into the field of temperament and more permanent behavioral traits. Geburt et al. (2015) indicated that this relationship may be reversed in terms of docility of cattle toward human handlers, with higher salivary testosterone levels being associated with greater docility. When docility was determined by leaving score, a numerical indication of ease in exiting the chute, saliva testosterone concentrations correlated

negatively with exit velocity, such that cattle with low testosterone leave the chute more quickly and exhibit flightier dispositions than those with higher testosterone. Salivary testosterone concentration measurements did not prove to have reliable repeatability, so the correlation between testosterone and temperament was weak. As a result, it was determined that testosterone levels did not provide a consistent method of estimating docility. Salivary cortisol concentrations instead proved to be a more dependable biomarker for predicting excitable or fearful temperament in cattle than testosterone, exhibiting a positive correlation between high cortisol and fearful temperament that was stronger than the testosterone-temperament correlation.

Elevation of circulating testosterone levels increases both primary and secondary sex characteristics, and therefore gives rise to the enhancement of reproductive traits as well as growth and carcass traits. It is well known that testosterone is directly related to fertility in males, as it guides reproductive development in fetal males, initiates puberty in adolescents, and ultimately produces reproductive capability through spermatogenesis and maintains libido in mature males (Mandal, 2014). Studies examining circulating testosterone levels have outlined the specific characteristics on which the hormone has an effect. Positive correlations have been documented to exist between serum testosterone concentration and scrotal circumference as well as between testosterone levels and ejaculatory volume in bulls (Sajjad et al., 2007).

In a recent study by Souza et al. (2011), blood testosterone levels in cattle were shown to have a low correlation to sperm motility; however, research by Asadpour et al. (2008) found no correlation between the two with seminal fluid testosterone. The mixed findings on the relationship between testosterone concentration and sperm motility indicate that it is weak, if it exists at all. Similarly, no relationship has been established in bulls between blood testosterone

levels and semen quality factors such as sperm morphology, abnormalities, or concentration (Souza et al., 2011). The absence of any direct associations among these factors is supported by the earlier findings of Asadpour et al. (2008), with one exception being when both testosterone levels and overall semen quality were shown to be higher during summer than winter. This anomaly may be attributed to other seasonal environmental factors, such as available feed sources or climate and temperature, which could support male fertility during summer over winter.

Testosterone also has major anabolic effects on the physical maturation of the male body, with a significant role in enhancing both the size and strength of muscles and bones (Mandal, 2014). By promoting higher protein metabolism, it augments the development of muscling. Unsurprisingly, testosterone concentrations measured in plasma of bulls were found to be positively correlated with average daily gain (Sitarz et al., 1977). Gortsema et al. (1974) evaluated carcass quality in steers and bulls and found that bulls reach market weight earlier than steers due to a greater concentration of circulating testosterone and resultant increased growth rate. Testosterone levels positively correlated with increased feed efficiency, as less trimmable fat and higher meat quantities were present in bulls than in steers, even though steers were kept on feed for 40 days longer to reach the same slaughter weight. Higher cold carcass weight per day of age was demonstrated in bulls than steers, with greater muscling in bulls yielding greater percent of total retail steaks and roasts.

In the study by Gortsema and coworkers, palatability was not compromised by elevated testosterone levels, but this result has not been consistent among researchers. Although steers showed more marbling, a factor that the authors attributed to the longer duration on feed, meat from bulls and steers was judged equal in flavor, juiciness, and consumer satisfaction, and

differences noted in tenderness were non-significant (Gortsema et al., 1974). Other studies have shown palatability measures to be significantly higher in steers, such as that reported by Reagan et al.'s (1971) indicating that flavor, tenderness, marbling, and overall consumer satisfaction are all lower in bulls than in steers. Still others report nonsignificant differences in quality.

Evidently, a consensus cannot be reached regarding subjective measures of taste due to the testosterone, but the literature on the role of testosterone in producing greater muscle volume and increasing the speed of muscle growth is undisputed.

Temperament also has a substantial effect on a range of facets of cattle production, from growth to meat quality. Cattle with fearful temperaments seem to be unfavorable not just for handling ease, but also for delays that reduce efficiency of operations. Several studies have shown that temperamental cattle undergo less average daily gain than calmer ones (Matheney, 2009), with animals scoring faster in flight speed exhibiting the smallest liveweight gain in the feedlot. Cattle with slower flight speeds also ultimately achieved significantly heavier slaughter and carcass weights than those with faster flight speeds (Burrow and Dillon, 1997).

Additionally, temperament scores have been found to be moderately correlated ( $r=0.54$ ,  $P<0.0001$ ) with ribeye area per 45.5 kg of body weight in cattle at weaning (Matheney, 2009). Aside from direct effects on weight gain, flighty temperament also leads to elevated stress levels with handling, which may indirectly have a negative impact on growth by reducing immunity (Matheney, 2009).

In terms of meat quality, fearful cattle temperament is equally undesirable. According to a study by Fordyce et al. (1988), the proportion of bruised carcass increased significantly as cattle temperament became more flighty, presumably due to the tendency of these cattle to have a magnified fright response to stimuli during travel. As compared to the most easygoing cattle,

the flightiest ones averaged a total of 1.5 kg more bruise trim that must be discarded per carcass. Moreover, the majority of additional bruising in temperamental cattle occurred along the back and rump, on or proximal to the most valuable carcass cuts. These results were not repeatable in frequently handled cattle, with temperament scores and bruising showing no relationship at all in this group. This suggests that environmental exposure to humans acts in conjunction with inherent temperament to modify overall bovine behavior. This study also suggested that excitable temperament may decrease meat tenderness, although the authors concede that this relationship cannot be determined conclusively from this experiment. Tenderness was compromised by the relatively high mean pH scores recorded in carcasses, but the heightened pH may have been caused by the stress of the long distances travelled to the slaughter plant rather than solely by physiological effects of flighty temperament. Likewise, borderline dark cutter carcasses were shown to be more prevalent in temperamental cattle (Matheney, 2009). Overall, the impacts of excitable cattle disposition on growth rates and meat quality ultimately contribute to major economic losses for producers at the time of harvest.

No direct link appears to exist between temperament and reproductive traits in bull cattle specifically. Since little research has been done on this relationship in bulls, further research may be warranted. The limited current published research available suggests that the relationship between temperament and physiological reproductive traits is weak in male cattle, if it exists at all. While a study by Kasimanickam et al. (2014) established that female beef cows with calmer temperament had significantly higher conception rates and smaller pregnancy loss rates than their more excitable herd-mates, temperament does not seem to have a similar physiological impact on bulls. Multiple experiments, including the work of Fordyce et al. (1996) and Matheney (2009), concur that scrotal circumference in particular is independent of

temperament. Only one study yielded results where scrotal size correlated with flight speed scores. Although the relationship was weak, it did persist across the bulls at all three times of measurement: at weaning, yearling, and final weights (Burrow, 2001). Research is lacking as to whether correlations exist between temperament scores and semen quality factors in bulls.

In spite of the apparent absence of a correlation between bull temperament and physiological sexual characteristics, connections have been found between temperament and initial libido (Petherick, 2005). A variety of research indicates that bulls with relatively fearful temperaments are more hesitant to engage in sexual activity than others, especially when placed in novel environments. For example, bulls with cautious temperaments exhibited significantly longer periods of inactivity before mating with estrous cows than did bulls with less reactive temperaments (Fraser, 1957). Likewise, in an experiment by Hafez and Bouissou (1975), certain flighty bulls required an adjustment period to adapt to new settings during artificial collection of semen. These bulls initially displayed reduced sexual interest but returned to normal sexual behavior after time was provided to acclimate to the situation.

A variety of phenotypic correlations between fertility traits and growth characteristics have been found in cattle. Research suggests that cattle reproductive development is positively linked with body growth but is negatively related to feed efficiency during growth periods. Growth of bulls' scrotal circumference positively correlated with average daily gains in a study performed by Hafla et al. (2015). Schenkel et al. (2004) similarly found that actual scrotal circumference values correlated with average daily gain across multiple breeds of growing bulls. Body weight measurements taken between 6 and 16 months of age are found to be positively correlated with testicular weight in bulls at 16 months of age (Brito et al., 2012). In contrast, Hafla et al. (2015) showed that gain in scrotal circumference in bulls was negatively correlated

with the physical weight gain to feed ratio. However, final scrotal circumference measurements were independent of the same gain to feed ratio and residual feed intake values. This indicates that although the rate of reproductive development may decrease feed efficiency, the actual values of fertility traits do not significantly impact it.

Semen quality parameters weakly associated with growth traits in growing bulls, provided that they are in a healthy body condition. Previous research by Barth and Waldner (2002) found that lack of body fat in bulls decreases scrotal circumference as well as semen motility and increases abnormal semen morphology. Excess nutrition and body fat also limits sperm production and quality, possibly because fat accumulation in the testes insulates them and precludes adequate cooling necessary for proper thermoregulation of sperm cells (Coulter and Bailey, 1988). Moreover, although scrotal circumference is higher in bulls fed high-energy diets than those with moderate-energy intake levels, this can be attributed to inflation of measured circumference values caused by fat reserves around the testes and not to greater testicular weight (Brito et al., 2012). Thus, it is well established that extremes of body condition play a negative role in reproductive development, but within a range of normal body condition scores, the relationships between growth and reproductive traits are weaker. In the recent study by Hafla et al. (2015), sperm morphology had a weak negative correlation with gain to feed ratio and a weak positive one with back fat thickness. However, there was no significant connection between morphology and average daily gain. This finding supports results reported earlier examining bull growth between 6 and 16 months of age (Brito et al., 2012). Progressive sperm motility was also unrelated to average daily gain, gain to feed ratio, and residual feed intake (Hafla et al., 2015).

Concentrations of steroid hormones in hair seem to be closely related to levels in blood serum. This may be because hormones are integrated into the growing hair from circulating



blood flow to the follicle during growth (Gleixner and Meyer, 1997). Hair progesterone levels in dairy cows proved to be an accurate indicator of pregnancy early in gestation, as reported by Liu et al., 1988, and from there, further investigation of steroid hormones measured in hair followed. An experiment by Yang et al. (1998) demonstrated that hair acts as a very reliable substitute for serum in measuring steroid hormone levels in humans. Hair and serum samples were collected at the same time of day to account for circadian pattern of fluctuating testosterone levels over the course of the day. Concentrations of testosterone in human serum were shown to be closely and significantly correlated ( $r=0.395$ ) with those in hair. Regardless of the use of varying segments from the top, middle, and basal regions of the hair, results were consistent across all sections (Yang et al., 1998).

In a study by Gleixner and Meyer (1997), concentrations of testosterone in hair were significantly higher in black-haired bulls than in white-haired ones. This result was particularly noteworthy because no similar trend existed for estradiol levels in black versus white hair. Based on this finding and previous research showing that testosterone binds melanin granules in vitro, the authors suggest that testosterone likely binds melanin in black hair to create these relatively elevated levels. The authors concluded hair color must be considered when evaluating testosterone concentrations in bovine hair across different breeds or phenotypes of cattle.

The use of hair as a non-invasive method of assessing hormone levels stands as a promising tool when conducting stress research in cattle. Hair analysis of hormones could be valuable in facilitating prediction of carcass traits and breeding value in beef cattle prior to slaughter. The non-invasive nature of hair collection minimizes stress and possibility of injury to both the animal and personnel collecting the samples, which will significantly improve animal well-being. Hormone analysis in hair also has advantages beyond safety implications. The

technique simplifies transportation and storage for collected samples, since hair does not require chilling or freezing to maintain freshness as blood and saliva do. Furthermore, hair is always available and easy to harvest, making it a strong solution for situations where minimalizing handling is necessary, especially in wildlife (Koren et al., 2001). Certainly, then, if hair analysis could provide an easier and safer, but equally reliable, method for data collection in agricultural teaching and research as compared to serum sampling, it would be a valuable tool.

With prior data and published literature as guidelines, the present research explores techniques for evaluating testosterone levels in bulls and what these concentrations may signify. More specifically, this study analyzes chronic testosterone concentrations in hair as a non-invasive method of assessing relationships between endocrine, production, behavioral, and reproductive traits in bulls. One objective is to observe how testosterone levels fixated in hair relate to growth and carcass traits, including weight and average daily gain as well as ribeye area and marbling. The research also aims to examine hair testosterone levels as they correspond with breeding characteristics, such as scrotal circumference and semen quality parameters. Additionally, the current study explores the relationship between testosterone and behavioral measures, including pen score and daily activity, exit velocity, and overall temperament in bulls. This would also present the opportunity for future publications on hair concentrations of testosterone as indicators of breeding performance and desirable carcass traits. Ideally, the study strives to determine whether hair testosterone measurements could serve as reliable, non-invasive alternatives to serum concentration measurements in cattle and whether hair analysis provides an accurate way to assess chronic testosterone as compared to the acute concentrations of circulating testosterone found in serum.

## Materials and Methods

All animal procedures in this study were reviewed and approved by the University of Tennessee Animal Care and Use Committee. Bulls (n=30) were housed at the Middle Tennessee Research and Education Center in Spring Hill, Tennessee. Most of the bulls were registered Angus cattle, except for one Santa Gertrudis, one Simmental, and one Simmangus bull. All were born between December 2012 and March 2013. The bulls were separated into three pens by body weight and age, with between 8 and 12 bulls in each pen, for an 84 day test period. All bulls were offered a pelleted feed as well as orchard grass and tall fescue blend hay *ad libitum*. Dataloggers on each bull tracked total steps taken, total lying time, number of lying bouts, and lying bout duration from d 0 to 28 and d 56 to 84. Body weight and scrotal circumference were measured upon arrival at the facility, and then bulls were allowed a 14-day habituation period to acclimate to the new environment.

After the habituation period, hair and serum data were collected at 28-day intervals from d 0 to 84. Hair was collected from a 20 × 30 cm rectangle over the area of the rump where the rump fat ultrasound was done. The same rectangle was clipped each time to show testosterone in only the hair that grew in since the previous hair collection. Thus, for each hair collection following the first, hair samples were representative only of testosterone levels relevant from the past 28 day period. Electric clippers with a #40 blade were used for the shaving and were cleaned with absolute ethanol after each bull was clipped. For blood sampling, a minimum of 10 mL of blood was drawn from the tail vein upon delivery and on hair collection dates.

Temperament data collected included pen score, order through the chute, time to leave the chute, and exit velocity. Pen scores ranging from 1 to 5 were assigned to all bulls by a single observer on d -1, 27, 55, and 83. After allotting individuals randomly within the pens in groups

of 3 to 5 bulls, the observer approached each bull for 30 s and determined its pen score, with 1 being the score assigned to the most docile or approachable animals and 5 being the score given to the most aggressive or fearful animals. The next day, bulls were run through the chute system, and the order of entrance was recorded, along with the amount of time it took for each bull to leave the chute after opening the head gate (Time 1). Exit velocity data were collected using two infrared sensor beams set at 1.83 m apart to clock the time it took each bull to cover that given distance. Overall temperament was calculated as a function of pen score and exit velocity on each test date (pen score + exit velocity / 2).

Body weight was measured on d 0, 28, 56, and 84. From these values average daily gain over each 28 day period was calculated, along with an overall average daily gain. Live animal ultrasound measures were recorded on d 85 and analyzed for backfat thickness, ribeye area, and intramuscular fat percentage on each bull. Final reproductive evaluation was also performed on d 85. Semen was collected by electroejaculation and assessed for percent normal sperm, primary defects, secondary defects, gross motility, and sperm motility. Scrotal circumference was measured on d 84.

For analysis of the data, competitive binding assays were used for analysis of both hair and serum testosterone samples. A commercial ELISA procedure (Salimetrics, Philadelphia, Pennsylvania, USA) was used for measuring low testosterone concentrations in the collected hair samples. Radioimmunoassay (RIA) kits (ICN Biomedicals, Inc., Costa Mesa, California) were employed for serum testosterone analysis. Statistical analysis of data was performed in SAS 9.3, with focus on Spearman correlations among variables. Correlations of  $r > 0.3$  or  $r < -0.3$  were considered statistically significant and reported as such.

## Results

Spearman correlations showed that bull hair testosterone concentrations significantly positively correlated with serum testosterone levels on day 0 ( $r=0.50$ ,  $P<0.005$ ), d 28 ( $r=0.50$ ,  $P=0.005$ ), and d 56 ( $r=0.30$ ,  $P=0.12$ ). Hair testosterone was not correlated with serum testosterone on d 84 ( $-0.3<r<0.3$ ). Significant results ( $P<0.05$ ) are summarized in Table 1 below.

*Table 1. Spearman correlations between measures of testosterone concentrations in serum and in hair.*

<b>Variable 1</b>	<b>Variable 2</b>	<b>Correlation</b>	<b>P-Value</b>
Serum d 0	Hair d 0	0.50	<0.01
Serum d 28	Hair d 28	0.50	<0.01

Neither hair nor serum testosterone concentrations were found to be significantly related to most of the experimental measures of temperament. Across all test dates, no correlations were reflected for either measure of testosterone with overall temperament, pen scores, latency to exit chute (Time 1), or order of entrance into the chute. Hair testosterone did exhibit a moderate negative correlation with exit velocity on d 56 ( $r= -0.40$ ,  $P<0.05$ ) and on d 84 ( $r= -0.34$ ,  $P=0.11$ ). Serum did not follow the same trend, as it was unrelated to exit velocity across all dates. These results are summarized in Table 2 below.

*Table 2. Relevant correlations between measures of temperament and testosterone production*

<b>Variable 1</b>	<b>Variable 2</b>	<b>Correlation</b>	<b>P-Value</b>
Exit velocity d 56	Hair testosterone d 56	-0.40	0.03

Overall, testosterone in hair and serum related moderately to a variety of growth and live ultrasound traits. Overall average daily gain (ADG) correlated positively with serum testosterone concentrations on d 0 ( $r=0.27$ ,  $P=0.15$ ), d 56 ( $r=0.44$ ,  $P=0.02$ ) and d 84 ( $r=0.25$ ,  $P=0.21$ ). Hair testosterone was not correlated with overall ADG on any of the test dates. Concentrations of both serum and hair testosterone also showed positive correlations ranging from weak to strong with body weight. Hair testosterone correlated weakly to moderately with BW on d 0 ( $r=0.44$ ,  $P=0.02$ ), d 28 ( $r=0.47$ ,  $P<0.01$ ), and d 56 ( $r=0.25$ ,  $P=0.19$ ). Serum testosterone was moderately to strongly correlated with BW on d 0 ( $r=0.66$ ,  $P<0.0001$ ), d 28 ( $r=0.59$ ,  $P<0.001$ ), and d 56 ( $r=0.47$ ,  $P=0.01$ ). Live ultrasound traits showed some overall relationships on d 84, when hair testosterone concentrations correlated negatively with backfat thickness ( $r= -0.29$ ,  $P=0.18$ ) and serum testosterone levels correlated negatively with intramuscular fat ( $r= -0.29$ ,  $P=0.16$ ).

Several measures of temperament also demonstrated links with growth traits, particularly average daily gain. Overall temperament on d 28 had a very weak positive correlation with ADG over the period from d 0 to d 28 ( $r=0.27$ ,  $P=0.14$ ), and temperament on d 84 similarly correlated with ADG between d 56 and 84 ( $r=0.27$ ,  $P=0.17$ ). Additionally, exit velocity was often linked with ADG. Overall ADG positively correlated with exit velocity on d 28 ( $r=0.40$ ,  $P=0.03$ ) and d 56 ( $r=0.45$ ,  $P=0.02$ ). Values for exit velocity on d 28 moderately correlated with ADG over the period from d 0 to 28 ( $r=0.46$ ,  $P=0.01$ ), and d 84 exit velocity weakly correlated with ADG from d 56 to 84 ( $r=0.28$ ,  $P=0.14$ ). Order of entrance into the chute system on d 0 displayed a moderate negative correlation with overall ADG ( $r= -0.45$ ,  $P=0.02$ ). Outside of ADG, a few other growth traits were also connected to gauges of docility. On d 56, more docile bulls tended to weigh more. This was indicated by the negative correlation between body weight

and temperament scores on d 56 ( $r = -0.30$ ,  $P = 0.11$ ) and, likewise, by the negative correlation between pen scores and BW on d 56 ( $r = -0.29$ ,  $P = 0.13$ ). Lastly, bulls deemed more docile according to lower assigned pen scores on d 84 showed greater ribeye area in the live ultrasounds ( $r = -0.48$ ,  $P < 0.01$ ). The results for these relationships are listed below in Table 3.

*Table 3. Relevant correlations between measures of temperament and growth performance.*

<b>Variable 1</b>	<b>Variable 2</b>	<b>Correlation</b>	<b>P-Value</b>
Exit velocity d 28	ADG d 0-28	0.46	0.01
Order of Entrance d 0	ADG d 0-84	-0.45	0.02
Exit velocity d 28	ADG d 0-84	0.40	0.03
Exit velocity d 56	ADG d 0-28	0.45	0.02
Pen score d 84	Ribeye area	-0.48	0.01

Reproductive parameters were inconsistent in their relationships with measures of temperament, with some being enhanced and others being depressed in temperamental bulls. Overall temperament on d 84 correlated positively with primary sperm defects ( $r = 0.27$ ,  $P = 0.19$ ) detected in the final breeding soundness examination but correlated negatively with secondary sperm defects ( $r = -0.36$ ,  $P = 0.07$ ). Pen scores on d 84 also significantly negatively correlated with secondary sperm defects ( $r = -0.31$ ,  $P = 0.13$ ). Similarly, pen scores on d 84 correlated negatively, although not quite significantly, with scrotal circumference on d 84 ( $r = -0.28$ ,  $P = 0.15$ ). Mixed results also occurred in relationships between time to exit the chute and breeding measures. On d 84, time to exit the chute correlated positively with percentage of normal sperm ( $r = 0.39$ ,  $P = 0.05$ ), negatively with primary sperm defects ( $r = -0.34$ ,  $P = 0.09$ ), and

positively with secondary sperm defects ( $r=0.37$ ,  $P=0.07$ ). These results are summarized in Table 4 below.

*Table 4. Relevant correlations between measures of temperament and reproductive soundness parameters.*

<b>Variable 1</b>	<b>Variable 2</b>	<b>Correlation</b>	<b>P-Value</b>
Temperament d 84	Secondary defects	-0.36	0.07
Order of entrance d 84	Sperm motility	0.40	0.05
Time to exit chute d 84	Normal sperm	0.39	0.05
Time to exit chute d 84	Secondary defects	0.37	0.07

Temperament traits were shown to be significantly linked to a few measures of behavior as well. Lying bout duration on d 84 positively correlated with d 84 EV ( $r=0.31$ ,  $P=0.10$ ), and lying bout duration on d 0 positively correlated with overall temperament scores that day ( $r=0.30$ ,  $P=0.17$ ). On d 84, total time spent lying down positively correlated with that day's pen scores ( $r=0.30$ ,  $P=0.12$ ) and overall temperament scores ( $r=0.30$ ,  $P=0.12$ ). Lastly, on d 56, pen scores positively correlated with total steps taken in a day ( $r=0.37$ ,  $P=0.04$ ). Table 5 below outlines these results.



*Table 5. Relevant correlations between measures of temperament and behavioral patterns.*

<b>Variable 1</b>	<b>Variable 2</b>	<b>Correlation</b>	<b>P-Value</b>
Pen score d 56	Step sum d 56	0.37	0.04

Growth performance also exhibited some associations with reproductive measures. SC on d 84 positively correlated with overall ADG ( $r=0.30$ ,  $P=0.13$ ), with ADG over the period from d 56 to 84 ( $r=0.36$ ,  $P=0.05$ ), and with BW on d 84 ( $r=0.46$ ,  $P=0.01$ ). BW on d 84 also positively correlated with the percentage of normal sperm ( $r=0.57$ ,  $P<0.01$ ) and negatively correlated with primary sperm defects ( $r= -0.51$ ,  $P<0.01$ ).

Scrotal circumference on d -14 strongly correlated with SC on d 84 ( $r=0.62$ ,  $P<0.01$ ). Hair testosterone had a strong negative correlation with secondary defects in semen ( $r= -0.58$ ,  $P<0.01$ ). Likewise, cumulative hair testosterone concentrations displayed a moderate negative correlation with secondary sperm defects ( $r= -0.37$ ,  $P=0.12$ ).

## **Discussion**

We found that testosterone concentrations in bull hair and blood serum correlated strongly on d 0 and 28, then moderately on d 56. Our results echo Yang's finding that steroid hormone levels can be as consistently measured in hair as they can in blood serum in humans (2008). In our study on cattle, although hair and serum testosterone data collected on d 84 showed no significant correlation, the results from the first three test dates suggest a clear trend of concentrations of hair testosterone increasing alongside those for serum in bulls. This

indicates that hair may serve as a non-invasive substitute for serum in assessing chronic testosterone concentrations in cattle, although more research would be necessary to confirm its reliability. By reducing risks for both animals and handlers, hair evaluation could potentially provide a superior method of endocrine analysis in cattle than those that have previously been employed.

Our study also showed that bulls with higher hair testosterone concentrations had significantly slower exit velocities on d 56 and 84. Geburt et al. saw the same pattern in their examination of relationships between cattle testosterone levels and temperament measures, where it was shown that elevated salivary testosterone concentrations correspond with slower exit velocities (2015). Thus, our results do agree with previous research, so perhaps hair testosterone concentrations could provide a predictor for cattle temperament as indicated by exit velocity, with higher hair testosterone suggesting more docile temperament in bulls. However, since serum testosterone did not significantly correlate with exit velocity on any of the test dates, further research would be needed to firmly establish that exit velocity consistently relates to measured testosterone concentrations across all different bodily substances.

With regard to relationships between growth performance and hair and serum testosterone, we found that bulls with higher serum testosterone concentrations had greater overall average daily gain from d 0 to 84, as expected. Sitarz et al. showed that the same trend exists between ADG and plasma testosterone concentrations in bulls (1977). We also found that heavier bulls had higher testosterone concentrations in both hair and serum. While this may have some implications about the effects of testosterone on muscle growth, we attribute this in part to the fact that our study examined yearling bulls, many of which may have reached puberty during the testing window. It is well established that pubertal changes include elevated testosterone

secretions (Amann and Walker, 1983). Therefore, in our study, it was unsurprising that bulls' body weight increased along with testosterone concentrations, as those bulls with higher testosterone may have been post-pubertal and much likely to be heavier than their immature contemporaries. In addition, our study showed that bulls with higher hair testosterone concentrations exhibited lower backfat thickness, and those with higher serum concentrations had lower intramuscular fat. Gortsema et al. also observed less trimmable fat in bulls than steers. In combination, the continued trend of these findings indicates that body fat is lower in the presence of higher testosterone. Interestingly, however, the only relationship between testosterone concentrations and reproductive characteristics that our study revealed was that bulls with higher cumulative hair testosterone concentrations had fewer secondary sperm defects. Although testosterone is often intimately linked to reproduction, the circa-pubertal age of the animals in this study may have confounded the results in this area, since most bulls were just approaching or had only recently undergone puberty at the time of the breeding soundness examination.

Contrary to prior literature demonstrating reduced growth in more temperamental cattle than in docile ones, our study found that bulls with both higher overall temperament scores and faster exit velocities achieved greater average daily gain. Here again, the pubertal age of bulls in this study may have played a role by affecting temperament over time. It is likely that immature, pre-pubertal bulls were more temperamental than those that had already reached puberty and matured more in terms of disposition. At the same time, pre-pubertal bulls may have experienced greater growth rates, or higher average daily gain, than post-pubertal bulls, who could have had less room left to grow being already closer to their mature weight. Additionally, with part of their energy being devoted to maintaining sexual function rather than physical

growth, perhaps post-pubertal bulls grew more slowly for this reason as well. This explanation would also make sense tying into our finding that bulls that were deemed more docile by lower overall temperament scores tended to weigh more.

Like Fordyce et al.'s finding that temperament is not associated with reproductive measures such as scrotal circumference (1996), we found that there were very few relationships between temperament and reproductive parameters. Even the significant relationships seemed to follow no consistent pattern. Primary sperm defects were higher in bulls that were more temperamental in terms of overall temperament score on d 84, perhaps again because more immature bulls, who would be more prone to produce defective sperm, exhibited less docile dispositions. Unexpectedly, however, bulls that were more temperamental according to d 84 pen scores had fewer secondary sperm defects, a result that appears inconsistent with the finding for primary sperm defects. Bulls assigned more temperamental pen scores on d 84 also tended to have lower scrotal circumference, although the figure was not quite statistically significant. This made sense in accordance with Burrow's study showing that scrotal circumference was lower in bulls with higher flight speed scores. Also, as predicted by the hypothesis that post-pubertal bulls were more docile in temperament, bulls that were more docile in being slower to exit the chute had higher percentages of normal sperm and lower prevalence of primary sperm defects. However, results deviated from expectation when bulls that were slower to exit the chute had a higher presence of secondary sperm defects. Overall, it appears that few relationships between bull temperament and reproduction exist, and those that do are not necessarily likely to persist over time. A great deal more research would be required to draw conclusions on these relationships in bull cattle.

Bull behavior tended to be less active, as indicated by longer lying bout duration, as disposition became more temperamental, as suggested by both exit velocity and temperament scores. Likewise, bulls that were more temperamental with regard to pen scores and overall temperament scores spent more total time lying during the day, possibly because those more temperamental bulls that spent more time lying were less mature and required more time resting. Our findings contradict Wierenga's experimental conclusion that more temperamental bulls spent less time lying (1987). However, because we did not see this relationship persist across multiple dates, it would perhaps be unsafe to draw conclusions on these relationships between cattle behavior and temperament without further research on the subject. In closer alignment with what we might expect, bulls in our study that were more temperamental by pen score took more steps within a day.

When examining relationships between growth performance and reproduction traits, our results supported our hypothesis. We found that bulls with higher scrotal circumference on d 84 tended to have greater periodic and overall average daily gain, implying that selecting bulls for larger SC may yield faster growing animals. This also coincides with Schenkel et al.'s experimental finding that SC values correlated with ADG across multiple breeds of growing bulls (2004). Additionally, in our study, bulls with greater body weights displayed greater percentages of normal sperm and fewer primary sperm defects, which makes sense in light of the fact that heavier bulls were likely more mature and thus more reliable in their sexual functioning.

Overall, our research suggests that hair analysis could serve as a noninvasive substitute for blood serum in evaluating testosterone concentrations in cattle. In addition, bull temperament in our study was better related to growth performance traits than to behavior and reproductive characteristics, which show more inconsistency among relationship patterns.

However, with the possibly confounding effects that the pubertal age of our bull subjects appeared to cause in our project, it would be beneficial to repeat a similar study in a group of older bulls that were known to have already attained puberty and test to see if the results were the same in senior bulls. Lastly, since testosterone concentrations were consistently lower in cattle who were deemed more temperamental with regard to exit velocity, it seems that hair testosterone may indeed provide an objective and noninvasive method of predicting cattle temperament.

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