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Use of endocrine and immune responses as predictors of bull sperm motility

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ABSTRACT

Research has shown that peripheral blood cell populations change in response to an immune challenge, and hormone concentrations directly affect sperm characteristics. The objective of this study was to utilize immune responses and hormone concentrations as biomarkers for yearling bull sperm motility. Seventeen Brahman-influenced bulls (mean age 1.1 ± 0.1 yr; body weight 478 ± 38 kg) were administered an intraperitoneal injection of lipopolysaccharide (*Salmonella typhimirium* 0.7 μg/kg of body weight). Blood was collected 0, 3, 6, 9, and 24 h after LPS injection then analyzed for differential cell count and endocrine concentrations of prolactin, insulin-like growth factor-1 (IGF), and cortisol. Semen was collected using electroejaculation every month for five months then analyzed for motility and morphology characteristics. Hormone concentrations and immune response had an effect on sperm characteristics. Number of sperm was correlated ($r > 0.65$; $P < 0.01$) with the IGF to prolactin ratio. Using stepwise regression analysis, we predicted that number of sperm = 172.43 + 12.8 (IGF:prolactin), $r^2 = 0.43$, and progressive sperm motility = -1469.6 + 1.63 (IGF:cortisol) + 14.41 (average temperature during immune challenge), $r^2 = 0.43$. This study showed that endocrine response to stress and activation of the immune system was associated with subsequent sperm motility characteristics. Our results suggest that endocrine and immune responses may be used as biomarkers for sperm motility. Those biomarkers may be useful in selecting replacement bulls.

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MEET THE STUDENT-AUTHOR

Lydia Mitchener

I am from St. Louis, Missouri and graduated from Kirkwood High School in 2011. After four years at the University of Arkansas, I graduated in 2015 with a Bachelor of Science degree in Animal Science and a minor in Spanish. This fall, I am moving to Columbia, Missouri to pursue a Doctor of Veterinary Medicine degree at the University of Missouri.

During my undergraduate career I was a member of the Pre-Veterinary Club, Delta Delta Delta sorority, Global Greeks, National Society of Leadership and Success, and the Racquetball Club. I also worked at a local veterinary clinic, Animal Medical Clinic.

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INTRODUCTION

Production animals, like bulls, in addition to experiencing considerable amounts of stress from the environment, experience stress internally due to infections that can negatively or positively affect their fertility. Research has shown that immune cells increase from an immune response, and hormone concentrations directly affect sperm characteristics (Jones and Mann, 1976; Hansson et al., 1989; Grattan et al., 2007). The objective of this study was to identify biomarkers for yearling bull sperm motility associated with endocrine response and activation of the immune system.

One of the most essential mechanisms in an animal's body is the immune system, a defensive composition of cells that maintain health (Erich, 2015). It protects animals against foreign pathogens caused by parasites, bacteria and viruses (Lin et al., 2014). There are various immune cells that make up the immune response. White blood cells, also referred to as leukocytes, are the primary immune cells. Leukocytosis can be an indicator that an animal has a disease, an infection, or cancer (Carroll and Burdick Sanchez, 2014). Types of leukocytes are lymphocytes, neutrophils, basophils, eosinophils, and monocytes. Leukocytes have various functions, including phagocytosis, inflammation, and regulation of adaptive immunity. In this study, an immune response was caused by lipopolysaccharide (LPS). Lipopolysaccharide is a membrane component from gram-negative bacteria that signals inflammation and destruction of tissues (Gao et al., 2015). The body interprets LPS as a microorganism invasion so the neutrophils move to tissues or lymph nodes (Carroll and Burdick Sanchez, 2014).

During stressful times, it is very important for the animal to maintain homeostasis using the stress axis. Cortisol is considered the primary stress steroid hormone and it is released from the adrenal cortex in response to environmental stress (Carroll and Burdick Sanchez, 2014; Hopster et al., 2002). Increased cortisol concentrations and white blood cell count is an acute stress response (Chase et al., 1995).

Insulin-like growth factor (IGF) is a hormone primarily involved in growth and development. Insulin-like growth factor is released by the liver under the direction of growth hormone (GH), which also plays a large role in protein synthesis (Mitra et al., 1972). Secretion of IGF varies depending on many factors, including age and stress. In bulls, it is at its highest concentration at birth, and decreases as the bull gets older (Purchas et al., 1970; Trenkle, 1971). During stress or immune challenge, GH is released, causing IGF to increase (Bernton et al., 1987). Testosterone is a sex steroid hormone that is immunosuppressive (Bernin and Lotter, 2014). The primary effect of testosterone and other sex hormones is the reduction of immature T lymphocytes (Giefing-Kroll et al., 2014). Prolactin is a hormone that decreases during stress or immune system activation (Bernton et al., 1987).

There are many traits that contribute to a bull's overall fertility including sperm characteristics. Scrotal circumference, sperm motility, and sperm morphology all largely affect a bull's reproductive capability (Sylla et al., 2007). Scrotal circumference has a positive relationship with sperm quality in beef cattle (Lunstra et al., 1978). Quality sperm is important in order to fertilize an egg. In one study, for instance, researchers found that the percentage of normal spermatozoa had the greatest influence on the calf crop percentage (Fitzpatrick et al., 2002). Sperm motility is important because in mammals, spermatozoa cannot fertilize an egg unless they achieve hyperactivated motility (Yanagimachi, 1994).

If bulls have reduced fertility, this negatively affects their reproductive and production value, creating economic strain for farmers. In order to improve production and selection processes, we need to have a better understanding of the relationships between immune response, hormone concentrations, and fertility in bulls. Our hypothesis was that a bull's immune response would serve as a predictor of future fertility.

MATERIALS AND METHODS

Description of Animals. The committee for animal welfare at the USDA-ARS, Dale Bumpers Small Farms Research Center in Booneville, Ark., and the University of Arkansas Institutional Animal Care and Use Committee approved the animal procedures used in this study. Seventeen Brahman-influenced bulls were kept near Booneville, Ark. at The Dale Bumpers Small Farms Research Center. They had a mean age of 1.1 ± 0.1 year and a mean body weight of 478 ± 34 kg at the time of immune challenge.

Blood Collection and Immune Challenge. An intraperitoneal injection of LPS derived from *Salmonella typhimirium* (0.7 µg/kg of body weight) was given in front of the right hip bone, pointed posteriorly and ventrally. Blood was collected 0, 3, 6, 9, and 24 h after LPS injection using EDTA vacuum tubes and serum separator tubes.

Assays. Whole blood samples were analyzed for differential cell count on a Cell-Dyn 3500 (Abbott Diagnostics, Abbott Park, Ill.). Concentrations of prolactin, insulin-like growth factor-1 (IGF), and cortisol were quantified using validated radioimmunoassays (D. Hallford, New Mexico St. Univ.). Hormone concentration ratios were calculated for IGF to cortisol, IGF to prolactin, and prolactin to cortisol.

Sperm Collection and Evaluation. Semen was collected by electroejaculation using an Electroejac IV (Ideal Instruments/Neogen Corp., Lansing, Mich.) every month beginning in February when the bulls were yearlings. Ejaculates were placed in a water bath maintained at 35.5 °C in 15-mL conical centrifuge tubes. Within 30 min of collection, semen samples were diluted 20:1 in Dulbecco's Phosphate-Buffered Saline and sperm motility evaluated using a Hamilton Thorne IVOS computerized sperm analysis system (Hamilton-Thorne Biosciences, Beverly, Mass.). We evaluated motility and morphology characteristics listed in Table 1 using Animal Motility Software, v. 12.1 in 10 different fields to determine averages for sperm characteristics. Thirty video frames were captured

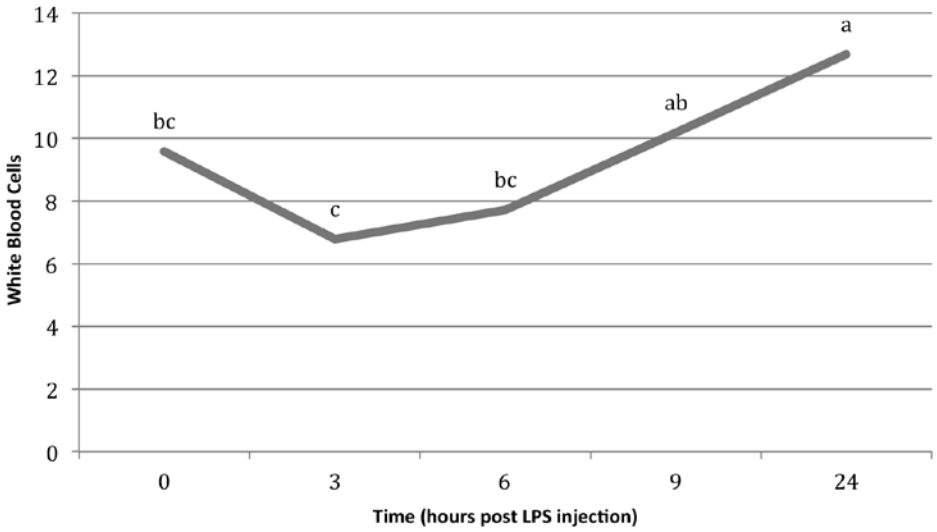

Fig. 1. Number (x 1000) of circulating white blood cells following LPS injection. Time affected ($P < 0.001$) white blood cell concentration. a,b,c = means without common superscript differ ($P < 0.05$; SE ± 1.06).

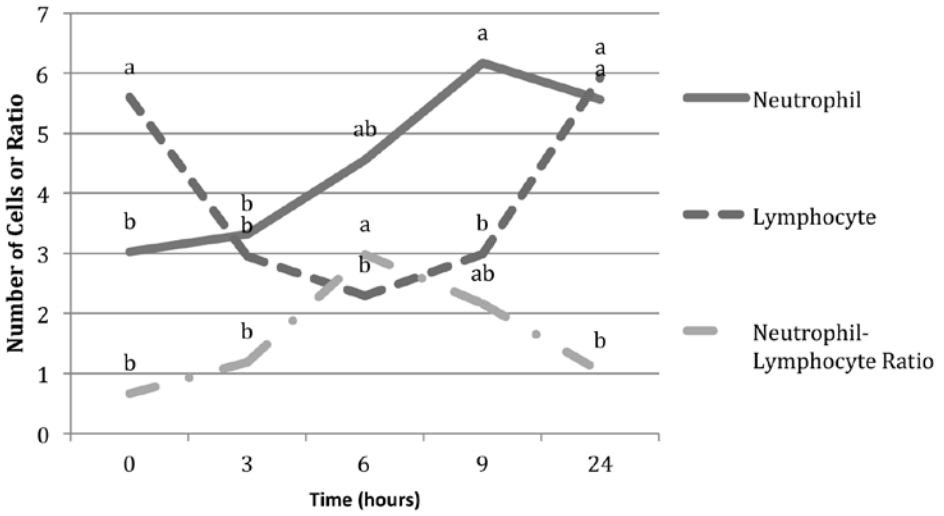

Fig. 2. Number (x 1000) of circulating neutrophils, lymphocytes, and their ratio following LPS injection. Time affected ($P < 0.001$) cell concentrations and ratio. a,b = means, within a cell type or ratio, without common superscript differ ($P < 0.05$; neutrophils SE ± 0.728; lymphocytes SE ± 0.473; neutrophil:lymphocyte SE ± 0.515).

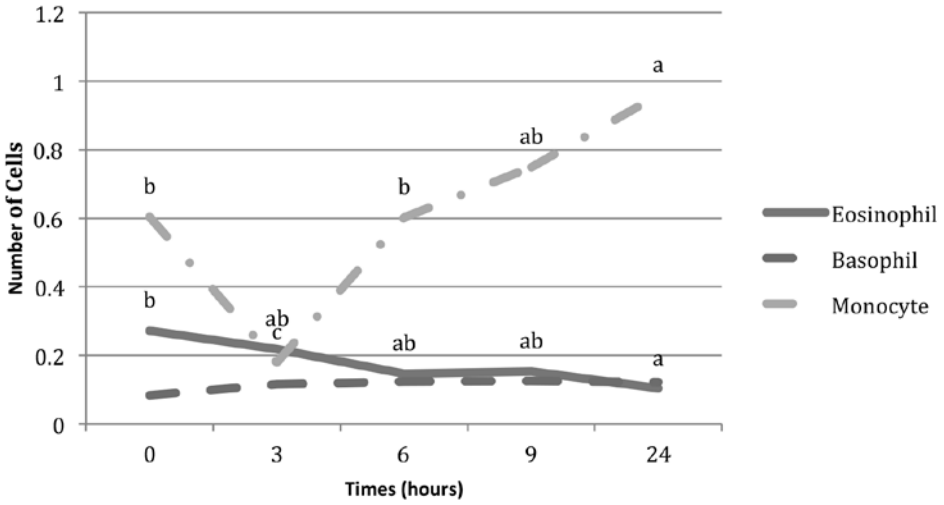

Fig. 3. Number (x 1000) of circulating eosinophils, basophils, and monocytes following LPS injection. Time affected ($P < 0.001$) eosinophil, and monocyte concentrations. a,b,c = means, within a cell type, without common superscript differ ($P < 0.05$; eosinophils SE ± 0.049; basophils SE ± 0.017; monocytes SE ± 0.09)..

Table 1. Effects of month of collection on spermatozoa characteristics.

	Month						
Item†	February	March	April	May	June	SE‡	*P* <
Sperm	229.9[b]	127.7[b]	171.7[b]	562.5[a]	609.5[a]	98.9	0.001
Area	5.2[a]	4.7[b]	4.8[b]	4.7[b]	4.7[b]	0.08	0.001
Live	38.3[a]	63.3[b]	62.2[b]	74.2[bc]	80.8[c]	4.9	0.001

† Sperm = millions of spermatozoa/mL of ejaculate); Area = size of sperm heads (μm^2); Live = percentage of live spermatozoa.

‡ SE = mean of standard errors.

[ab] Means within rows without common superscripts differ ($P < 0.05$).

within each field in order to analyze sperm motility. An eosin-nigrosin-based live-dead stain (Jorvet Stain, Jorgensen Laboratories, Lovelend, Colo.) was used to fix and evaluate spermatozoa for morphology. Each slide had approximately 100 spermatozoa and was analyzed for percentage live (dye exclusion) and dead.

Statistical Analysis. Data were analyzed using SAS procedures (SAS Institute, Inc., Cary, N.C.). Time was treated as a repeated measure and bull was the subject in the analysis of variance. Stepwise regression was used to determine the relationship between and among different measures of immune function and hormone concentrations on sperm characteristics.

RESULTS AND DISCUSSION

Time after LPS injection had an effect ($P < 0.05$) on the immune cell distribution. Figures 1-3 present the effects of time after LPS injection on immune response. White blood cells (WBC), neutrophils, lymphocytes, the neutrophil-lymphocyte ratio, monocytes, eosinophils, and basophils were affected ($P < 0.05$) by time after LPS.

Table 2. Correlations between weaning physiology and sperm characteristics.

		Sperm Characteristic†		
		Sperm	Progressive	Live
Item‡	Mean	448	44.7	72.3
Prolactin	23.1	-0.55*	-----	-----
I:P	22.5	0.65**	-----	-----
P:C	0.85	-0.53*	-----	-----
I:C	11.5	-----	0.53*	0.53*

*$P < 0.05$.

**$P < 0.01$.

† Sperm = millions of sperm per milliliter of ejaculate averaged over five collections; Progressive = percentage of spermatozoa moving at path velocity ≥ 50 µm/sec and straightness ≥ 70%; Live = percentage of spermatozoa that were deemed alive by excluding dye; dashed lines indicate that the correlation was not significant ($P > 0.1$).

‡ Prolactin (ng/mL), I:P = IGF1/prolactin; P:C = prolactin/cortisol; I:C = IGF1/cortisol

Table 1 shows the effects of time on sperm characteristics. As bulls aged and matured, both the number of spermatozoa and the percentage of live spermatozoa increased ($P < 0.001$). However, sperm head area decreased ($P < 0.001$) as the bulls aged.

Table 2 presents the correlation between traits collected at weaning and their relationship with average sperm characteristics. Number of sperm was correlated positively ($r > 0.65$; $P < 0.01$) with IGF:prolactin and negatively ($r \geq -0.53$; $P < 0.05$) with prolactin (ng/mL) and prolactin:cortisol. This coincides with research reporting that hyperprolactenemia caused infertility (Grattan et al., 2007). The percentage of spermatozoa that were alive, and had progressive motility was correlated ($r = 0.53$; $P < 0.05$) with IGF:cortisol. This corresponds with past research that states IGF enhances spermatogonial DNA production, therefore improving sperm number and function (Hansson et al., 1989). Few correlations existed between blood cell characteristics and sperm characteristics. Mean number of spermatozoa per ejaculate was correlated ($r > 0.51$; $P < 0.05$) with mean red blood cell hemoglobin concentration (MCHC). Research connecting MCHC with fertility is very limited. It is known that hemoglobin prevents oxidative damage, which damages sperm (Carroll and Burdick Sanchez, 2014). Therefore, an increase in MCHC should have a positive effect on fertility.

Using stepwise regression analysis, we predicted the variance for number of sperm, progressive, and path velocity (VAP). The following relationships were determined:

number of sperm = 172.43 + 12.8 (IGF:prolactin), $r^2 = 0.43$;

progressive sperm = -1469.6 + 1.63 (IGF:cortisol) + 14.41 (average temperature during immune challenge), $r^2 = 0.43$;

VAP = -337.52 + 0.846 (age, days at weaning) 0.41 (IGF, ng/mL) + 8.39 (cortisol, ng/mL) + 13.1 (IGF:cortisol) + 3.29 (lymphocyte number / 1000), $r^2 = 0.84$.

Just like all animals, cattle depend on their immune response in order to survive. The typical im-

mune response in bulls causes an increase in immune cells and changes in hormone concentrations that can affect their fertility. For example, spermatogonial DNA is synthesized in response to IGF; therefore, increases in IGF positively affect fertility (Hansson et al., 1989). On the other hand, previous findings show negative relationship between leukocytes and sperm function (Jones and Mann, 1976), and prolactin and fertility (Grattan et al., 2007). An increase in leukocytes damaged sperm (Jones and Mann, 1976) through reactive oxygen species (ROS) that inhibit ATP production (Villegas et al., 2005; De Lamirande and Gagnon, 1992). When ATP production is decreased, sperm function and motility are affected negatively, resulting in infertility (Pentyala et al., 2007). If an animal exhibits an overproduction of prolactin, it is said to have hyperprolactinemia, which is a cause of infertility (Grattan et al., 2007).

This study showed that endocrine response to stress and activation of the immune system was associated with subsequent number of spermatozoa and motility characteristics. Our research results suggest that hormone concentrations at weaning combined with bull response to an immune challenge may be useful in selecting replacement bulls that have greater fertility.

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