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Use of Endocrine and Immune Responses as Predictors of Bull Sperm Motility

An Undergraduate Honors Thesis

in the

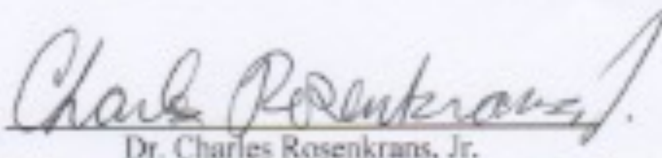
Department of Animal Science

Submitted in partial fulfillment of the requirements for the
University of Arkansas
Dale Bumpers College of Agricultural, Food and Life Sciences
Honors Program

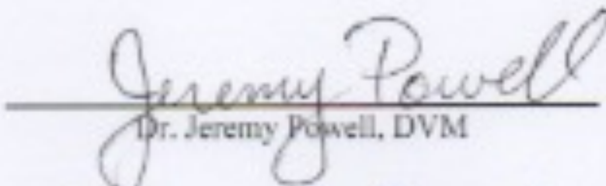
by

Lydia Mitchener

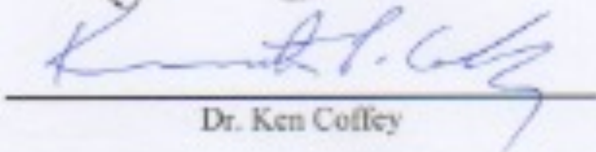
May 2015



Dr. Charles Rosenkrans, Jr.



Dr. Jeremy Powell, DVM



Dr. Ken Coffey

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Abstract

Research has shown that immune cells increased from an immune response, and endocrine concentrations directly affect sperm characteristics (Jones and Mann, 1976; Hansson et al., 1989; Grattan et al., 2007). Previous findings show a negative relationship between leukocytes and sperm function (Jones and Mann, 1976) and prolactin and fertility (Grattan et al., 2007). On the other hand, research has shown a positive relationship between insulin-like growth factor (**IGF**) and sperm characteristics (Hansson et al., 1989). The objective of this study is to identify biomarkers for yearling bull sperm associated with endocrine response and activation of the immune system.

Seventeen Brahman-influenced bulls (mean age 1.1 ± 0.1 yr; BW 478 ± 38 kg) were administered lipopolysaccharide (**LPS**) (*Salmonella typhimirium* 0.7 ug/kg of body weight) intraperitoneally. Blood was collected using EDTA vacuum tubes and serum separator tubes 0, 3, 6, 9, and 24 hours after LPS injection. The blood was analyzed for differential cell count on a Cell-Dyn 3500 (Abbott Diagnostics, Abbott Park, IL). Phase Haptoglobin Assay from Tridelta Development Ltd (Kit # TP 801) was used to determine Haptoglobin concentration. Concentration of the hormones prolactin, testosterone, insulin-like growth factor (**IGF**), and cortisol were quantified using validated radioimmunoassays (Hallford, New Mexico University).

Semen was collected using electroejaculation with an Electroejac IV (Ideal Instruments/Neogen Corp., Lansing, MI) every month for five months. Sperm was analyzed for motility and morphology characteristics listed in Table 1 using Animal Motility Software, version 12.1, in 10 different fields to analyze sperm motility. An eosin-nigrosin-based live-dead stain (Jorvet Stain, Jorgensen Laboratories, Loveland, CO) was used to fix and evaluate sperm for morphology. Data was then analyzed using SAS procedures (SAS Inst., Inc., Cary, NC).

Time was treated as a repeated measure and bull was the subject in the analysis of variance. Stepwise regression was used to predict sperm characteristics.

Endocrine responses to stress and immune response had an effect on sperm characteristics. At weaning, certain endocrine levels and sperm characteristics were correlated. Progressive, rapid, live, dead, and live % were correlated ($r > 0.51$; $P < 0.05$) with the IGF-1/cortisol ratio (**IC**). Number of sperm was correlated ($r > 0.65$; $P < 0.01$) with the IGF-1/prolactin ratio (**IP**). Medium speed was correlated ($r > 0.50$; $P < 0.05$) with the cortisol/testosterone ratio (**CT**). Number of sperm was negatively correlated with prolactin ($r < -0.55$; $P < 0.05$) and the prolactin/cortisol ratio (**PC**) ($r < -0.53$; $P < 0.05$).

When the immune challenge through LPS was administered, the immune response had an effect on sperm characteristics. Slow speed and area of sperm heads were correlated with total white blood cell count (**WBC**) ($r > 0.50$; $P < 0.05$). Slow speed was also correlated with neutrophil concentrations ($r > 0.58$; $P < 0.05$). Number of sperm was correlated ($r > 0.51$; $P < 0.05$) with mean cell hemoglobin concentration (**MCHC**). Straightness was negatively correlated ($r < -0.62$; $P < 0.01$) with WBC and neutrophils. Linearity was negatively correlated ($r < -0.53$; $P < 0.05$) with WBC and lymphocytes. Straightness was also negatively correlated ($r < -0.55$; $P < 0.05$) with lymphocytes.

Using regression analysis we predicted what caused 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), $r^2 = .43$; progressive sperm = -1469.6 + 1.63 (IGF/cortisol) + 14.41 (average temperature during immune challenge), $r^2 = .43$; VAP = -337.52 + 0.846 (age) - 0.41 (IGF, ng/mL) + 8.39 (cortisol, ng/mL) + 13.1 (IGF/cortisol) + 3.29 (lymphocyte number x 1000), $r^2 = .84$.

This study showed that endocrine response to stress and activation of the immune system caused differences in number of sperm, progressive sperm, amount of rapid, medium, and slow sperm, percentage of live and dead sperm, straightness and linearity of sperm, and area of sperm heads.

Introduction

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). The immune response is divided into two categories: innate and adaptive immunity (Medzhitov and Janeway, 1997). Innate immunity is nonspecific and is the body's first line of defense (Akira et al., 2006). It is comprised of humoral components and various immune cells (Benito-Martin et al., 2015). The innate immune response defends the body by signaling phagocytosis, cell lysis, and secretion of signaling molecules (Giefing-Kroll et al., 2014). Adaptive immunity is a group of specialized cells used to prevent or restrict specific pathogens (Lin et al., 2014). The adaptive immune response defends the body using cytokines and antibodies (Giefing-Kroll et al., 2014). The most important difference between innate and adaptive immunity is that adaptive immunity has a memory, meaning, upon secondary exposure to a pathogen, the body will have a better immune response (Lin et al., 2014).

Signaling molecules released to aid in communication during the immune response are called cytokines (Belardelli, 1995; Rothwell, 1997). Cytokines control the immune response based on the duration and intensity of exposure to a specific pathogen. There are two categories of cytokines: pro-inflammatory and anti-inflammatory (Banks et al., 1994; Navikas and Link, 1996). Anti-inflammatory cytokines return our bodies to normal while pro-inflammatory cytokines respond to infection and cause inflammation (Erich, 2015; Vels et al., 2009). Three pro-inflammatory classes of cytokines are tumor necrosis factors (**TNF**), interleukins (**IL**), and interferons. The function of TNFs is to destroy abnormal cells, activate other cytokines, promote

movement of lymphocytes to site of infection, and promote swelling and pain (Erich, 2015; Medzhitov et al., 1997). There are two responsibilities of ILs: to limit the spread of infection by causing a fever, and to encourage inflammation, thereby drawing specialized immune cells to the infection site (Erich, 2015). Interferons signal nearby tissue to be defensive and engage killer cells (Erich, 2015). Another cytokine involved in the immune response are chemokines. Chemokines are small cytokines that signal lymphocyte movement from the bloodstream to the inflammation site (Nibbs and Graham, 2013). Cytokines play a very important role in maintaining the health of an animal because they signal different immune responses.

There are various immune cells that make up the immune response. Immune cells have different types of receptors that recognize infection and respond with cytokine release (Asea et al., 2002). 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. Neutrophils are phagocytic, meaning they invade and digest foreign invaders (Shannon et al., 2015). Basophils, eosinophils, macrophages, and mast cells are responsible for inflammation (Benito-Martin et al., 2015). Eosinophils function in allergy, cytotoxicity, and regulate adaptive immunity (Benito-Martin et al., 2015). Lymphocytes are only present in the adaptive immune response and are differentiated into either T or B cells (Russel et al., 1988). They are antigen specific and are directed by cytokines to the infection site (Medzhitov et al., 1997). Once at the infection site, T and B cells control cell-mediated and humoral immunity

through different functions. Antibodies are produced by B cells while helper T cells secrete IL that activates natural killer cells, monocytes, and other T and B cells (Ohtsuka et al., 2011).

Other important immune cells include mast cells and macrophages. The innate immune response depends on basophils, mast cells, neutrophils, and macrophages (Benito-Martin et al., 2015). Macrophages serve an important function in both the innate and adaptive immune response. There are two forms of macrophages: M1 macrophages, which inhibit, and M2 macrophages that heal (Mills, 2012). In the innate immune response, M1 and M2 macrophages are important in signaling and directing immune responses (Mills et al., 2015). The role of M1 and M2 in adaptive immunity is the direction of T and B cell responses (Mills, 2012).

Lipopolysaccharide and *Salmonella typhimurium*

In this study, we injected Brahman-influenced bulls with *Salmonella typhimurium* lipopolysaccharide (LPS). Lipopolysaccharide is a 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). To limit inflammation, phagocytosis by neutrophils occurs within 24 hours after infection (Savill et al., 1989). Toll-like receptors, which induce a proinflammatory signal, recognize LPS (Asea et al., 2002, Vabulas et al., 2002). *Salmonella* is a species of bacteria that goes to the intestinal tract and then reach macrophages on the lining of mucosal epithelium (Vazquez-Torres and Fang, 2001). In defense against the *Salmonella*, active macrophages make bacterial substances and produce parts of the pathogen on their surface (Braukmann et al., 2015).

Endocrine Responses to Stress

Research by Carroll and Burdick Sanchez (2014) has demonstrated that stress is a common problem in modern livestock production. During stressful times, it is very important for the animal to maintain homeostasis using the stress axis. Physiologically, there is a direct relationship between the body's response to stress and its response to immune challenge. In the past, many believe there was a negative relationship between stress hormones and immune response. However, this idea is being rejected more and more as research is reported showing stress hormones are not immunosuppressive. In fact, many believe it is possible to use stress hormones to positively affect immune response in animals.

The stress axis can be both beneficial and harmful depending on the duration and frequency of stress (Carroll and Burdick Sanchez, 2014). Response of the stress axis depends on whether the stimulus is an acute or chronic stress. Acute stress causes energy to navigate towards organs and tissues needed for stress and preparing the immune system for secondary infections (Carroll and Burdick Sanchez, 2014). Chronic stress causes complete suppression of the immune system (Carroll and Burdick Sanchez, 2014), making an animal more prone to disease.

The stress and immune response have many common physiological consequences (Carroll and Burdick Sanchez, 2014). According to Apanius (1998) and Moberg (2000), body temperature, blood flow, digestive capabilities, respiration and heart rates are all involved in the stress and immune response. The primary reason for this is that endocrine responses to stress have an effect on immune cells. For example, castrated bulls had increased cortisol and white blood cell counts after surgery was performed (Chase et al., 1995).

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). Increase in cortisol levels and white blood cell count is an acute stress response (Chase et al., 1995). During an infection, white blood cell count increases along with cortisol levels (Chase et al., 1995). Cortisol also has a large effect on the immune system by preparing the body for secondary infection (Carroll and Burdick Sanchez, 2014).

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 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). Along with stress, IGF increases during sexual stimulation (Borg et al., 1991). Spermatogonial DNA is synthesized in response to IGF-1; therefore, increases in IGF positively affect fertility (Hansson et al., 1989).

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). Sex steroids also suppress B cells in the bone marrow (Giefing-Kroll et al., 2014). Fimmel and Zouboulis (2005) suggest there is also an inverse relationship between testosterone and wound healing.

Prolactin is a hormone that decreases during stress or immune system activation (Bernton et al., 1987). It serves an important role in immunity as well as fertility. Prolactin increases the expression of natural killer cells and has an important role in lymphocyte function by controlling the development of T and B lymphocytes (Mavoungou et al., 2004; Russel et al., 1988). If an

animal exhibits overproduction of prolactin, it is said to have hyperprolactinemia. Hyperprolactinemia is a cause of infertility (Grattan et al., 2007).

Acute Phase Response

Acute phase response plays an important role in the immune and stress response. During infection, it is responsible for inflammation, fever, and leukocyte mobilization (Vels et al., 2009). Cytokines, IL-6, and TNF are part of the acute phase response (Vels et al., 2009). Acute phase proteins are important regulators of the immune system because they participate in tissue repair and remodeling (Carroll and Burdick Sanchez, 2014). One of the major acute phase proteins is haptoglobin, which is secreted in the liver when activated by IL-6 and TNF (Vels et al., 2009). Haptoglobin prevents oxidative damage to organs because it binds to free hemoglobin instead of leaving it free for bacteria to use (Carroll and Burdick Sanchez, 2014). Increases in haptoglobin are directly related to increases in pro-inflammatory cytokines (Carroll and Burdick Sanchez, 2014). In cattle, an acute inflammation can be detected by the presence of pro-inflammatory cytokines like TNF and IL-6, as well as increases in haptoglobin secretion from the liver (Carroll et al., 2009b; Vels et al., 2009). According to Connor et al. (1988) and Arthington et al. (2003), haptoglobin indicated stress in cattle when they were transported, co-mingling and weaning. In one study, cows were exposed to corticotropin-releasing hormone and LPS to induce stress and immune responses and in both cases, serum concentrations of haptoglobin increased (Carroll et al., 2009a).

Sperm Characteristics

There are many traits that contribute to a bull's overall fertility including sperm characteristics. Table 1 presents the sperm characteristics we focused on along with their definitions. 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 percentage of normal spermatozoa had the greatest influence on the calf crop percentage (Fitzpatrick et al., 2002). Motility is also important because in mammals, sperm cannot reach the egg in order to fertilize it unless they achieve hyperactivated motility (Yanagimachi, 1994). Farmers use a breeding soundness exam to evaluate these characteristics in order to assess bull fertility (Irons et al., 2007). A breeding soundness exam includes a general physical exam of internal and external reproductive organs, scrotal circumference measurement, and collection and evaluation of a semen sample (Spitzer and Chenoweth, 2000; Chenoweth et al., 1992).

Cow's Immune System

Just like all animals, cows depend on their immune response in order to survive. The typical immune response in bulls causes an increase in immune cells, pro-inflammatory cytokines, acute-phase proteins, and endocrine levels.

In one study, LPS was injected intravenously into beef steers. One hour after the injection, there was a decrease in circulating leukocytes, lymphocytes, and neutrophils (Burdick Sanchez et al., 2014). This occurred because the leukocytes, lymphocytes and neutrophils were migrating to infected tissues to find the foreign organism and out of the blood stream. There are

various endocrine responses to pathogens in cattle. For example, when beef steers were exposed to LPS, cortisol levels increased (Burdick et al., 2012) and cortisol responded to LPS-induced stress to prepare the cattle's body for secondary infection.

An immune response in bulls can negatively affect their fertility. An increase in leukocytes damaged sperm (Jones et al., 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).

Significance and Objectives

This project is significant because 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. If bulls have reduced fertility, this negatively affects their reproductive value and production value. Economic strain is being placed on farmers when stressful production processes cause fertility problems in their livestock. In order to improve our production and selection processes, we need to have a better understanding of the relationships between immune response, endocrine levels, and fertility in bulls.

The objective of this project was to use weaning and immune response characteristics as predictors of sperm motility characteristics in bulls.

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 IACUC 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 (**BW**) of 478 ± 34 kg at the time of immune challenge.

Blood Collection and Immune Challenge. Salmonella typhimirium (LPS; 0.7 µg/kg of body weight) was administered intraperitoneally. The vaccination was given in front of the right hip bone, pointed posterior and ventral. Blood was collected 0, 3, 6, 9, and 24 hours after LPS vaccination using EDTA vacuum tubes and serum separator tubes.

Assays. The whole blood sample was analyzed for a differential cell count on a Cell-Dyn 3500 (Abbott Diagnostics, Abbott Park, IL). Phase Haptoglobin Assay from Tridelta Development Ltd (Kit # TP 801) was used to determine haptoglobin concentrations. Concentration of the hormones prolactin, testosterone, insulin-like growth factor (**IGF**), and cortisol were quantified using validated radioimmunoassays (Hallford, New Mexico St. Univ.).

Sperm Collection and Evaluation Semen was collected using electroejaculation with an Electroejac IV (Ideal Instruments/Neogen Corp., Lansing, MI) 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. Before evaluation, samples were diluted 20:1 in Dulbecco's Phosphate-Buffered Saline then evaluated no more than 30 minutes after collection. Evaluation was performed using Hamilton Thorne IVOS computerized sperm analysis system (Hamilton-Thorne Biosciences, Beverly, MA). We evaluated motility and morphology

characteristics listed in Table 1 using Animal Motility Software, version 12.1 in 10 different fields to determine averages for sperm characteristics. Thirty video frames were captured within each field in order to analyze sperm motility. An eosin-nigrosin-based live-dead stain (Jorvet Stain, Jorgensen Laboratories, Lovelend, CO) was used to fix and evaluate semen for morphology. Each slide had approximately 100 spermatozoa and analyzed for percentage live (dye exclusion) and dead.

Statistical Analysis. Data was analyzed using SAS procedures (SAS Inst., Inc., Cary, NC). 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

Immune Challenge Time Effects

Time after LPS injection had an effect ($P < 0.05$) on the immune response. Figures 1-Figure 6 present the effects of time after LPS on immune response. White blood cells (**WBC**), neutrophils, lymphocytes, the neutrophil-lymphocyte ratio, monocytes, eosinophils, basophils, red blood cells (**RBC**), hemoglobin, hematocrit, mean cell volume (**MCV**), mean cell hemoglobin (**MCH**), mean cell hemoglobin concentration (**MCHC**), red blood cell distribution width (**RDW**), platelet, and mean platelet volume (**MPV**) were affected ($P < 0.05$) by time after LPS (0, 3, 6, 9, and 24).

Sperm Characteristics

Table 1 gives the definitions of sperm characteristics and table 2 shows the effects of time on sperm characteristics. As bulls aged, sperm production and the percentage of live sperm increased ($P < 0.001$) and ALH tended to increase ($P < 0.07$). However, area decreased as the bulls aged ($P < 0.001$).

Weaning Physiology and Sperm Characteristics

Table 4 presents the correlation between traits collected at weaning and their relationship with average sperm characteristics. Progressive, rapid, live, dead, and live % were correlated ($r \geq 0.51$; $P < 0.05$) with IC. Number of sperm was correlated positively ($r > 0.65$; $P < 0.01$) with IP and negatively ($r \geq -0.53$; $P < 0.05$) with prolactin (ng/mL) and PC.

Immune Challenge and Sperm Characteristics

Table 5 presents the correlation between immune response and their relationship with average sperm characteristics. Slow speed was correlated positively ($r \geq 0.50$; $P < 0.05$) with WBC and neutrophils, and area of sperm heads was correlated positively ($r = 0.51$; $P < 0.05$) with WBC. Number of sperm was correlated positively ($r > 0.51$; $P < 0.05$) with mean cell hemoglobin concentration (**MCHC**). Straightness was correlated negatively ($r \leq -0.55$; $P < 0.05$) with WBC, neutrophils and lymphocytes. Linearity was negatively correlated ($r \leq -0.53$; $P < 0.05$) with WBC and lymphocytes.

Predictions

Using step-wise 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 = .43; progressive sperm = -1469.6 + 1.63 (IGF/cortisol) + 14.41 (average temperature during immune challenge), r^2 = .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 x 1000), r^2 = .84.

Discussion

Previously reported research using endocrine and immune responses to predict future sperm motility in bulls is limited. Time, endocrine levels, and immune response affected multiple sperm characteristics. In our research, prolactin alone had a negative relationship on sperm numbers. This coincides with research reporting that hyperprolactenemia caused infertility (Grattan et al., 2007). Our study showed IGF had a positive effect on sperm characteristics. This corresponds with past research that states IGF, which produces spermatogonial DNA, increases in response to stress and as a bull matures (Hansson et al., 1989; Purchas et al., 1970; Trenkle, 1971; Bernton et al., 1987). This study showed that changes in endocrine levels caused differences in the number of sperm, progressive sperm, amount of rapid and medium sperm, and percentage of live and dead sperm.

When an immune response was elicited, an increase in neutrophils and white blood cells had a negative effect on sperm characteristics. This coincides with research showing that leukocytes cause damage to sperm through oxidative stress (Jones et al., 1976). Mean cell hemoglobin concentration (**MCHC**) caused an increase in sperm and positively affected fertility.

Research connecting MCHC with fertility is very limited. It is known that hemoglobin prevents oxidative damage, which damages sperm (Carroll et al., 2014). Therefore, an increase in MCHC should have a positive effect on fertility. This study showed that different measures of immune response caused differences in number of sperm, amount of slow sperm, straightness and linearity of sperm, and area of sperm heads.

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Tables

Table 1 Sperm variables measured by the Hamilton-Thorne Sperm Analyzer (Hamilton-Thorne Biosciences, Beverly, MA)

Variable	Description
Motile	% of total sperm moving at path velocity ≥30 μm/sec and progressive velocity ≥15 μm/sec
Progressive	% of total sperm moving at path velocity ≥50 μm/sec and straightness ≥70%
Rapid	Progressive % with path velocity >50 μm/sec
Medium	Progressive % with path velocity <50 μm/sec but >30 μm/sec
Slow	% of total sperm moving at path velocity <30 μm/sec and progressive velocity <15 μm/sec
Static	Sperm not moving at all
Path velocity (VAP)	Average velocity of the smoothed cell path (μm/sec)
Progressive velocity (VSL)	Average velocity measured in a straight line from the beginning to the end of track
Track speed (VCL)	Average velocity measured over the actual point-to-point track
Lateral amplitude (ALH)	Mean width of the head oscillation as the sperm swims
Beat frequency (BCF)	Frequency of sperm head crossing the sperm average path in either direction
Straightness	Measures departure of average sperm path from straight line (ratio of VSL/VAP)
Linearity	Measures departure of actual sperm track from straight line (ratio of VSL/VCL)
Elongation	Ratio (%) of head width to head length
Area	Average size in square microns of all sperm heads

Table 2 Effects of time on sperm characteristics

Item[1]	Month					SE[1]	Prob. <
	February	March	April	May	June		
Sperm	229.9[b]	127.7[b]	171.7[b]	562.5[a]	609.5[a]	98.9	0.001
VAP	100.6	102.5	111.9	119.5	116.7	6.2	0.13
ALH	5.8	5.7	5.9	6.5	6.7	0.3	0.07
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

[1] Sperm= # of sperm (n/mL); VAP=Path velocity (µm/sec); ALH=Lateral amplitude; Area=size of sperm heads

[2] SE= mean of standard errors

Table 3 Weaning characteristics

Weaning Variable	Mean	SD[2]
Age, d	209	14.6
Weight	293	33.8
IGF1, ng/mL	287	100
Cortisol, ng/mL	28.4	9.75
Prolactin, ng/mL	23.1	16.2
Testosterone, ng/mL	4.31	4.19
IP[1], ng/mL	22.5	24.6
IC[1], ng/mL	11.5	5.73
CT[1], ng/mL	53.5	187
PC[1], ng/mL	0.85	0.61

[1] IP= IGF1/prolactin; IC= IGF1/cortisol; CT= cortisol/testosterone; PC=prolactin/cortisol

[2] SD= Standard Deviation

Table 4 Correlations between weaning physiology and sperm characteristics

		Sperm Characteristic						
		sperm #	Progressive	Rapid	Medium	Live	Dead	Live %
Item	Mean	448	44.7	50.6	5.54	72.3	27.5	0.72
Prolactin, ng/mL	23.1	-0.55*	-----	-----	-----	-----	-----	-----
IP[1],	22.5	0.65**	-----	-----	-----	-----	-----	-----
PC[1],	0.85	-0.53*	-----	------	-----	-----	-----	-----
IC[1],	11.5	-----	0.53*	0.51*	-----	0.53*	-0.55*	0.54*
CT[1],	53.5	-----	-----	-----	0.50*	-----	-----	-----

* *P* <0.05
** *P* <0.01
[1] IP= IGF1/prolactin; PC=prolactin/cortisol; IC= IGF1/cortisol; CT= cortisol/testosterone

Table 5 Correlations between immune response and sperm characteristics

		Sperm Characteristic				
		# of sperm	Slow	STR	LIN	Area
Item	Mean	448	10.4	87.3	60.7	4.75
WBC[1]	9.35	-----	0.50*	-0.66**	-0.53*	0.51*
Neutrophil	4.52	-----	0.58*	-0.62**	-----	-----
Lymphocyte	3.92	-----	-----	-0.55*	-0.54*	-----
MCHC[1]	35.1	0.51*	-----	-----	-----	-----

* *P* <0.05
** *P* <0.01
[1] WBC= white blood cell; MCHC= mean cell hemoglobin concentration

Figures

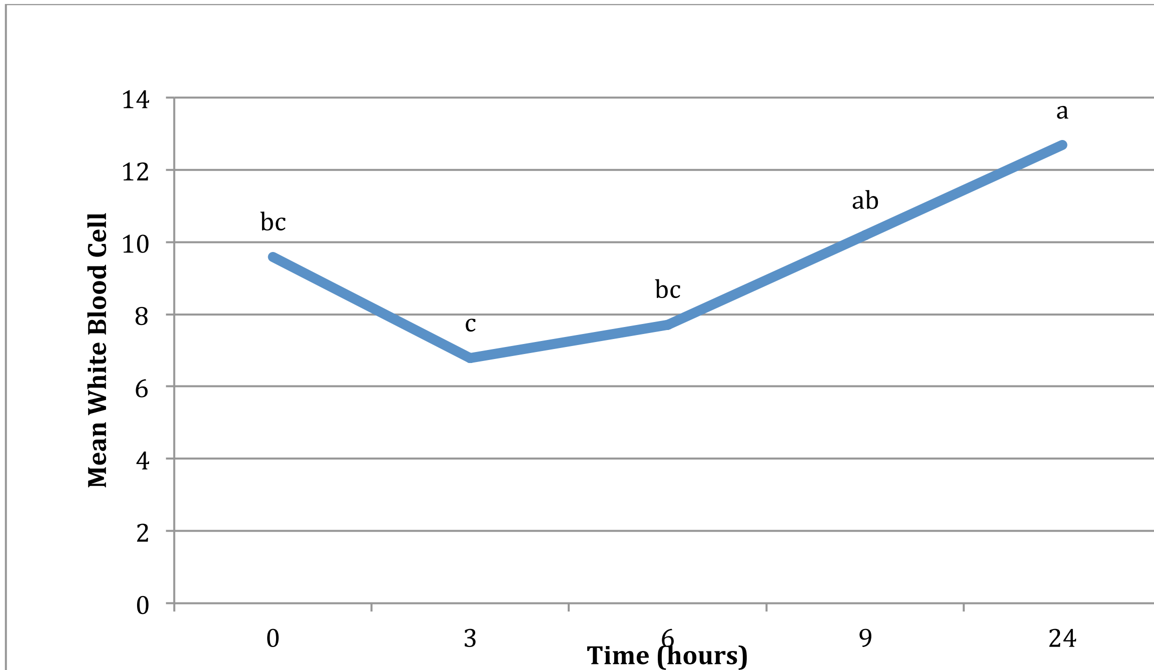

Figure 1 Effect of lipopolysaccharide on number of circulating white blood cells over time. Time affected white blood cell concentration ($P < 0.001$). a,b,c: $P < 0.05$. SE ± 1.06.

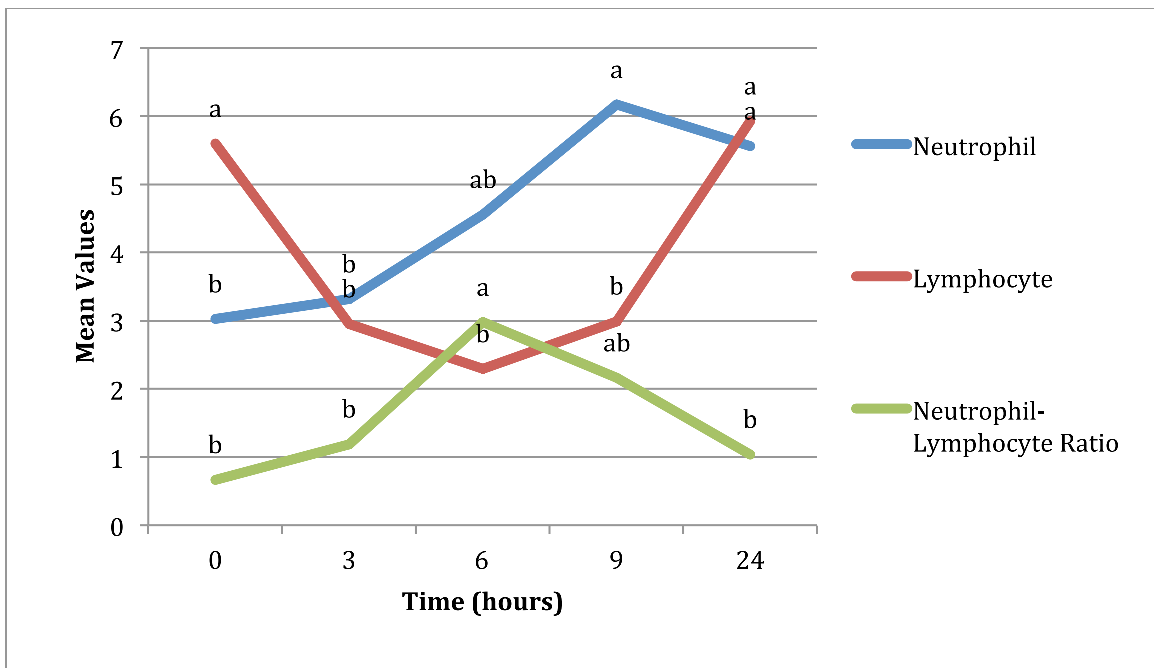

Figure 2 Effect of lipopolysaccharide on neutrophil and lymphocyte concentrations and neutrophil-lymphocyte ratio over time. Time affected neutrophil and lymphocyte ($P < 0.001$). Time (3,6,9,24 hrs) affected neutrophil-lymphocyte ($P < 0.05$). a,b: $P < 0.05$. SE ± .728, SE ± .473, SE ± .515.

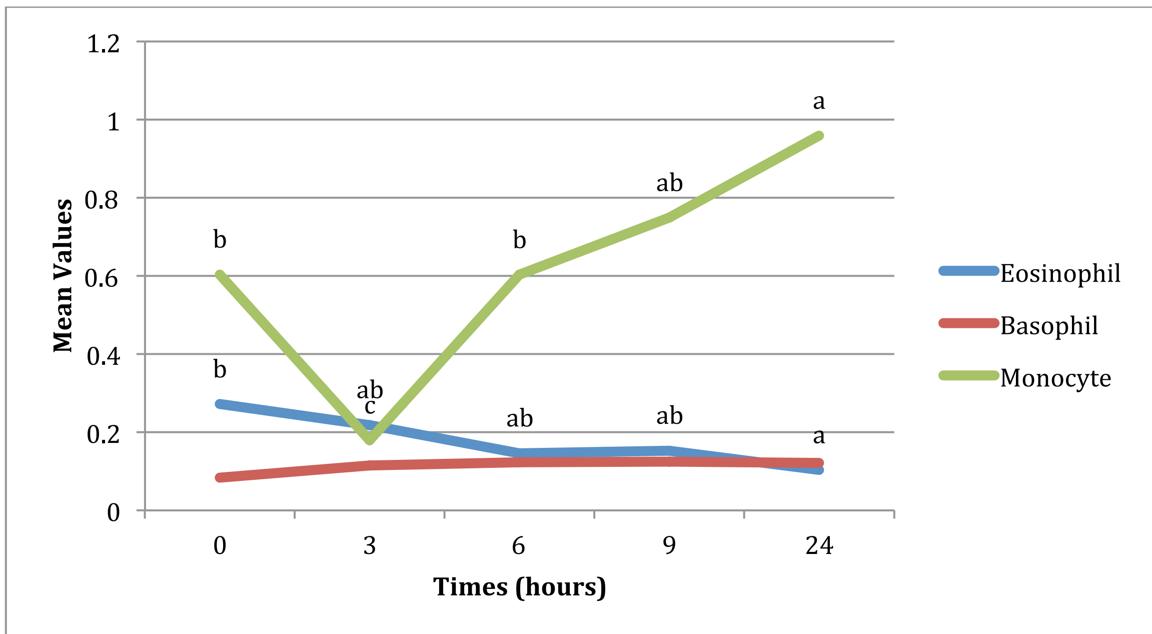

Figure 3 Effect of lipopolysaccharide on eosinophil, basophil, and monocyte concentrations over time. Time after lipopolysaccharide injection affected monocyte ($P < 0.001$). Time (3 hrs) affected monocyte ($P < 0.05$). Time (0,3,6,9 hrs) affected eosinophil ($P < 0.001$). Time (24 hrs) affected eosinophil ($P < 0.05$). Time affected basophil ($P < 0.001$). a,b,c: $P < 0.05$. SE ± .0486, SE ± .0174, SE ± .0903.

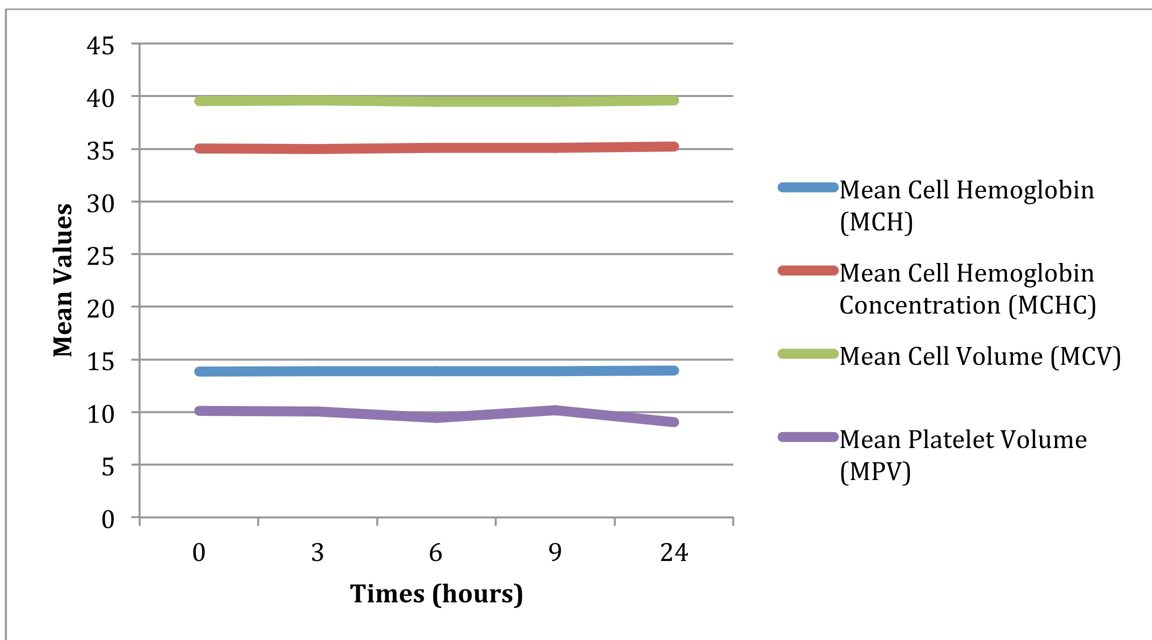

Figure 4 Effect of lipopolysaccharide on MCH, MCHC, MCV, and MPV concentrations over time. Time affected MCH, MCHC, MCV and MPV ($P < 0.001$). SE ± .233, SE ± .284, SE ± .585, SE ± .625.

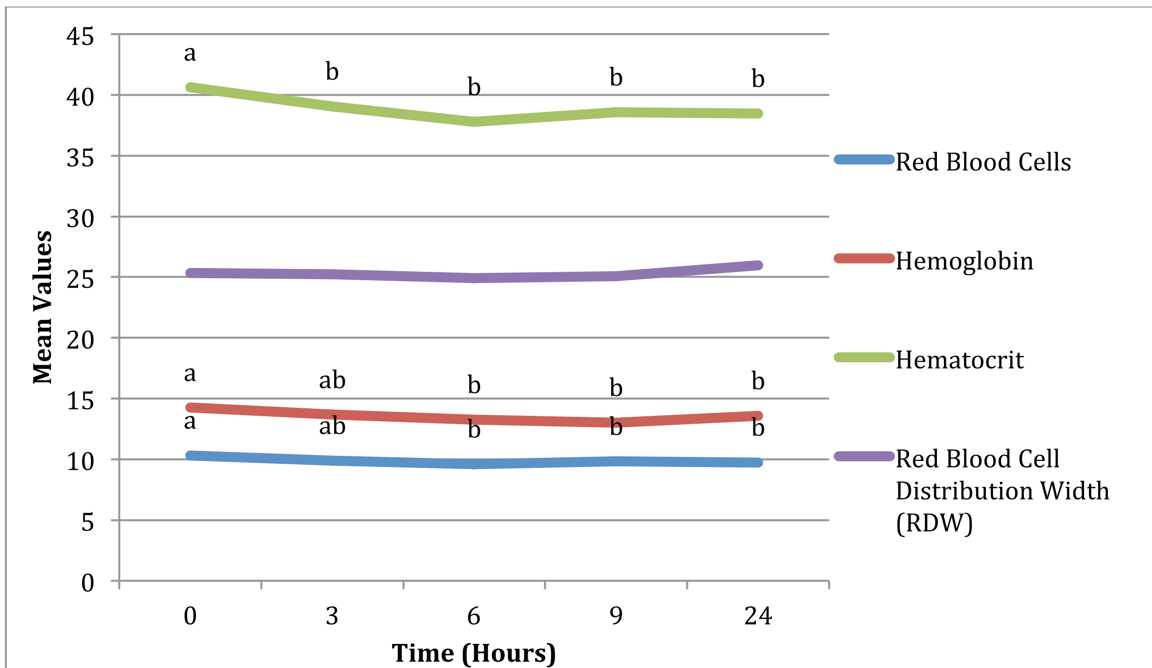

Figure 5 Effect of lipopolysaccharide on RBC, Hemoglobin, Hematocrit, and RDW concentrations over time. Time affected RBC, Hemoglobin, Hematocrit, and RDW ($P < 0.001$). a,b: $P < 0.05$ SE ± .179, SE ± .331, SE ± .512, SE ± .53.

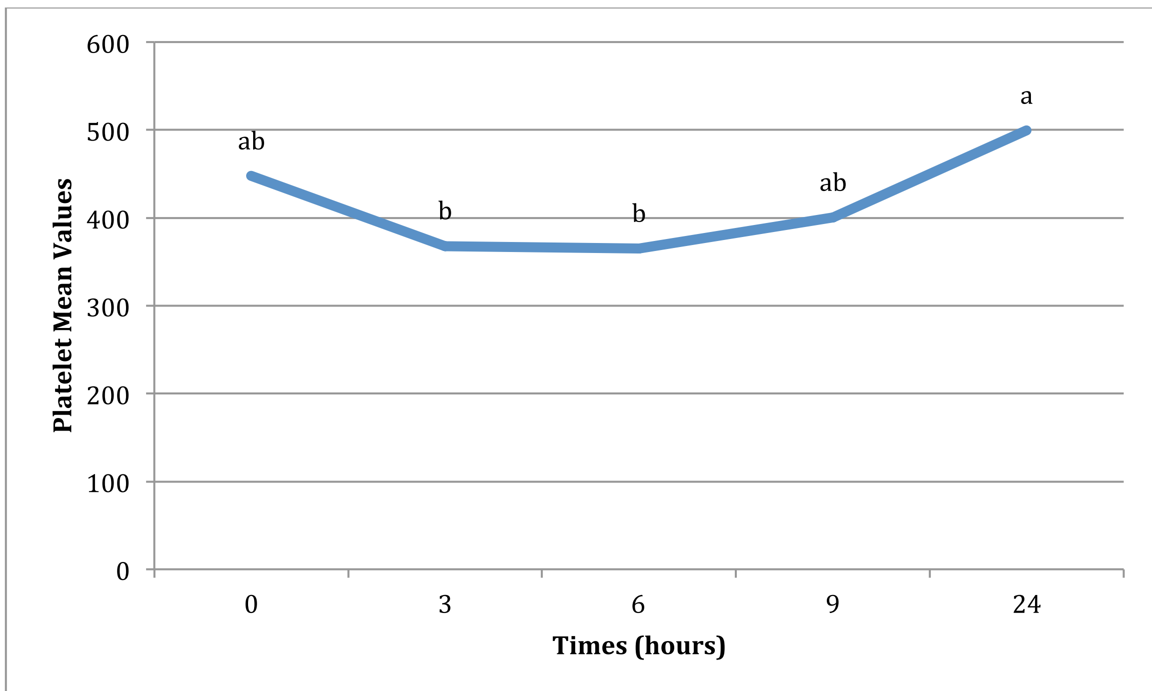

Figure 6 Effect of lipopolysaccharide on platelet concentrations over time. Time affected Platelets ($P < 0.001$). a,b: $P < 0.05$. SE ± 42.3.