Early and late gestation whitetail does embryo loss due to handling stress

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ABSTRACT: BioPRYN$_{wild}$ is an enzyme-linked immunosorbent assay for detection of pregnancy-specific protein B (PSPB) in the blood of ruminants (whitetail deer). The purpose of this research study is to determine the effect of handling does in early and late gestation on embryo loss. The data generated by this study support the following objectives.

1. To determine conception rate of does handled in a drop-floor chute at 10, 24, 40, 76 days post-breeding.
2. To determine fetus loss caused by handling does in late gestation – April 30, May 3, May14.
3. To determine the PSPB levels in the blood of whitetail does in early gestation as measured by the BioPRYN$_{wild}$ ELISA assay.
4. To measure the stress level of does as a result of handling.

The data in this study showed that the AI pregnancy rates were not significantly different among the three groups handled in a drop-floor chute at 10, 24, and/or 40, days post-breeding. In addition, the AI and buck bred pregnancy rates were not significantly different when does were handled at day 76 days post AI breeding and pregnancy status determined by the BioPRYN$_{wild}$ assay. When does were handled near fawning, Group 1 – May 14, Group 2 – May 3, and Group 3 – April 30, there were no apparent differences in fawning data. The data indicated that pregnant does can be handled during early (days 10 to 76 days post-breeding) or late (0-40 days pre-fawning) gestation with no more risk than handling deer at any other time.

Key Words: BioPRYN, PSPB, deer, pregnancy test, embryo and/or fetal loss

Introduction

Whitetail deer farmers have been concerned about handling pregnant does for many years. There are recommendations from deer farmers, reproductive management specialists, AI and laparoscopic breeders that caution producers not to handle does for a period of 10 days, 20 days and up to 55-60 days after breeding. The data to support these recommendations are almost non-existent.

The lack of a definite recommendation on handling pregnant does can affect the deer producer in making management decisions. Does may require treatment for health issues using the handling facility, and the decision to do so may rest on the risk of causing the death of the embryo if handled too early in gestation. The potential of losing an embryo affects when a doe may be transported or moved from one site to another. The decision to handle does to determine pregnancy status either by a blood test or ultrasound requires handling during early gestation.

When a deer producer makes the decision to handle a doe for a pregnancy determination, a concern is whether the pregnancy may be affected. The BioPRYN$_{wild}$ blood test can accurately determine pregnancy 40 days or more post-breeding. In addition, the test can be used as an indicator if a doe is bred to AI or to a follow-up buck by following these procedures: Expose the AI or laproscopically bred does to a follow-up buck 18-20 days after breeding and then draw a blood sample at 40 days after AI or laproscopic breeding. The buck-bred does should not be detected at the 20-22 days they had been exposed to the buck but the AI or laproscopic-bred does that have conceived will be identified. If this schedule is followed the AI and Lap does will be handled at 40 days post-breeding and the buck-bred does at 20-22 days post-breeding. Currently there are no data-based recommendations to determine if handling a doe at 20 to 40 days post-breeding has an adverse effect on the embryo.

In addition, when deer are sold at auction and moved to another location, or are due for vaccinations, it is not known what fetal losses might occur during late gestation. These management practices are affected by the lack of information regarding fetal loss during handling does in late gestation. The topic of embryonic loss and stress in whitetail does caused by handling is addressed in this paper.

Pregnancy-Specific Protein B

BioPRYN is an enzyme-linked immunosorbent assay (ELISA) for pregnancy in ruminant animals by detecting the presence of pregnancy-specific protein B (PSPB) in the serum or plasma. PSPB was discovered in the late 1970's by Dr. Garth Sasser's research group at the University of Idaho in the fetal placenta of cows (Butler et al., 1982 and Sasser et al., 1986).

A radioimmunoassay (RIA; Sasser et al., 1986) was developed for measuring PSPB in blood and tissues of cattle. The PSPB was in the blood of some pregnant heifers as early as 15 days after AI and in an increasing proportion of heifers for the next 13 days thereafter. Humblot et al., (1988) showed, on a herd basis, that application of the RIA at 30 days after conception was appropriate for detection of pregnancy. The protein remained in blood throughout pregnancy. In cattle, there is a low concentration of serum PSPB (2 to 5 ng/mL) from about 24 days until 70 days after conception. Then there is a linear increase in concentrations to a 20 ng/mL by 150 days and 73 ng/mL by 262 days. During the last three weeks of gestation, concentrations increase to 500 ng/mL (Sasser et al., 1986). The concentration of PSPB declines after parturition with a half-life of approximately 7 days and is non-detectable by RIA by 60 to 90 days postpartum (Kirakofe et al., 1993). Following these original studies, serum PSPB activity was measured using the double antibody RIA procedure developed for cattle (Sasser et al., 1986) adapted for use in deer (Haigh et al., 1988; Wilker et al., 1993; Willard et al., 1994, 1996, 1998).

The BioPRYN ELISA was developed to provide a convenient and inexpensive test for pregnancy based on the original PSPB RIA and has been commercially available for ten years for cattle and two years for wildlife.

Purpose and Objectives

The purpose of this research study is to determine the effect of handling does in early and late gestation on embryo loss. The data generated by this study support the following objectives.

1. To determine conception rate of does handled in a drop-floor chute at 10, 24, 40, 76 days post-breeding.
2. To determine fetus loss caused by handling does in late gestation – April 30, May 3, May14.
3. To determine the PSPB levels in the blood of whitetail does in early gestation as measured by the BioPRYN$_{wild}$ ELISA assay.
4. To measure the stress level of does as a result of handling.

Materials and Methods

BioPRYN$_{wild}$ assay:

This antigen-capture, or "sandwich," Enzyme-Linked ImmunoSorbent Assay (ELISA) detects Pregnancy Specific Protein B (PSPB) in wild ruminant sera greater than 40 days since breeding. Serum PSPB binds to antibodies coated in the wells and is detected by secondary binding of a labeled antibody (Detector). Binding of the labeled antibody conjugate is detected by the addition of a peroxidase linked streptavidin. The peroxidase enzyme reacts with 3,3́,5,5́-tetra methyl benzidine (TMB) and causes subsequent color development. Sulfuric acid was added to stop the reaction and optical density for each well was obtained from a plate reader (VersaMax, Molecular Devices, Inc). The cutoff OD for the assay

plate was determined from the mean OD (triplicate wells) of each of two PSPB standards. The cutoff for determining pregnancy or non-pregnancy status was equivalent to that of the RIA for testing pregnancy in cattle (Sasser et al., 1986; Humblot et al., 1988).

Animals and Management

The following investigation was conducted beginning October, 2011 through the spring of 2012 at the Sunset View Whitetail Farm (Chetek, WI), an intensive Whitetail deer broodstock and urine collection for scent farm. Sixty-one whitetail does (43 does selected from the Hanson breeding herd and 18 does that were purchased from a neighboring herd) were randomly assigned to three groups (Group 1 = 20 does; Group 2 = 20 does and Group 3 = 21 does). Random groups were generated using Random Sequence Generator found at: http://www.random.org/sequences/?min=1&max=60&col=3&format=html&rnd=new.

The age of the does in each group is summarized in Table 1.

Table 1. Age of Does in three experimental groups

			Age			
	2	3	4	5	8	Total
	N %*	N %	N %	N %	N %	
Group 1	14 70	0 0	5 29	1 12	0 0	20
Group 2	1 5	10 67	4 24	4 50	1 100	20
Group 3	5 25	5 33	8 47	3 38	0 0	21
Total	20 100	15 100	17 100	8 100	1 100	61

*Percent does of age category in group

The does on the Sunset View Whitetails farm are normally handled in the following manner. In late September or early October fawns are removed and all does to be AI bred are placed in the same pen. At this time the does are dewormed and vaccinated with 8 way, a/b clostridium. All does are run through the chute in late spring (March/April) for vaccination for E. coli to enable the antibodies to be in the colostrum. Every three years the does are tuberculosis tested in March or April. All deer are fed using a skid steer there is no walking in the pens. The deer are moved and checked during fawning season on a 4-wheeler.

Artificial Insemination

The eighteen purchased does were kept in a separate pen from the Hanson Breeding herd until October 26, 2011 when CIDRs were inserted in all animals. In addition to inserting CIDRs, the does were given a visual stress evaluation by Dr. Darrel Kesler. On November 8, 2011 the CIDRs were removed and the does were given an injection of 200iu of Pregnant mare serum gonadotropin (PMSG) in the muscle. The does were inseminated 58-60 hours after withdrawal of the CIDR and PMSG injection.

On November 10, 2011 the does were all AI'd to the Blue Sky Buck semen. The semen analysis was conducted by Minitube of America and the reported parameters were in the normal range of fertile semen.

On day 0 all the does were AI'd 2.5 days after CIDR removal and the PMSG injection. Laurie Seale (Maple Hill Farms, Gilman, WI) served as the AI breeder. The does were placed with the follow-up bucks on 24 days post breeding. The bucks were removed on December 21, 2011 allowing the does to be exposed to the bucks for 16 days.

Sample Collection and Animal Handling

For sample collection, does were herded from pastures to a working facility that was equipped with a drop-floor deer cradle (Delclayna) that restrained the does but minimized stress. Whole blood samples were collected in vacuum tubes containing no additives and sera were analyzed by BioPRYN.

Blood samples were collected by the herd veterinarian via jugular venipuncture using a 12 ml syringe. Tubes were allowed to clot and centrifuged off-site. One ml of serum was placed in a 3 ml red top tube (for cortisol analysis) and the remaining serum (for PSPB analysis) was placed in a 3 ml red top tube, frozen at -20°C until it was shipped to the BioTracking laboratory in Moscow, ID. The January 26, 2012 blood draw was shipped directly to the BioTracking laboratory and centrifuged and the serum was analyzed for PSPB.

The three groups were handled through the deer cradle on the shown in Table 2.

Table 2. Date does handled by group and treatment

Group	Date	Handling	PSPB	Cortisol	Visual Stress
1,2,3	Oct 26	CIDR's inserted	___	___	X
1,2,3	Nov 8	CIDR's removed & PMSG shots			
1,2,3	Nov 11	Does AI'd	___	___	___
1	Nov 21		X	X	X
2	Dec 5		X	X	---
1,2,3	Dec 21		X	X	X
1,2,3	Jan 26		X	---	---
1	May 14		X	---	---
2	May 3		X	---	---
3	April 30		X	---	---

On December 21, 2011 (40 days post-AI) all 60 does were PSPB tested to determine which does were AI bred. Again on January 26, 2012 (76 days post-AI) all does were PSPB tested as a confirmation of pregnancy test.

At the time of the 76 days post-AI blood draw, the does were divided into pens of 10 does depending on the results of the first BioPRYN test. Animals categorized pregnant by BioPRYN at 40 days post-AI were grouped together while animals categorized as not pregnant were grouped together. This was done to ensure a more consistent fawning date within a given pen.

Selected does were handled near their fawning date in order to determine if handling does near fawning caused fetal loss. A total of 28 does were selected from the three treatment groups. The selection process was partially dependent on the BioPRYN categorization 40 days post-AI. Animals categorized as Pregnant 40 days post-AI were classified as indicative of AI breeding while an animals categorized as Not Pregnant 40 days post-AI with a Pregnant categorization 76 days post-AI were classified as indicative of Buck breeding. Using this approach of the 28 selected animals 14 were indicative of AI breeding and 14 were indicative of Buck Breeding. All 59 of the does received a Clostridium perfringins type A vaccination and the 28 does that were also PSPB blood tested are identified in Table 3.

Table 3. Sampling date near fawning by group and whether AI or Buck bred

Group	Date PSPB	AI Bred*	Buck Bred*
1	May 14	5	3
2	May 3	6	5
3	April 30	4	5

*****Based on fawning data

Stress and Cortisol Measurement in Deer

One of the barriers to handling whitetail does is the fear that the stress caused can be detrimental to the doe and embryo or fetus. In this study stress was evaluated by a subjective assessment of temperament using a 5 point scale. This assessment was developed from previous research with cattle and Wapiti. Grandin (1993) suggested the use of a 5 point scale for analytical purposes.

In addition to the visual stress scores, blood cortisol was assessed. Blood cortisol is likely the best assessment of acute stress in most mammalian species; however, this technique is only available to researchers. Other methods have been used in other species with varying degrees of value. A less invasive method may very well be as effective as blood cortisol and would be most valuable to deer owners.

The visual assessments were conducted by Dr. Darrel Kesler, Prof Emeritus, University of Illinois with expertise in observing and measuring stress in animals. Each deer was given a number using the succinct descriptions as described in Figure 1. As the deer entered the handling facility, during blood collection, and as they exit the handling facility. Assessments (only whole numbers) were assigned by Dr. Kesler stress in animals.

Figure 1. Five-point Scale used in temperament assessment

	Stress Measurement	Description
1	Calm	Calm
2	Mild Stress	Minimal struggle and excitement
3	Moderate Stress	Some struggling and excitement
4	Markedly Stressed	Struggling, excitement, and vigorous movement
5	Extreme Stress	Very disturbed, vigorous/violent movement, and attempts to escape

All does in the study (Groups 1, 2, 3) were assigned a visual stress measurement score by Dr. Kesler when CIDRs were inserted on October 26, 2011. The does in Group 1 were assigned a visual stress score on November 21, 2011 and then on December 21, 2011 all does (Groups 1, 2, 3) were again assigned a visual score by Dr. Kesler.

The does in Group 1 were the only group that two blood samples were collected for the stress cortisol assay. They were tested using the cortisol assay from blood collected on November 21, 2011 and again on December 21, 2011. The does in Groups 2 and 3 were only tested for cortisol once using blood samples drawn on December 21, 2011.

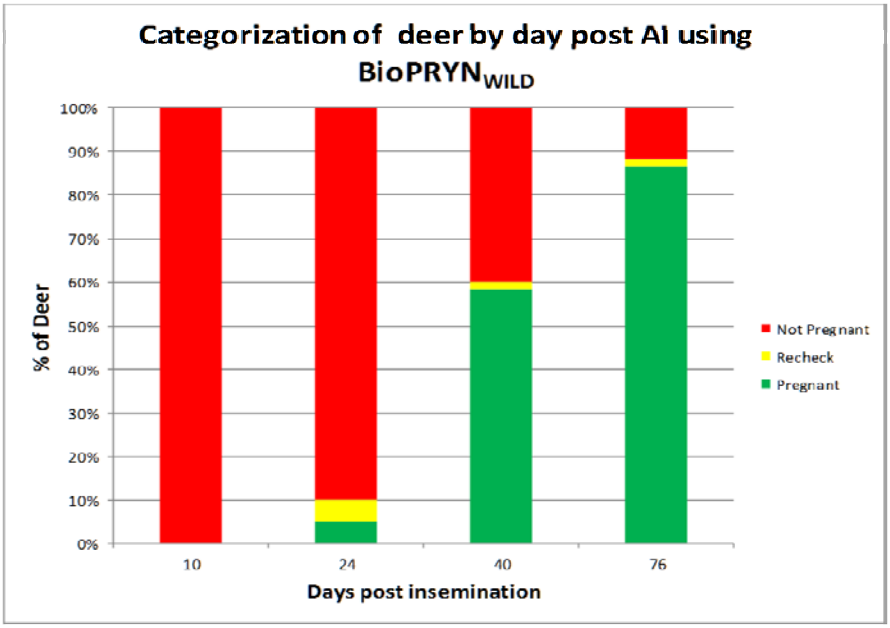
The blood samples in this study used for the cortisol analysis were received by the Biochemistry & Molecular Biology Department at Mississippi State University located in Mississippi State, MS on January 5, 2012. The samples were kept at -20°C until testing. The samples were then tested using a

direct competitive radioimmunoassay by Siemens and read from a Packared Cobra 2 Auto-Gamma Counter. A Siemens COAT-A-COUNT kit for Cortisol (catalog number TKCO5) was used for the analysis.

Results and Discussion

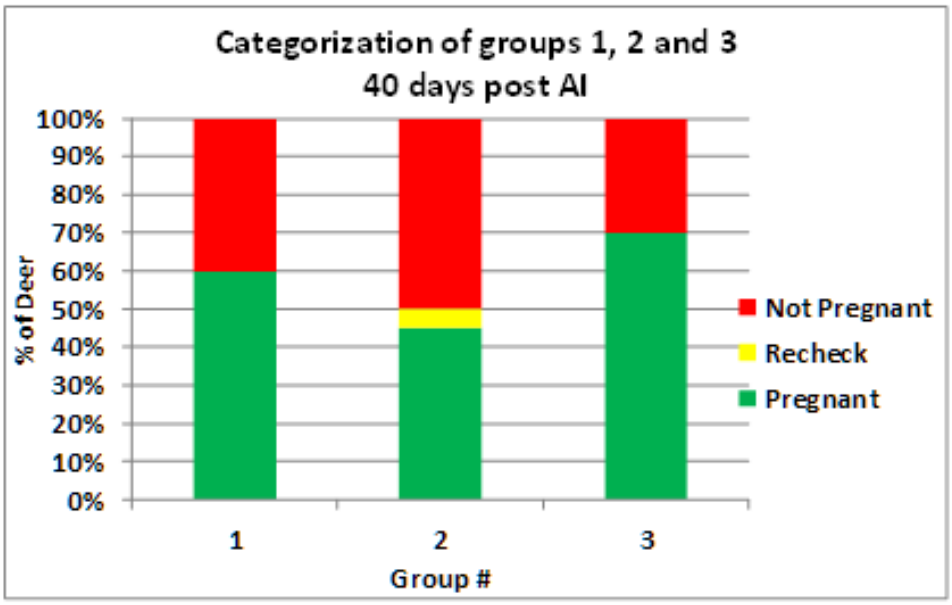
Figure 2 categorizes the deer by post AI using BioPRYN$_{wild}$. All does in Group 1 at 10 days post AI were open using the PSPB analysis. At day 24 post AI, Group 2 PSPB analysis categorized 18 does open and 1 pregnant and 1 recheck. It is possible that these two does were bred by a buck fawn prior to the AI breeding or this is an example or early expression of PSPB not seen with the other animals. At day 40 post AI, the PSPB analysis indicated that there were 24 does open and 36 does pregnant. Since the follow-up bucks were only with the does for 16 days there is a strong indication that the bred does were bred to the AI buck. At the confirmation check at day 76 post AI, there were 36 does indicative of AI breeding, 17 does indicative of follow-up buck breeding and 7 does open.

Figure 2.



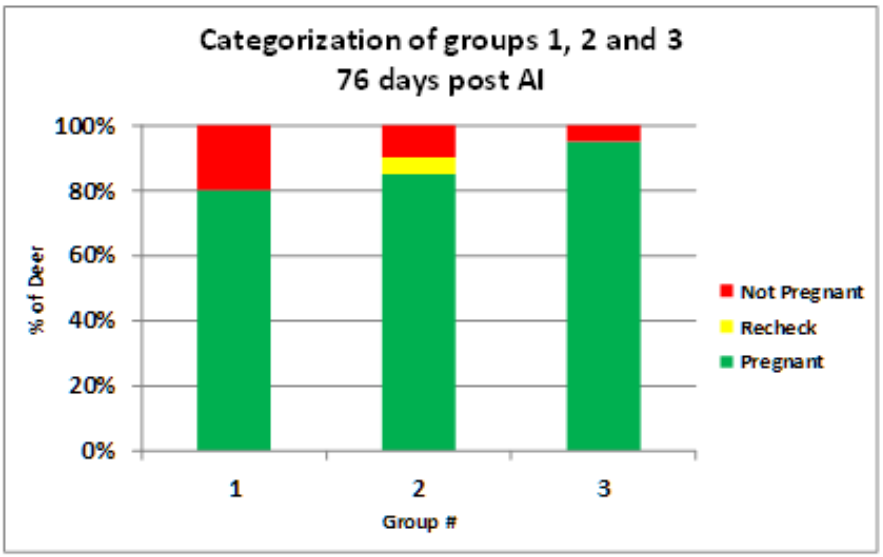
The three groups in Figure 3 display the number of does bred as detected 40 days post AI using the BioPRYN$_{wild}$ test. Group 1 was handled on November 21 and December 21 and the data indicated that 12 does were pregnant (AI bred) and 8 open. Group 2 was handled on December 5 and December 21 and the data indicated 9 pregnant (AI bred) and 10 opens. Group 3 was handled only on December 21 and the data indicated 14 pregnant (AI bred) and 6 open. Chi square analysis was used to determine if there were significant differences among the three groups at the 95% confidence level. There was no significant difference among the groups. Based on the BioPRYN rest results the AI pregnancy rate calculated across all three groups was 59 percent (Based on fawning data = 51.7%). The same farm in 2010 had an AI pregnancy rate of 56 percent and in 2009 it was 44 percent. This would indicate the handling within the study regardless of timing did not have an impact on overall AI pregnancy rate.

Figure 3.



In Figure 4, the three groups are categorized by pregnancy status at 76 days post AI breeding.

Figure 4.



Group 1 results showed that 16 does were pregnant and 4 does open. Group 2 revealed 17 pregnant, 1 recheck, and 2 open does. Group 3 indicated 19 pregnant does and 1 open. The recheck doe was the same doe that was a recheck at the 40 day post AI pregnancy check. Due to the fact that this animal continued to show a recheck status across gestation, it seems likely that this measureable but low level of PSPB may be a normal physiological state.

The overlay on Figure 5 shows the animals that were categorized as pregnant at 76 days post AI (Figure 4) but were categorized not pregnant at 40 days post AI. The animals under this overlay are indicative of the animals that were bred by the follow-up buck rather than the AI breeding. Based on using BioPRYN as an indicator of parentage: Group 1 there were 12 AI bred does and 4 follow-up buck does, Group 2 there were 9 AI bred does, 1 recheck doe, Group 3 had 14 AI bred does and 5 follow-up buck bred does.

Figure 5.

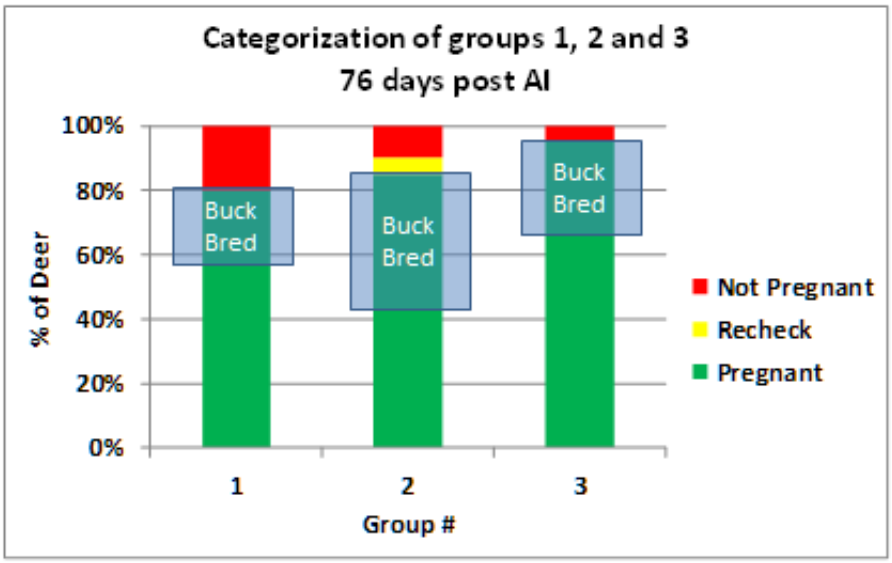
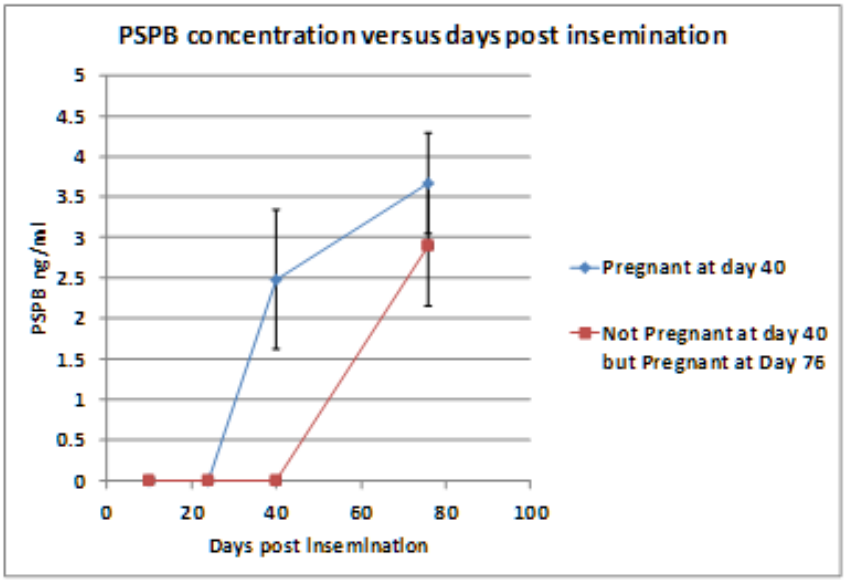


Figure 6 depicts the difference in PSPB concentration between those animals categorized as Pregnant at both the 40 day post AI check and the 76 day post AI check and those animals categorized as Not Pregnant 40 days pot AI and Pregnant 76 Days post AI. At the 76 day post AI check there was a non-statistically significant difference in PSPB concentration between the groups.

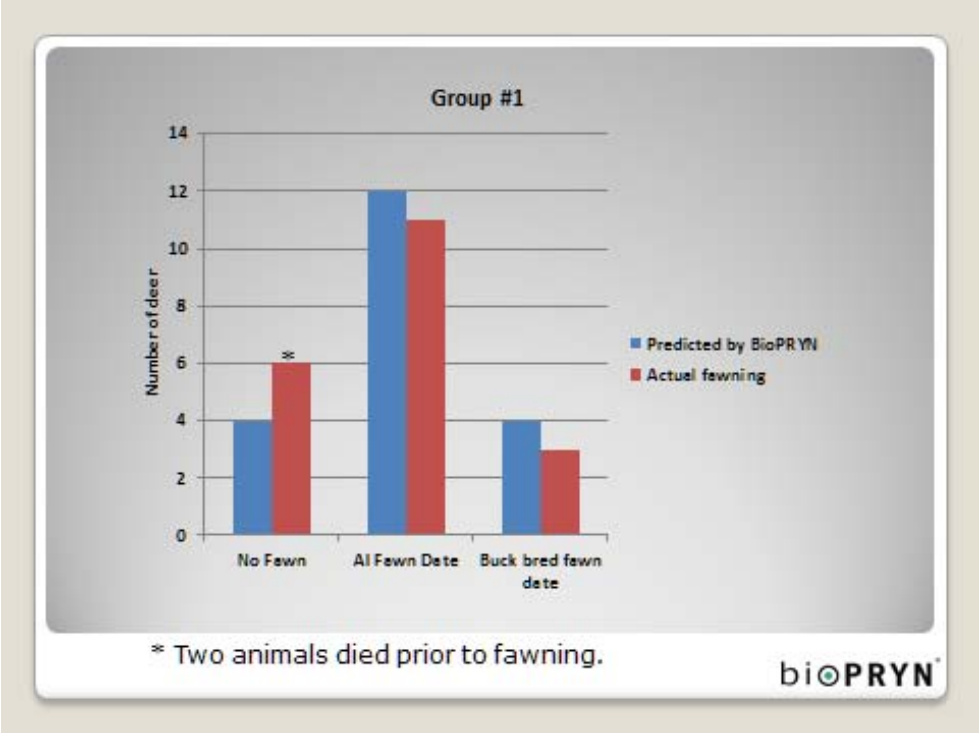
Figure 6.



This graph shows that the PSPB levels in follow-up buck bred does is not detectable at day 40 after AI breeding. However, the AI bred does are at approximately 2.5 ng/ml. Follow-up bucks were placed with the does on December 5, 2011 and taken out on December 21, 2011 exposing the does to the follow-up bucks for 16 days. The BioPRYN$_{wild}$ PSPB assay can be used as an indicator of fawning date if the procedure is followed (placing does with follow-up bucks 18 to 20 days post AI breeding and drawing the PSPB blood sample at 40 days post AI breeding). Using this approach only the AI bred does will be detected at 40 days post AI breeding.

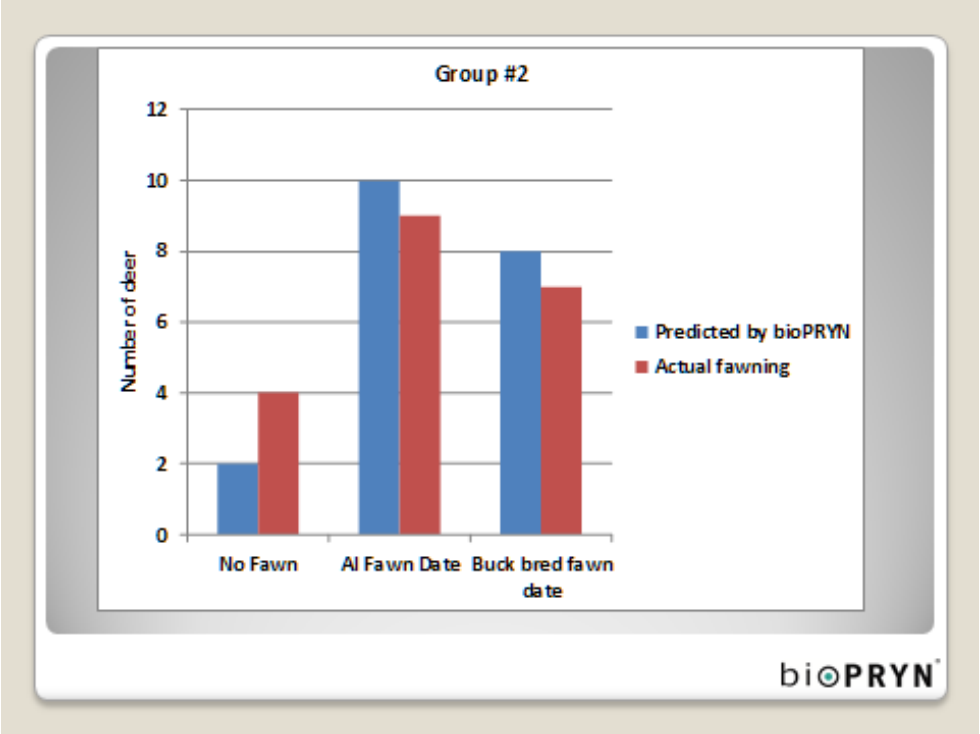
The data in Figures 7 through 10 are related to comparing how the BioPRYN$_{wild}$ predicted fawning compared to the actual fawning data. Figure 7 displays the PSPB predicted fawning versus the actual fawning data for Group 1. PSPB predicted 4 open does (1 died), 12 AI bred (1 aborted prior to fawning) and 4 (1 died prior to fawning) buck bred does. Actual fawning showed that 6 does were open, 11 fawned to the AI date, and 3 fawned to the buck bred date.

Figure 7.



Group 2 data are depicted in Figure 8. PSPB predicted 2 opens, 10 AI pregnant (one was recheck) (1 did not fawn) and 8 buck bred does (1 did not fawn). Fawning data indicated 4 open does, 9 fawned to the AI date (1 open) and 7 (1 open) fawned to the buck bred date.

Figure 8.



Group 3 data are summarized in Figure 9. PSPB predicted 1 open doe (1 culled), 14 AI bred does (2 abortions and 1 stillborn set of twins delivered to AI date), and 5 buck bred does (1 open). Actual fawning data showed 4 open does, 11 fawned to the AI date and 4 fawned to the buck bred date.

Figure 9.

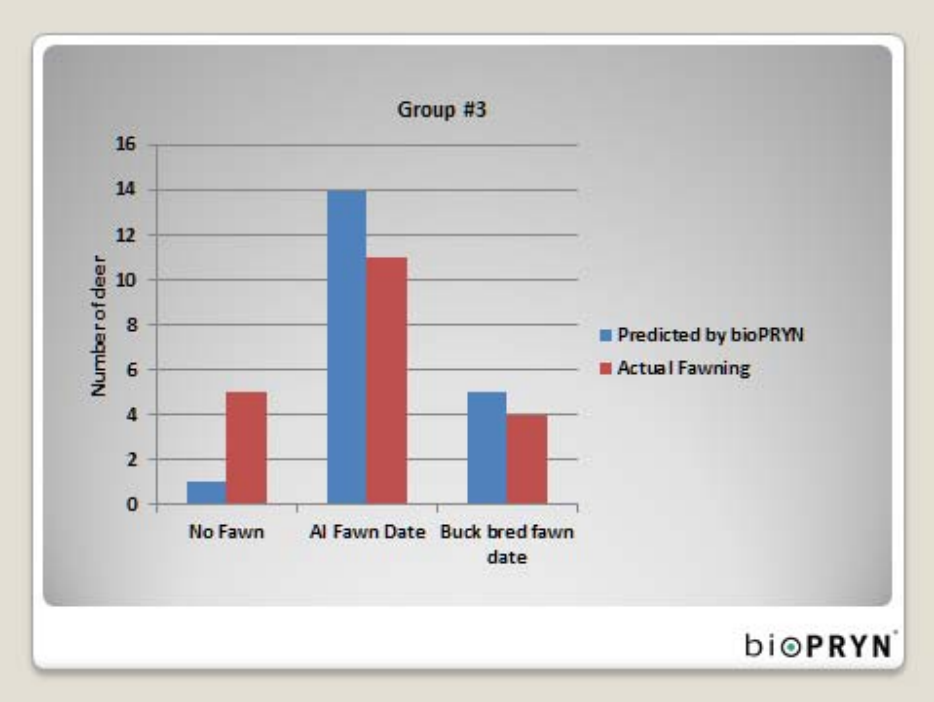
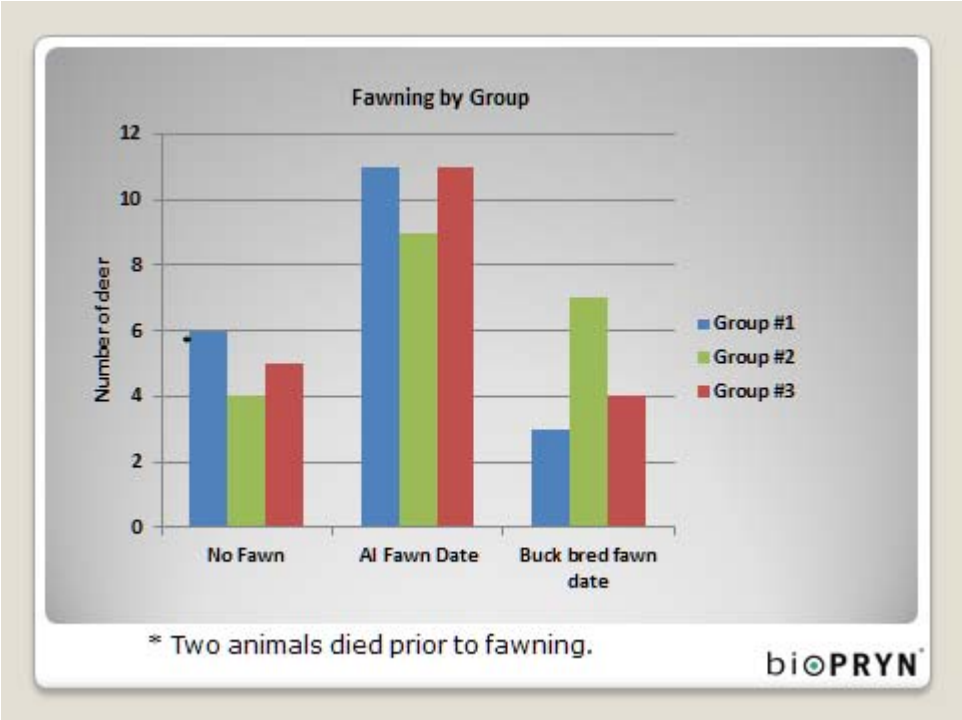


Figure 10 summarizes the fawning data among the three groups. In Group 1 there were 11 does fawned to the AI date and 3 does fawned to the buck bred date (14 total). Group 2, 9 AI does fawned to the AI date and 7 does fawned to the buck bred date (16 total). In Group 3, 11 does fawned to the AI date and 4 fawned to the buck bred date (14 total). There is no apparent difference between the groups based on this data. This would indicate the differences in handling between the does during gestation did not affect pregnancy. Groups 1 and 2 were handled more than Group 3. It was interesting to note that there were more buck bred does in Group 2 compared to Groups 1 and 2. It must be appreciated that all animals in this study were handled a minimum of 2 times. This does not appear to have changed the AI pregnancy rate of the herd based on prior herd data. However, without a control group with no handling this cannot be confirmed.

Figure 10.

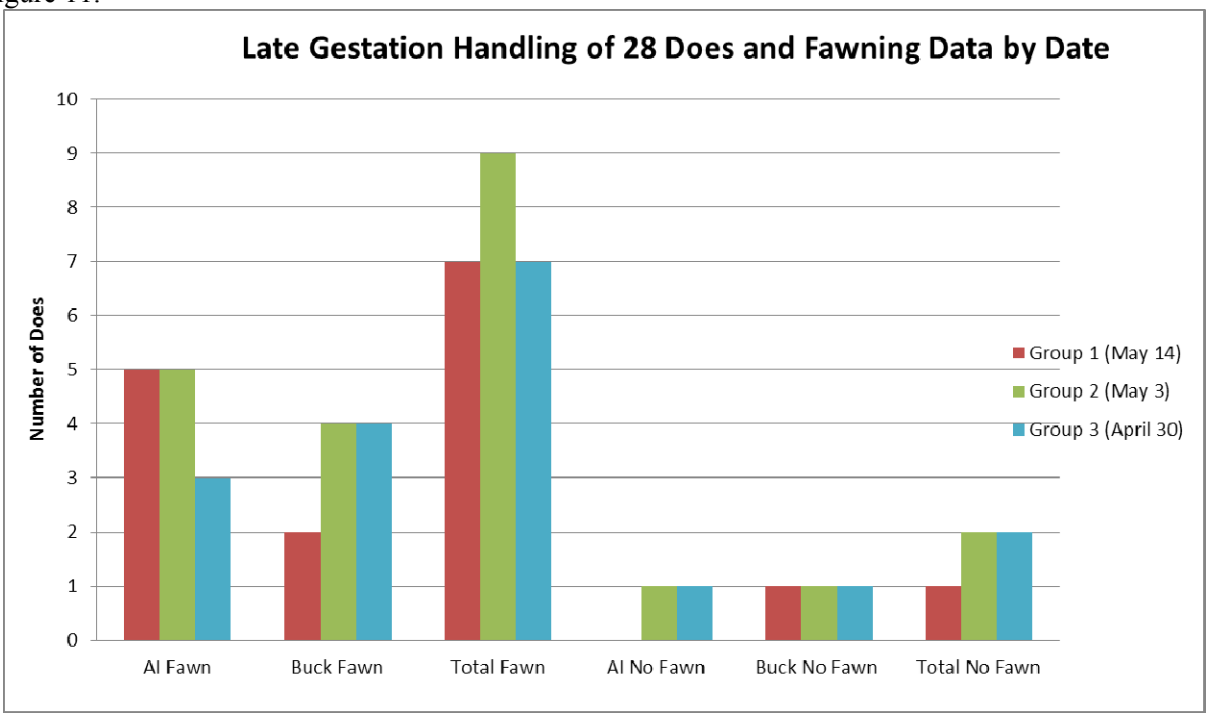


Late Gestation Handling Does Results

All 59 remaining does in Groups 1 (May 14), Group 2 (May 3), and Group 3 (April 30) were handled through the deer cradle near fawning and given vaccinations for Clostridium perfringins type A prior to fawning. In addition, 28 does of the 59 were also collected for a blood sample to measure PSPB. Table 3 summarized the number of does that were AI bred and follow-up buck bred used in each group that were vaccinated and also a PSPB blood sample was drawn. One might assume that there may have been some additional stress on the does that were also blood drawn but no stress was measured on these three dates. Actual fawning by the 28 does in the three groups that were vaccinated and blood drawn are exhibited in Figure 11.

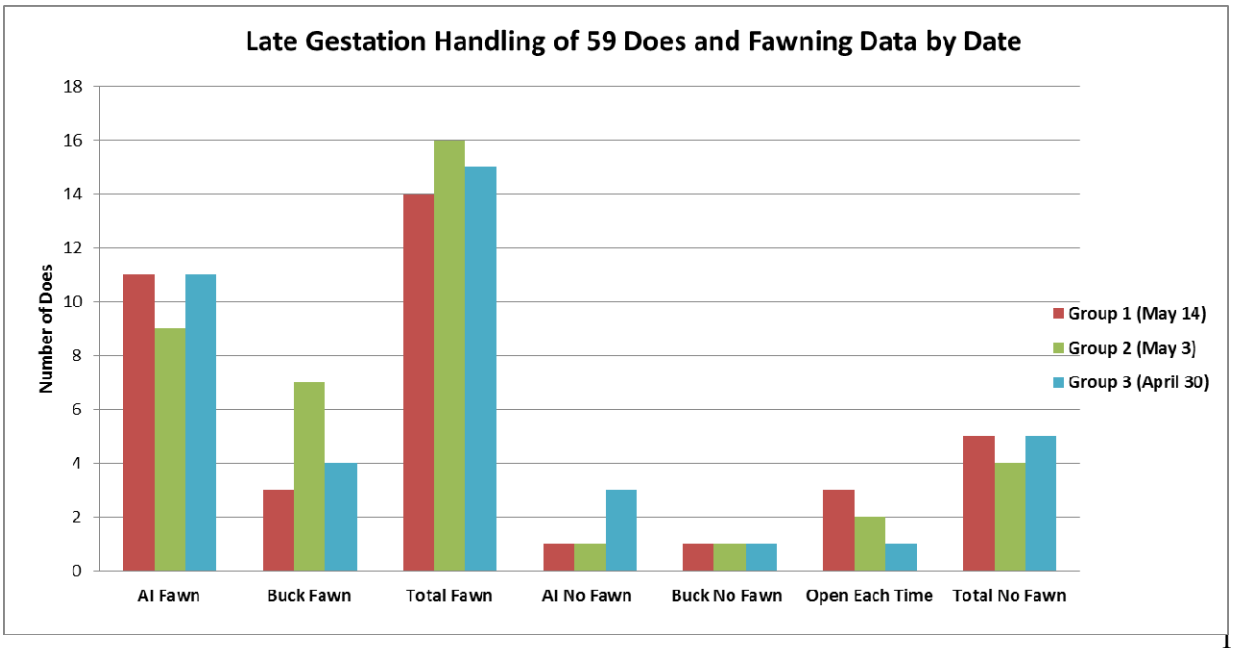
Blood samples were collected on 8 does in Group 1; 11 does in Group 2; and 9 does in Group 3. In Group 1, 7 does fawned and 1 did not fawn. In Group 2 9 does fawned and 2 does did not fawn and in Group 3 7 does fawned and 2 did not fawn. The doe not fawning in Group 1 died on May 27 thirteen days after handling. The two does not fawning in Group 2 were both open. The two does in Group 3 not fawning included one that was open and the other aborted on May 2, two days after handling. This particular doe ran into the fence and was injured causing her death. It was concluded that handling does near fawning was no more of a risk than handling them at any other time during the year.

Figure 11.



The data in Figure 12 depict the fawning result on all the does that were vaccinated and/or a blood sample taken by catching through the chute on the dates indicated. The total number of does fawning were14 out of 19 does in Group 1; 16 does fawned out of 20 does in Group 2; and 16 does fawned out of 20 does in Group 3. Although no statistics were used to analyze the data, visually there appears to be no difference among the three groups considering the number of times the three Groups of does were handled during the gestation period from breeding to fawning.

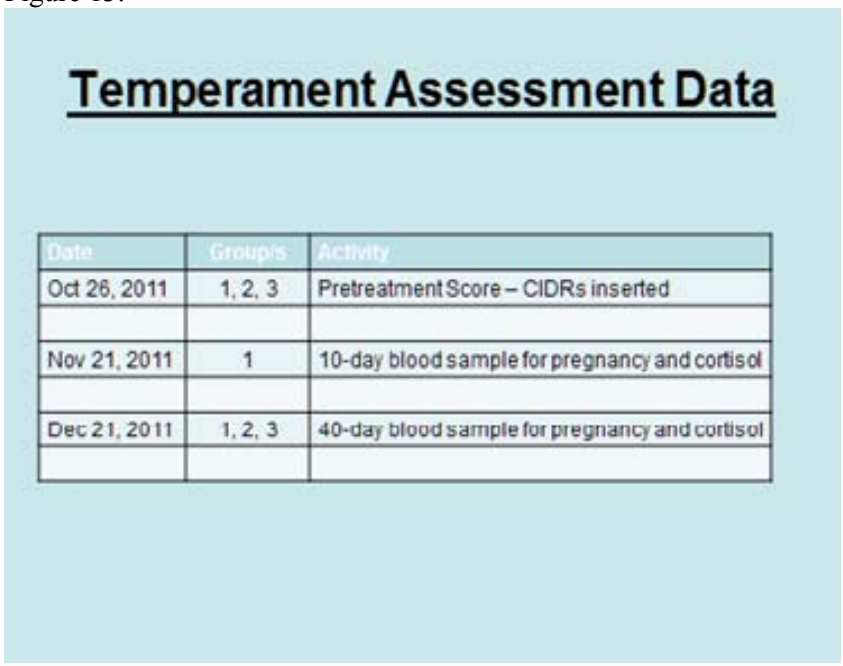
Figure 12.



Stress Evaluation using Visual Scores and Cortisol

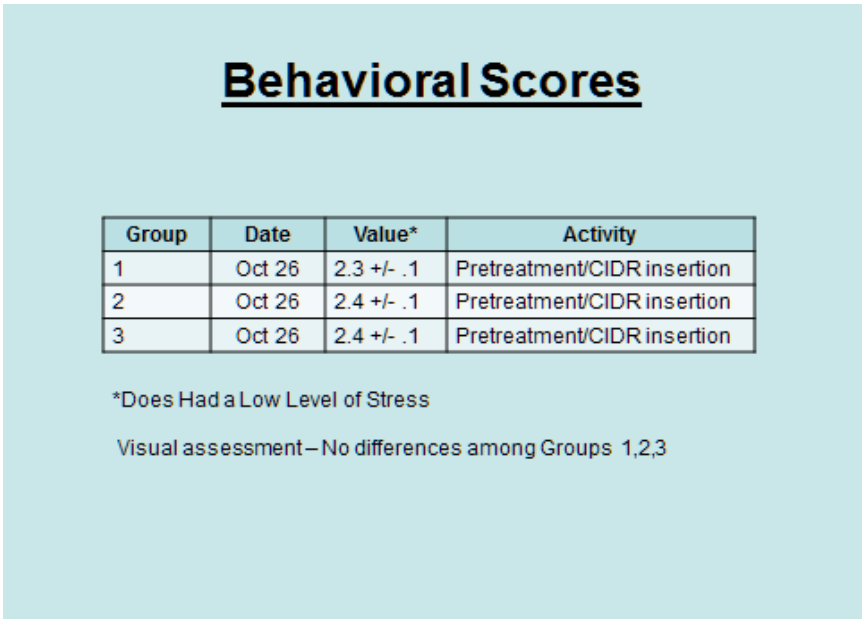
Data collected visually on the temperament of the does during handling are available in Figures 13, 14 and 15. In Figure 13 it identifies when the visual temperament assessment scores were assigned by Dr. Darrel Kesler.

Figure 13.



In Figure 14 summarizes the assigned Behavioral scores to does by the three groups. These data were assigned when the CIDRs were inserted on October 26, 2011. The combined values of the does in the three groups are quite similar. The value of 2.3 for Group 1 is not much different than the 2.4 for Groups 2 and 3. The scale used is found in Figure 1. The value of 2 correlated to Mild Stress and a value of 3 correlated to Moderate Stress. The does in this study had a low level of stress as assessed visually.

Figure 14.



In Figure 15, the data for Group 1 shows the behavioral scores were collected three times, Oct 26, Nov 21 and Dec 21. The values indicated that does were less stressed over time. In Groups 2 & 3 there were only two observation points and the scores between Group 2 and 3 were relatively the same. The scores between the two dates for the two Groups indicated there seemed to less stress on Dec 21 than on Oct 21.

Figure 15.

Behavioral Scores

Group	Dates		
	Oct 26	Nov 21	Dec 21
1*	2.3 +/- .1	2.2 +/- .1	1.5 +/- .1
2**	2.4 +/- .1	--	1.9 +/- .2
3**	2.4 =/- .1	--	1.9 +/- .1
Activity	CIDR Insertion	Blood Draw	Blood Draw

*Group 1 – Indication does were less stressed over time (r=.51)

** Only two observation points / Behavioral scores relatively the same

The cortisol values shown in Figure 16 indicated that there were no differences among groups or across time. It is difficult to determine if the cortisol was an accurate measurement of the stress in the does. From the Behavioral scores it seemed apparent the does were low stressed. This may also been the case with the cortisol scores. One additional thing to consider is by the time the does are in the handling chute they already have been stressed by getting them from the pen to the handling facility and into the chute. The value of having collected the visual behavioral assessment scores and utilizing the cortisol assay allows the producers who read this study to determine if their does might handle similar to the does in this study.

Figure 16.

Cortisol Values

Group	Dates			
	Oct 26	Nov 21	Dec 5	Dec 21
1*	--	7.63 +/- .62	--	8.45 +/- .85
2*	--	--	8.28 +/- .72	8.92 +/- .64
3*	--	--	--	8.32 +/- .68

*Indications there are no differences among groups or across time

Conclusions

The conclusions from this study are:

1. The data indicated that AI pregnancy rates (Group 1= 60%; Group 2=50%; Group 3=70%) handled at 10, 24, 40 days respectively (the 10 and 24 day groups were also handled at 40 days), were not significantly different among the three groups. Based on the results of the BioPRYN test the average pregnancy rate for the three groups was 59%. This compares to the last two years AI pregnancy rates of 56% in 2010 and 44% in 2009 for the Hanson deer herd.
2. Based on the results of the BioPRYN test the data indicated that pregnancy rates at 76 days post – AI were (Group 1=80%; Group 2=85%; Group 3=95%) handled 10, 24, 40 days respectively (the 10 and 24 day groups were also handled at 40 days), were not significantly different among the three groups. The average pregnancy rate for the three groups was 87% (when based on actual fawning data this figure was 75%).
3. The behavioral scores and cortisol values revealed that the does in this study had a low level of stress.
4. The PSPB levels detected at 10 and 24 days post-breeding indicated that the BioPRYN$_{wild}$ assay does not detect whitetail deer PSPB until after 24 days post-breeding. This means that the assay can indicate the AI and buck bred does at 40 days post-breeding with high reliability when the appropriate insemination and sampling procedure is followed.
    a. The current procedure is to place follow-up bucks with AI bred does 18-20 days post AI breeding and draw a blood sample at 40 days post-breeding.
5. The data in the study indicated that does can be handled anytime during gestation with no more risk in handling deer any other time.

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