AL-TR-1991-0123

ARMSTRONG LABORATORY

BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF HORSES TO SIMULATED AIRCRAFT NOISE

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JANUARY 1991

FINAL REPORT DECEMBER 1989 TO JANUARY 1991

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AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

NOTICES

TECHNICAL REVIEW AND APPROVAL

AL-TR-1991-0123

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
10 January 1991

3. REPORT TYPE AND DATES COVERED
Final Report Dec 89 to Jan 91

4. TITLE AND SUBTITLE
Behavioral and Physiological Responses of Horses to Simulated Aircraft Noise

5. FUNDING NUMBERS
89-COOP-2-4929
PE: 63723F
PR: 3037
TA: 05
WU: 05

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Large Animal Clinical Sciences
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Gainesville, FL 32610

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Noise and Sonic Boom Impact Technology
WPAFB, OH 45433-6573

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AL-TR-1991-0123

11. SUPPLEMENTARY NOTES
P.C. $19.50
MF. $9.00

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The effects of simulated F-4 aircraft noise on pregnancy outcome, behavior, rate of habituation, cardiac function, serum cortisol and progestogen concentrations of pregnant mares were determined. Eight experimental and 8 control mares were exposed to aircraft noise on days 320, 323, and 324 of pregnancy. The noise was heard over 47 sec with sound intensity increasing at 54.7 dB/sec to a maximum of 115 dB. Behavior was viewed using closed circuit TV and tapes were scored by a 5-member panel. All treatment mares delivered live, normal foals without assistance. Mean anxiety and movement scores were significantly different. Heart rate increased during noise periods, but without ectopic arrhythmias. Researchers observed some adaptation to the noise with less of a heart rate increase after successive episodes. Treatment mares experienced a significant rise in serum cortisol only after the first exposure to noise. Progesterone concentrations in serum varied greatly among individuals, but profiles were well within the normal range.

14. SUBJECT TERMS
Aircraft, Noise, Domestic Animals, Horses, Disturbance, Physiological Effects

15. NUMBER OF PAGES
70

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

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ABSTRACT

The effects of simulated F-4 aircraft noise on pregnancy outcome, behavior (startle reflex), rate of habituation, cardiac function, serum cortisol and progestogen concentrations of heavily pregnant mares were determined. Sixteen mares were assigned to treatment or control groups (8 each) according to foaling dates. From day 313 of gestation until parturition, mares were housed in box stalls between 1500 and 0900 hours. The remaining hours were spent in pasture. Mares were placed in stalls on day 313 to day 319 so they could become acclimated to stall environment prior to the start of the experiment on day 320. On days 320, 323, and 324 of gestation, the 8 treatment mares were exposed to four daily simulated F-4 aircraft noise events. The simulated noise was heard over 47 sec with sound intensity increasing at 54.7 dB/sec with a maximum of 115 dB. Three of the four noise exposures were administered randomly between 1630 and 2200 hours and the fourth was administered between 0600 and 0800 hours. On day 321 of gestation, treatment mares were exposed to 6 noise events spaced 10 min apart beginning at 2030.

Behavior of the mares was viewed through closed circuit security-type black and white TV cameras with wide angle lenses that were placed in each stall. The normal activities of all mares were videotape recorded for one minute on the hour and half-hour from 0530 to 0700 hours and again from 1630 to 2200 hours on days 315, 318, 326 and 328 of gestation. To record agonistic behavior due to noise, the mares were videotaped for one min each at 20, 10, and 1 min prior to each noise event, and continuously from 0 to 4 min after each noise event and again for one min each at 10, 20, and 40 min post noise. Tapes were reviewed by a panel of 5 members to evaluate anxiety and movement of treatment mares related to the noise event. The difference between the pre-noise and noise minute values was determined and statistically analyzed.

Long-term recording of the electrocardiogram by Holter recorders was performed on treatment mares to evaluate cardiac function. Each treatment mare was instrumented for 2 of the 4 nights that she was subjected to noise. Four control mares underwent identical procedures except exposures to aircraft noise. The cassette tapes were analyzed with a computer assisted Holter analyzer and screened for ECG-abnormalities, especially ectopic tachycardias, on full-disclosure printouts. The average hourly heart rate, the number of premature supraventricular contractions (APC), ventricular contractions (VPC), and sinus tachycardias were recorded and analyzed statistically.

To determine the stress response due to aircraft noise, cortisol was measured in serum collected prior to and after designated noise events. Blood was collected 5 min prior and 5, 15, 30, 60, 90, and 120 min after noise events 1 and 4 on day 320, 323 and 324. Control mares were bled on day 320 of gestation using the same blood collection schedule but were not subjected to aircraft noise. On day 321 blood was collected prior to noise events 1 and 3 and after noise event 6 following the same sequence as for day 320 (Table 9). Resting cortisol concentrations in all mares were determined prior to the start of the experiment on day 319 by collecting blood 15, 30 and 45 min after placement of a jugular catheter. Two weeks after the experiment was completed blood was again collected from treatment mares to determine if there were any residual effects from the experiment. Cortisol was measured in serum by a competitive binding radioimmunoassay kit (Gamma Coat, (I^{125}) Cortisol) validated in our laboratory. Differences between pre-noise treatment cortisol concentrations and times after noise events were analyzed by paired Student t-tests. Differences between treatment and control groups were evaluated by Student t-tests.

Progestogen concentrations in serum were evaluated to determine the effects of simulated aircraft noise on the feto-placental unit. Serum progestogen concentration was measured prior to noise exposure, during noise exposure (on days 323 and 324) and then every 3 days thereafter until 7 days before foaling at which time serum progestogen was measured daily. The same protocol was followed for control mares except they were not subjected to noise. Progestogens were measured in plasma by a solid-phase radioimmunoassay (Coat-a-count) previously validated in our laboratory.

None of the mares, treatment or control, aborted. Treatment mares all delivered live, normal foals without assistance, whereas, one of the control mares delivered a stillborn foal that required assistance.

We found that mean anxiety and movement scores (behavioral analysis) were significantly different by treatment day with the highest scores (highest degrees of anxiety and movement) seen following the first noise on day 320. As the mares continued to be subjected to the noise, scores decreased and it appears that the scores fell significantly after the 5th noise on day 321. All of the mares showed flight posture following the first noise but at no time did any mare strike or run into the stall walls or injure herself. Habituation rate varied among individuals with 7 of 8 mares habituating by the fifth noise on day 321 (anxiety score < 3 -mare showing alert stance only). The eighth mare habituated by the fourth noise on day 323.

There was a fairly consistent increase in heart rate (HR) during the noise periods, however, without any ectopic arrhythmias. A total of 55 noise episodes caused 21 "true sinus tachycardias" (HR > 100 beats/min for 20 sec), 8 "false sinus tachycardias" (HR > 100 beats/min for 5-19 sec) and 15 "transient increases in heart rate" (HR > 70 but < 100 beats/min). Control mares experienced only 2 true sinus tachycardias and no false sinus tachycardias or transient increases in heart. Treatment mares had significantly higher heart rates during sound hours than during no sound hours (57.4 ± 10.5 versus 54.8 ± 9.2, mean $\pm$ SD, respectively; $p < 0.05$). The difference in average heart rates for all hours, between control and treatment mares failed to reach statistical significance ($p > 0.05$). We noticed some adaptation to the noise with less of a heart rate increase with repeated noise episodes, however, we did not have sufficient numbers to prove it.

Mean cortisol concentrations in serum following catheterization of the jugular vein on day 319 rose in treatment mares but not in control mares. This rise was attributed to changes in cortisol concentrations in 3 of the 8 treatment mares. These three mares were accountable for 24 of the 26 serum samples containing > 60 ng/ml of cortisol. Treatment mares experienced a significant rise in serum cortisol only after the first exposure to simulated aircraft noise on day 320. This rise in cortisol was seen at 15, 30 and 120 min post noise ($p < 0.01$)

The progestogen concentrations in serum varied greatly among individuals, however, the progestogen profiles were well within the normal range. Because there were no abortions or major changes in progestogen concentration, we conclude that simulated aircraft noise does not affect adversely the feto-placental unit.

The result of this experiment suggests that mares exposed to jet noise while they are in the confines of a familiar stall are unlikely to injure themselves during a behavioral flight response. This experiment did not measure the responses of mares kept in larger open or novel spaces. Nor did it use live flyovers where the mares might see as well as hear the planes. However, since all of the mares in this experiment showed flight behavior during at least the first simulated jet noise event, it is likely that in a larger space the flight responses could result in a greater speed of gait (gallop) and distance covered. This increase in speed and distance may increase the risk of injury by running into objects. Therefore, it is suggested that, if new horses are brought into an environment that is subject to low level jet flyovers, they should remain stall confined until their level of behavioral reaction is identified and they habituate.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

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LIST OF TABLES

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1. INTRODUCTION

Since the 1950's over 40 studies conducted in the United States and overseas have addressed the acute effects of aircraft noise and sonic booms on domestic animals. These studies have been motivated both by public concerns and by claims leveled against the United States Air Force for damage done to farm animals by very low-level subsonic overflights. There are contradictions, however, among controlled studies and between anecdotal reports (claims against the USAF and popular accounts) and controlled studies. Numerous claims suggest that catastrophic responses by animals, such as stampedes, are possible, yet this is seldom seen by researchers. Reasons for these contradictions include the observational and short term nature of studies, the observation of subtle effects that can not be proven statistically, and the inability of researchers to design their project similar to the conditions under which substantial damages had been reported in claims.

Military pilots must become familiar with the aircraft that they are assigned to fly. Training flights are conducted to enable pilots to acquire basic flight skills, improve mobilization readiness, and fulfill National defense mission requirements. These Military Training Routes are conducted above rural areas. Many times these areas have commercially-run and noncommercial family farms, exotic animal farms, cattle ranches, and hunting preserves. Because it is impossible to avoid all of these areas the USAF is currently conducting studies to determine what is the environmental impact of aircraft overflights. Information gained from these studies will enable the USAF to avoid noise sensitive areas, identified with research as being such, and provide compensation for those facilities that are justifiably damaged.

This study was conducted to provide the USAF with a statistically designed study that evaluates the effects of simulated F-4 aircraft noise on pregnant mares near term. It was designed to determine the physiological and behavioral responses of mares to aircraft noise.

2. STUDY I: EFFECTS OF SIMULATED AIRCRAFT NOISE ON HEAVILY PREGNANT MARES

2.1 EXPERIMENTAL METHODS AND DESIGN

2.1.1 Specific Aim 1

Evaluate the effects of simulated F-4 aircraft noise on pregnancy outcome, behavior (startle reflex), rate of habituation, cardiac function, serum cortisol and progestogen concentrations of mares.

2.1.2 General Design

Sixteen pregnant mares were housed in pasture until day 313 of gestation. These mares were of light horse breeds and included: 5 Saddlebred, 4 Thoroughbred, 4 Quarter Horse, 2 Morgans and 1 Standardbred. From day 313 until parturition, all mares were housed in box stalls, 11 x 15 ft, from 1500 until 0900 hours. During the remaining hours, 0900-1500, mares were turned out to pasture. The 16 mares were assigned to a treatment or control group (8 each) according to foaling dates. Foaling dates of treatment mares were matched to those of control mares to reduce the effect of season on gestation length (Table 1). All mares were stalled in the same barn, however, in different sections to ensure that control mares were not subjected to noise events. The treatment and control mares were pastured in separate but similar pastures once stalling began. All mares were fed hay and grain and routine preventative veterinary management practices were followed.

Mares were acclimated to the stall environment from day 313 to day 319. On days 320, 323, and 324 of gestation, the eight treatment mares were exposed to four daily simulated F-4 aircraft noise events. The simulated noise was heard over 47 sec with sound intensity increasing at 54.7 dB/sec and with maximum of 115 dBA. Three of the four noise exposures were administered between

1630 and 2200 hours and the fourth was administered between 0600 and 0800 hours. Exposure times were chosen randomly to avoid a habituation response in mares. Exposure times were as follows: On day 320-0630, 1630, 2000 and 2100; on day 323-0600, 1630, 1800, and 2030; and on day 324-0600, 1730, 2000 and 2200. On day 321 of gestation, treatment mares were exposed to 6 noise events spaced 10 minutes apart beginning at 2030. This schedule was chosen to simulate a worse case flight scenario. The remaining eight control mares were not exposed to noise.

Table 1. Experimental Design

Assignment Length	Group	Due Date	Foaling Date	Gestation
T1	T1	2-16-90	2-24-90	348
T2		2-16-90	3-01-90	353
T3		2-18-90	3-02-90	352
T4		2-20-90	3-09-90	357
T5	T2	4-01-90	4-04-90	343
T6		4-08-90	4-02-90	334
T7		4-08-90	4-10-90	342
T8		4-08-90	4-14-90	346
C1	C1	1-03-90	1-13-90	350
C2	C2	3-13-90	3-15-90	342
C3		3-14-90	3-26-90	352
C4	C3	4-20-90	4-22-90	342
C5		4-22-90	4-19-90	337
C6	C4	5-11-90	5-20-90	349
C7		5-11-90	5-04-90	333
C8		5-15-90	5-11-90	336

2.2 PREGNANCY OUTCOME

None of the mares, treatment or control, aborted. Treatment mares all delivered live, normal foals without assistance, whereas, one of the control mares delivered a stillborn foal that required assistance. Gestation length of treatment mares was not significantly different from control mares. Gestation lengths for treatment and control mares were 346.9 ± 7.3 and 342.6 ± 7.1 days, respectively.

2.3 BEHAVIORAL ASPECTS (Startle Reflex and Habituation Rate)

The reaction of an animal to a threatening stimulus usually results in the animal moving quickly away from and future avoidance of the threatening stimulus. If the animal cannot avoid a threatening stimulus, as can be the case with penned domestic and zoo kept animals, then an adaptation to the stimulus may occur. This is called habituation.

Horses, as prey animals are well known for their flight behavior when a perceived threat occurs. If sufficiently alarmed during a perceived threat the horse can injure itself, other animals or people. The Air Force has received complaints of injury to horses and their riders following the overflight of military aircraft. This investigation was performed to determine the behavioral reaction of stalled horses to simulated F-4 aircraft noise and to determine the rate of habituation.

2.3.1 Experimental Design

Stalls (3.4 m x 4.6 m) were situated in pairs with one common wall where the mares could see and smell each other. The loud speakers were positioned directly over the common wall 3 m above the stall floor. The Noise Simulation System that was used, was designed to simulate a F-4 aircraft on a low-level Military Training Route, traveling at a speed for 569 kts/hr at 449 feet above the ground. The lateral distance between the recording station and the aircraft during overflight was 36 feet. This system utilized prerecorded noise samples from low-flying F-4s which were played back through a Panasonic Digital Audio Tape player. The aircraft noise event was generated via a loudspeaker cluster projected above the mares to simulate the flyover conditions. The stalls below the loudspeaker were calibrated into areas of known sound levels. Table 2 summarizes the values of the noise level descriptors for the F-4 in each of the subareas identified in Figure 1. Since the noise environment is not constant in each subarea, a noise level range is given in the last column. For example, The area designation 1 shows an Equivalent Noise Level (LEQ) of 105.3 dBA. The range of noise levels for this area is given as ± 2.0dB. This means that the LEQ level is Area 1 for the flyover sample is between 103.3 and 107.3 dBA with a mean value of 105.3 DBA.

Table 2. Summary of Noise Levels in Mare Observation Areas for the F-4

AIRCRAFT TYPE: F-4D				
AREA	A-Weighted Noise Level Descriptors (dBA)			Noise Level Range in Area (dB)
	LEQ[1]	MAX[2]	SEL[3]	
1	105.3	113.4	112.2	± 2.00
2	100.3	108.4	107.2	± 3.00

[1] LEQ Equivalent Sound Level. The LEQ is defined as the steady A-weighted sound level which produces the same A-weighted sound energy over a stated period of time as a specified time varying sound. In this case, the time period starts when the aircraft noise exceeds a level of 70 dB and ends when the level falls below 70dB

[2] MAX Maximum rms dB level achieved during flyover

[3] SEL Sound Exposure Level. SEL is the level in decibels of the time integral, relative to one second of the sound level, usually over a single event

Stalls were fitted with closed circuit security-type black and white TV cameras with wide angle lenses which allowed viewing of approximately 95% of the stall area. Coded cards and clocks were placed within view of each camera for tape identification. Mares within each group (treatment or control) had their normal activities videotape recorded for one minute on the hour and half-hour from 0530 to 0700 hours and again from 1630 to 2200 hours on days 315, 318, 326 and 328 of gestation. To record agonistic behavior due to noise treatment on days 320, 321, 323 and 324 of gestation, the mares were videotaped for one minute each at 20, 10 and 1 minute prior to each noise event, and continuously from 0 to 4 minutes after each noise event and again for one minute each at 10, 20 and 40 minutes post noise.

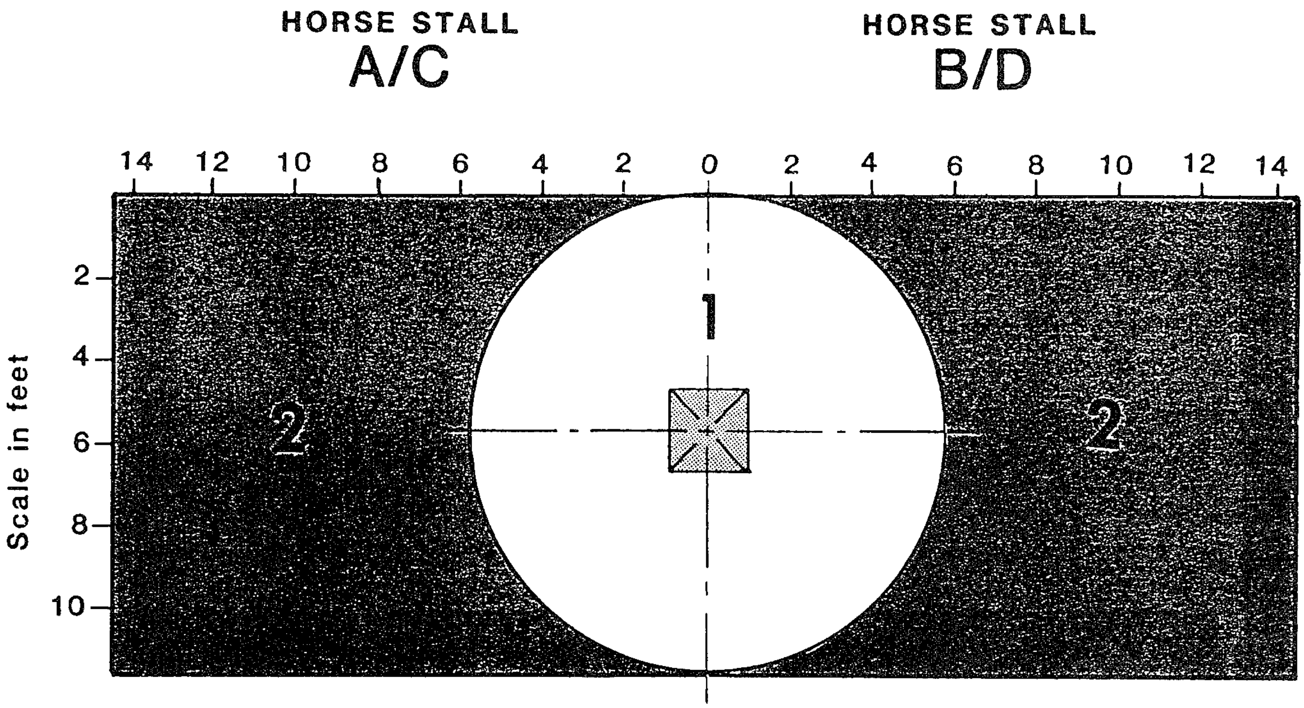

Figure 1. Observation Area Noise Sub-Area Map

A five member panel evaluated the tapes of the treatment mares for agonistic behavior during the one minute just prior to the noise event and during the minute starting with the beginning of the noise event. The noise event lasted 47 seconds. The panel members were experienced horse people and included a farrier, a trainer, a breeder, a veterinarian and a backyard owner. The horses were rated as follows: 2 = normal posture, 3 = alert an/or irritation postures, 4 = alarm posture and 5 = flight posture as described by Waring (1983). This was called the anxiety score, assumed to be of increasing intensity, and statistically analyzed as continuous data. The movement scores were determined by counting the total number of quadrants of the stall the horse occupied or moved through during any given one minute period.

2.3.2 Statistical Analysis of Behavioral Data

Two separate analysis of variances were performed. The first used data collected on days 320, 323, and 324 of gestation where the response variables of anxiety and movement are averaged over time and the second used day 321 of gestation only without averaging the response variables over time. In each case the difference is taken between before and during the noise treatment values and this difference is analyzed as a Randomized Complete Block design with horses as blocks.

2.3.3 Results

The results of effect of treatment day on mean anxiety and movement scores for days 320, 323 and 324 are presented in Table 3 and 4. For the response, anxiety, there was a significant difference in scores due to day of treatment (Figure 2). Anxiety scores on day 320 were significantly higher than those on day 323 ($p < 0.0001$). Scores did not differ, however, between days 323 and 324. All of the treatment mares received an anxiety score of 5 (flight) from four or more of the panel members on the very first noise exposure (day 320). For the response, movement, each of the days (days 320, 323, and 324) are significantly different from the others (Table 4; Figure 3, $p < 0.01$).

For day 321 (6 noise events in one hour), the mean anxiety score decreased linearly over time ($p < 0.0001$; Table 5, Figure 4) as did the movement score ($p < 0.0005$; Table 6, Figure 5). The mean anxiety score for day 321 was 3.7. By the fifth noise event 7 of the 8 mares had an anxiety score of 3 or less. The remaining mare had a score of three or less by the last noise event on day 323.

Table 3. Mean Panel Anxiety Scores* by Days Treatment Mares

	Day of Gestation		
	320	323	324
Pre-noise min.	2.09	2.05	2.01
Noise min.	4.50	3.07	2.95
Difference+	2.41	1.02	0.94

*2 = normal, 3 = alert, 4 = alarm, 5 = flight
+d320 vs d323 ($P < .0001$)

Table 4. Mean Movement Values* by Days Treatment Mares

	Day of Gestation		
	320	323	324
Pre-noise min.	1.81	1.84	1.47
Noise Min.	7.75	4.59	2.44
Difference+	5.94	2.75	0.97

* Stall quadrants traversed per min.
+d320 vs d323 ($P < .0003$); d323 vs d324 ($P < .019$)

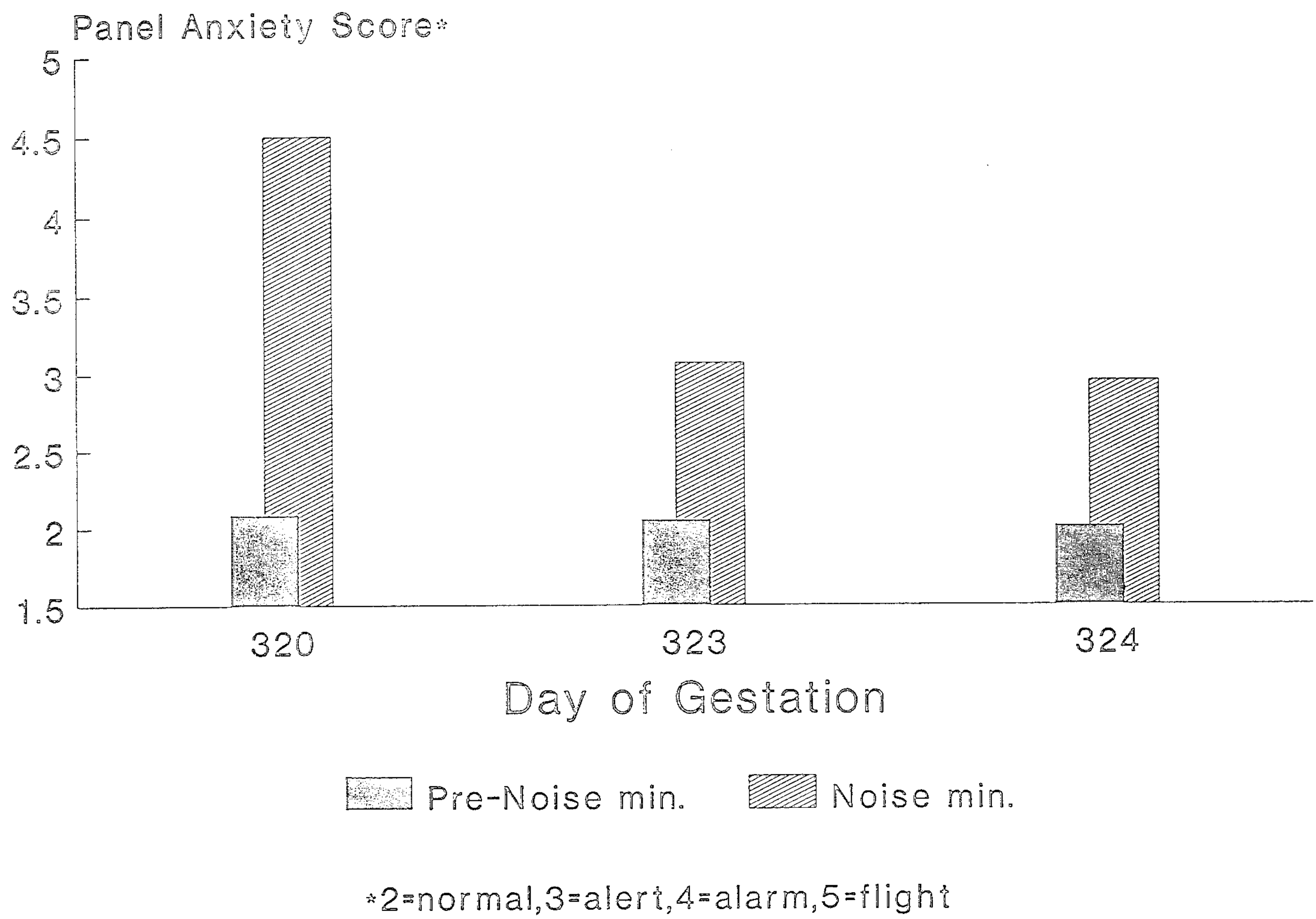

Figure 2. Mean anxiety score of mares subjected to simulated aircraft noise on days 320, 323, and 324. There was a significant effect of day on anxiety score ($p < 0.0001$; $F = 66.88$). Mean anxiety score on day 320 was higher than on day 323 ($p < 0.0001$; $F = 94.92$). Anxiety scores did not differ on days 323 and 324 ($p < 0.6067$; $F = 0.28$).

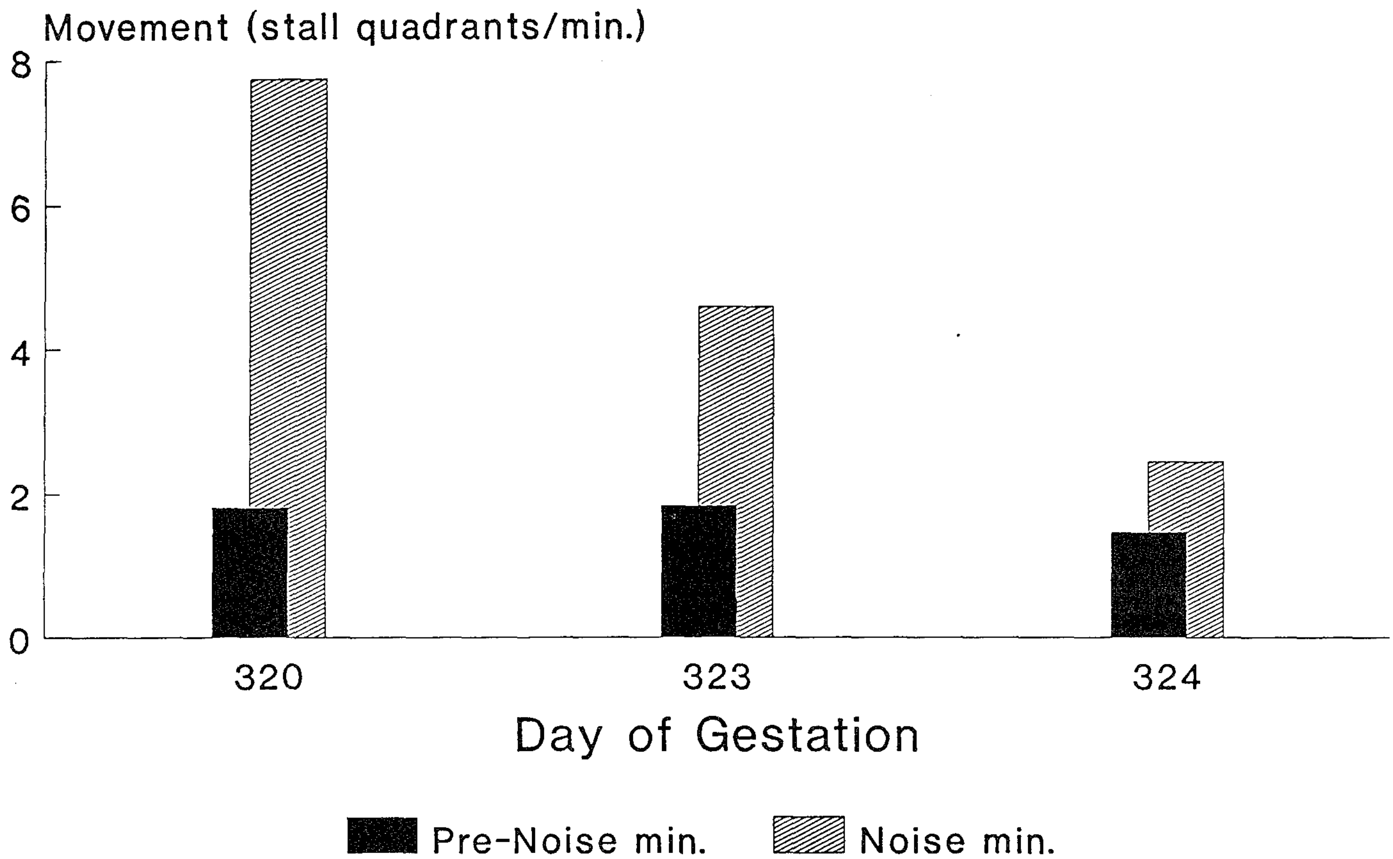

Figure 3. Mean movement scores of mares subjected to simulated aircraft noise on day 320, 323, and 324. The response is significantly different between each day. (effect of day $p < 0.0001$, $F = 28.13$; day 320 versus day 323 $p < 0.0003$, $F = 22.55$; day 323 versus day 324 $p < 0.0189$, $F = 7.04$).

Table 5. Mean Panel Anxiety Scores* by Event
D321 Treatment Mares

Event	Pre-noise min.	Noise min.	Difference+
1	2.00	4.05	2.05
2	2.05	3.78	1.72
3	2.00	3.90	1.90
4	2.12	3.72	1.60
5	2.05	3.62	1.58
6	2.00	3.10	1.10

*2 = normal, 3 = alert, 4 = alarm, 5 = flight
+linear trend ($P < .0001$)

All of the mares showed flight posture that included highly elevated head, wide open eye lids, dilated nostrils and forward or sideways movement faster than the walk on day 320, noise event 1. One or more complete circles of the stall were noted in most cases with some mares staying in motion for up to 20 seconds during the noise. At no time did any mare strike or run into the stall walls or injure herself.

Alarm and flight postures by treatment mares were observed only during actual noise exposure times. For one to two minutes after the noise event, alert posture, often including restless walking

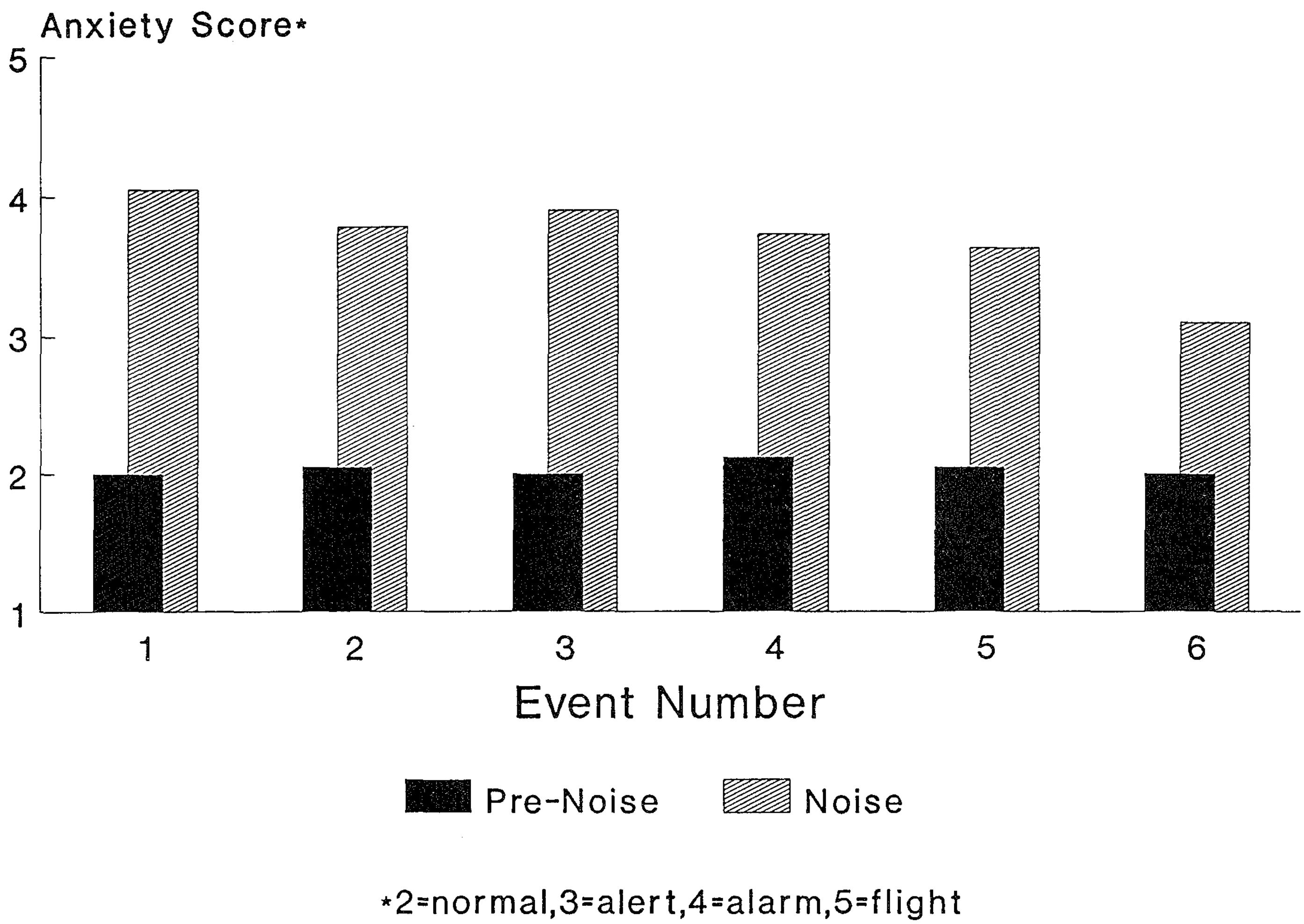

Figure 4. Mean anxiety scores of treatment mares on day 321 of gestation by noise event. Mean anxiety scores decreased linearly over time ($p < 0.0001$).

Table 6. Mean Movement Values* by Event
Day 321 Treatment Mares

Event	Pre-noise min.	Noise Min.	Difference+
1	1.00	7.25	6.25
2	1.00	6.88	5.88
3	1.12	7.25	6.12
4	1.12	6.50	5.38
5	1.62	5.00	3.38
6	1.00	4.38	3.38

* stall quadrants traversed/min.
\+ linear trend ($P < .0005$)

about the stall, was noted on days 320 and 321 in four of the eight mares but was rarely seen on days 323 and 324 in any of the mares. All mares were calm and easy to handle for the 5 minute post noise event blood collections.

Defecation or urination was frequently seen in the mares towards the end of the noise or during the minute immediately following the noise even through days 323 and 324. Most mares by the third day of noise exposure (day 323) were showing only an alert response of a moderately elevated head and if they moved, it was at a walk usually to move away from the speakers emitting the noise to stand at the opposite end of the stall. Four of the eight mares showed signs of irritation during some of the noise episodes on days 323 and 324 by shaking the head, pinning the

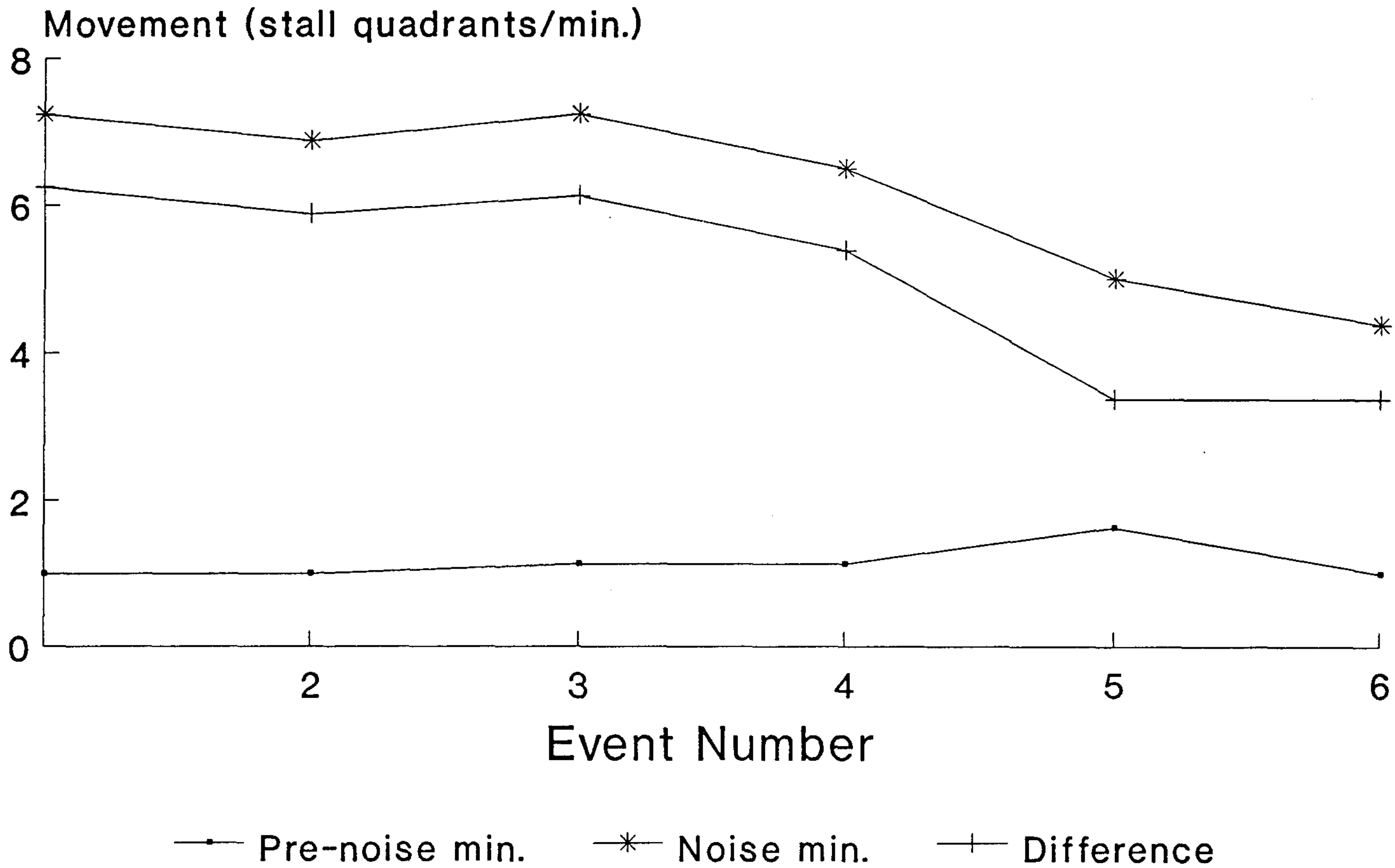

Figure 5. Mean movement scores of treatment mares on day 321 of gestation by noise event. Mean movement scores decreased linearly over time ($p < 0.0005$).

ears, backing up and/or wringing the tail. Uninterrupted chewing of grain or hay were observed in two mares during noise episodes on day 324.

A statistical comparison of the one minute video taping recorded on days 315,318, 326, and 328 of gestation (before and after noise event days) showed no significant differences between the time spent resting, alert or eating. Therefore, the four days of jet noise did not appear to alter the treatment mares' subsequent stall behavior.

2.3.4 Discussion

Waring (1983) describes three stages in the horses's fear-flight behavior response. The initial stage is characterized by the horse becoming alert, showing attempts to orient the sensory receptors (eyes and ears) of the head toward the stimulus. The horse may stop eating or walking, the head will be raised and the nostrils slightly dilated. During this phase the horse may actually become curious and approach the stimulus, however, if the stimulus is acute, the alert phase may be present only briefly before the next stage, alarm, is displayed.

During alarm, the eyelids open widely exposing white scleral tissue, the neck becomes fully elevated and the nostrils dilate. Any direct motion towards the stimulus ceases and defecation and nervous pawing may occur. Often an explosive blow of air is emitted from the nostrils. The last phase, flight, occurs with a speed, direction and distance relative to the stimulus with the horse moving away from the stimulus. An acute stimulus may result in alert, alarm and flight all occurring simultaneously. After an initial response the horse normally regulates its motion so as to not be excessive in its flight response.

Habituation is a reduction in reactivity of an animal to a stimulus over time. We found that the mares responded to the simulated aircraft noise by exhibiting all 3 stages of the fear-flight response. With repeated exposure to the noise, the flight phase was observed less frequently. The rate of habituation varied considerably among individuals, however, a decrease in the flight and alarm responses was seen in all mares by the 5th noise on day 321. It appears that the mares may have habituated more quickly during day 321 because of their repeated exposures to simulated noise over a short time. Differences in behavioral responses and in habituation rate among the mares may be due to different perceptions of the stimulus which are regulated by previous experience (Moberg 1985).

Krueger (1982) also reported adaptation in behavioral reaction and heart rate of mares by the 4th or 5th overflight of a particular aircraft. Erath (1984) exposed pregnant mares to similar aircraft flyovers (jets and helicopters) on three consecutive days (12 flyovers/day) and showed reductions in both heart rates and plasma cortisol concentrations from the first to the third day.

Horses, when compared with other grazing species, may show a more violent response to impulse noises and hot blooded breeds, such as the Thoroughbred, may not habituate as well as half-breeds (Cottereau 1978). From the results of the dairy cow study performed in conjunction with our study (Head HH 1991) it appears that horses do react more violently to simulated aircraft noise than dairy cows. Although this experiment used mares from several breeds some known as hot (Thoroughbred and Saddlebred mares) and some known as calm (Quarter Horse and Standardbred mares) there were insufficient numbers to detect breed or temperament differences. It has also been suggested that horses often show little reaction to an unusual noise unless they become afraid of the visually seen source (Schafer 1975). The mares in this study did not show fear of the speakers in the stalls that emitted the noise, but did respond to the noise.

2.3.5 Conclusion

Although all mares did show a flight response when subjected to simulated aircraft noise, they habituated very quickly, usually by the ninth noise treatment. It is very probable that if these mares were in a large paddock that they may respond by running when exposed to a loud, sudden jet noise for the first time. We suggest that if new horses are brought into an environment that is subject to repeated jet overflights, that they remain confined until they habituate. Observation of behavior would be the best method of evaluating habituation.

2.4 CARDIAC PARAMETERS

It is generally accepted that the noise generated by overflying aircraft constitutes an annoyance in humans, but may, particularly if occurring as a surprise, trigger a fright with the resulting "flight" reaction in fractious animals such as the horse. In horses, this "fright and flight" reaction appears to be particularly pronounced and characterized by very rapid elevation of the heart rate, induced by the release of endogenous catecholamines resulting in tachycardias and possible ectopic cardiac rhythms (Detweiler and Patterson 1972). These tachycardias, however, are likely to be short and transient in nature, and are unlikely to elevate the heart rate over prolonged periods of time (i.e. minutes or even hours) unless one is dealing with a particularly fractious, highstrung horse such as a thoroughbred in training. Because of the transient nature of the tachycardia, we do not believe of any resulting harmful effects, unless the horse runs away during a noise episode and injures itself during a stampede.

We hypothesize that the exposure to a sudden burst of aircraft noise triggers a relatively short-lasting bout of excitement and attempts to run away associated with only shortlasting (less than a minute) sinus tachycardia and possibly supraventricular ectopic tachyrhythmia in horses. In mares at late stages of their pregnancy however, such catecholamine mediated stress-responses, may be harmful and result in the induction of premature labor.

Long term recording of the electrocardiogram with so-called Holter recorders (slow speed analog recording of the amplified ECG-signal, and play-back with computer assisted processing) appears uniquely suitable for undisturbed, long term monitoring of the electrocardiogram of mares during their exposure to aircraft noise.

2.4.1 Experimental Design

Each treatment mare was instrumented for 2 separate nights with a holter ECG-recorder between their 320th and 324th day of gestation. Monitors were placed on mares prior to the first noise event of the evening and remained on the mares throughout the night. Monitors were removed approximately 60 minutes after the early morning noise event. Four pregnant mares served as controls and underwent identical procedures except exposures to aircraft noise. After clipping the hair and degreasing the skin with alcohol-soaked sponges, 5 permanent self-adhesive disk electrodes[1] were attached, 2 over the right scapula and 3 over the left apex of the heart near the ventral midline. This allowed the set-up of a bipolar base/apex lead (negative electrode over the right scapula, positive electrode over the left apex), a bipolar apex/base lead (negative electrode over the left apex, positive electrode over the right scapula) and placement of one additional electrode serving as the ground lead. The electrodes were attached to the patient cable and to a commercially available Holter recorder[2], and the entire set-up attached around the mare's chest with adhesive 2-inch tape and a surcingle.

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[1]AMI disp. electrodes

[2]Model 456A Electrocardiocorder, DelMar Avionics, Irvine CA 92714

The signal quality of the two leads was verified with a regular ECG machine, before a new battery and a regular tape recorder cassette were installed in the recorder and the recording start time was recorded on the internal digital clock of the instrument. The mares were allowed to roam free in their stalls during the night periods of recording and were disturbed as little as possible, except for the drawing of venous blood samples for hormonal analysis and cleaning out manure as indicated. Next morning, the Holter recorders were taken off the mares and the electrodes, leads and adhesive tape removed.

The cassette tapes were analyzed with a computer assisted Holter analyzer[3] with preset programs and additionally screened for ECG-abnormalities, especially ectopic tachycardias, on full-disclosure printouts, where 1 minute of analog ECG-signal (either of the two leads, apex/base or base/apex) is printed out at slow paperspeed and compressed to 1 line on regular 8 by 11 inch paper. In this mode, 1 hour of compressed ECG-data may be reviewed on a one page print-out (e.g. Figure 6). Additionally, computer-assisted retrieval of any desired time of recording and print-out of short strips at a regular ECG-recording speed (25 mm/sec) was done as needed, particularly during periods of tachycardias or poor ECG signal due to muscle tremor to rule-out artifacts.

The computer program was set up to count the average hourly heart rate (HR), and to detect and count the number of premature supraventricular contractions (APC), ventricular contractions (VPC) and pauses greater than 2 seconds. We defined episodes of "true sinus tachycardia" arbitrarily as episodes of sinus rhythm with the heart rate in excess of 100/min lasting for 20 seconds or longer, and episodes of "false sinus tachycardia" as sinus

[3]Model 152 Protrac, DelMar Avionics, Irvine CA 92714

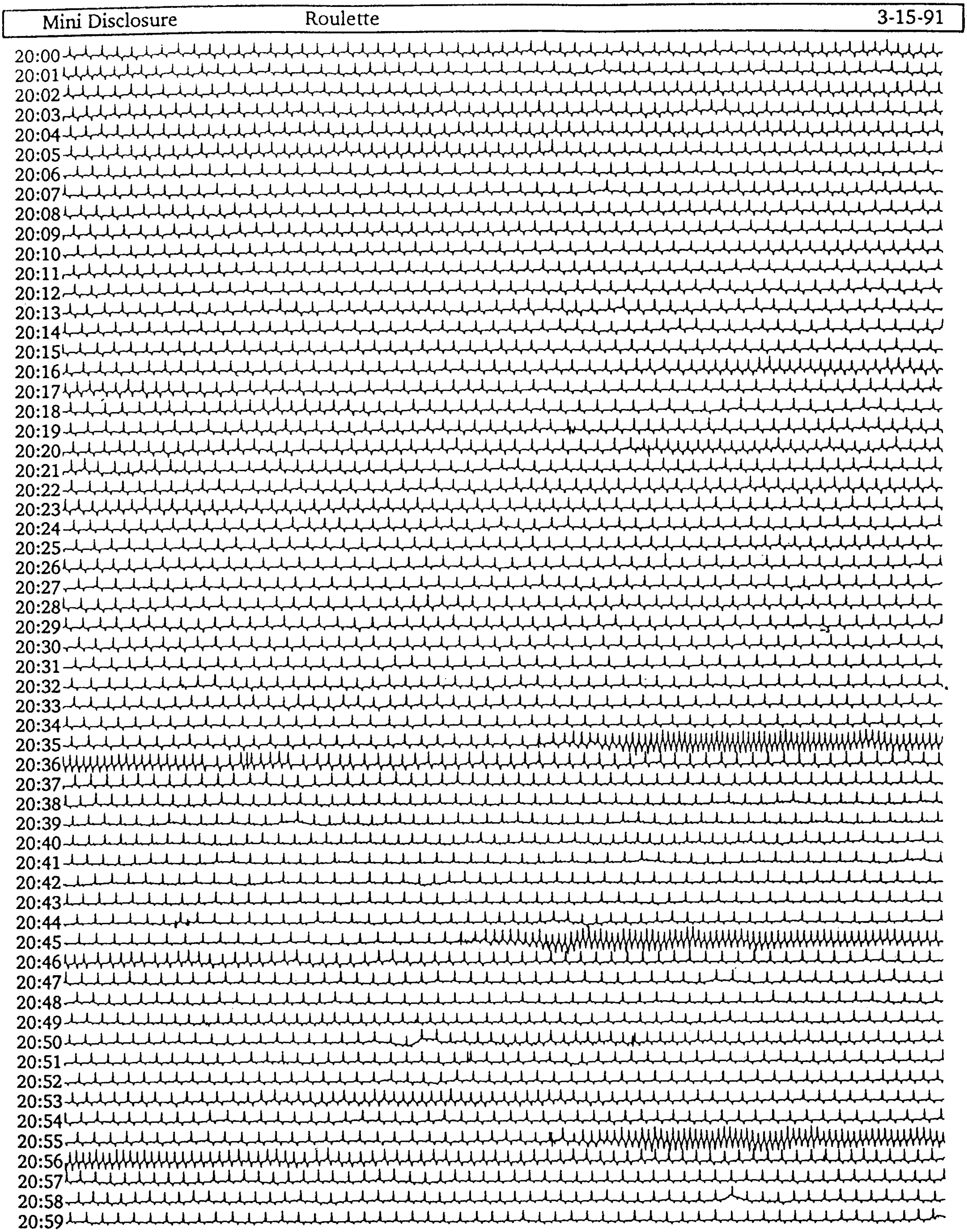

Figure 6. One Hour of Compressed ECG-data

rhythm over 100/min lasting from 5 to 19 seconds. Episodes of short rate increases (for less than 5 seconds) over 70/min, but less than 100/min, were defined as "transient HR increases".

2.4.2 Statistical Analysis

For the analysis of the results, we averaged the hourly heart rates for the entire recording time, exclusive of the hours with noise exposure, and compared them to the averaged hourly heart rates during which exposure to aircraft noise had occurred, with paired t-tests for each individual mare. The average heart rates of the control group of mares (without noise exposure) was compared with the average heart rates of the noise-exposed mares with Student's t-tests. Finally, we counted the episodes of "true sinus tachycardia", "false sinus tachycardia" and transient "HR increases" in relation to the episodes of aircraft noise, and counted any such episodes unrelated to noise.

2.4.3 Results

One recording was lost from the recorder due to technical malfunction of the equipment. One of the control mares foaled before the 2nd night of Holter recording could be performed. Otherwise, technically adequate recordings were generated from each mare and could be analyzed by the computer.

Table 7A shows the heart rate changes (episodes of "true sinus tachycardias", "false sinus tachycardia", and "transient heart rate increases") in relation to aircraft noise. We noticed a fairly consistent heart rate increase during the noise periods, however without any ectopic arrhythmias (Figure 6). We were surprised by the absence of any ectopic beats such as ventricular premature contractions. Figure 7 shows an ECG strip from a mare recorded at real speed of 25 mm/sec during an episode of true sinus tachycardia. A total of 55 noise episodes caused a total of 21 "true sinus tachycardias", a total of 8 "false sinus tachycardias", and a total of 15 "HR increases", resulting in a total of 44 periods with noise-induced heart rate changes.

Table 7: Heart Rate Changes in Relation to Aircraft Noise

Definitions:

TRUE SINUS TACH.	**= HR > 100 For 20 sec.**
FALSE SINUS TACH.	**= HR > 100 For 5-19 sec.**
HR INCREASES	**= HR > 70 but < 100**

Table 7A. Related to Noise

MARES #	Record. DATE	Number of Noise Episodes	Number of True Sinus Tachs (peak HR)		Number of False Tachs (peak HR)		Number of HR > 70 & < 100
T1	1-27-90	3	3	(120,120, 120)	0	-	0
T1	1-30-90	4	0	-	0	-	4
T2	1-27-90	3	3	(150,175, 175)	0	-	0
T2	1-30-90	4	0	-	0	-	1
T3	1-28-90	6	2	(110,100)	0	-	4
T4	1-26-90	3	1	(175)	0	-	0
T4	1-28-90	6	2	(130,130)	2	(120,110)	2
T5	3-15-90	6	4	(175,150, 150,120)	2	(120,120)	0
T5	3-17-90	4	3	(120,100, 110)	0	-	1
T6	3-15-90	6	0	-	3	(100,120 100)	2
T6	3-17-90	4	0	-	0	-	0
T7	3-14-90	2	2	(175,175)	0	-	0
T7	3-16-90	1	0	-	0	-	1
T8	3-14-90	2	1	(150)	1	(130)	0
T8	3-16-90	1	0	-	0	-	0
TOTAL		**55**	**21**		**8**	**-**	**15**

Table 7B. Unrelated to Noise

Mares #	Recording Date	Number of True Tachs. (peak HR)		Number of False Tachs. (peak HR)	
T1	1-27-90	2	(130,110)	0	-
T1	1-30-90	0	-	2	-
T2	1-27-90	0	-	1	(130)
T2	1-30-90	0	-	3	(100,100,100)
T3	1-28-90	0	-	0	-
T4	1-26-90	1	(130)	1	(130)
T4	1-28-90	0	-	0	-
T5	3-15-90	0	-	0	-
T5	3-17-90	2	(100,100)	1	(140)
T6	3-15-90	0	-	1	(100)
T6	3-17-90	0	-	0	-
T7	3-14-90	0	-	1	(100)
T7	3-16-90	0	-	3	(100,100,100)
T8	3-14-90	2	(120,150)	26	***
T8	3-16-90	0	-	3	(100,100,100)
Total		**7**		**45**	
Control Mares					
C1	4-21-90	1	(120)	0	-
C6	4-20-90	0	-	0	-
C6	4-22-90	0	-	0	-
C7	4-21-90	0	-	0	-
C7	4-24-90	1	(100)	0	-
C8	4-20-90	0	-	0	-
C8	4-22-90	0	-	0	-
Total		**2**	**-**	**0**	**-**

***(120,110,100,100,100,110,100,110,100,100,120,120,120,140,110,
120,120,130,120,100,110,110,100,110,110,110).

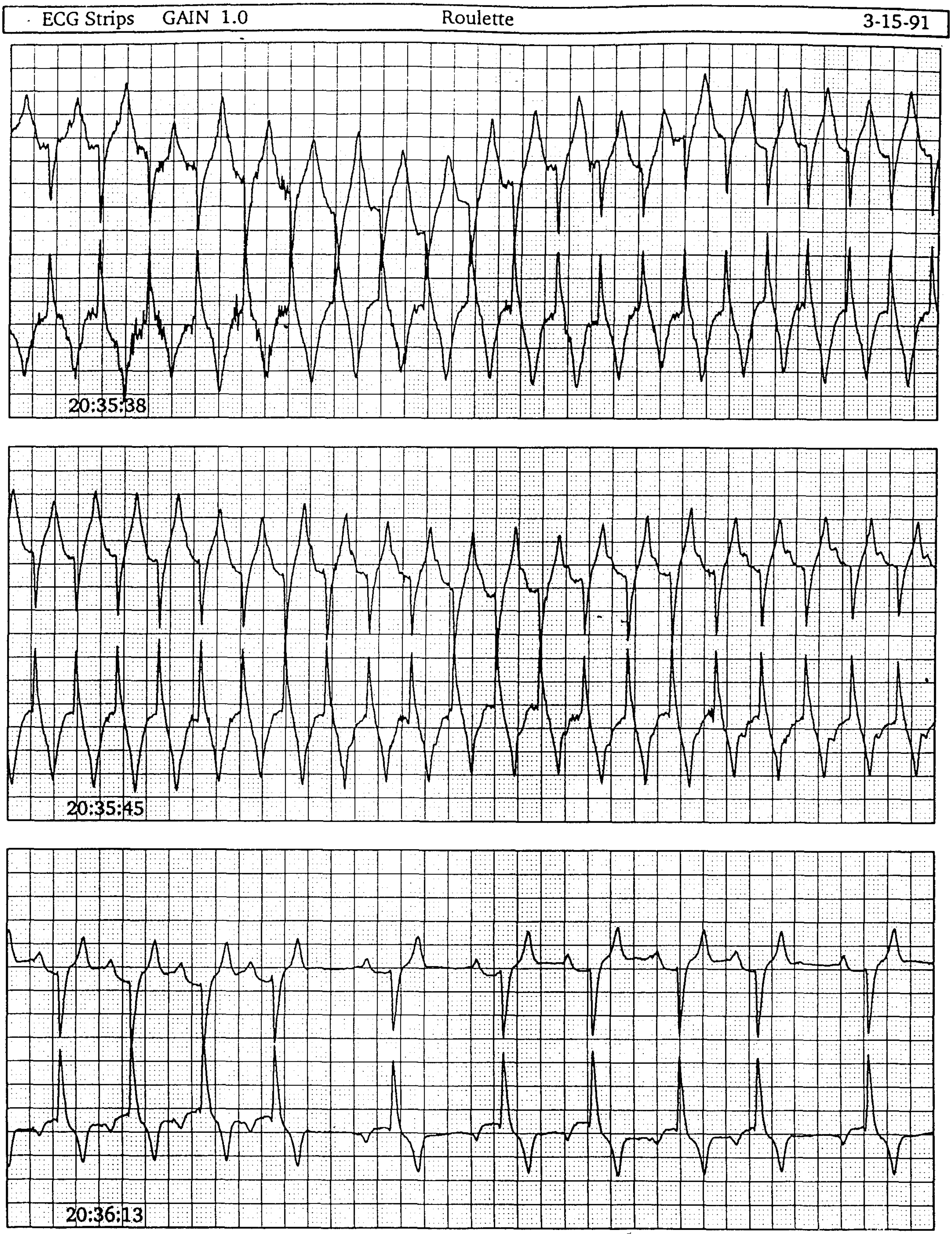

Figure 7. ECG Strip Demonstrating True Sinus Tachycardia

 Delta Holter™

In contrast, a total of 7 "true sinus tachycardias" and a total of 45 (26 in one mare alone) "false sinus tachycardias", resulting in a total of 52 heart rate changes unassociated with noise, occurred in treatment mares unrelated to aircraft noise, (Table 7B). One individual mare, T6 didn't seem to get disturbed very much and showed only mild heart rate increases without developing tachycardias during noise periods. Another mare, T8, appeared irritated during the experiment on the night of 3/14/90 and had frequent (a total of 26) episodes of "false sinus tachycardia" unrelated to aircraft noise. The peak heart rates reached during the episodes of tachycardia are also indicated in Table 7. They fell into the ranges previously published (120-180/min) (Detweiler and Patterson 1972) for sinus tachycardias in the horse and were not considered excessive.

There appears to be some adaptation to the noise with less of a heart rate increase with repeated noise episodes, however we did not have sufficient numbers to prove it. For example, mare T1 showed 3 noise-related episodes of "true sinus tachycardia" during the first night of recording, while only 4 noise-related episodes of "transient HR increases" were recorded during the second night of recording. There were no "true" and "false" sinus tachycardias observed in control mares.

In Table 8, the average hourly heart rates are reported for control and experimental mares, broken down into overall average hourly heart rates, average hourly heart rates during hours with aircraft noise and average hourly heart rates during hours without noise. The difference of average heart rates for all hours, between control and treatment mares failed to reach statistical significance ($p > 0.05$). Despite considerable overlap of the standard deviations, the means of the average heart rates during "no sound" hours (54.8/min) were significantly different from the "sound" hours (57.4/min), $p < 0.05$. When compared to the

Table 8. AVERAGE HOURLY HEART RATES

EXPERIMENTAL MARES

Name:	Rec. Date	Avg. HR ALL HOURS	Avg. HR NO SOUND HRS	Avg. HR SOUND HRS
T1	01/27/90	39.8	40.0	39.0
T1	01/30/90	77.6	77.5	78.0
T2	01/27/90	54.0	52.6	60.7
T2	01/30/90	54.3	53.8	56.0
T3	01/26/90	-	-	-
T3	01/28/90	58.9	58.2	62.0
T4	01/26/90	42.7	42.8	42.5
T4	01/28/90	43.6	43.8	43.0
T5	03/15/90	52.0	51.6	53.5
T5	03/17/90	51.2	50.8	53.0
T6	03/15/90	67.5	66.4	72.0
T6	03/17/90	62.5	62.2	64.0
T7	03/14/90	55.7	54.5	64.0
T7	03/16/90	60.1	60.2	60.0
T8	03/14/90	57.3	56.5	62.5
T8	03/16/90	50.6	50.8	50.0
AVG. ALL MARES		**55.2**	**54.8**[a]	**57.4**[a]
SD		**9.4**	**9.2**	**10.5**

CONTROL MARES

C1	04/21/90	50.5
C1	foaled	no data
C6	4/20/90	51.3
C6	4/22/90	52.0
C7	04/21/90	49.6
C7	04/24/90	48.3
C8	04/20/90	64.7
C8	04/22/90	65.0
AVG. ALL MARES		**54.5**
SD		**7.2**

[a] Values with similar superscripts are significantly different $p<0.05$

average heart rate for the entire recording period ("all hours"), the differences failed to reach statistical significance.

2.4.4 Discussion

To our knowledge, this experiment is the first to yield "true" resting heart rates (i.e. undisturbed by either ECG-recordings or manual pulse-taking) in the equine. Any intervention by man is bound to slightly influence (increase) the heart rate of the horse, which is considered a rather fractious animal. The recorded resting heart rates from the entire recording period ("all hours", Table 8) were well within the published normal range for warm-blooded horses (Detweiler and Patterson 1972). As expected, the initial noise exposure caused some fright-reactions with movement, characterized by muscle tremor on the baseline of the ECG-recording, as well as heart rate increases with true sinus tachycardia for longer than 20 seconds in all but one mare. The sinus tachycardias however were transient and lasted from 25 to 60 seconds, the longest being approximately 2 minutes.

The achieved peak rates in the range of 120-180/min were not considered excessive. Despite some compression of the ECG at such high heart rates, the P-waves could still be recognized on the down slopes of the T-waves of the previous heart beat and therefore the rhythm be identified as sinus tachycardia and not as an ectopic rhythm. Much to our surprise, no ectopic rhythms were recorded in any mare during any recording. It is possible that we dealt with a particularly "laid-back" subgroup of horses, or that pregnancy has some calming effect on cardiac rhythm, the latter being purely speculative. There are no published data on the true incidence of ectopic cardiac rhythms in apparently healthy equines, though anecdotal evidence of the increased presence of AV-Blocks 2nd degree, attributed to high vagal tone in well-trained thoroughbreds and standardbreds, exists among veterinary cardiologists. The used base-apex recording lead system served its purpose well and generated large P-waves and

QRS-complexes that were easily readable on the print-out and by the computer for analysis.

The significance of the recorded transient tachycardia episodes appears minimal. Though we were unable to prove fewer reactions with less of a heart rate increase with repeated noise exposure, it appeared that the mares were getting used to it and became less excited. Despite the significant difference of the average heart rates during "sound" hours from "no sound" hours, the effect of such a mild heart rate increase is not likely to have any effects on hemodynamics or altered myocardial metabolism.

2.5 HORMONAL PARAMETERS

2.5.1 Cortisol

Cortisol is the major corticosteroid in the horse (Hoffsis et al, 1970) and plasma concentrations have been shown to increase in stress (Taylor 1985; Rose et al 1983). We have shown (Santschi, et al 1991) that serum cortisol concentrations in pregnant mares undergoing severe medical or surgical crisis that subsequently aborted, were higher than in mares that did not abort after a crisis (mean $\pm$ SD 135 $\pm$ 35 and 83 $\pm$ 19 ng/ml, respectively). Therefore, a significant rise in serum cortisol may be associated with abortion. In this study, we determined if cortisol concentrations of heavily pregnant mares rose significantly after exposure to simulated aircraft noise and if this rise was associated with abortion.

2.5.1.1 Experimental Design

Blood for serum cortisol analysis was collected according to the following schedule. On day 319 of gestation, a catheter was inserted into the jugular vein of each mare. Fifteen, 30 and 45 minutes after catheter placement-times 0, 1 and 2, 10 ml of blood were collected and placed immediately on ice. Serum was harvested and stored at -20°C in labelled, dated, 2 ml vials until assayed. From days 320-324 blood was drawn from treatment mares according to the schedule in Table 8. Blood was drawn from control mares on days 320 of gestation according to the protocol listed for noise cycle 1- day 320. To ensure that any rise in serum cortisol concentration was due to noise, three treatment mares were bled according to the protocol listed for noise cycle 1 two weeks after noise treatment.

Table 9. Blood Collection in Treatment Mares for Serum Cortisol

			Time post-noise (min)					
Day	Noise cycle	Pre-noise (sample)[a]	5	15	30	60	90	120
320	1	x	x	x	x	x	x	x
	4	x	x	x	x	x	x	x
321	1	x						
	3		x					
	6		x	x	x	x	x	x
323	1	x						
	4	x	x	x	x	x	x	x
324	1	x						
	4	x	x	x	x	x	x	x

[a]Blood collected 5 min prior to noise.

Cortisol was measured in serum by a competitive binding radioimmunoassay kit (Gamma Coat, (I^{125}) Cortisol, Dade Baxter Travenol Diagnostics, Inc., Cambridge, MA 02139) validated in our laboratory. Sensitivity was 0.14 micrograms/dl. The antiserum cross reacted with cortisol 100%, prednisolone 73%, 6-methylprednisolone 18%, 11-deoxycortisol 4.4%, corticosterone 3.8%, prednisone 2.0%. Intra-assay coefficient of variation was 4.4 to 7.9% while the inter-assay coefficient of variation ranged from 5.0-9.7%.

2.5.1.2 Statistical Analysis of Cortisol Data

Differences between pre-noise treatment cortisol concentrations and times 5, 15, 30, 60, 90, and 120 after noise events were analyzed by paired Student t-tests with p values < 0.05 considered significant. The effect of repeated blood collection on serum cortisol concentrations on day 319 was analyzed by paired Student t-tests. Differences between treatment and control groups were evaluated by Student t-tests.

2.5.1.3 Results and Discussion

Cortisol results are shown in Table 10 and Figures 8 - 11. Mean cortisol concentrations in serum following catheterization of the jugular vein on day 319 rose in treatment mares but not in control mares (Table 10). Concentrations in treatment mares were statistically higher at the 30 minute sampling period (time 1) than at the 15 (time 0) and 45 minute (time 2) sampling period. Of the 8 treatment mares, three had significant rises in serum cortisol. The cortisol concentration in these 3 mares (T1, T3 and T4) ranged from 70-87.3 ng/ml at the 30 minute sampling period while the highest cortisol concentration observed in the remaining 5 mares was 43.5 ng/ml.

Table 10. Serum Cortisol Concentrations in Mares After Catheterization of Jugular Vein on Day 319

Time After Catheterization	Mean Cortisol Concentration ± SD Control Mares	Mean Cortisol Concentration ± SD Treatment Mares
15	23.6 ± 6.8	36.3 ± 14[a]
30	23.0 ± 6.8	52.2 ± 22[a]
45	24.7 ± 11.0	44.3 ± 23

[a] numbers with similar superscripts are significantly different

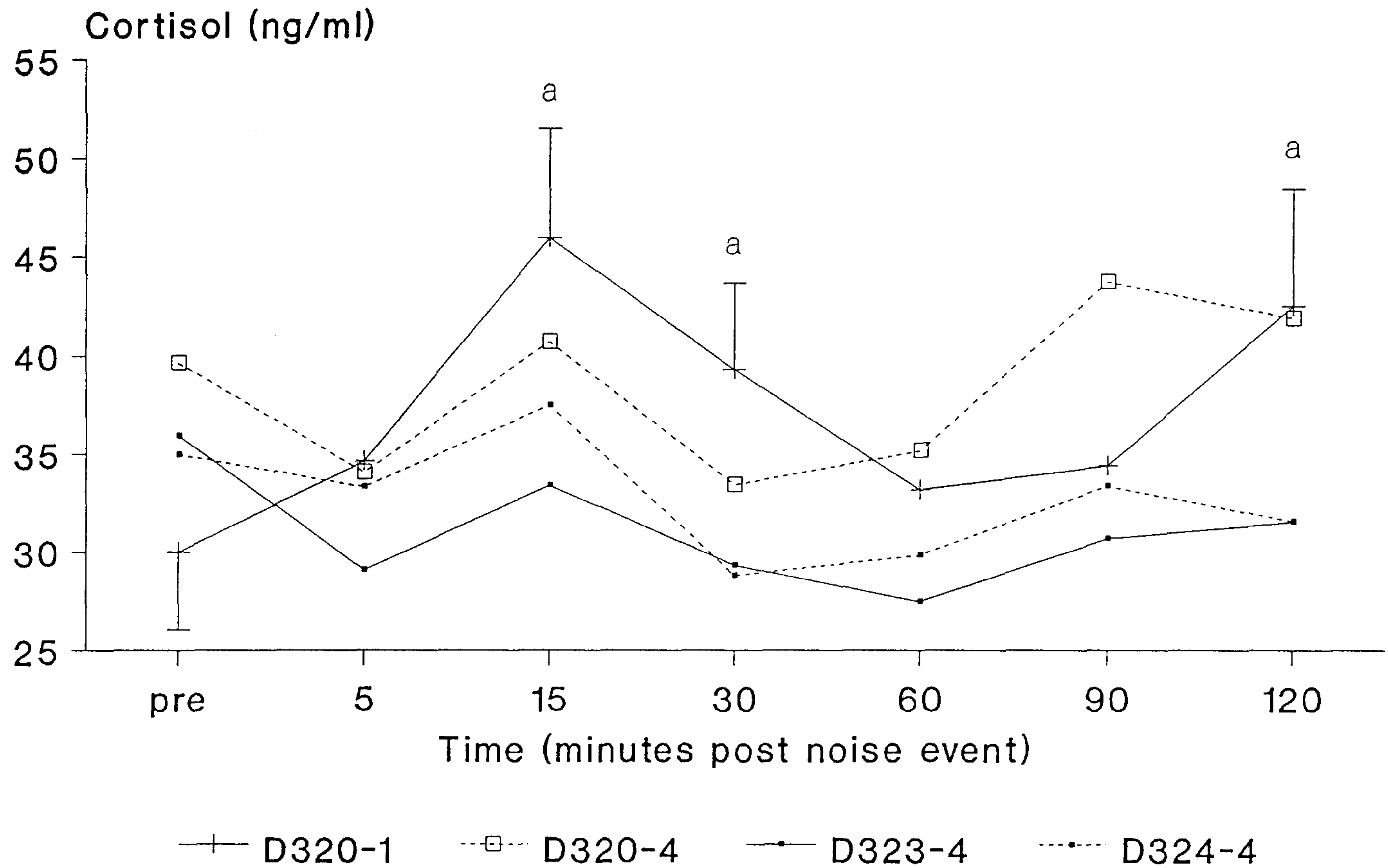

Figure 8. Mean serum cortisol concentration of mares subject to simulated aircraft noise on days 320, 323, and 324 of gestation. Blood samples were collected before and after simulated noise during noise event 1 on day 320, and event 4 on days 320, 323 and 324 of gestation. Mean cortisol concentration in serum of mares was significantly higher at 15, 30 and 120 min post noise (a, $p < 0.03$) than before subjection to noise only after noise event 1, day 320. The vertical bars represent the standard deviation at each time point.

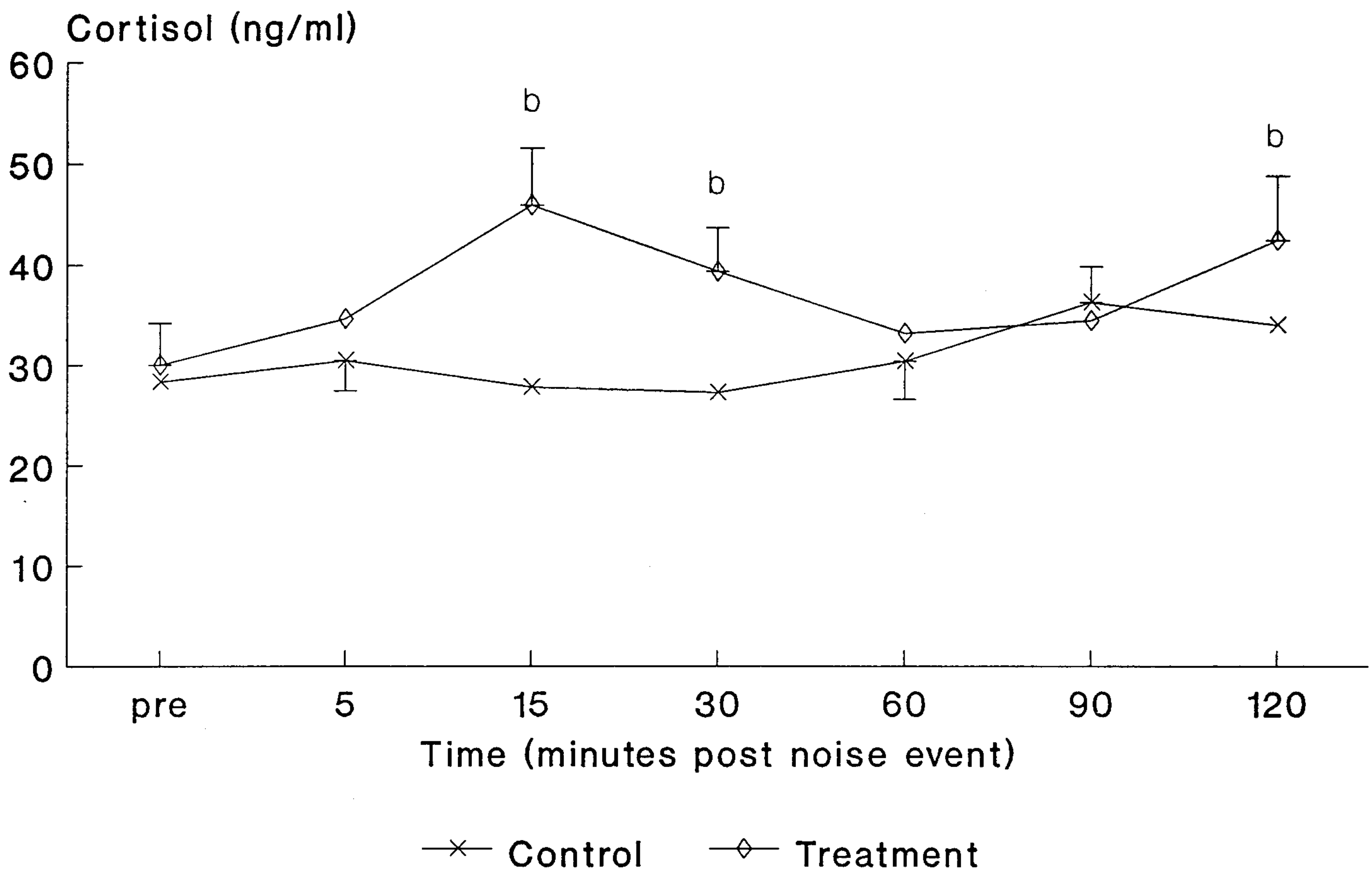

Figure 9. Mean serum cortisol concentrations on day 320 of gestation. Mean serum cortisol concentrations of treatment mares prior to and after simulated noise during event 1, day 320 are compared to control mares bled in a similar sequence but not subjected to noise. Mean cortisol concentrations n serum of treatment mares was significantly higher at 15, 30, and 120 min post noise (b, $p < 0.05$) than concentrations measured in control mares.

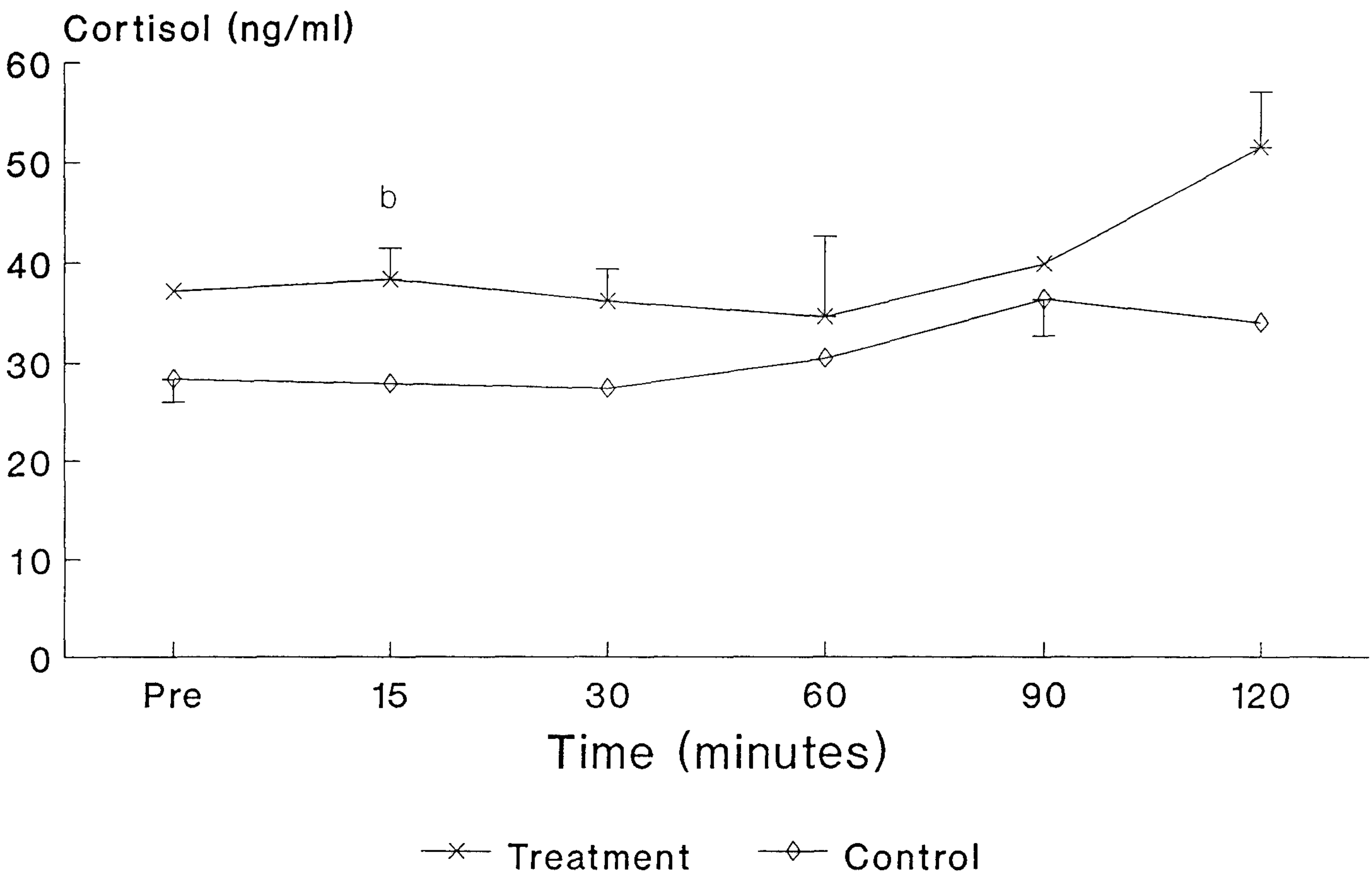

Figure 10. Comparison of serum cortisol concentrations of 3 treatment mares (T1, T2, and T3) bled 2 weeks after noise events with concentrations of control mares sampled on day 320 of gestation. Treatment mares had higher mean cortisol concentrations at the 15 min bleed than the control mares ($p < 0.05$).

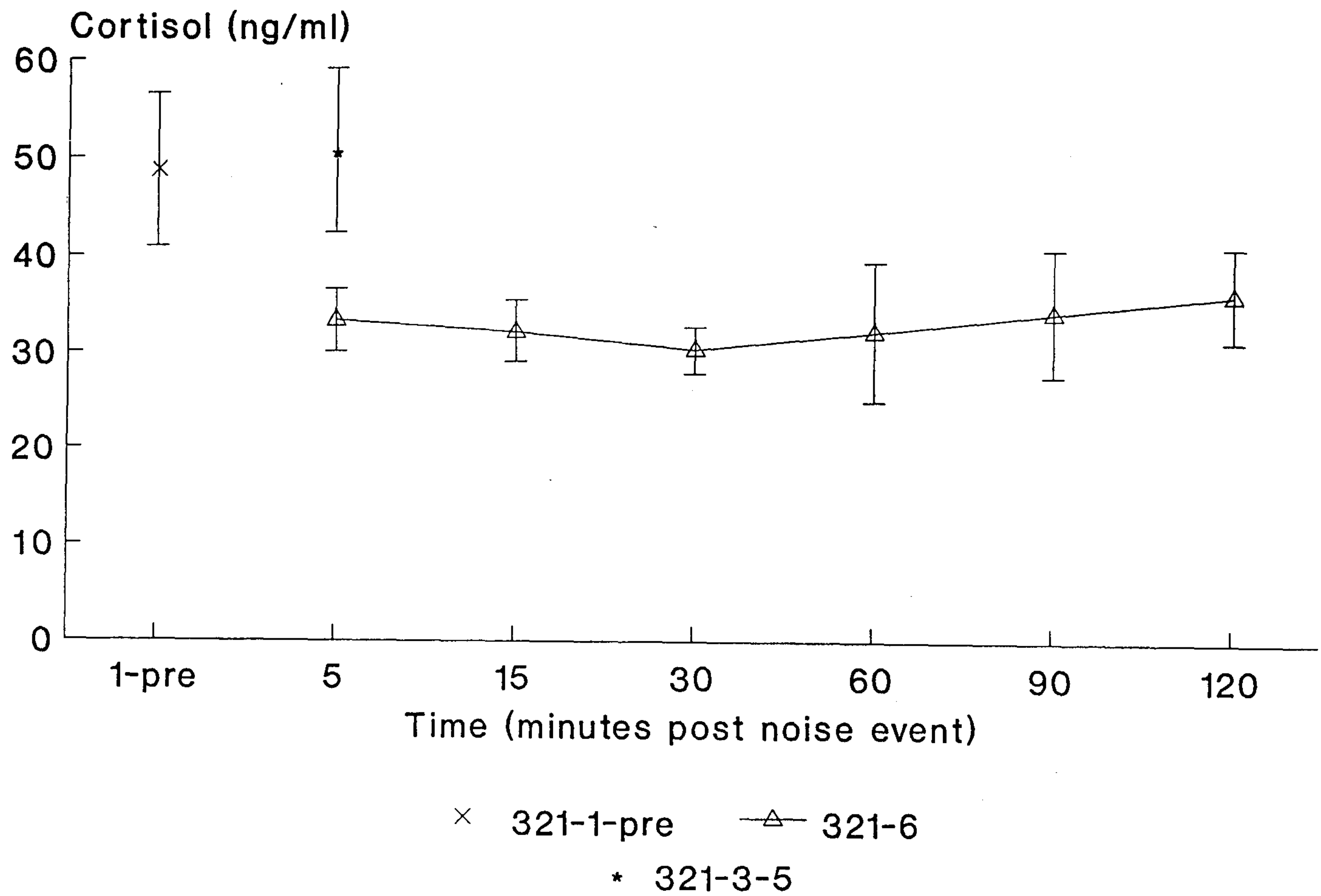

Figure 11. Mean serum cortisol concentrations of treatment mares before and after noise events on day 321. Blood was collected prior to the first (321-1-pre-x) and third noise event (321-3-5-*) and after the sixth noise event (321-6 -). Mean cortisol concentrations in serum after noise event 6 were significantly lower than levels prior to noise ($p < 0.05$).

Mares experienced a significant rise in serum cortisol only after the first exposure to simulated aircraft noise on day 320 (Figure 8). This cortisol rise was seen 15, 30 and 120 mins after noise event 1 (45.9 ± 5.6, $p < 0.0027$; 39.2 ± 4.6 ng/ml, $p < 0.0224$; and 42.5 ± 6.3 ng/ml, $p < 0.05$, respectively). There were no significant differences between the pre- and post noise event cortisol concentrations on day 320 noise treatment 4, days 323, and 324 (Figure 8).

Figure 9 depicts the cortisol concentrations of treatment mares before and after noise event 1 and that of control mares that were bled in a similar sequence on day 320. Mean cortisol concentration in serum of treatment mares was significantly higher at 15, 30, and 120 minutes post noise ($p < 0.05$) than concentrations measured in control mares. Mean serum cortisol concentration of the three treatment mares (T1, T2, and T3) that were bled two weeks after noise treatment are shown in Figure 10. Mean serum cortisol concentrations were higher in these treatment mares than in the control mares at the 15 minute bleeding time (control mares were bled in a similar sequence on day 320). The high cortisol concentration at the second bleed (15 minute) may reflect the anxiety of these mares to repeated bleeding. Two of the three mares (T1 and T3) were the mares that had increases in cortisol on day 319 prior to any noise events.

The mean serum cortisol concentration of mares prior to noise events 1 and 3 on day 321 (Figure 11) was higher than concentrations in serum collected prior to noise events on other test days (Figure 8). Mean cortisol concentrations in serum after noise event 6 were significantly lower (Figure 11) than the pre-noise event levels. Pre-noise treatment cortisol concentrations on day 321 may be high due to apprehension of blood collection or noise events. The decrease in serum cortisol concentrations, (in relation to the pre-treatment serum cortisol concentration), after noise event 6 on day 321 may be due to habituation. From the behavioral study it appears that all mares had a significant decrease in movement after the fifth noise on day 321 (ninth noise event of study). The low cortisol concentrations in treatment mares after noise event 6 on day 321 may reflect this behavioral change.

There were 26 blood samples with a cortisol concentration > 60 ng/ml during the noise events. Twenty four of the 26 samples were from the three mares (T1, T3 and T4) that had high plasma cortisol concentrations during the pre-noise bleed on day 319. The remaining 2 samples were taken from treatment mare 8, one on day 321-pre noise and the other on day 321 noise event 3 post 5 minutes.

In conclusion, a significant increase in cortisol was seen after the first exposure to noise. This increase was not seen after other noise episodes. Three mares were primarily responsible for this rise in cortisol indicating that the response of mares to noise varies considerably. From the cortisol and behavioral data it is evident that the mares habituate to repeated noise events. Until that occurs, care must be taken to avoid injury to the horse and to people.

2.5.2 Progestagens

Abortion has been associated with severe maternal disease in the horse, but the effect of maternal stress on the fetus and the mechanism of abortion is unknown. Endocrine studies of stressed pregnant mares suggest an effect on progestogen levels (van Niekerk & Morganthal 1982; Ousey et al, 1987; Santschi et al, 1991), but its association with abortion remains conjectural. High concentrations of progestagens are produced by the feto-placental unit during the last trimester of pregnancy (Barnes et al, 1975). A decrease in progestagens may reflect a decreased ability of the feto-placental unit to produce progestagens and transport them to the endometrium leading to the demise of the fetus. We have shown that serum levels in the dam drop dramatically if the fetus dies in-utero (Santschi, et al, 1991). It has been assumed that simulated aircraft noise is stressful to horses, particularly heavily pregnant mares. If the stress is severe, abortion may result. We measured progestagens in this study to determine if simulated aircraft noise affected the feto-placental unit adversely.

2.5.2.1 Experimental Design

Serum progestogen concentration was measured in the following blood samples collected from treatment mares: day 320 cycle 1 pre-noise event, days 323 and 324 cycle 4 120 minutes post-noise event, and every third day thereafter until 7 days before foaling at which time serum progestogen was measured daily. The same protocol was followed for control mares except they were not subjected to noise.

Progestagens were measured in plasma by a solid-phase radioimmunoassay (Coat-a-count, Diagnostic Products Corp., Los Angeles, CA) validated in our laboratory. Sensitivity was < 0.1 ng/ml. The antiserum crossreacted with progesterone (100%), 11-deoxycorticosterone 1.7%, 11-deoxycortisol 2.4%, 17 alpha-hydroxyprogesterone (0.3%), 20 alpha-dihydroprogesterone (2.0%), 5B-pregnane-3alpha-ol-20-one (0.2%), 5 alpha-Pregnane-3,20-dione (0.8%), and 5 b-Pregnane-3,20-dione (1.3%). Intra-assay coefficient of variation was 4.5-6.3% while the inter-assay coefficient of variation ranged from 10 to 12%. While most of the steroid measured early in pregnancy was probably progesterone, during the last trimester of pregnancy the assay would also be measuring the pregnanes, 17 alpha-hydroxprogesterone, and 20 alpha-dihydroprogesterone (Seren et al, 1981). The term progestagens is therefore used to refer to the steroids measured by this assay.

2.5.2.2 Results and Discussion

The progestogen concentrations varied greatly among individuals (Figures 12-15), however, the progestogen profiles were well within the normal range (Ginther 1979). During the last month of pregnancy, progestagens gradually increased in all mares, peaking 2 days prior to parturition and falling off to zero at parturition. In no mare did progestogen levels prior to parturition fall below 2 ng/ml.

The progestogen values we obtained from mares in this study differed from the studies performed in Germany by Krueger (1982) and by Erath (1984). These differences may be due to assay techniques. Progesterone is necessary for pregnancy maintenance in the mare. Until day 80 of gestation, the corpus luteum is the primary source of progesterone. At this time, the feto-placental unit begins to contribute to the pool of circulating maternal progestagens by producing 5 alpha-pregnanes. Plasma progesterone begins to decline by day 120 and only small amounts of progesterone are measurable in plasma by day 150. Presumably pregnancy is then maintained by placental progestogen production (Holtan et al, 1975). There is disagreement, however, on what is considered to be the normal concentration of plasma progestogen between Day 120 and parturition with daily progestogen values of 1 ng to 8 ng/ml reported as normal (Holtan et al, 1975; Squires et al, 1974). The discrepancy between reports can be attributed to cross-reaction of progesterone antiserum used in radioimmunoassays with other progestagens. Because of this cross-reactivity, interpretation of progesterone values obtained by RIA after 80 days of gestation must be interpreted with caution. A low plasma progesterone value may be due to a RIA that is extremely progesterone specific. Our progestogen values were always well above the minimum of 2 ng/ml. We conclude that simulated F-4 aircraft noise did not affect pregnancy adversely.

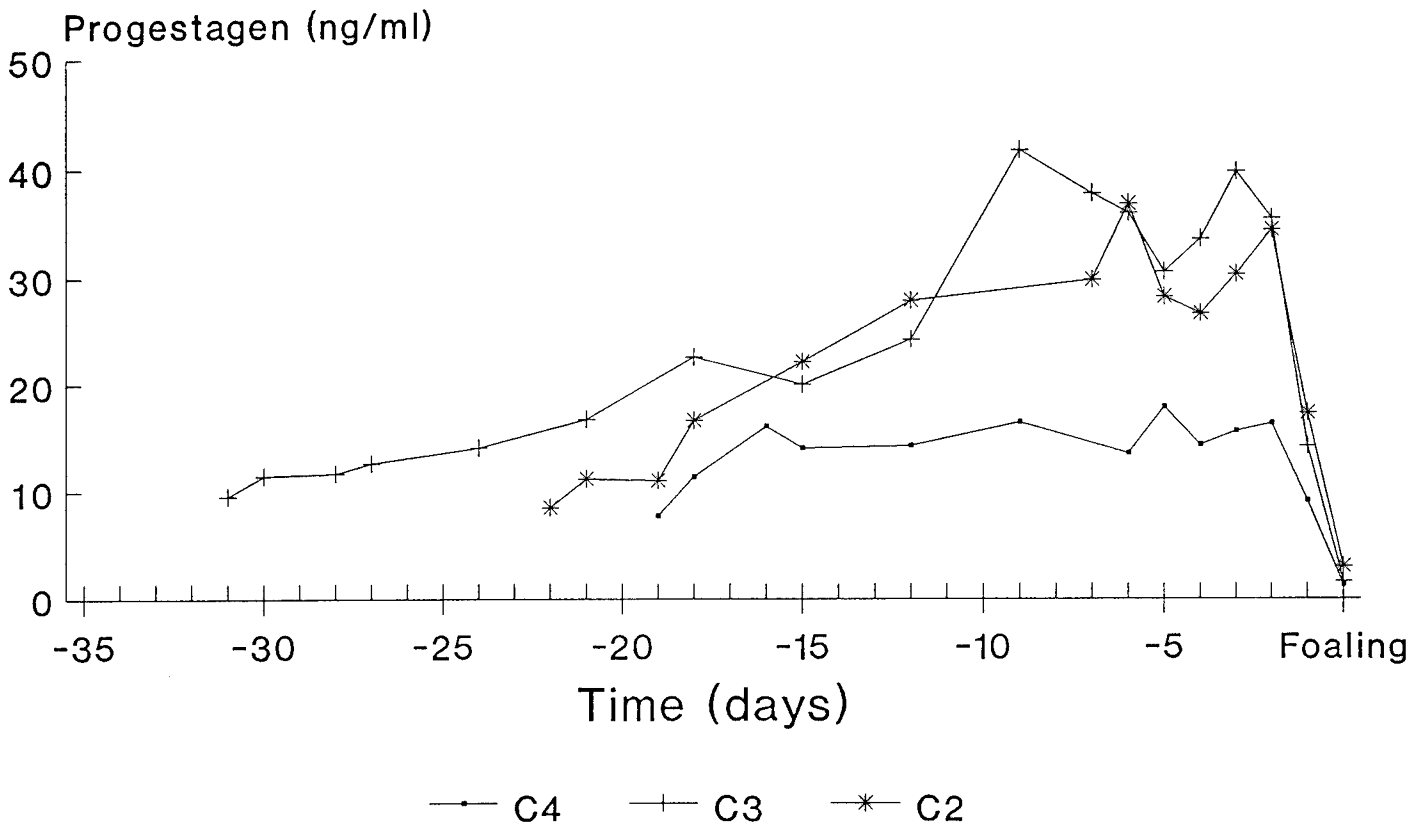

Figure 12. Serum progestogen concentrations in 3 control mares prior to foaling. Blood was collected from mares from day 319 until parturition. The profiles are within the normal published ranges.

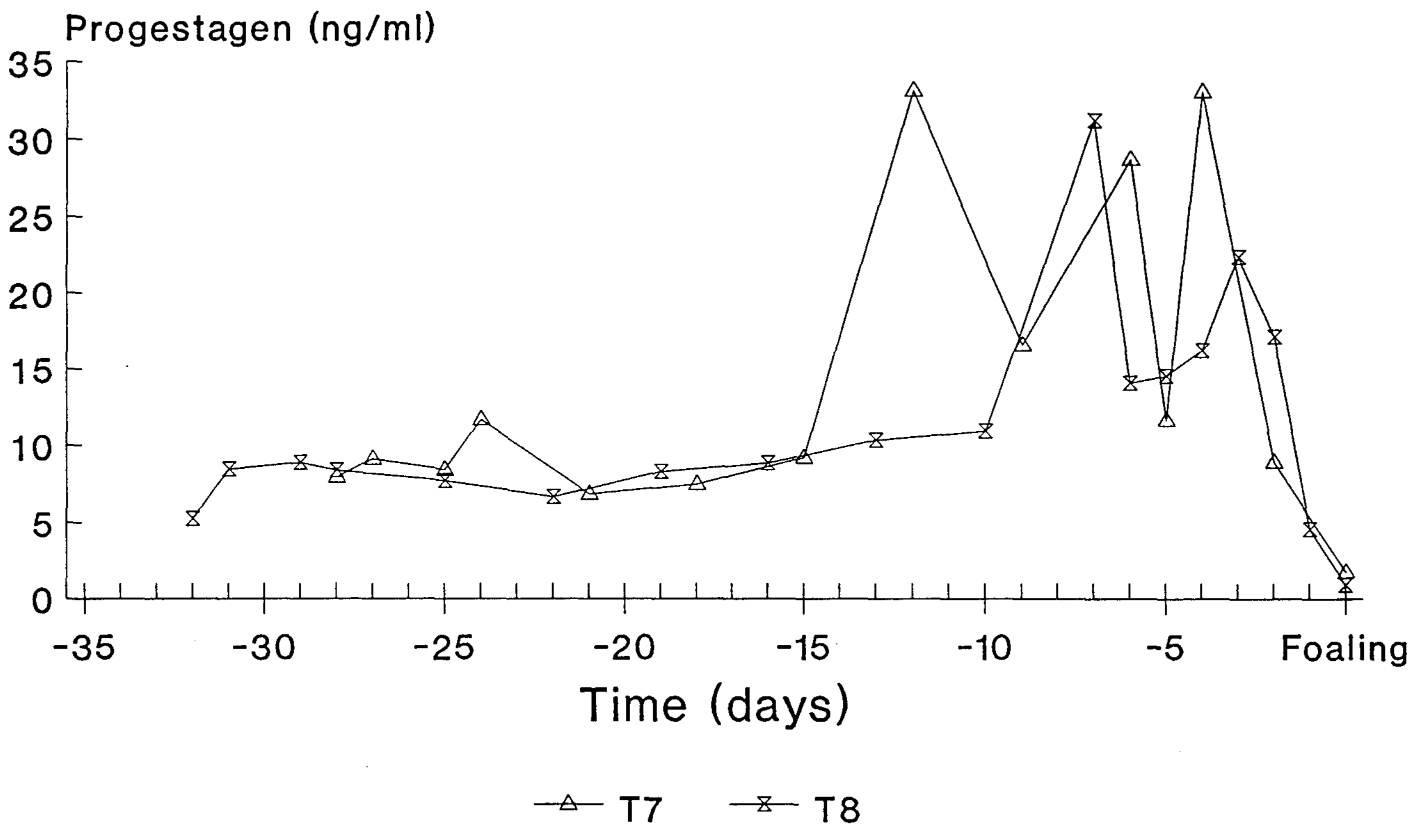

Figure 13. Serum Progestogen Levels Treatment Mares

Figures 13 through Figure 15. Serum progestogen profiles of treatment mares from day 319 until parturition. The progestogen pattern and concentrations are within normal limits.

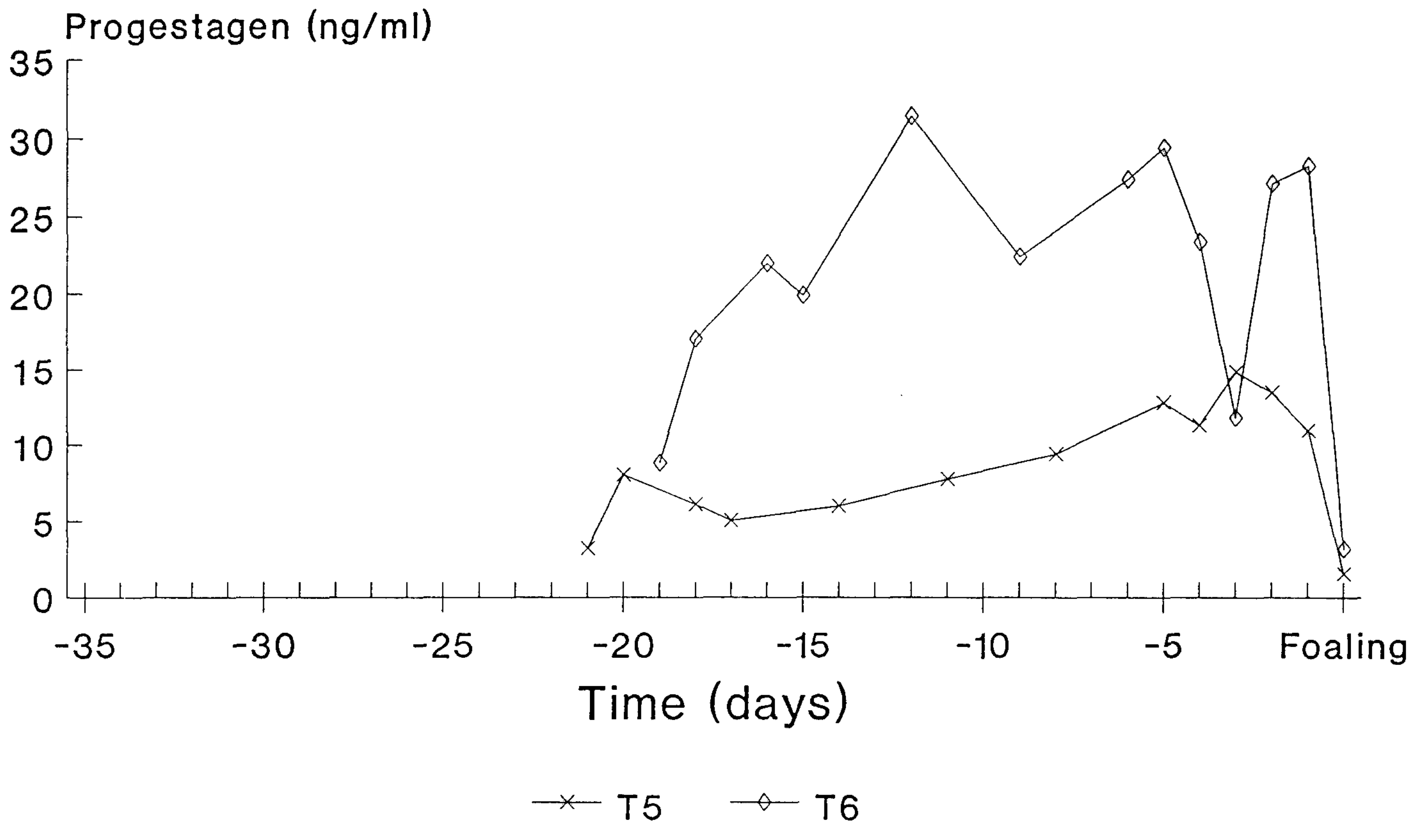

Figure 14. Serum Progestogen Levels
Treatment Mares

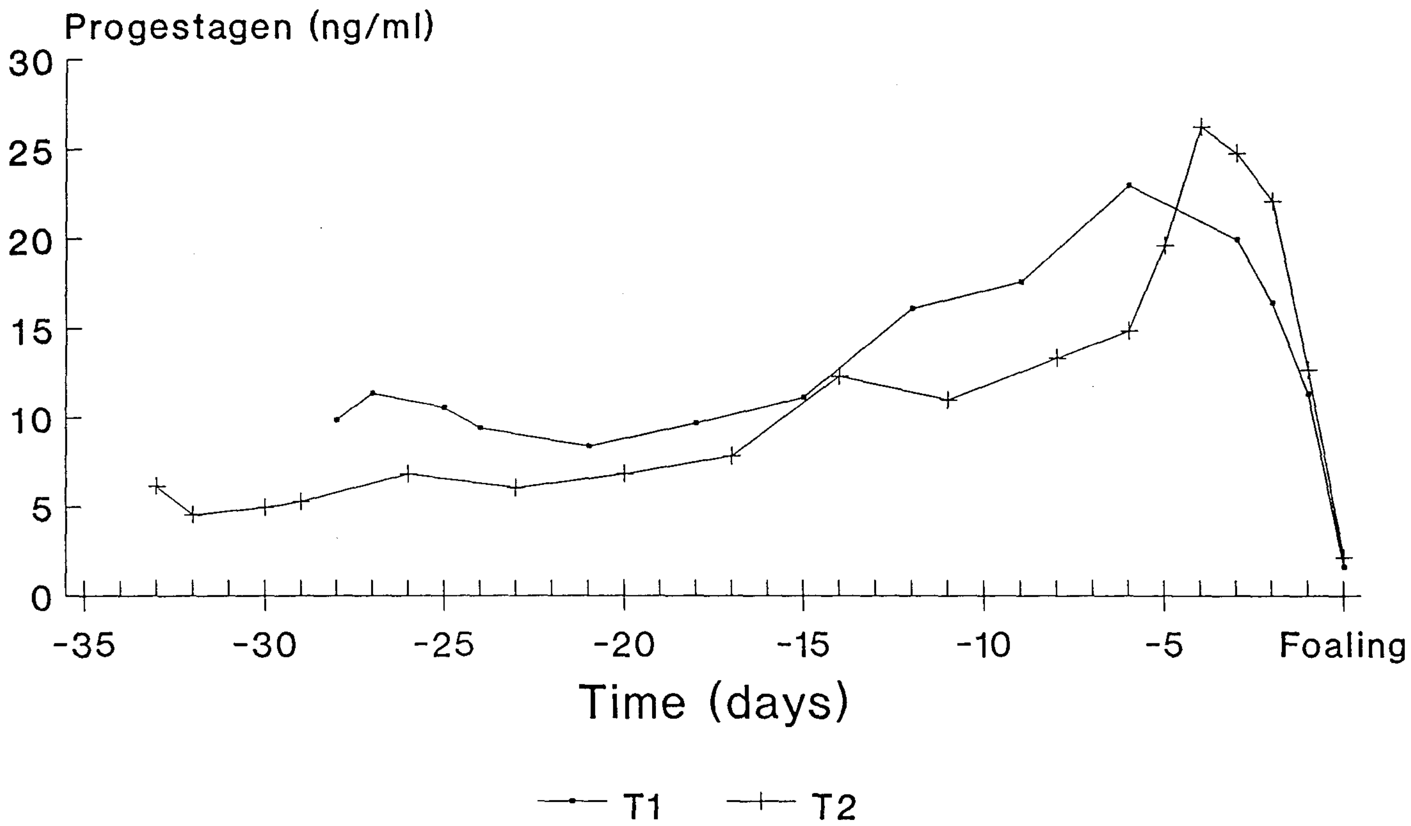

Figure 15. Serum Progestogen Levels
Treatment Mares

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3. STUDY II: EFFECTS OF SIMULATED AIRCRAFT NOISE ON COLOSTRUM PRODUCTION AND COLOSTRAL IMMUNOGLOBULIN ABSORPTION BY FOALS

3.1 SPECIFIC AIM 2

Determine if colostrum production was adversely affected in mares exposed to simulated aircraft noise two weeks prior to their expected foaling date. The health and serum antibody absorption of foals whose mares were subjected to simulated aircraft noise was also compared to a group of control foals.

3.2 COLOSTRUM PRODUCTION

Upon foaling, mares were milked manually every 30 minutes until the specific gravity of the mammary secretions was $\leq$ 1.04. Our previous work has shown that mares' milk has a specific gravity of 1.04 and it contains minimal amounts of IgG (< 100 mg of IgG/dl; LeBlanc et al 1986). The colostrum was stored in an equine colostrum bank in labelled 250 ml containers at -20°C. The IgG content, and specific gravity, of each container of colostrum was measured and the total amounts produced by each mare were quantitated. Differences between treatment and control mares were determined by Student-t tests.

3.2.1 Results and Discussion

The colostrum production of treatment and control mares is presented in Table 11 and Figure 16 and 17. There was no statistical difference in the parameters measured between the two groups. By 12 hours post foaling, no mares had detectable IgG levels.

TABLE 11. Colostrum Production

	TREATMENT MARES	CONTROL MARES
Volume produced[a] (ml)	2285 $\pm$ 617[b]	2313 $\pm$ 350
Total colostral IgG produced (g/L)	164.6 $\pm$ 40	186.2 $\pm$ 67
mg of colostral IgG produced per kg foal BW	3.39	3.8

[a] Volume of colostrum produced with a specific gravity $\geq$ 1.04.
[b] Means $\pm$ SD

We conclude that simulated aircraft noise did not adversely affect colostrum production (similar results were found in the cow; personal communication, HH Head 1991).

3.3 IMMUNOGLOBULIN ABSORPTION AND HEALTH OF FOALS

At birth physical exams were performed on foals and blood was taken for a complete blood count and for subsequent determination of serum IgG. Foals were muzzled and fed colostrum from an equine colostrum pool through a nipple bottle. Each foal received 1.5 g of colostral IgG/kg of BW as determined by the specific gravity of colostrum. Foals were fed colostrum when they began to search for the mare's udder, as observed on video camera. The volume suckled at each feeding was recorded. Foals were allowed to suckle their dam after they had ingested the prescribed dose of colostrum and the specific gravity of the dam's colostrum was $\leq$ 1.04. Five ml of blood was drawn from foals every 2 hours beginning at birth until 18 hours of age and at 24, 36, and 48 hours to determine serum IgG concentration. Serum was harvested and stored in 1 ml aliquots at -20°C until assayed. The time of peak absorption, and the maximum serum IgG

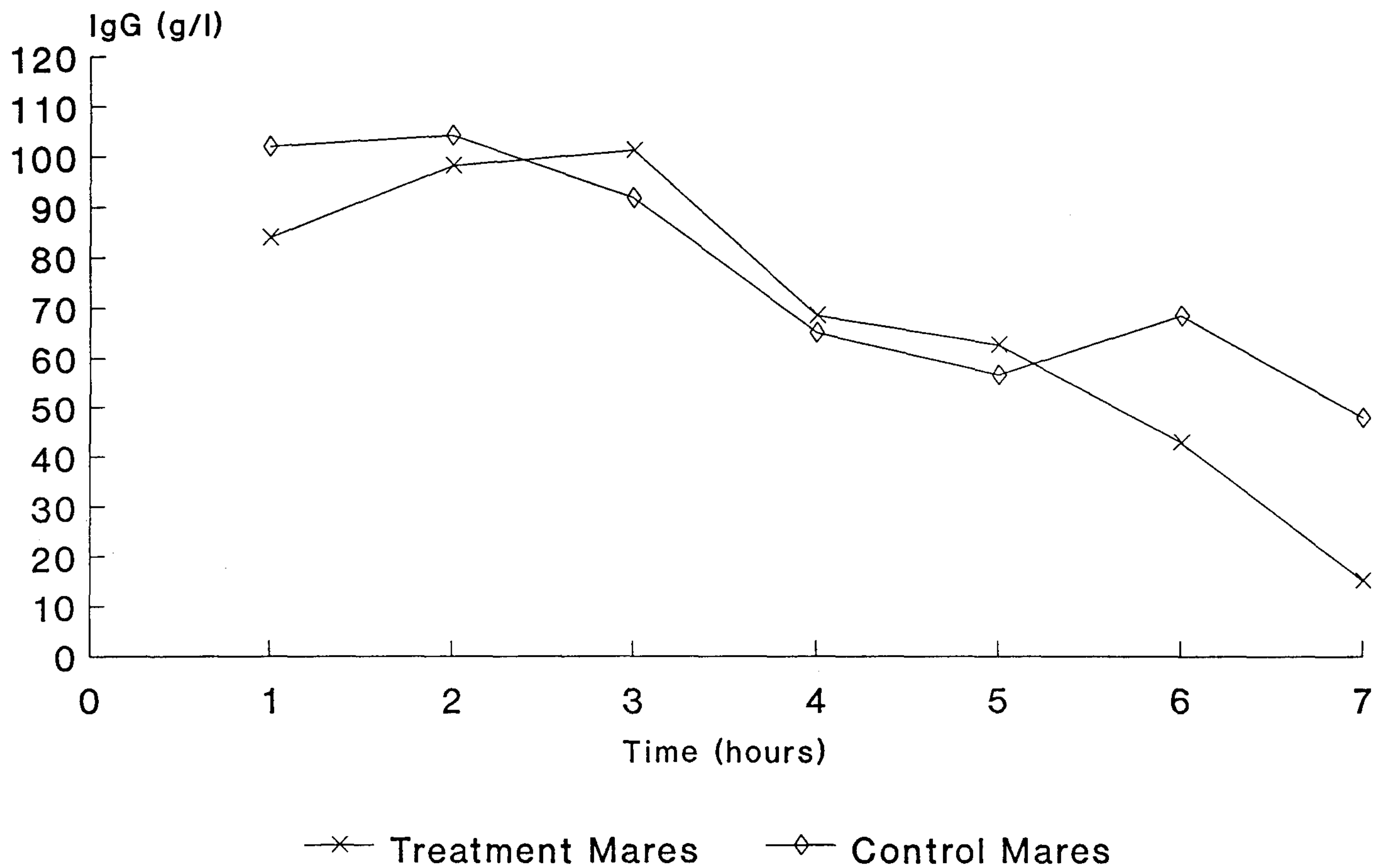

Figure 16. The IgG concentration in colostrum of mares during the first seven hours after parturition. There was no statistical difference between mares subjected to simulated aircraft noise (treatment) and control mares. Mares were hand milked until the colostral specific gravity was < 1.04, the specific gravity of milk.

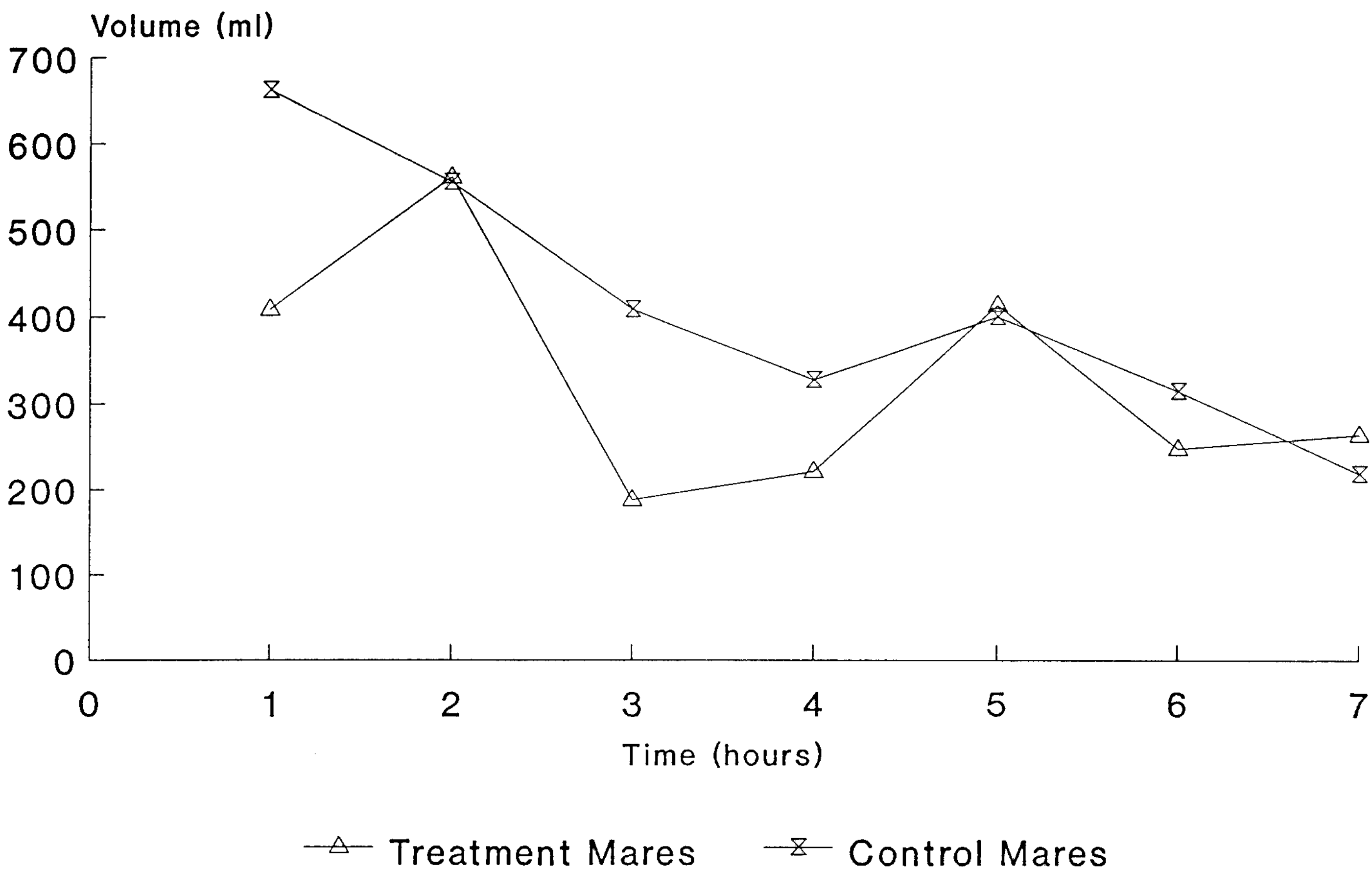

Figure 17. Volume of colostrum produced by treatment and control mares during the first seven hours after parturition. Mares were hand milked until the colostral specific gravity was < 1.04, the specific gravity of milk.

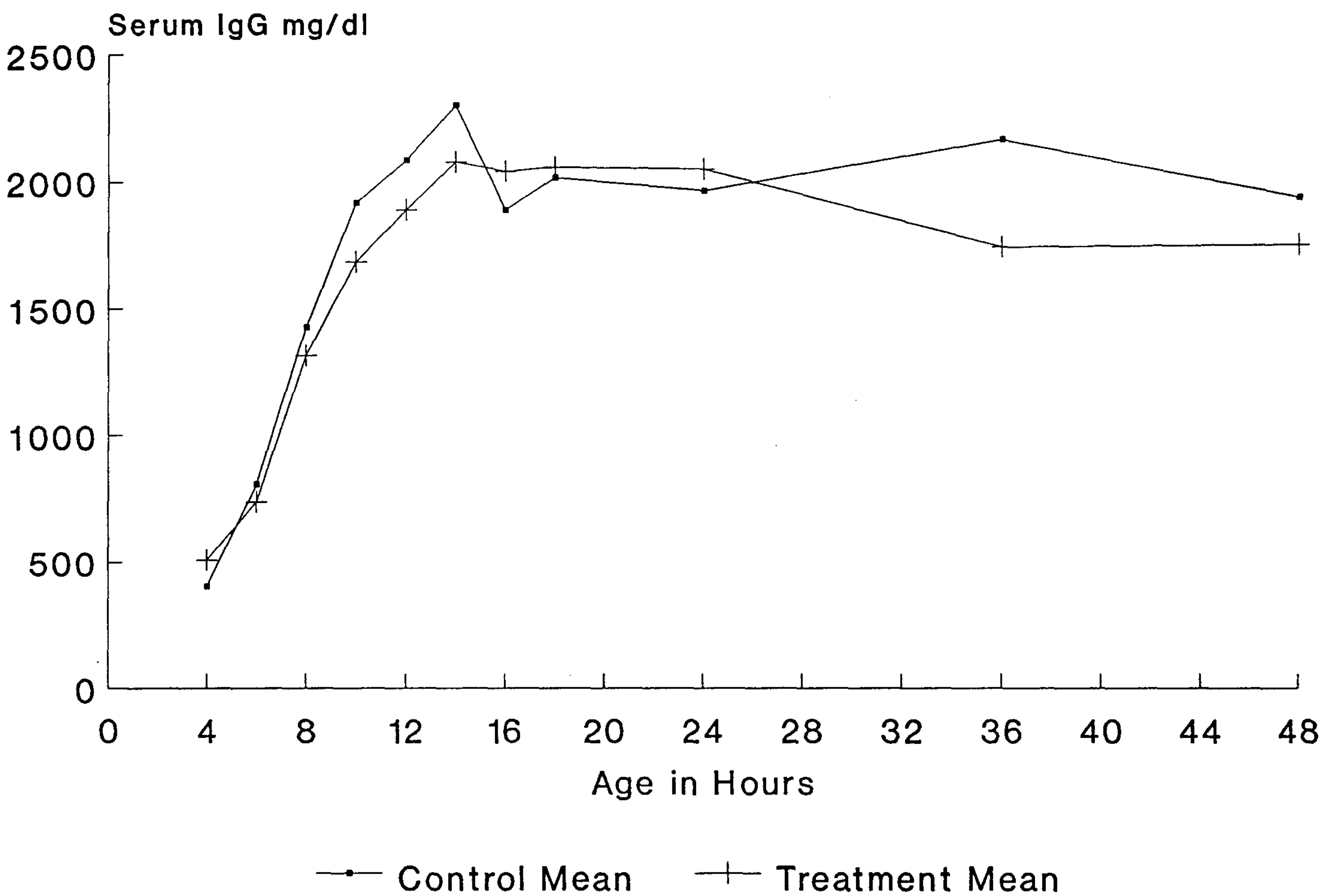

Figure 18. Serum IgG concentrations in foals during the first 48 hours of life. Serum IgG concentrations did not differ between treatment and control foals.

concentration was determined. The complete blood counts of experimental foals was compared to those of control foals. The health of the foals was monitored for 4 months to determine if experimental foals were more prone to infectious diseases.

3.3.1 Results and Discussion

No foal had detectable serum IgG levels prior to nursing. Seven of eight treatment and nine of nine control foals had serum IgG levels > 800 mg/dl by 8 hours of age (Figure 18). The maximum serum IgG concentrations attained by foals did not differ (2316.13 $\pm$ 571 mg/dl for treatment foals and 2598 $\pm$ 512 mg/dl, for control foals). Time to peak absorption for treatment foals was 13.5 $\pm$ 2.2 hours and 12 $\pm$ 2.8 hours for control foals. The complete blood counts of all foals were within normal limits. No foal experienced a disease that required antibiotic treatment during the first 4 months of life. One foal had diarrhea at 7 days of age which was corrected with antidiarrheal medication.

3.3.2 Conclusions

Simulated aircraft noise did not adversely affect the health of foals, or their ability to absorb colostrum during the first 12 hours of life.

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