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Abstract: We tested to see if semen collection interferes with fertility in naturally fertile pairs of cranes. We used 12 naturally fertile, Florida sandhill crane *(Grus canadensis pratensis)* pairs for this study, 6 control and 6 experimental. All pairs had previously produced fertile eggs. Semen was collected on Tuesday mornings and Friday afternoons from 26 February 1993 to 4 June 1993. We used standard artificial insemination methods to collect and to evaluate the semen and spermatozoa. Semen collection had minimal effect on semen quality and semen quantity. Semen volume, sperm density, sperm motility, sperm morphology, sperm viability, sperm number per collection, and male response to semen collection exhibited significant daily variation. Although semen collection began 13 days before the first egg in the experimental group, we did not observe differences in the date of first egg laid or in fertility between experimental and control groups. Also, we observed no statistically significant differences in the interval between clutches or in the percentage of broken eggs between experimental and control groups. However, 4 eggs were broken by adults during the disturbance associated with capturing birds for semen collection. We found that females with mates from which we consistently gathered better semen samples produced fewer fertile eggs than females with sires producing poorer semen samples (r = 0.60). We interpret these results to mean that males that were successfully breeding with their mates had little left at the time of our collection.

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Frozen sperm banks can theoretically store genetic material for centuries. As such they are especially important in maintaining genetic diversity of endangered species (Gee and Sexton 1980). Genetic diversity enables animal populations to adjust to changing environments and reduces the expression of harmful traits associated with progressive inbreeding in small captive populations (Gee and Sexton 1980). Also, frozen gene pools (semen or embryos) can be a valuable tool in captive propagation of wildlife (Gee 1986).

With improved husbandry, many new pairs produce fertile eggs without artificial insemination (AI). But, aviculturists may need to collect semen from males in naturally fertile pairs for cryopreservation and for insemination of nonproductive pairs. Our study was designed to determine if semen collection from males in these naturally fertile pairs reduce the fertility rate.

In chickens, sperm morphology has been related to fertility. Lake and Stewart (1978) reported that clumping of motile spermatozoa and increased numbers of morphologically abnormal spermatozoa appeared to decrease male fertility. Irrespective of semen dilution, Omprakash et al. (1992) found that sperm concentration, motility and percentage of live sperm exhibited a positive correlation with the fertilizing ability of semen. The percentage of abnormal sperm and a lower methylene blue reduction test (MBRT) value were negatively correlated with fertilizing ability of semen. Cooper and Rowell (1958) found that fertility was significantly correlated with the percentage of dead spermatozoa (r = -0.89), motility (r = 0.84), reduction time (r = -0.80), and live density (r = 0.55).

Investigators (Ansah et al. 1984; Hoolihan and Burnham 1985; Pfaff et al. 1990; Rutz et al. 1989,1991) have shown negative relationships between semen collection frequency and sperm quality, semen volume, and fertility. Birkhead (1991) showed that copulation reduces spermatozoa available in subsequent copulations and that repeated copulations are needed to obtain good fertility in the non-domestic Bengalese finch (*Lonchura striata*). We are unaware of studies to

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determine if semen collection interferes with production of fertile eggs from naturally fertile pairs.

In this study, we examined the relationship between semen collection and sperm quality, semen volume, and egg fertility. We compared the egg fertility of experimental and control groups and between this year's and last year's production for each experimental pair (1992 vs. 1993). Brief mention of our results was published earlier (Chen et al. 1997).

MATERIALS AND METHODS

Birds

We randomly divided 12 Florida sandhill crane pairs equally between experimental and control groups. We collected semen from the experimental group only. All pairs had produced fertile eggs in previous years. These pairs were in outdoor pens within auditory range but physically isolated from each other. Flight was restricted for each bird by tenotomy. Males were younger (7.2 ± 2.0 yr) than females (9.4 ± 2.9 yr) in the experimental group and about the same age (males 9.8 ± 1.0 yr: and females 9.5 ± 1.2 yr) in the control group. Each female had laid eggs for at least 2 years. In the previous year (1992), the 12 females laid at least 6 eggs each and fertility for each female was greater than 67%. We allowed 2 females that started laying later than those in the other 4 pairs to continue into the warmer weather (to complete a third clutch). We continued to collect semen from the males in those pairs but excluded the data collected after the second clutches from our semen analysis.

Semen Collection

We collected semen Tuesday mornings and Friday afternoons from 26 February 1993 to 4 June 1993. Semen collection began 13 days before the first egg was laid in the experimental group and ended after the female in each pair laid the last egg of the third clutch.

Whenever possible, the same team of 3 technicians collected semen. We used the methods and equipment for semen collection described by Gee (1983). The team entered the pen, immediately captured the male, and began the collection attempt. An assistant held and stimulated the crane by stroking the inner shanks. At that time, the person collecting the semen (operator) stimulated the region around the tail by stroking with the left hand, from the postdorsal region of the back to the interpelvic tail region, and then to the postlateral region below the tail. Then the operator lifted the tail with the left hand and stroked the abdominal and sternal regions from front to back with the right hand. Usually, the bird responded with a partial eversion of the cloaca and occasionally with ejaculation. The operator grasped the cloaca dorsally with the thumb and index finger of the left hand, and collected the semen in a small glass funnel (Fig. 1).

Bird's Response

We scored the male's response to stimulation on a scale from 0 to 4: 0 = no response, struggled; 1 = relaxed briefly; 2 = relaxed half of the time, raised tail; 3 = relaxed most of the time, raised tail, everted cloaca, occasionally vocalized; and 4 = relaxed, raised tail, everted cloaca, vocalized, and climaxed. Slightly different response combinations would lead to a plus (+) or minus (-) sign added to the numerical score. The 3 team members reached a consensus in determining the score.

Volume

To measure volume, we (immediately after collection) drew the semen, excluding feces, into a l-cc syringe and mixed it with crane semen extender (Gee et al. 1985). All syringes used contained 50 μl crane extender.

Fig. 1. Semen collection showing the position of hands and collecting device.

Sperm Motility

We collected a small sample (ca 5 μl) of undiluted semen in a plain (non-heparinized) microhematocrit tube before collecting the semen from the glass funnel. We examined semen motility in the laboratory within 2 minutes after placing the microhematocrit tube on the microscope. We scored the sample from 0 to 4: 0 = no motility; 1 = less than 25% motile; 2 = 25–49% motile; 3 = 50–74% motile; and 4 = more than 74% motile spermatozoa. All 3 team members examined sperm motility under the 10x objective and together assigned a score for each sample.

Sperm Concentration

We scored sperm concentration using the same microhematocrit sample used to score motility. We also scored sperm concentration on a scale from 0 to 4: 0 = no spermatozoa; 1 = few spermatozoa with large empty spaces; 2 = many spermatozoa with moderate spacing; 3 = numerous spermatozoa with little empty space; and 4 = packed with spermatozoa, hard to detect separate spermatozoa, no empty space. Slightly different combinations of these concentrations resulted in adding a plus (+) or minus (-) sign to the numerical scores.

Besides concentration scores, we counted the spermatozoa in each sample. We prepared semen samples for counting by diluting at 1:100 or 1:200 with crane extender containing 1% eosin and 10% formalin in white blood cell diluting pipettes. We diluted the samples that scored from 0 to 3+ at 1:100 and the others at 1:200. We discarded the first drops from the pipette and then filled the 2 sides of the counting chamber. We allowed 2–10 minutes for the sperm to settle, then counted the spermatozoa in the 4 large corner squares (each containing 16 small squares) of an Improved Neubauer chamber (Anonymous 1967) under high power (430x). We averaged the counts from the 2 chambers.

Sperm morphology

We examined sperm viability and morphology in semen smears stained with 10% nigrosin and 5% eosin (Quinn and Burrows 1936). We made 3 slides from each sample and examined them about 20 hours later. We examined at least 10 fields for each sample and 300 spermatozoa per slide under a high power (430x) microscope. We classified sperm cells into 6 distinct types: normal (N), bent (B), swollen (S), giant (G, very rare), droplet (DL), and dead (D) (Gee and Temple 1978). Also, we estimated the proportions of normal (live; i.e., eosin-impermeable) and abnormal (both eosin permeable and eosin-impermeable) spermatozoa appearing on the slide (Chaudhuri et al. 1988).

Egg fertility

We determined egg fertility by candling eggs each week with an incandescent light (Lyon Electric, Chula Vista, California, USA). We opened nonviable eggs to determine fertility. When we could not determine fertility (i.e., for broken or rotten eggs or eggs with a deteriorated blastodisk), we designated an egg as having no detectable embryo and deleted it from our analysis.

Statistics

We compared egg fertility, date of first egg laid, time between clutches, egg laying intervals within clutches, and percentage of eggs broken between experimental and control groups and for production from each experimental pair between 1992 and 1993. We used the t-Test, 1-way analysis of variance (ANOVA) and Kendall's Tau (Steel and Torrie 1960:110-111, 406-407; Lehner 1979:245-279) to test the relationships between semen collections and semen donors from 26 February 1993 and 27 April 1993. We also used Kendall's Tau test to compare averages for experimental and control males for (1) the quality of sperm (mean concentration and motility, mean percent normal and abnormal spermatozoa, live spermatozoa, dead spermatozoa, and the mean number of spermatozoa per collection), (2) the quantity of semen (mean volumes), and (3) responses of the male cranes to semen collections.

RESULTS AND DISCUSSION

Semen and Spermatozoa

We found little seasonal change in bird response, semen characteristics, and sperm morphology (Tables 1 and 2). Although the cranes had not been trained for AI, they responded well to stimulation (3.3 average response score), and we collected good semen samples from 80% of the attempts. Sperm density ($\bar{x}$ 1.9 million/mm^3), sperm motility ($\bar{x}$ 3.4 motility score), and sperm morphology were typical for the sandhill crane (Gee and Temple 1978). Semen volume averaged 37 μl and sperm concentration (density) averaged 1.9 million/mm^3 per collection. Gee and Temple (1978) and Gee et al. (1985) found similar results (mean semen volume 30 μl) from greater sandhill cranes *(G. c. tabida)* using the same collection technique.

The increased semen production during the course of this study may have been in response to training or a natural seasonal change. Semen volume increased as the season advanced from 29 ± 8 μl early in March (26 Feb 1993 to 12 Mar 1993) to 39 ± 9 μl late in March (16 Mar 1993 to 30 Mar 1993) to 46 ± 16 μl in April (2 Apr 1993 to 23 Apr 1993).

Table 1. Mean values for semen collections, semen properties, and male crane responses to each collection for experimental males in 1993.[a]

Collecting date	Successful collecting attempts	Semen volume (μl)	Sperm density (million/mm3)	Sperm motility score	Live spermato-zoa (%)	Spermatozoa per ejaculation	Response[b]
26 Feb	6/6	35 ±27		3.3 ±1.5	94.7 ±3.6		3.4 ±0.5
2 Mar	3/6	16 ±23	0.5 ±1.1	1.3 ±2.3	90.7 ±0.0	18.8 ±42.1	2.2 ±2.0
5 Mar	3/6	32 ±38	1.1 ±1.4	4.0 ±0.0	91.3 ±4.2	73.6 ±102.0	2.6 ±1.6
9 Mar	6/6	27 ±37	2.1 ±2.0	3.2 ±0.4	73.5 ±11.0	110.0 ±201.2	3.4 ±0.6
12 Mar	4/6	35 ±54	2.6 ±2.9	3.8 ±0.5	85.0 ±10.8	191.6 ±331.7	2.4 ±1.5
16 Mar	6/6	26 ±28	4.1 ±4.3	2.3 ±1.6	84.2 ±10.1	163.1 ±234.6	3.4 ±0.5
19 Mar	5/6	46 ±47	2.6 ±1.9	3.4 ±0.9	85.6 ±7.3	164.8 ±223.2	3.4 ±1.1
23 Mar	6/6	47 ±37	1.3 ±1.6	3.3 ±1.6	86.1 ±6.8	43.3 ±51.5	3.7 ±0.4
26 Mar	6/6	40 ±38	3.0 ±1.6	4.0 ±0.0	91.3 ±6.4	128.4 ±157.1	4.0 ±0.0
30 Mar	6/6	35 ±62	4.0 ±1.5	3.5 ±1.0	83.4 ±22.9	134.9 ±178.2	3.6 ±0.4
2 Apr	5/6	68 ±83	1.2 ±1.0	3.8 ±0.4	95.2 ±2.2	176.5 ±220.0	3.3 ±1.2
6 Apr	4/6	35 ±53	2.0 ±3.7	3.0 ±2.0	93.7 ±1.0	238.8 ±552.6	3.2 ±1.3
9 Apr	5/6	52 ±51	1.3 ±1.3	4.0 ±0.0	95.8 ±2.6	93.8 ±107.4	3.8 ±0.3
13 Apr	4/4	21 ±14	0.7 ±0.6	4.0 ±0.0	90.9 ±5.9	17.5 ±18.8	3.7 ±0.4
16 Apr	--[c]	--	--	--	--	--	--
20 Apr	2/4	55 ±71	1.2 ±1.5	4.0 ±0.0	97.2 ±0.3	113.2 ±130.8	3.7 ±0.4
23 Apr	3/3	43 ±66	1.8 ±1.3	2.7 ±2.3	94.6 ±6.0	116.7 ±192.6	3.0 ±1.0
27 Apr	2/2	30 ±14	1.3 ±0.3	4.0 ±0.0	91.3 ±0.0	36.9 ±7.6	4.0 ±0.0
30 Apr	0/2	0					0.7 ±1.0
4 May	0/2	0					1.3 ±1.2
7 May	2/2	48 ±60	0.6 ±0.9	4.0 ±0.0	96.1 ±0.0	54.8 ±77.5	3.5 ±0.7
11 May	1/1	90	3.7	4.0	98.0	332.2	3
14 May	0/1	0					1
18 May	1/1	40	1.7	4.0	91.5	66.8	3
21 May	0/1	0					0
25 May	1/1	100	2.7	4.0	97.7	265.9	3
28 May	0/1	0					3
1 Jun	1/1	80	11.9	4.0	96.3	945.5	3
4 Jun	0/1	0					1

[a] Mean ± standard deviation.
[b] Mean male crane response (score) to semen collection.

Also, the average number of spermatozoa per ejaculate increased from early March (98 million) to late March (127 million) and April (126 ± 75 million). The values for this parameter were so variable that even vast differences were not found to be statistically significant.

As expected, we found considerable variation in semen characteristics between birds (Cooper and Rowell 1958, Gee and Temple 1978, Sharlin et al. 1979). Semen volume, sperm density, sperm motility, sperm morphology (N, B, S, DL, D and G), sperm live, sperm number per collection, and male response to semen collection (Table 1) exhibited significant individual variation ($P < 0.05$). We also found considerable variation between collection days (Figs. 2–8 and Table 1). Despite these differences, we proceeded using pooled averages as the only manageable way to seek seasonal trends between experimental and control groups. The perhaps predictable result was that variability was so high that seasonal trends were masked.

Interestingly, we found consistently higher values for semen volume (33 versus 44 μl, Fig. 2), and motility (3.1 versus 3.6, Fig. 5) in the afternoon samples (Tuesday mornings versus Friday afternoons). Although these differences were not statistically significant (t-test), they are biologically important and probably relate to the birds having depleted their semen reserves by mid-morning each day.

Usually, semen volume is lower early and late in the

Table 2. Seasonal change in sperm morphology in 1993 (mean percent of all samples collected from six males).

Date	Normal	Bent	Giant	Swollen	Droplet	Dead	Other[a]
26 Feb	67.4	15.1	1.6	4.3	5.8	5.3	0.3
2 Mar	44.1	24.4	2.1	9.4	10.2	9.3	0.3
5 Mar	61.5	15.7	0.8	7.8	4.7	8.7	0.8
9 Mar	51.6	10.7	0.7	1.9	8.0	26.5	0.6
12 Mar	63.7	12.4	0.8	3.3	4.2	15.0	0.8
16 Mar	59.3	13.3	1.0	3.0	6.9	15.8	0.7
19 Mar	50.4	18.5	1.1	3.4	10.1	14.4	2.2
23 Mar	48.1	20.2	0.9	4.6	9.7	13.9	2.7
26 Mar	59.0	15.3	0.7	5.9	9.7	8.7	0.8
30 Mar	49.7	16.2	0.6	4.1	11.2	16.2	1.8
2 Apr	65.1	10.8	0.6	4.5	13.6	4.8	0.5
6 Apr	55.4	15.2	1.1	7.2	12.2	6.3	1.5
9 Apr	61.8	12.7	0.6	4.6	15.7	4.2	0.5
13 Apr	60.8	14.1	1.0	7.1	3.8	9.1	0.3
16 Apr	--	--	--	--	--	--	--
20 Apr	73.4	10.0	0.4	5.2	8.4	2.8	0.0
23 Apr	67.5	11.3	0.5	3.5	8.5	5.4	3.6
27 Apr	65.6	8.4	1.0	5.0	11.0	8.7	0.3

[a] Mean percent of the spermatozoa which do not belong to the morphology listed.

breeding season in cranes (Gee and Temple 1978) and in other birds (Takahashi et al. 1987, Temple 1972, Berry 1972, Grier 1973, Grier et al. 1973, Boyd et al. 1977). However, semen volumes may decrease during the height of the reproductive season when birds copulate or try to copulate with birds or other objects in the pen (Gee and Temple 1978). We did not observe significant seasonal variation in semen characteristics and sperm morphology because we postponed semen collection until 2 weeks before the first egg, and ended semen collection after the third clutch and well before the end of the season.

Egg Production

Semen collection did not apparently affect egg production. In comparison with 1992, the date of first egg in 1993 was later by 7 days in pairs not disturbed for semen collection and by 9 days in pairs where we captured the males for semen collection (a normal year-to-year variation). Semen collection did not affect egg intervals within clutches (Table 3). The average interval between eggs within the clutch for each group ranged from 2.6 to 3.2 days.

The interval between clutches was shorter ($P < 0.01$) for the experimental group (14.9 ± 5.0 days) than for the experimental group the previous year (21 ± 3.4 days) and for the control group (21 ± 2.5 days) (Table 3). The difference in the non-experimental groups resulted from leaving eggs in the nest for a longer time. We found about equal intervals between clutches from the day we removed the eggs from the nest to the first egg of the next clutch for the experimental group (13.5 ± 3.4 days), the experimental group the previous year (15.0 ± 3.2 days), and the control group (18.0 ± 4.4 days). Gee (1983) reported that when eggs were removed as laid from 9 greater sandhill cranes there was an average of 10.1 ± 4.1 days between 2 clutches and 3.0 ± 0.8 days between eggs in the same clutch. The difference in intervals between clutches in this study reflect changes in egg collection schedules at the completion of the clutch in the experimental group, 14.9 ± 5.0 days, and when eggs are left in the

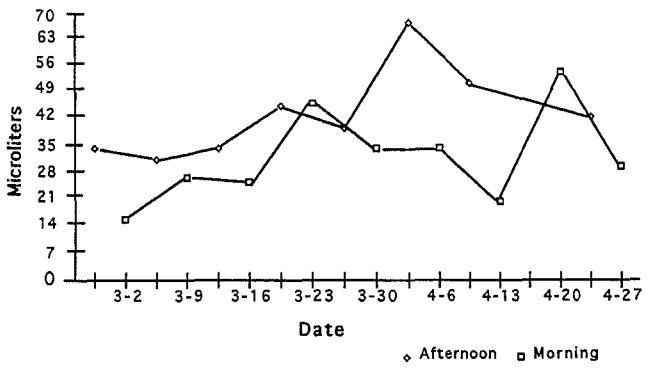

Fig. 2. Mean crane semen volume and time of collection.

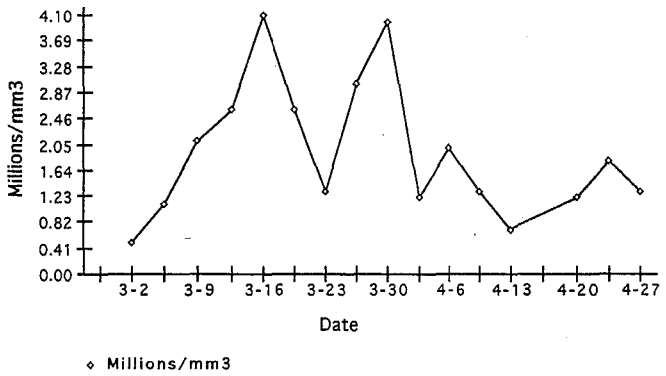

Fig. 3. Mean crane sperm density.

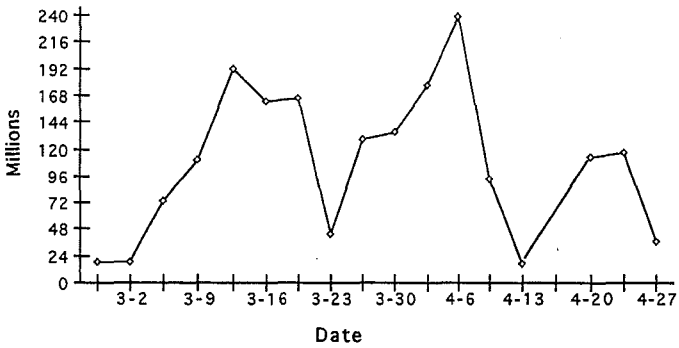

Fig. 4. Mean crane spermatozoa per collection.

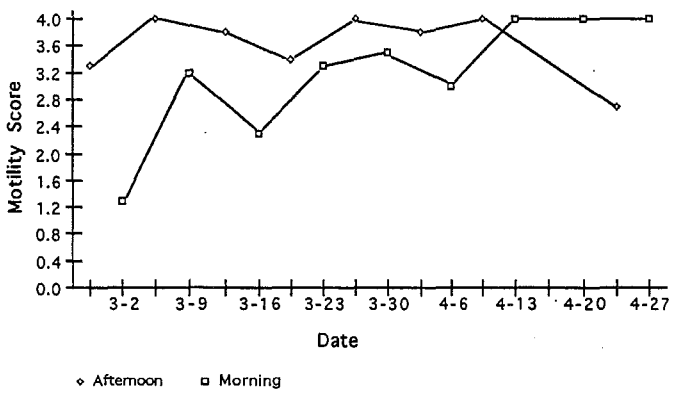

Fig. 5. Mean crane sperm motility and time of collection. Average motility score of spermatozoa from semen collected: 0 = no motility, 1 = less than 25% motile, 2 = 25–49% motile, 3 = 50–74% motile, 4 = more than 74% motile.

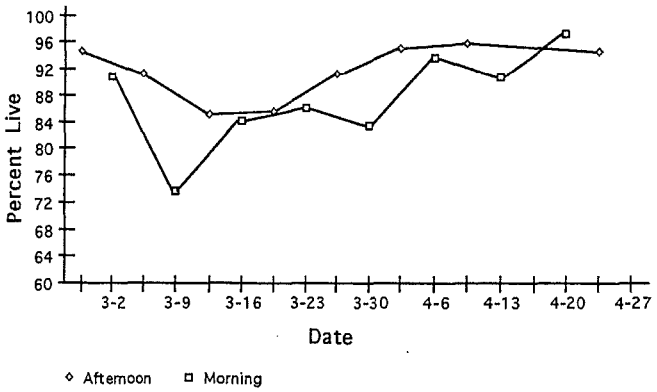

Fig. 6. Mean percent of live spermatozoa. Average from a live/dead count (300 cells per slide) of three 5% eosin/10% nigrosin stained slides.

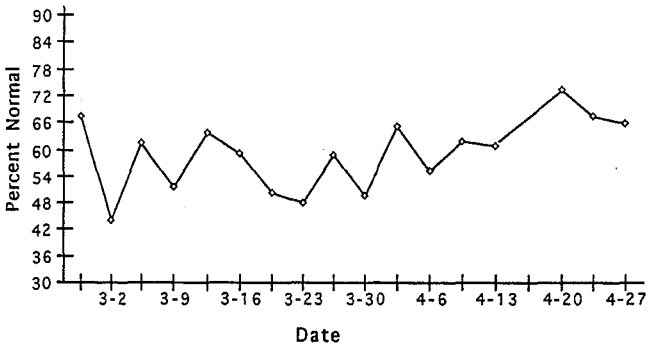

Fig. 7. Mean percent of normal spermatozoa. The average percent of normal live (eosin-impermeable) spermatozoa appearing on 5% eosin/10% nigrosin stained slides from 6 distinct cell types: normal, bent, swollen, giant, droplet, and dead.

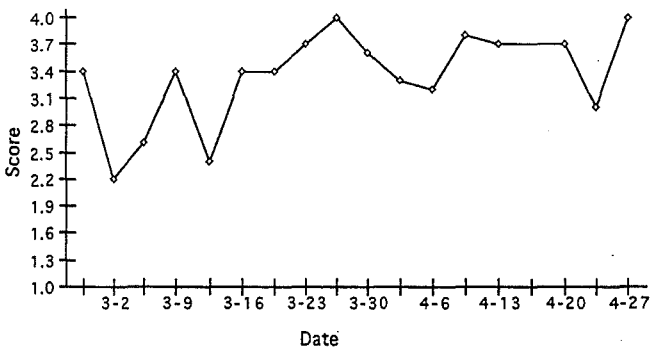

Fig. 8. Mean male crane response. Average response from collection attempts was scored as: 0 = no response, struggled; 1 = relaxed briefly; 2 = relaxed half of the time, raised tail; 3 = relaxed most of the time, raised tail, everted cloaca, occasionally vocalized; 4 = relaxed, raised tail, everted cloaca, vocalized, and climaxed.

Table 3. Egg laying dates[a] and clutch intervals[b] in captive sandhill crane pairs ($n = 6$).

	1992 (pre-experimental)	1993 (experiment)	1993 (control)
First clutch			
Egg 1	71.8 ± 8.9	80.8 ± 15.2	71.7 ± 15.5
Egg 2	74.8 ± 8.9	83.8 ± 15.2	74.3 ± 15.6
Nest[c]	(6.3 ± 2.0)	(3.5 ± 6.1)	(5.0 ± 4.3)
Interval	20.6 ± 3.4 (14.3 ± 2.1)[d]	13.5 ± 1.0 (12.8 ± 1.0)	21.8 ± 3.3 (17.2 ± 4.6)
Second clutch			
Egg 1	95.5 ± 9.5	97.3 ± 15.6	96.2 ± 14.2
Egg 2	98.2 ± 9.1	100.5 ± 16.0	99.0 ± 14.2
Nest	(5.5 ± 3.9)	(3.5 ± 6.1)	(1.5 ± 0.8)
Interval	21.3 ± 3.6 (16.0 ± 4.0)	16.3 ± 7.0 (13.7 ± 4.9)	20.8 ± 1.3 (17.4 ± 4.6)
Third clutch			
Egg 1	117.7 ± 15.5	116.8 ± 20.8	116.4 ± 12.2
Egg 2	122.3 ± 11.9	119.8 ± 21.2	119.0 ± 11.8

[a] Calendar days, Mean ±SD.
[b] Interval (days) between two clutches, Mean ±SD
[c] Number of days that eggs were kept in the nest.
[d] Interval (days) between two clutches from date that the former clutch was collected to the lay date of the first egg of the later clutch.

nest for 5.9 days, 21.4 ± 2.5 days (Table 3).

We detected no differences in egg production between groups (Table 4), but 1 bird in the control group did not lay a third clutch. In the experimental group, 7 eggs were broken. Three of these eggs were lost in a severe winter storm. The other 4 were broken when capturing the males in 2 crane pairs. Males in these pairs tended to be nervous and difficult to catch. We detected no differences in fertility of the eggs laid.

Fertility as Influenced by Semen Collection

In chickens and turkeys, semen quality was higher and semen volume and sperm concentration were lower for males that were penned with females (Jones and Leighton 1987). Repeated semen collections in the same day also reduces semen volume and sperm concentration (Gee and Temple 1978). In the non-domestic finch, natural copulations also decrease spermatozoa transfer (Birkhead 1991). Birkhead's studies (1991) of the Bengalese finch showed that not all behaviorally successful copulations transfer spermatozoa, and in reality, females may need several behaviorally successful copulations to ensure that they have enough spermatozoa to fertilize their eggs. Three copulations in 3 hours led to a 95% reduction of spermatozoa transferred, but the birds recovered from sperm depletion within 24 hours. Birkhead also found that some pairs would have to copulate 8 times to have a 95% chance of sperm transfer or 12 times to be 99% certain.

Semen quality is an important factor in determining the fertility of eggs resulting from AI (Lake 1989). In chickens, Wishart (1985) reported a relationship between increasing number of spermatozoa and probability of fertilization and the percentage of fertile eggs laid. At the point where more than 50 x 10^6 spermatozoa are inseminated, the increase in percentage of fertile eggs attributable to each additional dose of spermatozoa inseminated plateaus. Also, irrespective of semen dilution, Omprakash et al. (1992) found that sperm concentration, motility, and percentage of live sperm exhibited a positive correlation with the fertilizing ability of semen. In cranes, 16–20 million live spermatozoa per insemination and 3 inseminations per week are needed to get good fertility with frozen-thawed semen (Gee and Sexton 1979, Gee et al. 1985).

Besides sperm concentration, other factors in semen quality influence fertility. Larger than average mean sperm head length was found to be significantly correlated with improved fertility ($P > 0.04$, $r = 0.54$) in the sandhill crane (Sharlin et al. 1979). In chickens, Lake and Stewart (1978) reported that clumping of motile spermatozoa and increased numbers of morphologically abnormal spermatozoa appeared to decrease male fertility. Omprakash et al. (1992) also reported that the percentage of abnormal sperm and a lower methylene blue reduction test (MBRT) value were negatively correlated with the fertilizing ability of semen samples. Cooper and Rowell (1958) found that fertility was significantly correlated with the percentage of dead spermatozoa (-0.89), motility (0.84), resazurin reduction time (-0.80), and live density (0.55).

We ranked males from 1 to 6 (Table 5) according to the positive (e.g., greater number of spermatozoa) and negative influences (e.g., greater number of abnormal spermatozoa) of semen on fertility. Males of like rating were ranked at the same level. We gave the ranking a negative value when having a negative correlation with fertility (e.g., more dead spermatozoa) and a positive value when having a positive correlation with fertility (e.g., greater sperm motility). We found that the crane pairs with semen donors of the better semen samples produced fewer fertile eggs than donors of poorer semen samples ($r = 1.00$, $P < 0.05$). We believe the lower ranking of semen quality in pairs with higher fertility reflected a depletion of the semen from more frequent copulations. From previous studies, we know that chicken and turkey semen quality declined when males were penned with females (Jones and Leighton 1987). The decline in semen volume and sperm concentration was related to copu-

Table 4. Production and egg fertility.

ID	Eggs					Maximum fertility (%)[b]	Minimum fertility (%)[c]
	Laid	Fertile	Infertile	NDE[a]	Lost		
Pre-experimental group: 1992							
84030	6	5	0	1	0	100.0	83.3
85034	6	6	0	0	0	100.0	100.0
86007	6	5	1	0	0	83.3	83.3
85033	6	5	0	1	0	100.0	83.3
88035	6	4	2	0	0	66.7	66.7
83032	6	6	0	0	0	100.0	100.0
Total	36	31	3	2	0	91.7 ± 13.9	86.1 ± 12.5
Experimental group: 1993							
84030	6	2	0	3	1	100.0	40.0
85034	6	4	1	1	0	80.0	66.7
86007	7	3	2	0	2	60.0	60.0
85033	6	4	0	0	2	100.0	100.0
88035	6	2	2	0	2	50.0	50.0
83032	6 + 2[d]	6 + 2[d]	0	0	0	100.0	100.0
Total	37 + 2[d]	21 + 2[d]	5	4	7	81.7 ± 22.3	69.5 ± 25.3
Control group: 1993							
83036	4	2	2	0	0	50.0	50.0
83015	6	6	0	0	0	100.0	100.0
83034	6	6	0	0	0	100.0	100.0
83029	6	6	0	0	0	100.0	100.0
82027	6	6	0	0	0	100.0	100.0
86014	6	2	3	0	1	40.0	40.0
Total	34	28	5	0	1	81.7 ± 28.6	81.7 ± 28.6

[a] No Detectable Embryo.
[b] Maximum fertility = fertile / (fertile + infertile).
[c] Minimum fertility = fertile / (fertile + infertile + NDE).
[d] Two eggs were laid after we terminated semen collection.

lation and attempted copulation in the non-domestic finch (Birkhead 1991).

When the 2 infertile eggs from the second clutch are excluded from the data (Table 5), fertility changes from 60% to 100%, and the correlation coefficient of the ranking increases from r = -0.60 to -1.00 ($P < 0.05$). The male's left wing in pair R7 was injured and bandaged between the time the female laid her first and third clutches. Of the 3 eggs in the second clutch, 1 was broken and 2 were infertile. We believe the male's bandaged wing interfered with his balance, preventing normal copulation.

CONCLUSIONS

Our results are confused by the fact that variability between experimental and control groups for all parameters was much less than the variability for and among individual males and pairs within the experimental group and controls over time. Nevertheless, we can state with caution that repeated semen collection from males in naturally-mated pairs had little effect on successful collection attempts, semen characteristics, and sperm morphology. Semen volume, sperm density, sperm motility, sperm morphology , sperm viability, sperm number per collection, and male response to semen collection exhibited significant individual variation ($P < 0.05$). Also, semen collection from the males did not affect the date of first egg, intervals between and within clutches, egg production and egg fertility in the pairs. Semen quality from males in pens with higher fertility ranked lower than from males in pens with lower fertility. (This is consistent with the concept that males that are successfully copulating thereby deplete their semen reserves.) Disturbance associated

Table 5. Relationship between semen characteristics and fertility.

	Ranking[a]					
Pen Number	R7[b]	R30	R40	R44	S2	Y36
Fertility (%)[c]	100	40	67	100	50	100
Concentration[d]	+2	+5	+1	+4	+3	+1
Sperm motility	+1	+4	+3	+3	+4	+2
Dead sperm (%)	-6	-2	-3	-5	-1	-4
Abnormal sperm (%)[e]	-4	-3	-1	-6	-2	-5
Live sperm	+1	+6	+3	+2	+5	+4
Summary	-6	+10	+3	-2	+9	-2

[a] The most positive relationship between semen characteristics and fertility is +6 and the most negative is –6.
[b] The second clutch was excluded for this pair due to an injury to the male.
[c] Fertility = fertile/(fertile + infertile).
[d] Sperm concentration score: see text.
[e] Abnormal sperm = 100 - % normal sperm - % dead sperm.

with semen collection from a male can adversely affect behavior in some pairs. When collecting semen from males in naturally-fertile pairs, behavioral conditioning can reduce risk of egg breakage. Weigh the risk of egg losses against the need for the semen in sensitive pairs. We were able to collect semen from naturally-fertile pairs suitable for cryopreservation without interfering with fertility and egg production from the experimental pairs.

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