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Abstract: We analyzed fertility patterns of 339 eggs laid 1985–93 at the International Crane Foundation by cranes whose sole means of fertility was artificial insemination (AI). Ten of 15 crane species were studied. Fertility rates increased significantly ($P < 0.05$) with (1) higher sperm density, (2) greater sperm motility, and (3) 2 vs. 1 vs. 0 inseminations 4–7 days before oviposition. Semen sample size was inversely related to sperm density and had no strong relationship with egg fertility. Inseminations less than 2.5–3.0 days before an egg is laid are probably too late to contribute to fertilizing the egg but may contribute substantially to fertilizing the next egg. We also describe practical knowledge gained during the past 10 years that apply to certain species or situations. Wattled cranes (*Bugeranus carunculatus*) have a long fertile period post-AI (up to 16 days), but may require more careful methods and special AI scheduling strategies. We also describe techniques for AI just after oviposition that are especially beneficial for Siberian cranes (*Grus leucogeranus*), and methods for maximizing sample delivery to the female.

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At a captive breeding center, the managers' skill in prioritizing resources to maintain the health and propagation of the species is crucial. Artificial insemination (AI) is 1 means of species propagation that the International Crane Foundation (ICF) in Baraboo, Wisconsin, strategically utilizes. Natural copulation is preferred, but AI is necessary for the propagation of some cranes at ICF. Various physical disabilities, behavioral problems, and desired genetic outcomes, as described by Gee and Temple (1978), Gee and Sexton (1980), Russman (1980), and Gee (1983), necessitate this procedure.

Artificial insemination is risky for both staff and cranes. Errors in technique and timing can result in injuries or infertility. Although AI, with proper conditioning and management of cranes, often produces higher fertility rates than natural matings (Gee and Mirande 1996), it can be detrimental to breeding programs without adequate knowledge and preparation. For example, a few of our whooping cranes delay the onset of egg laying or stop laying eggs if we inseminate them more than twice per week. Our Siberian cranes do not behave in this manner, and to inseminate them less frequently than 3 times per week would result in lower egg fertility. Sources describing AI procedures include Archibald (1974), Gee and Temple (1978), Russman (1980), and Gee and Mirande (1996). This study focuses on when to inseminate females to maximize egg fertility and on the effects of semen variables on fertility rates. Russman (1987) determined that the optimal time to inseminate is 2–4 days before egg laying and just after oviposition. An ultrasonographic study of egg formation in cranes (*Grus* spp.) found that approximately 56–60+ hours elapse from ovulation to oviposition (Putnam 1983). Putnam estimated that the last chance to fertilize crane eggs is approximately 2.5 days before oviposition, and recommended that 1 insemination be scheduled 2.5 days before the earliest expected date of the female's next egg, based on past laying history.

We analyzed the effects of the following variables on crane egg fertility rates: (1) sperm density, (2) sperm motility, (3) semen volume, and (4) timing of insemination(s). This information might help managers improve their AI scheduling. Practical knowledge acquired by the ICF staff regarding AI technique will also be discussed, with the hope that other crane breeders may apply this knowledge.

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STUDY AREA AND METHODS

AI and egg records from 1985 to 1993 of captive cranes at ICF in southern Wisconsin (43°29'–43°32'N, 89°45'W) were incorporated into a data base. Inseminations that contained sperm and occurred 2–38 days prior to oviposition were considered "viable" and included in this study. We analyzed fertility effects of the last 2 inseminations only; including more inseminations exponentially increases the complexity of analysis, for no or questionable gain. Only cases in which AI was the sole means of egg fertilization were included; eggs whose fertility could not be determined

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(11/350, or 3.1% of eggs) were excluded.

Variables included in the data base were (1) egg sequence number, (2) year laid, (3) number of days between the last insemination and oviposition, (4) number of days between the second to last insemination and oviposition, (5) number of hours between the last inseminations and ovipositions which occurred 2–3 days later, (6) number of hours between the next to last inseminations and ovipositions which occurred 2–3 days later, (7) semen volume, (8) sperm density of semen sample, (9) sperm motility of sample, and (10) egg fertility. Ten of the world's 15 crane species were studied (Table 1).

SYSTAT software (version 4.0, 1988) was used for statistical analysis. We compared mean fertility rates (± 1 SD) among different groups of semen sample volume, sperm density, and motility, and for different times of insemination vs. oviposition date. We tested for significant differences in fertility rates ($P < 0.05$) using multiple linear regression, Student's *t*-test, Pearson's product-moment correlation matrix (Pearson correlation), Spearman rank correlation, and Chi-square goodness-of-fit tests. Because Spearman and Pearson tests yielded the same result in all but 2 analyses, we present only the more conservative Spearman correlation results except the times where the 2 disagreed.

Sperm Density

Sperm "quality" or density is a letter-graded scale (Russman 1980) in which "A" indicates the highest sperm density in a semen sample observed in the microscope field at 400×. "D" represents the lowest density, and "F" represents no sperm. We scored "A" as 4, "B" as 3, "C" as 2, and "D" as 1. We excluded all "F" samples.

Percentages of fertile eggs were calculated for each grade category (1–4) of sperm density. We did this separately for the last, and next to last, insemination prior to oviposition. *T*-tests were used to test for significant differences in fertility. Percent of fertile eggs was then calculated for combinations of sperm densities present in the last 2 inseminations prior to oviposition. We also analyzed the effect of 0 vs. 1 vs. 2 "A" or "B" graded samples in the last 2 inseminations by using Chi-square goodness-of-fit tests. A Spearman correlation determined the relationship between sperm density and resulting fertility.

Sperm Motility

Motility is the percentage of sperm exhibiting forward movement in a semen sample. Motility is rated from 0 to 95, with 0 meaning no sperm were moving forward. A Spearman correlation analyzed the relationship between sperm motility and resulting fertility. Percent of fertile eggs was calculated for the following scenarios: (1) sperm in both semen samples from the last 2 inseminations prior to oviposition were >50% motile, (2) 1 of the last 2 samples had >50% motility, (3) neither of the samples had motility >50%. We compared fertility rates of these groups by using *t*-tests.

Table 1. Eggs analyzed and fertility rates for 10 crane species (n = 339) at the International Crane Foundation, 1985–93.

Species	No. of eggs	% of total	% fertile
Grus leucogeranus (Siberian)	84	24.8	65.5
G. canadensis (sandhill)	61	18.0	77.7
G. japonensis (red-crowned)	54	15.9	38.9
G. antigone (sarus)	42	12.4	31.0
G. vipio (white-naped)	32	9.4	28.1
G. americana (whooping)	23	6.8	65.2
Bugeranus carunculatus (wattled)	13	3.8	30.8
G. monacha (hooded)	12	3.5	66.7
G. rubicunda (brolga)	12	3.5	50.0
Anthropoides paradisea (blue)	6	1.8	100.0

Semen Volume

Semen sample volumes are recorded at ICF on both male and female AI records. Semen volumes ranged from <0.01 ml to 1.1 ml. Those recorded as <0.01 ml were entered in the database as 0.005 ml. Some semen samples were split between 2 females. Before 1989 ICF rarely split samples between 2 females if the sample was ≤0.05 ml. Since then we have increasingly split samples <0.05 ml in AI on Siberian cranes. Semen volumes entered in the database were the amounts inseminated into the females.

Spearman and Pearson correlations were performed to determine the relationship between sample volume and resulting fertility. Pairwise comparisons of fertility rates (using *t*-tests) were performed between cases in which both semen volumes of the last 2 inseminations prior to oviposition were small (<0.01 ml), when both semen volumes were medium-sized (>0.01 and <0.10 ml), and when both were large (≥0.10 ml). The same was done for cases in which neither of the last 2 inseminations contained >0.025 ml of semen compared to cases in which both samples were at least 0.025 ml.

We used 2 types of analyses to assess the relationship of sperm density to sample volume. First, we used a *t*-test to compare mean sperm density of small (≤0.01 ml), medium (>0.01 ml and <0.10 ml), and large (≥0.10 ml) semen samples. This was done separately for the last and for the penultimate insemination prior to oviposition. Second, we

Table 2. Fertility (±SD) rate vs. sperm density of the last ($n = 334$) and second to last ($n = 292$) inseminations before oviposition for cranes at the International Crane Foundation, 1985-93.

	Last insemination		Second to last insemination	
Density	*n*	Fertility rate[a]	*n*	Fertility rate[a]
A	79	0.722 ± 0.451	66	0.758 ± 0.432
B	133	0.590 ± 0.494	118	0.602 ± 0.492
C	93	0.380 ± 0.487	88	0.420 ± 0.496
D	29	0.410 ± 0.501[b]	20	0.350 ± 0.489[c]

[a] Last insemination fertility rates were only statistically compared with one another, as were second to last insemination rates.

[b] Not significantly different ($P < 0.05$) from B or C; all other densities were significantly different from those below them.

[c] Not significantly different from C; all other densities were significantly different from those below them.

used Spearman and Pearson correlations to test whether sample volume and density covaried with each other for each of the last and next to last inseminations.

Semen Sample: Multivariate Analysis

A multiple regression (MGLH model) analyzed fertility effects of the last insemination before oviposition; egg fertility was the dependent variable, and sperm density, motility, and semen volume were independent variables.

Timing of Inseminations

In an attempt to minimize disturbance during the breeding season, ICF staff examines the birds once in the morning during servicing, and once in afternoon. Staff is not generally present at the time of oviposition. Therefore, to calculate the estimated number of hours between inseminations, and those ovipositions which occurred 2-3 days later, we estimated ranges of times for these ovipositions. This could help pinpoint the moment when it becomes too late to fertilize the next egg.

Percent of fertile eggs was calculated for each day (2 to >15) prior to oviposition in which the female was last inseminated. To search for combinations of last 2 insemination times that resulted in the highest fertility rates, we examined multiple scenarios. Egg fertility was compared for combinations in which the number of days between the last 2 inseminations was 1-2 vs. >2 days apart. Several more combinations which, in general, included both the greatest number of cases and the highest percentage of fertility were analyzed. We compared cases in which both inseminations occurred either 2-3 days, 4-7 days, or 8+ days prior to oviposition with a *t*-test. We used Chi-square tests to compare fertility rates when 0 vs. 1 vs. 2 inseminations were done 4-7 days prior to oviposition.

RESULTS

We analyzed a total of 339 eggs. Overall egg fertility was 54.3% (0.543 ± 0.499) (Table 1).

Sperm Density

"A" samples in the last 2 inseminations resulted in significantly higher egg fertility than "B", "C", or "D" samples (Table 2). Egg fertility of "B" samples was significantly higher than that of "C" samples. No significant fertility difference existed between "B" and "D", or between "C" and "D", samples.

Chi-square tests detected significantly higher fertility rates for cases in which both of the last 2 inseminations prior to oviposition contained "A" or "B" sperm density than when only 1 of the last 2 samples ($P < 0.001$), or neither of the 2 last insemination samples ($P < 0.001$), consisted of "A" or "B" density (Table 3). There was no significant difference between instances in which 1 of the 2 last insemination samples consisted of "A" or "B" density and cases in which neither of the samples contained "A" or "B" sperm density ($P > 0.1$).

Spearman rank correlation showed a significant positive relationship between egg fertility and the sperm density of both the last ($R = 0.257$, $n = 334$, $P < 0.001$) and second to last ($R = 0.270$, $n = 292$, $P < 0.001$) inseminations.

Sperm Motility

Spearman rank correlation showed a significant positive relationship between egg fertility and the sperm motility of both the last ($R = 0.263$, $n = 332$, $P < 0.001$) and second to last ($R = 0.381$, $n = 296$, $P < 0.001$) inseminations. The

Table 3. Fertility of eggs (±SD) at the International Crane Foundation, 1985-93, when both, 1, or neither of the last 2 inseminations had high (A or B) sperm density.

Last insemination	Second to last insemination	*n*	Fertility rate
A or B	A or B	146	0.719 ± 0.459
A or B	C or D	44	0.500 ± 0.506
C or D	A or B	41	0.488 ± 0.506
C or D	C or D	63	0.349 ± 0.481

more samples with high motility the female received, the higher the egg fertility. Significantly higher egg fertility was found for 2 vs. 1, 2 vs. 0, and 1 vs. 0 semen samples containing sperm motility >50% (Table 4).

Semen Volume

Spearman and Pearson correlations detected an inverse relationship between sample volume and egg fertility. This pattern was significant ($P < 0.05$) for the second to last insemination. For the last insemination, Spearman correlation revealed a marginally significant result ($R = -0.106$, $n = 339$, $P = 0.05$), whereas Pearson correlation showed no significant result.

Small- and medium-sized semen samples produced higher fertility than large samples. The fertility rate for eggs in which both of the last 2 semen volumes inseminated contained ≤0.01 ml (small volume) was 66.7% ($n = 27$); the fertility rate when both samples were >0.01 ml and <0.1 ml (medium volume) was 62.7% ($n = 133$); and the fertility rate when each of the last 2 samples inseminated contained ≥0.10 ml (large volume) was 40% ($n = 35$). Medium-sized samples produced significantly higher fertility than large samples (t-test, df = 166, $P = 0.017$) and small samples had nearly significantly higher fertility than large samples (t-test, df = 54, $P = 0.055$). In a separate test of smaller vs. larger semen volumes, there was no significant fertility difference (t-test, df = 202, $P = 0.234$) between cases in which neither of the last 2 sample volumes was >0.025 ml (66.7% fertility, $n = 39$) and cases in which both samples were ≥0.025 ml (55.9% fertility, $n = 152$).

The mean sperm density of the last insemination did not vary significantly among the 3 semen volume groups ≤0.01 ml, >0.01 and <0.10 ml, and ≥0.10 ml (Table 5). In the second to last insemination, however, medium volume semen samples >0.01 ml and <0.1 ml had significantly higher sperm density than either small or large semen samples (Table 5).

Table 4. Fertility of eggs (± SD) at the International Crane Foundation, 1985-93, when both, 1, or neither of the last 2 inseminations had relatively high (>50%) sperm motility.

>50% motility	n[a]	Fertility rate[b]
2	60	0.800 ± 0.403
1	89	0.629 ± 0.486
0	190	0.421 ± 0.495

[a] Includes some cases in which only 1 motility was recorded in the 2 inseminations, or where there was only 1 insemination, not 2.

[b] Fertility rates all significantly different from each other ($P < 0.05$).

Table 5. Sperm density (± SD) of small (≤0.01 ml), medium (>0.01 and <0.10 ml), and large (≥0.10 ml) crane semen volumes at the International Crane Foundation, 1985-93, in instances where both of the last 2 inseminations were small ($n = 21$), both were medium ($n = 118$), or both were large ($n = 35$). Density is measured on a scale of 1 (low) to 4 (high).

Semen volume (ml)[a]	Last insemination		Second to last insemination	
	n	Sperm density	n	Sperm density
Small	21	2.476 ± 1.209	21	2.500 ± 1.140
Medium	116	2.879 ± 0.876	118	2.958 ± 0.891
Large	35	2.886 ± 0.631	35	2.571 ± 0.655

[a] Small (≤0.01), medium (>0.01 and <0.10), and large (≥0.10).

The negative correlation between semen volume and egg fertility is consistent with a slightly negative relationship between semen volume and sperm density: this pattern was statistically significant for both the last 2 inseminations in the Pearson correlation ($P < 0.05$), but was not significant in the Spearman correlations.

Semen Sample: Multivariate Analysis

Initially, the timing variable in which the last insemination occurred >3 days prior to oviposition was included in the multiple regression along with the 3 semen variables. The timing variable had a strong non-linear relationship with fertility and was thus excluded when the final multiple regressions were run. Among semen variables, sperm density ($R = 0.168$, $t = 2.835$, $P = 0.005$) and motility ($R = 0.187$, $t = 3.150$, $P = 0.002$) were nearly equally important to egg fertility. Semen volume was not significantly related to egg fertility ($R = 0.025$, $t = 0.466$, $P = 0.641$). The ANOVA statistics were $n = 332$, df = 3,328, $F = 10.854$, and $P < 0.001$.

Timing of Inseminations

Attempts to determine the critical point (the number of hours) after which the egg cannot be fertilized by an insemination done 2-3 days before egg laying were of limited success due to the small sample of cases in which there was no prior insemination that could have contributed to egg fertility. Our limited results suggest a threshold near 2.5-3 days pre-oviposition after which AI is unlikely to fertilize the egg. There were 5 cases in which a lone insemination was done between 30.75 and 54.75 hours (approximately 2 days) prior to oviposition. All resulted in infertility. Sample sperm densities included 2 "A", 2 "B", and 1 "C". Likewise, none

Table 6. Fertility rate (±SD) of eggs at the International Crane Foundation, 1985–93, vs. time from last insemination to oviposition ($n = 336$).

Days since last insemination	*n*	Fertility rate
>15	20	0.050 ± 0.224
15	4	0.000 ± 0.000
14	1	0
13	1	1
12	3	0.333 ± 0.577
11	2	0.500 ± 0.707
10	1	0
9	9	0.333 ± 0.500
8	11	0.545 ± 0.522
7	8	0.750 ± 0.463
6	13	0.692 ± 0.480
5	24	0.500 ± 0.511
4	48	0.750 ± 0.438
3	102	0.578 ± 0.496
2	89	0.528 ± 0.502

of the 8 eggs in which a lone insemination was done approximately 3 days (49.25–72.75 hours) prior to oviposition was fertile. Sample sperm densities included 2 "A", 2 "B", 3 "C", and 1 "D".

For fertile cases in which there were 2 inseminations done 31.5–81.5 hours prior to oviposition, a third insemination existed (which had been done 5–11 days prior to oviposition) in 6 of these 7 fertile cases. Sperm densities of the 2–3 inseminations included 4 "A", 14 "B", and 2 "C". In those infertile cases in which there were 2 inseminations done between 29.75 and 78.75 hours prior to oviposition, a third insemination existed (which had been done 5–11 days prior to oviposition) in 7 of these 12 infertile cases. Sperm densities of the 2–3 inseminations included 1 "A", 8 "B", 18 "C", and 4 "D".

The smallest time period between the last insemination and oviposition and the next to last insemination and oviposition in which a fertile egg was produced, was approximately 2 and 3 days, respectively. Such cases, where both inseminations were ≤3 days before a fertile egg was laid, would usually have had a third prior insemination that could have fertilized the egg. Our study examined the contributions of a maximum of 2 inseminations. The longest time period in this study between the last 2 inseminations and oviposition of a fertile egg, was 16 and 18 days, respectively (wattled crane).

Tables 6 and 7 provide the groundwork for further analysis of insemination timing vs. fertility rates. For the last insemination, the highest percentage of fertility (where $n >$ 1 egg) was 75% and occurred when the female was last inseminated 4 ($n = 48$) and 7 ($n = 8$) days prior to oviposition (Table 6). In cases in which the last 2 inseminations occurred within 2 days of one another, 85.7% of 21 cases resulted in fertility when females were inseminated 5–6 days, and again 4 days, prior to oviposition (Table 7). When the last 2 inseminations occurred >2 days apart from one another, 71% of 21 cases resulted in fertility when females were inseminated >6 days, and again 4 days prior to oviposition (Table 7).

T-tests that compared differences in timing and resultant egg fertility showed no significant difference between cranes inseminated last 2–3 days vs. 4–7 days prior to oviposition ($P = 0.064$). There was significantly higher egg fertility, however, in those cranes inseminated 4–7 days vs. those inseminated 8+ days prior to oviposition, when only the last insemination was counted (Table 8).

When both of the last inseminations occurred 2–3 days, 4–7 days, or ≥8 days prior to oviposition, resultant egg fertility was significantly higher in those cranes inseminated 4–7 days prior to oviposition than 2–3 or ≥8 days before egg laying (Table 9).

Chi-square analysis further confirmed the fertilizing power of insemination(s) done 4–7 days prior to oviposition. Results show a significant increase in egg fertility with 2 inseminations in this time period (84.6% fertile, $n = 39$) compared with 1 (61.2%, $n = 178$) ($\chi^2 = 7.62$, df = 1, $P < 0.01$) or 0 (34.0%, $n = 97$) ($\chi^2 = 25.833$, df = 1, $P < 0.001$) inseminations. Similarly, 1 insemination done 4–7 days prior to oviposition yielded significantly higher fertility rates than if neither insemination occurred within this time period ($\chi^2 = 15.515$, df = 1, $P < 0.001$).

DISCUSSION

Semen Sample Characteristics

Our study found a strong relationship between sperm density and egg fertility. This is consistent with the findings of Gee and Temple (1978), who reported that a high percentage of living, active spermatozoa, and a high sperm concentration give excellent fertility rates after insemination. As sperm density improved, fertility improved.

Sperm density and motility had significantly greater effects on egg fertility than semen volume. Although one would expect larger semen volumes to contain more sperm, thus yielding higher egg fertility rates, our data do not support this. Although Hereford (1987) found a significant correlation between mean semen volume and "A" density of samples, our results (Pearson and Spearman rank correlations) indicate that as volume increases, sperm density decreases. These conflicting findings could be caused by

Table 7. Egg fertility rate (±SD) at the International Crane Foundation, 1985–93, vs. days to oviposition when the last 2 inseminations occurred within a 1–2-day period ($n = 132$) and when they occurred >2 days apart ($n = 144$).

	Last 2 inseminations 1–2 days apart			Last 2 inseminations >2 days apart		
Last insemination	Second to last insemination	*n*	Fertility rate	Second to last insemination	*n*	Fertility rate
9 days	10–11 days	4	0.500 ± 0.577	>11 days	5	0.200 ± 0.447
8 days	9–10 days	2	0.500 ± 0.707	>10 days	6	0.500 ± 0.548
7 days	8–9 days	3	0.667 ± 0.577	> 9 days	1	1
6 days	7–8 days	4	0.750 ± 0.500	> 8 days	7	0.714 ± 0.488
5 days	6–7 days	8	0.625 ± 0.518	> 7 days	13	0.385 ± 0.500
4 days	5–6 days	21	0.857 ± 0.359	> 6 days	21	0.714 ± 0.463
3 days	4–5 days	51	0.627 ± 0.488	> 5 days	45	0.600 ± 0.495
2 days	3–4 days	39	0.561 ± 0.502	> 4 days	46	0.540 ± 0.504

differences in the males used in the 2 studies, as well as the differences between our focus on the full range of sperm densities, and Hereford's (1987) on statistical patterns of "A" density samples. Our result may be explained, in part, by the presence of lymph, urates, or other contaminants, which are harmful to sperm (Smyth 1968, Lake 1971, Gee and Temple 1978) and more commonly found in larger sample volumes. We therefore suggest that once a sample as small as ≤ 0.01 ml is obtained from males that tend to give contaminated samples, attempts to obtain additional semen may be detrimental to the fertilizing capacity of the sample. To guard against this, use a second receptacle to attempt additional collection from a male if the original sample is at risk of further contamination. Furthermore, samples ≤ 0.05 ml may have sufficient sperm density to split in half and inseminate into 2 females. ICF has used this strategy successfully with Siberian cranes when female egg layers outnumber semen donors. Tiny semen samples (<0.01 ml) extended to 10–20× their volume by using semen extender had equal or greater fertilizing power as large (0.1 ml or larger) samples that were minimally extended. This suggests that fertility is not reduced when samples are extended to many times their volume.

Timing of Inseminations

Our limited results on lone inseminations close to the ovulation boundary of 2.5 days suggest that 1 or 2 inseminations less than 3 days before the egg is laid have only a small chance of contributing to the egg's fertility. Most fertile cases in which insemination(s) were done 2–3 days prior to oviposition would have had a second or third prior insemination that could have fertilized the egg.

Gee and Temple (1978) found when cranes were inseminated a few hours after oviposition, the fertility of subsequent eggs was greater than when birds were inseminated 2–3 days prior to oviposition. Likewise, we describe lower fertility rates when insemination(s) occurred 2–3 and 8+ days prior to oviposition. Our results indicate the optimal time to inseminate in order to fertilize the next egg is 4–7 days prior to oviposition, with 2 inseminations resulting in higher fertility than 1. In a similar finding, Gee and Temple (1978) found as the frequency of inseminations increased from 0 to 4 times in a 10-day period, the percentage of fertility increased as well. We believe it is very important to tailor insemination times to individual birds. It is helpful, however, to have a weekly schedule plan for your flock. Historically, semen collection and deposition was done at ICF and at Patuxent Wildlife Research Center every third day (Archibald 1974). Archibald and Viess (1979) subsequently reported an increase in frequency of inseminations to 3 times weekly at ICF. Gee (1983) also increased the recommended insemination frequency to 3 times per week to achieve the best fertility rate. In our study, 2 inseminations 4–7 days prior to oviposition translates into a schedule of 3 times per week for most crane species, with some adjustments for when cranes are expected to lay their next egg.

Although sperm host glands were discovered in birds 50 years ago (Van Drimmelen 1946) and have been confirmed in several species (e.g., Bakst and Bird 1987, Birkhead and

Table 8. Comparison of egg fertility rates (±SD) at the International Crane Foundation, 1985–93, during 3 time periods for the last insemination prior to oviposition.

Days before oviposition	*n*	Fertility rate
2–3	195	0.559 ± 0.498
4–7	92	0.674 ± 0.471
8–15	52	0.250 ± 0.437[a]

[a] Significantly different ($P < 0.05$) from other rates.

Table 9. Comparison of egg fertility rates (± SD) at the International Crane Foundation, 1985-93, when both of the last 2 inseminations occurred within selected time periods prior to oviposition.

Days before oviposition	*n*	Fertility rate
2-3	22	0.364 ± 0.492
4-7	39	0.846 ± 0.366[a]
8-15	35	0.229 ± 0.426

[a] Significantly different ($P < 0.05$) from other rates.

Hunter 1990), they have not yet been found in cranes (Russman 1987). But the lengthy fertile periods we observed (up to 16 days post-insemination) demonstrate sperm storage is occurring in cranes. Most females with the longest periods between AI and a fertile egg were inseminated infrequently. If they were inseminated frequently with high quality semen to fill up the putative sperm host glands, then left without any AI for some time, higher fertility rates ≥8 days post-insemination than we found might be detected (G. Gee, pers. commun.).

We summarize our analysis by stating that each bird is unique. While this is obvious, keep this in mind when using these study results to plan strategies. Our results may be skewed for several reasons: (1) only 10 of the 15 species were studied, (2) study species are not equally represented and therefore results are weighted more by species in which egg numbers are higher, (3) subjective variations between record keepers regarding sperm density and motility are likely, and (4) many more variables other than those analyzed in this study, such as weather, individual crane maturity, and changing pen arrangements, probably affected fertility rates.

Special AI Strategies and Techniques

Meticulous record keeping is crucial in enabling the crane propagator to detect quality patterns of semen samples obtained from the males, as well as egg laying patterns in females. Without such records it becomes much more difficult to strategically plan and execute artificial insemination effectively. Essential data on semen collections include date, time, temperature, sky conditions, person stroking crane, person teasing (operator) cloaca, person collecting semen, response of crane to stroking (struggled, relaxed, relaxed and raised tail, or purred), semen volume, apparent degree of semen contamination, amount of extension with semen extender, relative semen density, percent sperm motility, comments on any unusual crane behavior post-collection, and female inseminated. For females being inseminated, the same data should be collected, plus whether the insemination was placed in the oviduct or cloacally, the name of the male donor, dates of all subsequent eggs, and egg fertility. Optional data to collect include absolute sperm density and live/dead sperm ratios (both collected in lab after AI), distance between the points of the female's pubic bone (used to estimate when female will lay eggs), and appearance of blood spots on the cloaca (may indicate ovulation has occurred or will soon occur). Pubic bone spread is valuable in timing AI (or terminating/delaying it when it is not needed) if normals for the female have been previously established.

Wattled Cranes.—The following pertains especially to wild-caught or parent-reared wattled cranes, but the recommendations with regard to capture apply to any wild-caught crane. Less restrictive strategies may be used for hand-reared cranes.

1. Quick capture, by using 3 people, is essential. If the male becomes upset during a poorly executed capture, he is much less likely to provide a sample of acceptable quality. One month before egg-laying begins, prepare a capture area in the pen corner with the most acute fence angle. Visual barriers like reed mats or tennis wind netting (J. A. Cissel Co., P.O. Box 2025, Lakewood, NJ 08701, USA) attached to the fence, plus a soft material added to it, aids in safe captures. Used Christmas trees (with decorations removed) tied to the fence from the corner to 3 m out on each fence line work well. Dense trees retaining many needles are best.
2. Use the same team in their respective roles, once a semen sample has been successfully obtained by a particular team. Team choice can be based on an examination of past records. Vocalizations simulating crane copulatory calls may be executed by the "stroker" (handler) to enhance stimulation of the male. Because most of the sample will emerge when the "teaser" (operator) squeezes the cloaca the first time, the collection begins then. It may be necessary for the teaser to squeeze the everted cloaca twice before attempted collection in order to obtain very small samples (<0.02 ml). All team members should learn to perform each others' roles whenever possible, to increase chances for success in the event of a particular team member's absence.
3. Several of our wattled crane semen samples have been very small (0.005-0.01 ml) and contained acceptable sperm concentration and motility. Thus, no matter how small or discolored the sample appears, dilute the semen with semen extender and inseminate it immediately into the desired female.
4. Make a concerted effort to create a sense of security for

your pairs, especially for females during the breeding season. "Happy cranes lay eggs." We experimented during winter/spring 1994–95 by giving 1 particularly difficult pair daily rewards (approximately 5 newborn mice or 5 8-cm smelt, and a small handful of corn) several days before and after inseminations and before anticipated oviposition. Although we have no scientific evidence that this contributes to higher fertility rates, we believe it increased the chances for optimal insemination scheduling. The first season we tried this, the female appeared to lay eggs more reliably during the desired 15-day window after AI; she also laid 11 eggs that year instead of the usual 7. The additional protein provided by the smelt and newborn mice could increase the female crane's protein reserves for forming eggs. Furthermore, these tactics seem to calm the cranes and increase their comfort with our presence. We have observed these wild-caught cranes approach within 1.5–3 m of us (whereas previously they had fled) and dance upon provision of rewards. This positive association with humans also helps offset the presumably negative experience of being handled for AI.

5. Timing of insemination is difficult with wattled cranes. Our pairs usually lay 1-egg clutches anywhere from 10 to 50 days apart. However, 1 pair lays most eggs 12–18 days apart, and certain eggs (i.e., the fifth and seventh eggs) are usually clutched with the prior eggs and laid 3–6 days later. We do not recommend performing AI 3 times per week, as we do with certain other species. Minimal disturbance with wild caught birds is preferable.

We had 9/24 (38%) fertile wattled eggs during 1992–95 and of those, successful inseminations or pairs of insemination days prior to oviposition were 5, 6, 6, 6 + 13, 9, 9 + 11, 12, 12, and 16 + 18. Unsuccessful combinations included 2 + 29, 4, 6, 8, 9 + 16, 11, 15, 17, 17 + 29, 18, 19, 19 + 33, 20, 27 + 29, and 38.

Inseminations 4–16 days before oviposition resulted in 60% (9/15) fertility, while all 9 inseminations >16 days beforehand resulted in infertility. We aim for inseminating the female 6–8 days after oviposition so that most eggs will be laid 4–12 days later. Six of the 9 fertile eggs appear to have been fertilized by just 1 sample, because the next to last insemination in 6 cases was >38 days before oviposition, more than twice our longest confirmed fertile period. The other 3 fertile eggs may have been helped by their second to last inseminations 9–18 days pre-oviposition.

In our experience, however, wild-caught wattled cranes artificially inseminated more than once between 2 eggs cease laying eggs for 30–40 days and become unpredictable in their egg-laying pattern after that. Because predictability is the key to successful AI on these birds, we analyze the crane's past egg-laying record and try for 1 insemination between eggs. Sometimes the female clutches her eggs; when this happens we either have a chance at 2 fertile eggs or the second egg comes too late for fertility. It is best, however, to adhere to the most probable interval between eggs of 12–18 days for our pair.

Siberian Cranes.—Quick capture, once again, is vital. Use of 1 person (the assigned stroker) to capture the bird is recommended for birds who behave aggressively toward 1 individual but may be intimidated and become upset in the presence of >1 person. This type of crane usually advances toward the stroker to attack, at which time the stroker should capture the crane and immediately initiate stroking. Other persons must stay out of sight and remain quiet until the capture. After capture, the rest of the team should assume their respective roles and proceed.

Individual Siberian cranes consistently begin laying eggs the same time each year, so for most pairs we begin AI 1 week before that date to cover the first 2 eggs. Siberian cranes also have predictable laying patterns, particularly for the first 3–4 eggs of the season; 16/21 (76%) of the second eggs were laid 3 days after the first eggs. The other 5/21 were laid either 2 or 4 days after the first eggs. While this degree of predictability is good, the interval may frequently be too small to allow an insemination between the eggs to fertilize the next egg. Because it is necessary to inseminate at least 56–60+ hours prior to oviposition in order to fertilize the egg before shell membrane formation (Putnam 1983), it is essential to inseminate the female immediately after the first egg is laid (vs. found). On the anticipated lay date, we observe Siberian cranes more often during daylight hours to inseminate immediately after oviposition. Despite these efforts, it is still possible for the second egg to be infertile, particularly if the first egg is laid during the night, but not found until morning. A frequent consequence, however, of insemination just after the first egg is "found" is fertilization of the third egg. This is also a consequence of the short second-to-third egg laying interval in Siberian cranes.

Different females have individual patterns that differ from others of their species. Keeping a chart of the time intervals between eggs facilitates identification of patterns within the flock that provide the basis for selected insemination strategies (Table 10). For example, ICF routinely inseminates Siberian cranes 3 times per week in order to fertilize the next two eggs laid. Insemination times are tailored to individual birds, however, such that if a female were to lay on an alternate ("off") day, we inseminate her the same day, a few hours after she has laid.

AI After Oviposition.—There are techniques we use for cranes that exhibit one or both of the following behaviors,

Table 10. Examples of egg laying interval (in days) records kept for females at the International Crane Foundation. Grouping females by species (e.g., Siberian cranes here) assists in recognition of species-specific egg laying trends. In 1991, Ramsar's second egg was laid 3 days after the first egg, her third egg was laid 5 days after the second egg, etc. Ranjit's 12-day interval before her third egg in 1995 may have been caused by excessive AI.

Name (no.)	Year	Total no. of eggs	Season length	First lay date 1	Days between eggs 2	3	4	5	6	7	8	9	Last lay date
Ramsar	1991	3	9	2 May	3	5							10 May
6-14	1992	6	23	4 April	3	3	5	5	6				26 April
	1993	5	17	27 March	3	5	4	4					12 April
	1994	8	33	28 March	3	3	5	5	5	6			29 April
	1995	5	20	5 April	2	5	5	8					25 April
Ranjit	1992	6	21	10 April	3	3	4	5	5				30 April
6-22	1993	6	19	2 April	3	3	3	6	3				20 April
	1994	9	29	20 March	3	3	4	3	4	3	5	3	17 April
	1995	9	41	26 March	4	12	3	3	5	3	5	6	6 May

which frequently occur when we remove an egg from the cranes' nest (in an effort to keep the pair in egg production) and subsequently attempt to collect a semen sample to perform AI: (1) extreme aggression because we have taken the egg, and (2) significant fecal elimination during AI because cranes store up feces during incubation to avoid soiling the nest. These undesirable behaviors usually result in either a very poor sample or no sample at all. We recommend that personnel wait at least 3 hours after egg removal before collecting sperm and/or inseminating. This amount of time usually increases the possibility that birds will have "regrouped" and be more receptive to AI. This also enables incubating birds time to eliminate fecal matter and urates. Frequently, both birds have fecal retention. One crane may need to be restrained or locked indoors while the other is being handled for AI. Usually females become very aggressive when incubating. Take advantage of aggression to effect a quick capture.

One exception, in which AI should be done immediately, is when cranes will be keeping the first egg of the clutch and you hope to fertilize the next egg. The longer the cranes have the egg, the more difficult it will be to get a clean semen sample from the male. In Siberian cranes the chances are poor, but the best hope is to try immediately, reasoning that the egg may have just been laid and the male has not yet stored feces.

Delivering More of the Sample to the Female.—Because 0.04 ml of fluid remains in the tip of a tuberculin syringe (Russman 1980) after the plunger is pushed:

1. Extend all samples, no matter how small, to 0.10–0.15 ml by using semen extender. This ensures that at least 60–70% of the sample will be delivered, even if 0.04 ml remains in the syringe.
2. Position air behind the sample before inseminating, so that little of the sample remains after the plunger is pushed. To do this, pull the plunger back halfway with the syringe tip uncapped, place a cap on the syringe tip, and quickly flick the syringe forward so that the tip ends up down. Practice this with water in the syringe so that you can do it without making substantial semen go too far into the tip and adhere to the cap.
3. It is not necessary to save a semen sample before inseminating, because enough sample remains in the syringe for microscopic examination of quality even after the most successful insemination.

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