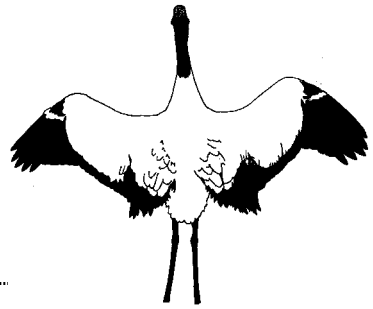
Special Techniques, Part A: Crane Artificial Insemination



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Captive breeding of nondomestic birds has increased dramatically since 1950, and captive crane production often exceeds that of wild birds in their native habitat. Artificial insemination (AI) is one propagation technique (Gee and Temple 1978; Gee 1983) used extensively with cranes. With proper conditioning and management of the cranes, AI often produces fertility better than that achieved through natural matings.

The most obvious need for an AI program is to reduce **infertility** (Archibald 1974; Gee and Temple 1978; Lake 1978; Sexton 1979). In some mated pairs natural copulation can be difficult because of injury (including wing impairment to deter flight), deformity, differences in body size, or behavioral difficulties. Sometimes females are kept in separate pens because of mate aggression, pair incompatibility, or the lack of a mate. Occasionally, a productive female may be in a distant location separate from the male, where transfer of semen is the only way to avoid infertility. Fertility in a mated pair can be improved by AI using the same or a different male.

AI is useful in carrying out the goals of **special breeding** programs for captive propagation. The genetic influence of one male in a population can be increased by using his semen to sire young from several females each season. Conversely, semen from several males can be used to increase female fertility. (Such practices can, of course, lead to questions of paternity; techniques for resolving paternity are still expensive and results are dependent upon the availability of suitable genetic markers.) **Hybridization** between behaviorally incompatible species is possible with AI, and although it should be avoided for propagation purposes, some research questions require hybridization. Patuxent used Whooping Crane semen to produce four Whooper-Sandhill Cranes to study hybrid characteristics.

AI has other **special uses**. For example, a male's potential for producing progeny with specific traits (chicks that grow more rapidly or possess superior disease resistance) or his potential fertility can be determined more quickly through AI than with natural matings. The collection of semen provides for other uses including laboratory studies evaluating reproductive potential (Sharlin et al. 1979), evaluating semen diluents (Sexton 1977), detecting disease (Thurston et al. 1975; Stipkovits et al. 1978; Ferrier et al. 1982), and separating species and subspecies through hybridization and sperm morphology (Sharlin et al. 1979; Russman and Harrison 1982).

Male Reproduction

Reproductive Anatomy

Crane anatomy (Fig. 7.2) is very different from mammal reproductive systems. For greater detail, also read Chapter 7. The paired testes lie deep in the body cavity of the crane, above the abdominal air sacs and below the anterior lobe of the kidneys. The vas deferens conduct the sperm from the testis to the cloaca. The vas deferens end in erectile papillae in the urodeum, the central chamber of the cloaca. Semen contains: (1) fluids secreted from the seminiferous tubules, (2) epithelial cells of the reproductive tract, (3) lymph from the lymph folds and erectile tissues in the cloaca, and (4) sperm (Mann 1964; Lake 1966; Buxton and Orcutt 1975; Nishiyama et al. 1976; Servouse et al. 1976; Burt and Chalovich 1978; Gasparska et al. 1981).

Semen Collection

Physiologists classify semen collection techniques according to levels of cooperation: **cooperative**, **massage**, and **electroejaculation**. Massage AI is more successful with cooperation, although stressed or aggressive cranes have also been stimulated to respond. Electroejaculation is successful with or without active cooperation from the bird.

Cooperative semen collection and insemination, pioneered with **sexually imprinted bir ds** of prey (Hamerstrom 1970; Temple 1972; Berry 1972; Grier 1973), requires careful timing to get an adequate number of samples and to get fertile eggs. Methods that intercept semen during natural copulation with other birds or dummy mounting devices are variations of the cooperative collection technique (Smyth 1968; Tan 1980).

The **massage** collection technique (Quinn and Burrows 1936) has been used for decades with domestic poultry and more recently with cranes (Archibald 1974; Gee and Temple 1978; Gee 1983). For this technique, the bird is restrained by an assistant while an operator collects the semen. This technique was first applied to cranes in 1969. The **basic technique** was altered to allow for the crane's long legs, sharp talons, and long beaks. The process generally takes 5 to 10 sec.

The following description will serve as a guide, but must be tailored to the unique behavioral and physical characteristics of each bird. It is important to reduce stress on the individual bird by capturing the desired bird quickly. It is also important for the AI team to attempt to stimulate cooperation when stroking.

An **assistant** , with help from two other members of the team, **captures** the male (if necessary guides him into the nearest corner) and holds him. Team capture reduces chase time and its resultant risk of injury. Adults which come to the gate and attack can often be safely and quickly captured by one person with less stress than if the bird flees. The assistant **cradles the bird** between the legs (Fig. 11A.1) with the bird's head protruding behind the assistant's back. The assistant rhythmically massages the bird's legs by grasping the shanks and stroking gently several times in a circular, inward and downward direction. The speed and pressure should be varied to coordinate with the calling and other responses from the bird.

The second person, the **operator** , kneeling behind the bird and facing the massage person, **strokes** the bird's back with the heel of the open hand toward the head and fingers directed toward the vent. Several strokes are given; each passes along the lower back to the base of the tail. With the other hand, another series of strokes passes from mid abdomen to vent. Both hands reach the tail region at the same time. The bird may respond to this stimulation by pushing forward against the assistant's thighs, emitting a low vocal growl or purr, and raising its tail. The operator then **pushes upon the tail** with the heel of the left hand (if right handed) and strokes the abdominal region with the right hand. Many birds respond to this stimulation by opening or even everting their cloacas. Next, the **cloaca is grasped** dorsally by the thumb and index finger and the semen is expressed.

The operator or a third person holds a small glass collection device (4-5 cm in diameter) in the right hand (if the person is right handed) for semen collection (Fig. 11A.2). Often, a spontaneous ejaculation occurs expelling the first drop of semen onto **the lip** of the glass (a sealed funnel or "shot glass"). Occasionally, the bird lifts its legs off the ground when it ejaculates and must be supported by the assistant's forearms. If the semen sample was small, sometimes the dorsal wall of the vent is again massaged to gently express the

FIG. 11A.1. *Whooping Crane AI: Brian Clauss injects sample as Jane Nicolich massages.* PHOTO DAVID H. ELLIS

FIG. 11A.2. *Collecting semen in a funnel. (Note protruding urodeum.)* PHOTO DAVID H. ELLIS

remainder of the semen which is then scooped onto the lip of the glass. The entire process is normally finished in 5-10 sec. However, some Siberian Cranes require stroking for 2-3 min before obtaining a sample, and some individuals of each species require similarly long massage periods.

Special Semen Collection Tips

Semen collection is something of an art. To avoid excessive stress, perform the task quickly, be relaxed, and talk only to facilitate successful coordination and collection. Semen collection techniques must be modified to each crane's unique anatomical, physiological, or behavioral characteristics. Birds respond differently to individual people. Note responses to the assistant and operator and assign people accordingly.

Semen volume and sperm concentration vary greatly between birds (see Chapter 3), and some cranes produce samples too small for insemination. However, with special care in collection, as little as 0.01 cc of semen can be diluted, drawn up into a 1-cc (TB) syringe and inseminated directly into the female. At ICF and Patuxent, significant numbers of eggs have been fertilized by 0.01-0.02 cc of semen. When semen samples are this small, it is helpful to extend the sample such that the total volume at insemination is approximately 0.1 cc. About 16 million sperm should be provided for an effective insemination (Gee and Sexton 1979).

Small samples can be aspirated into a microliter pipette or collected on the edge of a slide (Howell and Bartholomew 1952; Smyth 1968; Lake 1978; Gee and Sexton 1990). Because small samples dehydrate rapidly, protect them immediately by adding a drop of diluent. Although ejaculates produced by some males contain insufficient sperm to fertilize an egg, a pooled sample from several small collections have been used successfully (McDaniel and Sexton 1977; Gee and Temple 1978). Patuxent has collected semen from Sandhill Cranes daily, Monday through Friday, for extended periods, but we now try to **restrict collections to two to three times per w eek**. Extended periods of daily collections or rough treatment may lead to cloacal tissue damage, stress, and reduced cooperation.

Blood in a semen sample may indicate cloacal injury. Occasionally, the damage is only a superficial scratch on the vent. Avoid collecting semen from a bird with an injury for 7-10 days while the vent heals.

Fecal contamination of semen is common, but can often be reduced by conditioning the birds to a schedule. Although contaminated semen is normally discarded, egg fertilization is still possible if the semen is cleaned of the coarse contaminants and used for insemination immediately. To clean a sample, let the contaminants settle, then draw the semen from the top or side of the sample with the syringe. When using contaminated semen, deposit the sample in the cloaca not the vagina to avoid infections of the oviduct (Perek et al. 1969).

Female Reproduction

Reproductive Anatomy

The one functional ovary lies deep in the body cavity above the abdominal air sacs and below the anterior lobe of the left kidney. The oviduct carries the ovum through the infundibulum, magnum, isthmus, uterus, and vagina (see Fig. 7.3) to the vent in 3 to 4 days (M. Putnam, University of Wisconsin, Madison, personal communication). The infundibular region receives the egg from the ovary and is the site of fertilization (Olsen 1942). The vagina is the passageway for the egg from the uterus to the cloaca and for the semen into the oviduct (Sturkie 1965). Sperm storage sites (sperm host glands) are present in the infundibulum and the uterovaginal (UV) juncture (Bobr et al. 1962). The UV-sperm host glands enable birds to lay several fertile eggs following a single copulation (Smyth 1968).

Investigators have identified **sperm host glands** in domestic and nondomestic birds including cranes (B. C. Wentworth, University of Wisconsin, Madison, personal communication). They may be common to all birds. The host glands are thought to release sperm on a continuous basis (Compton et al. 1977, 1978; Compton and Van Krey 1979a, 1979b; Bakst 1980, 1981). However, some believe the release of spermatozoa is greatest at ovulation or oviposition. Bakst (1980) reported fewer sperm in the oviduct following the passage of an egg suggesting that sperm are sequestered or removed during egg formation.

Near laying time, the ends of the pubic bones become more pliable and spread apart, and the cloacal tissues enlarge and soften. The dorsal lip of the vent expands more than the ventral lip and creates the appearance of an inverted smile. It is possible to

predict when the bird will lay by the size of the enlarging vent and spread of the pubic bones. A history for each bird from previous years helps because some birds expand more than others. We measure the spread at the ends of the pubic bones by passing the fingers between the pubic bones while stroking the abdomen. Hold the palm of the hand against the abdomen and stroke from the abdomen to the base of thetail. In most cranes in winter, the distance between the ends of the pubic bones isless than one finger width.This distance is two or more fingers wide just prior to laying. By palpating pubic spread you can forecast egg laying and choose the best time to inseminate.

Insemination

At Patuxent, female cranes are massaged just as for the males. In the ICF method, the female's back and sides (posterior to the wings) are stroked to simulate the male's abdomen on the female's back during copulation. Semen can be deposited into the cloaca or vagina.

With effective stimulation, the crane opens the cloaca. The vagina, the distal end of the oviduct, appears as a red rosette on the left wall of the urodeum. To expose the vagina, push aside the dorsal wall of the vent separating the urodeum from the proctodeum with the syringe or other device. To avoid injury to the soft cloacal and oviductal tissues, the probing or inseminating device should be smooth, without abrasive edges. With practice the inseminating device can be inserted into the vagina (Fig. 11A.3) during the few seconds it is visible. If the vagina cannot be seen, gently probe with the end of the syringe on the left side of the urodeum. Next, allow the inserted syringe to drop to a relaxed position. When the cloaca contracts around the syringe, stop stroking and gently push the plunger to deposit the semen. Although the female is still being supported, she will frequently relax when stroking stops. Resume stroking gently if she starts to struggle.

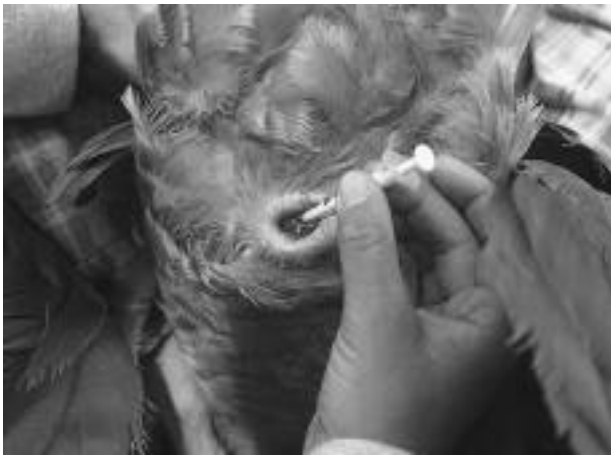


FIG. 11A.3. *Injecting semen into female with syringe.*
PHOTO DAVID H. ELLIS

In properly trained cranes, much of the manipulation of the cloaca can be avoided. At ICF, 12 species of cranes have been successfully inseminated after they assumed copulation posture and everted their oviducts in response to massage stimulation and handling. It is even possible to deposit semen in the oviduct of uncooperative cranes. If you are unable to locate the oviduct by palpation, the distal end of the oviduct can often be everted by placing firm pressure on the female's abdomen and the walls of the cloaca. We do not recommend that an inexperienced person evert the cloaca because force can cause injury and undue stress to the bird.

Placing semen directly in the oviduct has been the **preferred insemination technique** with most nondomestic birds (Smyth 1968; Bird et al. 1976; Boyd et al. 1977), although satisfactory results derive from simply depositing semen in the cloaca (Gee 1969 unpubl.; Temple 1972; Berry 1972; Grier 1973; Archibald 1974). In the Sandhill Crane, fertility rates above 80% were achieved with cloacal insemination. In this program, insemination was begun two to three weeks before the first egg was laid and continued throughout the season with at least two inseminations (each containing about 16×10^6 sperm) each week and within a few hours after each oviposition (Gee and Sexton 1979; Gee et al. 1985).

However, with the same insemination schedule, we achieved **better fertility** when semen was placed in the vagina. Deep vaginal insemination is preferred for other species (Lorenz 1969; Ogasawara and Fuqua 1972) because the storage site (sperm host glands) is in the utero-vaginal juncture (Bobr et al. 1962). Moderate depth vaginal inseminations also give satisfactory results (Smyth 1968; Wentworth et al. 1975; Bird et al. 1976; Boyd et al. 1977) and reduce the possibility of injury (Ogasawara and Fuqua 1972; Wentworth et al. 1975) that can result from forcing the inseminating device to the utero-vaginal juncture. When semen is deposited in the cloaca instead of the oviduct, inseminations should be more frequent and timed to follow oviposition (Gee 1969 unpubl.; Temple 1972; Berry 1972; Grier 1973; Archibald 1974; Gee and Temple 1978).

The cloaca should not contain **feces** when inseminating. When the cloaca is full, the bird will defecate soon after insemination and fecal bacteria can kill large numbers of sperm and reduce fertility. For some females, it may be necessary to withhold feed and water for 6 to 8 hours before AI (Smyth 1968), but normally, herding the bird (male or female) around the pen for a few seconds before capture will induce defecation.

The **volume of semen** that is required to produce a fertile egg depends upon the sperm concentration and the capacity of the reproductive tract to retain the semen. Often, a single semen sample is adequate to inseminate two or more females with the result that a sample may be diluted. However, if the volume of diluted semen exceeds the capacity of the bird's vagina, fertility rates can be reduced because some sperm are, as a result, expelled from the lumen of the vagina. More frequent inseminations are advisable when the number of sperm per insemination is low (Meyer et al. 1980). For example, a single Sandhill Crane ejaculate (200-300 million sperm/mL, 0.05 mL/ejaculate; Gee and Temple 1978) may not contain enough sperm to produce a satisfactory fertility rate. Based on more than 20 years of experience with cranes, we **recommend repeated insemination** every other day for two weeks before the crane lays her first egg, 2-3 times each week after that, and within 1-2 h after every oviposition to get the best fertility.

To get the highest fertility rates, we generally inseminate the entire ejaculate after diluting it 1:1 with a poultry semen extender or an extender modified for use in cranes (Gee et al. 1985). The dilution provides greater volume, thereby reducing the loss of sperm on the sides of collection, handling, and inseminating devices. Most inseminations contain 0.05 to 0.15 mL of good quality semen (see the Semen Protection and Evaluation section that follows). If we determine that a poor semen sample was used, we often return with another sample later in the same day.

Fertility Management

AI is only one of several methods used to correct infertility. AI of cranes is labor-intensive. Because natural copulation in properly mated cranes generally results in fertility equal to that in artificially inseminated birds (Gee 1969 unpubl.), a **change of mates** may prove sufficient to raise fertility and may be more cost effective than AI. Most flighted birds will breed naturally if properly reared with their own species and housed in large, net-covered pens (Ellis et al. 1991). For flight restricted birds, the number of males successfully copulating and the fertility rate are lower than for full-winged birds (Swengel and Archibald 1988; Ellis et al. 1991; Belterman and King 1993).

Other conditions that promote reproduction are treated in Chapters 6 and 7. Of the environmental conditions, light, temperature, and humidity are the three most important. In most cranes, semen production begins before, and continues until after, the end of egg production (see Chapter 3). However, the asynchronous production of semen and eggs does occur in cranes.

AI Training

When circumstances recommend AI, the **cranes should be trained** to accept the technique. Behavioral accommodations are of great importance in artificial insemination of cranes, especially those taken from the wild. **Stress** is difficult to control in crane AI, but can be reduced by using the same team, training birds to accept the procedures, and avoiding injury. Occasionally, the **training process** upsets rather than calms the bird and if continued, may interfere with the onset of egg production. In these cases, stop the training. Reinstate insemination only after the first egg is laid whereupon the bird may be more receptive. Another manipulation that may improve AI response is to place the bird or pair near other reproductively active birds. Visual and auditory displays by neighboring cranes sometimes stimulates reproductive activity (even in single birds).

Semen Protection and Evaluation

The following steps can be taken to **protect the semen** and to use it most effectively. Semen should not be exposed to direct sunlight. Store samples until use in a water bath or insulated container (ca 5° C) to reduce temperature fluctuations. A closed tube reduces dehydration and contamination. A diluent increases semen volume, reduces the risk of dehydration, and if sperm concentration is adequate, makes it possible to inseminate several birds from each ejaculate. A **diluent** was

developed especially for cranes (Gee et al. 1985), but most commercial poultry semen extenders (see Appendix) are adequate. Diluent reduces sperm concentration, bacterial contamination, provides energy, and controls pH and osmolality. All the tubes and **inseminating devices** that contact the semen should be clean and free of detergents. All equipment and supplies should be thoroughly rinsed with clean water before use. For reviews of factors harmful to sperm survival, see Mann (1964), Lake and Steward (1978), Lake (1969), and Smyth (1968).

Although the most **reliable semen test** is the production of **fertile eggs**, semen for use in AI can be evaluated immediately upon collection and later in the laboratory (see below). The **color** of good crane semen ranges from clear to milky white. **Fecal contamination** discolors the semen to shades of brown or green. Occasionally, flecks of blood may be present resulting from excessive force during collection or injury (Smyth 1968). Samples consistently contaminated with feces may need to be diluted with antibiotics to reduce the loss of sperm. The antibiotic, tobramycin, may even increase fertility when used as a diluent in "clean" semen (Sexton et al. 1980). Good crane semen is only **slightly thicker than water** . Samples that appear to be sticky or stringy are often contaminated with urates. Sometimes semen samples begin as a clear fluid in the collecting device and turn white as the urates precipitate out. **Watery semen** may result from collecting too much lymph in the sample because excessive force was used on the cloaca during collection. These watery fluids, like fecal and urate contaminants, adversely affect spermatozoa, especially if you hold the semen for some time before insemination (Smyth 1968; Lake 1971; Fujihara and Nishiyama 1976).

In the laboratory, samples of a crane's semen are examined for sperm number, motility, and morphology (Fig. 11A.4). A more extensive examination (i.e., an evaluation of metabolic rate and semen chemical composition) may be needed in special cases. The simplest **measures of semen quality** are sperm number and motility. Gross testing of semen quality is discussed later in this chapter.

Sperm number can be estimated from a semen score for density, a spermatocrit, or by counting in a hemocytometer or in an automated counter. Sperm concentration can be evaluated on a hanging drop slide, under a cover slip on a slide, or in a capillary tube (Putnam 1982). The scores can be calibrated by comparing them to actual sperm counts. The spermatocrit, a simple measure of sperm concentration, is useful in characterizing semen produced in quantity (>0.1 mL) and containing many sperm per mL ($>3x10^9$) (Arscott and Kuhns 1969). The semen sample is loaded into the standard microhematocrit capillary and centrifuged. To count sperm, the semen is diluted (if necessary), fixed, and the sperm counted in a hemocytometer or in an automated counter (Jones and Wilson 1967). Optical density of a diluted semen sample can also be measured and sperm number determined from an established standard curve (Kosin and Wheeler 1956; Carson et al. 1955).

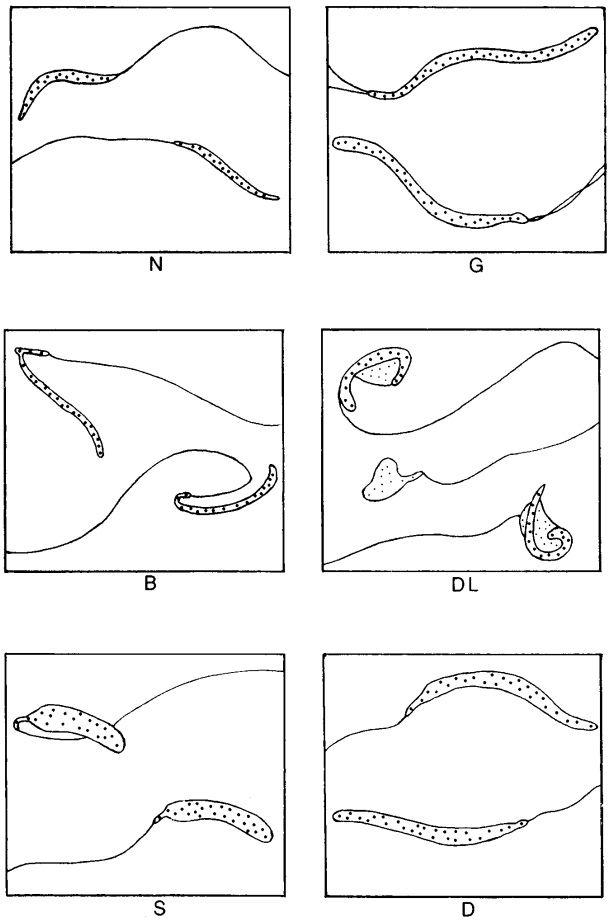


FIG. 11A.4. *Spermatozoa morphology, Greater Sandhill Crane: N, Normal; B, bent; S, swollen; G, giant; DL, droplet; D, dead. After Gee and Temple 1978.*

Sperm **progressive motility**, discussed in greater detail later, is an estimate of the percent of spermatozoa moving forward. Because some live cells are inactive, this measure is not an estimate of the percentage of live cells.

Sperm morphology can also provide information about the percentage of live cells in the semen as well as the frequency abnormalities and the size of cells.

One of the easiest determinations is a **live-dead count** from an eosin-nigrosin stained slide (Gee and Sexton 1979). Although this procedure is more time-consuming than progressive motility, it can be performed long after the insemination, usually without a loss in accuracy. However, excessive moisture in the atmosphere can make staining less definitive (Ogasawara et al. 1976).

Determine **abnormalities** from a variety of preparations including the eosin-nigrosin stained slide. Good slide staining techniques aid in delineating parts of the spermatozoa such as the head from the acrosomal cap and mid-piece (Sharlin et al. 1979; Russman and Harrison 1982). Abnormalities in sperm help in evaluating semen from the males and in determining effects of diluents and storage. Sperm head size as determined from properly stained slides can also help distinguish subspecies (Sharlin et al. 1979; Russman and Harrison 1982) and predict fecundity (Sharlin et al. 1979). Electron microscopy can also be useful in detecting membrane and fine structure abnormalities in spermatozoa.

Although laboratory tests are useful in evaluating semen, satisfactory fertility rates have come from semen that scored poorly in the laboratory, especially frozen-thawed semen (Sexton 1976). Cryoprotectants and freezing can affect sperm motility and morphology without destroying the ability of the frozen-thawed semen to produce fertile eggs.

Short-term Semen Storage

Although the best fertility rates come from using semen immediately following collection, crane semen can be stored for several hours without significantly reducing fertility. **Semen storage** for an hour or more calls for temperature control and protection from contamination and drying. Sperm of most species survive best at near freezing temperatures (Gee and Temple 1978; Sexton 1979). We use a wide-mouth thermos, ice water, and a submerged dry container for samples. Because bacterial contamination can rapidly destroy sperm cells, avoid contamination or use diluents to introduce antibacterial agents, stabilize pH and osmolality, and in other ways extend the life of a semen sample (Smyth 1968; Gee and Temple 1978; Sexton et al. 1980). Semen pH and osmolality vary from species to species. Semen pH ranges from 6.0 for a duck to 8.0 for a crane.

Any of the **semen extenders** used for domestic poultry are adequate for short-term storage (Lake and Steward 1978, Ogasawara and Ernst 1970, Sexton 1977, 1978). The Beltsville Poultry Semen Extender has been adapted for dilution of Sandhill and Whooping Crane semen (Gee et al. 1985, see Table 11A.1) by raising the pH to 7.8. You can use this extender for fresh storage and for freezing (see Chapter 11B). Semen from each crane species has a characteristic pH and osmolality (Gale 1987). Extender pH and osmolality should be tailored to the species for long term storage (fresh or frozen). By adapting **sperm preservation** techniques to a species, semen can be kept in the frozen state indefinitely (Sexton and Gee 1978; Watanabe and Terada 1980; Watanabe et al. 1981).

TABLE 11A.1

Crane Semen Extender

1000 mL distilled water
5.0 g D-fructose (MW = 180.16)
0.34 g magnesium chloride ($MgCl_2$) (FW = 203.32)
0.65 g potassium phosphate (monobasic) (FW = 136.1)
12.7 g potassium phosphate (dibasic) (FW = 228.2)
0.64 g citric acid (MW = 306.4)
1.95 g N-[2-hydroxyl-1,1-bis(hydroxy-methyl)ethyl] taurine (MW = 229.25)
8.67 g L-glutamic acid (anhydrous) (MW = 169.1)
4.26 g sodium acetate (MW = 136.085)
Adjust pH to 7.8 with sodium hydroxide. Adjust to 310 mosM with distilled water.

Equipment, Facilities, and Supplies

The **basic equipment** used in AI is simple and inexpensive (Corten 1973, see Appendix). The record book is most important (see Chapter 10) and must be kept for comparisons during the year and from year to year. Devices used to collect semen include glass or plastic cups, "shot glasses," sealed funnels, syringes, test tubes, capillary tubes, and pipettes (Smyth 1968). A thermometer is needed for the semen storage case. Inseminating equipment includes syringes, needleless syringe caps, pipettes, straws, or eye droppers and

devices to hold or add diluents. Chaps should be worn by the massage person to help prevent injury from the crane's bill and talons. Goggles should be worn by all members of the AI team. In the AI kit (see Table 11A.2), put the AI supplies on one side of the kit and provide a separate area for first aid supplies to treat minor abrasions.

Crane facilities should be free from obstructions to reduce the chances of injury and to facilitate a quick, less stressful capture. A cloth or tennis netted corner (capture corner) in the pen reduces abrasion to the crane's wings during handling for AI. Also, clean facilities reduce the risk of semen contamination and soiling of the bird during capture.

Laboratory equipment, including a microscope, is needed for the routine examination of semen. Progressive motility estimates require only a clean slide and cover slip, but a hanging drop slide is useful for lengthy microscopic studies of living samples. It is desirable, but not necessary, to do additional evaluations of semen. A variety of stains are required if you are to do live-dead counts or special morphological studies. A balance with 0.01 g accuracy is needed to weigh out chemicals and to prepare stains and other supplies. Other supporting pieces of equipment and supplies include cell counters, slide trays, and photographic attachments. Also needed are pH paper or a pH meter, and to determine osmolality from small samples, a vapor point osmometer. Respirometers or spectrophotometers are needed to test semen metabolic activity.

TABLE 11A.2

AI Kit

AI funnels
1 cc syringes
semen extender
square gauze pads
roll gauze
adhesive tape
vet wrap
critoseal
small labels
capillary tubes
ruler
pencils
"sharpie" (indelible) marker

AI Program: Preparations for the AI Season

AI is labor intensive and calls for **planned and coordinated activities**. Form your **AI crew** a few weeks before the AI season to establish work schedules and to train personnel. Because birds respond to different people in different ways, choose people based on the bird's response to them. To avoid disturbance, maintain an established routine (same sequence each day, same time, with the same people) and move quietly with the least disturbance to the birds.

Write a short **reproductive history** for each bird that includes typical behavior, health, lay dates, semen characteristics, and fertility with AI. Also, prepare the proper **records** (AI book, species egg logs, dam reproductive records, and egg cards) before the season starts (see Chapter 10).

The AI Routine

Gradually introduce each pair to the collection and insemination routine. For the first two days (i.e., Monday and Wednesday), acclimate the birds to the crew by merely capturing, handling, and releasing them. On the third day (Friday), start stimulation and try to collect semen samples on every AI day thereafter. Begin insemination when the female is ready (i.e., responsive to handling, shows a widening in the distance between pubic bones, exhibits cloacal expansion, begins nest building, performs certain social displays such as Bill-down and Bill-down-growl, and becomes more aggressive). In a few birds the process may take longer. Do not proceed if the bird does not respond to stimulation. Use the crane's reproductive history as a guide and try to complete three inseminations before the first egg.

Males should be **rated** on a scale of 0 to 4 for their response to AI. We use the following score:

- 0 = No positive response to AI. Bird struggles and shows no sign of stimulation.
- 1 = Bird relaxes briefly but struggles most of the time.
- 2 = Neutral to slightly positive response. Doesn't struggle. Raises tail.
- 3 = Bird is relaxed. Raises tail. Everts cloaca.
- 4 = Responds strongly by raising tail, everting cloaca, vocalizes during massage. May climax.

The **female's** response to massage is **scored** as for the male. It is very important to measure the **pubic spread** prior to massage and insemination; otherwise semen could be expelled from the cloaca while taking the measurements. To measure the pubic spread, stroke the base of the tail with one hand, and with the other hand massage the lower abdomen below the vent in an upward motion. The spread of the pubic bones and the size of the cloaca is measured by finger widths. A pubic score of 2 means two fingers can fit between the pubic bones. Convert finger widths to mm after leaving the pen. A few days before laying, the pubic bones spread significantly (e.g., a female may change from 2 to 3.5 fingers) and the lips of the vent enlarge. Use the bird's previous records as a guide to condition and to help forecast egg laying.

EachAIday fill anadequatenumber of **syringes** before startingAIwith 0.05 mLextender (ortheaveragesemen volumecollectedfrom yourbirds). Fill a few syringes with 0.02 mLextender tobe readyforthefew smallejaculatescollected. In analternative method, collectthesemen, addanequalamount(adroportwo) ofextender, drawthedilutedsemenintotheinseminatingsyringe,andthenproceedwiththeinsemination.

To **evaluate** a sample of **semen**, draw a small part of the semen (about 5 uL) from the collecting funnel into a microcapillary tube (tube). Move the sample away from the tip of the tube by gently tapping the far end, then seal both ends with a putty ("Critoseal" or equivalent). Label the sample with pen number and place it in a small thermos (filled with ice and maintained between 0° and 5° C). Draw the rest of the semen into a 1 cc tuberculin syringe (Fig. 11A.5) already loaded with an equal volume of semen extender for intravaginal insemination. If the sample is very small, add enough extender to bring the volume up to 0.05-0.1 cc. An alternate method used by ICF is to use a microscope slide to examine the sample remaining in the syringe after insemination.

When semen is too contaminated to use, or the sample is too small, inseminate the female later in the day or the next day with the same donor. If paternity is of concern, it is better not to inseminate than to use another donor.

If a **contaminated semen sample** is to be used, draw the clean portion into the syringe, but use it only for cloacal, not vaginal, insemination. Large semen samples (0.08 mL and above) with good sperm concentration can be split and used to inseminate two females. Do not inseminate when a hard shelled egg is present in the oviduct.

Fig. 11A.5. *Semen is drawn from the collecting funnel into a syringe.* Photo David H. Ellis.

Once you collect a female's first egg of the season, visually **check for eggs** at least twice daily, 0800 and 1600 h. During the laying season, check for eggs at 0700, 1200, and 1600 h, and during peak season, add another check later in the evening. When you expect a crane to lay an egg (calculated or felt in abdomen), make checks of that pen every few hours, so you can inseminate immediately after laying. **Insemination** soon after oviposition produces higher fertility of the next eggs. Schedule an AI team on holidays and odd hours to enable timely response. If the birds are multiple clutched (see Chapter 3), the interval between eggs increases, and checks twice each day are generally adequate.

Reproduction stresses birds, and AI compounds the problem. Some medical practices can help. Keep your breeding birds healthy. Put **padded protectors** on the leading edge of the wrist (see Chapter 8) for birds that have a tendency to scrape their wrists on the fence during capture. Report sick or injured birds to the

veterinary staff for evaluation and treatment. During handling for AI, inspect the cloaca for soreness, inflammation, and infection. Stop AI for a week or more when such conditions appear. When a sick bird has been handled, avoid spreading the infection to other birds.

Details of Semen Evaluation

At the lab, **examine the small semen** samples collected in capillary tubes or from the tips of the labeled TB syringes used for insemination earlier in the day. Remove the tube or syringe from the thermos and score the sample within two minutes after placing it under the 10x objective of the microscope. For a more detailed view, the contents from the tip of the syringe can be plunged onto a slide with a coverslip and viewed in the microscope field at 430×. Focus up and down at a point near the meniscus to find the sperm.

First, estimate **progressive motility** (percent of spermatozoa moving forward) and record in AI book. Score as follows:

0 = no motile sperm
1 = less than 25% motile
2 = 25% to 49% motile
3 = 50% to 74% motile
4 = more than 75% motile

Second, estimate **sperm concentration** (Fig. 11A.6) on a score of 0 to 4 and record in AI book. Score as follows:

0 = no sperm
1 = few sperm with large empty spaces
2 = many sperm with moderate spacing between them
3 = sperm numerous with little space between them
4 = packed sperm, hard to detect single sperm

Use the photographs of typical concentration scores (Fig. 11A.6) and display them above the microscope for comparison to help standardize scores between individuals and across years. In ICF's method, remember to **multiply** the sperm concentration seen in your field of view by your dilution factor. For example, if you extended the sperm sample 1:1, thus creating twice as much volume, you would multiply the sperm concentration by 2.

For males producing semen for the first time, in addition to the routine semen scores, make three complete **laboratory examinations** (early, mid, and late in the season) from an eosin-nigrosin stained slide. To prepare an **eosin-nigrosin (live-dead) slide**, place one

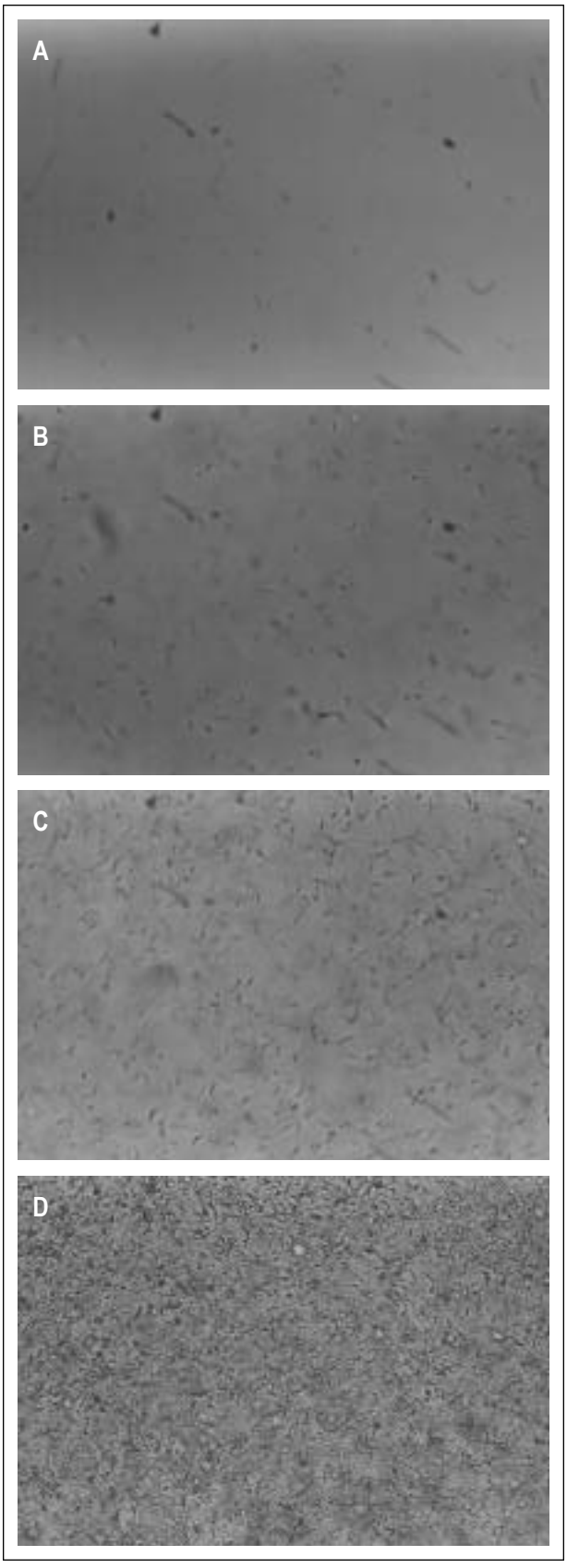


FIG. 11A.6. *Crane sperm concentration scores: A (15x) = 1, B (15x) = 2, C (10x) = 3, D (5x) = 4.*

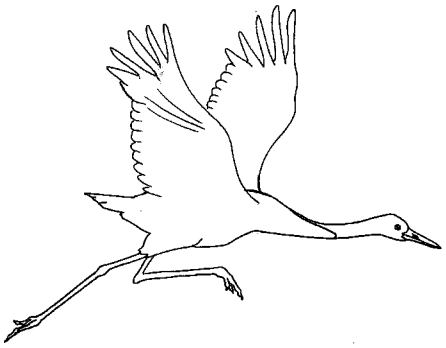
drop of 5% eosin (by weight) on the upper right hand corner of the slide. Place 3-4 drops of 10% nigrosin (by weight) directly below the eosin. Break off each end of the capillary tube and place one drop of semen from the tube on the slide close to but to the left of the eosin. Using a small glass rod, mix the eosin and semen, and then after 10 seconds, mix both with the nigrosin.

Place the end of another slide over the eosin-nigrosin stained semen sample at a 45° angle to the sample slide. Draw the upper slide toward the sample until the sample spreads along the underside of the upper slide. Next, push the upper slide away from the sample, along the surface of the lower slide, dragging out a thin uniform smear on the surface of the lower slide. Dry the slide quickly by waving in the air or, in damp weather, by exposing it to a warm air current.

For a permanent specimen, apply mounting medium when dry and mount a cover slip. You can view the slide under the microscope then or later when the mounting medium has dried. Count **clear cells as live** and **red or purple cells as dead** . Also, separate the live cells into five other categories: normal, bent, swollen, giant and droplet and compare them to normals (Fig. 11A.4) for other birds in your collection (Sharlin 1976; Gee and Temple 1978; Russman 1981; Sharlin et al. 1979).

AI As a Tool

First used in cranes in 1969, AI is now a practical propagation tool. The technique combines cooperative and massage methods to increase semen yield and egg fertility. Although semen charaacteristics are useful indicators of semen quality, the most reliable test is fertility rate.



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