

# Psittacine Artificial Insemination

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## Introduction

Ideally, all captive birds will be able to reproduce by natural means. There are instances where it may be necessary, or at least desirable, to use artificial assistance. The Raptor Rehabilitation Propagation Project, Inc. maintains 32 different species of birds of prey and a wide variety of psittacine species. Some of these birds are being utilized in an artificial insemination study. This article deals with some of the successes of our program as well as some of the needs we have observed.

Semen collection and subsequent insemination is routinely used in the domestic poultry industry, particularly with turkeys. The original work with artificial insemination in poultry was reported by Quinn and Burroughs (1936). Other techniques have been developed over the years, but most are based on that original procedure. The subject of artificial insemination of non-domestic avian species has been well reviewed (Gee and Temple, 1978). Many avian species have been successfully produced by artificial means. Those mentioned in the above review include ducks, geese, pigeons, doves, cranes, peafowl, hawks, falcons, eagles, curassows and turkeys. There are no psittacine species mentioned and as one checks the available literature, no reports of successful psittacine artificial insemination have been found.

There are several facets of avian artificial insemination that warrant investigation. These include semen collection, short term semen storage, long term semen storage, and insemination of the female.

## Semen Collection

### Massage Techniques

As stated, massage techniques utilize adaptations of the procedure developed by Quinn and Burroughs (1936). At our project, several Maroon-bellied Conure (*Pyrrhura frontalis*) and Monk Parakeet (*Myiopsitta monachus*) males have been used to develop a manual method of semen collection. Non-imprinted,

unhandled birds were selected and then sexed with a laproscope.

Our semen collection technique is an adaptation of one previously published (Irwin, et al., 1986) and requires two persons — one to hold the bird and the other to actually stimulate the bird and collect the semen. The holder places and holds the bird in the left hand, with the head of the bird toward the holder. The right hand is placed below the left hand and holds the feet. The holder may choose to hold the bird with one hand, depending on hand size and bird size. Of course, it is necessary for the holder to wear proper gloves.

Stimulation involves stroking the back to the tail with the thumb and index finger of the right hand. After several strokes (eight to ten usually), the tail is pushed back dorsally with the right hand. Semen is then obtained by gently squeezing around the vent with the thumb and index finger of the right hand. Be careful not to injure the bird by using excessive pressure. The male should respond by engorging the tissues of the cloaca causing it to evert. The semen typically appears as a minute droplet appearing to little more than moisten the cloacal tissues. The semen is immediately collected into a 20 ul or smaller blood capillary tube. Semen volumes are typically 1 to 2 ul and it is nearly clear, although some samples have a slight golden color. Because of this small volume, it has been desirable to point the tail of the bird toward a light source, such as a surgical lamp, for better visibility. Microscopic examination of the semen will indicate if spermatozoa are present, although the concentration may vary with each collection and between males.

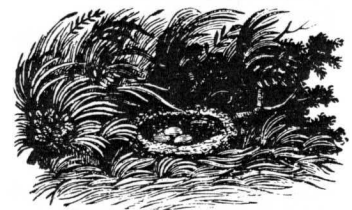
Semen samples that we have collected have the typical avian shape. Avian sperm cells usually have long, narrow head and midpieces. They also have very long tails. However, under normal magnification, the tails may not be visible.

One should be able to collect

semen on a daily basis; in fact, it is best to attempt daily collections to help condition the bird as a semen donor. If a male bleeds as a result of excessive pressure, he should be rested for three days before collections are resumed. Then reduce the amount of pressure used.

## Cooperative Semen Collection

Cooperative semen collection seems to be an area that merits exploration for psittacine breeding. Cooperative semen collection typically involves birds that are sexually imprinted on one or more humans. Such birds choose their human caretaker as their partner and will choose to "mate" with that partner, which simply means that they voluntarily ejaculate semen on the hand, head, shoulder or back of the person. Semen produced from cooperative donors is typically of better quality and volume than those samples collected by the massage method. Many instances of cooperative semen collections from non-domestic birds are found in the literature: Red-tailed



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Hawk, Temple (1972); Goshawks, Berry (1972); Golden Eagles, Grier (1973); and Prairie Falcons, Boyd and Boyd (1976).

Of course, the phenomenon of sexual imprinting is common in psittacine species. Imprinting most often occurs in birds that have been hand reared from hatching, in isolation from conspecifics. No references to cooperative semen collections with psittacines have been found in the literature. But, the possibility of locating cooperative semen donors seems to have real promise for psittacines since so many birds are hand reared.

### Short Term Semen Storage

In most instances, it is desirable to inseminate the female as soon as the semen has been collected from the male. However, there will be other instances when it is necessary to store semen for a short time. Short term storage should allow breeders to hold semen for at least two days. This will allow time for shipping from one breeding facility to another. Also, semen can be collected on two or three successive days and then pooled for one insemination. This increases the number of sperm cells that will be in the semen sample which, in turn, should improve the insemination success rate.

If the semen is to be stored for more than an hour or up to a few days, it will be necessary to extend the semen with a diluent. There are several diluents available. They contain the correct balance of electrolytes and nutrients for the sperm to survive. They also contain buffers to maintain the proper pH. The available diluents have been developed by the commercial poultry industry and include: Beltsville Poultry Semen Extender, Lakes Diluent, and Minnesota Turkey Growers Association extender (with and without Gentamycin). These have been tested in our laboratory and been found suitable for non-domestic species semen storage. We have found that best viability is maintained when the semen is stored at 5°C which is near the typical refrigerator temperature.

### Long Term Semen Storage

The prospect of long term or indefinite storage of bird semen is on the horizon. The technique has been widely used with many mammalian species and provides fertility equivalent to natural breeding. Chicken semen has also been frozen and

stored under liquid nitrogen (Lake et al., 1981). However, with avian species, fertility has not been as good as that obtained with fresh semen. Although it is known that fertility is not as high with frozen semen, there is still great research interest in the subject because of its potential.

In order to freeze semen from any species, a cryoprotectant must be added to the semen. In mammals, glycerol based cryoprotectants are commonly used. However, in the bird, glycerol has a contraceptive action and must, therefore, be removed prior to insemination (Lake et al., 1980). With the small semen volumes that are typical of most non-domestic avian species, separation of the glycerol from the semen does not appear to be practical. Therefore, other cryoprotectants have been studied (Lake and Ravie, 1984 and Brock, 1985).

One of these, containing Dimethylacetamide (DMA) has been tested in our laboratories with chickens, Bald Eagles, Harris Hawks, Peregrine Falcons and Red-tailed Hawks. Samples have been diluted with the DMA cryoprotectant and frozen in a programmable freezer as per Lake and Ravie, 1984.

Frozen samples are thawed at 0.5°C for seven minutes as per Duplaix and Sexton, 1984. Upon microscopic examination, some motility has remained in some samples. Research in this area is still in its infancy, but freezing bird semen may be a tool for breeders of the future.

### Insemination of the Female

The technique used to inseminate the female will be either cooperative or manual. This, like semen collection, will depend upon the bird to be inseminated.

#### Cooperative Insemination

Cooperative insemination may be possible for those breeders who have a sexually imprinted female. Such a female may solicit and posture for the human partner. The person may then place a light pressure on the bird's back and raise the tail so that the person can deposit semen from a small syringe or medicine dropper. Cooperative inseminations are very easy and under ideal conditions, should result in excellent fertility.

#### Manual Insemination

Unfortunately, many captive females will not cooperate to allow an insemination. Therefore, a more forcible procedure may be necessary.

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Before attempting a forced insemination, be aware of the following: 1) The female must be sexually mature and ready to lay eggs, 2) The female should not have a hard shelled egg in her oviduct.

It is simply impossible to inseminate a female that is not in egg-laying condition. In order for the insemination to occur properly, the female will have to evert her oviduct and if she is not laying, she cannot do so. Attempting to force a non-laying female to evert may injure her and could render her sterile or useless for future attempts. Many breeders wait until the first egg of a clutch has been laid before an insemination is attempted.

If the female has a hard shelled egg in her oviduct and she is handled excessively, there is a distinct danger that the egg may be cracked or broken, injuring the female in the process. This is likely to occur during a forced insemination because pressure must be applied to the abdominal area in order to evert the oviduct. Many persons will be able to tell that an egg is present in their female simply by the distended appearance of her abdominal area. In other cases, it may be necessary to handle the bird. The hard shelled egg is easily felt by gently laying the fingers flat on her abdomen. Breeders are also cautioned to remember that the tissues of the oviduct are extremely tender and easily punctured.

If a forcible insemination is deemed necessary, two persons will need to work together. One will hold the bird and evert the oviduct and the other will inseminate her. In most smaller species, eversion is accomplished by the holder applying pressure to the abdominal area beneath the vent with the separated fingers of the left hand. Pressure is also exerted to the areas to the sides of the vent with the fingers of the right hand from above. When the oviduct has been everted, the semen is then deposited as described in the previous section. The entire process is typically very quick, with the task being completed in less than one minute. Prolonged handling at this time may cause excessive stress, causing the female to absorb the ova and not lay again. The female is also much more easily injured at this time.

One good insemination should ensure fertility for seven to ten days. In most situations with non-domestic species, where semen quality and volume are poor and where there are

doubts as to the insemination success, it is often desirable to inseminate more often — every third day, or four to twelve hours after each egg in the clutch is laid.

### Conclusion

We are not attempting to say we are experts on the subject of psittacine breeding. We have had limited success with semen collection from two psittacine species. We also have wide ranges of experience and success relating to both domestic and non-domestic avian species. We feel this gives us the perspective to see numerous opportunities and needs for research in the psittacine area.

The possibilities of storing semen from numerous psittacine species, especially endangered species, could allow us to create captive gene pools for future breeding projects. With more and more species becoming threatened and endangered, it makes good sense to continue research into this area of captive breeding.

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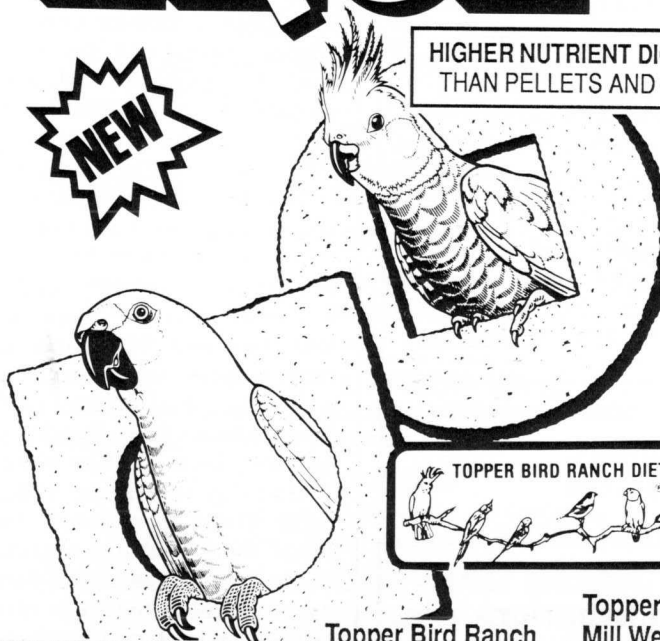
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