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## SPECIAL CONFERENCE ON CAPTIVITY BREEDING OF RAPTORS—A REPORT

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### Part F. Artificial Insemination (Panel 7)

edited by

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*Panel Members:* Robert B. Berry, Chairman, Frances Hamerstrom, Walter Morgan, Stanley A. Temple.

HUNTER. If there are no more questions I think perhaps we can move on to the next thing on our program which is artificial insemination. One of our guests who is here to give us a demonstration has a limited amount of time so we will proceed to that. Now, Bob Berry.

BERRY. I'll be the chairman for this artificial insemination panel discussion. I want to pass out some papers that I have prepared. For those of you who don't get them, you might get them from someone else or write to me and I will send you a copy. Now, if Bill Halliwell is ready with the slide projector.

HALLIWELL. Yes, I'll turn it on for you.

BERRY. Well, I'm not quite ready. I want to say that what we hope to cover here basically is the two techniques of artificial insemination. Both Stan Temple and Dr. Walter Morgan from State University of South Dakota, will demonstrate the massage technique on chickens. I will talk about cooperative insemination in the American Goshawk, a technique that does not require massage or manipulation of the gonads. Fran Hamerstrom is also on the committee and she will discuss the techniques that she has applied to the Golden Eagle. Perhaps the easiest way for me to show you or explain the technique of cooperative insemination is to show you some very few slides. If we can have the lights now we

can begin.

Before there can be any cooperative insemination there must be a pair bond established between the researcher and the experimental subject, in this case a female Goshawk which was raised in captivity as a household pet. She is four years old at this time, and she is accepting a stick from me in probably the same fashion as she would accept sticks or food items from a male Goshawk. She builds her own nest. She fulfills all the requirements for a natural mating except, of course, she does not have a natural mate. All during this nest building stage and during the period of copulation which precedes the eggs, incidentally, by about three weeks, she is extremely defensive about other people, other human beings than myself, and against conspecifics. In this case the male Goshawk, which happens to be housed in an adjoining chamber and is also what we can refer to as a conditioned or imprinted eyass bird. The male also builds a nest and carries out at least the prenesting cycle. I have never given him youngsters to raise. But I presume he would probably incubate eggs in the same fashion as the female. The male was conditioned to ejaculate semen voluntarily. I started in February to condition the male. He was four years old and had never displayed any indication of sexual response. There was no display in previous years, no chirping, and no territorial aggression. I merely started feeding him from the fist. He was a typical eyass Goshawk. He mantled violently and he attempted to escape with the food. Within two weeks he stopped mantling and within another week he started chirping towards me. Within a month when I gave him the food he would hop off two or three feet, and he would hop back to me and give the food back. He would step off, look at it and chirp. He would stick his tail up in the air and chirp some more. So I knew that he was coming into breeding vigor. When I approached the cage, his tail would be straight up in the air as I think you will see in another picture and he was chirping violently—all the time being completely and totally hostile towards the female. The female was doing the same thing at the same time in the adjoining pen.

VOICE. They could see one another, is that right?

BERRY. They could see one another during the 1970 year. This was duplicated during the second year with no visual contact between the two birds, and they behaved precisely the same at the precise same time of year. They displayed vigorously. He was obviously very excited. One day I was in there feeding him in the early evening which was my general practice and I put a paper bag full of food on the ground next to me and I went to feed the female. He came down and attempted to copulate with the paper bag. That evening I was devising all sorts of artificial female Goshawks in my mind, trying to figure out how the devil to get semen from him. I didn't want to handle him physically because one of the very important aspects of maturation of the gonads is the absence of stress. I feel that if you grab a bird you are going to have one chance and only one chance to take semen. So I was trying to devise a technique whereby semen could be taken without shaking him up. These are actual photographs of his first clumsy attempt to copulate with me as the mate. This is the bird landing

on my leg. Notice how his feet are balled up so that they do not damage the mate. He is calling violently. He kind of works his way down to my shoe. This is a trifle bizzare, but it works [laughter]. Now he is down on the shoe. He is just about to copulate with the shoe as you can see. This is actual copulation. The wings are down. He is on his tarsi, he is straddling the shoe. And his abdomen is revolving in a circular fashion and pressed down against the shoe. This forces the papilla to penetrate the panel feathers, the papilla being the rudimentary copulating organ in a bird of prey. They have no structure comparable to the mammalian penis. So this papilla is forced down against the shoe which of course would be pressed against the female's cloaca or the everted oviduct in natural mating. This is immediately after—I guess this is a withdrawal process, a sort of post-copulating depressive mood [laughter]. It wasn't very many days until I devised a workable technique and sort of mutually agreed that my hand would be the desired copulating object. I would go in to his chamber, place my hand and fingers on this block which was his feeding block at about a 45 degree angle and he would fly to the block, placing one foot on one side of my hand and the other foot on the other side and copulate with the back of my hand.

VOICE. Facing your arm?

BERRY. Facing my arm. Exactly the way it occurred right here. He literally works his way right up on the hand, the papilla penetrating the panel feathers. I guess it's about a quarter of an inch long. It's merely a lymph fold. I imagine Stan could probably fill you in on this.

VOICE. Is it just blood that distends it?

TEMPLE. It develops during the breeding season. Not blood.

BERRY. But it has some erectile tissue in it. It is not very much. It is quite short. But anyway this lodges between the fingers, and he ejaculates. The semen is collected from the fingers in a tuberculine syringe and taken to the female. Maybe you can see it better in this picture, a better idea of the way the feet are balled up and he gets down on his tarsi and so forth. During the 1970-71 season we went through this routine 117 times. We only got semen 15 times during this whole process. There are many, many, many dry runs preceding a one-week period during which you get most of your semen. And the week period corresponds precisely to the egg laying period of the female. Collectable amounts of semen were only secured three times during the 1970-71 season. Three times for each season.

VOICE. How much is collectable, cc-wise?

BERRY. Collectable semen amounted to about 0.02 cc which is equivalent to about a good sized drop of water. Very, very small amounts were ejaculated. However, examination with a microscope disclosed the semen was very concen-

trated and very motile. When I approached the female Goshawk, she assumed a precopulatory posture. This is taken with a telephoto lens. When I was 40 or 50 yards away the female would become quite excited and fly to the door of the pen. She assumed this position with her head down and her wings out a little bit. The only physical part of this whole routine by me is the stroking of the female on the back which stimulates her to spread her panel feathers and expose the cloaca. Actually right there I am not really working with the cloaca, I am palpating the bird to find out just where the eggs really are in the bird. You can feel an egg as the abdomen extends. It becomes enlarged about two to three days before an egg is laid. You know when that egg is coming. For maximum fertilization, insemination should be 36 to 48 hours preceding that first egg. This photo illustrates the minimal stimulation on her back. You notice the tail is going the other direction and unfortunately the wing in this particular picture is drooped down to support the conspecific male, obscuring the division in the panel feathers. She is actually exposing her cloaca. She just wouldn't tolerate anybody else around when this whole thing was going on; I can understand that. Generally I would do this with the left hand, the minimal amount of stroking of the back, then I would grasp the tail in the left hand and lift it up, exposing the cloaca. In this picture there is no eversion of the oviduct. In the year 1970 there was no eversion of the oviduct. The syringe was placed in the upper left hand portion of the cloaca and inserted about  $\frac{3}{4}$  inch. The left side corresponds to the functional left oviduct in a raptor. In the 1971 year the cloaca turned inside out on the bottom exposing the small orifice of the oviduct. In the 1971 year the semen was placed about  $\frac{1}{2}$  inch into the actual oviduct. It didn't seem to make any difference because there were four fertile eggs in 1970 and three fertile eggs in 1971, 100% fertility in both years.

THACKER. Bob, when you took the semen specimen, did you immediately run into the next pen and implant it?

BERRY. Immediately. The semen was immediately transferred and was not diluted. There is always residue. If you collect perhaps .03 cc, there is always some residue in the syringe which was mixed with physiological saline and given to the bird about 15 to 20 minutes after the original insemination.

VOICE. Did you give it three times in a row then for three days?

BERRY. No, if you got one of those papers, it shows that the maximum, the optimum insemination occurs a day or two before each of the eggs. With probably any one of those inseminations the whole clutch would have been fertilized.

THACKER. Did you do any motility work on the semen, any work like that?

BERRY. Yes, Motility with undiluted semen did not seem drastically altered after three hours at room temperatures. After six hours motility obviously de-

creased and perhaps half the sperm no longer moved. At nine hours, 90% of the sperm were immobile and the rest were not very active.

TEMPLE. I have got a lot of data on that.

BERRY. OK. Let Stan worry about the motility. This is the first chick hatching. The chicks were hatched in an incubator and given back to the adult female to raise. At just one day of age here is the huge Goshawk feeding the tiny little chick. She was allowed to incubate one of the eggs for 31 days. I gave her this chick and took the egg away from her. The next chick was hatching three days later and she was given the second chick, and six days after that the third chick hatched. I allowed the third egg to remain in my study for several days hoping to get her to recycle. So perhaps a rather bizarre technique. It's something that you certainly can't practice with the ordinary raptor. But I do believe it can be practiced with most any imprinted raptor that is properly conditioned to accept you as mate. And I think probably the only requirement for this is total and complete isolation from other raptors and of course a lot of communication between you and the bird during the normal process of maturation of the gonads.

Stan Temple, would you present the massage technique of artificial insemination?

TEMPLE. I think I'll comment a little on my work with cooperative insemination first. This was done with Red-tailed Hawks. It's the same basic type of procedure—there's not much you can vary in the procedure. I would comment on this imprinting technique. Imprinting in birds is accomplished very early in their life. And when we use "imprinting," we hear that the bird has become fixed on some object during its early development in the nest; and it will come to regard that object as a conspecific, something it should associate with. We do this—falconers do this all the time—when we take a young eyass from the nest and handraise it. The bird sees you and thinks of you as its parent. It becomes imprinted to you, it thinks that you are the type of object that it should regard as a parent image. The next thing, when a bird has regarded you as a parent image—as long as they are kept away from other members of their same species—the next step in this progression is for them to assume that you are a social partner, and falconers do this by hunting with their birds, by manning them and by hunting them. The bird cooperates in a social interaction, hunting, which is a partnership between you and the bird. The next logical step from a social interaction, when the bird becomes sexually mature, is a sexual interaction. And this imprint is nothing unique to birds of prey; all birds will do this. Most of you, I think, have probably read some of the popular accounts that have come out on territoriality, like Konrad Lorenz's *On Aggression* and so on. Ducks, for instance, have a following response when they're imprinted. You raise a duck from hatching and it sees you and thinks you're its mother, and it'll follow you around. These ducks, when they're imprinted, will also do exactly the same thing that we have happening with birds of prey—they'll become socially and

sexually fixed on human beings.

I repeated essentially the same type of technique that Bob had. I had a male that was 14 years old. He started copulating on my gloved hand, putting semen on the glove when he was four years old. It was way back when I was in high school. However, I was never able to get a female that would lay eggs—and of course, this is one thing we should stress right off the bat in artificial insemination. Artificial insemination isn't worth a darn unless you have a bird that has fully functional gonads. So the male would copulate fine with the glove. We could collect semen. I might say that the male Red-tail produced semen volumes and frequencies that far exceeded Bob Berry's Goshawk. I collected semen daily and I had volumes that ranged from .1 up to almost .4 cc. This is a big drop, not a little drop. I injected it daily into the female. The female responded the same way to stroking her back. This female was a little different, she'd let anyone stroke her. This may have been because Bob was the only person who handled his Goshawk, whereas this bird was handled by many people. Two fertile eggs were produced. One hatched by artificial incubation. The other was put under a broody hen. I'll have more about that later. So basically what I would like to emphasize is that falconers as a group probably have many birds that, given the right amount of handling, are likely candidates for this type of work with artificial insemination. Most of these birds have been taken as eyasses trained for falconry and then retired. You saw Bob Berry handing a stick to the female. It is very important that you go in there daily and help them build their nest, stroke them. Be around so you strengthen the pair bond. The other thing that I tried last spring was manual ejaculation of the male. I knew that my male Red-tail was in full reproductive condition. Therefore, I thought it would be valuable to try a forced ejaculation technique. Now this massage technique is a little deceptive using it on a bird such as this Red-tail that will ejaculate voluntarily, because it doesn't take much to get him to ejaculate. But I decided I would go all the way and try doing just like you would have to do to a bird that wasn't going to ejaculate. I trained the bird so I could lift him up holding him by the legs. Unlike Bob Berry's bird I did get my bird right in the middle of production. It didn't seem to bother him at all. He had to be trained very much like you train a Cooper's Hawk to sit on the hand. The training technique involved first holding his wings closed; forcing him down on my lap; holding him there for a few minutes and offering him a bit of food as I let him up. Much like breaking a bird to a hood. After about a week and a half of this there was no stress at all involved with picking him up right off the perch and holding him. He just didn't seem to mind it at all, once he had been broken in to it. The bird was held by the legs; the cloaca was exposed, and the technique that we will show you after we are finished here with chickens, because we don't have any raptors in breeding condition, worked very adequately on him. The technique involved physically pulling the papilla out and what you do is strip or pull the semen out of the very end of the sperm duct—the seminal glomulus. This is where the sperm is stored before ejaculating. Using this technique, this forced technique, I wasn't able to get as large an ejaculate as I was with the voluntary

technique. The ejaculates were much smaller. They ranged down to less than 0.1 cc, more in the range of what Bob was able to get using the voluntary technique, but I was able to get perfectly viable semen that looked in every way healthy, just like it would in a normal ejaculate from a hawk. There is one other technique that can be used for collecting semen, and this involves the technique called electro-ejaculation. This technique is used frequently with farm animals, bulls and rams and hogs. It involves giving the bird an electric shock. What you are trying to do is cause the muscles that squeeze the seminal glomulus to cause an ejaculation. You are causing those muscles to contract violently and force an ejaculation. You all know that you get a muscle spasm when you get shocked. The machine that was used is right over here. Basically, it is a pair of electrodes. You control the voltage and the amperage of the shock that you give the bird. They found doing work of this type with chickens that approximately 30 volts at a range of about 1/10 or lower amperage, given to the bird at three second intervals, three to five times, three seconds off, three seconds on, etc., usually stimulated an ejaculation. The electrodes are attached at two points. One electrode is attached to the rump area just above where the testes would be or just above the leg joint. The other electrode is a probe and the probe is inserted right into the cloaca. Give him the shock. This is one drawback perhaps with the birds of prey. It does cause muscle spasms in the hip region, but the bird seems to recover right away. My work has only been with chickens using this technique so far. It seems to be a very good technique for getting semen from a male that is in full reproductive condition but that would not cooperate like our imprinted birds would. I don't think it is going to be necessary to apply this with birds of prey. But I present it here. I have circuit diagrams for anyone who would like to build one and try it on some bird. You can have a crack at it, and see what happens. I haven't yet tried it.

SWARTZ. You found that placement of the cloacal electrode was not critical. I failed to succeed with an adapted ranger electrode stimulator. And I think maybe Skip Walker and Jim unsuccessfully tried it with pigeons or discovered that placement was critical.

TEMPLE. Placement is critical, yes. What I did was bypass the papilla. If I had a blackboard, I could make a diagram. The papilla is on the ventral side, the lower side of the cloaca. You can think of the cloaca as being like a rubber glove and the papilla rolling out like you sticking your finger through the rubber glove. Sticking it out through the cuff, sort of like that, the papilla comes out; you insert the electrode back almost into the rectum of the bird. It's behind the papilla so that the placement of the electrode isn't hindering the papilla from being exposed.

VOICE. Are you trying in essence to get it as close to the testes as you can?

TEMPLE. No, no. The idea is not to get it close to the testes. That's not it at all. You are trying for the muscles that control the seminal glomulus which is the area of the sperm duct just before it gets to the cloaca.

SMYLIE. Do you use any kind of anesthetic while you are doing this?

TEMPLE. You can. If you put it on your own hand you can feel what it's like. When you get it in contact with the muscles as you are by clamping it, you get muscle spasms. It's not the type of thing that is . . .

SMYLIE. What about trapping a wild raptor. Anesthesia . . .

TEMPLE. I wouldn't recommend it, no.

SMYLIE. I was just wondering if any experimenting has been done with birds that are not tame.

TEMPLE. Yes. This technique is one way. They have used it with uncooperative and untrained birds. But the thing I'm saying is that even for an untrained bird, you can get the bird to ejaculate using the massage technique that we will demonstrate later on. In fact something that Fran Hamerstrom alluded to in a little note in *Raptor Research News*, it can be used effectively. The one other thing that I've got some information on I think is rather unique information, since it's probably the only case where we've gotten enough semen from a bird of prey to try to use a certain diluent for preserving the semen. Let me put this slide on. I was able to get enough semen from a male Red-tail that it was worthwhile to try some very simple experiments on how to extend the life and to dilute the semen so it might be able to go around to between seven or eight more birds. There are two—think of them as things you can dilute the semen with or perhaps preservative—that are very easy for you to prepare. Most extenders or diluents that are used by the poultry industry are not the type of things that it would be very easy for the average person to put together. To make up I tried two that are very easy. I tried Ringer's Solution which is a physiological saline type solution, the mixture of which you can get out of any standard physiology book. And one that has worked with chicken semen and turkey semen is sterilized whole milk. I diluted the semen in 1:1 dilutions—one part of semen to one part of my diluent in each of these trials, and I compared it at two different temperatures: ice box temperature about six degrees C and body temperature at about 40 degrees C.

What I looked at here was how well the semen survived under these conditions as compared to undiluted semen. Can these techniques be used perhaps to extend the life of the semen sample so that we could ship semen around the country to different people. What I did: The semen in a good ejaculate is very dense, and the individual sperm cells are very active. It's a very simple-minded type of experiment. In a microscope field (under 430 power) I placed samples of each of the different treatments described above. In each field of view I counted 100 sperm cells. Out of the 100 I counted how many of these sperm cells were dead, the ones that were not at all motile, the ones that were not moving at all. I then did that, initially. The first column here is undiluted



**Table 1.** Semen Extension Experiments (after Temple).

	Percentage of Dead Cells		
	0 hrs.	3 hrs.	6 hrs.
Undiluted at 6 C	0	21	30
Undiluted at 40 C	0	39	54

semen (Table 1). This is fresh semen that has just been collected. Zero hours of collection I found no dead cells of the 100 cells counted; three hours later undiluted semen at six degrees 21; at six hours 30. So that by six hours after collection at six degrees about a third of the sperm cells are no longer functional. At 40 degrees body temperature, this is much worse. The three samples were 0 at collection time, 39% at three hours, over 50% at the end of six hours 54% were dead. Obviously, keeping it cool but not frozen is the best way to do it. I also looked at sperm motility. What I did here is a very simple thing. I picked 10 random sperm cells in the microscope field and scored their motility—how fast they were moving, how vigorously they were moving. I scored it 0, 1 or 2. Two if it was very active; 1 if it was moderately active; 0 if it was practically inactive. I then got a percent of what full activity would be for those ten. It's 100% right away after collection. It drops off to 40% activity, which is pretty low really. If you get anything less than half of that motility, you might as well forget it. The activity is probably so low that the sperm is not active—even worse with warm semen, 100% to start out with. It goes down to six hours with only 10% activity of the sperm cells. That's what happens when you are using undiluted semen just as you collect it. If you dilute it with Ringer's Solution, the same thing occurs, generally. I think I won't go through all these data. You see that in both cases it is worse. Ringer's Solution didn't do the trick. It didn't extend the life of anything. The sperm motility drops right off, much sharper than it does with undiluted semen. Actually, approximately the same for warm semen, but this is a small sample size. There is only one sample, so you can't really say. Sterilized milk is merely milk that has been heated to destroy the lactic acid in it, which is harmful to semen. And here the results are roughly comparable to the Ringer's Solution. Still nowhere near as good as undiluted semen. The only thing you can say is that at higher temperatures these diluents did seem to preserve motility of the sperm a little bit better. OK, you can have the light on now. I want to emphasize that it is probably not going to be very practical to send or distribute avian semen, raptor semen, through the mail. In other words, if someone has a female in California that is laying eggs and someone has a male in New York that they can collect semen from, it is probably not practical to send it to the fellow. Probably if you are going to get hawks together the way to do it is to bring the two birds together somehow.

HUNTER. You didn't try freezing it immediately in liquid nitrogen?

TEMPLE. I was advised by the poultry people that it would have been much worse. Freezing avian semen is not like mammalian semen where you can quick freeze it and preserve it. Quick freezing avian semen for some reason is very, very hard; it kills something like 90% of the sperm cells right off the bat.

HUNTER. One wonders why.

TEMPLE. Who knows. I might say the literature on artificial insemination in poultry is voluminous. People are just working on this. The publications are in the hundreds every year. They are actively working on it with some of the wildest diluents you could ever imagine. Stuff that is carbonated and aerated. It's unbelievable what they have tried. So I might also say if any of you want to try this massage technique, we are going to show it in a few minutes, on any of your birds that you think might be in reproductive condition, there are a couple of things that you can look at in the microscope to tell whether it's a good semen sample. We're going to show you a couple of these things.

BERRY. We are going to look at some of the semen under the microscope in back after Dr. Morgan shows us the technique. If you're done, Stan, thank you very much. I think we have to hold questions, we just don't have the time. Dr. Morgan has a tight schedule. Fran, could you take the floor and tell us your experiences with the eagles.

HAMERSTROM. Bob Berry asked me to talk on the massage technique. I had never heard of it. We always called it rough artificial insemination. I have successfully gotten semen from four species: Horned Owls, Red-tails, Golden Eagles and Broad-winged Hawks. Some of these birds were prepared in advance. Others were fresh caught wild birds. We just grabbed them and got semen. I'll tell you how one does this assuming you all know how to catch them in the first place. You grab their legs. If it's a Red-tail or smaller you put the legs between your knees with the bird's back up. Then you start stroking its back and putting pressure on its belly until the tail comes up. Then you slip your fingers on either side of the vent. If it's a male, out comes semen, if that male was just about ready to copulate. With the Golden Eagle one prefers to have several assistants [laughter]. I tried it alone for quite a long time. And my eagle kept mounting me and fortunately not my little hand. I would rip off my jacket and look at it carefully to see if there was semen on it. I had some disadvantages that you didn't all have.

I would like to talk about some of the things that work up to semen production. You can use a combination of these things. You can do all this coaxing at the beginning and then if you need semen because you know your female is about to lay and you can't get it by the cooperative technique, why you may wish to resort quickly to the massage technique. What leads up to this? In the birds that build stick nests, it's playing with sticks. Tease them. Don't let them have sticks all the time or you will lose your advantage. This works with owls, and I don't think it works with falcons. They don't build stick nests. They are

not fascinated by sticks. I think they are fascinated by things like wings of pigeons. I use my voice working with the eagle and neck stroking. My Red-tail copulates first on my ankle and after that he much prefers my head. My Horned Owl copulated only once and that was on my head at night [laughter]. My diurnal raptors tend to copulate first thing in the morning. Then they're keen on it and then again late in the afternoon but their enthusiasm wanes near noon. With the Golden Eagle you have to wait about 20 minutes between attempts to get semen. If you fail the first time, go get coffee—spend 20 minutes doing something else and then go again. With rough artificial insemination I am using the equivalent of that little box but it is cheaper. You just take the bird and you keep stroking and you keep working and you get exhausted both emotionally and physically, because you think you are being rough on the bird. And just about the time you want to give up, the bird bates. That's when the semen comes, right at that bate. That's when you keep going. This is the quick shot treatment. When that bird has had enough of this monkey business and wants to get away is when he is going to ejaculate semen. I'd like to clear up one thing. I have a paper in *Raptor Research News* (5(3):91, 1971) on semen extenders—a recipe for it. My purpose for semen extender was totally different. It was, if I didn't get enough semen, to extend the quantity rather than the time. Now if we could show my slides, that would be very fine. I might mention that I have a paper coming out in *Die Vogelwarte* on the male Red-tail's capabilities. And the Golden Eagle material is largely in my book. (I would like to give one bit of advice to breeders. When you go in for any of these monkey-shines, take the bird away from the breeding pen if you are going in for rough artificial insemination and then walk the bird back to the pen. The bird will forgive you in about three paces while you are carrying it. There is nothing more soothing to a bird than to be carried.) Could I show the slides, please. Here is my Golden Eagle, Grendel. He is a little uncertain but it is early in the season. He is preparing to mount. You cannot imagine the conversation in the Hamerstrom household. This happened every day after breakfast. I would go out and get mounted. I'm the very absent-minded type. Sometimes I would go running out in a little thin sweater or something or other and my husband says, "Fran, did you remember your copulation jacket?" [laughter]. The bird is really cued in on that jacket. At any time of year I believe he will mount me or show considerable interest if I have that on. Next. Here he comes flying. And eventually he gets up onto my head. I think that's next or there aren't any more. I don't know which.

Oh, yes, preparation for artificial insemination. I go and catch the bird. This poor chap comes to our place, and he had never seen an eagle near to before and he said, "Oh, I wouldn't want to get near that;" and in about five minutes he was helping because we were short-handed. Next. He is picking up the bird. This is the female, and I always run away because I don't want to spoil my beautiful relationship with that female eagle, Chris. So I watch from the window. I may be foolish but there is no point in taking a chance. Next. I've gotten five eggs out of her in one clutch. And it brings me to a point I would like to make. Consider the possibility of not just recycling. Consider egg stealing. My hunch is birds of prey are far less determinate than we have supposed. And you may get

more eggs. Put the first eggs in an incubator or under something else. But consider this possibility, too. Some experimental work needs to be done on it. Artificial insemination. Two professors from the poultry division of the University of Wisconsin. I always admire their courage. They handle eagles as though they are turkeys. Until finally they saw my man get nailed. And then they had a very different viewpoint. Next. This is about two minutes after a really rough go. Two attempts at getting semen. The second was successful. Everybody was exhausted. And if you notice the eagle is perfectly placid. They get over this right away. And so do my other birds. Next; good, that's the end.

BERRY. Thank you, Fran. Can we now have Dr. Walter Morgan come up here for a minute. He has hopefully a few comments or recommendations and suggestions.

MORGAN. I'm happy to be with you today. Brookings is about 60 miles north of here, and my schedule isn't really so tight. There are a few comments I'd like to make before the demonstration. Actually I don't know if I am going to stand on these chairs or what. I was sitting in the back row, and I am a little sympathetic. It's kind of difficult to see the demonstration; I am afraid it will be quickly done, and we will have the result from it, and we won't have gotten enough information from it. First, we might ask when is artificial insemination used. And we use it in poultry for special single sire mating when we want a particular male, a lot of progeny from that and known identity. We use it if we have cages. If we have birds in cages which are breeding birds. Normally we had floor matings for many years. Now as we use cages more, we do not mix the sexes often. And we can use artificial insemination as a fine tool here for when the sexes are separated. We can keep a pen of males. When you put a group of males together after they are sexually mature normally they are going to start fighting. But if you rear them together, the males will be harmonious and you can take one male out, collect semen, and put him back in. Nothing will happen. There are more copies of this if anyone needs them. Another kind of artificial insemination that is used and commercially it is used very much now in turkey production. Practically all of our turkey producers use this. The reason for this is that the geneticists have done such a good job in breeding for big full breasts and for short legs and for meaty legs that the male Tom turkeys are not able to mount the hens any more. This is kind of ridiculous to breed for one specific desire in our economy to the extent that natural matings are not likely. The more successful breeders I think have the natural breeding plus artificial insemination. Some of them use only artificial insemination. Considering when it's used, we consider when there is maturity. What Stan said was true about having to have mature birds before you can collect any semen or before it is worthwhile to inseminate them; at least they should be inseminated a short period before they come into production. Now with turkeys for instance, the pens of the males are lighted for about a month—they have artificial illumination. The day is lengthened artificially about a month before the semen is collected. This way spermatogenesis is complete and mature sperms are produced.

With the females, about the same time is needed.

Why is artificial insemination used? One reason for using it is precision so that you know exactly what male has been mated with what female. I think that is important to all of us here. The raptor breeders. For establishing genotypes, for establishing what particular lines for selection you might want to have. To overcome natural mating obstacles. And it seems to me that that is what the whole bag of tricks is about here. And to conserve space by using artificial insemination if you have them, for instance, in cages.

As far as storage of the semen is concerned, the duration of potency for chicken semen is normally about three or four hours, if it is in vitro. After you have collected the semen you have two or three hours, three hours perhaps, to inseminate. You can carry it around and move it if you want to. But as Stan indicated, you cannot ship it long distances. We do not yet have means of preserving semen the way they can, for instance, bull semen, where they can use it a year and a half, perhaps 20 years after. I don't know how much longer, after they have collected the semen. But we can only use it a very short period with poultry semen. I think that the longest that it has been demonstrated is some semen was sent from Maryland, College Park, over to England. They inseminated some hens there. But it was a very rapid trip in less than two days.

One of the cautions that I would mention to you, and this stems from some work which I did in Belgium a couple years ago. I had an opportunity to work quite a bit with semen motility at that time. First I mention that, in order to be potent, good semen has to have good motility. But the opposite is not true. If it has good motility, this does not mean that it is potent semen. I was interested in what dead semen was in your slides. A lot of us think of semen that has lost its motility as dead semen. We had some irradiation studies over there. When semen is irradiated with over 4,000 roentgens, semen that had been ejaculated, it lost its potency but it had good motility. We had semen that was control semen that had not been treated that continued its motility for up to 10-12 days but it was not potent the first day after it had been collected. So you can see motility, and it's not necessarily a criterion for saying that it's good semen. Some of the cautions that we take with artificial insemination are that we have to watch for the quick ejaculator. I think you have been exposed to that in the discussion that we have had before. Sometimes after you have been training the male, training the roosters for a while, you have them in the cage. You reach in for them, if you happen to hit them the wrong way—it's gone like that. You haven't even put your receptacles, your collector up there to get the semen.

Another caution would be cleanliness of your operation. Now I have distributed a page telling what the procedures are going to be. I also have here the equipment that is necessary for artificial insemination. Normally we use a pair of scissors, any pair of scissors, to cut the feathers off the rooster around the vent on the abdomen. The reason for this and the only reason for this is visibility, so you can see what you are doing and can have a clear field to work with. For our chickens this is a good type of collector to use. It's a plastic container, for turkeys and for chickens also. The normal amount of semen that we collect from a well trained male will be up to one cc. We need approximately, or nor-

mally use, .2 cc for each insemination. So that if we have a good producer of semen, we can take care of five hens without using an extender or extending the semen. The semen in hens will be effective for a week. Now I don't think there are any cases with good semen where you need to inseminate more frequently than a week. A week apart for inseminations is good. They can last up to two weeks, possibly to four weeks. In the turkey the semen insemination will last for practically the whole breeding period. So we have differences in species. And this is something you are concerned with, but you have such a small clutch with your raptors. I would suggest that a good insemination a day or two before they start laying would last for the whole clutch. There is no reason to be concerned about multiple inseminations. I'm saying this off the top of my head without a lot of knowledge, but from the experience we have had with chickens. The amount of semen that is inseminated is important because you probably won't have too little semen; you may have too little semen in chickens. If you have 0.2 cc, mainly you have enough. It is protected and maintained within pockets which are in the oviduct. And the actual insemination takes place as the egg is ovulated from the ovary as most of you know, as it starts down the oviduct. And these little individual bits of semen are available to fertilize if another egg is coming. So it is a matter of livability, if you want to use that term—viability. I would rather use viability of the semen after it is in the oviduct. In turkeys we find it lasts a long time. In chickens it doesn't last for such a long time. Now the tools that we use for collecting the semen. We collect it in a small tube. After it has been collected (this is ABC on your hand-out) we transfer the semen from the tube that we collect it in, into a syringe. And normally what I do in practice is take out .2 ml for each injection insemination. because if you have .8 out and you try to inseminate .2 down to .6, it is quite precarious because you have to try to look at it, it's pretty difficult. If you just have .2 in there and make a complete plunge you are all set. Those are really the only instruments that you use. In addition to those today, if we can we will set up a demonstration of motility and for that we use cover slips and depression slides, and I have the slide here. It will probably be five or ten minutes after I finish the demonstration that we will have the sperm available to look at the motility.

BERRY. Dr. Morgan, would you comment on temperature shock?

MORGAN. Temperature shock on semen?

BERRY. The outside temperature when you collect semen. Should you take precautions before transferring it to the female?

MORGAN. No. I would reiterate that in storage the coldest semen retains its motility much longer. In the samples that we used we had it refrigerated temperature. But retaining motility doesn't mean retaining viability. They are more heat susceptible than they are cold susceptible. I would make that comment coming from body temperature they go to the cooler room temperature and

this is fine. So I don't think any precautions need to be taken there, if you do it within a couple of hours.

THACKER. Dr. Morgan, would you recommend sterilized equipment?

MORGAN. I would, yes. I would be sympathetic toward the posture of the veterinarians and I would say that sterilized equipment is highly desirable. I do recommend it. In practice I don't follow it at all times. I've been fortunate not to have problems. I could use those chickens if somebody wants to bring those boxes up and that lab coat that's there.

BERRY. I was looking at semen under a microscope. Extended over a period of hours, I noticed that some sort of growth appeared in the semen. It was almost like they became too crowded to swim after a while. Something was growing in there. Was that bacteria?

MORGAN. There is a clumping of the semen which I've noticed, they clump around the semen.

BERRY. They clump around that, what is that?

MORGAN. It's some kind of starch artifact, I don't know what it is.

HUNTER. Greg Thomas is going to take this on video tape. We are going to kill two birds with one stone if you will excuse the expression, demonstrating closed circuit TV.

MORGAN. What has been done previously to these birds—you recognize that they do not have all their feathers. The first two here are trained males. I hope they produce. They have been clipped. The third one I will clip and it hasn't been trained. I have less hope for good results with the others. Now you will notice on your sheet that this can be done with two operators or one operator. Frequently they have one person hold the bird and the other person stroke it and collect the semen. I have come to do it as a single person operation, and it normally works pretty well. The testes as you know are in this area of the bird up near the back. Stroking of the back like so produces the ejaculate. Now there is some fecal material there. And there is a sort of stripping of the duct that I think Stan mentioned in some of his birds, too. Usually after you have tried it three times and if you haven't gotten anything, then you might as well give up. He produced a pretty good supply of semen and there was some fecal material in there as well. So this sample you would have to let the fecal material sediment down to the bottom. And I will remove it with a syringe that I have here. As I said, it is anticlimactic because it is all over. I'll remove the semen. Now that one produced about 40 hundredths cc of semen.

GRAHAM. How much pressure did you use on that?

MORGAN. I put a lot of pressure.

TEMPLE. I have an untrained rooster that any of you can practice with.

HUNTER. I have 300 of them.

MORGAN. OK, there are some things, the first was without telling many of the particulars about the technique. I think with your raptors you'll have different types of reactions than we would have here. Reactions in terms of methods of holding them. For this I put my little finger between the legs, hold the legs and have the container near the cloaca. Now, actually, I stroke him two or three times, and then I push down; and I'm kind of pushing up on the abdomen, too. If you can watch and see that perhaps, stroke him like that. Now that is fecal matter, that's not semen. I hope you didn't get a picture of that.

VOICE. Is this a trained bird again?

MORGAN. Yes, this is one of the two trained ones. There is quite a bit of fecal material but there is a good supply of semen, too. We'll set this semen up under the microscope.

I will demonstrate now the insemination into a hen. And for that purpose I will return some of the semen into the container. It is a very simple process. The semen is not as clear as I would like it to be. So whether it's our personal relationship I'm not sure. Now this rooster has all of its feathers, you see. He hasn't been clipped or anything. It's possible to collect semen without clipping them, but I like to clip the feathers off. Whoops [laughter]. It is a little bloody. There is a little bit of semen, there is a very little bit. But there is some. This is normally what we would expect. You can see where it drained down the sides here. We would expect to get a little bit the first time and an increase with daily trials. I'll set up some of this semen.

TEMPLE. While you have that chicken, there is a technique that I use on other birds such as pigeons. This seems to work a little better for me, a little better to have someone hold it and hold the tail up and massage the back. Then for actually stripping the bird that is going to be very uncooperative, like for instance a hawk that's not imprinted. Using the other hand and approaching ventrally instead of dorsally is to reach in until you can feel the end of the papilla and bringing it out; he has just ejaculated and just sort of letting your fingers slide along. I don't know, for me that seems to have gotten more from an untrained bird than just the stroking of it. You might want to comment on that technique. Is that common to pull the papilla out?

MORGAN. The chicken has about as little papilla as any of your raptors would. They are practically without it, too. If you have a duck or goose, they have a nice long one. You can really strip that. There is not much to strip a raptor. And I think there isn't much to stripping chickens.



TEMPLE. It's small, pulling it out.

MORGAN. I put it in my notes here—manipulation is stripping of the duct, so we have that in common. I would be optimistic about these techniques with your birds particularly after hearing what we've heard with previous two speakers. It's a really simple technique. It's something that you work on—well, if you know when the female is going to start laying, you would start on your male a week to 10 days or maybe two weeks before that. Then if you inseminate, if you're able to inseminate her, just before she starts laying, you are through in two weeks. So it's not a long tedious process. And if you can collect semen, the insemination of the female is extremely easy.

GRAHAM. Would you explain in more detail just the pressure you used and exactly where when you stripped.

MORGAN. With the hens there is quite frequently, there usually is, an expulsion of fecal material. Now, for holding the hen normally, I rest her on my thigh like this, too, and her feet will go right on my thigh. And I will press down on her back when I get ready because I will need all my hands to manipulate here. The hen has as mentioned previously, an oviduct. With the chickens the oviduct everts. There is quite a large opening. It is just a matter of putting the tube into the everted oviduct. That's mentioned on your sheet; it's really mentioned there better than I can say now because you relax after you have inserted the syringe. And when the hen has relaxed, I push the plunger. And in all this we also realize the sensitivity of the birds we are working with and we are trying to avoid stress. I think I use the word effort in the first line there; several places I said without too much effort, or without much effort. Without stress, I am trying to say. I encase her head under my arm and go in like this. There is some fecal material there which is interfering with the oviduct. There are two openings. You see there is an oviduct and there is also an anal opening. You have to insert it into the oviduct. We have had some chickens in Brookings which had two oviducts. This is a genetic difference where they have a right and left oviduct. Very unusual. Normally, they just have the left. If they have two oviducts and we have inseminated into the right oviduct, we have never had any fertility. So it should be into the left functional oviduct. And I noticed you said you went on the left direction when you were into the raptors so this I would expect, so this is as it should be. If anyone else would like to practice with these, why they are welcome to, I consider it kind of a challenge for Don saying that he wanted one that was untrained and I didn't know they were going to bring one; so I have one that is untrained, or I'll take his.

In the training period these have been trained for about a week. I have a student who works with me on this. And the first time that he tried it, there were 15 roosters, and four of them ejaculated semen. So it is not unusual to ejaculate the first time, to get semen the first time.

EBERLY. How do you train them?

MORGAN. Just by trying to collect semen from them. This is what the training is. I would expect this would be true in raptors. You would have to train them. I have heard several people speak of the very precise and close association people have with raptors. Perhaps this is a necessary part of it. But at least with chickens any of us who try it can usually get results if we use the proper technique.

OK, as I strip this male, I've got the tube in my hand so make it all realistic. I like to have him kind of sitting down like that just like the hen was sitting down. Then I can bring pressure to bear up here and with this hand I am pushing down here. I am pushing on the abdomen and with these two fingers I am stripping in here. There is quite a bit of pressure that is given in the excitement. Here I think one of the dangers I might be suggesting, that we might be reluctant to squeeze them too much. You do squeeze them quite a bit. I've found with students that they think maybe I am hurting the bird.

VOICE. As hard as a hand shake? [laughter]

MORGAN. Yes, at least.

VOICE. When you get a small amount of semen coming down the side, do you use a dilution material to get that out?

MORGAN. This I let settle for quite a while. This will eventually settle, maybe after two minutes. Then I try to pull what I can up into the syringe. I haven't diluted it. We don't practice dilution. I'm for dilution. I think it's OK. But we haven't needed to use dilution.

VOICE. You get just that little bit that you got out of that first ejaculation. Will that be enough to draw up?

MORGAN. No, on this one I didn't have enough.

VOICE. But that's just about as much as you are going to get out of a small raptor.

TEMPLE. Look at a Red-tailed Hawk, a Red-tail gives as much. Bob, you have never stripped your bird to see whether you could get more out.

BERRY. No.

VOICE. Is it too viscous to come up in capillary tubes?

TEMPLE. I tried doing this on Starlings which are roughly comparable to kestrels as to size. I could bring the papilla out and get an ejaculation. And that is what I used in capillary tubes. I just touched it and it was sucked up into the capillary tube. I'm almost certain that if you have a raptor in full reproductive

condition using the stripping technique, you should get plenty. I don't think you have to worry about not getting enough.

VOICE. What about [inaudible].

TEMPLE. I've used a pipette for the insemination; I didn't use a syringe, I used a pipette.

HUNTER. For whatever it is worth, about four years ago he told me that Pheasants were very difficult. I didn't happen to have any chickens at home, but I did have some Pheasants. It was breeding season. A cock pheasant was obviously in breeding condition. I went over and picked him up, got semen from him immediately, it surprised me, quite easily. I never really tried it on chickens.

MORGAN. Some people use a little tube to get a greater distance in insertion; this is particularly true in turkeys, where there is a longer oviduct distance and the sperm really has to go down to the area of the ovary, but usually the sperm is potent enough to do it without this assistance. I am finished then, unless there are any questions

GRAHAM. What about injection of fecal material with the sperm?

MORGAN. We like to be cautious and avoid that but if you do, that will not kill the sperm.

VOICE. [inaudible] bird that is laying eggs already and then you inseminate?

MORGAN. OK. The question is about when there is a delay in when you inseminate, and if I don't give the answer to this, come back to it. With laying hens particularly if there is an egg in the oviduct, this egg will not be fertilized, but the egg that she lays day after tomorrow will be fertilized. The most desirable time to do this in chickens is right after they have laid an egg, because their oviduct is free then. It takes about 24 hours for a new egg to come through. If there is an egg in the oviduct, this does not prevent the sperm from going up though. So it can be done when there is an egg in there. If you do make an insemination today and your raptor lays an egg tomorrow, I don't think the egg is going to be fertile.

VOICE. You mentioned that if you can feel the egg in the bird, it is already in the oviduct, right?

MORGAN. Somebody mentioned that.

BERRY. I think I mentioned that. If you can feel an egg in the oviduct, you could probably get that egg. That's before the shell. You can feel it enlarge long before the shell is laid down around the soft part of the egg. So you can get that

egg after you feel it.

MORGAN. In chickens after the egg is ovulated, it's the yolk which is ovulated, the actual size it's going to be when it's laid. After the yolk is ovulated and has started down the oviduct, it does not become fertile at any time. In chickens we can palpate the egg. You can feel in the cloaca. If there is a hard shelled egg, you know it right now. And that egg will never be fertilized by an insemination today.

MENG. Have you experimented with insemination after an egg has been laid? Drilling a small hole in the shell and putting the sperm in.

MORGAN. I haven't and I think it's impossible.

TEMPLE. Very difficult. People have tried to with chickens and it's very, very difficult to do. What happens is the membrane that's laid down around the yolk as it goes down through the oviduct is very difficult.

BERRY. I think it has been done though.

VOICE. A fellow out in California is doing a lot of work on it right now.

TEMPLE. It has been done. Your chances are pretty slim. I might add, when is the right time to start insemination if you have a hen that is going to lay eggs? On the female Red-tail you can feel the pubic symphysis which is where the two halves of the pelvic girdle come together. It's between the legs almost to the vent. That separates and widens as the female comes to the time when she is going to lay an egg. This is a real easy thing if you have handled your bird when she is not in reproductive condition—you can feel those two bones very close together. When she comes into reproductive condition and approaches the time she is going to ovulate (in the female Red-tail it is about a week before she ovulated) those bones come really wide apart. In the female Red-tail you could put three fingers in between the halves of the pubis. Really opens right up, but you feel it coming apart. Once you can feel it coming apart that is probably the time you want to start your insemination. That is a sure sign she is going to be laying real soon.

**APPENDIX: Summary Issued at Conference**

## **PRACTICAL CONSIDERATIONS IN ARTIFICIAL INSEMINATION**

by Robert B. Berry

*Anatomy.* Female: In most hawks, both ovaries are functional but only the left oviduct is functional. Anatomically, the oviduct is comprised of five parts:

the infundibulum (fertilization occurs here), the magnum, the isthmus, the shell gland and the vagina.

Male: The genital tract consists of paired testes, epididymides, deferent ducts and in hawks, a rudimentary copulatory organ called a papillae. Sperm is stored at the base of the deferent ducts (Petraik, 1969).

#### *Artificial Insemination.*

##### *Advantages*

1. Improved fertility.
2. Cross breeding.
3. Reduction in males.
4. Determine potency in individuals.

##### *Disadvantages*

1. Stress.
2. Urates reduce viability of sperm.
3. Requires a skilled operator.

#### *Semen Production.*

1. Vitamin A and E deficiency affect spermatogenesis adversely.
2. Coccidiostat at 0.0125% in feed does not affect semen production.
3. While semen can generally be taken from a male fowl at any time of year, one cannot expect similar results from a raptor since maturation of the gonads is a cyclic occurrence (they may expand 200 to 500 times their size during the mating season).
4. Greater semen yields occur in the late afternoon—domestic fowl.
5. The greater the frequency, the lower the yield.

#### *Collection of Semen.*

1. Optimum response is secured from a fowl if he is picked up quickly and immediately manipulated—ostensibly before surprise turns to fear.
2. Do not allow semen to cool quickly, even though fowl semen is more resistant to temperature shock than the semen of other domestic animals.
3. The best fertility is achieved within one hour after collection.
4. Maximum fertility in a chicken requires insemination every seven days.
5. Duration of fertility in a chicken is 10-13 days.

#### *Semen Quality.*

1. Fertility appears to be correlated with motility, percentage of dead sperm, methylene blue reduction time and numbers of live sperm per unit volume of semen.
2. Fertility does not appear to be correlated with pH, density of sperm and volume of ejaculation.

#### *Semen Quantity.*

1. Chicken (large) 1 to 4 cc.
2. Turkey 0.3 to 1 cc.
3. Goshawk 0.01 to 0.05 cc.
4. Red-tailed Hawk .10 to .40 cc.

*Dilution and Storage.*

1. Many attempts have been made to arrive at a diluent medium and ideal temperature and other physical conditions for the prolonged storage of semen, all without success. Storage beyond 48 hours produces such reduced fertility as to be impractical.

*Insemination.*

1. Optimum time to inseminate is just following oviposition (egg laying).
2. Fertilization will generally occur the second day following insemination.
3. Maximum fertility in chickens requires insemination every seven days.
4. Duration of fertility in chickens—10 to 13 days.
5. A hard-shelled egg in the shell gland reduces the chances of good fertility for other eggs (easily discovered by palpating the abdomen just behind the breastbone).

Is artificial insemination the panacea we are looking for in captive raptor reproduction? Certainly not! Artificial insemination should be attempted when all else has failed, and then probably only when intraspecific hostility endangers the life of one of a pair. Maturation of the gonads must take place before sperm or egg production is possible—a complex and lengthy process brought about by a combination of external stimuli, including the weather, photoperiod, territory, absence of stress, stimulation by a mate. Therefore, if a pair of birds is physiologically capable of mating successfully, a prerequisite for artificial insemination, then they should be allowed to do so. Exceptions might involve a laying female with a displaying male that appears on the threshold of copulation, but for some unknown reason, cannot take that last step. Extreme care, however, must be taken in the introduction of stress which might destroy the reproductive cycle.

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