

## Intrauterine artificial insemination by vaginal endoscopy

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Intrauterine deposition not only of frozen-thawed semen but also of fresh and chilled semen, results in significant better conception rates than deposition in the cranial vagina (Linde-Forsberg, 1999). This is achieved very reliably by inserting the semen into both uterine horns after laparotomy under general anaesthesia. In several European countries this procedure is not allowed and in general, there are concerns because of the invasiveness and for ethical reasons. Transcervical catheterisation and artificial insemination by the use of a rigid endoscope, as described by Wilson 1993, is a well accepted method with the advantage of good visualisation of the female genital tract. In a modified technique, a balloon around the endoscopic sheath should provide a better sight in the cranial vagina (Kong, 2003).

The author uses the equipment as provided by the Dr. Fritz company, Tuttlingen, Germany ([www.dr-fritz.de](http://www.dr-fritz.de)). It has a modified tip of the cystoscope that makes the passage of the pseudocervix easier and provides more accurate guidance for the catheter which is passed through the cervix (Blendinger, 2006). A human urethral catheter with a curved tip and opening at the end of the catheter is used for the cervical passage.

Under the close guidance of the endoscopic sheath and using the mandrin, this catheter is rigid enough not to deviate in an undesired way from the area of interest. In order to facilitate the introduction of the catheter into the cervix, it is useful to bring the cervix in a more horizontal position. This is best achieved when the cranial end of the endoscope is

first protruded under the cervix into the blind sac and then slowly drawn back. As soon as the uterine portio is visualized, the scope can direct it by small movements forward and backward and to the side. Once the external opening of the cervical canal is seen in front of the optic, the catheter is carefully pushed forward until the tip is in the view. By rotation of the curved tip, the catheter can be directed in several directions giving a better chance to enter the external cervical opening. The black marks on the catheter show how far it is inside the uterus.

After some time of training, this technique is appropriate for the use in routine practice.

Some considerations using canine fresh, chilled or frozen semen are demonstrated by the recommendations of three different companies.

### References

- BLENDINGER K (2006): Videovaginoscopy and transcervical insemination in the bitch – practical tips. *Kleintierprax* 51 (7/8): 388-393.
- KONG IK, YU DJ, JEONG SR, OH IS, YONG CJ, CHO SG, BAE IH, OH DH, KOM HR, CHO SK, PARK CS (2003): A new device for intrauterine artificial insemination in the dog. *Asian-Australian Journal of Animal Sciences*, 16 (2): 180-184.
- LINDE-FORSBERG C, HOLST BS, GOVETTE G (1999): Comparison of fertility data from vaginal vs intrauterine insemination of frozen-thawed dog semen: A retrospective study. *Theriogenology*, 53 (1): 11-23.
- WILSON BS (1993): Non-surgical intrauterine artificial insemination in bitches using frozen semen. *J Rerod Fertil Suppl* 47: 307-311.

## Appendix 1): Recommendations of the Synbiotics Corporation

### PERFORMING FRESH CHILLED SEMEN A.I. IN THE BITCH

In fresh chilled semen breedings, 2 inseminations should be performed during the fertile period usually on days 4 and 6 post-serum LH-peak. By properly planning multiple inseminations, you'll optimize the chances of success.

(the *Synbiotics Fresh Chilled Semen Breeding Training Manual* contains more details on timing procedures and insemination technique).

1. The semen should be inseminated within 15 minutes after removal from the chilled package. Gently rotate the tube to resuspend the sperm cells, as they tend to settle auto. Evaluate a drop of semen for motility.
2. Draw 3 ml of air into the syringe (to clear the pipette at the end of the insemination).
3. Draw all the semen into the syringe and attach the pipette.
4. Have the bitch on a table or on the floor, depending on her size and how you and the dog are most comfortable. Make sure an assistant is available to restrain her as necessary.
5. Spread the labia and pass the tip of the insemination pipette dorsally toward the vaginal opening, ventral to the rectum. The pipette should be in an almost vertical direction at this stage. To further pass the pipette, redirect it in a horizontal position and gently advance it toward the cervix. The vaginal folds may produce temporary resistance, but may be avoided by spinning and rolling the pipette as it is advanced.
6. The pipette tip will usually stop at the dorsal fold of the anterior vagina. It is recommended, but not necessary to advance beyond the dorsal fold. Do this by applying slight pressure in a ventral direction while advancing the pipette. The insemination pipettes are marked at 5 cm intervals. The pipette should be advanced as far as possible. Generally, in most giant bitches, the pipette should be passes a full 25cm. In large bitches, pass the pipette 25 – 24 cm.
7. In small to medium bitches, advance the pipette 15-18 cm. In toy breeds, pass the pipette 5-10 cm, or just past the first mark. Check the position of the pipette by abdominal palpation. In many bitches you can feel the cervix, which should be just cranial to the tip of the insemination pipette.
8. Elevate the hindquarters of the bitch.
9. Keeping the hindquarters elevated, turn the syringe vertically with the plunger in the uppermost position, and inject the inseminant, finishing with the air left in the syringe to clear the pipette.
10. Withdraw the pipette, keeping the bitch's hindquarters elevated.
11. Gently stimulate the vaginal Wall with gloved finger for 5 minutes ("feathering").
12. Keep the hindquarters elevated for an additional 5 to 10 minutes after stimulation is completed.

SYNBIOTICS CORPORATION 11011 Via Frontera San Diego, California 92127.

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Chilled semen AI 11/00

## Appendix 2): Recommendations of the Minitube Company

**Canipro™ is a liquid medium for long term preservation of canine semen and adequate for conservation at +4°C to +6°C for approx. 7 days. Canipro™ is delivered in a 20 cc bottle and is a clear solution. It requires adding of 4 cc fresh egg yolk.**

### 1. COMPOSITION

**Canipro™** consists of the following components:

- Purified water
- Sodium Citrate
- TRIS
- Glucose
- Fructose
- Proprietary factors
- Gentamycin

### 2. PACKING SIZE

**Canipro™** with Gentamycin sulphate, 20 cc Ref. 13700/0050

### 3. APPLICATION

#### 3.1. Preparation

Warm **Canipro™ Culture Medium for Chilled Canine Semen** to room temperature. Add 4 cc fresh egg-yolk. Gently mix.

#### 3.2. Preparation and Addition of Egg Yolk for Canipro™ Extenders

1. Obtain fresh eggs.
2. Wash, rinse, dry and store in a refrigerator until use.
3. Prior to use, wipe clean using a 70% solution of isopropyl alcohol and a brush. Allow to air dry.
4. Crack the egg and separate the yolk using a metal egg separator of manual technique.
5. When the yolk and white have separated sufficiently, place the yolk with membrane intact on a folded square of paper towel (non-recycled product).
6. Roll the yolk very gently to remove any excess white. The egg yolk can be aspirated from the membrane using a 5 cc syringe and a 16-18 gauge needle. Alternatively the yolk membrane can be lanced and the yolk drained into a beaker or measuring cylinder. It is very important that the yolk membrane not be used in the extender, as it may be harmful.
7. Carefully add the exact amount of egg yolk required by the extender using a calibrated instrument (syringe, pipette or balance).

### Appendix 3): Recommendations of the CLONE Company

#### CLONE CHILLED SEMEN PREPARATION

Phone: 610-458- Fax: 610-458-1102 Web Site: cloneusa.com E-mail: cloneusa@aol.com

**!Keep Vials of Solutions & Ice packs Frozen until ready to use!**

(Vials may be thawed but cold when received, just re-freeze them immediately.)

**Objective:** To collect the sperm rich fraction only with no contaminates  
(There is a chart on the of these instructions to guide you with the semen preparation.)

1. Thaw the **FROZEN EXTENDER VIAL (orange or salmon color vial.)** It must be at room temperature when it is added to the semen after you have collect it.
2. Assemble the Plastic Collection Cone onto the centrifuge tube as directed on the instructions on the back side of this form if you are using this collection system.
3. Tease the stud with an estrus bitch if possible. When stud has full erection, let two squirts of the first fraction be expelled to wash out the urethra before collecting the sperm portion.
4. Collect the sperm rich fraction only, stopping when you notice any clear fluid being ejaculated. (Check step #1 on the chart on back of these directions for the approximate amount to collect.)
5. If you collected in a plastic bag and gently pour into the plastic centrifuge tube.
6. **Ask the bitch owner how is the insemination going to be performed?**  
You must know how the insemination is to be done to prepare the semen for shipment. Check the Extender Addition Voumes on the table on the back of this form.If a Trans-cervical or a surgical as insemination is to be done you must have a condensed pellet of semen so you have a final insemination volume as shown in the table.
7. **If step # 4** above can't be accomplished without collecting a large amount of prostate fluid you must then centrifuge the semen at urine speed (700g; very slow) for 3-4 min. Draw off the prostate fluid with a syringe very slowly, leaving about 1/4 to 1/2 ml of prostate behind. There should be no more fluid than .50 ml for small dogs & 6-7 mls large dogs to add the Extender for a regular insemination and no more than .25 and 1 ml to add the extender for a Trans-cervical or a surgical insemination.
8. Re-suspend the sperm cells into the remaining prostate fluid by gently rocking the centrifuge tube until sperm cells are totally back into solution.
9. Add the right amount of orange or salmon colored extender to semen at this time, slowly. (Follow step # 2 located on the chart on the back of these directions for approximate volumes.)
10. Attach the centrifuge tube to its Styrofoam holder with a rubber band.  
(You could use 4 wraps of paper towels in lieu of the Styrofoam holder.)
11. Set the frozen ice pack next to Styrofoam semen holder, keeping the tube of semen from touching the ice pack by facing it away from ice pack.
12. Make sure the Semen **Activator vial (clear solution), Insemination Instruction s, Syringe & Insemination Pipet** are included in the box for the inseminator of the bitch. Tape the box closed and ship by way of Next Day Air or faster. Do not use the US Postal service. The semen will cool on it's way and be ready to reactivate when it arrives at it's destination.

Chilled Semen Preparation	Preparation Volumes for Regular AI					Preparation Volumes for Trans-Cervical or Surgical Insemination				
	Suggested Inseminate Volumes	Collector Step 1 – Step 2		Inseminator Step 1 – Step 2		Suggested Inseminate Volumes	Collector Step 1 – Step 2		Inseminator Step 3 – Step 4	
		Collected Volume	Orange Buffer Addition	Activator Addition	Final Insem. Volume		Collected Volume Centrifuged	Orange Buffer	Activator Addition	Final Insem. Volume
Maltese 1-10	1-2 ml	1/2 ml	3/4 ml	3/4 ml	2 ml	1/2 - 1 ml	1/4	1/2	1/2	1 – 1 1/4
Whippet 10-25	2-3 ml	1	1	1	3 ml	1 – 1 1/2	1/2	75	75	1 1/2 - 2
Corgi 25-60	4-5 ml	1 1/2	1 1/2	1 1/2	4-5 ml	1 1/2 - 2 ml	1/2	1	1	2 1/2
Labrador 60-80	8-10 ml	2	4	4	10 ml	2-3 ml	1/2	1 1/2	1 1/2	3 1/2 - 4
Rottweiler 80-120	8-12 ml	3 – 4	4	4	12 ml	2 1/2 - 3 ml	1.0	1 1/2	1 1/2	3 1/2 - 4
Mastiff 120-180	12-16 ml	6 – 7	4	4	15 ml	3 1/2 - 4 ml	1.0	2	2	4 - 5

### Collection Directions:

**There is now a Plastic Zip Bag for the semen collection that replaces the old plastic cone or funnel.**

1. Have a sharp pair of scissors on hand.
2. At the top of the bag zip or pull off the sealed closure at the perforation just above the yellow wire band.
3. Open the bag and fold back the yellow tabs that stick out from the bag along each side of the bag.
4. Now fold or roll down over the outside of the bag the yellow tab with the wire stiffener one or two times like you would roll up the cuffs on your shirt at your wrist. This will make a softer entrance into the bag by covering the wire stiffener with more of the plastic of the bag and will also help keep the bag open.
5. When collecting the semen make sure the dogs penis dose not go into the bag more then half way as not to contaminate the lower portion of the interior sides of the bag from the mucus and bacteria of his penis. His mucus and/or contaminate will stick to the sides of the bag but will not run down into the semen you collected if you are careful.
6. After collecting the right amount of semen, hold the bag in front of you at eye level and let all the semen run into one of the two corners of the bottom of the bag. Never let the semen run above the lowest portion of the bag so it does not pick up any contamination from where his penis was during the collection.
7. With a pair of scissors cut off on a diagonal the other corner of the bottom of the bag and pore all the semen into the orange covered plastic centrifuge tube.

## INSEMINATION INSTRUCTIONS CLONE CHILLED SYSTEMS

1. When semen arrives, keep refrigerated, (**not in the freezer**) until ready to use. (at this pint the semen is in an almost motionless state)
2. One half to one hour before the insemination bring the semen and the activator (**the clear or blue solution vial**) out of the refrigerator and let them both come up to room temperature, and until they are both at the same temperature.
3. Prepare a water bath of approximately 1 gallon of water, in a container such as a Styrofoam box or bucket. Have the water or bath pre-warmed to 85 to 90 degrees F.
4. Add the activator solution slowly to the semen which is in the centrifuge tube at this time. (**when both are the same temperature**)
5. Put the cap back on tightly, then gently rock the semen tube back and forth a few times very slowly until all the solutions are mixed.
6. It's a good idea at this point to lower the semen tube so that the semen portion is submerged in the warm 85 to 90 degree water bath 8 – 10 minutes to activate the semen to almost normal temperature, speed and activity. At this point you may then examine the semen to check motility and forward speed of progression.
7. Assemble the breeding tube or rod to the syringe and draw the semen up the breeding tube into the syringe in one column with no air bubbles.
8. Inseminate the bitch in the elevated position, and keep her rear elevated for 8 to 10 minutes after the insemination is performed.

**P.S. NEVER WARM SEMEN ABOVE THE TEMPERATURE OF 90 DEGREES FAHRENHEIT. INSEMINATE THE SEMEN AS SOON AS YOU REMOVE IT FROM THE WATER BATH. INSEMINATE AT ROOM TEMPERATURE AND NOT IN ANY COLD AREA AFTER THE SEMEN HAS BEEN PREPARED AND WARMED.**