

BACKGROUND AND HISTORY OF PARSEMUS FOUNDATION RESEARCH ON FEMALE STERILIZATION USING ULTRASOUND GUIDED INJECTION

This is the story of how our research into whether ultrasound-guided ovarian injection could be used to nonsurgically sterilize female dogs and cats morphed into a related approach that we think will work much better: ultrasound-guided uterine injection for sterilization/contraception/treatment of female dogs, cats, and other mammals, including humans.

BACKGROUND AND RATIONALE FOR STUDIES

Dog and cat overpopulation is a problem worldwide, and only the wealthiest communities have been successful in getting ahead of the problem with surgical sterilization (castration for males and ovariectomy or ovariohysterectomy for females).

Since March 2010, Parsemus Foundation has been exploring whether the nonsurgical dog sterilization work using calcium chloride (“Calchlorin”) in males can be extended to females.

The use of calcium chloride dihydrate in solution with ethyl or isopropyl alcohol or ethanol to sterilize MALE dogs was reported in the literature as far back as 1977/1978 (e.g. Koger LM). An Indian team had been studying the technique (using calcium chloride dihydrate in normal saline) and publishing detailed studies from 1998 to present (Samanta, Jana, et al.). Based on their studies and the ones before them, calcium chloride injection (in alcohol or saline) appears safe and effective for sterilizing male animals. Parsemus Foundation has been helping these researchers bring their work to a wider audience, and supported new research to identify the optimal formula for male dog sterilization (see <http://www.parsemusfoundation.org/calcium-chloride-for-males/>)

If anybody has thought about the idea of using calcium chloride in the ovaries, it has been commonly assumed that it wouldn't work— for example, Parsemus Foundation's medical research director assumed that it would affect nearby organs if used in the ovaries, which are inside the abdominal cavity (unlike in the testes, which are quite separate from the rest of the body), and the Indian team using calcium chloride in males saw little point in trying to inject calcium chloride into the ovaries, believing it necessary to remove the uterus, not just the ovaries, to avoid health

problems. We have also been told categorically by a vet that the ovaries cannot be seen on ultrasound (ultrasound guidance would be the modern twist that we thought could make this work). However, when Parsemus Foundation explored the literature in March 2010, after the foundation's manufacturing and regulatory consultant asked whether the Indian team had ever tried it in females and expressed interest in that concept, four interesting facts emerged that, taken together, made it seem that such injection would work:

1) The ovaries are apparently somewhat separate from other organs in the abdomen, tucked away in the peritoneal cavity (from Wikipedia: "Thus, the ovary is the only organ in the human body which is totally invaginated into the peritonium, making it the only interperitoneal organ"), making it seem that ovarian injection could be better contained than imagined.

Nobody seemed to have tried injecting calcium chloride solution into the ovaries— but what about alcohol? Was alcohol, which could be one component of an injection, as it was in the 1978 Koger LM calcium chloride publication, being used to ablate anything besides testes and the foundation's initiative in breast ducts? And it turns out that yes,

2) Alcohol injection under ultrasound guidance is commonly used to ablate many types of tumors, such as hepatic tumors.

So, that was some indication that alcohol might work even in an internal area, without damaging nearby tissues. But had alcohol or injection been used any closer to the ovaries? No research was found (even back in the 1960's or 1970's) trying to use alcohol injection to ablate the ovaries, but we found that

3) Researchers in Egypt, in a 2000 publication, had carefully described a procedure for using injection of hot saline solution into the stroma (outer covering) of the ovaries to treat Polycystic Ovarian Syndrome. They were successful, and concluded that transvaginal ultrasound-guided injection of hot saline into the ovarian stroma in polycystic ovarian syndrome can be an office procedure under local (rather than general) anesthesia. They also describe a more modern way of injecting, with ultrasound guidance.

Then the foundation's medical research director thought about some other MALE dog sterilization research that was done in India in the 1970's, using CADMIUM chloride instead of CALCIUM

chloride. It took a long time for this work to be dropped– the original work was done in the 1970's, but it was still being talked about as a possibility in the 1990's. The foundation arrived at a definitive answer on cadmium chloride in males: it is nasty stuff with toxicity issues and side effects and there was no reason to use it instead of calcium chloride.

Would the researchers in the 1960's have tried cadmium chloride in females, too, while they were at it? And sure enough, they had! The Indian journals from the 1960's revealed that, yes,

4) CADMIUM chloride was successfully used in 1960's publications to sterilize female animals (Chatterjee and Kar).

CADMIUM chloride is not an acceptable candidate for a female sterilant, because of the toxicity and potential injection site reactions noted above regarding males, but it's interesting that it DID work for the 1960's researchers in females– apparently well enough that they did studies in several different species (guinea pigs, goats, and scrub cows). And they were already using transvaginal injection (using a specially-designed instrument), like the saline-injection-for-PCOS team in Egypt many years later, so it seemed that is a good approach (and the researchers in the 1960's were even able to manage to get the injection into the ovaries without ultrasound guidance, which is a good sign for practicality of use in communities without ultrasound available).

So, putting all that together, the foundation concluded that there is a good chance that calcium chloride in saline and/or alcohol solution would work to safely ablate the ovaries, given that

- this combination is being used in the testes, a similar organ from an evolutionary standpoint
- part of this combination (saline) has been successfully used to dissolve growths on the outside of the ovaries
- an injection technique to the ovaries is well-defined, both in a low-tech version for larger animals (1960's papers) and an ultrasound-guided version (2000 paper)
- a similar (but toxic) solution was tried successfully to accomplish the purpose in the 1960s

At that point Parsemus Foundation submitted a patent application on the ovarian injection approach to protect it from being patented by for-profit entities.

USE OF ULTRASOUND GUIDANCE

Unlike in the cow, however, in dog and cat the ovaries are too far from the vagina and the vagina too small (and the rectum is too small for inserting an arm and stabilizing the ovaries from the other side as is done in cows!), so we decided to take a dorsal injection approach (i.e. closer up by the back). This brilliant suggestion was the idea of Dr. Michelle Kutzler, who pointed out that in dogs and cats, the ovaries lie all the way up by the back and are attached there by the ovarian ligament.

(Of course, everything described here could also be done without the ultrasound guidance, by just making an incision to open things up and injecting under direct visualization, or with a minimally-invasive laparoscopic approach, but then you don't get the additional benefits of a completely nonsurgical procedure.)

The latest high-resolution ultrasound equipment will be needed to be able see the ovaries. (Vets who say the ovaries cannot be seen on ultrasound are probably trying to image them from the abdominal direction, in which there are too many other organs in the way, and they may have a lower-resolution \$10,000 ultrasound machine instead of the latest \$30,000 machine. This too was pointed out by Dr. Kutzler, who has remained a key part of the brainstorming process and the process of developing this method; it would not have gotten to where it is without her input.)

In June 2010 we confirmed that the ovaries are visible with this equipment. (*Footnote 1*). However, we ran into a roadblock: the needle was not visible! After trying fruitlessly to get it to show up on the ultrasound, we discovered the problem: we needed an echogenically-enhanced needle!

So several questions remained: would the ovarian suspensory ligament hold the ovaries in place enough that an injection will be possible by a skilled vet (and HOW skilled— will extreme skill be necessary)? Would the calcium chloride solution stay in the ovaries, or leak out and create problems?

OVARIAN INJECTIONS PROVE TO BE CHALLENGING

The foundation got a chance to further test ovarian injection the second week of December—in a goat cadaver, from the butcher. No ultrasound was available, but a clever suggestion from Dr. Sujoy K. Guha of India was used to attempt the injection with the belly open and the ovary identified. A small pump was used with a tube attached to it; the tube gripped hold of the ovary like the end of a vacuum cleaner tube to stabilize it, and then a needle was injected with a quick jab.

(As many people had warned us, it was indeed nearly impossible to inject the ovary unless Prof. Guha's suggestion of a quick jab was used; thanks are due to Prof. Guha and his engineering background for the jab suggestion.) Encouragingly, the ovary could be injected freehand even without the vacuum pressure holding it, as long as the jab was judged correctly. Less encouragingly, the dye that was injected didn't tend to stay completely in the ovary (although perhaps it was coming out of counterpunctures and previous holes made during the experiment). We also tried injecting the uterine horns with dye before finishing, just to see what would happen; it seemed to go in, although by that time there was enough dye everywhere that it was hard to tell what was what.

On December 16, 2010 the foundation got a chance for another test with ultrasound in a cadaver dog. (*Footnote 2*) The first ovary was visible on the ultrasound to our collaborator, who is a skilled ultrasonographer. And then, success: the echogenically-enhanced needle lit right up on the screen!

However, from there it got discouraging. The ovarian ligament was very stretched out, probably because the poor dog had just had a litter of 11 puppies (!), so it did not hold the ovary tight against the dorsal wall as it usually does in dogs. When our collaborator would go to insert the needle into the ovary, it would push and push against the ovary and then slip off. This could perhaps be addressed with a spring-loaded injector of the sort of design proposed by Prof. Sujoy K. Guha, but we did not have that available and were discouraged at the extreme toughness of the ovarian capsule. We did find, upon opening the abdomen, that when holding the ovary it is somewhat easier to inject into its head or tail than the middle of the body, probably because one is injecting in line with the fibers instead of across them. However, it wouldn't be possible to do that under ultrasound guidance—you can't see enough detail to know where the head or tail is, and you wouldn't necessarily be able to get a needle lined up that particular direction anyway.

One encouraging thing was that on one ovary, the dye got out one of the holes we had made and went into the bursa (the sack around the ovary), and it seemed to stay quite well in the bursa. So that is encouraging—that the bursa may protect the rest of the body pretty well from an ovarian injection.

However, what was not encouraging was that on the other side, the injection seemed to have entered blood vessels in the ovary, and you could see the dye fanning out from the ovary several inches through the blood vessel system. Normally this would not happen in a live dog, since there

would be pressure in the blood vessels. Unless the injection were made with more pressure than is in those blood vessels, the pressure in the blood vessels would beat back the injection fluid. But it was still a little bit of a concern, given that those blood vessels carry their contents straight to the heart. Researchers wishing more information on these results in order to avoid duplication of effort and unnecessary experimentation are welcome to contact the foundation.

In this dog, our collaborator was not able to distinguish the second ovary on ultrasound, despite a high skill level. It would be easier in a live dog, because the motion of other organs makes the ovary easier to distinguish from them; but this also was discouraging.

INJECTING THE UTERINE HORNS

Finally, towards the end, we tried something else before finishing: injecting the uterine horns just to see, since the ovaries and uterus were already exposed.

And this was where our collaborator's experience came in particularly handy. She pointed out that the dye was going in quite nicely. It was not difficult, even for a less experienced hand, to get the needle into the lumen of the ovarian horn, and to tell when it was inside and even move it several inches; the feel of it moving freely within the lumen (as opposed to hitting the walls) is quite distinct. We injected dye, and it stayed perfectly within the ovarian horn. Quite a bit can be injected, too: we injected probably 0.4 ml, and there was room for more.

Could this be a nonsurgical sterilization? We think so. Our collaborator pointed out that veterinarians routinely do intrauterine insemination by doing uterine injection of the semen after making a small surgical hole so they can view the uterus. Is the recovery time for that intrauterine insemination process quicker than for a spay? Apparently substantially so. And according to Dr. Kutzler, any reasonably-skilled ultrasonographer should be able to do a uterine injection. So, in that case, why don't vets with ultrasound machines and advanced skills offer their IUI clients an ultrasound-guided insemination? And if so, why wouldn't someone have thought to do that for some sort of injectable sterilization? Because according to Dr. Kutzler, there is no need to do ultrasound-guided IUI; skilled veterinary reproductive specialists such as her are quite handy with threading a catheter through the cervix and inserting the sperm that way. So they don't need to do an ultrasound-guided procedure. (Unlike most of the time, when a dog is ready to be inseminated the cervix becomes open. At other times, the dog cervix is extremely difficult to get through; Prof. Sujoy K. Guha appears to be the first one, in a poster presented at the 2010 conference of the

Alliance for Contraception in Cats and Dogs, to design a device and system that will get through the non-estrous dog cervix.)

So, IUI vets don't need to do ultrasound guidance, because the cervix is open for a short while and they can go that route instead. But, the foundation's director asked, could one use ultrasound-guided uterine injection for sterilization the rest of the year, when the cervix is closed? Dr. Kutzler says there's no reason why not. In her words December 17, 2010: "Yes, I don't see any reason why a skilled ultrasonographer could not administer an injection into the uterus. There has just never been a reason to do so."

Well, now that we've put two and two together, there's definitely a reason to do so! So we planned to test this further in the coming weeks and months.

INTRA-UTERINE STERILIZATION INJECTION CONCEPT

There are several substances which could potentially be used with this ultrasound-guided intrauterine injection/sterilization/injectable contraception (UG-IUI / UG-IUS / UGIUIS / UGIIS or UGIIC) technique. One is calcium chloride which has been used as a successful male intra-testicular sterilant. One is alcohol, such as that used to ablate tumors (e.g. liver and pancreatic tumors). Others are polymer contraceptives such as Vasalgel (being developed in the US by Parsemus Foundation), RISUG or a variant on RISUG such as FerroCept (developed by Prof. Sujoy K. Guha of the Indian Institute of Technology).

The advantages of calcium chloride solution and alcohol are that they are readily available (calcium chloride 10% in saline is a standard emergency room item), and they would probably destroy the endometrium (the uterine lining), both preventing the nuisance of dogs' heat bleeding and reducing the chance of uterine lining infection (pyometra). In humans, this could reduce or eliminate menstrual bleeding and be similar to the effect of endometrial ablation. It might be a good idea to mix a thickener with the calcium chloride solution or alcohol; one would want to be sure it didn't get all the way out the cervix into the vagina. The advantages of RISUG or FerroCept are that they are antibacterial, so they might also reduce the risk of uterine infection too; they are more viscous, so they would probably be easier to place along the lumen of the uterine horns and stay put; they have active sperm-killing properties, so they wouldn't depend on blocking every bit of the

way to be reliable; and they might be reversible like they are in male animals (in the vas deferens in males, RISUG can be dissolved and flushed out with an injection of its solvent).

So sometimes failure leads to success! We hope that is the case here. As we were warned, ovarian injection was indeed quite difficult (although not always for the reasons people told us it would be; and nobody warned us that it would be the hardness of the ovarian capsule that would finally make us admit defeat). But out of it came a more clear focus on the possibility of ultrasound-guided uterine injection.

Uterine injection is not a brand new idea. Professor Sujoy K. Guha of the Indian Institute of Technology had considered the idea of injecting FerroCept into the fallopian tubes in women—perhaps with laparoscopic visualization of the fallopian tubes—in case regulators end up preferring that approach to the transcervical approach. But Parsemus Foundation’s director always assumed that for the dog and cat work it would be better to go after the ovaries, because in dogs and cats if you leave the ovaries you still have potential problems with mammary tumors and pyometra. The health negatives of removing the ovaries generally outweigh the health positives especially in big dogs (the only items in the positive column are mammary tumor and pyometra reduction), but that is not the generally accepted understanding among the public in the U.S. after decades of animal lovers successfully encouraging ovariohysterectomy sterilization. We are pursuing this idea anyway because we think it will be effective in street dog situations—most street dogs will not live long enough to get mammary tumors and pyometra—and as an option for the segment of the U.S. population which is still accustomed to heat cycles, may consider that more “natural,” and represents the largest unmet need for spay in the U.S. Also, of course, because of the potential applicability to sterilization and contraception for human women.

We will be exploring collaboration with researchers to test ultrasound-guided intrauterine injectable sterilization (UGIS), either by sponsoring pilot studies or by bringing it to the attention of larger funders, or both. We encourage veterinary researchers to contact us who might be interested in trying this technique, as there are some details to be aware of (for example, it is important to use an echogenically-enhanced needle). We think it would be appropriate to test this procedure in cadavers (animals already euthanized due to cancer or other illnesses) so that skill levels are high before seeking approvals to test it in living animals.

Update February 9, 2011: We've decided that uterine injection is pretty hard, though we haven't entirely given up. So on Monday this week we tried a different approach: transcervical injection of a sclerosant (in this case, Sotradecol) on a dog scheduled for euthanasia due to aggression, tumors, and age. It went in well, although we didn't give ourselves the full challenge, since she was in heat and her cervix was very open. She's doing well and is happy— as if nothing had happened. The procedure didn't even require sedation (although it likely would in a dog that wasn't in heat, since they are not so open and receptive).

Update November 2011: The sclerosant was well-tolerated, but didn't seem to do much of anything to the uterine lining (perhaps because of the coating of heat blood). Dr. Jeffrey Jensen at Oregon Health Sciences University is trying a similar approach with a similar sclerosant, Polidocanol, in the Fallopian tubes in monkeys, with more encouraging results. But we're not so hot on the sclerosant idea anymore after seeing the lack of effect in the one we tried. We think the polymer approach, such as polymer in the Fallopian tubes as is being tested by Prof. Sujoy K. Guha of the Indian Institute of Technology, Kharagpur, or in the uterus as we have been testing (with Prof. Guha's help and guidance), is more promising. We've mixed the polymer with silver-coated hollow glass microbeads (Cospheric LLC) to make it show up on ultrasound. More information is available in the prior art declaration.

FOOTNOTES

1- Using a cadaver dog—a dog which had already been euthanized because of failing the temperament/aggression test for adoptability and being rejected by the rescue organization for its adoption program.

2- Using a cadaver dog—a dog which had already been euthanized because of overcrowding at the shelter and being rejected by the rescue organization for its adoption program—one of the 4 million dogs and cats which are euthanized in shelters every year. This figure used to be 12-20 million per year, and we hope that our work will help make it someday much, much lower. To quote the Humane Society of the United States: "In the 1970s, American shelters euthanized 12-20 million dogs and cats, at a time when there were 67 million pets in homes. Today, shelters euthanize around 4 million animals, while there are more than 135 million dogs and cats in homes.

This enormous decline in euthanasia numbers—from around 25 percent of American dogs and cats euthanized every year to about 3 percent—represents substantial progress. We will make still greater progress by working together to strike at the roots of animal overpopulation.”

This information was added to the prior art database at IP.com (www.PriorArtDatabase.com) on December 21, 2010 so that it is in the public domain. Researchers may study this approach without barriers now, as it is no longer patentable. Permission from Parsemus Foundation is not required.