

New Advances in Assisted Reproduction

OFA President Frances Smith, DVM, PhD, DACVP, of the Smith Veterinary Clinic discussed three aspects of canine reproduction, beginning with the use of drugs to induce estrus. Previous induction regimes “were very, very ineffective, and many of them were dangerous and resulted in pyometra, rather than pregnancy,” she said.

The main treatments were gonadatropins like FSH and LH, pregnant mare serum gonadatropin, and estradiol compounds like DES.

The bitch has a relatively unique reproductive cycle, characterized by a period of progesterone stimulation, or diestrus, which lasts 63 days in the pregnant animal, even longer with no pregnancy. By contrast, the greatest inroads in reproductive medicine have occurred in species that cycle more frequently—a lot is known about human reproduction, and cattle which cycle every 21 days.

Drawing on research by Margaret V. Root-Kustritz, DVM, PhD, Dr. Smith traced hormone levels at different stages in the reproductive cycle, before listing two new groups of drugs that appear effective in manipulating the cycle: dopamine agonists, and gonadatropin-releasing hormones. The first group acts by suppressing prolactin secretion, which appears to affect FSH, LH, and the way the ovary responds to them. The second can be released in minute amounts through an implanted pump to induce a safe heat, although the pump is essentially a laboratory mechanism and costly to install.

The drugs have pros and cons, Dr. Smith said. The advantage is that they can be used to manipulate the reproductive cycle, to schedule litters around national specialty events or hold off estrus for long periods of time. But there are problems, as well: None of the drugs are approved for use in dogs, and the only one that is commercially available in the United States is a compounded drug, so that users must be warned that it may not be effective or safe.

“Those are big issues,” she said. “If I were to use this on a client’s dog, I would have them sign a release saying that based on endocrine knowledge and based on my belief that this drug does contain what I think it does, we have a pretty good shot at having your bitch cycle normally, conceive, and whelp a litter.” But the drug could not be used without a release.

The treatments are also expensive, ranging from \$130.00 to \$770.00 per treatment for a 70-pound dog, not including shipping and veterinary fees. Some require 30- to 40-day courses of daily treatment, and the least expensive option, Buserilin, has a pregnancy rate of only 20%.

Deslorelin offers a 62.5% pregnancy rate with one injection, at a cost of \$250 for the single dose. Dr. Smith said she was trying it out with one of her bitches, and had recently called her clinic for an update. “What I can also tell you is that she achieved near-ovulation, she showed no external signs of heat, and the boys had not a clue that she was near ovulation. So this has been a treatment failure so far. The good news is that it did not make her ill in any way.”

Breeders can also use low-dose prostaglandin 15 minutes before collecting sperm from stud dogs that are reluctant or have low sperm counts. The drug is quite safe, but users should not ask to bring it home. Prostaglandins are a dangerous class of drugs that can be fatal to both handlers and dogs if they’re not used properly.

Embryo transfer is another exciting option. The difficulty in canines is that both the donor and the recipient must be within about a day of ovulation, but the procedure can be and has been successful. Synchronization can theoretically be used to facilitate the transfer, but the other option is to maintain larger breeding colonies with many bitches at different stages in the cycle.

For canine embryos in vitro, the limiting step is the maturation of non-ovulated eggs, or oocytes. The eggs can be collected at any point in the cycle, though the procedure is simplest and most straightforward when the bitch is already in estrus. In Japan, a research team at Tottori University harvested mature oocytes from bitches with pyometras. The procedure has a low success rate, but is still promising for a breeder with a spectacular bitch that must be spayed.

Canine cloning has been attempted, but Dr. Smith described one very expensive effort that was unsuccessful, as well a successful clone in South Korea that required three years, 123 embryo transfers, and enough money to take the work beyond the point where it was financially feasible.

Dr. Smith said the central question is whether breeders should adopt new techniques, just because they can. "In my reproductive practice, the biggest single reason for failed conception is still poor timing," she said. "Most of our dogs are still very normal. We just don't get them together at the right time."

A participant asked about treatment options for bitches whose progesterone levels drop during pregnancy, so that their litters are lost. Dr. Smith said premature luteolysis was suspected for some time but can be proven now, thanks to the use of ultrasound. Researchers don't know whether the problem is caused by infection or genetic issues, and the cause will have to be identified before breeders can decide whether to treat the event. Breeders who opt for treatment can use Regumate, a synthetic progestational agent designed for horses. The product carries the risk of masculinization of female fetuses, leading to "puppies who look like little girls but internally don't have normal reproductive organs," but the risk to the bitch is minimal.

Another attendee asked about the best timing for implanting frozen semen. Dr. Smith said the ideal is to start from the day that the progesterone reaches a level of 2, follow the levels daily, and breed four to six days later, usually on day five. But the specifics will vary from one animal to the next.

A participant warned that some reproductive problems can be 30% heritable, and that breeders should consider whether they want to pass those characteristics on to future generations. He added that falling progesterone levels may not always dictate treatment: sometimes, a bitch with low levels will abort one puppy, then carry the rest to term.

In response to another question, Dr. Smith said the sole bitch puppy in a normal-sized litter often looks very mature, but never cycles. The suspicion is that the female puppies are influenced in utero by placental leakage of male hormones. She said she would welcome a research grant proposal to the CHF to try to confirm this clinical observation.

A participant asked Dr. Smith whether she prefers pellets or straws for artificial insemination. Dr. Smith replied that pellets are the most biologically appropriate way to freeze sperm and offer 10% better sperm motility in good dogs, but can be more difficult to identify than straws. Freezing ovaries to harvest the oocytes requires advance planning, and the procedure depends on a university or some other facility that is set up to conduct embryo transfers. "The boys are much more simple because of the availability of the sample," she said, though male canine infertility is far more common than many people appreciate. She urged breeders to collect sperm as soon as their dogs are sexually mature, and audience members who had bred older dogs agreed.