

Health: Dreams of Alice in a Wonderland

By: Catherine Emanuel and contributing article by D. Caroline Coile, Ph.D

Awhile back, an active Club member had requested that the ASDCA consider publishing several articles by Dr. Caroline Coile as they applied to health. Her works have been published in many dog magazines, i.e. DogWorld and Dog Fancy. Dr. Coile was generous enough to allow us to use her articles; however, some are definitely more applicable than others. The article that we have selected for this issue of the Times, is very interesting to me as I recently had an experience that is very applicable to the article and with an Anatolian. In order to give this article more meaning to the Anatolian world, I have incorporated some of my experiences into the text. Please note that my additions are italicized.

Who's Your Daddy?

D. Caroline Coile, Ph.D.

Maddie's puppies have three daddies. Or at least, three possible daddies. If they were all on the Jerry Springer show, it would be time for the boys to deny ever touching her and blame the situation on another loose dog. But Maddie isn't a loose dog with loose morals. Maddie is DC Gavril Blonde Ambition, SC, LCM3, an overachieving show and lure-coursing Borzoi whose breeder-owners, Michael and Shirley Rehberg and Rachel Rehberg Gongre of Lake City, FL, bred to three different sires. At once. On purpose.

Alice was a retired show Anatolian Shepherd. She was never considered for breeding until she over five years of age due to timing conflicts. We didn't want multiple litters and her heat cycle always coincided with other bitches. Before we knew if we looked and Alice was five. From the time Alice was conceived, we knew that she would eventually be bred back to one of our dogs that had been dead for many years. Sadly, due to our error in judgment (waiting to breed her), we decreased the chance of her obtaining a viable pregnancy. After the age of five we decided that we couldn't wait any longer. We induced her heat cycle using a cervical implant; however, due to an unfortunate accident Alice was injured and the implant was not removed on time, nor completely due to the breakage of the implant. At this point we were advised to wait and see if she even has a normal heat cycle due to the implant breakage. It could have delayed her cycle or even worse prevented Alice for cycling again. Now Alice was six. Tic-tock. Tic-tock.

Her next heat came towards the end of her sixth year. From the perspective of fertility, breeding a bitch for the first time at almost seven significantly decreases the odds of attaining a regular pregnancy. We worked with our reproductive specialist to try to make our dreams happen. Alice and Cody. Now, Cody's semen was collected back when the technology was new (80's) and it was collected just prior to his death. So after further consultation and semen analysis we realized we were dealing with a poor semen sample and not a lot of it.

We weighed the risk of losing the semen on a possibly infertile older bitch and realized the risk was too great. So we opted for a live breeding to prove her fertility and maternal instincts, as well as produce a quality litter. She was bred twice, whelped five healthy puppies with ease and demonstrated excellent motherhood instincts. At this point Alice was almost eight and understanding the risk of losing our semen samples planned to breed her during her next heat (when she would be most fertile) to Cody. This was done under the supervision of a Board Certified Reproductive Specialist. While we wanted the breeding to take, Alice's well-being and health was paramount and never rated second to breeding. Tic-tock. Tic-tock.

Just a decade ago such actions would be blasphemy. But now more breeders are acknowledging the usefulness—and responsibility—of multiple-sire litters. Shirley Rehberg explains: “We chose to use multiple sires with Maddie because she was 8 years old and we felt her age would preclude any future breedings; in addition, she would not breed naturally, and although she had been bred unsuccessfully by artificial insemination several times, we decided to try a uterine implant. Since this was our last attempt, we elected to use multiple sires as well.”

Besides giving breeders a last ditch chance to try for those litters their bitch will never have time for, multi-sire litters have other advantages. Many hound breeds tend to produce larger litters than there are good hound-savvy homes available. Many of these same breeds are losing bloodlines because breeders just can’t breed every litter they would like to. Multiple sired litters give breeders the chance to incorporate more genetic variety in one litter, often allowing them to achieve in one litter what may have taken three litters to achieve before.

Alice was older than we had wanted and we were using semen that was not of the highest quality. So we weighed many factors. Did we want Alice X Cody puppies? Yes. Did we want another litter out of Alice? Yes. Which was more important to us? At this point we wanted another litter out of Alice. Cody would have provided us with an outcross, but we had other options to achieve the same goal. We had semen on one of Cody’s progeny. This semen sample was of similar quantity, but much better quality.

The Rehbergs used three males—one a self-colored, one a tri-colored, and one white with spots—with Maddie, who is also white with spots. All of the males were collected by Beverly Brimacomb, DVM, of Lakeland, FL, who also performed the implant. They were rewarded with a litter of all self-colored pups plus one tri-colored pup. The Rehbergs could give an educated guess about each puppy’s sire based on color, but only a paternity test could verify who was whose.

We decided to use both Cody and one of his sons to increase our semen sample and increase the chance of Alice conceiving. By doing this we hoped that one of the puppies was out of Cody, but really we just hoped for a healthy pregnancy.

DNA and the AKC: Just as DNA is used to settle human paternity suits, it can be used to settle canine paternity questions. By comparing the DNA of the dam, puppies, and each of the possible sires, each puppy can be attributed to its biological father. The AKC approved the use of DNA paternity testing for multiple sired litters in 1998.

It’s not just planned breedings, of course. Accidents do happen, and with DNA testing, the resulting puppies no longer have to be sold as unregistered pets—or registered illicitly based on guesswork and wishful thinking.

DNA parentage testing makes matters dicey for the less than scrupulous breeders out there: AKC can randomly inspect large-scale breeding operations to determine if their pedigrees are accurate by comparing what pedigrees claim and DNA disclaims. By 2002 more than 13,000 litters had been tested. In 2002, 94% of the litters tested had correct parentage, and of those that were incorrect, 27% were corrected using DNA parentage testing.

DNA testing also allows the AKC to respond to complaints regarding suspicions that parents or sires are not as alleged. Does that puppy you paid big money for resemble the breeder’s pet bitch more than their top-winning bitch? Did your bitch whelp a litter that looks more like the stud owner’s over-sexed youngster than his older dog? Did a bitch owner claim her bitch was covered by her own dog once you sent her home, and that your stud is not the sire of her litter? Now you can demand to know the truth. But it will cost you if you’re wrong. To discourage frivolous allegations, a written complaint and \$500 deposit (returned if the charges are validated) must be submitted by the complainant.

Prior to making this decision we were forced to reconcile one minor glitch. Both stud dogs selected died prior to the use of DNA analysis in dogs. Actually, both dogs died prior to AKC acceptance. So we had to determine if we could analyze the DNA of the hopeful puppies when we did not have DNA collected on either sire. This led us to the AKC/DNA monitoring office. They guided us to a laboratory that had a current protocol to analyze the semen and determine parentage of the individual puppies after birth. We were now ready.

Costs: Planning a multiple sire litter does require extra budgeting. The logistics of natural breedings or fresh artificial inseminations to more than one stud can be difficult, and the expense of uterine implants can be comparatively high. If chilled or frozen semen is used, the bitch owner must bear the costs of shipping and possibly collecting the semen— from more than one stud! DNA profiling costs about \$40 for each dog, and must be done for the dam, all puppies, and each possible sire. The AKC will analyze the results for you for \$20 plus \$5 per additional sire. If additional markers are needed in the case of ambiguous results, it costs an extra \$20 per dog. The AKC charges \$200 to register litters from more than one sire on top of the regular registration fees for each litter (each different sire-dam combination counts as one litter). Stud fees are a private matter, but could become complicated if every contingency isn't planned ahead of time.

In our case, we were not paying stud fees. However, we were paying for the progesterone testing, the implantations and due to the DNA having to be analyzed from the semen samples the cost of analysis would have been significantly increased. Unfortunately, we were not as successful as the Rehbergs. Alice never conceived. We did use an unrecoverable semen sample, which can not ever be given a dollar equivalent.

Is it worth it? "I imagine that we will use multiple sires again," says Rehberg. "When we breed, we are doing it for ourselves and never advertise puppies for sale. By using multiple sires, we can breed a bitch just once instead of twice and have fewer puppies than had we bred two litters. There's less risk for the bitch with one litter, and let's face it, our dogs are first and foremost our pets, so reducing risks to health is probably the primary reason we will do it again. "Having a litter of puppies is a lot like opening Christmas presents— you're never sure exactly what you're going to get in terms of color and sex, but having a litter of multiple sired puppies is like opening Christmas presents when you have to figure out who they're from. We look forward to our next multiple sire litter!"

Would we do it again? We took a risk and that came with consequences regarding the loss of the semen. However, we knew that Alice's age was working against us. Luckily, we still have a happy and healthy Alice. The process was one that was well-planned heavily calculated. The financial investment was challenging, but it would weed out anyone that does not fully-vest themselves in their litters. Yes. We would try it again, especially on an older bitch. However, we probably wouldn't use frozen semen on a bitch older than five again, unless we had a large quantity. The benefit of being able to create the genetics of two crosses without risking the bitch twice truly makes this process a viable option. After all, Alice and our other girls are family. Anything that can be done to decrease the risks to our dams should be considered.

Sidebar: Multiple-sire testing is as easy as 1-2-3

Here's how it works: A cheek-swab sample is collected from each dog and submitted to the AKC for DNA profiling. The DNA profile is a collection of genetic markers, which are

segments of DNA that have many different forms in the dog population, and which are reliably passed from parent to offspring. Each parent has a pair of possible markers at each marker location, and each parent randomly contributes a copy of one of those markers to each offspring. This means each puppy also has a pair of markers, one inherited from the sire and one from the dam, at each location. One marker can easily be traced to the dam; the remaining marker must match one of the proposed sires or that male is unlikely to be the sire. Say the dam's markers at one location are AB, and the sire's are BC. Puppy #1 is AB, puppy #2 is BB, and puppy #3 is BD. Puppies 1 and 2 could have been sired by that sire, but puppy #3 could not have been because the sire had no D marker to contribute.

But not so fast. It's possible that puppy #3's D marker arose from a mutation, especially if the D marker is qualitatively similar to the sire's C marker. It's also possible that puppy 1 and 2 had the same markers as the sire through coincidence. Thus, decisions are never made on the basis of a single marker. That's why the AKC expanded its DNA profile to 14 markers last year. At least two non-matching markers must be present to exclude a dog as a possible sire, and that decision also depends on how qualitatively different the markers are.

The plan seems simple enough if none of the sires are related, but what if they are brothers or worse, highly inbred brothers? Two of the sires the Rehbergs used were closely related, but the DNA profiles had no problems differentiating between them. Only rarely has a situation occurred in which the AKC's former 10 marker test produced inconclusive results with closely related sires. In those cases the use of additional markers cleared things up. Now that the AKC regularly uses 14 markers such situations should be even more rare.

How did DNA testing work for the Rehberg's multiple sired litter? Rehberg reports that determining the sire of each puppy with the DNA profiles was simple and the results were what they predicted based on colors: the tri-colored pup was sired by the tri-colored sire and the rest were sired by the self-colored sire, with the spotted sire not fathering any pups.

--parts of this article previously appeared in *Dog World* magazine