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SUBFERTILITY IN STUD DOGS

Study Evaluates the Effects of Age on Sperm Quality



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GREAT DANES ARE FOCUS OF LARGEST MALE FERTILITY STUDY IN A SINGLE BREED

A powerful, muscled fawn Great Dane named “Logan” (BISS CH Surfside Lera Wave Ryder AOM) sired 11 champions out of 27 puppies whelped from his early natural breedings. At age 5 when he could have been earning points



A Best in Specialty Show winner, “Logan,” photographed at 7 years of age, began having fertility issues when he was 5, an age generally considered the prime for breeding male Great Danes.

for Top Stud Dog, Logan was no longer able to produce puppies.

Vicki Kimbell and her late husband, John Kimbell, of Sierra Vista, Arizona, lamented the premature loss of fertility in their handsome male. “Logan passed

on to his offspring beautiful fronts, and they had a true shoulder layback even when he was bred to bitches that didn’t have this,” says Kimbell, a longtime owner of Great Danes.

“As more people wanted to breed their bitches to Logan, we began using fresh chilled or frozen semen,” she says. “The semen quality as he got older was getting poorer and poorer. I describe him as having ‘delicate sperm.’ When it was apparent that he was not able to reproduce, I had to turn people away from breeding to him.”

Joy DeGruccio of Costa Mesa, California, was a co-owner of Logan. A longtime breeder of Great Danes under the Tydwind prefix, DeGruccio, who is a trustee of the Great Dane Club of America (GDCA) Charitable Trust, learned that other stud dog owners were likewise experiencing early reproduction problems in their dogs and were concerned about the emerging health issue.

“Our dogs hit 5 years of age and start having fertility difficulties,” DeGruccio says. “We have no idea why this happens. They should be in their breeding prime at that age.”

Volunteering to research studies of stud dog reproductive health

for the GDCA Charitable Trust, DeGrucco found an expert at the University of California-Davis. Stuart Meyers, DVM, PhD, DACT, professor of anatomy, physiology and cell biology, specializes in male fertility and sperm biology. His work evaluating the semen of healthy male Labrador Retrievers in the breeding program at Guide Dogs for the Blind in San Rafael, California, was the first comprehensive study for a single breed to evaluate canine semen. The research conducted from 2015 to 2017 [was funded by the AKC Canine Health Foundation](#). The findings were published in July 2019 in *Theriogenology*.

DeGrucco reached out to Dr. Meyers to learn if he would do a fertility study in male Great Danes. Dr. Meyers welcomed the opportunity to continue exploring factors that affect sperm function in dogs and how they differ among breeds and age groups.

“Extrapolating between breeds and age groups is challenging because the normal distribution of semen and fertility characteristics varies among breeds,” Dr. Meyers explains. “This is a study of sperm quality. We do not know how fertile these males have been, but this is an important first step in sorting out how factors underlying sperm function can drive age-related sperm quality losses in Great Danes.”

With funding support of \$150,000 from GDCA and the GDCA Charitable Trust, Dr. Meyers and his team began collecting semen samples. They drew 25 semen samples at the 2018 GDCA National Specialty in Topeka,

GREAT DANE CLUB OF AMERICA CHARITABLE TRUST HELPS FUND MALE FERTILITY STUDY



The Great Dane Club of America (GDCA) Charitable Trust, working closely with GDCA, is a significant source of funding for research that helps to advance the health and well-being of Great Danes. The male fertility study featured in this issue of the *Great Dane Update* is an example of the two organizations working together to fund important research.

GDCA president Jason Hoke notes that male fertility issues have increased over the past five years. “Back in the ‘80s and ‘90s, it was rare for males to have a fertility issue unless the dog had an infection,” he says. “Now, it is not uncommon to see dogs that have been tremendous show winners suddenly become unable to reproduce. The Great Dane community owes it to the breed to work together and sort this problem out so we can understand what happens as males age and what can be done about it.”

Founded in 2002, the nonprofit GDCA Charitable Trust also supports breed rescue, helping to rescue and rehome 1,800 Great Danes in 2018, and breed education. A disaster relief fund was begun in 2018, and a college scholarship program provides financial awards to young people involved with Great Danes.

Great Dane breeders and owners are encouraged to contribute to the GDCA Charitable Trust. To learn more, please visit gdca.org/charitable-trust/trustees/.

Kansas, 25 samples at the 2019 Northern California Great Dane Specialty Show in Lodi, and 50 samples at the 2019 GDCA National Specialty in Virginia Beach, Virginia. Owners received a complete analysis of their individual dog’s semen quality report.

The study is the largest male fertility study conducted in a single breed. The aim is to determine whether there is a relationship between a dog’s age and sperm parameters that determine his viability as a reproducer. “As a giant breed, Great Danes age rapidly and have a relatively short life span,” Dr. Meyers says.

“When males become subfertile in middle age, years before they should, it is concerning.”

DANE FERTILITY STUDY

“The production of sperm is a dynamic, constantly occurring biological process,” Dr. Meyers explains. “Any semen sample we assess is considered valid only for that day and that time of collection. Many factors can alter sperm production. Since it takes two months to make a sperm cell, factors such as fever, trauma and infection can manifest after we assess a given sample.”

The decline in male fertility that occurs with aging is well-known, he says. “The degree to which male fertility declines is highly dependent on the individual male and is likely linked to a genetic influence,” says Dr. Meyers. “This decline in fertility may cause lower sperm numbers, lower sperm quality and declining libido.”

Dr. Meyers and his team are evaluating these measures of



Sperm morphology, the size and shape of sperm, is used to help predict a dog's fertility. Dogs with normal sperm quality typically have at least 60 percent or more morphologically normal cells in an ejaculate.

sperm health and how they relate to a dog's sperm production capabilities:

- sperm motility, the total number of moving sperm and the progressive motility of sperm moving in a straight line
- sperm morphology, the size and shape of sperm, which reflects the sperm manufacturing process inside the testicles
- testicular volume, or sperm density
- DNA damage
- velocity, or the speed at which sperm travels

Although testicular volume generally correlates with total sperm count, this was not a predictor of the total number of sperm in Great Dane ejaculates. Additionally, analysis of the Great Dane sperm samples showed a significant loss of total sperm motility with aging.

“There is an approximate 8.35 percent decrease in total motility with every one year of age in the Great Dane males we've studied,” Dr. Meyers says. “This means that as a male ages, there is a decrease in the ability of his sperm to reach the oocyte and fertilize an egg resulting in pregnancy. Increasing age is also associated negatively with sperm concentration and progressive motility.

“These age-related decreases in motility parameters and sperm concentration highlight a progressive reduction in the fertilizing potential of Great Danes. Exactly how they impact or affect breeding success is not known.”

As dogs age, there is less lateral sperm head motion, a process

TIPS FOR OWNERS OF STUD DOGS

- Semen evaluations should be done annually to assess a stud dog's fertility. More frequent evaluations are advised if a dog misses with one or more bitches or goes from having normal-sized litters to small litters. A semen evaluation also is warranted before a chilled semen breeding or before freezing semen.
- A breeding soundness examination (BSE) should be performed annually to monitor declines in semen quality or libido. A BSE documents semen quality, libido, and testicular and penile health, and includes an assessment of sperm morphology and motility. It also establishes a baseline to track changes in an individual dog. An abnormal result should be repeated in two to three months to give the testes time to make new sperm.
- Stud dogs should be current on vaccinations.
- Good body condition, defined as having a 4 or 5 rating on a 9-point body condition score, is important for health and vigor.
- Brucellosis testing is recommended every three to six months for males that are bred frequently, particularly in areas of the country known to have the bacterium *Brucellosis canis*. Brucellosis is a highly infectious, devastating disease and a leading cause of infertility in dogs.

whereby the sperm head moves back and forth laterally that is thought to be important for penetrating and fertilizing an egg. Sperm use reactive oxygen species (ROS), also known as oxygen-free radicals, to make the energy molecule adenosine triphosphate (ATP) that powers the sperm's flagellum and propels sperm toward the eggs for fertilization. This is what occurs just after mating inside the female's genital tract. During this process, sperm can over-produce oxygen-free radicals, which can damage sperm cell membranes and contribute to infertility.

In Great Danes, there is a reverse ROS relationship, according to the findings. "We attribute high ROS levels in Great Danes with greater total and progressive sperm motility. This positive relationship between ROS and motility highlights a delicate balance between fertility and cellular damage," Dr. Meyers says.

"As we gain better understanding of how these factors influence sperm function, we can look at possible effects from genetic background, coat color and environmental factors such as diet and toxins," he says.

ENVIRONMENTAL EFFECTS

A U.K. study conducted from 1988 to 2014 reported on the potential effects of environmental chemicals on stud dog fertility. Investigators at the University of Nottingham found a significant fallout in sperm quality and a concurrent increased incidence of cryptorchidism, or undescended testicle(s), in males across all ages over the 26 years of the study.

Published in August 2016 in [Scientific Reports](#), the study indicated a possible relationship between environmental toxins and the decline in canine semen quality. Noted particularly were the effects from diethylhexyl phthalate (DEHP), a substance added to plastic to increase

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flexibility, and polychlorinated biphenol 153 (PCB-153), a manmade chemical used commercially until 1977 in insulation, coolants, lubricants, and electrical equipment.

Breeds included in the study were Border Collies, Curly Coated Retrievers, German Shepherd Dogs, Golden Retrievers, and Labrador Retrievers. The researchers evaluated the morphology and progressive motility of the stud dogs' sperm and found environmental contaminants in their sperm and testes that directly affected sperm function and vitality.

The investigators associated a widely reported [significant decline in human semen quality over the past 70 years](#) from exposure to environmental chemicals during development with evidence that [canine testicular cancer over the past 40 years has increased](#) in parallel with the changes seen in humans. Importantly, since dogs share our environment and develop similar diseases such as testicular dysgenesis syndrome, which includes cryptorchidism coupled with declining sperm quality, this provides a One Health opportunity in which dogs may contribute valuable information about environmental influences on fertility in men.

Meanwhile, Dr. Meyers' research of the Labrador Retrievers in the breeding program of Guide Dogs for the Blind found declines in progressive motility and morphologically normal sperm in senior dogs 7 years of age and older compared to young dogs

from 1 to 3 years of age or middle-aged dogs from 4 to 6 years of age. Velocity also decreased with increasing age and was lower in chilled semen compared to fresh or frozen semen.

"Overall, the magnitude of changes was small given that our sample consisted of healthy stud dogs relative to what might be expected from dogs with significant fertility problems," Dr. Meyers says. "Our hypothesis was that oxidative damage to sperm membranes and mitochondrial DNA could provide another tool to help assess fertility and longevity of sperm.

"Oxidative damage occurs during spermatogenesis inside the testis because of exposure to oxygen and oxygen-free radicals that result from mitosis and meiosis in the testicular tubules. It also can occur when sperm is being processed for transporting or cryopreservation. As dogs age, the process of mitosis, or cell division, becomes more error-prone and the cellular mechanisms of sperm formation become less efficient in generating cellular energy for making sperm."

The aim of the Labrador Retriever study was to evaluate the relationship between fresh and post-thaw sperm quality and age as they relate to total motility, progressive motility, velocity, viability, morphology, and the presence of reactive oxygen species. Dr. Meyers and his team reported that freezing and thawing of sperm is likely to have a significant decrease in sperm motility, viability and membrane integrity. "Sperm



motility is generally a more sensitive indicator of cellular damage caused by the freezing-thawing process than any other parameter measured in this study," he says.

SEMEN SAMPLES NEEDED

Stud dogs are not always the source of reproduction problems. "Poor semen quality comes in second to poor ovulation timing," Dr. Meyers says. "Although it is impossible to catch all stud dog reproductive problems before a dog is bred, owners can take steps to lessen the likelihood of disappointment by having annual semen evaluations and breeding soundness examinations."

As for the Great Dane study, Dr. Meyers hopes to gather more semen samples from senior dogs, noting that the oldest dog thus far was 5 years of age. "Bringing older Great Danes into the study should shed more light on the impact of ROS levels and poten-

tial damage caused by oxygen-free radicals," he says. "It is very important to recruit senior dogs to increase the power of the study. The broad use of artificial insemination today underscores the need to better understand ways to optimize sperm function for storage as well as for maintaining stud dog fertility."

The ultimate goal is to gain knowledge that will help breeders keep their male Great Danes fertile as they grow older. "We are on our way to understanding the complex nature of fertility and how that will benefit our dogs today and in the years to come," Dr. Meyers says. ■

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