Theriogenology 87 (2017) 115–123

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Semen evaluation and fertility assessment in a purebred dog breeding facility

Andrea Hesser [a], Christa Darr [b], Kris Gonzales [c], Heather Power [c], Tawny Scanlan [b], James Thompson [d], Charles Love [d], Bruce Christensen [a], Stuart Meyers [b,*]

[a] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California, USA
[b] Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, California, USA
[c] Guide Dogs for the Blind, California Campus, San Rafael, California, USA
[d] Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA

ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 21 July 2016
Accepted 9 August 2016

Keywords:
Canine sperm
Male fertility
Reproductive senescence

ABSTRACT

Semen quality in dogs has not been assessed in a longitudinal study that includes endpoints of female fertility and pregnancy. Although use of artificial insemination with chilled semen is increasingly used in canine reproduction, the resultant level of predictability and odds of fertile matings for dogs is still not fully understood. This research provides, for the first time, comprehensive semen evaluation in a large population of dogs in which fertility has been tracked. Duplicate ejaculates were obtained from 39 Labrador retriever males of the Guide Dogs for the Blind (San Rafael, CA, USA) breeding program. Sperm endpoints were determined in fresh semen and extended chilled semen at 48 hour after collection. Evaluation included total and progressive motility, average path velocity, morphology, membrane lipid peroxidation, presence of sperm reactive oxygen species, sperm chromatin structure, and mitochondrial DNA copy number. Male age ranged from 1 to 10 years and were grouped as young (Y; 1–3 years, n = 21), middle aged (M; 4–6 years, n = 13), and senior (S; 7 years or greater, n = 5) for analysis. The effects of age and sperm state (fresh vs. chilled) on the above sperm endpoints were determined using a linear mixed effects model. Semen endpoint values for all parameters were established for this group of fertile males. Progressive motility was only lower in the senior male chilled samples compared to all other groups, fresh and chilled ($P < 0.05$). Velocity decreased with increasing age and was lower overall in chilled samples ($P < 0.05$). Percent morphologically normal sperm was lower in senior dogs compared with the other age groups ($P < 0.05$). The presence of reactive oxygen species was lower in chilled samples compared with fresh ($P < 0.05$). For sperm chromatin structure, the senior-aged group had a higher %COMPα_t than the middle-aged group ($P < 0.05$). Bayesian analysis determined that no differences were seen in total motility, membrane lipid peroxidation, and mitochondrial DNA copy number, with regard to conception rate or average litter size between age groups or between fresh and chilled samples. We observed no effects from semen quality on fertility or fecundity regardless of age, despite the differences found in semen quality. The use of advanced laboratory tests to evaluate sperm parameters beyond the standard motility, morphology, and concentration will open investigation to more specific and sensitive fertility tests in canine reproduction.

* Corresponding author. Tel.: +1-530-752-9511; fax: +1-530-752-7690.
E-mail address: smeyers@ucdavis.edu (S. Meyers).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.08.012

1. Introduction

Measures of semen quality in stud dogs have not been subjected to a longitudinal study that includes endpoints of mating and fecundity. Consequently, components of semen that affect pregnancy rates have not been sufficiently identified in dogs, in comparison to other domestic species [1]. Despite growth in the clinical use of artificial insemination (AI) and increasing use of chilled semen in dog breeding programs, the resultant level of predictability or odds of fertile mating for dogs is not sufficiently understood. Sperm fertility parameters in male dogs are largely extrapolations of research and clinical observation from other species. In addition, the effects of canine age on semen quality and fertility have not yet been investigated.

In contrast to livestock species, few large-scale canine breeding operations exist in the United States in which significant fertility data can be generated [1]. Canine semen parameters associated with female pregnancy and varying fecundity have not been studied in depth. Factors associated with sperm quality such as motility, morphology, semen volume, sperm concentration, membrane oxidation status, oxidative stress, and other endpoints have not been investigated in a large group of stud dogs. Several studies have reported dog sperm morphometric and oxidative status, but only a few studies have used more than 4 to 6 stud dogs [2–7], and no studies have related sperm quality to fertility status and age of males and females in a commercial breeding program [8–12]. Further study is necessary to assess the characteristics of ejaculated sperm, and to monitor and predict successful AI and fertilization rates for dogs.

Despite the first use of AI in the dog by Spallanzani in 1788, AI in dog breeding has only recently increased in clinical usage. Intravaginal AI using fresh and chilled (4 °C) semen is selected often for dogs that cannot copulate for medical, behavioral, or geographic reasons [13]. In recent years, intrauterine insemination has become more popular for fresh and chilled semen as a result of the development and increased availability of the transcervical insemination technique. Conception rates have been investigated using most of these modalities. Natural breeding in fertile dogs bred once between 4 days before to 3 days after ovulation resulted in 95% conception rates in one study [14]. In other studies, conception rates for vaginal insemination of fresh semen yielded between 60% and 95% pregnancy rates [13]. Chilled semen success rates when compared to fresh semen are seen to be very similar; with 94% and 95% pregnancy rates in one study [7].

Sperm are highly susceptible to oxidative damage that ultimately impairs sperm function and interferes with fertility [15–20]. A limited ability to store antioxidant enzymes combined with a membrane rich in unsaturated fatty acids makes sperm particularly susceptible to oxidative stress and peroxidative attack by reactive oxygen species (ROS), specifically superoxide anion and hydrogen peroxide [16,21,22]. Under normal conditions, mitochondria are the main ROS-producing organelle in somatic cells and sperm [15,23–25]. Nuclear and mitochondrial DNA (mtDNA) are targets of such oxidative attack, and it is likely that "surviving" mitochondria in sperm would be exposed to elevated oxygen concentrations, leading to higher ROS production and further damage to cell organelles [15,16]. In addition, given the limited content of mtDNA repair enzymes present in sperm, cumulative damage to this mtDNA may result in lower mitochondrial production of ATP (if not compensated by glycolysis) leading to lower motility and, possibly, lower fertilization rate. It is unlikely that mtDNA is transcribed after sperm complete spermatogenesis, but it is not known whether oxidative attack on mtDNA results in downstream sperm dysfunction. Most likely, mtDNA levels in sperm are a result of insufficient destruction of mtDNA during spermiogenesis and represents "leftover" DNA packaged into sperm. Surprisingly, very little research has been aimed at understanding sperm mitochondrial function particularly at the level of DNA, despite the fact that sperm rely on mitochondria for motility and delivery to the site of fertilization. Mitochondria contain a unique double-stranded DNA genome that codes for 13 polypeptides including four of the five major enzyme complexes involved in oxidative phosphorylation. Further, it is known that age of the father influences fertility in humans and that paternal age is associated with a number of diseases that have mitochondrial etiologies or components [26,27]. It is thought that age-dependent accumulation of testicular oxidative insult very likely leads to mutations in nuclear and mtDNA and, although not determined for dogs, paternal age and oxidative load may be a mechanism for declining fertility in aging stud dogs [28,29].

The objective of this research was to establish expected reference values for normal canine sperm using a consistently managed group of stud dogs with extensive fertility records. We sought to define sperm parameters for commonly used testing methods as well as emerging methods that have found application in other species. In addition, we sought to evaluate the effect of age of stud on sperm parameters and fertility. We hypothesized that oxidative status of sperm membranes, mtDNA copy number, and other novel endpoints could be adjunctive to existing methods for assessing fertility and longevity in cooled conditions.

2. Materials and methods

2.1. Chemicals and reagents

The fluorochromes C_{11}-BODIPY (4,4-difluro-5-[4-phenyl-1,3-butadienyl]-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid) and propidium iodide were obtained from Invitrogen (Eugene, OR, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

2.2. Animals

Thirty-nine Labrador retriever males were evaluated at Guide Dogs for the Blind (GDB), San Rafael, California from October 2014 to February 2015. The dogs represent all the actively breeding Labrador stud dogs in their program at that time point. Dogs ranged from 1 to 10 years of age and