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Investigation of captive red wolf ejaculate characteristics in relation to age and inbreeding

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ABSTRACT

An evaluation of a large database of red wolf fresh ejaculate characteristics (n = 427 ejaculates from 64 wolves) was undertaken to increase knowledge of seminal characteristics in the red wolf and evaluate possible relationships between inbreeding, age, and seminal quality. Phase microscopy analysis of electroejaculates collected over 14 natural breeding seasons was compared with animal ages and inbreeding coefficients. Ejaculate volume increased and sperm concentration and total count decreased as wolves aged ($P < 0.01$, 0.001, and 0.05, respectively), and the proportion of sperm cell morphological abnormalities was greater in animals with higher coefficients of inbreeding ($P < 0.001$), particularly for older animals ($P < 0.001$). Moreover, the mean coefficient of inbreeding of animals that had failed to reproduce given at least one opportunity during their lifetimes was significantly greater than that of wolves with proven fertility, and wolves of proven fertility exhibited higher sperm concentrations and total counts than nonproven wolves. Thus, as the captive red wolf population becomes more inbred, the maximum age of reproduction is likely to decrease; an important finding to consider when projecting population dynamics and determining pairing recommendations.

1. Introduction

The red wolf once ranged throughout the south-eastern United States and possibly as far north as Maine [1,2]. However, numerous factors including private and government-based persecution, habitat loss, and hybridization with the coyote (*Canis latrans*) combined to cause a severe and rapid decline in the red wolf population during the 19th and 20th centuries. Drainage of marshlands and clearing of forests for agriculture and oil exploration reduced and fragmented the available habitat for red wolves and their primary prey species [2–4]. At the same time, urbanization created a niche suitable for the coyote [2]. The coyote was able to significantly expand its range eastward [2,5,6], whereas fragmentation of the red wolf population into ecologically isolated patches compromised their ability to disperse and locate appropriate mates, leading to the occurrence of interspecific breeding in areas in which red wolves and coyotes cohabitated [3,7,8]. Owing to the morphological similarity between the red wolf and coyote and the fact that coyotes were not historically known in the region [2,9], coyotes and hybrids were often misidentified as red wolves [2], and the decline of red wolves went virtually unnoticed until the species was facing extinction. Finally, in the late 1960s, the rarity of the red wolf was recognized, and it was listed as endangered [10,11].

Once it became clear that red wolves were a minority within their range relative to coyotes, and that the pressures of habitat loss, hybridization, and local antiwolf sentiment were not solvable in the near term, efforts at

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preserving the species in the wild were abandoned in favour of planned extirpation with the long-term goal of reestablishing the species in protected portions of its historical range [12]. Of over 400 animals evaluated, 43 met the morphological standards to be considered nonhybrids. Of those, ultimately only 14 became founders for the captive breeding program [12–16]. Because the founder group for the extant red wolf population is small, and because the zoo-based population has been skewed toward older animals [17], there is a need to understand the implications of both inbreeding and age on reproductive success in this species to make optimal breeding decisions for the future of the species. Currently, the captive red wolf population is managed through a zoo-based Species Survival Plan (SSP) in combination with the Red Wolf Recovery Plan, administered by the United States Fish and Wildlife Service.

Little information exists on the specific effects of aging and inbreeding on ejaculate characteristics and/or sperm quality in wild canids. There have been two previous reports of seminal traits in the red wolf (*Canis rufus*) [18,19]. However, these studies considered relatively small numbers of animals sampled over one and two breeding seasons, respectively. The semen parameters and sperm characteristics for the red wolf reported in these studies, while comparable to other canids, tended to be on the extreme ends of the canid spectrum and exhibited a high range of variability both within and among wolves. The significance of these findings in regard to red wolf fertility has not been established.

An evaluation of a large database of red wolf fresh ejaculate characteristics compiled over a 14 year span and including multiple samples from individual wolves, was undertaken. The objective of this study was to improve and build upon current knowledge of seminal characteristics in the red wolf and evaluate possible relationships between fertility, age, inbreeding, and seminal quality.

2. Materials and methods

2.1. Animals

Adult, male red wolves (*C. rufus*; n = 64) were maintained at 10 facilities in various geographical locations across the United States. All housing facilities adhered to husbandry protocols set by the Red Wolf SSP. Wolves ranged in age from 1 to 14 years. All wolves were housed singly, in conspecific pairs, or in family units. Animals were exposed to natural photoperiod and housed in pens that contained natural substrate, foliage, and sheltered dens with bedding material. Wolves were fed a commercially available dry dog food daily and provided water *ad libitum*. Animals that sired at least one litter during their lifetimes were considered to be of proven fertility, whereas males that did not produce a litter given at least one opportunity to breed during their lifetime (i.e. housed with a female conspecific for breeding purposes for the duration of one or more natural breeding seasons) were considered nonproven. Eight of the 64 study animals were of indeterminate fertility (i.e. unpaired) and as such were excluded from fertility-based analyses.

2.2. Semen collection and evaluation

Semen collection was performed during 14 natural breeding seasons; from mid December until early April 1990 to 2004. One to 10 collections were performed per animal per year, for a total of 427 ejaculates from 64 animals. Wherever possible, care was taken to ensure that collections were not aligned with mating events. Consistency in collection procedures and evaluation parameters was assured in that all technicians were trained by the same individual and used standardized operating procedures and a specific data collection form designed for this study.

Wolves were fasted 1 day before collection. On the day of collection, animals were anesthetized using Telazol (teletamine hycrochloride and zolazepam; 6.5 mg kg^{-1}) administered by hand syringe. Before semen collection, the penis was cleaned and the bladder drained of urine *via* catheterization with a five Fr, 55.8-cm long polypropylene catheter (Sherwood Medical, St. Louis, MO, USA). Electroejaculation was performed using a PT Electronics model 302 ejaculator (no. 4 probe: 1.6-cm diameter; Boring, OR, USA). Using previously described methods [18,20,21], ejaculation was achieved through a set of three to five stimulation series, each consisting of multiple on-off stimuli in increasing voltages ranging from 3 to 8 volts. A rest period of 5 to 7 minutes was allowed between each series. Semen was collected into plastic containers.

For each ejaculate, fresh semen from all series was pooled and the total volume, pH, concentration, and percent motile cells were determined using previously described methods for this species [18,20,21]. Specifically, concentration was measured and percent motile cells estimated using a hemocytometer [18,20,21]. The forward progressive status of motile cells was rated on a scale from 0 to 5 (0 = no motility, 1 = side-to-side flipping without forward progression, 2 = slow meandering progression, 3 = moderate meandering progression, 4 = moderate linear progression, 5 = rapid linear progression). To assess morphology and evaluate acrosome integrity, aliquots of 5 μL of each ejaculate were smeared on separate, clean glass slides, and allowed to dry before fixation in methyl alcohol for 60 seconds. Beginning in 1993, fixed slides were stained with Spermac (FertiPro, Belgium; supplied by Meditech first Canada, Inc.) and examined using phase microscopy as described by Goodrowe et al. [18]. A total of 300 spermatozoa from each ejaculate were evaluated, and the percentages of normal spermatozoa, each abnormality type, and spermatozoa with intact, partial, and missing acrosomes were determined. Intact acrosomes were determined by a uniform blue color in the distal portion of the sperm head, whereas the postacrosomal region was stained pink, as described by Goodrowe et al. [22]. Partial acrosomes were identified as those in which the blue stain in the distal portion of the sperm head was disrupted or irregular in appearance. Missing acrosomes were identified by blue color in the equatorial region only, or by even pink color in the acrosomal region. Morphological abnormalities were categorized as those involving the head, midpiece, or flagellum. Neither urine-contaminated nor aspermic samples were included for analysis.

2.3. Data analyses

Values are reported as means ± standard error of the mean. Individual coefficients of inbreeding (f) were generated using complete population pedigree records [23] and PM2000 software. Differences in ejaculate and sperm characteristics between wolves of proven and nonproven fertility were assessed using Student t tests. Effects of increasing age among and within wolves and f among wolves on ejaculate and sperm cell characteristics were assessed using multiple linear regressions. Post hoc analyses of the data indicated that, despite large effect sizes (all ds were ≥1.2), statistical power was below the conventionally acceptable level of ~0.8 [24] due to sample size limitations. In such cases, it has been suggested that a more lenient α level (0.10 or 0.15) should be adopted [25,26].

3. Results

3.1. Semen characteristics

Summary data from semen analyses are presented in Tables 1 and 2. A high level of variation in electroejaculate characteristics was observed both within and between wolves. The greatest proportion of ejaculates (69%) exhibited between 70 and 90% motile spermatozoa. Nearly half of all ejaculates (45%) contained between 70 and 93% morphologically normal cells, 34% contained 50%–70% normal cells, and 21% contained less than 50% the normal cells. Of the mean of 33± 0.9% abnormal spermatozoa, the predominant abnormalities were bent and coiled flagella.

3.2. Fertility status

Subsets of the data were constructed on the basis of ejaculate characteristics from wolves of proven (n = 32; 248 ejaculates) and nonproven (n = 24; 148 ejaculates) fertility. There were no differences between males of proven and nonproven fertility in terms of ejaculate volume, pH, % motility, or forward progressive status ($P > 0.15$). Neither were there significant differences between fertility subsets in the proportions of morphologically normal spermatozoa, or the relative proportions of intact, partial, and absent acrosomes. However, nonproven animals did exhibit lower mean sperm cell concentration and total cell counts ($P < 0.10$) than animals of proven fertility. Interestingly, the mean inbreeding coefficient of nonproven males in this study (0.0281 ± 0.004, range 0–0.125) was significantly greater ($P < 0.01$) than that of proven males (0.0172 ± 0.002, range 0–0.0605).

3.3. Effects of wolf age and inbreeding

Ages of donor animals were normally distributed ($P < 0.05$) around a mean of 6.9 ± 0.1 years. The greatest proportion of donors (63%) had inbreeding coefficients of 0, with the remainder normally distributed ($P < 0.05$) around a mean of 0.0546 ± 0.002.

Although variability was high both between and within wolves, there was a modest overall increase in ejaculate volume in older animals ($P < 0.01$), whereas sperm concentration and total cell counts both decreased with age ($P < 0.001$ and 0.01 respectively). These trends were not apparent before the age of 6 years (Fig. 1). Decreases in total cell count over time were also observed at the individual level. Of n = 17 animals sampled over 4 or more consecutive years, 11 were aged 6 years or older at the beginning of sampling, four were aged 6 years or less for the entire sampling period, and two spanned ages 4 to 13 years during sampling, for a total of n = 13 individuals tracked beyond the age of 6 years and n = 6 individuals tracked before the age of 6. Of these, 11/13 individuals exhibited statistically significant declines in total sperm count ($P < 0.15$) beyond the age of 6, whereas 0/6 individuals exhibited declines before the age of 6. Spermatozoal concentration and total count both decreased in association with higher f ($P < 0.05$ and 0.01 respectively), an effect that was more pronounced in combination with higher f ($P < 0.001$ and 0.10 respectively).

Changes in the morphology of fresh red wolf spermatozoa tended to be associated primarily with inbreeding, with age becoming a contributing factor for some characteristics. Overall, the proportion of morphologically normal spermatozoa decreased in association with increased f ($P < 0.001$), with a greater effect in older animals ($P < 0.001$). The specific morphological characteristics showing the most pronounced associations with f were coiled and bent flagella. The incidence of both increased sharply with increasing f, particularly for older animals ($P < 0.001$), although age alone did not show any association with these traits.

Biflagellism increased mildly with increasing f ($P < 0.01$), and very slightly with increasing age ($P < 0.01$),

Table 1
Mean (±standard error of the mean) semen parameters for red wolf fresh ejaculate characteristics.

Parameter	All samples[a]	Proven fertility[b]	Unknown fertility[c]
Volume (mL)	5.3 ± 0.2 (0.1–32.5)	5.3 ± 0.3 (0.1–32.5)	5.4 ± 0.3 (0.4–18)
pH	6.5 ± 0.04 (6–8)	6.5 ± 0.1 (6–7.6)	6.6 ± 0.1 (6–8)
% Motile spermatozoa	72 ± 1.2 (5–95)	73 ± 1.4 (10–95)	70 ± 2.0 (5–95)
Forward progressive status (0–5)	3.6 ± 0.1 (1.5–5)	3.6 ± 0.1 (2–5)	3.7 ± 0.1 (2.5–4.5)
Sperm concentration ($\times 10^6$ mL^{-1})	104 ± 5.9 (0–664)	110 ± 7.8 (0–592)	89 ± 8.5 (0.9–664)
Total number of spermatozoa ($\times 10^6$)	469 ± 30.0 (0–4262)	485 ± 40.5 (0–4262)	433 ± 43.2 (5–3280)

Numbers in parentheses represent ranges.
[a] Values for all samples (n = 427 ejaculates from 64 wolves).
[b] Values for animals of proven fertility (n = 248 ejaculates from 32 wolves).
[c] Values for animals of unknown fertility (n = 148 ejaculates from 24 wolves).

Table 2
Mean (±standard error of the mean) proportion (%) of sperm cell morphological characteristics and acrosomal status from fresh red wolf ejaculates, determined by phase microscopy.

Parameter	All samples[a]	Proven fertility[b]	Unknown fertility[c]
Normal	65 ± 0.9 (13–93)	65 ± 1.1 (13–93)	64 ± 1.4 (20–90)
Head abnormalities			
Macrocephaly	0.3 ± 0.05 (0–7)	0.4 ± 0.07 (0–6)	0.3 ± 0.08 (0–7)
Microcephaly	1.5 ± 0.2 (0–39.7)	1.2 ± 0.1 (0–14)	1.9 ± 0.4 (0–39.7)
Knobbed acrosome	4.5 ± 0.8 (0–23.3)	4.9 ± 1 (0–23.3)	3.3 ± 1 (0–12.3)
Detached head	3.6 ± 0.3 (0–70)	3.5 ± 0.4 (0–70)	3.9 ± 0.4 (0–28)
Midpiece abnormalities			
Abnormal midpiece	0.2 ± 0.05 (0–9)	0.2 ± 0.06 (0–9)	0.2 ± 0.07 (0–6.5)
Bent midpiece	3.2 ± 0.6 (0–36.3)	3.1 ± 0.4 (0–20)	3.5 ± 0.5 (0–36.3)
Tail abnormalities			
Coiled flagellum	6.6 ± 0.4 (0–44.7)	6.7 ± 0.5 (0–41.7)	6.5 ± 0.7 (0–44.7)
Biflagellate	0.4 ± 0.07 (0–17)	0.5 ± 0.1 (0–17)	0.3 ± 0.07 (0–4)
Bent flagellum	12 ± 0.6 (0–75)	13 ± 0.7 (0–75)	11 ± 0.8 (0–46.7)
Bent neck	2.5 ± 0.3 (0–74)	2.6 ± 0.5 (0–74)	2.3 ± 0.4 (0–21)
Acrosome			
Intact	57 ± 2.6 (2.3–94.3)	59 ± 3.0 (4.7–94.3)	52 ± 5.4 (2.3–88.3)
Partial	35 ± 2.4 (6.5–90.7)	34 ± 2.7 (6.5–90.7)	38 ± 4.8 (7–83.7)
Absent	7.2 ± 1.0 (0–67)	6.4 ± 1.2 (0–67)	9.2 ± 1.9 (1–38.7)
Ballooned	8.9 ± 1.0 (0.3–22.7)	8.6 ± 1.3 (0.3–22.7)	9.7 ± 1.7 (3.6–19)

Numbers in parentheses represent ranges.
[a] Values for all samples (n = 427 ejaculates from 64 wolves).
[b] Values for animals of proven fertility (n = 248 ejaculates from 32 wolves).
[c] Values for animals of unknown fertility (n = 148 ejaculates from 24 wolves).

with intensified effects for age and *f* in combination ($P < 0.01$). The proportions of detached heads and microcephaly were very slightly increased in association with increasing *f* ($P < 0.05$ and 0.01, respectively) but were unaffected by variation in age. The incidence of knobbed acrosomes and the proportion of abnormal midpieces also increased in association with increasing *f* ($P < 0.05$ and 0.001, respectively), but not with increasing age. The relative proportions of intact, partial, and missing acrosomes were not affected by either *f*, age, or both in combination.

The proportion of motile cells decreased with increasing *f* ($P < 0.001$), but did not appear to be associated with wolf age. The forward progressive status of motile spermatozoa exhibited a slight reduction in older animals ($P < 0.01$), and this effect was more pronounced in animals with higher *f* ($P < 0.001$). Finally, ejaculate pH increased in association with increasing *f* but not wolf age ($P < 0.001$).

4. Discussion

Remarkably little information exists on the specific effects of aging on ejaculate characteristics and sperm quality in nondomestic canids. In a natural environment, individuals rarely survive long enough to experience reproductive senescence; however, in captivity, animals benefit from good nutrition, veterinary care, and reduced

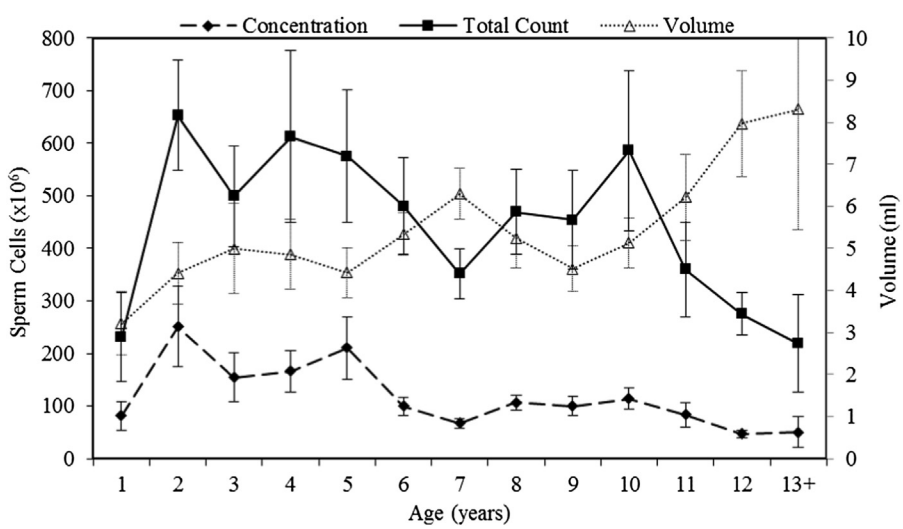

Fig. 1. Changes in red wolf (*Canis rufus*) mean sperm concentration (10^6 cells/mL), total sperm count, and ejaculate volume in relation to wolf age.

risk of traumatic death. Therefore, for captive populations, and particularly for captive breeding populations of endangered species, understanding the effects of age on reproductive success may be of critical importance for the optimization of breeding and management decisions.

It is interesting to note that red wolf seminal volume was found to be nearly twice as much as that typically reported for other canids, a result consistent with previous research [18]. Goodrowe et al. [18] suggested that the relatively high volume of red wolf ejaculates may be due to increased accessory gland secretion provoked by electroejaculation. Mean electroejaculate volumes of the gray wolf (*Canis lupus*) [27] and coyote (*C. latrans*) [28] are similar to that of the domestic dog, and approximately half that of the red wolf. Although it is difficult to draw conclusions given the high level of variability observed, these data suggest that the relatively large volume of fluid in red wolf ejaculates could be a natural seminal trait particular to the species, or possibly a species-specific response to electroejaculation. However, the specific implications of increased ejaculate volume for breeding success in the red wolf remain unknown. No correlation was found herein between ejaculate volume and fertility. Moreover, similarly high variation in ejaculate volume existed across both proven and nonproven animals. Clearly, further study is needed to elucidate a practical relationship of ejaculate volume changes to reproductive success, if indeed one exists.

Mean sperm concentration and total sperm count were similar to those previously reported data for the red wolf by Koehler et al. [19] but lower than those reported by Goodrowe et al. [18]. Furthermore, both increasing age and higher inbreeding coefficients were associated with a decrease in sperm concentration, with more highly inbred animals showing the most marked decreases with age. However, given that ejaculate volume increased with age, the fact that concentration decreased is not necessarily unexpected. Perhaps of more interest is the observation that total sperm count per ejaculate also decreased with age, again with more highly inbred animals showing the most marked decreases. Moreover, the decreases seen herein were not apparent before the age of 6 years but were consistently observed within individuals beyond that age. Therefore, it appears that the effects of age on spermatozoal output is not manifest until after approximately 6 years of age. In dogs, spermatozoal output has been shown to decrease with inbreeding [29] and age [30]. In this study, animals with nonproven fertility tended to be older and more inbred than those with proven fertility, and also had lower sperm concentration and total cell counts than proven animals.

The emphasis of the red wolf SSP on maintaining genetic diversity has resulted in the preferential selection of older animals for mating [31]. This, coupled with our finding that total sperm count decreases with age, implies a case for the increased use of artificial reproductive techniques that would allow for the maximization of sperm to be used in artificial insemination as an alternative or corollary to natural breeding. To this end, *in vitro* sperm handling and cryopreservation techniques have been developed for the red wolf [18,22,32], although further study is required to fully optimize the processes involved. Artificial insemination trials using noninvasive means of predicting ovulation timing have also been conducted, and preliminary results are promising [17].

The proportion of red wolf spermatozoa with abnormal morphology reported here is higher than that reported by Goodrowe et al. [18] but in line with that reported by Koehler [19]. Of the types of abnormalities measured, the most pronounced associations with inbreeding coefficients were incidence of tightly coiled flagella and bent flagella. Also affected, though to a lesser degree, were the incidences of knobbed acrosomes, biflagellism, detached spermatozoal heads, and microcephaly. With the exception of bent flagella, these are considered to be abnormalities associated with spermatogenesis [33,34]. The percentage and forward progressive status of motile cells decreased with increasing inbreeding coefficients, a result probably attributable to the increased prevalence of spermatozoa with flagellar abnormalities within these samples.

Although we did not observe a measurable difference in the proportion of morphologically normal spermatozoa between proven and nonproven breeders, the seminal traits described here are consistent with those found in inbred populations of other mammalian carnivores [29,33,34]. For the domestic dog, inbreeding has been associated with reduced ejaculate quality in terms of concentration, count, volume, and motility [29]. Inbreeding effects reported for the gray wolf (*C. lupus*) include decreased spermatozoal concentration and motility, and increased incidence of morphological abnormalities in spermatozoa [35,36]. It has been previously suggested that the semen parameters of the red wolf are consistent with inbreeding effects related to the small number of founders [19,37]. In an examination of semen characteristics and cryopreservation protocols, Goodrowe et al. [18] did not find evidence of inbreeding depression in seminal characteristics; however, both the sample size and the range of inbreeding coefficients of animals examined by those studies were smaller than those considered herein, and as such may be less representative.

Furthermore, red wolf ejaculate characteristics tend to be on the extreme edges of typical canid parameters. The proportions of morphologically abnormal spermatozoa reported here and by Koehler et al. [19] are substantially larger than those reported for the domestic dog [38–40], red fox (*Vulpes vulpes*) [41], or gray wolf [27], whereas sperm concentration and total cell count are lower. Abnormalities reflective of spermatogenic dysfunction constitute a greater proportion of the total abnormalities in red wolves than domestic dogs [18,38]. Also, there was a high level of variability observed in these characteristics in the red wolf; similar levels of variability typically are not observed in other canids but have been noted among inbred domestic dogs [29]. Perhaps most telling, red wolf ejaculate characteristics compare poorly with those of the coyote, a species that is very closely related, but that has not been subject to a severe population bottleneck or inbreeding. Compared to coyote ejaculate characteristics [28], red wolf semen has lower sperm concentration and cell count, lower motility, higher volume, and larger ranges in semen characteristics. Red wolf semen also contains

more morphologically abnormal cells and more cells with acrosomal damage than coyote semen, including abnormalities consistent with inbreeding.

It is important to note that the range of inbreeding coefficients of sperm donors within the present study (0–0.125) was relatively small and low compared with inbreeding coefficients listed in reports of other canid species in which suboptimal sperm quality is observed as a manifestation of inbreeding depression [33,35]. The range for the entire captive red wolf population (0–0.3046) is larger than that encompassed by sperm donors for this study. Thus the trends toward suboptimal semen quality detected here are likely more pronounced within the population at large.

On the basis of a comparative evaluation of evidence for 20 mammalian species, Fitzpatrick and Evans [42] found that impaired sperm quality is linked to inbreeding, and is particularly apparent in endangered species—a distinction based largely on population reduction. In addition, Asa et al. [43] found a significant effect of inbreeding on sperm quality in Mexican gray wolves (*C. l. baileyi*), and related both inbreeding and sperm quality to reproductive success in that species. The Florida panther (*Felis concolor*) is similarly low in genetic variation to the red wolf and shows significant incidence of male sterility because of spermatozoal abnormality [44]. In dogs, Oettlé [40] determined that if the percentage of normal spermatozoa falls below 60%, the fertility of the individual is compromised. Mean percentages of normal spermatozoa in all wolf subsets in this study were above 60%; however, those percentages may fall as the population becomes more inbred.

In fact, reduced total counts, sperm cell motility, and high levels of morphological abnormalities may already be affecting red wolf fertility, as evidenced by the differences observed between proven and nonproven breeders. Although earlier studies did not find definitive evidence of inbreeding depression [18,45], Goodrowe et al. [18] suggested that the semen parameters of the red wolf were consistent with inbreeding effects related to the small number of founders, and Koehler et al. [19] found nonsignificant trends in their data consistent with inbreeding depression. More recent examinations of demographic data have indicated that although breeding success within the zoo-based population had not been significantly reduced at the time of analysis, litter size had been negatively affected by paternal levels of inbreeding [46], and both increasing male age and inbreeding reduce the probability of a successful mating [31,47]. Thus, it seems clear that more research focused on male reproductive capacity is warranted for this species. Functional assessment based on gamete interactions would shed more light on the relationships between inbreeding, age, and fertility in male red wolves, and there is an increasing need for the continued development of assisted reproductive techniques such as sperm cryopreservation and artificial insemination.

4.1. Conclusions

A number of conclusions may be reached from these data. First, semen parameters for the red wolf are generally less robust and more highly variable than those of other canid species investigated. Second, ejaculate characteristics change as individuals age: volume increases, sperm concentration decreases, and total numbers of sperm per ejaculate is reduced, particularly after the age of 6 years. However, the age at which fertility is impaired is undetermined and may not be reached within the lifetime of the average male red wolf in captivity. Third, there is some indication of inbreeding depression within the captive red wolf population in terms of ejaculate sperm cell concentration and total sperm count, and in the increased incidence of specific morphological spermatozoal abnormalities. Finally, the suboptimal semen characteristics associated with age and inbreeding tend to be exacerbated by these factors in combination, that is, inbred wolves are more likely to experience reduced fertility as they age. While this seems obvious, it means that as the captive population inevitably becomes more inbred, the maximum age of reproduction, and the likelihood of successful matings for older animals will decrease; a conclusion to keep in mind when projecting population dynamics and determining pairing recommendations.

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