COLLECTING AND FREEZING SEMEN FOR ARTIFICIAL BREEDING OF CERVUS ELAPHUS

J.C. HAIGH B.V.M.S., M.SC., M.R.C.V.S.
Department of Herd Medicine & Theriogenology,
Western College of Veterinary Medicine,
University of Saskatchewan,
SASKATOON, SASK. S7N OWO, CANADA

INTRODUCTION

Interest in the artificial breeding of captive wapiti and red deer (Cervus elaphus) has stemmed from three different viewpoints. In the first semen was collected, evaluated, frozen and used for the insemination as a scientific exercise (Krzywinski & Jaczewski 1978). The second arose from the interest of New Zealand deer farmers in the use of semen from Canadian wapiti in the breeding programs of farmed deer. This was especially intended for the upgrading of wapiti type animals. These animals have emerged over the last 70 years from the interbreeding of a small number of wapiti introduced from the U.S.A. in 1908 and red deer introduced over many years. The third has been the fairly recent enlightened interest among members of the zoo community in the breeding of captive species without further depletion of wild resources. As the gene pool of many species in captivity is small, and the risks and cost of moving animals from one zoo to

another may be great, it has been recognised that artificial breeding offers a genuine alternative as a means of captive propagation. Limited success has been reported in these efforts (Haigh, in press). Although wapiti and red deer cannot be classed as endangered, it may be that information acquired while studying them will be applicable to other species.

The continued interest among New Zealand deer farmers in the importation of red deer from Europe and the high costs involved makes the potential for artificial breeding considerable. Not only can semen from suitable stags be imported, but all potential import stags can be evaluated before export. Furthermore, all stags and especially new imports undergoing adjustments to antipodean light regimes, can be evaluated prior to use.

MATERIAL AND METHODS

Semen was collected in Canada from twelve wapiti bulls over four seasons. The bulls varied in age from 1 to 12 years.

Apart for five collections in the first year (1980) and ten in June and July of 1981 all collections were made on wapiti physically restrained in a specially designed squeeze chute. This was modified during the study.

In the first year only five isolated collections were made. In the second year when collections were made twice monthly from July 1981 to May 1982 only one attempt was made to ejaculate each bull on each

date. The next two collection seasons were during the period 15 September to 15 January (1982/83 and 1983/84). In the first year collections were made twice monthly (Haigh, Cates, Glover & Rawlings, 1984). In the remaining years collections were made two or three times a week and ejaculation was attempted 1, 2 or 3 times at 10 minute intervals.

The equipment used for collection and the methods employed have been described (Haigh, Barth, Cates & Glover, 1985).

In the field laboratory ejaculates were evaluated for colour (graded in comparison to milk), volume and wave motion. Wave motion was rated on a subjective scale from very good (ejaculates showing vigorous swirls and eddies) to nil. Semen smears were made at this time and stained with aniline blue-eosin stain.

Ejaculates with good or better motility, the colour of milk or cream and at least 0.3 ml volume were placed in a 100 ml bottle containing extender that had been incubated at 37°C. These bottles were then placed in a 1 litre beaker containing 500 (later 300) ml of 37°C water and placed in refrigerator at 4°C. The beakers were transported in an insulated box to another laboratory and placed in a cool room (4°C) for further evaluation and extension

In the first year of the study the extenders were either 20% egg yolk citrate or 2% fat milk. In the 82/83 season a vegetable protein extender was tested and in the third year only the milk extender was used. Comparative data among the three extenders are reported

elsewhere (Haigh & Barth in prep.).

Sperm cell density of the extended semen was measured with a haemocytometer. Stained smears were evaluated for sperm morphology and live/dead ratio.

The final volume of semen was calculated to provide 40-60 x 10^6 live normal cells/ml before freezing. Fifty percent of this volume containing twice the final glycerol concentration was added in seven equal aliquots at 10 min intervals. The final glycerol concentration was 10% for the milk extender, 7% for the egg yolk citrate extender and 7% for the vegetable protein extender.

The percentage of individually motile sperm in the extended ejaculates was estimated by examination under phase contrast microscopy at 400X after a small quantity had been removed from the cooling room and rewarmed to 37°C for 5 min. A minimum of 300 cells from six fields as counted.

The minimum criteria for freezing of the ejaculates after equilibration were 80% normal cells, 80% individual motility (rate 3/5). The straws were then placed five to a mini-goblet, two mini-goblets to a cane. The canes were placed in a single layer on a metal tray and suspended in nitrogen vapour 4 cm above the fluid in a liquid nitrogen tank for 209 min. The canes were then plunged into the liquid nitrogen.

From one to three straws from each batch of semen were thawed in a water bath at 37°C for 2 min. As soon as possible after thawing the semen was evaluated for percent motile sperm and percent intact acrosomes. The semen was maintained at 37°C for a further two hours and the motility and acrosome evaluations repeated. The minimum criteria for satisfactory rating have been described (Haigh et al., 1985).

RESULTS

Two hundred and thirty five ejaculates were collected from eight of the bulls between 1 September, 1981 and 2 March, 1982; 15 September, 1982 and 16 November, 1982; and 15 october, 1983 and 15 January, 1984. Two of the bulls could not be trained to the chute system and were removed from the study. Ejaculates collected in late March, April, May, June, July and August were either azospermic or contained a large proportion of abnormal sperm (Haigh, Cates, Glover & Rawlings, 1984).

Erection usually occurred during the early stages of stimulation. After erection a few spurts of clear fluid (which we tried to avoid collecting) were followed by a small volume of a milky to creamy sperm rich fluid. Mean ejaculate volume was 1.65 ± 0.72 ml (range 0.3 - 5.0, n = 207). This fraction was delivered over two to five stimulations. Further stimulation sometimes produced a viscid bright yellow material resembling the secretion found in the vesicular glands of wapiti culled during the rut (Haigh, 1982). This material may be the same as that seen in red deer from which semen was collected by

artificial vagina (A.V.) (Krzywinski & Jaczewski, 1978). The vesicular gland secretion was produced most frequently by two of the bulls (11/36 and 8/35 ejaculates), sometimes being the sole component of the ejaculate and sometimes mixed with the sperm rich fraction. On 44 occasions only a clear or dirty brown fluid was obtained.

Ejaculate colour provided only a crude estimate of density. The difference between thin milk and a thick "clotted cream" appearance could readily be used to distinguish between ejaculates with less than 0.5×10^9 or more than 2.5×10^9 sperm/ml. In the 1981/82 season the density of all ejaculates measured by haemocytometer ranged from 0 to 4.6×10^9 sperm/ml. After the 1981/82 season haemocytometer measurements of density were only conducted on ejaculates having a density of thin milk or better. The mean density of these ejaculates was 1.77×10^9 sperm/ml ($\pm$ 1.25, n - 102 range 0.3 - 6.3).

Sixty percent of the 235 ejaculates exhibited wave motion graded as good, good to very good or very good. In the three years of the study these categories comprised 62%, 57% and 62% of all ejaculates respectively (Text-Table 1a). When the quality of the ejaculates based upon the wave motion was compared among bulls there were significant differences (Text-Table 1b).

Table 1a

Motility characteristics of all wapiti ejaculates collected over three seasons (September 1st, 1981 and March 2nd, 1982), September 15th, 1982 and 16th November, 1982), 15th October, 1983 and 15th January, 1984).

Categories N=no motility; P-poor; F-fair; G-Good; VG=very good (see text for explanation) 60.5% of all ejaculates were graded good or better.

Season	1981/82	1982/83	1983/84	Total
N	-	31	-	31
N-P	2	6	14	22
P	6	7	3	16
P-F	1	-	3	4
F	2	4	-	6
F-G	9	-	4	13
G	13	14	11	38
G-VG	13	19	14	46
VG	8	33	16	57
Total	54	114	65	233

Table 1b

Comparison of individual bulls over same period as in table 1a. Each ejaculate was assigned a number from 1-9. 1=VG; 2=G-VG; 3=G; ...9=N. Bulls with subscripts not in common are significantly different from one another ($p < 0.05$).

Bull No.	76	4	6	78	95	40	82	80
$\bar{x}$ score	2.70	3.03	3.36	3.50	4.19	4.50	4.94	5.09
	a	a	a	a				
		b	b	b	b			
			c	c	c	c	c	c

Wapiti sperm had the same general appearance as those of domestic ruminants (Haigh et al, 1984). The variation in percent normal sperm with season has been described (Haigh et al., 1984). Of 122 sperm smears made between 15 September and 15 January from seven of the eight bulls 107 had >85% normal cells ($\bar{x}$ = 91.6, s = 6.18). The exception was one bull that consistently produced sperm with a high (13-56%) proportion of midpiece or head defects. None of the semen from this bull was frozen.

There was no significant difference in sperm motility pre-freeze between the egg yolk citrate and the milk extenders, but the vegetable protein extender was significantly ($p > 0.05$) less able to support motility of sperm.

Of the three extenders tested, both egg-yolk citrate and milk provided satisfactory cryo-protection but the soya bean extract proved to be unsatisfactory. The data on semen extended in either egg-yolk citrate or milk are presented in Text-Table 2.

DISCUSSION

The semen of C. elaphus has been collected both by the use of an artificial vagina (A.V.) (Krzywinski & Jaczewski, 1978) and by electroejaculation (E-E) (Jaczewski & Jasiorowski, 1974, Haigh, Cates, Glover & Rawlings, 1984).

Text-Table 2

Collection, extension, evaluation, freezing and thawing data from Wapiti semen extended in either 20% egg-yolk citrate or 2% homogenized milk and frozen between 15 September and 15 December in each year.

Season	1981	1982	1983	Total
No. collected	33	100	65	198
No. exhibiting inadequate motility	14	42	26	82
< minimum density	3	6	8	17
< 80% normal morphology	1	4	1	6
< 80% motile	4	17	13	34
No. frozen/thawed	11	31	17	59
No. satisfactory	5	16	11	32
No. questionable	-	3	-	3
No. unsatisfactory	6	12	6	24

Both methods have advantages and disadvantages relative to one another. Collection by A.V. involves the use of either very tame (halter trained) females, or the use of dummies sprayed with estrous urine to simulate the female in estrus. In either case a specially designed A.V. is used (Krzywinski, 1976). One of four stags in the Polish studies could not be collected by this methods due to excessive aggressiveness (Krzywinski, 1976).

Although no estrous female is required to collect stags or bulls by E-E not all wapiti bulls can be satisfactorily collected by this method without resorting to immobilization. Immobilization offers an alternative for the occasional collection of semen from a given individual, and has been widely used in zoos for semen collection of many dfferent species (Seager et al., 1980); however, it is not a suitable method for the long term collection of semen due to its disruptive nature. Two of 12 animals studied in our tests proved to be impossible to handle in chutes and could not be trained for E-E.

A disadvantage of E-E, no doubt due to the non-physiological nature of the method, is that occasionally one fails to collect a good quality semen sample from a bull wapiti even in the midst of the rut. A subsequent collection within a few minutes, or the following day may yield a satisfactory sample.

For wapiti the E-E method offers a reasonably reliable and safe method of collection for both the animal and the handler, provided that the chute facilities are of an adequate standard of design and construction.

It is likely that semen will have to be collected in adverse weather conditions (by whatever method) and means must be devised to protect the semen from thermal shock (Krzywinski & Jaczewski, 1976; Haigh, 1982).

Semen quality reaches a peak in the late summer or early autumn, just before the onset of the rut. The quality remains high for about six months, although there are variations from one collection to another. In the northern hemisphere, no sperm are present in ejaculates from about mid-April until mid-July (Haigh et al., 1984).

In cattle it is considered that semen collected by A.V. is denser, but of a lesser volume than that collected by E-E (Ball, 1980). Comparison of the data from red deer and our own data shows that this may not be the case with Cervus. The difference between the two methods may lie in the proportion of samples that are of poor quality with E-E (Text table 1a). Densities of samples collected by both methods fluctuated widely. The most dense of the red deer semen samples contained 3.95×10^9 cells/ml (Krzywinski & Jaczewski, 1978). The densest wapiti sample contained 5.61×10^9 cells/ml.

Wapiti and red deer semen has been frozen either in pellets on dry ice, which were then transferred to liquid nitrogen (Krzywinski & Jaczewski, 1978), or in liquid nitrogen vapour using 0.5 ml straws (Haigh et al., 1984). The latter method has considerable advantages, especially in regards to the subsequent identification of the specimen and is likely top be the only acceptable method when semen is to be imported.

Frozen semen has been successfully used in both red deer and wapiti (Cooper, pers. comm.; Krzywinski & Jaczewski, 1978; Kelly & Moore, 1981; Haigh, Shadbolt & Glover, 1984) and with improving techniques of estrous synchronisation may provide a real alternative to the high cost importation of live animals.

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