Storage of semen and artificial insemination in deer

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Abstract

Methods of collection and freezing of semen of some deer species and aspects of controlled reproduction associated with the use of frozen–thawed semen by artificial insemination (AI) are discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cervid; Deer; Freezing of semen; Oestrus synchronisation; Artificial insemination

1. Introduction: the species

Geographically and phenotypically, the cervids are an extraordinarily diverse group of ungulates, with approximately 43 species and 206 subspecies presently described, ranging in size from the Alaskan moose (*Alces alces gigas*; 700 kg) to the Northern Pudu (*Pudu mephistophiles*; 9 kg) (Whitehead, 1993). The various taxonomic groups are distributed naturally across all major landmasses except Australia and Antarctica, although various species have been naturalised in Australia and New Zealand within the last 150 years (Wodzicki, 1950; Bentley, 1978). The habitats occupied by cervids are many and varied, and diversity of behavioural strategies reflects this, ranging from solitary territoriality of musk deer (*Moschus* spp.) to nomadic gregariousness of Barren-ground Caribou (*Rangifer tarandus groenlandicus*) (Whitehead, 1993).

It is clear, therefore, that no one taxon of cervid can be considered to be typical of deer in general. This is also true of their reproductive biology; some cervids exhibit highly seasonal patterns of births in cool temperature climes, while others are completely aseasonal in equatorial regions. Furthermore, many species are strictly monovular and bear single offspring annually, while others are normally polyovular and bear

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multiple offspring. Even embryonic development and placentation vary enormously between species (Lincoln, 1985).

A review of the development of assisted reproductive technologies for cervids reveals that information is limited and available on only a few species. Most cervids have relatively little association with man other than as prey; this is particularly so for solitary forest-dwelling species. In contrast, a few select species, particularly the larger-bodied, gregarious deer that inhabit open range or the forest–pasture interface, have a long history of close association with man. These (dozen or so) species account for > 95% of recent research effort. Emphasis on assisted reproductive technologies, such as artificial insemination (AI), multiple ovulation–embryo transfer (MOET) and in vitro embryo production (IVP) for propagation and genetic management of cervids, has concentrated largely on species with either high commercial value (e.g. farmed red deer, *Cervus elaphus*, and fallow deer, *Dama dama*), high recreational/environmental value (e.g. white-tailed deer, *Odocoileus virginianus*, in North America) or a high reliance on man for species survival in captivity (e.g. brow-antlered Eld's deer, *C. eldi thamin*). Even in these species, information is very limited in comparison with that derived from other domestic species such as sheep, cattle and goats.

This section is principally concerned with collection and storage of semen in deer, but as these processes are centred around the application of AI and gene resource cryobanking, other aspects of controlled reproduction associated with the use of stored semen are also discussed.

2. Seasonality constraints

Most cervid species of commercial or recreational value, particularly those of northern temperate origin, exhibit highly seasonal patterns of reproduction entrained by prevailing photoperiod regimens (Lincoln, 1985). This poses a major constraint on both the harvesting of gametes and the application of AI. The males of these species (e.g. red deer, fallow deer) exhibit alternating periods of fertility and infertility related to dramatic changes in testis size and function (Lincoln, 1971; Mirachi et al., 1977; Haigh et al., 1984; Asher et al., 1987. 1996b; Gosch and Fischer, 1989). This is reflected in dramatic seasonal changes in semen characteristics (Fig. 1), whereby aspermatogenesis in the spring/summer antler regeneration period results in semen completely devoid of spermatozoa at this time. This severely limits opportunities for semen harvest, often necessitating collection operations during or immediately after the autumn rut, a period when male deer are highly aggressive. Further limitations of pronounced seasonality relate to the timing of AI to optimise conception rates. Female cervids of temperate species exhibit deep anoestrum over spring and summer months, and generally fail to show ovulatory responses to oestrous synchronisation treatments until the onset of the rut in autumn (Morrow et al., 1992). Thus, most AIs are performed only during the immediate rut and post-rut periods (Asher et al., 1993).

Tropical cervid species are generally subject to fewer seasonal constraints. Some species are completely aseasonal in their calving patterns, although individual males generally exhibit annual cyclicity in their antler cycles (e.g. axis deer, *Axis axis*; Loudon
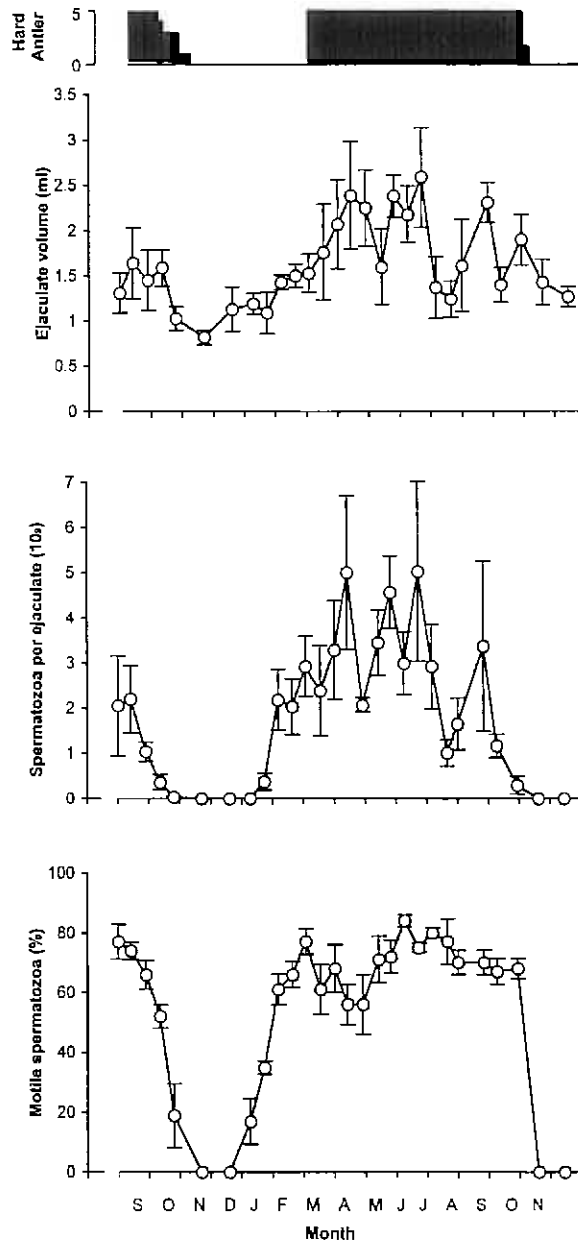
Fig. 1. Profiles of hard antler status and of mean ($\pm$ SEM) ejaculate volume. spermatozoa per ejaculate and percent motile spermatozoa for semen collected from five adult male European fallow deer (*D. dama dama*) over a 15-month period (from Asher et al.. 1996a).

and Curlewis, 1988). Other species exhibit rather loosely defined patterns of herd synchrony in calving and antler cycles (e.g. sambar deer. *C. unicolor*; Semiadi et al.,

1994), while others are highly seasonal with an autumn/winter calving synchrony when located in temperate climes (e.g. Eld's deer; Monfort et al.. 1991). Whereas males of temperate species are invariably infertile during the antler regrowth period, viable spermatozoa have been harvested throughout the entire antler cycle of tropical species such as axis deer (Loudon and Curlewis. 1988; Mylrea, 1992) and Eld's deer (Monfort et al.. 1993b).

3. Methods of semen collection

Cervid behaviour and physiology present some unique challenges when faced with the task of semen collection. Various combinations of donor intractability, volatility and outright aggression have often impacted severely on the general welfare of the collector. Collection of semen from cervids centres around three methods: natural service into an artificial vagina (AV), electroejaculation, and post-mortem epididymal recovery.

3.1. Natural service

Polish researchers have pioneered studies on the use of natural service semen collection methods for red deer (Krzywinski, 1976; Krzywinski and Bobek, 1984; Krzywinski and Jaczewski, 1978; Strzezek et al., 1985) and European moose (Krzywinski, 1985). They designed a variety of AVs that were either hand-held or worn externally by the oestrous hinds or dummy hinds treated with oestrous hind urine. While they were repeatedly successful in obtaining ejaculates with the AV, the techniques generally required a high level of stag training and habituation. As such, these techniques have limited application for most deer farming operations because of limited opportunities to train stags of high genetic merit. Furthermore, the extreme aggression of the larger bodied cervids towards humans renders this exercise extremely hazardous to the handlers. More domesticated and docile species, such as reindeer (*R. tarandus tarandus*), are probably more amenable to this form of semen collection, as demonstrated by Dott and Utsi (1971).

Jabbour and Asher (1991) describe the development of a prototype internal AV, primarily for semen collection from fallow deer. Ovariectomised does were treated with controlled internal drug releasing (CDIR) devices (EAZI breed CIDR™ G, Inter-Ag, Hamilton, New Zealand) for 6 days and 0.05 mg oestradiol benzoate (ODB) 24 h after CIDR device withdrawal. They were fitted with the internal AV during oestrus, 18–24 h after ODB injection, and exposed to the buck within their pastoral environment. After an observed mating, the AV was removed and the semen aspirated for assessment. While this technique may have obvious advantages in terms of operator safety, it proved to be a slow and involved process which required considerable labour input.

3.2. Electroejaculation

By far, the majority of semen collections from deer are performed by electroejaculation of anaesthetised or sedated donors (Asher et al., 1993). While there are risks to the donors associated with the use of anaesthetics, when compared to natural service

methods of semen collection, electroejaculation has the advantage of being generally safer for handlers, it can be used across all individuals rather than a select few trained animals, and is often less time-consuming. Offset against this is the often repeated argument that natural ejaculates are generally of better quality than electroejaculates, although this has not been demonstrated for deer. To date, semen has been collected successfully by electroejaculation from a wide range of cervid species, often with no noticeable difference in quality or quantity of ejaculate compared with natural service collections.

3.3. Post-mortem epididymal recovery

Post-mortem recovery of epididymal spermatozoa has been employed for a number of cervid species for genetic salvage, particularly from harvested trophy males. The techniques are well described for red deer and European moose (*A. alces alces*; Krzywinski, 1981) and for white-tailed deer (Platz et al., 1982; Jacobson et al., 1989). Spermatozoa capable of fertilisation have been collected in all cases.

4. Storage of semen

A variety of semen storage/preservation techniques have been applied to cervid species, but few direct comparisons have been made between different diluents and processing methods within species, or for a particular method across species. Development of protective diluents and methodology has lagged behind that for other domestic ruminants; nevertheless, much has been learned from the experience in sheep and goats in particular. Most diluents used successfully in deer have been adapted from the commonly used sugar-based tris and/or citrate-buffered ones developed for other small ruminants, using egg yolk for protection against cold shock and glycerol as cryoprotectant (for review, see Salamon and Maxwell, 1995). Although egg yolk coagulating enzyme contained in seminal plasma has proved problematical when high proportions of egg yolk have been used in goats (Ritar and Salamon, 1982), there is no reported evidence of such problems in any of the semen of the deer species tested. Two of the most commonly used diluents for freezing deer semen, namely sodium citrate–egg yolk–glycerol (Krzywinski and Jaczewski, 1978) and tris–glucose–citrate–egg yolk–glycerol (Evans and Maxwell, 1987), are detailed in Table 1.

It can be said with some justification that there has been little original research on formulation of diluents specifically for deer semen, probably because the existing diluents have given acceptable results. However, some investigators have, at least, compared several semen diluents developed for other ruminants in order to find the most suitable for a particular deer species. The following sections deal with the various diluents and storage techniques on a species by species basis.

4.1. Red deer and wapiti (C. elaphus spp.)

In comparison to all other cervid species, a large number of studies have investigated aspects of collection, physiology and storage of semen in red deer (*C. elaphus scotius*)

Table 1
Formulations of two commonly used diluents for freezing deer semen

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Citrate-based diluent (Krzywinski and Jaczewski, 1978)

72 ml 2.9% sodium citrate
1.25 g fructose
0.1 g streptomycin sulphate
20 ml egg yolk
8 ml glycerol

The ejaculate is collected into, or immediately diluted with 5 ml diluent at 37°C, cooled to 5°C over 2 hr. and diluted further to the required concentration, before freezing in pellets on dry ice.

Tris-based diluent (Evans and Maxwell, 1987)

3.63 g tris (hydroxymethyl) aminomethane
0.50 g fructose
1.99 g citric acid
15 ml egg yolk
5 ml glycerol
0.06 g penicillin G (sodium salt)
0.1 g streptomycin sulphate

Made up to 100 ml with glass distilled water.
Semen is diluted 1 + 4 (semen + diluent) and cooled slowly (1.5–2 h) before freezing in pellets on dry ice or in LN2 vapour in straws.

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and wapiti (*C. elaphus nelsoni*, *C. elaphus rooseveltii*, *C. elaphus manitobensis*). The use of liquid-stored semen (i.e. stored at ambient temperatures) in red deer AI programs is reasonably common practice in New Zealand, particularly when donor stags are located on the same property or have a history of producing semen that does not survive well after freezing. Red deer semen can be successfully maintained at ambient temperatures (15–20°C) for periods of between 2 and 24 h before insemination. Early extension with protective buffers (usually normal freezing diluents without cryoprotective agents), followed by slow (4 h) cooling from 30°C to 15°C, reduces the incidence of cold shock (G. Shackell, personal communication). Inseminates of fresh semen for intrauterine insemination normally contain 2–10 × 10^6 motile spermatozoa, a factor of 2–3 lower than frozen–thawed inseminates.

Freezing of red deer and wapiti semen has tended to follow protocols established for sheep and cattle. It appears that red deer and wapiti spermatozoa withstand freeze-thawing and have generally acceptable levels of post-thaw survival (30–70%) in various extender/cryoprotectant formulations (Haigh et al. 1985, 1986; Asher et al 1988b; Fennessy et al., 1990; Veldhuizen, 1994; Zomborszky et al., 1999) The most commonly used diluent/freezing regimen is based on that of Krzywinski and Jaczewski (1978). Minor modifications of this have been subsequently used widely in experimental and commercial programs with acceptable results (Asher et al., 1986; Fennessy et al., 1990, 1991a,b; Haigh and Bowen, 1991).

Until recently, few attempts have been made to evaluate alternative extenders for red deer and wapiti. Haigh et al. (1986) evaluated three diluents for straw-freezing of wapiti semen, namely, 20% egg yolk–citrate, low-fat milk, and a vegetable protein extender, all using glycerol as cryoprotectant (7–10%) and all with or without 0.2% sodium EDTA. Both the egg yolk–citrate diluent and the milk diluent were used with some

success in terms of preserving motility and acrosome integrity of semen, particularly when EDTA was present, but the vegetable protein diluent proved inadequate. Veldhuizen (1994) has provided a more detailed comparison of alternative diluents for use with red deer semen. The study examined the combination of five diluents using three different cryoprotectants. The diluents evaluated were sodium citrate–egg yolk (Krzywinski and Jaczewski, 1978), tris–glucose–citrate–egg yolk (Evans and Maxwell, 1987), skim cow's milk–egg yolk (Vinha and Coughrough, 1972), lactose–egg yolk (Dott and Utsi, 1973) and a synthetic diluent for ram semen, RSD-1 (Upreti et al., 1995). Cryoprotectants evaluated were glycerol, propan-1,2-diol (PROH) and dimethyl sulphoxide (DMSO) at both normal rates (as per literature) and 50% normal rates. In all cases, pooled semen from four stags was diluted to a final concentration of 100×10^6 spermatozoa/ml and frozen in 0.25 ml straws in an automated chamber freezer.

The semen exhibited the highest post-thaw motility ($56.7 \pm 8.4\%$) and incubation performance with the tris–citrate–egg yolk–glycerol (half rate) extender (Fig. 2a). Generally, for any extender, glycerol outperformed other cryoprotectants except for RSD-1, which performed better with DMSO. The lactose–egg yolk–glycerol combination was the superior buffer for maintenance of acrosomal integrity. However, despite these apparent differences, in vivo evaluation, by assessment of conception rate after laparoscopic intrauterine AI, showed no practical differences between sodium citrate, tris–citrate and lactose based extenders (40–50% hinds pregnant; $P > 0.10$) (Veldhuizen, 1994). The generally low conception rates suggest that factors other than semen quality may be important.

Recent developments in in vitro fertilisation (IVF) in deer, particularly red deer (Berg et al., 1995), have stimulated some further interest in semen storage for this special purpose. For IVF, there must be sufficient numbers of spermatozoa for fertilisation, but an excess results in polyspermy. This requires that semen frozen in unit doses contains fewer spermatozoa than for normal AI, possibly also requiring special precautions to combat the dilution effect (Ashworth et al., 1994). The sperm cells should also be free of epididymal or seminal plasma since these contain "decapacitation factors" (Eng and Oliphant, 1978; Hunter et al., 1978; Fraser et al., 1990). Further, bovine seminal plasma has been reported to lyse bovine oocytes (Ijaz et al., 1989). A percoll gradient is routinely used to remove the extraneous material from the sperm after thawing. The diluent in which spermatozoa are frozen may also have an effect on the IVF process, e.g. skim milk is very difficult to separate from spermatozoa even through a percoll gradient (D. Saywell, personal communication). A modified TEST buffer (Graham et al., 1972; "Universal Buffer", E.F. Graham, personal communication) has been successfully used to freeze red deer sperm for use in IVF. Preliminary results show that spermatozoa frozen in Universal Buffer improve post-thaw motility and increase the longevity of the thawed spermatozoa, in comparison with sodium citrate- and tris-based buffers, when using red deer ejaculates that freeze moderately and poorly (D. Berg and S. Beaumont, unpublished results).

4.2. Fallow deer (D. dama)

By far, the majority of semen collections from fallow deer have been by electroejaculation. In a recent study, Asher et al. (1996b) were successful in collecting ejaculates on
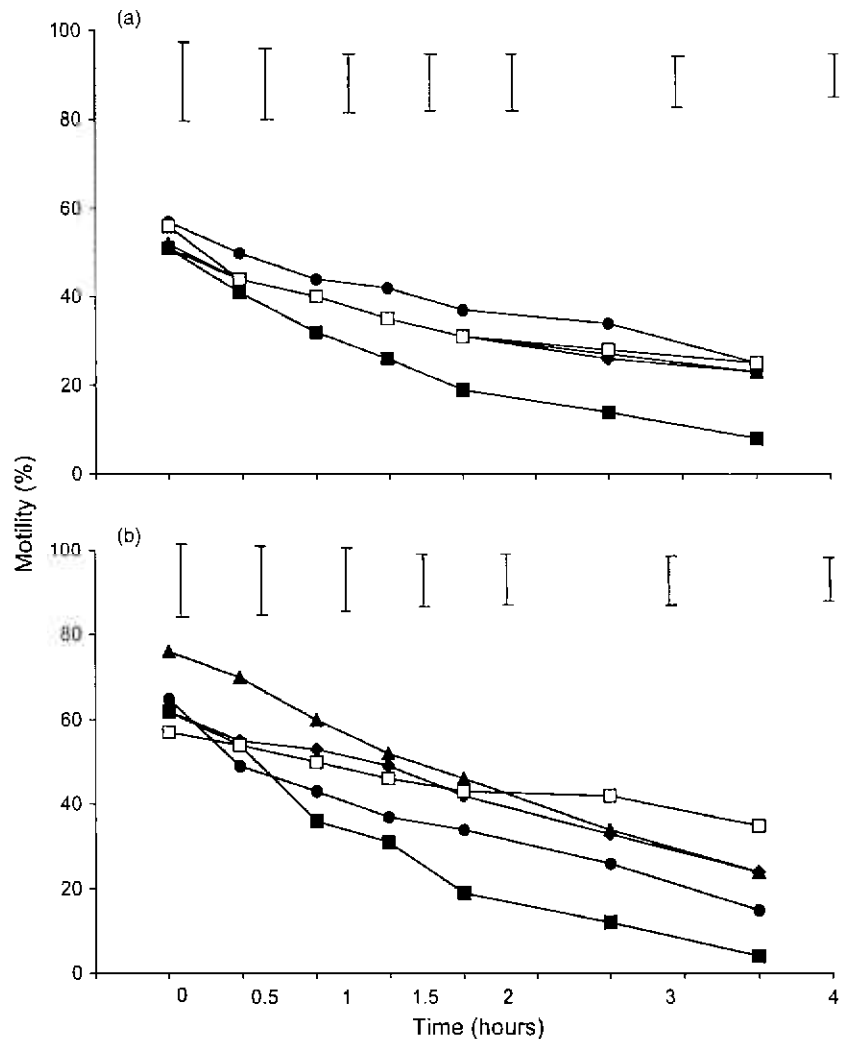
Fig. 2. Mean post-thaw motility (%) and incubation performance for semen from red deer (a) and fallow deer (b) frozen with various extenders. The results are for the five best performing extender combinations out of a total of 30 combinations tested for each species. (■) Sodium citrate/glycerol; (●) tris citrate–glycerol; (▲) skim milk–glycerol; (◆) lactose–glycerol. Ram synthetic diluent/DMSO. Vertical bars denote the least significant difference (LSD 0.05) (from Veldhuizen. 1994).

272/277 (98%) attempts throughout the year, although those collected during the summer were devoid of motile spermatozoa (Fig. 1). In general, fallow deer spermatozoa appear to be considerably more robust than red deer spermatozoa (Asher et al., 1993). Furthermore, semen from hybrid Mesopotamian × European fallow bucks (i.e. *D. dama mesopotamica* × *D. dama dama*) is generally of higher quality than that from the parental genotypes (Mylrea et al., 1991; Asher et al., 1992a).

As with farmed red deer, the utilisation of fresh or liquid-stored semen in AI programs has presented no major problems (Asher et al., 1988a, 1992a; Jabbour et al., 1993a,b). Spermatozoa numbers per intrauterine inseminate as low as 2.5×10^6 have resulted in acceptable conception rates in fresh insemination programs (Asher et al., 1992a).

Frozen storage of fallow deer semen has, in some cases, been spectacularly successful, with post-thaw motilities often in excess of 70% (Asher et al., 1993). As with red deer, most workers have used the 2.9% sodium citrate–20% egg yolk–8% glycerol extender (after Krzywinski and Jaczewski, 1978) formulation for freezing in straws (Asher et al., 1992a). Mulley et al. (1988) have also successfully frozen fallow deer semen as pellets on dry ice following extension in an egg yolk (2.25% v/v)–tris–glucose–citric acid buffer with 6% (v/v) glycerol (developed for goats by Ritar and Salamon, 1983), although post-thaw motilities were highly variable between ejaculates (10–70%).

In an attempt to improve post-thaw viability of fallow deer spermatozoa, Veldhuizen (1994) investigated the same range and combination of extenders and cryoprotectants that were evaluated for red deer (see above). For pooled semen obtained from four bucks, the highest post-thaw motility (76.7 ± 8.0%) and initial (2 h) incubation performance favoured the skim milk–egg yolk–glycerol extender (Fig. 2b). As for red deer, glycerol outperformed other cryoprotectants except for RSD-1, which was better with DMSO. However, apparent in vitro differences between the diluents were not reflected in any significant differences in conception rate after laparoscopic intrauterine AI (range 65–71%; $P > 0.10$) (Veldhuizen, 1994).

4.3. Père David (PD)'s deer (Elaphurus davidianus) and its hybrids

Recent investigations into the hybridisation of PD's deer and red deer have utilised AI to overcome barriers to natural matings between the species (Asher et al., 1988b; Fennessy et al., 1991b; Argo et al., 1994). Freezing of PD deer semen in 2.9% sodium citrate–20% egg yolk–8% glycerol has yielded modest (40–55%) post-thaw recovery of spermatozoa (Asher et al., 1988b; Argo et al., 1994). Although conception rates after laparoscopic intrauterine AI of red deer hinds have been consistently low (5%), this is more a reflection of the hybridisation process than semen quality (Tate et al., 1997). Interestingly, attempts to freeze semen from the resultant F^1 hybrid males, using the same protocols, have been largely unsuccessful. Despite initial motilities of > 70%, post-thaw recoveries have seldom exceeded 0–2% (G.W. Asher and G. Shackell, unpublished observations). However, fertility of hybrid semen is not in doubt, as fresh semen AI programs have yielded conception rates > 50% in red deer hinds (Fennessy et al., 1991a; Tate et al., 1997). The apparent fragility of hybrid semen during freezing has yet to be explained.

4.4. Axis (chital) deer (A. axis)

There have been few attempts to develop artificial breeding programs for this species. Semen collected by electroejaculation has been successfully frozen in pellet form after

extension in various diluents, including tris–egg yolk–citrate–glycerol diluent (Evans and Maxwell, 1987; Mylrea 1992), zwitterion-buffered egg yolk diluent (Molinia et al, 1994) and the commercial diluent Triladyl (Minitüb, Germany). Comparisons of the various diluents (Haigh et al., 1993; Dradjat, 1997), and of egg yolk levels of 2–20%. and pellet and straw-freezing methods (Dradjat, 1997) have revealed no consistent difference in semen quality after freezing; post-thaw motility rates were generally high, in excess of 50%, and often in the region of 60–70%.

Despite the apparently successful freezing of semen in a variety of diluents, attempts at AI with either fresh or frozen–thawed semen have been met with disappointing results. Mylrea et al. (1992) described the use of both fresh and frozen–thawed semen for intracervical AI of 19 hinds. Conception rates of 44% were obtained for fresh semen and 10% for frozen–thawed semen, with an overall calving rate of only 26%. Intrauterine AI of frozen semen using a laparoscope yielded only a slightly better result with 7/31 (23%) hinds calving (Mylrea, 1992). Dradjat (1997), likewise, in small trials with cervical or intrauterine insemination of either fresh or frozen–thawed semen, obtained conception rates less than 30% with no differences between fresh and frozen semen. Together, these results indicate that there are more problems associated with the ovulation induction or insemination techniques, or perhaps with handling stress, than there are with semen freezing in this timid species.

4.5. Eld's deer (C. eldi thamin)

Monfort et al. (1993a,b) extended electroejaculated semen in a modified BF_5 diluent (Pursel and Johnson, 1972) that comprised 20% egg yolk, 1.6% glucose, 1.6% fructose, 1.2% Tes, 0.2% Tris, 1000 i.u./ml penicillin G, 1000 mg/ml streptomycin sulfate and 0.5% surfactant mixture of sodium and triethanolamine lauryl sulfate. Glycerol was added after initial dilution and cooling to 8°C (16% glycerol contained in the same diluent and added 1:1 giving a final concentration of 8%). Straws were frozen on dry ice for 10 min before transfer into liquid nitrogen. Semen quality from six donor stags was high (60–75% initial post-thaw motility and 50–55% after 3 h of incubation) and nine pregnancies were obtained from 20 hinds after laparoscopic intrauterine AI (Monfort et al., 1993a,b).

4.6. White-tailed deer (O. virginianus)

There have been few studies on AI procedures for this species. Semen collection by electroejaculation (Bierschwal et al., 1970; Platz et al., 1982) and post-mortem epididymal recovery (Platz et al., 1982; Jacobson et al., 1989) has been attempted successfully, and pregnancies achieved after insemination with fresh semen (two pregnant/two inseminated; Haigh, 1984). Freezing in an egg yolk–tris bovine semen extender (Schermerhorn et al., 1980), a lactose–egg yolk–glycerol (Platz et al., 1982) or tris–fructose–egg yolk–glycerol (Jacobson et al., 1989) diluent resulted in acceptable post-thaw survival (> 50% motility) after pellet or straw-freezing. In two studies, in which does received intravaginal or intracervical inseminations with frozen–thawed

semen 40/53 (Jacobson et al., 1989) and 6/9 (Magyar et al., 1989), pregnancies were confirmed.

4.7. Reindeer (R. tarandus)

While reindeer have been domesticated for several millennia, AI has seldom been featured in their management. Early attempts at semen collection by AV (Dott and Utsi, 1971) and intracervical insemination with fresh or pellet-frozen semen (Dott and Utsi, 1973) produced poor results (two pregnancies from 16 inseminations). More encouraging results were obtained from semen diluted with lactose–citrate–yolk extender and frozen in straws: four out of 11 females conceived after vaginal insemination (Mkrtchyan and Rombe, 1973). There seems to be no further interest in developing AI procedures for reindeer.

5. Application of AI

AI is a tool for genetic management which allows wider and more rapid dissemination of desirable genetic material than would be remotely possible by natural mating strategies. For farmed deer, emphasis has been largely on increasing selection intensity of males of high genetic merit, accessing genes from rare genotypes (e.g. Mesopotamian fallow deer), allowing interspecies hybridisation in the face of biological limitations to natural mating (e.g. PD's deer × red deer), and as a safe and cost-effective means of international exchange of genetic material (Asher et al., 1993).

Developments in techniques of collection and freezing of semen, synchronisation of oestrus, and insemination have occurred mostly within the last 15 years, largely from studies on farmed red deer and fallow deer in New Zealand, Australia and USA (Asher et al., 1988a, 1990a, 1992a; Mulley et al., 1988; Fennessy et al., 1990, 1991a; Haigh and Bowen, 1991), although some pioneering work on red deer was conducted in Poland a decade earlier (Krzywinski and Jaczewski, 1978). Overall success in terms of conception rates to synchronised AI is in the order of 55–70% for red deer and 60–75% for fallow deer after laparoscopic intrauterine insemination with fresh or frozen–thawed semen (Asher et al., 1993). Present use of AI in these species is based largely in New Zealand, with up to 10,000 females (mainly red deer) inseminated commercially per annum. As this represents < 1% of the national breeding herd, it is clear that application is still in its infancy in the deer farming industry. However, application of AI will greatly exceed this figure once formalised genetic recording and improvement programs (e.g. sire-referencing) are implemented either nationally or internationally.

Genetic salvage also features in the adoption of AI techniques for cervids. Post-mortem recovery of spermatozoa from the epididymides of various species has been generally successful, including moose and red deer (Krzywinski, 1981) and white-tailed deer (Platz et al., 1982; Jacobson et al., 1989). On the assumption that the harvest of trophy males divests the population of individuals of high genetic merit (for trophy production), gamete recovery post-harvest allows valuable genetic material to be reinvested in the population or to be transferred to other populations (Platz et al., 1982).

There has been considerable speculation about the role of AI in genetic management of endangered cervid species in captivity, particularly as an aid to elimination of inbreeding depression. AI allows for the insemination of females with semen of unrelated males from distant populations (Wildt, 1992; Holt et al., 1996). While it seems to have become fashionable to quote this as an outcome of any study on AI in cervids, in reality, specific research and practical applications are rare. Perhaps the best example of the principle in practice is that of Eld's deer in North American zoos, whereby hinds were inseminated laparoscopically with semen from males pre-selected on the basis of underrepresented genotype (Monfort et al., 1993a).

6. Synchronisation of oestrus and AI

The review of synchronisation of oestrus and AI of deer by Asher et al. (1993) provides considerable detail on procedures for red deer and fallow deer, species that probably account for > 95% of all inseminations performed in cervids. Recent studies on other cervid species have tended to use similar procedures to those established for fallow deer. Inevitably, there must be considerable variation in response to treatment according to species, season, type of progestagen device used, and type and dose of gonadotrophin used. However, there have been few extensive controlled experiments to evaluate the effects of these factors in most deer species, particularly with regard to timing of oestrus and ovulation, which is particularly critical in AI programs with frozen–thawed semen.

6.1. Oestrous synchronisation

Synchronisation of oestrus in deer has been generally adopted as a more efficient and cost-effective alternative to detection of natural oestrous. As with other domestic ruminants, synchronisation can be achieved either by simulating the activity of the corpus luteum through the administration of progestagens, or by shortening the luteal phase of the oestrous cycle by administration of a luteolysin (prostaglandin $F_{2\alpha}$ or its analogues). For some species such as fallow deer, it is also possible to obtain a high degree of synchrony of a return oestrus after artificial synchronisation of the first oestrus (Asher and Thompson, 1989; Asher et al., 1986).

A large number of studies have investigated the use of the CIDR device, containing progesterone, for efficacy of control of oestrous in deer. Intravaginal insertion of CIDR devices for periods of between 12 and 14 days during the breeding season is generally effective in promoting a synchronised oestrus within 48 h of device withdrawal in fallow deer (Morrow et al., 1992) and red deer (Asher et al., 1992b). This regimen has also proven successful for chital deer (Mylrea, 1992), Eld's deer (Monfort et al., 1993a) and sambar deer (Muir et al., 1997). However, the additional administration of equine chorionic gonadotrophin (eCG or PMSG) at or near CIDR device withdrawal is generally performed for red deer, particularly if synchronisation is attempted close to the onset of the breeding season (Fennessy et al., 1990, 1991a). The use of eCG is contraindicated in fallow deer due to increased incidence of both anovulation and

multiple ovulation, as well as fertilisation failure (Asher and Smith, 1987; Jabbour et al., 1993a).

A limited number of studies have investigated the efficacy of intravaginal sponges impregnated with either fluorogestone acetate or medroxyprogesterone acetate for control of oestrus and ovulation in red deer (Kelly et al., 1982; Haigh et al., 1988) and fallow deer (Mulley et al., 1988). While synthetic progestagens are able to control ovulatory activity, some studies were plagued by excessive sponge loss, mitigating against their general effectiveness.

The ability of prostaglandins to synchronise oestrus is dependent on the presence of an active corpus luteum at the time of treatment, which limits synchronisation programs to the period after the onset of natural ovulatory activity. Generally, the cervine corpus luteum is responsive to the exogenous prostaglandin during the latter half of the oestrous cycle, although the precise timing of the transition from refractoriness to responsiveness has varied across studies on red deer, ranging from Day 6 (Asher et al., 1995) to Day 11 (Fisher et al., 1994). Oestrous synchronisation after injections of prostaglandin analogue has been reported for red deer (Asher et al., 1995). wapiti (Glover, 1985), fallow deer (Asher and Thompson, 1989; Jabbour et al., 1993a). PD's deer (Curlewis et al., 1988). white-tailed deer (Magyar et al., 1988) and reindeer (Ropstad et al., 1996). However, fertility of prostaglandin-induced ovulation has not been widely evaluated. In fallow deer, administration of prostaglandin analogue was observed to induce tight synchrony but poor fertility when compared with CIDR devices (Jabbour et al., 1993a).

6.2. Insemination procedures

Red and fallow deer together account for the vast majority of AIs performed in cervids, particularly in New Zealand. Australia. USA and Canada. Laparoscopic intra-uterine insemination is presently the preferred method of AI in these two species (Asher et al., 1993), as it allows precise placement of relatively small quantities of semen close to the site of fertilisation and effective use of frozen–thawed semen. Detailed descriptions of the technique in deer are provided by Fennessy et al. (1991a,b) and Haigh and Bowen (1991). Attempts at intravaginal (Krzywinski and Jaczewski, 1978; Jabbour et al., 1993a) and transcervical intrauterine (Asher et al., 1990a) inseminations in red or fallow deer have generally yielded variable, and often low (< 40%), conception rates, especially with the use of frozen–thawed semen (Asher et al., 1993). Laparoscopic procedures have been adopted for other cervid species, including Eld's deer (Monfort et al., 1993a), axis deer (Mylrea et al., 1992) and sambar deer (Muir et al., 1997).

7. Conclusion

In comparison with other domestic ruminant species, understanding of freezing of deer semen is limited in its depth and breadth. Many factors contribute to this, including limited research resources, the wide variety of species, and scarcity and intractability of many of the species. All of these render it difficult to perform properly controlled extensive investigations into short term or frozen storage of semen, including fertility testing, of most species.

Semen and AI technology developed for sheep and goats, and in some cases cattle, has been successfully adapted to several deer species, particularly to the commonly farmed fallow and red deer (Asher et al, 1993). However, even among the relatively well studied species, it is clear that there is considerable variation in viability of frozen–thawed spermatozoa. There is also variation in the fertility of females and difficulty in determining appropriate insemination times in different species, particularly in the rare or endangered species. It must, therefore, be concluded that knowledge of semen storage and AI in cervids is limited and there is much still to be learned.

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