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Review article

Potential applications of assisted reproductive technologies (ART) in reindeer (*Rangifer tarandus*)

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ABSTRACT

Interest in the use of assisted reproductive technology in reindeer husbandry has gradually increased during the last decades. This article reviews Western and Russian literature on reindeer semen collection, semen cryopreservation and artificial insemination. In addition, literature on the synchronisation of the stage of reindeer oestrous cycle among females, recovery of *in vivo* embryos, embryo transfer, the production of *in vitro*-produced embryos and pregnancy diagnosis is reviewed.

1. Introduction

Interest in and the need for the development of assisted reproductive technologies (ART) in reindeer has gradually increased. One reason for the lack of progress in development of ART in reindeer herding has been the fact that in domestic reindeer herds there has been little need to utilise procedures to enhance reproduction, because pregnancy rates are reported to be 90–95% (Laaksonen, 2016) and may approach 100% (Tyler, 1987). The ART technologies have been exploited to a greater extent in other deer species (Garde et al., 2006; Pintus and Ros-Santaella, 2014; Asher, 2018, 2019) than in reindeer. The benefits of ART in reindeer, however, have been acknowledged in a review by Ropstad (2000) and by Rowell and Blake (2019).

In 2015, the International Union for Conservation of Nature's Red List of Threatened Species placed *Rangifer tarandus* in Europe in the group of "least concern"; however, on a global scale, the species was classified as "vulnerable" (Gunn, 2016). Several reindeer subspecies are, however, regarded as threatened. In Norway, wild mountain reindeer (*Rangifer tarandus tarandus*) are now located in more than 20 virtually isolated sub-populations due to industrial development mainly occurring along valley bottom lands, leading to the loss, fragmentation and degradation of mountain reindeer habitat (Panzacchi et al., 2015). Several forest and tundra reindeer populations in the European part of northern Russia are regarded as vulnerable or near threatened (Vors and Boyce, 2009; Mizin et al., 2018). Furthermore, the status of *Rangifer* populations in Novaya Zemlya (Mizin et al., 2018), Finland (Antti Paasivaara, personal

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communication, https://www.suomenpeura.fi/en/wild-forest-reindeer/conservation-status.html; Danilov et al., 2018) and Canada (Gunn, 2016; COSEWIC, 2017) is considered uncertain, near threatened and endangered, respectively. Furthermore, the effects of climate change in the Arctic, such as extreme warm spells and rain-on-snow events, are becoming more frequent (Hansen et al., 2014), and there have been marked decreases in populations, as reported for *Rangifer tarandus* subspecies (Kohler and Aanes, 2004; Forbes et al., 2016; Langlois et al., 2017). In northern Mongolia, traditional reindeer pastoralism, as well as the distinct wild reindeer population of the region, is being affected by direct and indirect effects of climate change (Taylor et al., 2019). The number of domesticated reindeer in Mongolia decreased from 2275 to about 650 between 1977 and 2006 (Smith, 2006). According to Tyler et al. (2021), the effects of human intervention and development of infrastructure in Norway are causing environmental variation and ultimately leading to a loss of reindeer pasture that exceeds the effects of current climate change. Circumstances such as the recent anthrax outbreak, after which 200,000 reindeer were culled on the Yamal Peninsula in Siberia, can further exacerbate decreases of non-domesticated and domesticated reindeer populations (Hueffer et al., 2020), which emphasise the importance of genome resource banking. Furthermore, in North America and Scandinavia, small reindeer herds are confined for either rental business purposes or as pets and consist of females only, excluding males, because of the aggressive nature of males and consequential increased probability of danger to humans during the mating season. Thus, to sustain and increase numbers of animals in the reindeer herds without male inclusions while avoiding purchases of animals from other sources, there is a need for artificial breeding.

In recent decades, reindeer husbandry has increasingly become directed towards intensive farming (Rowell et al., 2004; Blake et al., 2007), such as using supplementary feeding and confining reindeer in large enclosures. Even though there has been this progress in deer management for human purposes, mating is mainly uncontrolled and the sires of calves are unknown (Rönnegård et al., 2003). In Finland, record-keeping of the maternal pedigree and animal identification are practiced by one-third of managers (Muuttoranta and Mäki-Tanila, 2012). Selection is currently based on the phenotypes of the animals rather than on records or pedigrees, the main selection criteria being calf size and dam phenotype. According to Muuttoranta (2014), there is an ever-increasing need for use of selective breeding in reindeer husbandry. Before any selection of animals for enhanced productivity is feasible, it is essential to have a system for animal identification, sire ascertainment and a pedigree database.

Aspects of the basic reproductive physiology of female and male reindeer (*Rangifer tarandus*), respectively, have been reviewed by Ropstad (2000) and Nagy et al. (2021). Reindeer are seasonal breeders, with the breeding season extending from late August until early February. In most free-ranging females and males, sexual maturity is reached at the age of approximately 16 and 18 months, respectively. Breeding occurs to the greatest extent during the rut in September and October (Laaksonen, 2016). The length of the female oestrous cycle is between 13 and 33 days, averaging approximately 24 days. Females are polyestrous spontaneous ovulators and there is generally production of a single calf after a gestation period ranging considerably in length from 198 to 240 days (Rowell and Shipka, 2009; Rowell and Blake, 2019).

The benefits of the utilisation of ART in reindeer husbandry are considerable making it possible to produce offspring from parents with favourable traits and establish breeding regimens accordingly. Furthermore, developing semen and embryo technologies for reindeer and genome resource banking would be possible for reviving endangered deer species where extinction is a possibility, a concept previously described by Wildt et al. (1997). Domestic herds are an excellent resource for development of species-specific ART for endangered, and non-domesticated subspecies. Difficulties in traversing the reindeer cervix during insemination or embryo recovery and the lack of capacity of the spermatozoa of many males to survive the freezing–thawing process have complicated the development of the ART in reindeer. The aim of the present review is to provide an overview of Western and Russian literature on ART in reindeer (semen collection, semen evaluation, semen cryopreservation, artificial insemination, oestrous synchronisation, embryo recovery, embryo transfer and *in vitro* embryo production). In addition, research findings related to the detection of pregnancy in reindeer are described.

2. Application of assisted reproductive technologies in reindeer husbandry

2.1. Semen collection, cryopreservation and artificial insemination

In Russia, according to Ivanova (2001), the first studies on reindeer semen collection were conducted at the Murmansk State Regional Agricultural Experimental Station in 1931 by A.A. Pokrovsky et al. with semen being collected from reindeer using a boar artificial vagina (AV). The semen quality of males was evaluated before the rut and males with lesser quality semen were not utilised for breeding. In the early development of reindeer semen technology in Russia, various boar AV devices were evaluated (Ivanova, 2001). A shortened AV for bulls was subsequently used by E.K. Borozdin in the 1960s and by M.E. Mkrtchan and V.I. Deryazhentsev in the 1970s to collect reindeer semen (Ivanova, 2001).

Borozdin (1966) collected semen from 20 reindeer males using a shortened bull AV. To accomplish this, the males were separated from the herd at the beginning of the rut and trained to ejaculate into the AV. Semen characteristics were not described. In the earlier studies, Borozdin (1963) collected epididymal semen and estimated the total quantity of epididymal spermatozoa of 3-year-old males as 4032×10^6, and that of adult males ($\geq$ 4 years) to be 5106×10^6. There, however, was not further detailed descriptive statistics provided. This quantity of spermatozoa was estimated to be a sufficient number for the insemination of 18–25 females. The progressive motility of spermatozoa collected from the head and cauda epididymis, respectively, was 10% and 80%.

Preobrazhensky (1968) collected 207 ejaculates using a boar AV. The average ejaculate volume was 0.22 mL (range 0.05–1.00 mL), and the concentration was 920×10^6 (range 200 to 3600×10^6) spermatozoa per 1 mL. Based on the motility of spermatozoa, semen was characterised into five categories. The majority of the semen samples (33.6%) was graded in the second-quality category of 4, with 18.6% of samples in category 5, 25.3% in category 3 and 18.8% in categories 2 and 1. There were 3.7% of samples in which there was

very little sperm motility or in which there were only non-motile sperm.

There are few well-documented cases of the application of AI in reindeer husbandry reported in the Western literature. Dieterich and Luick (1971) in Alaska and Dott and Utsi (1973) in Scotland conducted some "ground-breaking" experiments, but there was no subsequent utilisation of these findings to improve the efficacy of AI for breeding of reindeer. Dieterich and Luick (1971) noted that semen collection is possible but technically difficult, because the ejaculate volume is small (0.5–1.5 mL). There was successful utilisation of a ram probe for electroejaculation. Semen was processed in a commercial extender for bull spermatozoa, and when cooled to 5 °C, sperm viability was acceptable (34%) after 15 days of storage. Although it was reported that AI were conducted, the results were not reported.

In Scotland, Dott and Utsi (1971) reported that electroejaculation was not an effective method for reindeer semen collection and focused on semen collection with an AV developed for sheep. Males descending mainly from Swedish mountain reindeer easily became accustomed to semen collection, and it was not necessary to use "teaser" females in oestrus for conducting these collections. Semen had a varying colour from amber to brown, and varied in appearance from almost clear to cloudy and milky. Ejaculates from vasectomised males had little viscosity and were of a yellowish colour. According to Dott and Utsi (1971), epididymal semen showed low viscosity and had a milky consistency, but there was not reporting of the method for collecting epididymal semen. It was determined (Grenville Foster, personal communication) that there was a peristaltic method utilised that was described by Dott et al. (1979). The average volume of semen collected utilising the AV was 0.5 mL, with there being a mean density of 467×10^6 spermatozoa/mL. Mass sperm motility was usually very little but was greater after dilution in an extender that had been previously utilised for bull spermatozoa. Viability was generally very little: the mean percentage of dead spermatozoa as determined using eosin-nigrosin staining was 74.5%, but individual results varied from 15% to over 90% dead spermatozoa. Most of the samples contained 20–30% of morphologically abnormal spermatozoa and in some samples there were large percentages of tailless heads, indicating that the testes were probably exposed to high ambient temperatures (Dott and Utsi, 1971). A similar condition can also develop due to other factors, such as testicular hypoplasia, laminitis due to excessive grain feeding, fever or senescent sperm in the epididymis (Barth and Oko, 1989). Such spermiograms indicate there is relatively lesser than desirable semen quality, with the quality control standards of domestic animals not being met. Dott and Utsi (1973) conducted AI with freshly collected, undiluted reindeer semen, as well as with diluted semen and frozen–thawed semen. Four different extenders (a lactose, skim milk, glucose and inositol-based extender) and three different glycerol percentages varying from 2.7% to 3.5% were used. The insemination dose with diluted semen was 20×10^6 live sperm. Diluted semen remaining after inseminations was frozen in pellets after 5–6 h in + 4 °C. Inseminations were conducted with a glass tube and semen was deposited at the external os of the cervix. Two calves were born after insemination with fresh undiluted semen (Anon, 1972; letter to Nature). All the other 14 females inseminated with either fresh diluted ($n = 5$) or frozen–thawed semen ($n = 9$) subsequently returned to oestrus, and were mated and produced offspring the following spring. It was concluded that either the extender (and the glycerol within it) or the dilution caused a reduction in fertility.

In Russia, Mkrtchan and Deryazhentsev (1973a) were the first to use electroejaculation on un-sedated restrained males to collect semen. The volume of ejaculates varied from 0.2 to 0.7 mL. Other semen characteristics were not described. In their second study, Mkrtchan and Deryazhentsev (1973b) cryopreserved reindeer semen and reported that spermatozoa were viable after freezing and thawing. Semen was loaded into tubes of 0.1 mL and equilibrated for 5–6 h at + 4 °C. The tubes were subsequently maintained for 3 min in liquid nitrogen (LN_2) vapour and plunged into LN_2. The post-thaw motility varied from 0% to 35%. No further information was provided about semen collection, the extender, the dilution rate or thawing method.

To the best of our knowledge, Russian researchers Mkrtchan and Rombe (1973) were the first to succeed in producing reindeer calves after AI with frozen–thawed semen. Semen was collected using electroejaculation and for freezing, semen was diluted four to ten times with lactose-citrate-yolk diluent containing an unknown concentration of glycerol. There was 0.1–0.15 mL of the diluted semen loaded into straws of 5–6 cm in length. The semen straws were placed on a layer of cotton wool in a Petri dish and transferred to a thermos with ice for 5–6 h. The straws were subsequently placed on a float in LN_2 vapour for 4–5 min and transferred to LN_2. For AI, the straws were thawed by placing straws under the arm (*i.e.*, close to body temperature). Progressive motility was 50% of initial motility. Immediately after thawing, semen was used for AI. Eleven females were inseminated with frozen–thawed semen during 1971 and 1972. Before the inseminations, four herdsmen were continuously observing the herd and detecting the females for onset of oestrus. As soon as a male began to chase a female, she was caught using a rope. The female was restrained, and the inseminator washed the genitals and inserted a vaginal dilator, holding the cervix in place. With utilisation of a light source, the semen volume of 0.1–0.2 mL of frozen–thawed semen was deposited in the cervix at a depth of 2–3 cm. The AI was repeated 12–14 h later. After inseminations, the females were housed in a closed pen for 40 days, until the end of the rut. Four healthy calves were born the following spring.

Further experiments with frozen–thawed semen were conducted by H. Dott and M. Utsi in Scotland, but results remain unpublished. Pedigree records and the archived Minutes of the Reindeer Council of the United Kingdom at the Cairngorm Reindeer Herd (https://www.cairngormreindeer.co.uk) indicated that two male calves were born after artificial insemination with frozen–thawed semen pellets in 1973 and 1975 (Tilly Smith and Lotti Papastavrou-Brooks, personal communication).

Deryazhentsev (1974) described fresh AI of 11 reindeer. The semen of two males was collected using electroejaculation procedures. The volume of the ejaculate ranged from 0.58 to 0.9 mL, motility was approximately 70% and sperm concentration varied from 1800 to 2150×10^6/mL. Semen diluted 1:4 with either glucose-yolk-sodium citrate or glycol-yolk-sodium citrate extender was stored in a refrigerator at 2–4 °C. The motility of sperm cells was analysed daily by warming a sample to 38–40 °C, and daily evaluations of the refrigerated semen dilution were continued until the motility of spermatozoa was zero. Deryazhentsev (1974) varied the composition of the extenders based on the amount of sodium citric acid and determined a measure termed the "absolute survival of semen" by using the formula $S = t_1A_1 + t_2A_{2\ldots} + t_nA_n$, where t_n is the time period in a refrigerator and A_n is the motility of spermatozoa in points on a scale from 0 to 10, where 0, 1, 2…10 equals 0%, 10%, 20%...100% of spermatozoal motility (Milovanov, 1962). The optimal

concentrations of sodium citric acid were reported as 2.4–2.6 g per 100 mL in the two extenders, and the absolute survival of sperm varied from 114 to 140. For fresh AI, a third, aminoacetic acid-yolk-sodium citrate extender was used due to the greater efficacy of this extender compared to the previously described extenders for storing and maintaining spermatozoa viable at 0 °C after storage. For fresh semen AI, a syringe and a catheter for cows shortened to 10–12 cm was used along with a vaginal dilator without an external light source. The AI volume and dose were not reported, and neither was the treatment of the females prior to and after AI. As a result of using these previously described procedures, seven calves were born.

Serdtsev (1980) studied the time of ovulation and the duration of sperm motility in the reproductive tract of the female. Females were killed at different times after mating (30 min and 1, 12 and 24 h) and the vagina, uterus and ovaries were evaluated. At 1 h after mating, the spermatozoa were in the uterine horns and had progressive motility. At 12 h after mating, 50–60% of spermatozoa had considerable motility in the body and horns of the uterus, and after 24 h, spermatozoa were only detected in the cervix and had an oscillatory motility pattern.

A series of experiments in Finland from 1998 to 2000 was conducted and results were summarised by Lindeberg et al. (2005), with the aim of the study being to develop a genome resource bank for the endangered wild forest reindeer (*Rangifer tarandus fennicus*). During this period, both electroejaculation on anaesthetised males (Lindeberg et al., 2000) and epididymal semen collection from slaughtered males (Lindeberg and Valtonen, 1998) were applied. The latter method did not result in collection of sperm with acceptable quality for cryopreservation. With electroejaculation, however, there was collections of a total of 12 ejaculates during October and November 1999 from five semi-domesticated reindeer (*Rangifer tarandus tarandus*) and two wild forest reindeer (*Rangifer tarandus fennicus*) males on which anaesthesia was induced before semen collection (Lindeberg et al., 2000). The average (± SD) ejaculate volume was 1.5 ± 1.3 mL and sperm concentration was 554 ± 267 × 10^6/mL. These were greater than the respective values reported by Glover (2012), Dott and Utsi (1971), Nikitkina et al. (2019) and Rowell et al. (2019), except for there being a greater average sperm concentration recorded by Rowell et al. (2019) for males that were administered no medroxyprogesterone (MPA) treatment. The average (± SD) sperm motility in the 12 ejaculated samples was 41 ± 21%. Six ejaculates passing 60–70% pre-freeze motility were cryopreserved, and the post-thaw motility varied from 0% to 30%. No inseminations were conducted with the frozen–thawed semen. During October 1999, however, AI was conducted on three successive days with both fresh undiluted (2 days) and diluted (1 day) semen and reported by Vahtiala et al. (2003). Transvaginal AI was performed using an optical fibre (Gourley Scope method, Elite Visions, Waukon, IA, USA) which included an insemination catheter and a light source to view the portio vaginalis when conducting the AI procedures. A semen dose of 28 to 92 × 10^6 motile spermatozoa was released into the cavity of the uterine body. Pregnancies were diagnosed using transrectal ultrasonography 34–35 days subsequent to the time of AI. Five of 12 of the transvaginally inseminated females were confirmed pregnant and calved the following spring (Vahtiala et al., 2003).

Goncharov (2002) collected semen for evaluation from five males using a laboratory personnel-constructed electroejaculator. Pulses were applied for 4–5 s, followed by a pause of 4–5 s. The voltage was increased from 2 to between 6 and 8 volts. Males were restrained while lying down. The average volume of collected semen was 0.7 mL, the concentration ranged from 200 to 800 × 10^6/mL and sperm motility ranged from 60% to 80%.

Experiments using frozen–thawed semen were more recently conducted in North America using oestrous synchronisation and timed AI (Shipka et al., 2010; Bott et al., 2011). Shipka et al. (2010) collected semen from a 15-month-old reindeer male at one location, processed semen to a final dilution of 350 × 10^6 spermatozoa/0.5 mL straw, and froze and shipped the straws to another location for conducting AI. The spermatozoal pre-freeze motility was 70%, post-thaw motility was 45%, and 68% of spermatozoa were morphologically normal. One of the seven intra-cervically inseminated reindeer females was detected to be pregnant using a pregnancy-specific glycoprotein B (PSPB) assay of serum collected 11 weeks post-insemination. This female gave birth to a male calf the subsequent spring from when AI occurred (http://news.uaf.edu/fairbanks-reindeer-birth-makes-agricultural-history/, Rowell and Blake, 2019).

Bott et al. (2011) used a minimum of 10 × 10^6 progressively motile frozen–thawed sperm in the insemination dose utilised in a study. A speculum-guided standard French-style AI instrument was used to perform transcervical insemination. With this procedure, a clear speculum was inserted into the vaginal canal and the os cervix located visually with the aid of a fibre-optic light source connected to the speculum. The AI instrument was then inserted into the os cervix and manipulated through the cervical canal. Semen deposition occurred at the internal os, or into the uterine body area proximal to the cervix, in all females. Four of the six females were pregnant when diagnosed using transrectal ultrasonograpy as well as pregnancy-specific protein concentrations determined in blood samples 48 days after conducting the AI procedure. In the conference abstract of Bott et al. (2011), the semen collection methods, semen cryopreservation methods and calving rates after positive pregnancy diagnoses were not reported.

Plemyashov et al. (2017) collected 19 ejaculates for semen evaluation from ten males by electroejaculation in Taimyr, Evenkia and St. Petersburg. Males were either restrained using a rope tied around the legs and antlers or sedated using 0.5–0.8 mg/kg of xylazine hydrochloride (Xyla 20 mg/mL, Interchemie werken 'De Adelaar' B.V., the Netherlands or XylaVET professional 20 mg/cm^3, Alpha-Vet Veterinary Ltd., Hungary). The ejaculate volume varied from 0.28 to 0.90 mL, sperm concentration from 280 to 1100 × 10^6/mL, sperm motility from 40% to 85% and pH from 6.7 to 7.2. Findings using phase contrast microscopy indicated that 11.5 ± 1.2% (± SD) of spermatozoa had damaged acrosomes and 6.8 ± 1.1% had injuries in the tail and neck. The following perturbations could be identified in the acrosome: swelling (loosening), wrinkling (up to 3.9%) and absence (up to 6.5%).

Nikitkina et al. (2019) collected semen for cryopreservation and post-thaw evaluation by electroejaculation of 11 males or post-mortem by flushing the epididymides of six males using a commercial semen extender (Steridyl, Minitüb, Germany). After thawing, the average (± SD) total and progressive sperm motility was 35.6 ± 3.6% (range 0–68%) and 19.3 ± 2.3% (0–45%), respectively.

Rowell et al. (2019) collected semen using electroejaculation procedures from eight rutting males aged 1–4 years with or without

medroxyprogesterone (MPA) treatment. This is the first report in reindeer in which semen collection and evaluation were utilised to review the outcomes of an experimental treatment. The MPA treatment reduced rut-associated body weight loss and rut-associated hypophagia, interfered with velvet antler cleaning and resulted in a lack of aggressive rut-related behaviour. Semen characteristics did not differ between treatment groups, except for a lesser sperm concentration and total sperm production in MPA-treated males.

Lindeberg et al. (2019) harvested spermatozoa directly from the cauda epididymis utilising the method described by Zomborszky et al. (2005) for cryopreservation. The results were positive, even though the cauda epididymis of reindeer appeared to be considerably smaller than that of red deer. Testicles with the scrotum of six reindeer (*Rangifer tarandus tarandus*) males were collected from the reindeer slaughterhouse and transported to the laboratory in an open plastic bag placed in a Styrofoam box. Caudae epididymides were incised in several locations with a surgical blade and semen was collected with a pipette tip into a commercial extender (Steridyl, Minitüb, Germany). The initial total motility in microscopic evaluation varied from 50% to 80%. Semen was diluted to a concentration of 100×10^6/mL and equilibrated for 2–6 h before loading into 0.25-mL straws. The semen was then cryopreserved in LN_2 vapour on a rack in a Styrofoam box 4 cm above the surface of LN_2 for 10 min, plunged and then stored in LN_2. Post-thaw motility in microscopic evaluation varied from 25% to 60%. Semen with 60% post-thaw motility was successfully used for the *in vitro* production of reindeer blastocysts (Peippo et al., 2019) described in Section 2.4 of this manuscript.

2.2. Oestrous synchronisation and superovulatory treatment

In the earliest studies on AI in reindeer (Preobrazhensky, 1968; Dott and Utsi, 1973), no oestrous synchronisation regimens were used. Females expressing natural oestrous symptoms were detected using either a vasectomised male (Dott and Utsi, 1973) or young fertile males with aprons attached to prevent mating with the oestrous females (Preobrazhensky, 1968).

2.2.1. Prostaglandins

Prostaglandins (PG; *i.e.*, $PGF_{2\alpha}$, cloprostenol) appear to be effective in inducing luteolysis in non-pregnant oestrous-cycling reindeer (Ropstad, 2000; Rowell et al., 2000). The effectiveness of prostaglandins was first reported by Ropstad and Lenvik (1991) in a study on 91 7-month-old female calves and there was further thorough investigations of efficacy of prostaglandin use by Ropstad et al. (1996) in seven 1.5-year-old reindeer (*Rangifer tarandus tarandus*) females in which reproductive steroid hormone profiles had been evaluated. Synthetic prostaglandins appear to be more efficacious than natural prostaglandins (Ropstad and Lenvik, 1991). Ropstad et al. (1996) recognised oestrous symptoms 56–69 h after cloprostenol (Estrumat vet, Pitman-Moore, UK) treatment. Standing oestrous behaviours lasted on average 27 h (range 24–30 h).

The stage of the oestrous cycles was synchronised among six multiparous reindeer females utilising three treatments of 0.25 mg cloprostenol (Estrumat 0.25 mg/mL, Mallinckrodt Veterinary Ltd., UK) on days 0, 14 and 24 (Lindeberg et al., 1998). After the treatments, three of the six females mated with a fertile male 57–77 h after the last PG treatment (*i.e.*, there was 50% success in oestrous synchronisation). The three mated females were slaughtered 8 days after the last PG treatment. There was a corpus luteum in the ovaries and the uteri and oviducts were flushed post-mortem for collection of single embryos. One 8-cell stage embryo from the oviduct of one female and one blastocyst stage embryo from the uterus of another female were recovered, therefore, the recovery rate was 66.7% (two embryos/three flushings).

In Alaska, two treatments with $PGF_{2\alpha}$ (15 mg Lutalyse) 10 d apart were efficacious for synchronising stage of the oestrous cycle among eight of ten reindeer females (Rowell et al., 2004). Oestrus occurred 48–60 h after the administrations of the second PG treatment. According to the same researchers, placement of a bull with a group of females resulted in synchronisation and advancement in the average time of oestrous onset by 2 weeks (Shipka et al., 2002).

Säkkinen et al. (2003) and Vahtiala et al. (2005) conducted treatments to synchronise the stage of oestrous cycles among five multiparous reindeer (*Rangifer tarandus tarandus*) females using 0.25 mg cloprostenol (Estrumat 0.25 mg/mL, Pitman Moore, Germany) to study ovarian follicular dynamics utilising ultrasonography, and there was collection of blood samples *via* permanent jugular vein cannulas (Aalto et al., 2003) every 3 h for 5 days after PG treatment. In four of five females (the fifth female had no ovarian structures indicating lack of oestrous cycles), plasma progesterone decreased to baseline values and LH peaks occurred 52–60 h after PG treatment.

In Russia, Nikitkina and Goncharov (2015) conducted treatment regimens to synchronise stage of oestrous cycles among 25 Evenk and 10 Nenets reindeer females by administering 52.5 mg of D-cloprostenol (PG; Galapán, Invesa, Spain) intramuscularly at the end of September ($n = 25$ Evenk females) and at the beginning of October ($n = 10$ Nenets females). Greater than 80% of both Evenk ($n = 21$) and Nenets ($n = 8$) reindeer females expressed symptoms of oestrus at 72–96 h after PG treatment. The females were mated naturally and mating occurred at an average of 96 h after PG treatment. Pregnancy diagnosis was not conducted. During the next spring, during a period of 7 days, there were parturitions of 33 of the 35 females, while the remaining two females did not produce offspring. It was concluded that four females were possibly mated after 96 h from when PG treatment occurred (Nikitkina and Goncharov, 2015).

2.2.2. Gestagens and superovulation

During the breeding season, in non-pregnant oestrous cycling reindeer females, gestagens have been reported to be effective for inducing oestrous synchronisation. Dieterich and Luick (1971) induced synchronous expression of oestrus among female reindeer by using intravaginal progestin-containing pessaries (Syncro-Mate, G.D. Searle and Co., Chicago, Illinois). Females usually expressed oestrus 3 days after the removal of the pessaries. Oestrus was detected by using a vasectomised male and females were inseminated as soon as females were marked by the male.

In the studies of Valtonen et al. (1996) and Rainio et al. (1997), three non-pregnant non-oestrous cycling reindeer females were

intravaginally administered fluorogestone acetate sponges (Chrono-Gest 40 mg, Intervet International BV, Boxmeer, Holland) for 13–16 days for inducing oestrous synchronisation with there being a combined administration of PMSG (Folligon, Intervet International BV, Boxmeer, Holland) 1500 IU (first year) and 800 IU (second year) intramuscularly for superovulation 1 day before sponge removal. Cloprostenol (Estrumat 0.25 mg/mL, Pitman Moore, Germany) treatment of 0.25 mg was included 6 h before sponge removal during the first year of the study only. In the experiments conducted both years, the previously described treatment regimens when utilised were not effective for purposes of synchronising stage of the oestrous cycle among females or inducing super-ovulations. The cause for these lack of responses was thought to be due to the females being in an anoestrus-state of reproduction at the time treatments were administered (retrospectively confirmed by serum progesterone concentrations in sampling at 4- to 5-day intervals).

In studies of non-pregnant reindeer females during the breeding season in October and November, controlled intravaginal drug release devices (CIDR) were determined to be effective for management of stage of oestrous cycles among females. A 14-day treatment with intravaginal progesterone release devices (two CIDRs for 14 days, a second CIDR was inserted on day 11 of treatment in Vahtiala et al. (2003): Eazi-breed™ CIDR® containing 0.3 g of progesterone, Pfizer Animal Health, New Zealand, and a second CIDR was inserted on day 10 in Lindeberg et al. (1999): CIDR-G containing 0.33 g of progesterone, Inter Ag, New Zealand) was used to synchronise the stage of the oestrous cycles among 17 multiparous reindeer (*Rangifer tarandus tarandus*) females for conducting an AI programme (Vahtiala et al., 2003) and in ten females for conducting an embryo recovery programme (Lindeberg et al., 1999). According to Vahtiala et al. (2003), 12 of the 17 females (70.6% success in oestrous synchronisation) were confirmed to be in oestrus as a result of the observed mating with a vasectomised male and were artificially inseminated on the day of oestrus.

In the Lindeberg et al. (1999) study, a superovulatory treatment was included, starting 72 h before the second CIDR removal, and consisted of twice-daily injections for 4 days with 0.55 mg of follicle stimulating hormone (FSH) (Ovagen™, Immuno Chemical Products, Auckland, New Zealand) in each dose. All females were treated with 0.25 mg cloprostenol (Estrumat 0.25 mg/mL, Pitman Moore, Germany) on days 3 (evening) and 4 (morning) of the 4-day FSH treatment period. A proven fertile reindeer male mated with the females during the induced period of oestrus. Two of ten females were not mated and were removed from the embryo recovery programme. Embryos of four females were recovered post-mortem and embryos of the remaining four females were recovered using 3:2 medetomidine (Domitor 5 mg/mL, Orion-Farmos, Finland, 16.5–22 mg/female) – ketamine (Ketalar 50 mg/mL, Parke-Davis, S.A., Spain, 90–145 mg/female) anaesthesia, 0.75 mL of combination/10 kg body weight. For embryo recovery, a catheter used for heifers (Ch 15, Wörrlein, Germany) was inserted into the uterus through the cervical canal while there was guiding with the surgeon's hand inserted into the peritoneal cavity through an incision in the linea alba. The recovery rates of post-mortem and surgical techniques were, respectively, 23.7% (nine recovered unfertilised oocytes and embryos/38 corpora lutea) and 23.1% (three embryos/13 corpora lutea). It was concluded that the protocols utilised to conduct this study needed to be improved.

At the onset of the breeding season at the end of August, Rowell et al. (2004) successfully used shortened CIDRs typically used for cattle (EAZI-BREED™, Pharmacia & Upjohn, Kalamazoo, MI, USA) to synchronise the stage of the oestrous cycle among 11 females that were placed with a male approximately 24 h after CIDR removal (inserted 12 days earlier). Copulations occurred 48–60 h after CIDR removal. There were 10 of 11 females mated and that became pregnant as a result of mating at the synchronised oestrus following conducting of this treatment regimen.

In one of the two most recent studies where there was reporting of successful AI outcomes with use of frozen–thawed reindeer semen, Shipka et al. (2010) synchronised the stage of oestrous cycles among eight females using a CIDR-b, modified to fit the smaller reindeer vagina, using two CIDR-bs for 14 days. At the time of removal of the second CIDR-b, the females were administered 200 IU PMSG (either P.G. 600®, Intervet or PMSG, Sigma Chemicals) to induce oestrus and ovulation. Intra-cervical inseminations occurred 55 h after the second CIDR-b removal. Seven females expressed symptoms of behavioural oestrus at the time of insemination.

In the study of Bott et al. (2011), six adult non-mated female reindeer 2–6 years of age were selected and housed separately from male animals prior to the initiation of the fall breeding season. Females were confined in an area where there was a controlled photoperiod (10 h of light; 14 h of darkness) for 3 weeks beginning the last week of August. The stages of the oestrous cycle among females were synchronised using a 14-day controlled drug release device typically used in sheep (CIDR), and were administered an injection of cloprostenol (250 μg, im) at the time of CIDR removal. At 44 h following CIDR removal, a timed transcervical AI was performed using two 0.5-mL straws of frozen–thawed semen. Each straw contained a minimum of 10×10^6 progressively motile sperm. A GnRH injection (100 μg, im) was also administered at the time of AI. At 48 days after AI, four of the six females (66%) were diagnosed pregnant using procedures for both transrectal ultrasonography and optical density determination of blood PSPB.

2.3. Embryo transfer

One successful surgical transfer of fresh *in vivo*-produced embryos in a domestic reindeer (*Rangifer tarandus tarandus*) has been reported (Lindeberg et al., 2003). The stages of the oestrous cycles among three reindeer donor females were synchronised using two CIDR devices for 15 days (the first CIDR 10 days and the second 5 days in the vagina, CIDR-G containing 0.33 g of progesterone, Inter Ag, New Zealand) during the breeding season of 1999. After the removal of the second CIDR, the donor females were allowed to mate with a vasectomised male during the following oestrus to confirm the efficacy of the oestrous synchronisation treatment regimen.

Starting on day 6 (day 0 = day of mating of donor with the vasectomised male), the donors were treated with FSH (Ovagen™, Immuno Chemical Products, Auckland, New Zealand) twice daily during a 4-day period with a decreasing dose regimen (1 + 1 + 1 + 1 + 0.75 + 0.75 + 0.5 + 0.5 NIH-FSH-SI units) to induce superovulation. Luteolysis was induced using 250 μg of cloprostenol (Estrumat 0.25 mg/mL, Pitman Moore, Germany) at 72 h (250 μg) and at 84 h (250 μg) after commencing superovulation. The donors were mated with a fertile male during the PG-induced oestrus and embryos were recovered after inducing anaesthesia on day 8 or 9 (day 0 = day of mating with a fertile male) using the surgical-transcervical technique described by Lindeberg et al. (1999). Three

preimplantation-stage embryos were recovered and transferred in the fresh state into the uteri of two reindeer recipients on which anaesthesia was induced on day 7 or 8 (day 0 = day of mating of the recipient with the vasectomised male) with a Cassou pistolette using the surgical-transcervical technique. The stages of the oestrous cycles among recipient females were synchronised using CIDRs for 14 days. In one recipient, there was transfer of two blastocyst stage embryos and in the other recipient there was transfer of one blastocyst. The recipient into the one blastocyst was transferred conceived and this recipient was diagnosed pregnant at the time of an ultrasonographic examination 60 days after the embryo transfer. This recipient female gave birth to a male calf 212 days after the embryo transfer occurred.

2.4. *In vitro* embryo production

In two published papers (Krogenæs et al., 1993, 1994) and one conference abstract (Peippo et al., 2019) there is a description of *in vitro* embryo production (IVP) in domestic reindeer using a cattle IVP treatment regimen. Krogenæs et al. (1993) collected the ovaries of reindeer calves (4–6 months old) from slaughterhouses during the non-breeding season in August/September and recovered 2.1 oocytes/ovary. After 24 h of *in vitro* maturation (IVM), 71% of the oocytes had developed to the metaphase II stage with extrusion of the first polar body. In the second experiment, Krogenæs et al. (1994) collected semen for *in vitro* fertilisation (IVF) from epididymides of testicles collected from the carcasses of two slaughtered reindeer males. The fertilisation rate based on oocytes fixed and stained 24 h after insemination was 36.0%. Of the presumptive zygotes, 31.8% cleaved in *in vitro* culture (IVC) and the majority developed to the 2- to 4-cell stage. Two embryos developed to the morula stage after 7 days in culture.

Peippo et al. (2019) were the first to produce reindeer blastocysts derived using IVP procedures. Oocytes were collected from slaughtered prepubertal and adult females (*Rangifer tarandus tarandus*) during the breeding season in November 2018 to January 2019. Cumulus–oocyte complexes (COCs) were washed two times in Emcare solution and once in IVM medium. After 24 h of maturation, the COCs were placed in tubes containing IVM media and transported to the laboratory in a portable incubator in 38.5 °C. For the 20-h IVF procedure, sperm-TALP -washed frozen–thawed semen cryopreserved after epididymal collection from a 4-year-old male (Lindeberg et al., 2019) was used. Denuded zygotes were cultured in G1/G2 medium supplemented with FAFBSA and L-carnitine for 7 and 8 days. Four day-7 and two day-8 blastocysts were produced and cryopreserved for possible later use.

2.5. *Reproductive ultrasonographic examinations and hormonal pregnancy detection*

Ultrasonographic examinations of the reproductive tract can be conducted. The small body size (the weights of multiparous reindeer females vary from 70 to over 100 kg) of females, however, prevents insertion of the hand with the transducer inside the rectum. During the examination, the females need to be restrained in a crate to prevent them from moving too vigorously or lying down while the linear transducer with a rigid holder is positioned inside the rectum. In the study of Säkkinen et al. (2003) both ovaries were located in 67% of the 165 examinations with a 7.5-MHz linear transducer, only one ovary in 21% of the examinations and both ovaries were not located in 12% of the examinations. Transrectal ultrasonographic examinations, therefore, are much more practical for pregnancy diagnosis than for ovarian follicular dynamics assessments. Results from studies conducted for technique-efficacy of pregnancy detection in reindeer (*Rangifer tarandus tarandus*) indicate the transrectal ultrasonographic method as being 92% accurate in diagnosing pregnancy at week 6 of the gestational period (Vahtiala et al., 2004) and 99.5% accurate at weeks 7–16 (Savela et al., 2009). The foetal growth curves obtained when conducting ultrasonographic examinations in the study of Vahtiala et al. (2004) resembled those obtained when there were previous morphological studies with post-mortem tissues in the same subspecies (Roine, 1974; Roine et al., 1982). In a field study conducted by Savela et al. (2009), foetal measurements were obtained when the foetal position allowed for such evaluations and were used for estimation of the stage of gestation.

Hormonal pregnancy determination, analysing the concentration of pregnancy-associated glycoproteins (PAGs) in milk, has become commercially available for early pregnancy diagnostics in dairy cattle. For reindeer, there were the first reports of hormonal pregnancy determination where there was analysis of PSPB in the blood of semi-domestic (*Rangifer tarandus tarandus*) and Svalbard (*Rangifer tarandus platyrhynchus*) reindeer by Ropstad et al. (1999) and in the blood of reindeer and caribou (Rowell et al., 1999). Results from further studies indicated there were increased concentrations of plasma PAGs as early as 21–30 days after the estimated conception date (median date: 10 November) (Ropstad et al., 2005; *Rangifer tarandus tarandus*), and it was concluded that the plasma concentration of PAGs provided a reliable pregnancy detection method when measured > 30 days post-conception and in herds when there were mid-December evaluations. In Savela et al. (2009), only one incorrect negative pregnancy diagnosis was made using plasma PAGs at the threshold level 0.5 ng/mL. For the commercial milk pregnancy test in cattle, the cut-off value is 0.25 ng/mL. Ropstad et al. (1999) reported the need for cost-efficient, early and accurate methods for pregnancy testing in the reindeer industry to be able to slaughter barren females and manage reindeer herds more efficiently. For pregnancy status assessments in reindeer herds, a small number of pregnancies are confirmed by ultrasonographic assessments, while pregnancy diagnosis using plasma PAGs is only used for research.

3. Summary and conclusions

A large amount of reindeer-specific information has been published on health, diseases and veterinary care (Laaksonen, 2016; Tryland and Kutz, 2019; Bott, 2021), including literature on reindeer reproductive management (Blake et al., 2007; Bott, 2018) and reproductive physiology (Rowell and Blake, 2019). This will hopefully increase understanding of reindeer management issues and enable the productivity of reindeer herds to be markedly improved. Reindeer semen has been successfully cryopreserved and live

offspring produced after AI a total of five times: once in Russia and twice in Scotland and twice in North America about 5, 5 and 2 decades ago, respectively. In Finland, one successful fresh embryo transfer has been reported since 2000, and the first successful production of reindeer IVP blastocysts was reported in 2019. The few research successes are indicative of the possibilities for use of the ART in reindeer husbandry. In general, cryopreservation technologies provide for new opportunities for improved breeding regimens. Semen and embryo technologies may also be utilised to revive some of the endangered *Rangifer* populations.

CRediT authorship contribution statement

H. Lindeberg: Writing – original draft, Writing – review & editing. **E. Nikitkina:** Writing – original draft, Writing – review & editing. **Sz. Nagy:** Writing – original draft, Writing – review & editing. **A. Musidray:** Writing – original draft. **G. Shiryaev:** Writing – original draft. **J. Kumpula:** Writing – review & editing. **Ø. Holand:** Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anireprosci.2021.106890.

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