ASSISTED REPRODUCTIVE TECHNOLOGIES IN DEER (ARTIODACTYLA, CERVIDAE): A REVIEW*

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The application of the Assisted Reproductive Technologies (ARTs) in wildlife is crucial not only for conservation and management purposes, but also to address important clues in human reproduction. Nevertheless, the knowledge of reproductive biology of deer is still limited to few species, thus the successful application of ARTs. This review deals with reproductive biology and ARTs mainly focused on male deer providing a deep insight into the most recent literature published on the following topics: sperm collection, storage, and artificial insemination.

cervids; reproductive biology; sperm collection; sperm storage; artificial insemination

INTRODUCTION

Cervids are a remarkably diverse group of ungulates, with approximately 43 species and 206 subspecies presently described, greatly ranging in size from the Alaskan moose (*Alces alces gigas*; 700 kg) to the Northern Pudu (*Pudu mephistophiles*; 9 kg) (Whitehead, 1993; Asher et al., 2000; Morrow et al., 2009). Antlers are the most widely distributed sexual trait of male Cervidae, with the exception of reindeer (*Rangifer tarandus*, where also females have antlers) and the genus *Hydropotes* (where antlers are replaced by developed upper canine teeth).

With the exception of Antarctica and Australia, deer are naturally distributed in every habitat and latitude (Asher et al., 2000). Adaptation to such various environmental conditions is responsible of the diverse reproductive biology of deer ranging from highly seasonal species typical of the arctic latitudes (e.g. *Rangifer tarandus*) to the aseasonal species of the equatorial regions (e.g. *Axis axis*). Moreover, several deer species show unusual reproductive features like e.g. the phenomenon of diapause in the roe deer (*Capreolus capreolus*; for a review see Renfree, Shaw, 2000). Embryonic diapause, or delayed implantation as it is also called, occurs when the conceptus enters a state of suspended animation at the blastocyst stage of development (Renfree, Shaw, 2000). Despite the fact the embryonic diapause in roe deer was described more than a century ago, the mechanisms of this phenomenon are still poorly understood (Hermes et al., 2000). Moreover, although diapause has been described in seven mammalian orders, the roe deer is the only case within the Artiodactyla (Renfre, Shaw, 2000).

Deep knowledge of reproductive biology of deer is crucial for the successful application of Assisted Reproductive Technologies (ARTs) (Asher et al., 1993; Jabbour et al., 1997; Berg, Asher, 2003). Despite its utter importance, the reproductive biology of several deer species still remains unknown (Pukazhenthi et al., 2006; Fickel et al., 2007). Undoubtedly, ARTs will be most effective at present for species whose reproductive physiology is well characterized, and where appropriate techniques exist for manipulating or monitoring the female reproductive cycle (Holt et al., 1996). Knowledge of reproductive biology in wildlife is valuable not only for recovering and genetically managing rare species, but also for addressing certain reproductive issues in humans (Wildt et al., 2010). For instance, owing to its remarkable seasonality, the roe deer has been suggested as a model to study testicular functions (Klonisch et al., 2006). The authors stated that the determination of the molecular pathways controlling the seasonal changes in testis function might provide important clues to the regulatory mechanisms involved in spermatogenesis, which are applicable not only to

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the roe deer but also to fertility/infertility in other mammals including human.

On the other hand, seasonal pattern of involution and recrudescence of spermatogenesis poses a major constraint on both the harvesting of gametes and the application of artificial insemination (Asher et al., 2000). In fact, in seasonal breeders, sperm collection is limited to the mating season.

Deer have also been used as a model in evolutionary biology and sexual selection studies due to their exaggerated sexual dimorphism, which has led to different mating strategies in the two sexes (e.g. Clutton-Brock et al., 1982). Malo et al. (2005) found that in Iberian red deer antler size is positively related to sperm production and quality, whereas Gomendio et al., (2006) showed that the fertility of Iberian red deer stags is positively associated with the proportion of male offspring, providing evidence that also males could bias sex-ratio in the context of the Trivers, Willard's (1973) hypothesis. Moreover, sperm morphological traits and proportions between components of sperm flagellum in Iberian red deer stags are greatly associated with sperm velocity (Malo et al., 2006), cryoresistance, and lifespan (Ros-Santaella, 2012).

It is noteworthy that several deer species are threatened or endangered with extinction. According to the IUCN red list (IUCN, 2013), within the Cervidae family 16 species are classified as vulnerable, 2 near threatened, 7 endangered, 1 critically endangered (*Axis kuhlii*), 1 extinct in the wild (*Elaphurus davidianus*), and 1 extinct (*Rucervus schomburgki*). Successful conservation of any species requires a multidisciplinary approach, which integrates both biochemical, cellular, physiological, and ecological aspects of the biology of the species to be conserved (Jabbour et al., 1997). Moreover, conservation efforts require a comprehensive understanding of the biology of deer in order to better preserve them for the next generations to come (Jabbour et al., 1997). Both *in situ* and *ex situ* conservation programs for endangered mammalian species can benefit from modern reproductive biotechnologies or assisted reproductive techniques including semen/oocyte collection and storage, artificial insemination (AI), embryo transfer (ET), *in vitro* fertilization (IVF) and embryo production (IVP), gamete/embryo micromanipulation, semen/embryo sexing, and genome resource banking (GRB) (Andrabi, Maxwell, 2007).

The case of Sardinian red deer (*Cervus elaphus corsicanus*), one of the smallest subspecies of red deer, is a representative example. Sardinian red deer is an endemic species of Sardinian and Corse and it became almost extinct in the 1970s (around 200 individuals left in the wild) (Schenk, 1976). Nowadays, thanks to both *in-situ* and *ex-situ* programs it is safe from extinction and more around 3000 individuals are currently censused in Sardinia (Casula et al., 2013). Recently, advanced ARTs such as *in vitro* embryo production and interspecies somatic-cell nuclear transfer (iSCNT) have been performed in this species (Ucchedda, 2012), although to date no blastocysts have been obtained *in vitro*. The deer-sheep iSCNT embryos did not develop indeed beyond the 8–16 cells stage (Ucchedda, 2012).

Further, successful application of ARTs allows more offspring to be obtained from selected parents to ensure genetic diversity, reduce the interval between generations and avoid depression in small and fragmented populations (Comizzoli et al., 2000, 2010; Andrabi, Maxwell, 2007). To date, ARTs for cervids have been limited and available only for a few species (Asher et al., 2000). Although ARTs have been greatly improved over the last decades in the farming deer industry, their application within natural populations is in its infancy (Garde et al., 2006). Nevertheless, its future potential is enormous, not only in relation to genetic management or conservation (Asher et al., 1993; Loskutoff et al., 1995; Garde et al., 2006), but also for sport based on trophy hunting (Long et al., 2003).

This short review deals with the past and future goals of ARTs on cervids. The methods here discussed are basics of semen collection, handling, storage, and AI in cervids with a deep analysis of the most recent literature published on this topic.

Sperm collection

To date, three methods of sperm collection have been described in cervids: natural service into artificial vagina (AV), electroejaculation (EE), and *post-mortem* epididymal recovery (e.g. Garde et al., 1997; Asher et al., 2000; Soler et al., 2003a; Gizejewski, 2004). Semen characteristics are greatly influenced by the method of collection. In fact, while electroejaculation results in ejaculates of high volume (due to the increased volumes of fluids from accessory glands and epididymides), higher quality sperm samples can be obtained using artificial vagina (Gizejewski, 2004; Ungerfeld et al., 2008). However, to date neither EE nor AV can successfully separate ejaculate in sperm fractions. Other methods like internal AV (Gizejewski, 1991) or modified AV (Gizejewski, 2000, 2004) have been considered in order to separate sperm fractions.

In red deer, three fractions of ejaculates could be clearly identified: grey, white, and yellow fractions (Gizejewski et al., 2003). All fractions contained spermatozoa, with the exception of the yellow one, and vary greatly during the reproductive cycle (Gizejewski et al., 2003). In fact, the sticky and viscous yellow fraction ("honey fraction"), a result of vesicular glands secretion, could be found only during the rut and its presence may affect sperm quality (Gizejewski et al., 2003; Garde et al., 2006).

Spermatozoa that come in contact with yellow fraction lose indeed their motility (pers. observation). Role and function of the yellow fraction are still unknown but it might be involved in post-copulatory sexual selection.

Polish researchers have pioneered studies on the use of natural service semen collection methods for red deer (Krzywinski, 1976; Strzezek et al., 1985; Giżejewski, 2004). In spite of the minimally invasiveness, natural service into an artificial vagina requires a high level of stag training and habituation. Moreover, this method is quite dangerous due to the high aggressiveness of stags during the breeding season (Asher et al., 2000). On the contrary, Dott, Utsi (1973) have shown that reindeer is a more domesticated and docile species that could be easily trained to this method.

Electroejaculation is the most widespread method of sperm collection in cervids and it must be used when the objective is either to obtain a representative number of samples from natural populations not subjected to hunting, or to repeatedly recover samples from selected males (Garde et al., 2006). White-tailed deer (*Odoicoleus virginianus*) was the first deer where electroejaculation has been performed (Bierschw et al., 1970). Usually, a combination of xylazine hydrochloride and ketamine hydrochloride injected intramuscularly (Asher et al., 1996; Umapathy et al. 2007; Ungerfeld et al., 2008, Favoretto et al., 2012; Rittem et al., 2012) or intravenously (Martinez-Pastor et al., 2006b, 2009; Martínez et al., 2008; Ungerfeld et al., 2008), or a combination of xylazine hydrochloride and tiletamine/zolazepam injected intramuscularly (Pintus, 2012) are used as anaesthetic protocols. Yohimbine hydrochloride (Martinez-Pastor et al., 2006b, 2009; Umapathy et al., 2007; Martínez et al., 2008) or atipamezole hydrochloride (Pintus, 2012) are generally used to revert anaesthesia.

Despite the risks associated with the use of anaesthetics, electroejaculation does not require training and it is less time-consuming. Nevertheless, the risks of contamination by urine or accessory glands secretion are inevitably higher. In order to avoid urine contamination, it is a good strategy to change the collection tube between semen emissions (Martinez-Pastor et al., 2009). Electroejaculated semen has a different composition compared to semen collected by natural methods (i.e. artificial vagina), because of different stimulation of seminal glands by the electroejaculation probe and the difficulty of achieve repeatability between electroejaculation sessions (Garde et al., 2006).

Post-mortem sperm recovery is the most practical option to obtain samples from wild populations of red deer with hunting representing a constant source of harvested animals (Garde et al., 2006). On the assumption that the harvest of trophy males divests the population of individuals of high genetic merit or 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).

Post-mortem recovery of spermatozoa from the epididymis of various species has been generally successful, including moose and red deer (Krzywinski, 1981), white-tailed deer (Jacobson et al., 1989), sika deer (Hishinuma et al., 2003), and Iberian red deer (Garde et al., 1997; Soler et al., 2003a), although time lapse between animal's death and sperm collection could affect sperm parameters. In Iberian red deer, epididymal sperm characteristics, except the percentage of morphological abnormalities, significantly decrease after 12–24 h post-mortem (Garde et al., 1998). Recently Malcotti et al. (2012) found that acceptable sperm motility after thawing could be obtained even after 30 h of storage at 20°C. In Iberian red deer, Soler et al. (2003a) observed that temporary storage of testes at 5°C may help to preserve plasma membrane up to 4 days post-mortem, whereas the percentage of morphologically normal spermatozoa remained unaffected during the first 3 days of storage. In a later study, the same authors found that the motility of spermatozoa stored in the epididymis for up to 96 h did not decrease significantly but, after cryopreservation, a decline in sperm motility was seen in spermatozoa stored for 48 h, or later (Soler et al., 2005). To complete the work by Soler et al. (2005), Fernández-Santos et al. (2009a) evaluated sperm motility by CASA system at 0 and 2 h post-thawing. The study revealed that even after 96 h of storage at 5°C, sperm motility after cryopreservation was still good enough to consider the salvage of spermatozoa from samples with long post-mortem time (Fernández-Santos et al., 2009a).

Epididymal sperm could be collected by cutting the cauda of epididymis with a surgical blade (Soler et al., 2003a, 2005; Zomborszky et al., 2005) or by retrograde flushing of the vas deferens and the proximal cauda (Garde et al., 1997; Comizzoli et al., 2001). These methods have been recently compared finding that the total amount of sperm recovered is equivalent between the two techniques (Martinez-Pastor et al., 2006c). Although epididymal spermatozoa lack the seminal plasma (SP) containing the secretions of the accessory glands, they have the advantage of being collectable even post-mortem or post-orchidectomy. Martinez-Pastor et al. (2006a) found that supplementing epididymal sperm with seminal plasma from the same species improves cryopreservation of Iberian red deer spermatozoa. However, the use of epididymal spermatozoa for AI or *in vitro* fertilization (IVF) procedures requires conditions other than for SP-embedded spermatozoa from ejaculates (Fickel et al., 2007).

Martínez et al. (2008) compared physiological parameters between electroejaculated and epididymal

Table 1. Sperm cryopreservation protocols in deer - part 1

Species	CM	Diluent	CPA	T° of CPA addition	Final SC (Spz ml^{-1})	ET	CR	Thawing	Reference
Cervus elaphus hispanicus	ES	Triladyl® – 20% EY	G (6%)	RT	400 × 10^6	2 h at 5°C	from RT to 5°C in 1.5 h; frozen in liquid nitrogen vapour for 10 min	70°C for 5 s, 60°C for 8 s, 37°C for 20 s	Soler et al., 2003c
	ES	TES-Tris-fructose – 10% EY	G (4%)	37°C, 5°C	100 × 10^6	2 h at 5°C	–20°C/min from 5 to –100°C	65°C for 6 s	Martinez-Pastor et al., 2006a
	EE, ES	TES-Tris-fructose – 20% EY (320–380–430 mOsm kg^{-1})	G (4% for EE, 8% for ES)	RT for EE, 5°C for ES	100 × 10^6	2 h at 5°C	–20°C/min from 5 to –100°C	65°C for 6 s	Martinez-Pastor et al., 2006b
	ES	TES-Tris-fructose – 10% EY	G (4%)	5°C	100 × 10^6	2 h at 5°C	–20°C/min from 5 to –100°C	65°C for 6 s	Martinez-Pastor et al., 2006c
	ES	TCF – 20% EY	G, EG, PG (all at 0–3–6–12%)	22°C, 5°C	400 × 10^6			37°C for 30 s	Fernández-Santos et al., 2006a
	ES	TCF (0–5–10–20%), WE or CE	G (3–6%)	RT	200 × 10^6	2 h at 5°C	from RT to 5°C at –0.23°C/min or –4.2°C/min; frozen in liquid nitrogen vapour for 10 min	37°C for 20 s	Fernández-Santos et al., 2006b
	ES	Tris-sugar (fructose, manose, glucose, maltose, sucrose, trehalose, rafinose) based medium – 20% CE (360–600 mOsm kg^{-1})	G (6%)	22°C	200 × 10^6	2 h at 5°C	from RT to 5°C in 10 min; frozen in liquid nitrogen vapour for 10 min	37°C for 30 s	Fernández-Santos et al., 2007a
	ES	TCF – 20% CE enzymatic and nonenzymatic antioxidants	G (6%)	22°C	200 × 10^6	2 h at 5°C	from RT to 5°C in 10 min; frozen in liquid nitrogen vapour for 10 min	37°C for 30 s	Fernández-Santos et al., 2007b
	EE	Andromed® – soybean extract, Bioxcell® – soybean extract, Triladyl® – 20% EY, UL – 15% EY, UL – 8% LDL	G (7%), G (6.4%), G (6%), G (4%), G (4%)	RT, 5°C	160 × 10^6	2 h at 5°C	slow cooling (from RT to 5°C in 90 min); frozen in liquid nitrogen vapour for 10 min	65°C for 6 s	Martinez-Pastor et al., 2009
Cervus elaphus	ES	Triladyl® – EY, Triladyl® + Trehalose	G		400 × 10^6		–1°C/min	37°C for 20 s	Malcotti et al., 2012
Cervus elaphus hippelaphus, Dama dama	ES	Triladyl®, Bioxcell®			45–158 × 10^6, 90–235 × 10^6	2.5–10 h at 4°C, 3–6 h at 4°C	frozen in liquid nitrogen vapour for 10 min	38°C for 1 min	Zomborszky et al., 2005
Cervus elaphus, Dama dama, Elaphurus davidianus	EE	Tris – 2.5% EY	G (5%)		200 × 10^6	4 h at 5°C	cooling to 5°C in 1.5 h; frozen in liquid nitrogen vapour at –80°C for 15 min	70°C for 5 s, 50°C for 8 s, 37°C for 10 s	Soler et al., 2003b
Cervus nippon	ES	Tris-glucose-citric acid – 10% EY	G (8%)	RT	5 × 10^7	1 h at 4°C	–5°C/min from 5 to –20°C; –23°C/min from –20 to –130°C	35°C for 40 s	Hishinuma et al., 2003

Table 1. Sperm cryopreservation protocols in deer - part 2

Species	CM	Diluent	CPA	T° of CPA addition	Final SC (Spz ml^{-1})	ET	CR	Thawing	Reference
Cervus nippon taiouanua, Cervus unicolor swinhoei	EE	five different extenders (2.25–20% EY)	G (5–6–8%)		150×10^6		slow cooling to 5°C in 2.5 h; –5°C/min from 5 to –20°C; –20°C/min from –20 to –150°C	37°C	Cheng et al., 2004
Cervus eldii siamensis, Cervus eldii thamin	EE	Tris modified BF5F	G (5%)	5°C	100×10^6	1 h at 5°C		37°C for 30 s	Rittem et al., 2012
Axis axis	EE	TALP and TCF – 20% EY	G (4–8%)	37°C	$200–250 \times 10^6$		–1°C/min from 24 to 4°C ; –6°C/min from 4 to –80°C	37°C per 1 min	Umapathy et al., 2007
Dama dama	EE	2.9% sodium citrate – 20% EY	G (8%)		400×10^6		–6°C/min	37°C	Jabbour et al., 1993
Mazama americana	EE	Tris-EY, TES-Tris-EY, TES-Tris-EY – Equex®	G (6%)		30×10^6		–0.25°C/min from RT to 5°C; –5°C/min from 5 to –120°C	37°C for 20 s	Favoretto et al., 2012
Rucervus eldii siamensis	ES	Tris-fructose-citric acid – 20% EY	G (8%)		200×10^6	4 h at 4°C		37°C for 30 s	Thuwanut et al., 2013

CE = clarified egg Yolk; CM = collection method; CPA = cryoprotectant; CR = cooling rate; EE = electroejaculated semen; EG = ethylene glycol; ES = epididymal sperm; ET = equilibration time; EY = egg yolk; G = glycerol; LDL = low density lipoproteins; PG = propylene glycol; RT = room temperature; SC = sperm concentration; TCF = Tris-citrate-fructose; UL = Tes-Tris-fructose; WE= whole egg yolk

sperm samples finding that the latter results in higher concentration, osmolality, and lower pH. As a result, higher glycerol concentration and osmolality of extender for epididymal sperm give better results in sperm cryopreservation protocols while lower glycerol concentration and osmolality are required for electroejaculated sperm samples (Martinez-Pastor et al., 2006b; see next paragraph). Moreover, although percentage of motile spermatozoa is roughly similar between epididymal and ejaculated samples, the quality of movement together with the percentages of normal sperm and intact acrosome are higher in the latter (Martínez et al., 2008).

Semen storage

The cryopreservation of the seminal doses is a fundamental step in the management of germplasm banks, and one of the most critical ones (Garde et al., 2006).

It can be said with some justification that, since the last decade, there has been little original research on formulation of diluents specifically for deer semen, probably because the existing diluents have given acceptable results (Asher et al., 2000; Garde et al., 2006; Martinez-Pastor et al., 2009). All diluents tested in cervids have been adapted from studies on livestock (i.e. bull, ram, and goat). Most of diluents used successfully in deer have been adjusted 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. In recent years, there has been a growing interest on studies regarding cryopreservation of deer semen and several diluents, cryoprotectants, and egg yolk concentrations have been evaluated (see Table 1). In Iberian red deer, both self-made (e.g. TES-Tris-fructose egg yolk) and commercial extenders (e.g. Triladyl®, Andromed®, and Bioxcell®) have been tested (Martinez-Pastor et al., 2009). Triladyl® and Andromed® are commercial extenders that well preserve quality of red deer sperm after cryopreservation (Martinez-Pastor et al., 2009). Moreover, the same authors found that the sperm-rich ejaculate fraction not only renders more motile and viable spermatozoa but also shows higher freezability (higher motile spermatozoa recovery) (Martinez-Pastor et al., 2009). Several cryoprotectants (glycerol, ethylene glycol, propylene glycol, and DMSO, all at 3%) have been employed in Iberian red deer epididymal spermatozoa finding that glycerol better preserves sperm quality, while DMSO shows the highest toxicity

(Fernández-Santos et al., 2005). In a later study, Fernández-Santos et al. (2006a) compared the effects of different concentrations of glycerol, ethylene glycol, and propylene glycol on sperm cryoresistance. The authors found that 12% of any cryoprotectant was toxic to red deer epididymal spermatozoa membrane integrity, whereas an improvement in sperm parameters was obtained when the TCF diluents contained 6% of glycerol (Fernández-Santos et al., 2006a).

By contrast, in spotted deer (*Axis axis*), Umapathy et al. (2007) found that Tris-citrate containing 4% glycerol with 20% egg yolk is the best extender for cryopreservation while increasing glycerol to 8% or using TALP significantly decreases percentages of both motile and progressively motile sperm after thawing. In red brocket deer (*Mazama americana*), classified by the IUCN (2013) as a data deficient species, Favoretto et al. (2012) found that TES-Tris-Yolk-Equex® better preserves sperm motility and vigour after cryopreservation compared to Tris-Yolk and TES-Tris-Yolk.

Different egg yolk concentration and cooling rates have been evaluated in epididymal spermatozoa of Iberian red deer finding that rapid cooling rate (4.2°C/min) with 20% egg yolk better preserve sperm characteristics both after cryopreservation and refrigeration (Fernández-Santos et al., 2006b, 2006c).

Moreover, several antioxidants have been tested in Iberian red deer during the different phases of sperm cryopreservation such as refrigeration (Fernández-Santos et al., 2009b), cryopreservation (Fernández-Santos et al., 2007b) and post-thawing incubation (Domínguez-Rebolledo et al., 2010). Recently, Anel-López et al. (2012) have evaluated the effects of vitamin E analogue (Trolox) and reduced glutathione (GSH) on epididymal sperm cryopreservation finding that the latter gives promising results. On the contrary, Trolox does not seem suitable as a supplement for the cryopreservation extender, although lower concentrations thereof need to be evaluated (Anel-López et al., 2012).

Artificial insemination

Artificial insemination (AI) is the most widely applied ART (Comizzoli et al., 2000; Cseh, Solti, 2000) and is a crucial tool for genetic management because it allows for a wider and more rapid dissemination of desirable genetic material that would be remotely possible by natural mating strategies (Asher et al., 2000). Cryopreservation of spermatozoa combined with AI is the method of ART that has been most extensively applied to deer species (Asher et al., 2000). By the mid-1980s, AI with cryopreserved semen was commercially available to New Zealand deer farmers (Asher et al., 2000). The application of AI to non-domestic species presents a number of challenges, including: (1) precise knowledge of genetics and reproductive biology of the species; (2) ability to manipulate the animals for AI without undue stress or injury; and (3) ability to manage the animals to promote establishment and maintenance of pregnancy and minimize neonate losses (Morrow et al., 2009).

It is widely known that success of AI requires an optimal system of oestrus detection or oestrus synchronization in order to perform the insemination as close as possible to the ovulation. Small errors in insemination timing, perhaps only by a few hours, could easily mean that fertile spermatozoa and oocytes are never present at the same time (Holt et al., 1996). In the last decades, ultrasonography has received an increasing attention as a tool for assessing reproductive fitness of captive and free-living wild species (Hildebrandt et al., 2003). In deer, this powerful method has been extensively applied for early pregnancy detection (in red deer: Revol, Wilson, 1991; in sika deer: Willard et al., 1996; in fallow deer: Willard et al., 1998a; in reindeer: Savela et al., 2009; in Iberian red deer: Gomez-Nieto et al., 2011), but also for monitoring of ovarian follicular dynamics (in red deer: McCorkell et al., 2006, 2007, 2008) and *in vivo* oocytes collection (in red deer and sika deer: Comizzoli et al., 2001; but see also the review by Berg, Asher, 2003).

The most common method for oestrus synchronization is the use of controlled intravaginal drug-releasing device (CIDR) containing progesterone, followed by an injection of equine chorionic gonadotropin (eCG) after CIDR withdrawal or before performing the AI. Unfortunately, the use of PGF2α as a luteolytic agent in deer is limited to the cycling females, which poses a major constraint in seasonal breeder species (Morrow et al., 2009).

As alternative to CIDR for oestrus synchronization treatment, Umapathy et al. (2007) have recently used an implant containing 3 mg norgestomet that was inserted intradermally in the ear, with a further solution containing 3 mg norgestomet and 5 mg estradiol valerate injected intramuscularly at implant insertion in adult female spotted deer. Moreover, Gentry et al. (2012) have found that eCG may not be essential for acceptable pregnancy rates in white-tailed deer. In fact, increased pregnancy rates may result when fixed time artificial insemination (FTAI) is done ≥60.5 h after progesterone device removal (Gentry et al. 2012).

To date, AI inseminations have been performed only on seven deer species and the live offspring born as a result of AI is still low (for references see Morrow et al., 2009).

Spermatozoa number per intrauterine insemination usually ranges between 2.5 and 10×10^6 (Asher et al., 1992, 2000), whereas conception rates to AI in deer is generally in the 50–80% range (Soler et al., 2003c; Morrow et al., 2009). Several factors may affect conception rate ranging from the insemination method to the optimal time of insemination. Transcervical and

laparoscopic intrauterine insemination are the most common methods for AI in deer. However, A l l e r et al. (2009) have successfully used the rectal-transcervical AI method (similar to that in cattle) in red deer does.

M e l l a d o et al. (2013) have recently examined the effects of insemination either transcervically or by laparoscopy on several factors such as fawning rate, litter size, litter weight, and neonatal fawn mortality in white-tailed deer. The study showed that fawning rate and litter size did not differ as a result of intrauterine deposition of semen by laparoscopy compared with the transcervical insemination technique (M e l l a d o et al., 2013). By contrast, A l l e r et al. (2009) found that pregnancy rate was significantly higher by laparoscopic intrauterine insemination whereas W i l l a r d et al. (1998b) stated no differences between the two techniques.

CONCLUSION

Successful application of ARTs in cervids has been slowly increasing in the recent decades. Nevertheless, reproductive biology of several deer species still remains unknown, despite the fact that they are critically endangered or even extinct in the wild. Deep knowledge of reproductive biology in deer is necessary not only for management and conservation purposes, but also for solving important clues in human reproduction.

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