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Artificial insemination in canids: A useful tool in breeding and conservation

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Abstract

Artificial insemination (AI) and semen freezing have become services available to dog owners worldwide, and the demand for services to freeze semen is increasing. In other canids such as the fox, the fur industry utilizes fresh or frozen semen to artificially inseminate vixens to produce pelts. Clearly, AI facilitates the use of a male to sire several females by diluting the ejaculate, increases breeding hygiene, and allows crossing between species with slightly different breeding seasons. The African wild dog (*Lycaon pictus*) is currently considered by the World Conservation Union (IUCN) as one of most endangered canids. In captive populations of African wild dogs, semen has been frozen with encouraging results, using a standard cryopreservation protocol for domestic dogs, but successful AI has not been reported. In wolves, there is one report regarding the live birth of an offspring after intravaginal AI of a deslorelin-induced estrous female. In 2005, three Mexican gray wolf females were artificially bred by intrauterine insemination with freshly collected semen from unrelated males, and all females whelped. Artificial insemination may be vaginal, intrauterine or intratubal, and the semen may be fresh, fresh and chilled (diluted), or frozen–thawed, and the source of semen may be epididymal or ejaculated. In the domestic dog, the results are good to excellent for AI with all three types of processed semen when the source is ejaculated semen, whereas epididymal sperm still yields poorer results. Species differences in female physiology, as well as differences in the cryotolerance of the sperm from various canid species, warrant further research and development.

Keywords: Artificial insemination; Techniques; Frozen semen; Conservation; Canids

1. Introduction

Artificial insemination and semen freezing are available to dog owners worldwide, and the demand for semen freezing during large international dog shows is increasing. Semen from dogs originating from continental Europe, as well as the Americas and Australia, was cryopreserved during the 2008 World Dog Show in Stockholm, Sweden, to be used to artificially inseminate bitches in Scandinavia at a later date. Interested breeders in the UK purchase semen from UK dogs exported to Scandinavia, whose semen had been cryopreserved and kept frozen for a number of years, to 'get their breeding lines back'. Field trial and competition dogs are watched and evaluated in their home countries by traveling breeders, and semen from these elite dogs are requested to import superior working stock. In the guide dog services for the blind and in the military, customs and police (odor detection) dogs are becoming an enterprise worldwide, and semen from valuable sires are requested to minimize costs of keeping or transporting live animals. Fresh, chilled semen is increasing as an alternative to mating or use for

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AI with frozen semen when time is short (e.g. the female is already in estrus) and transport distances are not too far. New commercial diluents for fresh semen are being developed that may enable storage of chilled semen for several days, which eliminates transport distance as an obstacle to AI with fresh semen [1].

In other canids, such as the fox, the fur industry utilizes fresh or frozen semen to artificially inseminate vixens to produce pelts. In 2007, 65,000 female foxes in Norway were inseminated with fresh semen, and approximately 80% of the 155,000 marketed pelts were produced from AI offspring (2007 records of the Norwegian Fur Breeders' Association). In The International Fur Trade Federation's state of the industry report for 2007, it was stated that worldwide sales figures for all fur clothing rose to $15 billion in 2007 (up by 11% in 2006 [2]). Foxes constitute a substantial part of the fur-farmed species. Both farmed species, the red fox and its color variants (*Vulpes vulpes*) and the blue fox (*Alopex lagopus*) are also bred naturally, but AI facilitates the use of a male for several females by semen dilution, increases breeding hygiene, and allows crossing between the two species, which have a slightly different breeding season.

The African wild dog (*Lycaon pictus*) is currently considered by the World Conservation Union (IUCN) as one of most endangered canids in the world [3]. Implementation of assisted breeding in the captive African wild dog is restricted by a lack of knowledge regarding their reproductive physiology and the apparent difficulty of manipulating the complex social dynamic of the pack in order to conduct reproductive procedures. In captive populations of African wild dogs, semen has been frozen with encouraging results using a standard cryopreservation protocol for domestic dogs [4,5]. So far, however, there are no reports of live offspring as a result of AI.

Several wolf species around the world are threatened by inbreeding and by human intervention. In addition to protecting the animals from poaching and securing their habitats, artificial breeding techniques may be one of several solutions to exchange genetic material between wild or captive populations. In wolves (*Canis lupus* spp.), there are reports regarding sampling and cryopreservation of semen from, for example, the subspecies gray wolf (*Canis lupus*), red wolf (*Canis rufus*) [6,7], and Mexican gray wolf (*Canis lupus bayleyi*) [8]. One account of live birth offspring after intravaginal AI of one female wolf inseminated in a deslorelin-induced estrus is reported in the scientific literature [9]. In 2005, the first author of the current review was involved in cooperation with the AZA Wildlife Contraception Center at the St. Louis Zoo (St. Louis, MO, USA) in an effort to carry out non-surgical intrauterine AI of female Mexican gray wolves in spontaneous estrus. In February 2005, three Mexican gray wolf females were artificially inseminated with freshly collected semen from unrelated males (Fig. 1). All females whelped. The experiment attracted media interest and was cited in an article by the New York Times. One successful pregnancy following insemination with frozen semen was reported in the gray wolf (*Canis lupus*) by Seager and his group [10].

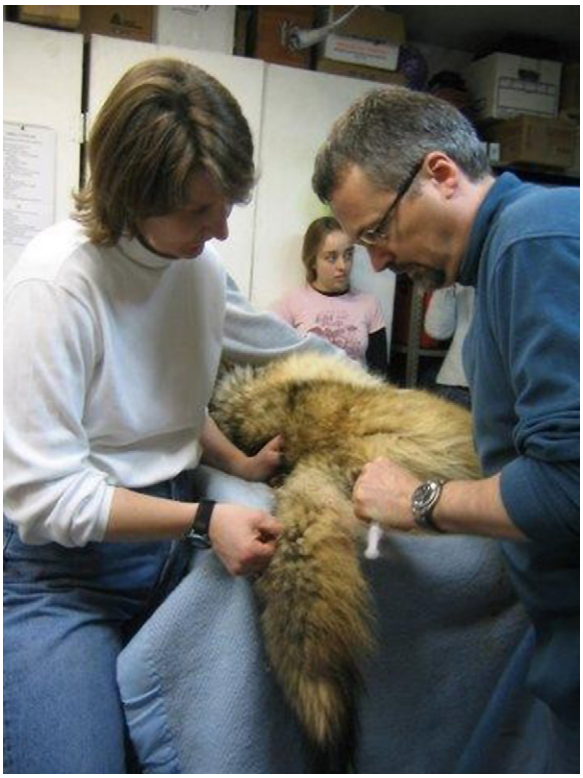

Fig. 1. Karen Bauman from the AZA Wildlife Contraception Center at the St. Louis Zoo assists the author (Ragnar Thomassen) in the intrauterine AI of a sedated Gray wolf female. (Photo: AZA).

The objective of this review is a state of the art survey of the techniques used for AI, and results and challenges we meet when applying AI to canid species for breeding or conservation purposes.

2. Source and type of spermatozoa

The source of sperm may be either from the epididymus, in some cases from the testis, or more commonly from an ejaculate. Testicular sperm is still highly experimental, but some success has been obtained in mice and humans [11]. Epididymal sperm

may be collected post-mortem. Most commonly, semen is collected from ejaculates obtained by digital manipulation, or in some cases by electroejaculation, and inseminated undiluted into a female that is either present at the time of collection, or is kept in close proximity to the male. Fresh, diluted and chilled semen may be sent for longer distances or kept for $\geq$1–2 d before insemination, or the semen may be cryopreserved for long-term storage.

2.1. Epididymal sperm

Although epididymal spermatozoa lack the seminal plasma containing the secretions of the accessory glands, the advantage of being able to sample such sperm post-mortem or after castration warrants the possibility of using epididymal sperm for AI. However, their use for AI procedures may require conditions other than those for spermatozoa from ejaculates, which have been exposed to sperm plasma, since sperm plasma modulates both sperm surface and function during transit through the male reproductive duct system [12].

2.1.1. Fresh epididymal sperm AI

Benign prostatic hyperplasia was diagnosed in an American Staffordshire Terrier of high breeding value, presenting with concurrent hematuria. After castration, the caudae epididymides were flushed with semen extender and the spermatozoa collected were inseminated intravaginally in a bitch using an insemination catheter designed for dogs (Kruuse, Marslev, Denmark). The AI resulted in eight live-born puppies 63 d after insemination. This is the first report of a normal pregnancy and birth of puppies from a bitch inseminated with fresh epididymal semen obtained from a dog affected by benign prostate hyperplasia [13].

2.1.2. Cryopreserved epididymal sperm

Freeze-storage of epididymal sperm is an important technique for the preservation of gametes in animals, including endangered species, since epididymal sperm may be collected post-mortem, e.g. after the accidental death of a valuable animal. Hori et al. [14] froze canine sperm recovered from the caudae epididymides and investigated fertility. The quality of sperm from the caudae epididymides before freezing was significantly higher than that of sperm from the caput-corpus of the epididymis. Freezing was used only for sperm recovered from the cauda epididymis. The sperm motility and viability after thawing were 19.5 $\pm$ 2.5 and 53.1 $\pm$ 3.3%, respectively. In this study, these parameters were slightly lower than those of frozen–thawed ejaculated sperm, but the differences were not significant. However, although the subjective qualities of epididymal sperm after thawing were similar to those of ejaculated sperm, the conception rate obtained with frozen–thawed epididymal sperm was low.

2.2. Ejaculated semen

In most domestic dogs, semen collection is possible using simple digital manipulation. Similarly, in farmed foxes, the males are trained to accept manipulation when fixed in a particular collection box and readily ejaculate into collection funnels after a masturbation technique adapted for each fox species. Nervous, obstinate, and aggressive males that resist handling in these boxes, making masturbation impossible, are not used for further breeding. In wild canids, their natural suspicion and fear of humans may make digital manipulation impossible, and this may necessitate the use of electroejaculation. Electroejaculation has been done in the domestic dog [15], African wild dog [5], Mexican gray wolf, and gray wolf [8], based on methods used in cats [16].

Ejaculated dog semen has been used fresh, fresh chilled or frozen–thawed. The first puppies derived from frozen–thawed semen were born after intravaginal AI [17]. van Gemert [18] produced the first frozen–thawed puppies after intrauterine AI and Andersen followed in 1972 with the introduction of a new AI technique and freezing procedures for dog semen [19].

3. AI techniques

For successful use of AI, it is important to perform the insemination at the optimal time, use semen of good quality, and deposit the semen at the optimum site for fertilization. Natural mating in dogs ensures intrauterine deposition of a considerable portion of the ejaculate by transport of spermatozoa from the vagina through the cervical canal during the coital tie.

There are three or four principal techniques to artificially inseminate canids. The semen may be deposited deeply into the vagina or into the uterus, either by transcervical catheterization or by surgery, and finally by intratubal AI, which also requires surgery.

3.1. Vaginal AI

Vaginal AI may be performed using a simple plastic catheter, such as a bovine uterine flushing catheter cut to an appropriate length, to which a simple plastic disposable syringe containing the semen is attached,

or ready-made catheters may be obtained from a commercial supplier. The insertion of the pipette is done with the bitch in a standing position on an examination table and the pipette is inserted to the base of the vagina, close to the false cervix. During AI and up to 10 min after AI, the bitch is held with the hindquarters up and head down to ensure that the semen will not be expelled through backflow. However, reducing the interval of elevated hindquarters to 1 min did not affect fertility [20]. Some commercial catheters have a flexible latex tube lining which has an inflatable part at the tip, which when fully inflated, forms a ball that prevents semen backflow. Such catheters are constructed to imitate the dog's erect penile bulb and are meant to increase the probability for intrauterine transport of the semen [21,22]. A similar device, the Articop instrument, has been used to inseminate farmed foxes in Finland [23].

3.2. Intrauterine AI

The first to compare intrauterine to vaginal AI using the Norwegian catheter for both was Farstad [24], who showed that intrauterine AI yielded significantly higher whelping rates than vaginal AI when frozen semen was used. These results were later substantiated by Linde-Forsberg et al. [25,26] who reported that significantly higher whelping rates and litter sizes were obtained not only with frozen–thawed semen, but also with fresh, as well as chilled, extended semen when the semen had been deposited in the uterus rather than in the vagina, and by others [27,28]. Some clinics have obtained consistently good results with intravaginal AI of frozen–thawed semen using several daily inseminations and have also found that addition of homologous prostatic fluid to the semen after thawing improved whelping results [29]. Silva et al. [27] found no differences between uterine laparoscopic AI and vaginal AI with the Osiris catheter. In another study, the results with vaginal deposition of frozen–thawed semen were not encouraging even when prostatic fluid was added to the inseminate [30]. Hence, with fewer or single inseminations, reduced longevity of the spermatozoa and scarcity of semen, intrauterine AI yields better results than intravaginal AI when frozen semen is used.

3.2.1. Transcervical AI

3.2.1.1. The Norwegian AI catheter. The insemination equipment consists of a stainless steel catheter and a plastic guiding tube. The guiding tube is used to protect and steady the catheter and to stretch the vagina and protect the vaginal mucosa against damage during insertion of the catheter. The cervix is fixed through abdominal palpation, and the semen is deposited by intrauterine insemination by insertion of the catheter through the cervix. This is a non-surgical method, which requires meticulous training, but once learned, allows AI in non-sedated standing bitches in a matter of a few minutes. In a trial from our clinic, altogether 665 bitches were inseminated into the uterus, and in 20 bitches the semen was deposited deeply into the vagina after the attempt to insert the catheter into the uterus had failed (less than 3%) [31]. Inseminations were performed on standing bitches. Only a small number of the bitches may need sedation to allow the deep palpation of the abdomen necessary to hold the cervix. Less than 10% of females were sedated using 2–6 mg xylazine iv (Rompun®, Bayer, Leverkusen, Germany) [31,32].

The catheter consists of a stainless steel hollow rod with a larger base and a smaller neck, ending in a ball tip. The catheter is produced in three sizes for large, medium and small bitches, respectively. Cranially, the catheter ends in a small rounded ball tip with a small hole at the tip, and at the caudal end, it has a luer standard adapted lock to fit a disposable syringe (with a plastic, *not* rubber, stopper, since some types of rubber may be toxic to sperm). To protect the vaginal mucosa from the pointed tip during insertion through the vagina, and also to protect the steel catheter from vaginal contamination, a plastic guiding tube is fitted around the catheter during insertion (Fig. 2).

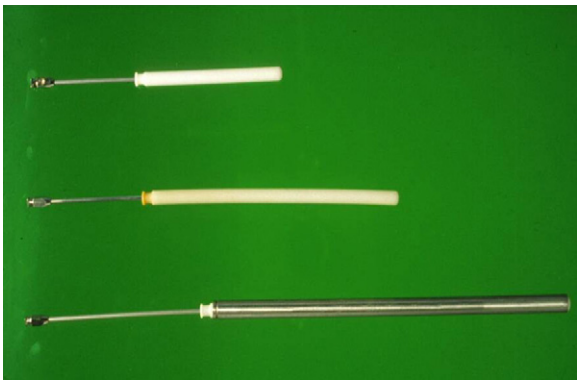

Fig. 2. The transcervical stainless steel catheter (Lima A/S, Sandnes, Norway) comes in three sizes: large (bottom, with a stainless steel guiding tube), medium (middle), and small (top) with plastic guiding tubes. Catheter dimensions: large, medium, and small dogs: length: 46, 31 and 21 cm; diameter of main part of catheter: 3, 3, and 2.8 mm; length of smaller neck 4.5, 4.3, and 2.4 cm; neck diameter: 1.2, 1.2, and 1.0 mm; and ball tip diameter: 1.8, 1.8, and 1.3 mm. The length of the guiding tubes are 31, 22, and 12.5 cm, respectively, and the external diameter is 9 mm.

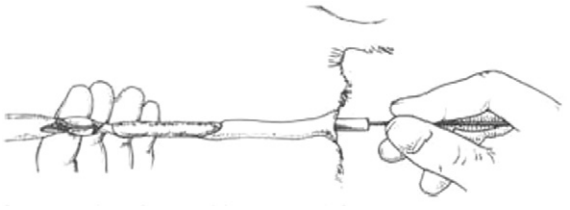

Fig. 3. Abdominal fixation of the cervix is necessary to insert the stainless steel catheter into the cervical canal and uterus of the bitch [19]. The cervical orifice is located at the end of the dorsal vaginal fold.

The guiding tube, hiding the cranial part of the catheter, is thus inserted into the vestibulum, guided through the vagina as far as to the smaller neck of the vagina called the pseudocervix, by gentle pressure with the operator's one hand. Then the cervix is located by means of the index finger and thumb of the operator's one hand, and the cervix is held in a firm grip. By the other hand, the catheter is inserted further along the dorsal vaginal fold until the cervical opening is located. Then the catheter is inserted into the external orifice and by gentle pressure, guided through the cervical canal, and the semen is deposited into the uterine lumen (Fig. 3).

3.2.1.2. The endoscope-guided procedure. The endoscope method involves the use of flexible, hollow plastic tubing within a rigid endoscope to enter the cervix through the vagina, and to pass through the cervical canal. The endoscopic AI technique was first described by Wilson [33]. A method of endoscope-guided catheterization of the canine cervix was developed specifically for use in the bitch, permitting visualization of the entire vaginal lumen, and vaginal portion of the cervix (external orifice). This technique uses an elongated cysto-urethroscope (Storz Extended Length Cysto-urethroscope: Telescope 30° 325 B, 3.5 mm. Sheath 027KL Bridge 027NL) for the procedure. The cysto-urethroscope, which is comprised of a 3.5 mm forward oblique telescope with 30° viewing angle, a 22 Fr. protective sheath with two luer-lock adaptors and obterator, a telescope bridge with one 10 Fr. instrument channel and a cold light source. The working length of the scope is 29 cm. The 30° angle is essential for visualization of the dorsally oriented cervix. A rigid polypropylene catheter is passed through the endoscope into the caudal (vaginal) cervical os, and advanced through the cervix into the uterine lumen. The rigidity of the scope permits its insertion beyond the pseudocervix into the cranial vagina, and enables positioning of the polypropylene catheter into and through the cervical canal. Endoscopic biopsy and culture instruments can also be passed into the uterine lumen using this method [34].

Endoscope-guided AI has gained increasing popularity in small animal hospitals, and the endosccope provides additional advantages, e.g. intrauterine examination, sampling and the potential for computerized digital image storage (for student and owner education). The technique also requires practice, but once mastered provides good conception results [34–37]. In a study by Linde-Forsberg et al. [25], the insemination results with the Norwegian IU catheter, intrauterine AI by fiberoptic endoscope, or vaginal AI were compared, with significantly higher results for the Norwegian IU catheter method. Unfortunately, so far few if any fertility results are available on a large clinical scale that can compare statistically the relative results of the two non-surgical techniques.

Non-surgical AI offers the possibility to inseminate several times in an attempt to increase whelping rates further. In our laboratory, we found that conducting AI twice yielded a higher whelping rate and mean litter size in the overall study. When AI was performed at the apparently optimal time, whelping rates were not different between one or two AIs. Mean litter size differed, however, significantly by 0.7 pups per litter [32]. Results from single AI are no longer necessarily poorer than those from multiple AIs. In a study on single, endoscope-guided AI with frozen semen a conception rate of 89.4% and a whelping rate of 87.5% was obtained in 137 Greyhound bitches [37]. However, in a retrospective clinical trial of 70 purebred Labrador retriever bitches (1.5–6 y of age) from the Norwegian guide dog's association ''Veiviseren'' and 30 privately owned pet Labradors (2–7 y) inseminated with frozen–thawed semen using the Norwegian intrauterine catheter from January 1994 to May 2008, the whelping rate using either three (3 bitches), two (20 bitches) or one AI (77 bitches) was 100, 95, and 88%, respectively. In this trial, the difference in whelping rates between one and two AIs was not significant ($P = 0.34$, Fisher–Irwin test). Also, there were no significant differences in the mean litter sizes (6.3 and 6.3 pups per litter for two and one AI, respectively) [38].

3.2.2. Surgical intrauterine AI

Surgical and laparoscopic AI are also procedures to ensure that the semen is properly deposited into the uterus [39–43]. A laparoscopic technique is described which has been adapted and used in zoological medicine for various mammals, birds, and reptiles for

reproductive and diagnostic studies, as well as clinically related research. Laparoscopy in zoo animals was effective for evaluating reproductive status, particularly ovarian anatomy and function, direct visual biopsy of internal organs, sex determination in selected birds, and as a surgical means of fertility control [44]. This method seems to be the method of choice in veterinary clinics of North America and some practices in Europe. This usually involves semen deposition only once. The results for surgical AI are claimed to be excellent, but scientific publications presenting results from surgical AI are also scarce. One recent example is the AI of 157 Greyhounds in a private clinic in the UK. The bitches had a whelping rate of 92% and the mean litter size was seven pups with one AI, compared to the mean average whelping rate recorded for natural service, 81% and five pups per litter [45].

It may be argued that since anesthesia is routinely required for most manipulative procedures in zoo animals as well as in small animal clinics, and since laparoscopy adds little additional risk, the use of this technique when indicated provided an additional diagnostic aid. When done correctly by an experienced surgeon, this method should be safe, with a relatively small risk of post-surgical complications. It does, however, necessitate general anesthesia and thus poses a risk of unwanted side effects of anesthesia in older females or sensitive animals. Furthermore, if anything goes wrong (e.g. peritonitis or wound infection) monitoring of wild animals is usually more demanding and conditions may go unnoticed until the animal's health is seriously compromised. Hence in these cases, the complications may require another additional anesthesia for treatment.

In Europe most countries refer to welfare considerations if invasive procedures are used when non-invasive procedures are available. The (UK) Kennel Club has published the following policy statement on its internet site [46]: ''The Kennel Club is advised by the Royal College of Veterinary Surgeons that surgical insemination as described in b), has ''many disadvantages for the bitch'', and so has a higher 'welfare debt'. This means that the benefits accrued from its use will have to be considerable to offset this 'welfare debt'. The RCVS advises veterinary surgeons that surgical AI is justified ''only for exceptional reasons'', and requires the vet to ''record in the bitch's clinical records why transcervical insemination is not a practical option and the justification for the invasive procedure''. The Kennel Club will therefore require justification of the net benefit to the bitch in conceiving as a result of this ''invasive procedure.''

3.3. Intratubal AI

The number of spermatozoa required to obtain conception by intratubal insemination in dogs was examined. Three groups of five, eight, and eight bitches received 0.5×10^6, 2.0×10^6 and 4.0×10^6 spermatozoa, respectively, into each uterine tube. No conception occurred in the five dogs inseminated with 0.5×10^6 spermatozoa, but conception occurred in 6/8 (75.0%) and 3/8 (37.5%) dogs inseminated with 2.0×10^6 and 4.0×10^6 spermatozoa, respectively. Among the pregnant animals that had been inseminated surgically into the oviducts, three aborted (33.3%) and the mean (±SEM) number of puppies was small, 2.5 ± 0.5 [47].

4. Time of insemination

In domestic dogs and farmed foxes the timing of insemination is based on a thorough knowledge of female reproductive physiology [48–50]. The domestic bitch is monoestrous and non-seasonal and ovulates in early estrus after a preovulatory LH peak of approximately 48 h duration. Since both foxes and dogs ovulate oocytes that have arrested in the germinal vesicle stage or the prophase of the first meiotic division, a maturation period of 2–3 d after ovulation is necessary to reach MII. Timing of the AI in relation to the preovulatory LH peak or anticipated ovulation time is usually done by measuring LH or progesterone in serum, or by observation of ovarian follicles by ultrasonography. Clinical and behavioral cues (vulva swelling, color of vaginal discharge, vaginal smear morphology, lordosis and tail flexing) may provide information when hormone assays are not available, particularly when fresh semen is used. Clinical estrus symptoms are less reliable for timing AI with frozen–thawed semen, because individual display of these symptoms in relation to ovulation time varies greatly. Since freshly ejaculated sperm or fresh diluted sperm inseminated shortly after ejaculation may be fertile for several days, natural mating or AI carried out from the time of anticipated ovulation until the day before diestrus (Day-1) are usually successful. Artificial insemination with frozen–thawed semen requires accurate timing due to the shorter fertile lifespan of frozen spermatozoa. Insemination with frozen–thawed semen is therefore carried out 2–3 d after anticipated ovulation, based on measurement of serum progesterone concentrations [32].

Red and blue foxes are monoestrous seasonal breeders with increasing ovarian activity at increasing

daylength. In the Northern hemisphere, red foxes come into season in January to March and the blue fox slightly later, in March to May. Estrus detection is carried out using a modified ohm meter (SLI Heat detector, Lima a/s, Sandnes, Norway) that measures electrical resistance in the vaginal mucus; AI is performed on the first and second day after the distinct maximum reading of the ohm meter (peak of electrical resistance) after a decline by a minimum of 50 ohm [50]. For fresh semen, AI clinical and behavioral clues are used, particularly tail flexing and vulva swelling and color. The display of estrus in foxes is naturally synchronized, and the majority of females in a particular farm location come in estrus within 1–21 d of the first female showing estrus. Older females come in estrus earlier, display more obvious and longer estrus signs than pubertal vixens. Males are rotated between cages and females to enhance the display of behavioral estrus signs (e.g. tail flexing, lordosis). In dogs and some wild canids, the GnRH agonist deslorelin has been used successfully to induce fertile estrus [9].

When attempting to develop a successful AI procedure for wild canids, such as the dhole (Asian wild dog, *Cuon alpinus*), the Ethiopian wolf (*Canis simensis*) and the island fox (*Urocyon littoralis*), it is necessary to learn as much as possible about normal physiology and pack behavior. Hormonal and behavioral cues are important to be able to assess whether reproduction is seasonal or not, and if the presence of males or other females, as well as the hierarchy between mature females, is affecting the display of estrus signs and behavior. Collection of fecal samples to assess the sex steroid hormones is a non-invasive procedure that may even be applied to individual animals [51,52]. If females can be captured and kept in enclosures for some time, vaginal smears and blood sampling can be used to monitor ovarian activity, and ovulation detection can be used to optimize timing of AI. Female anatomy can be elucidated using non-invasive (e.g. ultrasonography to observe ovaries, or vaginal speculum and endoscope to observe vaginal crenulation), or invasive methods, such as laparoscopic surgery and post-mortem examination. Catheters for AI can be modified to fit the anatomy of the female genital tract. As an example, fox intrauterine catheters are slightly bowed at the tip compared with the straight canine catheters, due to the particular anatomy of the external cervical orifice and cervical canal in vixens [53]. In wild canids, pack structure may also influence male reproduction. It has been shown that both social structure of the pack and time of the year may influence the possibility to collect semen from males by electroejaculation [5].

5. Discussion and conclusion

Current reliable methods for AI in the bitch have been available for nearly 40 y. Semen deposition varies, but for fresh or fresh and chilled semen vaginal AI may give good results and facilitate the use of AI in routine clinical practices, outside clinics, or under field conditions. However, with the current freezing methods and AI techniques, it is apparent that better results are obtained with intrauterine AI when using frozen semen. The ethical concerns over surgical AI will encourage the use of non-surgical techniques further, but training to perform these techniques is a prerequisite for those offering such services. In wild canids, surgical intervention may be the only option in some cases, but surgery necessitates careful consideration due to the value of the animals. Non-surgical AI can be carried out in non-sedated or sedated animals, but surgery requires general anesthesia. In dogs, one AI may be as successful as two inseminations, even when frozen semen is used. However, two inseminations are often not an option in wild canids, particularly if surgical AI is necessary.

The use of frozen–thawed canine semen is beneficial to help saving the genetic potential of endangered breeds, as well as working domestic dogs, salvaging genetic material prior to a medical treatment in a diseased male animal, or for sanitary reasons, since frozen semen may be processed to prevent disease transmission. Not all microorganisms can be eliminated through processing and dilution, but the stud dog may be screened for diseases prior to and after semen collection [54]. One of the major applications of AI with frozen semen in conservation is to avoid genetic depression caused by fragmentation of groups in free-living species. For some species living in small populations, it has been suggested to capture females for short periods for AI with sperm collected from zoo-maintained healthy males. The females could then be returned to the original habitat to whelp. Alternatively, capture of free-ranging males for a limited time for semen collection may be possible for subsequent AI of females kept in zoos or reserves [55]. Another possible application of AI, for in situ or zoo-based conservation, is to overcome poor natural mating behavior or male–female aggression. This is done, for example, when hybrid offspring (bluefrost) of the two farmed fox species are produced, since silver fox males do not normally mate blue fox females. Additionally, AI can be applied in captive non-domestic species to avoid the stress inflicted on the animals by transporting them to breed at a different location, or to avoid high transport costs [56].

Sperm cryobanking is necessary to have sperm available at the time when the female is in estrus, or when genetically suitable females only arise from generations past the reproductive lifetime of the male. The semen bank at The Leibniz Institute for Zoo and Wildlife Research (IZW; Berlin, Germany), claims to store epididymal and ejaculated spermatozoa of more than 40 mammalian species, including the domestic dog, the red fox, and the African wild dog [12]. In Norway, a semen cryobank exists for elite -and rare mutant (pelt color) foxes of the two farmed species blue- and red fox administered by the Norwegian Fur Breeders' Association, and a canine bank for pedigree dogs is located at the Norwegian School of Veterinary Science (Norwegian Kennel Club semen bank). In Europe and the USA, several commercial or university-based canine semen banks have been established.

From the fertility results recently published on AI with frozen dog semen, as well as those from our own laboratory, we inferred that the fertility from AI with frozen–thawed ejaculated semen in domestic dogs is generally very good. Whelping rates in the range of 70–95% require access to good semen from males of high fertility, rapid, but accurate hormone analyses, good reproductive management, and high fertility of the female populations, as well as skilled operators. Freezing dog semen is no longer a problem for stud dogs with superior semen quality, although great individual differences exist among males and individual ejaculates within males. In inbred or older individuals from wild or captive populations of canids, there may be a very large range in semen quality and female fertility. Also, the use of epididymal sperm for freezing and AI still needs to be further developed to improve fertility. Lack of successful IVF, embryo culture and freezing systems for dogs does not yet facilitate the use of artificial fertilization techniques such as ICSI.

When optimizing semen processing protocols for a particular canid species that has not been studied with respect to semen conservation, one may come across species-specific differences in sperm physiology. In addition to the individual differences in semen quality post-thaw, there are still challenges to be met concerning freezing procedures for semen from wild canid species, since differences can exist in cryotolerance between closely related species [57]. The real challenge is to find a suitable extender, freeze–thaw protocol and storage conditions for semen, if known protocols for dog and fox semen cannot be used for the species in question. A series of laboratory tests may be necessary, e.g. of ''physiological'' solutions and diluents that have been used for dog and fox semen, type of vial (straw, pellet), and effect of cooling, freezing and thawing on the morphology, motility, speed, survival and longevity of spermatozoa. Supplementary physiological sperm tests, such as in vitro zona penetration test, and flow cytometry to assess sperm chromatin structure state and acrosome integrity, may be feasible if laboratory facilities are available [57]. The use of AI in wild or captive species for conservation purposes also requires a thorough knowledge on the reproductive physiology of the female to be able to accurately time AI, as well as is currently possible with the domestic dog.

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