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Artificial Insemination in Veterinary Science

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1. Introduction

Artificial insemination (AI) is widely used by veterinarians and veterinary specialists in most domestic as well as wild animal species. Reasons for use of AI instead of natural matings are diverse and different for individual species. As in humans, AI can be used as a tool to increase conception rate in animals that have fertility problems. This is usually performed in companion animals, like horses and dogs, where individuals are important as breeding animals. In these cases, breeding animals are mostly chosen for performance and pedigree instead of breeding soundness. Breeders go through a lot of effort to produce offspring of a specific female or male animal irrespective of potential reproductive problems.

In production animals, AI is a way to increase reproductive efficiency and production. AI has proven to be a very effective reproductive technology that selectively increases genetic gain through increased selection pressure on males. In Holstein cattle, for example, AI supported selection for the milk production trait and within 40 years milk production has nearly doubled. Farm animals, males as well as females, are usually chosen for breeding programs based on breeding soundness examinations (BSEs). These BSEs determine suitability and likelihood of females or males to participate successfully in breeding programs. Animals that do not fulfill certain criteria are identified. These "problem" animals are excluded from insemination programs. Estrus cycles of females can be manipulated to institute efficient insemination programs. With the use of these estrus synchronization programs, large groups of females can be inseminated at the same time. This does not only have the advantage of concentrating work on specific days during breeding, but will ultimately also simplify the herd management before and after the offspring are born. Group feeding of pregnant animals, partus observation, vaccination programs for calves and tail docking of lambs are just a few examples of improved herd management areas through the group effect achieved through AI.

Another reason for AI is to ensure effective use of semen. An increased number of offspring from a superior sire can be produced when AI is employed. For example, a stallion's ejaculate can be sufficient to inseminate 5-10 mares at the same time when split into doses instead of one live cover on a mare. Ram ejaculates can also be split into up to 15 fresh AI doses. Freezing bull semen can provide up to 200 straws of frozen semen from one ejaculate, equaling 200 AI doses. Overuse of males is prevented and commercial distribution

facilitated. Other important aspects are the prevention of venereal disease transmission that plays a major role in the economic system of offspring production, and increased safety for valuable breeding animals as mating related injuries are avoided.

Venereal diseases that play a major economic role in cattle production are for example Trichomonosis and Campylobacteriosis both of which decrease reproductive efficiency through decreased pregnancy rates, high return rates to estrus and increased pregnancy losses. In general, shipping fresh and frozen semen nationally and internationally involves fewer health risks and welfare implications than transporting live animals.

Import and export of frozen semen is a huge economic market. Semen of a specific bloodline, a specific individual male or breed is imported. This is especially important in countries where breeds were introduced and are somewhat isolated with a small genetic pool. To eventually prevent breeding setbacks due to inbreeding and to expand the genetic pool, imported semen is used. Furthermore, AI can be used for frozen semen from males that have died or are not physically available for matings due to distance or physical inability. A great advantage of frozen semen in general is that it can be stored indefinitely and has the potential to outlive the male donor animal by years.

2. First developments and milestones in the field of veterinary Artificial Insemination

Artificial insemination (AI) was the first assisted reproductive technique applied to control and improve reproduction as well as genetics. The first successful insemination was performed by the Italian physiologist and priest Abbe Lazzaro Spallanzani (1784) in a dog which whelped three pups 62 days later (Foote, 2002). The establishment of AI as a practical procedure was initiated in Russia in 1899 by Ivanov who studied AI in domestic farm animals, dogs, foxes, rabbits and poultry. He also developed semen extenders. Milanov, another Russian scientist and successor of Ivanov, started large scale breeding programs for cattle and sheep, and designed and made artificial vaginas. Horse breeding programs and research was initiated at the same time in Japan even though translations of the original research only became available to the western world after 1958. Some AI work, especially in horses and cattle, had been done in Denmark in the early 1900s. It was Danish veterinarians who established the method of rectovaginal fixation of the cervix for insemination in cattle which enabled semen deposition deep into the cervix or into the uterine body (Foote, 2002). This technique is still used today. Another Danish invention was the straw for packaging semen. These straws have been further developed and modified by the French and are now used worldwide for processing and storage of frozen semen. Research on artificial insemination in Italy led to the development of an artificial vagina for dogs in 1914 and to the establishment of the "International Congress on AI and Animal Reproduction" in 1948. This congress is held every four years since (Foote, 2002). Rapid development of AI in dairy cattle occurred in the USA in the 1940s. One of the important milestones was the establishment of the "Dairy breeding research centre" on the campus of the Pennsylvania State University in 1949 to assist in the development of artificial insemination in dairy cattle. Interest in and development of frozen semen started with successful cryopreservation of gametes from a variety of animal species after discovery of the protective action of glycerol by scientists in Cambridge, England, in 1949 (Amann & Pickett, 1987). Early research focused on bull spermatozoa; methods successful to cryopreserve bull spermatozoa have

been less successful for other species. Barker and Gandier reported the first pregnancy from frozen stallion semen in 1957 (Barker & GaPndier, 1957). Poor pregnancy results with the use of frozen-thawed stallion semen limited research funding for breeding trials in this species (Amann & Pickett, 1987). Only later the Chinese inseminated more than 110'000 mares with frozen-thawed stallion semen (between 1980 and 1985). The United States also did not have interest in developing improved procedures for freezing stallion spermatozoa in the seventies. Only in 1980 the Animal Reproduction Laboratory of Colorado State University began a long-range research program aimed at developing satisfactory procedures to freeze stallion semen. The acceptance of frozen semen as a method to produce registered foals by two of the largest breed associations, the American Quarter Horse and the American Paint Horse association in 2001 has furthermore stimulated new interest in the frozen semen technology (Loomis, 2001).

3. AI techniques in different species

Insemination techniques used depend on species, type of semen, breeding system, availability of equipment and expertise. Intravaginal (dogs), intracervical (sheep), transcervical intrauterine (cattle, horses, dogs, sheep), transcervical deep horn intrauterine (horses, cattle, pigs), laparoscopic (sheep), surgical intrauterine (dogs) insemination as well as endoscopic semen deposition at the uterotubal junction (horses) is available.

3.1 AI techniques in dogs

In dogs, fresh, chilled extended and frozen semen can be used. AI using fresh semen is usually performed if the animals will not or cannot copulate naturally. There are certain breeds, like English bulldogs and other brachycephalic breeds that almost always require AI due to their anatomical incompatibility to mate naturally. AI is also used if time constraints are an issue as fresh semen AI in the bitch is much quicker than a natural mating where the coital tie between male and female can last up to 40 minutes or more. AI can be used in bitches with congenital vaginal abnormalities like vaginal strictures or septae which might cause copulation failure. These cases often have ethical implications as well. Owners need to understand that even though AI can be performed easily these bitches might require Cesarian sections as the vaginal abnormalities might also impair natural delivery or that certain vaginal abnormalities are heritable and offspring with the same problem might be produced. AI is also used if the semen quality of the specific male is poor and addition of semen extender or pooling of more than one ejaculate is required for one insemination dose. Fresh semen is deposited into the cranial vagina of the bitch using an insemination pipette that is inserted through the vulva and directed into the vagina. Once the semen has been deposited, the hindquarters of the bitch are usually elevated for a few minutes to facilitate movement of the semen from the vagina through the cervix into the uterus. Vaginal contractions can be elicited for the same reason at the same time by either tickling the vaginal wall or massaging the clitoris. An insemination dose of at least 150 x 10^6 sperm is recommended and most commonly the whole ejaculate is used which may contain 250-2500 x 10^6 sperm (Johnston et al., 2001).

Pregnancy rates with the use of fresh semen for insemination is reported to vary between 65-84% on average depending on semen quality, timing of insemination and correct site of semen deposition (Johnston et al., 2001; Linde-Forsberg & Forsberg, 1989).

Chilled extended semen is usually used if the male and female are geographically separated. An extender is added to the collected semen before shipment in order to prolong the lifespan of sperm. For the same reason, the extended semen is slowly cooled down to 4 degrees Celsius. The entire ejaculate is usually used for the AI but an insemination dose of at least 150 x 10^6 sperm is recommended. Average pregnancy rate for chilled extended semen is 50% but can vary between 33-89% (Johnston et al., 2001). Extended semen can be used for up to 4 days after collection but should be inseminated as soon as possible. The insemination technique is the same as described above for fresh semen or transcervical intrauterine insemination can be used. Transcervical AI is performed using special catheters (e.g. Norwegian catheters) or endoscopes. The anatomy and location of the cervix in the bitch is such that it is difficult to penetrate the cervix and perform transcervical intrauterine inseminations due to a vaginal fold that is obscurring direct access to the cervix. Therefore, special equipment is needed for transcervical intrauterine AI. Norwegian catheters have been developed in the 1970s (Andersen, 1972, 1975) and are available in three different sizes to accommodate different sized bitches. For this technique, the cervix must be fixed through abdominal palpation and the catheter is moved slowly to find the cervical canal while the cervix is pulled downward. This method requires a lot of practice and there is a potential danger that inexperienced veterinarians might traumatize the cervix. Used by experienced clinicians this technique offers an inexpensive, fast way of inseminating non anesthetized bitches. Using a rigid endoscope for transcervical AI enables the operator to visualize the cervix, straighten the cervical canal and insert a catheter into the uterus to deposit the semen. The advantage of this technique is that it is not blind and the danger of trauma to the reproductive tract is minimal. Bitches do not usually have to be sedated for the procedure and AI can be repeated a few times if necessary.

For frozen semen, deposition of the semen into the uterine lumen is recommended in order to achieve good pregnancy rates (Thomasse et al., 2001; Thomassen et al., 2006). Even though the first report of successful AI with frozen semen in dogs was a result of intravaginal deposition of the semen (Seager, 1969) it has been shown that success rates with intrauterine inseminations are far superior (Thomassen et al., 2001; Thomassen et al., 2006). Intrauterine insemination can be performed by transcervical intrauterine AI as described above or surgical AI via laparotomy. In cases where transcervical intrauterine AI is unsuccessful or the equipment is not readily available, surgical intrauterine AI is performed. It is usually a quick procedure that requires general anesthesia for about 20 minutes. A small abdominal incision is made, the uterus exteriorized and the semen injected directly into the uterine lumen through the uterine wall. Disadvantages of this procedure are the relatively high costs as well as the anesthetic and surgical risk. Surgical insemination is considered an unethical procedure in some countries (Linde-Forsberg, 1991). Insemination doses are usually composed of 100-150 x 10^6 motile spermatozoa. Whelping rates for frozen semen AI are reported to be as high as 70-75% if the timing of insemination is accurate, good quality frozen-thawed semen is used and the semen deposition is correct (Thomassen et al., 2001; Thomassen et al., 2006).

3.2 AI techniques in horses

AI is a widely practiced breeding method in most sport horse breeds worldwide. Fresh, chilled extended and frozen-thawed semen can be used in horses. Common reasons for the use of insemination for fresh semen instead of natural mating are the reduced risk of injury

to the often very valuable stallion by avoiding live coverings, insemination of mares that do not want to allow a mating or splitting of an ejaculate into several insemination doses if more than one mare is to be inseminated at the same time. Generally accepted insemination doses for fresh semen as supported by the World Breeding Federation of Sport Horses (WBFSH) are 300 x 10^6 straight forward swimming (progressively motile) spermatozoa (Katila, 2005) which is slightly lower than the originally recommended fresh semen AI dose of 500 x 10^6 progressively motile spermatozoa (PMS) (Pickett & Voss, 1975). First cycle pregnancy rate for fresh semen AI is approximately 60%. Chilled extended semen is usually used if the mare and the stallion are in distant places. Semen is collected, extended with a suitable semen extender to provide buffers, nutrients, antibiotics etc. in order to prolong lifespan of the spermatozoa and slowly cooled down to 4 degrees Celsius. Usually, special transport containers that form cooling units (e.g. Equitainer®, Hamilton Thorne) are used and the semen of most stallions is viable for at least 48 hours. Most insemination centres try to inseminate mares with chilled extended semen within 24 hours of collection. One billion spermatozoa or 600 x 10^6 progressively motile spermatozoa (according to WBFSH (Katila, 2005)) form one insemination dose and pregnancy rates of about 60 % (Sieme et al., 2003) can be expected depending on timing of insemination relative to ovulation, AI dose and semen quality after chilling. Some stallions' semen is not suitable for chilling and the semen quality deteriorates very rapidly. It is therefore recommended to perform a trial cooling of equine semen before a shipping is done and to evaluate semen quality parameters over time to assess longevity.

The insemination technique for fresh and chilled extended semen is similar in the horse. A transcervical intrauterine insemination is performed where the semen is deposited into the uterine body. A commercially available insemination pipette is inserted manually into the vagina, the external opening of the cervix is located using the index finger and the pipette is guided through the cervical canal into the uterus. The penetration of the cervix is generally very easy as the cervical tissue is smooth muscle that relaxes under estrogen influence. That allows easy access to the equine uterus at the time of insemination. Frozen-thawed semen has first been used to inseminate mares in 1957 (Barker & Gandier, 1957) even though it only gained increasing popularity over the last 15-20 years. Using frozen semen has a lot of benefits: accessibility to semen from stallions in competition or stallions that become ill, injured or overbooked during the breeding season. Using frozen semen eliminates the need to organize stallion availability at the optimum time for breeding of the mare and disease transmission. Chances for injury are decreased as direct mare-stallion contact is avoided. Pregnancy rate per cycle for frozen semen varies between 30-50% on average (Leipold et al., 1998; Metcalf, 2007; Sieme et al., 2003; Vidament, 2005; Vidament et al., 1997). There are, however, some disadvantages regarding frozen semen. The management of mares during estrus is more intense which increases the costs involved for frozen semen inseminations. This is necessary as inseminations with frozen semen should be done as close to ovulation as possible since frozen-thawed spermatozoa have a shortened lifespan. Frozen-thawed spermatozoa survive for about 12 hours in the reproductive tract of mares whereas fresh semen can survive for 48-72 hours. Frozen-thawed equine semen can be inseminated into the uterine body as described for fresh and chilled distended semen if an insemination dose of 250 x 10^6 progressively motile spermatozoa is available (Katila, 2005). If the insemination dose is lower or a very small volume is to be inseminated (0.25-0.5ml), the semen can be deposited deeply into the uterine horn ipsilateral to the ovary where ovulation will take place or has taken place (Katila, 2005). Other possibilities for low dose (5-25 x 10^6

progressively motile sperm) or very low volume (0.02-0.2 ml) inseminations (Katila, 2005) are endoscopic deposition of semen at the utero-tubal junction right in the tip of the uterine horn ipsilateral to the ovary where ovulation will take place (Lindsey et al. , 2001; Lindsey et al. , 2002; L. H. A. Morris, 2004), and surgical deposition of spermatozoa directly into the oviduct (McCue et al. , 2000).

3.3 AI techniques in dairy cattle

In Britain, AI in dairy cattle began to be available in 1942, and by 1950 20% of dairy cattle were being inseminated. By 1960, more than 2 million cows were inseminated yearly, which was about 80% of the maximum level that AI would reach (Brassley, 2007). The established procedure for AI in cattle since the 1960s is transcervical deposition of semen into the uterine body. This technique replaced the original vaginal or shallow cervical insemination performed in the 1940s as the intrauterine method proved to be more efficient and resulted in higher fertility (Lopez-Gatius, 2000). Transcervical intrauterine AI involves the technique of cervical fixation per rectum to facilitate easier penetration of the cervical rings with a stainless steel Cassou device (AI pistolette) (Noakes et al. , 2001). Other techniques like unicornual or bicornual insemination where semen is deposited into one or both uterine horns, and intraperitoneal insemination have been investigated (Lopez-Gatius, 2000) but could not replace the transcervical intrauterine AI with semen deposition into the uterine body. For commercial AI in cattle, frozen-thawed semen is routinely used and a generally accepted insemination dose contains 10-20 x 10^6 spermatozoa. Deep horn AI close to the uterotubal junction has been investigated and facilitates AI with a conventional number of spermatozoa reduced x 100 or if very small volumes of semen (0.1-0.25 ml) are to be used (Hunter, 2003). Potential advantages of deep horn AI include: raising fertility of genetically valuable bulls whose non-return rates to estrus are sub-optimal, reducing the number of sperm per AI dose, facilitating the use of limited numbers of sex selected sperm cells available from flow cytometry, and breeding from valuable but oligospermic (too few sperm in ejaculate) bulls (Hunter, 2003).

3.4 AI techniques in pigs

Even though AI in pigs has been used since the 1930s, wider commercial application only started in the 1980s, with a dramatic increase in the use of AI in pork production in the last 20 years. International comparison shows that in 1990, 80% of pigs in former Eastern Germany were bred by AI, compared to only 23% in West Germany, while the figures for Norway were 71% and for the Netherlands 51%. France and the UK were about the same with 10% and the USA was only 7% (Brassley, 2007). By the end of the 1990s, close to 50% of the worldwide gilts and sows were inseminated (Roca et al., 2006). Nowadays, AI is used routinely in pig companies. In some European countries, like Belgium, Italy, the Netherlands, Norway and Spain, more than 80% of females are bred by AI and in North America and Brazil the percentage has reached 75% (Roca et al., 2006). One reason for the slow take-off for AI in pigs were initial conception rates which were as low as 18.1% in 1955 rising to 62% in 1961 while natural conception rates were expected to be close to 90% (Brassley, 2007). Conception rates could be increased to about 80% by the mid 1970s. Consistent 80-90% fertility rates with the use of liquid extended semen are common on many farms by now with an overall fertility success (measured as farrowing rates and litter

sizes) similar to or better than those resulting from natural matings (Roca et al., 2006). Apart from good conception rates, there are other reasons for the dramatic increase in AI utilization for pig breeding systems. On one hand there was the change of breeding and farrowing units that became larger and more specialized, and that the application of AI mating technologies became more feasible and cost effective on the other hand (Singleton, 2001). At the same time, the pork industry initiated payment programs based on actual carcass value instead of live weight basis. Genetic evaluation programs were used to implement genetic improvement programs through the use of AI to produce higher quality pork carcasses. For most commercial breeders it was the prospect of genetic improvement that was the major incentive to engage in AI (Brassley, 2007). Semen processing centres (boar studs) or on-farm collection facilities provide boar semen used for AI programs. More than 99% of AIs are performed with semen extended in a liquid state that can be stored at 15-20°C for up to 3 days (cooled semen)(Roca et al., 2006). The number of viable sperm per dose ranges between 2.5- 4 billion motile spermatozoa. Dose volumes range from 80-100ml for fresh liquid semen. The insemination procedure, called intra-cervical insemination (intra-CAI), involves the deposition of spermatozoa into the posterior part of the cervix using a catheter that engages with the folds of the cervix, stimulating the corkscrew tie of the boar's penis (Roca et al., 2006). This easy and quick procedure has been developed in the mid 1950s, standardized in the 1970s and is still used worldwide today (Roca et al., 2006). The remaining 1% of AIs utilizes frozen-thawed semen at doses of 5-6 x 10^9 spermatozoa. Even though large sperm numbers are used, fertility is substantially lower than that obtained with cooled semen. Due to the lowered reproductive performance, frozen-thawed boar semen is mostly limited to specialized breeding programs, research and for export puposes (Singleton, 2001; Wongtawan et al. , 2006). There are two insemination procedures available that allow insemination with low numbers of spermatozoa: post-cervical insemination (post-CAI) and deep uterine insemination (DUI). Both techniques facilitate deposition of semen into the uterus. Using post-CAI, semen is placed into the uterine body while DUI facilitates placement of semen in the proximal 1/3 of one uterine horn (Martinez et al., 2005). Similar fertility results can be expected for DUI using 600 x 10^6 spermatozoa and 1-1.5 x 10^9 spermatozoa for post-CAI as compared to intra-CAI using 3000 x 10^9 sperm per dose (Roca et al., 2006). Or in other words, a threefold reduction of fresh sperm numbers using post-cervical AI, and for DUI a 20-fold reduction for fresh and sixfold reduction for frozen semen can achieve acceptable pregnancy rates (J. M. Vazquez et al., 2008). Other possibilities for inseminations with low sperm numbers are surgical intrauterine inseminations where high pregnancy rates (89%) can be obtained with as few as 10 x 10^6 cooled stored spermatozoa that are placed on the uterotubal junction (Krueger & Rath, 2000; Krueger et al. , 1999). A further reduction in sperm numbers to 5 x 10^6 can be achieved if spermatozoa are placed close to the uterotubal junction by laparoscopy (Fantinati et al., 2005). A new procedure where semen is placed into the oviduct via laparoscopy also displays an opportunity for the use of diluted and sex sorted spermatozoa (Vazquez et al., 2008). Other insemination techniques like intraperitoneal insemination, where semen was deposited directly into the abdominal cavity, have been used to investigate insemination possibilities other than intra-CAI (Hunter, 1978). This technique, however, has never come to use in the AI industry due to ineffective sperm transport to the oviducts, reduced fertility and difficulty of the procedure when compared to intra-CAI. It has also been shown that

specific boar stimuli (such as olfactory and tactile stimuli) at or around the time of AI positively influence reproductive performance through an effect on reproductive processes like sperm transport and ovulation (Soede, 1993).

3.5 AI in sheep (Youngquist & Threlfall, 2007)

Fresh, chilled and frozen semen is utilized for AI in sheep. Methods used are vaginal, cervical, laparoscopic intrauterine and transcervical intrauterine insemination. Vaginal insemination can be used for fresh and chilled semen and is also referred to as the "shot in the dark", or SID, method as the semen is blindly deposited into the cranial vagina. Pregnancy rates performing vaginal AI for fresh and chilled semen are acceptable while they are not for frozen semen. The technique is very fast but the use of semen is inefficient as AI doses have to contain large numbers of sperm. For cervical insemination, the hindquarters are elevated while the ewe is restrained "over the rail". The cervix is visualized using a speculum and a light source. Semen is deposited into the cervix with an angled tip insemination gun.

In contrast to horses and cattle, a successful method for transcervical intrauterine insemination in sheep has not been well established due to the specific anatomy of the ovine cervix (Halbert et al. , 1990). The cervical canal is approximately 7 cm long with a series of 6-8 rearward facing, offset rings that make transcervical insemination difficult to impossible. The Guelph system for transcervical AI (GST-AI) has been developed which requires special positioning of the ewe, cervical retraction and stabilization, and the use of specially designed instruments. Trained, experienced inseminators may penetrate the cervix in as much as 75-85% of ewes. Cervical injury, abscesses, infections and poor pregnancy rates are associated with this technique. Surgical AIs (i.e. via midventral laparotomy) are effective, but they are costly, time consuming, require technical proficiency, limit the number of times ewes can be used and require anesthesia (Evans & Maxwell, 1987). Laparoscopic intrauterine AI is used for frozen semen, and requires laparoscopic equipment as well as technical expertise. Animals are usually sedated and restrained in a laparoscopic cradle in dorsal recumbency. The laparoscope is used to identify the uterus and a loaded insemination pipette directed to the uterus to facilitate intrauterine deposition of the semen. Three hundred or more ewes can be inseminated per day using this technique if an experienced team works in a well-equipped and organized operation. Insemination doses for fresh as well as frozen-thawed semen are 400×10^6, 200×10^6, 20×10^6, 100×10^6 progressively motile spermatozoa for vaginal, cervical, laparoscopic and transcervical AI, respectively. Expected lambing rates for fresh semen are 20-60%, 40-80%, 70-100%, 40-80%, and 5-20%, 25-60%, 40-80%, 30-70% for frozen semen for vaginal, cervical, laparoscopic and transcervical AI, respectively.

3.6 AI in other species

Since the 1920s, AI has been a reproductive tool in commercial rabbitries that permits more controlled management and better planning (e.g. in batch parturition and weaning) than natural mating (Morrell, 1995). Conception rates after AI can be equivalent to or better than that achieved with natural breeding (Morrell, 1995). Natural matings in healthy individuals can result in conception rates of 85%. A single ejaculate can be split into 20-50 insemination doses and used for AI (Lavara et al. , 2005). For the AI procedure, the female is placed into a restraining box, the tail is lifted and an insemination pipette with a bend approximately 8cm from the end is inserted into the vagina at an angle of 45^o in order progress beyond the

pelvic rim (Morrell, 1995). Semen is deposited intravaginally. Due to the fact that rabbits have two separate uterine horns and cervices, intracervical AI is not performed as it would be required to release semen into both cervices. Other peculiarities regarding AI in rabbits are that females should be kept separate from males for about 19 days before breeding to exclude pseudopregnancy, that timing of AI is based on general behaviour and vulva colour of the doe, and that rabbits are induced ovulators that always require induction of ovulation when AI is used. Three methods for induction of ovulation are available: 1) mating with a vasectomized buck, 2) administration of human chorionic gonadotropin (hCG) or 3) administration of gonadotropin releasing hormone (GnRH) analogues (Morrell, 1995).

AI in non-human primates is based on captive colony management and propagation of endangered or valuable founder animals. Its application, (although limited due to high costs, substantial technical requirements and limited available captive populations), has found use in Great Apes as well as Old World and New World Macaques (Wolf, 2009). Initially, intravaginal AI was performed while intrauterine insemination is the technique of choice today.

Application of AI in South American camelids has been challenging due to the inconsistent success in collecting semen from males. Llamas as well as alpacas copulate for extended periods of time (10-60 min), display a recumbent mating posture, deposit semen into the uterus, and the semen is very viscous (Adams et al., 2009). Camelids are not as easily trained to mount an AV as rams, bulls or stallions. It is necessary to maintain a stable AV temperature during prolonged copulation. Other semen collection attempts using condoms or intravaginal sacs, vaginal sponges, electro-ejaculation, post-coital vaginal aspiration, and fistulation of the penile urethra were associated with recovery of poor semen samples and contamination of semen samples with blood (Adams et al., 2009). For AI, semen is deposited into the uterus transcervically or via laparoscopy. Reported pregnancy rates after AI vary widely between 2-68% (Adams et al., 2009).

4. Use of epididymal spermatozoa for AI

Another interesting field for application of AI is the use of epididymal spermatozoa. Harvesting of epididymal sperm enables storage and usage of valuable genetic material of males after death or shortly before death if unexpected accidents or health problems occur. The epididymis is part of the male reproductive tract, is connected to the testis and forms a site for sperm maturation and storage. The epididymis can be dissected free from the testis after castration and epididymal spermatozoa can be harvested by flushing or slice-and-dice techniques (Bruemmer, 2006). Aspiration of spermatozoa from the epididymis has also been performed. Interestingly, the first reported pregnancy in a mare after AI was achieved with frozen-thawed epididymal stallion spermatozoa in 1957 (Barker & Gandier, 1957).

Epididymal spermatozoa have been harvested from a variety of species like cats (Filliers et al., 2008; Hermansson & AxnÈr, 2007), dogs (Garcia-Macias et al., 2006; Hewitt et al., 2001; ¤Nothling et al., 2007; Ponglowhapan et al., 2006), rats (Yamashiro et al., 2007), horses (Braun et al., 1994; Bruemmer, 2006; Heise et al., 2011; Johnson & Coutinho da Silva, 2008; Melo et al., 2008), cattle (Goovaerts et al., 2006; Martins, Rumpf, Pereira, & Dode, 2007), pigs (Ikeda et al., 2002), sheep (Garcia-Macias et al., 2006), goats (Blash, Melican, & Gavin, 2000), red deer (Fernandez-Santos et al., 2006; Garcia-Macias et al., 2006; MartÌnez-Pastor et al.,

2006), Spanish Ibex (Santiago-Moreno et al., 2006), African buffalo (Herold et al., 2006), North American buffalo (Lessard at al., 2009) and monkeys (Goff et al., 2009; Ng et al., 2002). Pregnancies and offspring after AI with epididymal spermatozoa have been produced amongst others in horses (Barker & Gandier, 1957; Heise et al., 2010; Morris et al., 2002; Papa et al., 2008), dogs (Hori, Hagiuda, Kawakami, & Tsutsui, 2005) and Spanish Ibex (Santiago-Moreno et al., 2006). Application of AI for epididymal spermatozoa holds tremendous potential for future use of valuable genetics not only in domestic but also especially in wild animal species.

5. Use of sexed semen for AI

Another aspect of artificial insemination in animals is the use of sex sorted spermatozoa. Separation of the X and Y bearing sperm is desirable in animals as one sex has significantly more value than the other in certain species. For dairy cattle for example, cows as the "milk producers" are the main source of income for the industry while bull calves are of less value as lactation is limited to females. For other food producing animals a higher percentage of male offspring might be beneficial as they grow faster and produce more meat. In many large, long-lived species like elephants, rhinoceroses, dolphins that are kept in captivity, sex selection could ease and avoid housing problems with males of these species (Durrant, 2009).

It has first been established for human spermatids in 1979 that there is a difference in DNA content between the mammalian X-chromosome-bearing spermatozoa and the Y-chromosome (Otto et al., 1979). Since then, DNA content measurements have been used to identify the sex-chromosome bearing sperm populations with good accuracy in semen from at least 23 mammalian species (Garner, 2006; Garner et al., 1983; Lu et al., 2010; Pinkel et al., 1982), and offspring have been produced from sexed sperm of at least seven species, including rabbits (Johnson et al., 1989), humans (Levinson et al., 1995), cattle (Cran et al., 1993), horses (Buchanan et al., 2000), sheep (Catt et al., 1996), dogs (Meyers et al., 2008), cats (Pope et al., 2008), elk (Schenk & DeGrofft, 2003), buffalo (Presicce et al., 2005) and dolphins (O'Brien & Robeck, 2006). The first offspring born with flow cytometrically sex sorted spermatozoa was in rabbits after surgical AI into the oviduct (Johnson et al., 1989).

The Beltsville Sperm Sexing technology (Garner, 2006) uses the difference in DNA content of the X- and Y-chromosome to sort the sex-determining gametes. The procedure is called fluorescence-activated cell separation (FACS) where ejaculated spermatozoa are treated with a DNA stain (called a flourochrome) and due to the fact that X-chromosomes contain more DNA, the stain take-up will be higher for the X-chromosome bearing spermatozoa than the Y-chromosome bearing spermatozoa. This difference in stain absorption is used in a flow cytometer chamber where the fluorescent stain in the spermatozoa is excited by a laser. Each live sperm produces an emission with an intensity that is directly related to the quantity of DNA within the sperm head. The X bearing spermatozoa emit more intense light than the Y bearing spermatozoa. A high speed computer is used to analyze the relative fluorescence of the X- and Y-sperm populations as they flow through a cytometer chamber. Spermatozoa are then assigned either a negative or positive charge depending on the DNA content while passing by charged plates. An electromagnetic field separates the X- and Y-chromosome bearing spermatozoa (Senger, 2003). This separation technology has a 85-95% success rate (Garner, 2006).

Not all mammalian sperm are equally suitable for sex sorting of spermatozoa. Apart from the DNA content difference of the X- and Y-bearing spermatozoa, the head shape of the spermatozoa plays a role as well. Flattened, oval shaped sperm heads (e.g. bull, boar, ram spermatozoa) are more readily oriented in a sperm sorter using hydrodynamics than those gametes with more round or angular head shapes (rodent spermatozoa) (Garner, 2006). The area of the flat profile of the sperm head can be multiplied times the difference in DNA content of the X-and Y-chromosome bearing sperm to give the sorting index. This index suggests that bull and boar sperm are well suited for separation in a flow sorter.

Initially, the use of sex sorted spermatozoa was limited due to the slow separation process where only a few hundred thousand sperm per hour could be sorted. Newer sperm sorter systems are able to sort 20,000 sperm/s resulting in up to 6000 or more sperm/s each of X- and Y-sperm at 90% accuracy (Garner & Seidel Jr, 2008). Due to low sperm numbers acquired with sex sorting, initial efforts to predetermine the sex required surgical insemination. Later, with improvement of the equipment, quantities were sufficient for in vitro fertilization (IVF). Today, sexed sperm are commercially available for cattle where the standard insemination doses of 2×10^6 sexed sperm achieve 70-80% of the pregnancy rates achieved with non-sorted sperm in doses of $10\text{-}20 \times 10^6$ (Bodmer et al., 2005; Garner, 2006). In pigs, low dose (70×10^6 spermatozoa) AI with flow cytometrically sorted sperm deep into the uterine horn resulted in pregnancy rates of 35-45.6% (Vazquez et al., 2003). In horses, hysteroscopic insemination into the uterine horn (Lindsey et al., 2002; Morris & Allen, 2002) and ultrasound guided deep uterine AI were performed using sex sorted spermatozoa in low concentrations (5×10^6 sperm cells/ dose).

6. Conclusion

AI has been and still is the most used reproductive technique in animals. A lot of research has been done over the last few decades, constantly improving techniques, methods and applications of AI. Routine AI procedures as well as specialized techniques like low-dose inseminations or use of sexed semen offer a wide variety for application of AI in domestic as well as wild and endangered animal species.

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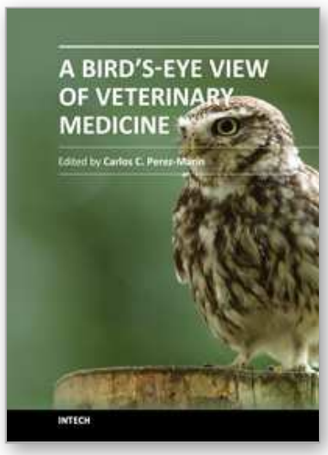

A Bird's-Eye View of Veterinary Medicine

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Veterinary medicine is advancing at a very rapid pace, particularly given the breadth of the discipline. This book examines new developments covering a wide range of issues from health and welfare in livestock, pets, and wild animals to public health supervision and biomedical research. As well as containing reviews offering fresh insight into specific issues, this book includes a selection of scientific articles which help to chart the advance of this science. The book is divided into several sections. The opening chapters cover the veterinary profession and veterinary science in general, while later chapters look at specific aspects of applied veterinary medicine in pets and in livestock. Finally, research papers are grouped by specialisms with a view to exploring progress in areas such as organ transplantation, therapeutic use of natural substances, and the use of new diagnostic techniques for disease control. This book was produced during World Veterinary Year 2011, which marked the 250th anniversary of the veterinary profession. It provides a fittingly concise and enjoyable overview of the whole science of veterinary medicine.

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