Artificial Iinsemination

The successful use of artificial insemination (AI) as a means of animal breeding relies upon three major premises:

- Firstly, that spermatozoa can survive outside the body.
- Secondly, that they can be reintroduced into the female genital tract in a way that results in an acceptable conception rate.
- Thirdly, that the fertile period of the female can be identified.

AI regimes have been developed for most domestic and many semi-domestic species. It is routinely practiced in cattle, sheep, pigs, goats, fowl, turkeys, salmon and trout, and is used in dogs, domestic foxes, buffalo, horses and even bees.

ADVANTAGES AND DISADVANTAGES OF AI

Artificial insemination offers several potential advantages over natural service. The most commonly is genetic improvement. Each ejaculate can be divided into many insemination doses, such that each AI sire can potentially be used to breed a very large number of females. Hence, the total number of sires needed is reduced. The major advantage to AI is the control of venereal disease specially the epizootic venereal pathogens *Trichomonas fetus* and *Campylobacter fetus*.

Nevertheless, the detection of the fertile period in the female oestrous cycle is potentially the most problematic aspect of AI programmes. In cattle, the prominent homosexual behavior of oestrous females allows relatively accurate human identification of the fertile period, but in most other species its detection is less easy. In such species, detection of oestrus therefore requires the presence of infertile (e.g. vasectomised) males, or the timing of oestrus must be controlled by pharmacological (e.g. oestrus synchronization/induction regimens). The process of insemination also requires trained personnel, which may require a limited degree of technical proficiency, as is the case of laparoscopic intrauterine insemination of ewes.

It is also necessary to log insemination dates in an adequate recording system, in order to allow birth dates to be calculated and so that expected dates of return to oestrus are known, thereby allowing appropriate observations to be made. The identities of the sires need to be recorded to avoid inbreeding. The value of AI as a rapid means of transmission of the genes of superior sires has already been identified. Hence, AI programmes should be underpinned by an efficient reporting system for monitoring abnormalities in the progeny, with clearly defined criteria for the withdrawal from use of sires that carry deleterious genes.

PREPARATION OF SEMEN FOR USE IN AI

In most AI regimes, semen evaluation is limited to measuring sperm numbers, motility and, usually, morphology. Direct inseminations are performed most commonly in the bitch, usually in response to some incapacity of the sire that precludes normal mating or in the mare with chronic endometritis.

Dilution

The ejaculates of most domestic animals contain more sperm than are needed for achieving a pregnancy. Hence, by diluting the semen, it can potentially be used for several inseminations. In species such as the dog and the horse, the whole sperm rich fraction of the ejaculate is diluted and chilled, then used. The maximum degree of dilution is determined from the minimum number of spermatozoa and the volume of inseminate that is required to achieve acceptable pregnancy rates. These factors are themselves determined by the site of insemination, the survival of sperm in diluents. In general, where an intrauterine insemination can be achieved, the minimum numbers of sperm are one or two orders of magnitude lower than for an intracervical insemination. In the ewe, this requires as complex a procedure as laparoscopic insemination.

The major properties of semen diluents are:

1. *Addition of volume*. Insemination doses must be prepared in a volume, which is a compromise between ease of handling and an appropriate volume for the site of insemination. This phenomenon, known as the 'dilution effect', represents a loss of cell viability, probably through leaching of structural components of the cell membrane.
2. *Buffers*. Spermatozoa have a narrow range of tolerance to changes in pH, so provision of buffering capacity is necessary.
3. *Maintenance of osmotic pressure*. Seminal plasma has an osmotic pressure of 285 mm. Sugars, which are added to provide nutrition for the sperm or to contribute to the cryoprotective properties of the diluents.
4. *Energy substrate*. Most diluents make some provision of energy substrates for sperm. In general, simple sugars such as glucose, fructose, mannose and arabinose are suitable substrates. Lactose, which is present in milk-based diluents, is not metabolisable to any appreciable extent. However, egg yolk, also a component of many diluents, provides many substrates for sperm metabolism.
5. *Antimicrobial activity*. Antibiotics are added to most semen diluents as a prophylactic against the pathogenic bacteria and to reduce the load of non-pathogenic organisms that contaminate the semen.

The life span of spermatozoa at ambient temperatures is generally short, but can be extended by inhibiting their metabolism and motility with carbon dioxide. Furthermore, some of the constituents of egg are toxic to the sperm of some species, notably the goat, in which a toxic interaction occurs between yolk and components of the seminal plasma, causing sperm death. Moreover, whole milk is also toxic to sperm, for it contains a protein, 'lactenin', which is spermicidal. The fertility of bovine semen stored at 5°C in such a diluents remains acceptable for 2–4 days, although that of ram semen only persists for 12–24 hours.

Cryopreservation

Longer-term storage of semen is achieved through cryopreservation. For sperm to survive freezing, they need to be extended in a diluents that contains not only substances that protect them against cold shock, but also cryoprotectants, such as glycerol, which protect them from the deleterious consequences of freezing.

DISEASES TRANSMISSIBLE IN SEMEN

Many infectious agents can be transmitted through semen. Foot and mouth virus can be transmitted in the semen of all species that are susceptible to its infection. Control of diseases in other species is generally much less stringent than in cattle. For example, for dogs, control of *Brucella canis* and leptospira infection is generally required for international shipments of semen.

AI OF CATTLE

Collection, handling and storage of semen

Semen is usually collected by an artificial vagina, although electroejaculation is occasionally used. After assessment for motility, density and morphology, the semen is diluted into insemination doses. Most semen is cryopreserved, although some is used after simple extension and chilling to 4°C. For cryopreservation, or for use at 4°C, the semen is first extended with a diluents based upon either egg yolk or skimmed milk, which contains antibiotics for the control of contaminating bacteria. The semen is then cooled to 4°C. If it is destined for use in this form, the motility of the sperm will be reassessed, then the semen released for use. If the semen is destined for cryopreservation, glycerol is also added, then the semen packed into 0.25 or 0.5 ml paillettes, or 0.5 or 1.0 ml glass ampoules. The semen is then equilibrated for 1–4 hours. The semen is then frozen in the vapour of liquid nitrogen or in a microprocessor-controlled freezer. The semen thereafter remains in liquid nitrogen until thawed for use. The ability to perform an intrauterine insemination in cattle means that a relatively low dose of sperm is required to achieve acceptable pregnancy rates. Typically, of the 20–30 million sperm that are required in each insemination dose, 6–7 million survive freezing. Lower numbers of sperm can be used where unfrozen semen is use. However, it has many advantages over other methods of extension, for, whereas cryopreserved semen requires about 20 million spermatozoa per insemination, semen extended in ambient temperature diluents can achieve acceptable fertility with less than 2.5 million sperm per insemination.

Insemination

Cows ovulate at about 12 hours after the end of the oestrus period. The ideal time for insemination is therefore 6–24 hours prior to ovulation. Where the technician service provided by an AI centre is used, the optimum insemination times achieved in practice are on the same day (morning or afternoon) where oestrus is first observed in the morning, or on the morning of the next day, where oestrus is first observed in the afternoon. However, it is claimed that it is possible to achieve a better timing of insemination in relation to the most fertile period of oestrus by farmers inseminating their own cattle at appropriate intervals after the first observation of oestrus. Cows are inseminated just into the short uterine body. Insemination into the cervix produces a lower fertilisation rate, while insemination deeper into the uterus runs the risks of either inseminating into the uterine horn contralateral to the

ovulation site, or scoring the endometrium with the tip of the insemination catheter. Reduced fertility is the consequence of both of the latter two errors. The standard technique of insemination is to grasp the cervix through the rectum with the left hand. A catheter, into the tip of which a paillette of semen has been inserted, is then passed into the vagina and manipulated into and through the cervix by the right hand. This technique, the rectovaginal method of insemination, requires considerable practice for success. The vulval lips are opened by downwards pressure from the arm in the rectum, while the circular folds of vaginal mucosa are obliterated by pushing the cervix forward. The catheter is initially inserted pointing upwards at an angle of about 30° to avoid entering the urethral meatus or fossa, and is then moved horizontally until it engages in the external os of the cervix. The left hand squeezes the anterior vagina on to the caudally projecting external os of the cervix, thereby obliterating the fornix of the vagina and facilitating entry of the catheter into the cervix. Entry into the external os is accompanied by a characteristic 'gritty' sensation. The catheter is then introduced through the convoluted cervical canal by manipulation of the cervix through the rectal wall. One finger is placed over the internal os of the cervix, so that the tip of the catheter can be palpated as it emerges from the cervical canal. As soon as the catheter has emerged, deposition of semen into the uterus begins; the catheter is advanced no deeper into the uterus. In this way, semen should be equally distributed between the two uterine horns. The most common fault of insemination is twisting the cervix in the left hand, so that one uterine horn is partly occluded. Alternatively, the catheter may be partly withdrawn during the deposition of semen, resulting in a partially intracervical insemination. Penetration of the cervical canal of maiden cattle is difficult at oestrus, and virtually impossible at other stages of the oestrous cycle; such animals are therefore often beyond the capabilities of inexperienced inseminators. It is therefore imperative that it is known whether an animal is likely to be pregnant before insemination is attempted, for abortion can be induced if an insemination catheter penetrates the fetal membranes or if infection is introduced into a pregnant uterus by poor insemination hygiene.

Management of insemination

Insemination can be performed at an observed or induced oestrus. Oestrus can be induced and synchronized by the use of prostaglandin F2α (PGF2α) or its analogues, progesterone-like hormones, or combinations of progesterone and a luteolytic agent. Most such regimens require the use of fixed-time insemination and reinsemination performed if animal's exhibit sign of oestrus after fixed-time insemination has occurred. Fertility to AI is generally very similar to that achieved at natural service, with a calving rate to a single insemination of around 50%. The true fertilization rate is much higher than this, at around 90%, but subsequent embryonic losses bring the apparent figure to the lower value. In practice, AI companies are generally unable to obtain complete data on the calving rates, so they estimate fertility from the proportion of cows which are represented for insemination by either 49 or 60–90 days after the initial service.

Control of infectious diseases

Control is exercised over the health status of donor bulls and the hygiene of technicians who, by travelling between farms, offer considerable risk as disease vectors. Most of the serious viral diseases of cattle (foot and mouth, rinderpest, etc.) can potentially be transmitted through AI. Legislative regulation of bovine AI has, for many years, been based upon the primary precept of preventing such transmission from occurring. Many of the somewhat less serious viral diseases, such as infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (IBR–IPV) can also be transmitted thus. Recently, it has become apparent that the bovine viral diarrhea (BVD) virus can be present in the semen of bulls, potentially causing early embryonic death and abortions in inseminated cows, as well as its better known ability to cause the birth of persistently infected progeny. A number of bacterial diseases are transmissible in semen, including tuberculosis, brucellosis, leptospirosis and, possibly, Johne's disease. *Haemophilus somnus*, to which the possibility of causing reproductive failure has been attributed, may also be present in semen. Many species of *Mycoplasma* and *Ureaplasma* are present as commensals of the prepuce of the bull, and are harmless when inseminated into cows, but some species are responsible for a granulomatous vaginitis in cows, which causes infertility and unwillingness to mate. Most importantly, the classic venereal pathogens of cattle, *T. fetus* and *C. fetus* are transmissible by AI; control of these two organisms remains the second major precept upon which legislation governing cattle AI is based. In cattle, control of these diseases rests upon three major strategies. Diseases that can be detected by serology, such as brucellosis, IBR, EBL, Q fever, etc., are controlled by exclusion of seropositive bulls from AI studs. Likewise, tuberculosis is controlled by exclusion of bulls that react to tuberculin testing. *Leptospira spp.* may be killed by freezing and thawing. The antibiotics that are added to semen diluents are intended to kill both pathogenic bacteria (including *C. fetus*) and the contaminant bacteria that originate from the penis and prepuce during semen. Antibiotics are also used to control *Mycoplasma* and *Ureaplasma* species. The final and most potent means of control of disease is the quarantine of semen after its collection. After semen has been frozen, it is placed in a container where it remains untouched for 28 days. If, during that period, the donor bull develops any disease, the semen is destroyed. If not, it is released for use.

AI OF SHEEP

The sheep is less amenable to artificial insemination than is the cow, since oestrus cannot readily be detected without the presence of rams, insemination is less straightforward and ovine semen is less easy to freeze than bovine semen. Ewes normally display oestrous behavior only in the presence of a ram. In order to determine the time at which AI should be performed, it is therefore necessary either to control the timing of oestrus or to detect it with male animals. In the former situation, pharmacological methods are used to induce and synchronize oestrus, so that the time of the fertile period is defined. In the latter situation, either raddled, vasectomised rams or intact rams with an abdominal apron to prevent intromission are used to detect oestrus. However, the most important limitation of the use of

AI in sheep is in the method of insemination, since it is difficult to achieve an intrauterine insemination because the cervical canal of the ewe is so tortuous. Since intracervical AI results in both a lower conception rate and a lower number of lambs born per ewe than with natural service, a number of methods of insemination have been devised that try to circumvent the cervix. The methods in widespread use are:

- intravaginal
- intracervical
- transcervical intrauterine
- laparoscopic intrauterine.

Collection, handling and storage of semen

Most inseminations of ewes are performed using semen that was collected on the day of insemination. Such semen is normally extended by the addition of simple diluents, although direct insemination of raw semen is still practiced in some regions. The semen is collected by an artificial vagina or electroejaculation and subjected to routine examination for motility and density. It is then diluted with simple diluents, to a final volume and sperm content that depend upon the route by which it is to be inseminated. The number of sperm and the volume of diluted semen used for insemination depend upon the route of insemination, whether the insemination is undertaken during the natural breeding season or after induction of out of season breeding, and whether the semen is cryopreserved. After dilution, the semen is cooled and stored at either +15°C or +4°C until used. The semen has to be used within 8 hours of collection, as fertility declines substantially after this time. These arise as a relatively large number of sperm (150–200 million total sperm) have to be contained within the limited volume of inseminate that can be placed within the ovine cervix. Since the anatomy of the cervical canal limits the insemination volume to below about 0.25 ml the dilution rates are limited to between 1:1 and 1:4. In consequence, insufficient protection can be afforded to the sperm by the diluents against cold shock and freezing damage, generally resulting in mediocre post-freezing survival of functional sperm. For intrauterine insemination, in which lower numbers of spermatozoa are required, far more satisfactory dilution rates of semen can be achieved, so cryopreservation is more successful. Conception rates of 65–80% are typical when this method is used in the ewes' breeding season, with a somewhat lower figure when for out-of-season breeding regimes. Embryonic mortality is generally considered to be similar with frozen and fresh semen.

Insemination

Vaginal insemination deposits semen into the cranial part of the vagina, without attempting to locate the cervix. However, as previously described, this method requires large numbers of spermatozoa per insemination and is not really amenable for use with stored semen. Moreover, conception rates are also poor after pharmacological oestrus synchronization, so intravaginal insemination is best suited to use after oestrus detection during the natural breeding season. The ideal timing of insemination is before ovulation, i.e. 12–18 hours after the onset of oestrus. Highest conception rates are therefore achieved when

the timing of insemination is optimized by drafting ewes for insemination twice per day. Intracervical insemination is best achieved with the hindquarters of the ewe elevated. After cleaning of the perineum, the vagina is opened with a duck-billed speculum and the cervix located. The insemination catheter is then inserted as far as possible into the cervix. Penetration of the cervix is typically 0–2 cm. The conception rate is highly correlated with the depth of penetration and, hence, the technical proficiency of the inseminator. Conception rates achieved with the use of unfrozen semen by this method are adequate after pharmacological methods of oestrus synchronisation. The ideal time for insemination is 1 hour after removal of progesterone sponges, or 15–17 hours after the onset of detected oestrus. The method of direct intrauterine, laparoscopic insemination was developed to overcome many of the difficulties of intravaginal and intracervical insemination. In this method, ewes are restrained in a cradle and laparoscopy is performed close to the udder, whereupon the uterus is located and semen injected into the uterine lumen. The semen can be introduced to the uterus via a simple pipette or by the use of specialized insemination equipment. With oestrus-synchronised ewes, the ideal timing of insemination is between 68 and 72 hours after withdrawal of progesterone sponges. Conception rates to frozen semen inseminated by this method are higher than for intracervical insemination, because of better cryopreservation of sperm and a site of insemination that avoids sperm having to traverse the cervix. However, laparoscopic intrauterine insemination of ewes is undoubtedly the most significant development in sheep AI, for it circumvents many of the problems of the traditional methods. The numbers of sperm required for insemination are lower and the volume of inseminate is proportionally greater, allowing more appropriate dilution rates and, therefore, better preservation of sperm. Hence, conception rates are closer than those of natural service, and embryonic mortality is reduced to an acceptable level. The method also allows for the possibility of genuine progeny testing of rams, as semen from an individual sire can be used in many flocks, over prolonged periods of time. Transcervical intrauterine insemination, the cervix is fixed by being grasped with forceps, and an insemination needle is introduced as far as possible through its lumen. Although better conception rates can be achieved by this method than by conventional intracervical insemination, both the retraction of the cervix and its penetration of the cervical lumen are associated with significant levels of damage.

AI OF GOATS

Artificial insemination of goats is generally very similar to that of sheep. However, it is much easier to achieve an intrauterine insemination via the cervix of the goat than in the ewe. Moreover, conception rates to frozen semen tend to be much higher in the goat than in the sheep, since, in addition to the better site of insemination, caprine semen appears to survive cryopreservation better than ovine semen. Cryopreservation of goat semen differs from that of the ram in one important aspect: that the seminal plasma must be removed before dilution in media that contain egg yolk. A phospholipase that is secreted by the bulbourethral gland coagulates egg yolk media and hydrolyses lecithin to fatty acids and

spermicidal lysolecithins. Therefore, diluents for goat semen have either been based upon skimmed milk, in which the sperm survive adequately, or the seminal plasma has been removed before using egg yolk-based diluents. Where yolk-based diluents are to be used, washing the spermatozoa is achieved by dilution in Krebs–Ringer phosphate solution and centrifugation. For direct insemination, caprine semen can be extended in a skimmed milk–glucose diluents or, after removal of seminal plasma, in an egg yolk–citrate or egg yolk–tris–fructose diluents. For intracervical insemination, there are similar problems of sperm numbers in relation to the volume of inseminate to those that apply in the sheep. Although overall doses of 50 million motile frozen thawed sperm, inseminated into the uterus, are comparable with those of natural service. Intravaginal insemination is not widely used, so intracervical insemination is the most widely practiced method. In a significant number of does, an intrauterine insemination can be achieved via the intracervical route, since the caprine cervix is relatively easier to traverse than the ovine cervix. Because the intracervical route is relatively successful, the impetus to the use of laparoscopic intrauterine insemination has been far less compelling in the doe than in the ewe.

AI OF HORSES

AI of horses has been practiced for many years. The lack of use of AI in this breed is due to the refusal of the Thoroughbred Breeders' Association to allow registration of foals conceived by AI. Even in the face of venereal disease, such as the outbreak of contagious equine metritis, the restriction on the use of AI in thoroughbreds was not relaxed. In other breeds, AI, after appropriate treatment of the semen with antibiotics, has proved a most useful method of achieving pregnancies in mares that regularly have post-covering endometritis. The technical demands of AI of horses differ somewhat from those of cattle. Firstly, it might be considered that the collection of semen from stallions is more difficult than from bulls, for coitus is both more prolonged and more violent in horses than in cattle. Secondly, the stallion produces a fractionated ejaculate, from which the viscous, post-sperm-rich fraction has to be separated and discarded. Thirdly, stallion semen has proved more difficult to store than has that of bulls, with cryopreservation proving difficult to achieve consistently. Finally, the peculiarities of the oestrus period of the mare make pinpointing the moment of peak fertility more difficult than in the cow. Mares do not reliably display visible signs of oestrus in the absence of a stallion and, furthermore, the oestrous period is prolonged, so that unless the ovaries of the mare are palpated to determine the time of ovulation, repeated insemination is likely to be required to ensure that the fertile period is covered. However, insemination of the mare is much easier than insemination of the cow, for the equine cervix is soft and does not have a convoluted luminal canal.

Handling, dilution and storage of semen

After collection, equine semen requires careful temperature control to prevent damage to the sperm by cold shock. If the mares that are to be inseminated are close by, insemination

can be performed directly, using raw semen. Alternatively, the semen can be diluted (at a ratio of about 1:3) in a simple diluents (e.g. skimmed milk plus antibiotics) and stored at 4°C for 12–48 hours before insemination. However the use of more complex diluents, together with the removal of seminal plasma by centrifugation, may extend the storage life of semen for up to 72 hours. Stallion semen has been successfully cryopreserved using low concentrations (4%) of glycerol, in diluents containing both glucose skimmed milk and egg yolk, or lactose, EDTA and egg yolk.

Insemination

Unless palpation of the ovaries of the mare is undertaken to determine the timing of ovulation, however, if the presence of an ovulable follicle is determined, and insemination of frozenthawed semen performed within 6 hours of ovulation acceptable success rates can be achieved. Rather more latitude exists for chilled semen; insemination with semen from stallions of high fertility within 48 hours of ovulation produces acceptable pregnancy rates, although inseminations of semen from less fertile stallions need to be within 12–24 hours. After applying a tail bandage and cleaning the perineal area, a hand is inserted into the vagina and the cervix located. The index finger is inserted into the cervix and an insemination catheter passed through the vagina, then alongside the index finger and so into the uterus. Initially, inseminations with chilled, extended semen used $1–2\times10^9$ sperm in a volume of 10–50 ml. With an insemination dose of 1×10^9 sperm, conception rates of 73–75% have been recorded for this method.

Control of infectious diseases

A considerable number of pathogens are transmissible through equine semen. Most importantly, this includes equine viral arteritis, which can be shed into the semen of viraemic and recovered stallions. Many other viruses can be transmitted through semen, including EHV-III, equine infectious anaemia and, EHV-II and vesicular stomatitis. Many non-specific bacterial contaminants of semen may cause infertility in inseminated mares. The presence of β-haemolytic streptococci in semen is associated with reduced fertility of mares, as are haemolytic *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Klebsiella spp.* are of more clear-cut significance as a cause of infertility, while *Taylorella equigenitalis*, the causal organism of contagious equine metritis, is an important venereal pathogen, *Trypanosoma equiperdum*, the protozoon responsible for dourine, is venereally transmitted. Legislative control exists in many countries to control the spread of equine viral arteritis, equine infectious anaemia, contagious equine metritis and dourine, at least in the blood-stock industries, but control of venereal diseases in ponies and riding horses is often minimal. The simple precautions of serological examination of stallions for the presence of viral infection and serial bacteriological examination of their external genitalia before using the animals as AI (or, indeed, natural service) sires are all that is required to control most venereal pathogens.

AI OF DOGS

AI of dogs is limited to two main circumstances. Firstly, it is used where copulation is not possible. Secondly, AI is employed as a means of using sires that are geographically remote from the bitch, particularly where these reside in a different country. For many breed societies, this latter circumstance is the only one under which they will allow registration of puppies conceived by AI.

Collection handling and storage of semen

Semen is collected from the dog by digital manipulation or by artificial vagina. The pre-ejaculatory fluid, sperm-rich fraction and a little of the post ejaculatory (prostatic) fluid are collected. The whole ejaculate may be immediately inseminated into the bitch's vagina, but it is more common to dilute the semen so that multiple inseminations can be performed. Dog semen can be stored in a chilled condition for 24–72 hours at 4°C, after dilution in simple diluents (e.g. skimmed milk). Cryopreservation of dog semen is more difficult than for many species, but success has been improving over recent years. Egg yolk appears to be an important component of most diluents.

Insemination

The bitch has a prolonged period of receptivity to the male, but a relatively short fertile period. When natural service is used, many breeders allow bitches only a single mating, which typically occurs 12 days after the onset of pre-oestrous bleeding. For successful AI, much closer attention to the time of the fertile period is needed, with the timing of ovulation predicted from vaginal cytology or the preovulatory rise in circulating progesterone concentrations. Thus, AI of dogs is limited to two main circumstances. Firstly, it is used where copulation is not possible. Secondly, AI is employed as a means of using sires that are geographically remote from the bitch, particularly where these reside in a different country.

Collection handling and storage of semen

Once inseminated, the hindquarters of the bitch should be raised for a few minutes, to prevent retrograde loss of semen. Some authors recommend inserting one or two fingers into the vulva after insemination, in order to promote the motility of the female genital tract that normally occurs during the copulatory tie. The fertility achieved in canine AI primarily depends upon achieving correct insemination timing in relation to that of ovulation in the bitch. Dogs with long-lived sperm can achieve pregnancies even if the timing of insemination is not optimal, whereas with dogs whose sperm have poor survival, sperm death is more likely to have occurred prior to ovulation under such circumstances. Pregnancy rates of 39.0% with frozen semen, compared to 54.7% with fresh (diluted or raw) semen, with litter sizes of 4.1 and 5.9 pups, respectively. Intrauterine insemination was recommended for use with cryopreserved semen. Non-surgical, transcervical insemination was used to achieve good pregnancy rates (85% pregnancy rate, 7.8 pups per litter).