ARTIFICIAL INSEMINATION WITH FROZEN SEMEN IN DOGS: PROCEDURES AND INTERNATIONAL SEMEN EXCHANGE

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Introduction

Fertility results with frozen canine semen are improving rapidly, and many semen banks and university clinics achieve high pregnancy rates with frozen canine semen; however, the opposite may also be the case if the facility has little experience with this type of service. An increasing interest is observed among dog owners for semen banking of valuable dogs, since this so far is the only workable solution available at present to preserving ex situ canine genetic material. There is a need to coordinate regulation both for import export of canine semen and for the legal use of frozen semen in various countries, since some kennel clubs will register "frozen -thawed" puppies, whereas others will not. In the following presentation some aspects of AI procedures, results and international semen exchange will be discussed.

Timing of AI with frozen semen

The vaginal smear is less reliable for timing the events of the LH peak and ovulation, especially when frozen semen will be used, due to the reduced longevity of frozen –thawed semen. Also, ovulation precedes the final maturation of the oocyte by 2-3 days due to the peculiarity of the postovulatory meiosis in canine oocytes. Timing is accomplished via identification of the LH peak, or indirectly its effect, as displayed by the increase in progesterone to approximately 2 ng/mL at the time of the LH peak, or the progesterone concentration (P4) at ovulation (approximately 5ng/mL) (Concannon, 2000, England and Concannon, 2002, Rivière et al, 2004). A rapid canine LH test, which is commercially available in the USA, is the Canine ovulation timing test, ICG Status-LH™ (Synbiotics Corporation, San Diego, California 92127 USA ,US Vet License No 312). One package contains 6 tests. Quantitative procedures for serum progesterone determination, such as Enzyme immunoassays (EIAs) or radioimmunoassays (RIAs), are available at many universities and hospitals, but an evaluation of the assay should be done before using these for dog serum, since the results may vary between laboratories. Rapid tests are also available for P4 but may sometimes present inaccurate results, which makes them less well suited for determining the timing for AI with frozen semen. When quantitative assays are used, a single numerical value could either be presented as ng/mL (frequently used in English and American literature) or nmol/L (international SI units). The mole weight of progesterone is 3.18, and thus to convert from ng/mL to nmol/L, the ng/mL value should be multiplied with 3.18. In our laboratory we use the following rules of thumb for AI with frozen semen based on our previous experience:

Canine oocytes are mature 2-3 days after ovulation, 4-5 d after the LH-peak. The optimum days for AI with frozen semen are those closest to the fertilization time during the time when ova are still alive, but preferably before the closure of the cervix, if a transcervical intrauterine method is to be used to deposit the semen. Progesterone tends to double its value in two

days. If P4 is less than 1ng/mL or 3nmol/L: there are at lest 4 days before ovulation occurs, and a new test for P4 is necessary. If 3-4 ng/mL or 10nmol/L, ovulation will occur in 1-2 days. The mean serum progesterone concentration at ovulation is approximately 15-20 nmol/L (Concannon and others 1975). Therefore, a progesterone concentration of 15-25 nmol/L indicates insemination two and three days later, 25-35 nmol/L one and two days later, 35-60 nmol/L on the same day and the following day, and concentration >60 nmol/L on the same day only (Thomassen and others 2001). An insemination interval of 24 hours with frozen-thawed semen assures a continuous high number of live spermatozoa during the fertile time interval.

Freezing of semen

Freezing of canine sperm has employed a variety of extenders and protocols (for review see Farstad, 1996). However, glycerolated (3-6vol/vol %) TRIS-egg yolk (10-20%) (Andersen, 1975) with or without the addition of membrane protective agents, such as sodium dodecyl laureate (Orvis or Equex-STM paste), are more common and have been evaluated with respect to fertility (Farstad, 1984; Linde-Forsberg, 1998; Nöthling et al, 1997; Rota et al., 1999; Peña and Linde- Forsberg, 2000; Thomassen et al., 2001; Stornelli et al., 2002).

Artificial insemination

In the early 1970s Kjell Andersen in Norway developed an intrauterine AI technique for dogs, and with this procedure Andersen in 1972 produced the first litter of puppies born after AI with frozen semen in Scandinavia (1). Thus, this technique is often referred to as the Norwegian method. 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 intrauterine by insertion of the catheter through the cervix. This is a non- surgical method, which requires training, but once learned, allows AI in non-sedated standing bitches in a matter of a few minutes.

Artificial insemination using frozen dog semen may routinely yield 70-75% whelping rate and 6 pups per litter on average in unselected females after non-surgical intrauterine AI using cervical catheterization with a stainless steel catheter. Pregnancy rates may increase above 80 % with repeated (2-3) inseminations and increasing post-thaw motility of the semen (>60% post-thaw) (Thomassen et al, 2001). Endoscope-assisted AI yields similar whelping rates (Wilson, 1993; Wilson, 2001). The number of spermatozoa used for non-surgical inseminations is normally from 100-150 million total sperm per mL. Intrauterine, or even intrafallopian, surgical insemination with smaller volumes and number of spermatozoa may be attractive if semen samples are few and precious, given that such invasive surgical techniques are accepted by the bitch owner and the public. Even repeated (daily from first day of standing estrus until met estrus) intravaginal AI have resulted in good whelping rates when homologous prostate fluid was added to the semen after thawing, yielding larger inseminate volumes of 7-9ml (Nöthling and Volkmann, 1993). Addition of prostate fluid during freezing, however, was found to adversely affect the motility and viability of canine frozen semen (Sirivaidyapong et al., 2001). An evaluation of vaginal versus two different AI methods, favored intrauterine AI (Rota et al, 1999).

International semen exchange

Dog semen can be shipped as extended and chilled, or frozen. Whether to use fresh chilled or frozen semen will depend on a number of factors, such as shipping distance (transcontinental transport usually necessitates the use of frozen semen), sanitary regulations such as testing the semen donor for specific diseases prior to import, and whether the shipped semen is intended for artificial insemination (AI) of one or of several bitches, as well as any additional rules and regulations of the country in question (for review see Linde- Forsberg et al, 2001). These regulations may change relatively rapidly. Lately, Australia has changed its importation rules for dry-shippers and they now accept cleansing with Vircon™ or radiation as opposed to the previous requirement, which included that the LN_2 tank containing the canine semen had to be new and unused prior to transport to Australia. One factor to consider when inseminating is that some semen banks in the USA freeze semen with relatively low density (million spermatozoa/mL), since the use of surgical AI is more common in this country. When the semen is to be inseminated using either the intravaginal or the transcervical intrauterine route, a larger number of sperm may have to be used. Furthermore, it is imperative to adhere strictly to the thawing instructions provided by the laboratory that froze the semen. In many countries, such as Norway, keeping one vial for later identification of the sire is mandatory, so one should allow for this extra vial when freezing the semen.

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