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Artificial insemination with frozen semen in dogs: A retrospective study of 10 years using a non-surgical approach

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Abstract

From 1994 to 2003, a total of 526 bitches of 99 different breeds were artificially inseminated in 685 estrus cycles with domestic ($n = 353$) or imported ($n = 332$) frozen-thawed semen from 368 males. The overall whelping rate was 73.1% and mean (±S.E.M.) litter size 5.7 ± 0.1 pups. The whelping rate was higher after intrauterine insemination (75.0%; $n = 665$) than after intravaginal insemination (10.0%, $n = 20$; $P < 0.05$). Insemination at the optimal time resulted in a higher whelping rate (78%, $n = 559$; $P < 0.01$) and larger litter size (5.8 ± 0.2; $P < 0.05$) than inseminations performed late or too late (55.7% and 4.5 ± 0.5, $n = 61$). Two inseminations ($n = 384$) yielded a higher whelping rate ($P < 0.05$) and mean litter size ($P < 0.01$) than one insemination ($n = 241$), 78.1% and 6.0 ± 0.2 and 70.5% and 5.1 ± 0.2, respectively. For inseminations performed at the optimal time, however, the whelping rate was not significantly different for bitches inseminated twice (79.3%, $n = 358$) versus once (76.8%, $n = 168$), but the litter size was larger (6.0 ± 0.2 and 5.3 ± 0.3). Semen classified as of poor quality (progressive motility <50% or percentage abnormal sperm >20%) resulted in a lower whelping rate ($P < 0.01$) than semen classified as of good quality (progressive motility ≥50% and percentage abnormal sperm ≤20%), 61 and 77%, respectively. Small breeds ($n = 50$) had a smaller litter size (3.9 ± 0.3; $P < 0.01$) than larger breeds (medium [5.7 ± 0.3, $n = 94$], large [5.9 ± 0.2, $n = 295$] or giant breeds [6.1 ± 0.5, $n = 62$] [$P < 0.01$]). Bitches older than 6 years had a lower whelping rate (68.2%) than younger ones (77.0%; $P < 0.05$). The duration of pregnancy was longer ($P < 0.01$) for bitches with a litter size of <3 pups (61.7 ± 0. 4 days, $n = 30$) than for bitches with larger litters (60.5 ± 0.1 days, $n = 177$). These results show the potential of transcervical intrauterine insemination for routine artificial insemination in dogs. The results with frozen semen inseminations were optimised by inseminating bitches ≤6 years old 2 and 3 days after ovulation with semen of good quality from males ≤8 years old.

Keywords: Dog; Artificial insemination; Frozen semen; Transcervical insemination

1. Introduction

More than 30 years have elapsed since the first transcervical insemination technique for dogs was developed in the early 1970s [1,2]. This technique is often referred to as the Norwegian or Scandinavian method and has proven to be very successful in canine breeding [2–9]. The technique enables semen to be deposited non-surgically into the uterus in standing, usually non-sedated bitches. Thus, frozen-thawed semen, in which both the number of fertile sperm and the longevity of the sperm are reduced (compared to

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freshly ejaculated semen), may be deposited closer to the site of fertilisation. During the last 10 years, we have inseminated 685 bitches with frozen semen from a variety of sources; to our knowledge, this represents the largest database of AI with frozen semen so far published. The aims of this retrospective study were to review the results of AI with frozen-thawed semen in dogs at our clinic over a 10-year interval (1994–2003), and to discuss factors that may influence the success rate with frozen-thawed canine semen.

2. Material and methods

2.1. Animals

A total of 526 bitches were inseminated in 685 estrus cycles: 476 in one, 41 in two and nine bitches in three estrus cycles each. These cases are referred to as 685 bitches, and they were inseminated with frozen-thawed semen from 368 males of 99 different breeds at our clinic from 1994 to 2003. The inseminations were performed by experienced personnel (>5 years practice with the technique), and all inseminations with frozen-thawed semen performed during this period are included in the material. The population of bitches was unselected, since the owners wanted frozen semen to be used in accordance with their breeding plans. A total of 353 bitches were inseminated with semen frozen at our clinic, and 332 bitches were inseminated with imported semen from a variety of sources. The results of the inseminations were obtained by personal contacts with the bitch owners. The animals were grouped according to the weight of the female into small, medium, large and giant breeds. Small breeds weighed up to 12 kg, medium breeds ranged from 13 to 25 kg, large breeds from 26 to 40 and giant breeds weighed >40 kg.

2.2. Semen evaluation and processing

The domestic semen was collected by digital manipulation in the presence of a bitch in estrus. Only the sperm-rich fraction was used for evaluation and processing. Motility was evaluated subjectively at 37 °C under a light microscope and an aliquot of the semen sample was evaluated for morphology. The evaluation of morphology was done on fixed semen samples using Hayems fixative. Several fields (10–15) in the microscope were viewed, estimating the percentage of abnormal spermatozoa. Sperm with primary defects [10] and proximal droplets were counted as abnormal. Semen with a motility of ≥90% and a proportion of abnormal sperm of ≤20% was regarded suitable for freezing. Semen with motility ≥75 and ≤30% abnormal sperm was frozen, but if post-thaw motility was <40%, the owner of the dog was strongly encouraged to allow us to dispose of the semen. Semen of poorer quality (motility <75% or abnormal sperm >30%) was not frozen. The sperm-rich fraction was diluted in a prewarmed (35–37 °C) Tris–fructose–citric acid extender containing 8% (v/v) glycerol and 20% (v/v) egg yolk to a final concentration of approximately 1.0×10^8 spermatozoa/mL [1]. The diluted semen was wrapped in paper to ensure slow cooling and placed in a refrigerated room at 5 °C. After an equilibration time of 2–3 h, the semen was filled in 0.5 mL medium PVC straws (Minitüb, Tiefenbach, Germany), frozen in N_2 vapour [1] and stored in liquid nitrogen containers. The volume of each insemination dose was 2–2.5 mL with a total of approximately 2.0×10^8 spermatozoa. Imported semen was received from various private or university clinics, or from commercial agencies whose cryopreservation procedures were proprietary. The insemination dose, therefore, may differ from the ones using domestic semen.

2.3. Semen thawing and post-thaw evaluation

The domestic semen was thawed in a 70 °C water bath for 8 s, and the imported semen according to recommendations from the veterinarian or agency that had processed the semen; either in water bath at 70 °C for 8 s, or at 37 °C for at least 30 s. Before insemination semen was evaluated subjectively for sperm motility and morphology (at 37 °C using a light microscope). A small drop of semen was placed on a slide and evaluated for motility. Morphology was assessed from the same sample either at the borders of the coverslip or after the motility had ceased. Progressive motility ≥50% was classified as ''good'', and <50% as ''poor'' semen quality. If the percentage of morphologically abnormal spermatozoa was >20%, semen quality was classified as ''poor'', regardless of semen motility [11]. If the semen was of poor quality, the insemination dose may have been increased, for example, by adding an extra straw.

2.4. Timing of the insemination

For all bitches, the timing of the AI was based on a clinical evaluation, including a vaginal smear and serum progesterone concentration. Cells for the vaginal smears were collected with a plastic catheter (Bovivet®, Kruse, Marslev) inserted deeply into the vagina. Cells that