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Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen

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ABSTRACT

The aim of this prospective cohort study was to utilize multivariable statistical methods to identify factors that significantly affected whelping rate, litter size and gestation length in a large population of bitches of many different breeds, presented for routine breeding management. In addition, we aimed to determine the incidence of dystocia and the proportion of bitches undergoing a caesarean section procedure. A total of 1146 individual bitches representing 84 different breeds contributed 1203 inseminations over the 9 year (2007–2015) study period. Bitches were inseminated with either frozen-thawed (n = 645), fresh (n = 543) or chilled (n = 15) semen from 1371 different males. The mean (SD) whelping rate was 74± 4% and the mean litter size was 5.8 ± 3.1 pups per litter for all bitches in the study. The whelping rate was significantly lower in bitches inseminated with frozen-thawed semen compared with bitches inseminated with fresh semen (71% vs 80% respectively; P < 0.001). Semen that was classified as having poor motility (<30% progressive) resulted in a significantly lower whelping rate (37%) than semen classified as good (30–65% progressive; whelping rate = 67%) or excellent (>65% progressive; whelping rate = 79%). There was a linear decline in whelping rate with advancing age. Greyhounds and Labradors demonstrated a significantly higher whelping rate (88% and 94% respectively) compared with all other breeds (71.3%, P < 0.001). Bitches inseminated with frozen-thawed semen had significantly smaller litter sizes than bitches inseminated with fresh semen (5.4 ± 3.1 vs 6.2 ± 3.0 pups per litter respectively; P = 0.02). Smaller breeds had significantly smaller litters (4.4 ± 2.1 pups) than medium (5.2 ± 2.9 pups), large (5.9 ± 2.9 pups) or giant (6.7 ± 3.8 pups) breeds. For each advancing year of age, litter size decreased by 0.13 pups per litter. The mean (SD) gestation length from LH0 was 65 ± 1.9 d. Greyhounds had a significantly longer pregnancy duration (68.0 ± 1.5 d) than other breeds. For each additional year of bitch age, gestation length increased by 0.11 days (P < 0.01), and for each additional pup per litter, gestation length was reduced by 0.08 days (P < 0.05). Of the 890 bitches for which whelping outcomes were recorded; 409 (46%) whelped normally without assistance, 249 (28%) had an elective C-section, 205 (23%) underwent an emergency C-section and 27 (3%) were medically managed or required veterinary assistance for dystocia. Brachycephalic breeds were 11.3 (95CI = 9.3 −17.9; P < 0.001) times more likely to have a C-section compared to all other breeds. Bitches with litter sizes of one or two pups had a C-section rate of 83%, whereas bitches with litter sizes of three or more pups had a C-section rate of 43% (P < 0.001). This study provides important clinical information to optimise whelping rates, litter size and the prediction of whelping in certain breeds for clinicians working in canine reproduction.

1. Introduction

Many factors can potentially influence reproductive success in bitches after artificial insemination (AI). The two most important measures of reproductive performance in the bitch are whelping rate and litter size. Many factors have been suggested to influence

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both of these parameters after AI with fresh, chilled or frozen-thawed semen. These include semen type, semen quality, method of semen cryopreservation, site of semen deposition, type of AI, number of AIs per cycle, AI operator, bitch age at time of AI, age of the dog at the time of AI or semen freezing, breed, bitch body weight, parity of the bitch at the time of AI, season and the timing of AI in relation to the LH surge (LH0) [1–5]. However, most studies describing the reproductive performance of canids have evaluated factors which vary at a single level (univariate analysis). Univariate analysis involves examining the effect of a single explanatory variable, for example bitch age, on the outcome of interest assuming conditions of independence [6]. Univariate analysis does not take into account that some variables influencing reproductive outcomes are related to each other, for example bitch age and parity, nor does it determine which exposure variables are the most significant predictors of reproductive success. Given that many independent variables can contribute to reproductive success, multivariable analyses are required to determine the relative contribution of each on the outcome of interest [7].

Several large-scale studies have investigated factors affecting reproductive patterns and performance in the bitch, analyzed retrospectively, using data acquired from kennel club or breed registries [4,5,8]. Such databases rely on the timely and accurate recording of information by private breeders and as such are limited by inaccurate recording and missing data [4]. Previous studies of this kind have only examined one measure of reproductive performance such as litter size [5], or have only involved a single breed [4].

The objective of this large-scale prospective cohort study was to utilize appropriate statistical methods to examine the effects of multiple exposure variables and their interrelationships, in order to determine which factors significantly affect whelping rate, litter size, gestation length, and C-section rate in the bitch after AI.

2. Materials and methods

2.1. Animals

A total of 1146 individual bitches representing 84 different breeds contributed 1203 inseminations over the 9-year (2007–2015) study period. Bitches were inseminated with either frozen-thawed (n = 645) or fresh (n = 558) semen from 1094 different males. Frozen semen used for AI in this study was either frozen by GlenBred (n = 239) during the period from 1988 to 2015, or frozen by technicians and veterinarians from a large number of organizations either within New Zealand or internationally, and stored at GlenBred from 1993 to 2015 (n = 406). Chilled semen originated from within New Zealand, or was imported from Australia (n = 10). All inseminations were performed by one of two experienced operators in a private referral practice ("GlenBred"). The mean (SD) age of bitches at the time of AI was 3.9 ± 1.7 years with a range of 8 months–10 years. The mean parity of bitches at the time of AI was 0.7 ± 0.2 litters with a range of 0–5 litters. All bitches presented to GlenBred for routine breeding management during the study period were included in the analysis, including those referred with a history of infertility. The owners of each bitch selected the semen type, sire and the age at which the bitch was inseminated as part of their breeding program. A significant number of the bitches were referrals from general practitioners with a history of failing to conceive in the past, or having a history of uterine disease (pyometra, cystic endometrial hyperplasia or endometritis). Classification and identification of all of these 'infertility cases' became difficult, so all bitches were included in the analysis. Bitches were allocated into groups according to body weight as classified by the New Zealand kennel Club; small (<12 kg), medium (13–25 kg), large (26–40 kg) and giant (>40 kg).

2.2. Semen processing and handling

Regardless of the final use (frozen, chilled shipment or fresh semen for AI) all semen samples collected at GlenBred were obtained using a manual open hand technique with the presence of a bitch in standing heat (teaser). Only the sperm rich fraction was used and evaluated for motility, morphology and concentration prior to dilution in canine semen extender.

2.2.1. Preparation of fresh ejaculated semen for AI

After semen samples were taken for microscopic evaluation, the sperm rich fraction was immediately and slowly diluted with pre-warmed (37 °C) extender (Uppsala Equex System EYT/1; [9]) to a final volume of approximately 2–4 ml depending on the size and body weight of the bitch for intrauterine AI. If a vaginal AI was performed, the sperm rich fraction was diluted with pre-warmed extender (Uppsala Equex System EYT/1; [9]) to a volume of 5–8 ml depending on the concentration of the ejaculate (to achieve a final concentration of 100–400 × 10^6/ml), and the size and body weight of the bitch to be inseminated.

2.2.2. Semen frozen at GlenBred

Ejaculates with a motility ≥85% and a proportion of abnormal sperm ≤20% were considered suitable for freezing. Ejaculates with sperm motility ≤70% and low numbers of morphologically normal sperm (≥30% morphologically abnormal sperm) were frozen only in special circumstances. Owners of dogs with a post thaw motility ≤40% were strongly encouraged to not keep or use the semen. In these cases, the semen was destroyed and a repeat collection would often be carried out at a later date. Canine semen frozen at GlenBred prior to 2007 was processed by Dr Marion Wilson [10]. Briefly, all ejaculates were extended and frozen using a Tris-citric acid-fructose extender containing 20% egg yolk (v/v) and 8% glycerol (v/v) to a concentration of 100 × 10^6 spermatozoa/ml. A one-step freezing method was used whereby the extended semen was cooled and equilibrated for 2 h at 5 °C prior to loading into pre-cooled 0.5 ml straws which were then placed on a pre-cooled rack and suspended 4 cm above liquid nitrogen for 10 min. The straws were then plunged into liquid nitrogen and transferred into a long-term liquid nitrogen storage tank. As the minimum recommended insemination dose for frozen-thawed canine semen is 100 × 10^6 motile spermatozoa [11], 4–5 straws were used per AI dose depending on post thaw motility and the proportion of morphologically normal spermatozoa. Straws were thawed by immersion in a water bath at 37 °C for 30 s. No extender was added after thawing. Therefore, the final volume of each insemination dose of semen frozen at GlenBred prior to 2007 was approximately 2 ml containing a total of ≥200 × 10^6 spermatozoa.

All canine semen frozen from 2007 onwards at GlenBred was performed by a single operator (F. Hollinshead) using the following protocol developed by Rota et al. [12]. The sperm rich fraction was slowly diluted 1:1 (v/v) with a pre-warmed (35–37 °C) Tris-citric acid-fructose extender containing egg yolk (20% v/v; Uppsala Equex-2 system; [9]) and then centrifuged at 650 × g for 10 min. The supernatant was removed and a Tris-citric acid-fructose extender containing 20% egg yolk (v/v) and 3% glycerol (v/v; Uppsala UE-2/1 [9]) was added to the remaining sperm pellet at room temperature (21 °C) to make a concentration of 400 × 10^6 spermatozoa/ml. The diluted semen was then placed at 5 °C and equilibrated for 2 h. After this equilibration time an equal volume of pre-cooled (to 5 °C) Tris-citric acid-fructose extender containing 20% egg yolk (v/v), 7% glycerol (v/v) and 1% equex paste (v/v; STM Nova Chemical Sales Inc., Scituate, MA, US; Uppsala UE-2/2; [9])