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 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.

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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
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 (univariable analysis). Univariable analysis involves examining the effect of a single explanatory variable, for example, bitch age, on the outcome of interest assuming the analysis involves examining the effect of a single explanatory variable, for example, bitch age, on the outcome of interest. 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 success, and have determined 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].

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 method of semen collection, chilled semen 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 extenders.

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 not to 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 precooled 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 was 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])
was added at a dilution rate of 1:1 (v/v) making the final concentration 200 × 10^6 spermatozoa/ml. The chilled semen was equilibrated for a further 15 min at 5 °C before loading into 0.5 ml PVC straws (Minitüb®, Germany) which were placed on a pre-cooled stainless steel rack 4 cm over the surface of liquid nitrogen in a specialized steel canister (described by Wilson [10]) for 10 min before plunging into liquid nitrogen. Afterwards, all straws were 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], two straws were used per AI dose if the post thaw motility was >50%. Straws were thawed by immersion in a water bath at 37 °C for 30 s. A pre-warmed Tris-citric acid-fructose ‘thawing extender’ without any egg yolk or glycerol (Uppsala: UE-2 Thaw medium; [9]) was slowly added to the thawed semen at a ratio of 1:1 to 1:2 depending on the size of the bitch. Therefore, the final volume of each insemination dose was approximately 2–3 ml containing a total of 200 × 10^6 spermatozoa and greater than 100 × 10^6 motile spermatozoa.

2.2.3. Imported frozen semen

Imported frozen semen came from a number of different sources and was frozen using many different types of extenders and cryopreservation techniques which were mostly commercial and proprietary. All imported frozen semen was thawed according to the freezing center’s instructions when provided (usually in a water bath at 70 °C for 8 s or at 37 °C for 30 s). The total volume of the insemination dose with imported semen varied considerably, from 0.5 to 4 ml, depending on the concentration at which the semen was frozen, the semen quality and whether thaw media was provided.

2.3. Semen evaluation

All semen samples, regardless of origin (domestic or imported) or type (fresh, frozen-thawed or chilled) were evaluated immediately prior to AI. Assessments of percentage of motile spermatozoa (to the nearest 5%) and FPM rating (FPM: 0 ┼ 5 scale; 0 = no movement, 5 = rapid forward progression; modified from Howard [13]) were subjectively made by examining several different movement fields under light microscopy (X200 and X400) at 37 °C. For the purposes of statistical analysis, progressive motility was further classified as ≥65% (‘excellent’), 30–65% (‘good’) and <30% (‘poor’). Morphology and cytological evaluations were performed on all semen samples collected at GlenBred used for either immediate insemination or freezing but not on imported frozen semen due to limitations in freezing but not on imported frozen semen due to limitations in the freezing center’s instructions when provided (usually in a water bath at 70 °C for 8 s or at 37 °C for 30 s). A pre-warmed Tris-citric acid-fructose ‘thawing extender’ without any egg yolk or glycerol (Uppsala: UE-2 Thaw medium; [9]) was slowly added to the thawed semen at a ratio of 1:1 to 1:2 depending on the size of the bitch. Therefore, the final volume of each insemination dose was approximately 2–3 ml containing a total of 200 × 10^6 spermatozoa and greater than 100 × 10^6 motile spermatozoa.

2.4. Timing of insemination

The timing of insemination for each bitch was based on estrous behavior, vulval changes, vaginal cytology, and serum progesterone concentrations [17]. Starting from Day 5–7 of proestrus (based on the onset of a bloody vulval discharge, vulval swelling and attractiveness to other dogs), bitches were presented initially every 2nd – 4th day depending on the stage of the estrous cycle and individual progression through the estrous cycle, then every 2 days after determination of the LH surge (LH 0), and every 1–2 days after ovulation until the day of AI, for the following tests: vaginal cytology, vaginoscopy and collection of blood for determination of serum progesterone concentration.

Vaginal smears were collected from the cranial vagina using a modified Perspex tube (vaginoscope), and cotton tipped swab attached to an extension handle. Smears were made by rolling the swab onto a glass microscope slide. Slides were air dried and stained using a simple modified Wrights-Giemsa stain (Diff-Quik: New Zealand Veterinary Pathology, Hamilton, NZ). Each vaginal smear was evaluated under light microscopy (X100–X200 magnification) for the type of vaginal epithelial cells present in order to determine the stage of the estrus cycle [18]. Particular attention was paid to the presence of neutrophils only when 100% late superficial or cornified cells (i.e during cytological estrus) were present as an indication of a potential uterine infection or endometritis [19].

Vaginoscopy was performed using a modified Perspex tube and light source. Visualization of changes in the vaginal mucosa (folds and color) facilitated determination of ovulation timing and the optimal day(s) for insemination, as described by England and Concannon [17].

Blood was collected for progesterone analysis by jugular or cephalic venipuncture into a plain glass or plastic tube (with no additives). All samples were submitted to the same commercial laboratory for determination of progesterone concentration (ng/ml) by electrochemiluminescence (ECL) using a Roche Modular E170 analyzer (Roche Diagnostics New Zealand, Auckland) with an intra- and inter-assay coefficient of variation at 2 ng/ml of 1.2% and 8.2% respectively.

Time of AI was based on determination of both LH 0 and ovulation, primarily using progesterone concentrations (>2 ng/ml and/or double its previous value and > 5–10 ng/ml for LH 0 and ovulation respectively) supplemented with vaginoscopy (maximal crenulation: angular and shrunken vaginal folds, pale/white vaginal mucous membranes and large vaginal lumen). Importantly, determination of the day of the estimated LH surge was based on a significant rise in progesterone concentration, usually greater than 2 ng/ml. Vaginal cytology was always performed at the time of AI to confirm that the bitch was in cytological estrus (100% superficial/cornified cells) and that there were no neutrophils present indicating potential endometritis [19]. With late insemination (>LH6), clumps of vaginal epithelial cells and the appearance of round nucleated cells indicating the start of ‘vaginal epithelial dumping’ and imminent onset of diestrus day 1, were often seen and recorded. Owners of bitches where the progesterone concentration did not rise above 10 ng/ml or only slowly increased over several days prior to AI were advised not to proceed with the insemination, especially if valuable frozen semen was to be used. Frozen-thawed

be the gold standard for determining sperm concentrations in domestic species [16]. Total numbers of sperm per AI dose of semen frozen outside of GlenBred was not determined, as accurate measurement of the inseminate volume after thawing was not achievable. Furthermore, it was often difficult to achieve a dilution rate with samples after AI that would allow a minimum of 200 spermatozoa to be counted in the chamber.
semen was inseminated 5 and/or 6 days after LH 0 (or 3 and/or 4 days after ovulation) and inseminations with fresh or chilled semen were mostly performed on the 3rd, 4th or 5th day after LH 0 (2nd, 3rd and 4th day after ovulation).

The number of inseminations performed per estrous cycle with frozen semen depended on a number of factors. Due to the costs involved in collecting, purchasing and importing frozen canine semen to New Zealand, especially from the Northern Hemisphere, it is common to use just one dose of frozen semen per estrous cycle. From 2007 to 2012 often a single dose was split so that two AI's were performed on Day 5 and 6 after LH 0. Analysis of over 500 inseminations during this time period revealed that there was no benefit in carrying out two inseminations with a split dose, compared to one insemination when only one dose of frozen semen was used per estrous cycle [20], so from 2012 onwards, in the majority of cases, only a single AI was performed when only a single dose of frozen semen was available.

The season in which each AI was performed was recorded as either Winter (June–August), Spring (September–November), Summer (December–February) and Autumn (March–May).

2.5. Artificial insemination technique

Two experienced veterinarians performed all inseminations during the study period. Frozen-thawed semen was deposited into the uterus using either a surgical (celiotomy) approach (n = 36) or an endoscopic transcervical insemination (TCI, n = 609) technique and fresh or chilled semen was deposited into either the vagina (n = 57) or the uterus using TCI (n = 494) or surgery (n = 7).

2.5.1. Vaginal AI

All vaginal inseminations were performed using a flexible, silicone pipette with an in-built stilette and inflatable balloon (MAVIC catheter, Minitube of America, MOFA®, Verona, USA). The size of the AI catheter used depended on the size and body weight of the bitch (MAVIC mini®: 18 FR and 120 mm length: small breeds, MAVIC 250®- 18FR and 250 mm length: medium sized breeds or MAVIC 400®- 36FR and 400 mm length: large and giant breeds). After semen collection and assessment all ejaculates were diluted as described above. The diluted semen was first deposited into the cranial vagina and was then followed by slow infusion of 5–25 ml (depending on the size of the bitch) of warm ‘flush’ media (Upsalla Equex System EYT/1 [9]) over a 15 min period to mimic what occurs during natural mating (flushing effect of prostatic fluid). ‘Feathering’ (stimulation of the vulval/clitoral area) was performed during infusion of both the diluted semen and flush extended to stimulate vaginal contractions and facilitate sperm transport [21] to mimic what occurs during natural mating (stimulation of vaginal and uterine contractions during the ‘tie’).

2.5.2. Intrauterine AI

Two techniques were used for the deposition of semen into the uterus. The majority of cases (n = 1103) were inseminated using the TCI technique. It was not possible to catheterize the os cervix in only 7 bitches during the study period, and these bitches had to then be surgically inseminated. Interestingly, the Golden Retriever (n = 4) and the Bernese Mountain Dog (n = 3) were the predominant breeds represented in this group of bitches. A small number of clients elected for surgical insemination to be performed (n = 36). These were predominantly owners of greyhounds using frozen semen sold to them with the proviso that a surgical AI was performed or there would be no semen replacement if the bitch was not pregnant.

2.5.2.1. Endoscopic transcervical insemination (TCI). All TCIs were performed using a modified 43 cm ureterorenoscope (Karl Storz®, Tuttlingen, Germany) equipped with air inflation (30020 rectal insufflation bulb, WelchAllyn®), a xenon cold light source (Karl Storz®, Tuttlingen, Germany) and video camera (Karl Storz®, Tuttlingen, Germany), with images displayed on a flat screen monitor (Sony®, Tokyo, Japan) for client and operator viewing as described previously [22]. Briefly, all bitches were placed in a standing position on a hydraulic table, and as they were in standing heat, sedation was rarely used (n = 1 over the study period). After visualization of the cervix, a CH-4 or CH-5 transcervical catheter (Minitube of America, MOFA®, Verona, USA) was inserted through the cervix and into the uterine body, at the base of the uterine horns. Semen was slowly infused into the uterus over a 2–4 min period (depending on inseminate volume). Backflow of semen out of the cervix was recorded but not scored. When backflow was observed, the insemination was paused and the catheter repositioned. The use of large volume inseminates (>8–10 ml) infused slowly over time, was not performed in this study. A small amount of backflow from the cervical os at the end of the insemination was noted in >80% of cases and had no effect on whelping rate. The procedure was completed in the majority of bitches in under 5–10 min.

2.5.2.2. Surgical insemination via celiotomy. All bitches that were surgically inseminated underwent a general anaesthetic, midline celiotomy, and exteriorization of the uterus to facilitate deposition of semen via a 22-ga intravaginal catheter inserted into the uterine lumen. The anesthetic protocol and surgical and insemination technique used were as described by Burgess [3]. Deposition of semen was performed at the base of the uterine horns. Manipulation and massage of the uterine horns was not performed as this was considered to be detrimental, potentially damaging to the endometrium and also unnecessary as it has been well documented in the bitch and other species that sperm distribution along both uterine horns is independent of the site of semen deposition [22–24].

2.6. Pregnancy diagnosis and whelping data

In some bitches (n = 369) confirmation of pregnancy, number of fetuses, presence of resorptions, gestational aging and uterine pathology was determined at GlenBred by abdominal ultrasonography with bitches in a standing position using B-mode and a curvilinear 3–9 MHz probe (My Lab 30 VetGold®, Genova, Italy) 28–35 days after LH 0. For the remaining bitches in the study, that were located at a considerable distance from our clinic, pregnancy outcome was determined from whelping information. For the purposes of analyses, only the whelping rate was used as a measure of reproductive success.

The outcomes of each insemination were obtained from a questionnaire sent out to every client two months after the insemination had taken place. If a questionnaire was not returned within one month, a follow up phone call and/or e-mail was carried-out, in order to obtain the whelping information. The compliance rate was high, with complete whelping information retrieved from 96% of clients. Information obtained from the questionnaire included the date the bitch whelped, the number of bitches, presence of resorptions, gestational aging and uterine pathology was determined at GlenBred by abdominal ultrasonography with bitches in a standing position using B-mode and a curvilinear 3–9 MHz probe (My Lab 30 VetGold®, Genova, Italy) 28–35 days after LH 0. For the remaining bitches in the study, that were located at a considerable distance from our clinic, pregnancy outcome was determined from whelping information. For the purposes of analyses, only the whelping rate was used as a measure of reproductive success.

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were seen by their local referring veterinarian. In these cases, where possible, veterinary records were obtained from the referring veterinarian and the nature of the intervention was classified, coded for analysis purposes and recorded in our database. Determination of the timing of an elective C-section performed by referring veterinarians, was primarily based on the interval from LH0 to 64–65 days.

2.7. Statistical analysis

The three main measures of reproductive performance (dependent variables) of interest were whelping rate, litter size and gestation length. The whelping rate was defined as the total number of bitches producing at least one pup (alive or dead) divided by the total number of bitches inseminated x 100. Litter size was defined as the total number of pups born in each litter (alive or dead). Gestation length was defined as the number of days from LH0 until the date that the bitch whelped. Gestation length was measured from LH0 rather than other biological events, such as ovulation or the first day of cytological diestrus, as this has been shown to be the most reliable physiological event on which to determine gestation length [25]. Bitches that underwent an elective C-section were not included in the analysis of gestation length.

Whelping outcomes were classified as: a) whelped normally, with no intervention, b) an elective C-section, c) an emergency C-section, or d) required medical treatment and/or assistance during whelping. Further classification of dystocia into maternal and fetal causes was not possible from the information provided. The C-section rate was defined as the number of bitches that had an emergency or elective C-section, as a proportion of the total number of bitches whelping x 100.

The independent variables included in each multivariable model of whelping rate, litter size and gestation length were: semen type (fresh, chilled and frozen-thawed), frozen semen source (imported, frozen at Glenbred), sperm motility, type of AI (TCI, surgical, vaginal), number of AIs per cycle, AI operator, bitch age at time of AI, bodyweight representation was as follows; small (n = 132), medium (n = 167), large (n = 671) and giant (n = 233). The mean age of bitches in the study at the time of insemination was 3.9 ± 1.7 years.

The mean P4 concentration at LH0 was 2.7 ± 0.6 ng/ml and at the time of ovulation, which has been confirmed to occur between LH2 and LH3 [26], it was 4.8 ± 0.9 and 7.2 ± 1.3 respectively.

The mean whelping rate was 74± 4% for all bitches in the study. The results of the multivariable analysis revealed that the only significant predictors of whelping rate were semen type, sperm motility, semen source, age of the bitch and breed (Table 1). The whelping rate was significantly lower in bitches inseminated with frozen semen compared with bitches inseminated with fresh semen (71% (95CI = 65–75) vs 80% (95CI = 77–84) respectively; P < 0.001). Semen that was classified as having poor motility (<30% resulted in a lower whelping rate (37% (95CI = 23–52) than semen classified as good (30–65%; whelping rate = 67% (95CI = 62–72)) or excellent (>65%; whelping rate = 79% (95CI = 76–82)). The whelping rate was significantly lower for frozen-thawed semen imported into GlenBred compared with semen processed at GlenBred (whelping rate of 64% vs 77%)

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Coefficient (SE)</th>
<th>OR (95% CI)</th>
<th>P -Value</th>
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<tbody>
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<tr>
<td></td>
<td>good</td>
<td>1.71(0.34)</td>
<td>3.72 (1.94–7.36)</td>
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<td></td>
<td>excellent</td>
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<td>0.66 (0.49–0.91)</td>
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<td>Labrador</td>
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<td>6.57 (3.42–12.6)</td>
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a Interpretation: Bitches inseminated with semen of ‘good’ quality were 3.72 times more likely to whelp, compared with bitches inseminated with ‘poor’ quality semen.

b Results for all 84 different breeds are not shown.
respectively; $P < 0.001$). There was a linear decline in whelping rate with advancing age. For each additional year of age, the odds of whelping declined by 0.93 (95CI = 0.86–0.99). Greyhounds and Labradors demonstrated a significantly higher whelping rate (88.4% and 94% respectively) compared with all other breeds (71.3%, $P < 0.001$). The following variables were not significant predictors of whelping rate in the multivariable model: type of AI, bitch body weight, bitch parity, season, and operator and the number of AI’s per cycle.

The mean litter size for all bitches in the study was 5.8 ± 3.1 pups per litter. The results of the multivariable analysis revealed that the only significant predictors of litter size were semen type, body weight, and age of the bitch (Table 2). Bitches inseminated with frozen semen had significantly smaller litter sizes than bitches inseminated with fresh semen (5.4 ± 3.1 vs 6.2 ± 3.0 pups per litter for bitches inseminated with frozen and fresh semen 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 following variables were not significant predictors of litter size in the multivariable model: type of AI, breed, parity, season, sperm motility, semen source, operator and the number of AI’s per cycle.

The mean gestation length from LH0 was 65 ± 1.9 d. The results of the multivariable analysis revealed that the only significant predictors of gestation length were breed, age of the bitch and litter size (Table 3). 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 ($P < 0.01$). The following variables were not significant predictors of gestation length in the multivariable model: type of AI, body weight, parity, season, semen type, sperm motility, semen source, operator and the number of AI’s per cycle.

Of the 890 bitches for which whelping outcomes were obtained; 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. The total number of bitches classified as having dystocia was 232 (26%). The results of the multivariable analysis revealed that the only significant predictors of C-section rate were breed of the bitch and litter size. Of bitches classified as brachycephalic, 87% underwent a C-section compared with 38% in all other breeds ($P < 0.001$). Brachycephalic breeds were 11.3 (95CI = 9.3–17.9; $P < 0.001$) times more likely to have a C-section than all other breeds. Bitches with litter sizes of 1 or 2 pups had a C-section rate of 83%, whereas bitches with litter sizes of 3 or more pups had a C-section rate of 43% ($P < 0.001$). The following variables were not significant predictors of C-section rate in the multivariable model: body weight, parity, season and gestation length.

### Table 2
Significant fixed-effects variables retained in a multivariable linear regression model of litter size following 1203 inseminations with fresh and frozen-thawed semen in bitches of various breeds.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Coefficient (SE)</th>
<th>t value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(intercept)</td>
<td>Ref</td>
<td>7.49 (0.37)</td>
<td>20.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Semen type</td>
<td>Fresh</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen-thawed</td>
<td>−0.85 (0.22)</td>
<td>−3.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age of bitch</td>
<td>Year</td>
<td>−0.13(0.06)</td>
<td>−1.95</td>
<td>0.05</td>
</tr>
<tr>
<td>Body weight</td>
<td>Giant</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>−2.13(0.43)</td>
<td>−5.47</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>−1.36(0.40)</td>
<td>−3.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>−0.54(0.31)</td>
<td>−1.75</td>
<td>0.08</td>
</tr>
</tbody>
</table>

4. Discussion

To the authors’ knowledge, this is the largest prospective cohort study to investigate factors that influence reproductive outcomes in bitches that have undergone AI with fresh or frozen-thawed semen. More importantly, this is the first clinical study of its size to use appropriate statistical techniques (multivariable analysis) to identify the most significant predictors of reproductive performance in a large population of bitches of mixed breeds and ages, and to include multiple exposure factors.

Whilst there have been larger scale canine studies published that included the analysis of reproductive data from thousands of bitches, the data for these large retrospective studies was obtained from kennel club registries in Sweden [4] and Norway [5]. An important limitation of these large-scale retrospective studies is the source of the information. These kennel club databases rely on private breeders accurately registering the details of each breeding and any subsequent litter born, usually several weeks, or even months after the pups have been born or a failed mating is diagnosed. This can result in inaccurate information as well as missing data. In the study by Gavrilovic [4], due to incomplete data in the Swedish Kennel Club Registry, it was not possible to obtain information on whelping rate, litter size at birth, gestation length or dystocia rate. Therefore, whilst these studies can provide large numbers of bitches for analyses, they are limited in the reproductive outcomes that can be measured, compared to studies based on clinical information.

Retrospective canine reproductive performance studies based on clinical data have been published. However, most of these studies have been limited to the investigation of using only frozen-thawed semen [1,2,7,28] and/or performing intrauterine insemination (IUAI) using a single technique (surgical IUAI [3], IUAI with the Norwegian Elk catheter [1], or TCI [27]). In addition, most of these studies were analyzed using univariate analysis of the data [1,2,7,28]. Mason and Rous [28] is the only study to compare the two IUAI techniques, TCI and surgical AI. However, the numbers in their study were very low (TCI: n = 78; Surgical AI, n = 40) and the method of analysis limited.

An important point of difference between our study compared to all past studies on canine reproductive performance is that the collection of our data was prospective rather than retrospective. Prospective cohort studies provide the opportunity for more detailed data collection and attention to recording the details of interest [7]. In our study, this facilitated the compilation of not only more accurate and detailed information, but we had very little missing data as a result of client communication immediately after whelping. Additionally, our database included all types of canine semen (frozen-thawed, chilled and fresh) and both vaginal and intruterine (TCI and surgical) insemination.

The overall whelping rate after AI in our study of 74% was similar to the whelping rate reported in previous clinical studies involving large numbers of bitches (range 71–73% [1–3]). In contrast to the study by Mason and Rous [28], referral infertility cases were not

### Table 3
Significant fixed-effects variables retained in a multivariable linear regression model of gestation length following 1203 inseminations with fresh and frozen-thawed semen in bitches of various breeds.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Coefficient (SE)</th>
<th>t value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(intercept)</td>
<td>Ref</td>
<td>64.39 (1.57)</td>
<td>40.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Litter size</td>
<td>Year</td>
<td>0.08 (0.02)</td>
<td>−3.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age of bitch</td>
<td>Year</td>
<td>0.11 (0.04)</td>
<td>2.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Breed</td>
<td>Greyhound</td>
<td>3.41 (1.57)</td>
<td>2.17</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Results for all 84 different breeds are not shown.
excluded from our analysis of whelping rate. We found that the whelping rate was significantly affected by: sperm motility, type of semen (fresh/frozen), semen source, breed of the bitch and age of the bitch.

Insemination of sperm, regardless of semen type (fresh, frozen or chilled), with a total forward progressive motility of less than 30% resulted in a lower whelping rate (37%) than after insemination of sperm with a total forward progressive motility of greater than 65% (whelping rate = 79%). Similar findings were reported by Linde Forsberg [2] and Thomassen [1] in which bitches inseminated with semen with a total sperm motility greater than 40% or 50% resulted in significantly higher whelping rates than those inseminated with semen with less than 50% motility. Burgess [3] also found that bitches inseminated with semen of 50–100% sperm motility had a four-fold greater odds of whelping compared to those inseminated with semen with a motility of less than 50%. Generally, the subjective assessment of sperm motility alone has limited value in predicting the subsequent fertility of a semen sample regardless of the species [29,30]. However, when in vitro semen assessments are performed under controlled conditions by a single, experienced operator or a group of operators from the same laboratory where regular calibration and quality control assessments are routinely carried out, as in our study, motility has been shown to be a significant predictor of sperm functional capacity in various species, for example, elephants [31], humans [32], cattle [30], pigs [33] and horses [34].

There are few studies in which all types of semen: fresh, chilled and frozen-thawed were included and compared. Most studies have examined whelping rate after insemination with only frozen-thawed semen or after natural mating. Previous studies which have compared whelping rates after insemination with fresh or frozen-thawed semen, interestingly, did not find a significant effect of semen type on whelping rate [3,8]. This is in contrast to our study in which the whelping rate after insemination with fresh semen (80%) was significantly higher than with frozen-thawed semen (71%). This difference in whelping rate may be due to the larger number of bitches in our study compared to Burgess [3] and the controlled nature of our study with only two operators carrying out the semen collections and AI procedures, unlike data obtained from kennel club registries in which there are a number of veterinarians with different levels of skills and knowledge carrying out these procedures [8]. It is expected and well documented in many livestock species that insemination of a greater number of ‘freshly ejaculated’ sperm will result in higher pregnancy rates, than after insemination with a low number of frozen-thawed sperm which have been exposed to the damaging processes of cooling, freezing and thawing, ultimately resulting in reduced longevity and viability in the female reproductive tract (reviewed by Ref. [35]).

There was also a significant difference in whelping rate between frozen-thawed semen collected, processed and frozen by GlenBred compared to semen collected, processed, frozen and imported to GlenBred from an outside semen freezing facility. Fertility studies in which the semen was frozen by a single operator [2,10] or frozen using a very standardized methodology [27] resulted in higher whelping rates (>80%) than the reported whelping rate for studies involving semen from many different centers (73.1% [1], 58.5% [28]). However, another potential reason for a higher whelping rate with our in-house frozen semen is that we generally do not collect and freeze semen from dogs older than 8 years of age. Similar to that reported by Thomassen et al. [1], we have found that semen collected from older dogs, in particular maiden older dogs, results in lower whelping rates and smaller litter sizes. It is a limitation of this study that we were not able to collect enough data in regards to the age of the dog at the time of freezing. Some of our semen was collected over 35 years ago and this information was not available, in addition a lot of the older imported semen did not contain this information. Also, contributing further bias towards a higher whelping rate for in-house frozen semen is that ejaculates of poor quality or sperm that did not tolerate the cooling and freeze-thawing processes (post thaw motility less than 40%) were generally discarded. For semen frozen at GlenBred, the total number of frozen-thawed sperm per AI dose was 200 × 10^6. This number of sperm per AI dose is similar to the total numbers of frozen-thawed sperm used in the studies of Thomassen et al. [1] (200 × 10^6) and Linde Forsberg et al. [2] (183.6 × 10^6). However, an important limitation of our study and that of Thomassen et al. [1] is that for frozen semen imported from other semen freezing facilities, both domestically and internationally, the total number of frozen-thawed sperm was not confirmed at the time of AI. Determination of the total number of sperm in imported frozen semen after thawing and AI could not be accurately achieved, especially with semen frozen in pellets. This was particularly so for dilute samples of frozen semen, which were common. Burgess et al. [3] used a Makler counting chamber to determine sperm concentration which may have provided more accurate information in regards to sperm numbers, or at least sperm concentration than a hemocytometer in these cases. Despite the recommended minimum number of motile frozen-thawed canine sperm per AI dose being >100 × 10^6 [1], it has been shown in two found clinical studies that when greater numbers of motile frozen-thawed canine sperm are inseminated, a higher whelping rate is achieved [3,28]. Burgess et al. [3] concluded that insemination with more than 200 × 10^6 motile frozen-thawed sperm increased the odds of whelping, and that the minimum standard international recommendation for motile frozen-thawed sperm should be closer to 200 × 10^6 motile spermatozoa, not >100 × 10^6 motile spermatozoa per insemination. Our study, even with limited information on the number of motile frozen-thawed sperm inseminated in imported semen, supports this conclusion.

It is well known that the timing of insemination, especially with frozen-thawed semen is critical to achieving successful whelping results. Furthermore, the deposition of frozen-thawed semen closer to the site of fertilization (intrauterine deposition), also increases the likelihood of pregnancy [2]. However, in our study, both of these factors; time of AI in relation to LH0, and site of insemination, did not affect whelping rate. This is because they were confounded by semen type. Vaginal inseminations with frozen-thawed semen were not performed in any bitch in the study. All frozen-thawed semen was deposited into the uterus either endoscopically (TCI) or surgically. If catheterization of the cervix was not possible during a TCI procedure, surgical insemination was performed. This only occurred in 7 bitches. Further confounding the effect of site of sperm deposition on whelping rate was that compromised ejaculated fresh semen (low sperm numbers or a high proportion of morphological defects), or ejaculated semen from older dogs (>8 years) was often deposited directly into the uterus using TCI, rather than the vagina, to increase the likelihood of pregnancy. Insemination with frozen-thawed semen was mostly always performed on LH +5 and/or LH +6. If a bitch had a slow or unusual progression of progesterone concentrations, coupled with an abnormal cytology and vaginoscopy, it was recommended to abandon the AI rather than perform an insemination after LH +6.

Interestingly, we found no difference in whelping rate after intrauterine insemination using either the non-invasive endoscopic IUAI technique (TCI) or surgical deposition of semen into the uterus (surgical AI). This is in contrast to the only other study that has directly compared these two intrauterine insemination techniques, where it was found that a higher whelping rate resulted after deposition of frozen-thawed semen into the uterus using the TCI technique compared to surgical AI [28]. However, in that study the number of surgical AI’s performed was low (n = 40) and racing greyhounds, which are predominantly surgically inseminated,
were not included. In the past, in countries such as Australia and the USA, there has been significant debate about whether whelping rates are higher after surgical AI compared with TCI. Theoretically, there should be no difference in whelping rate between these two techniques, as the site of deposition is the same i.e. intrauterine. Our study was the first large scale study to objectively demonstrate that there is no difference in whelping rate after either TCI or surgical AI and that semen quality and other factors are significantly more important to achieving pregnancy than the IUAI technique used. These findings will no doubt contribute to the ongoing ethical debate as to whether invasive surgery, and the associated general anesthetic risks and potential post-operative complications, should be a technique used to inseminate bitches, when other non-invasive techniques are available that achieve the same whelping rates [36].

In previous studies where two inseminations were performed per estrous cycle, higher whelping rates and larger litter sizes were obtained compared to performing only one insemination per cycle [1,2,37,38]. However, in these studies, an entire dose of at least 100 × 106 motile frozen-thawed sperm was used at each AI. This is in contrast to our study whereby when two inseminations were carried out in one estrous cycle, only half a dose of frozen-thawed semen was used at each AI. This probably explains why no difference in whelping rate was seen in our study after two AIs compared to one AI per cycle. In agreement with Thomassen et al. [1], there should be no difference in whelping rate between one or two inseminations per cycle, if optimal timing of a single insemination is carried out with at least 100 × 106 motile frozen-thawed sperm of high quality (>50% progressively motile; > 80% morphologically normal).

Breed of the bitch has not been found to affect whelping rate in previous studies [1–3]. This finding may be associated with an insufficient number of bitches in each breed category. A previous study, investigating whelping rates after TCI with frozen-thawed semen in greyhounds, indicated that racing greyhounds appear to be more fertile than other breeds (87% whelping rate [27]). As our study included 1146 individual bitches of 84 different breeds, we were able to demonstrate for the first time a breed effect on whelping rate, with Greyhounds and Labrador Retrievers having significantly higher whelping rates (88% and 94% respectively) after AI compared to all other breeds.

We also found that the age of the bitch had a significant effect on whelping rate, with a linear decline in whelping rate with each advancing year of age. Thomassen et al. [1] similarly found that bitch age had an effect on whelping rate, with bitches older than 6 years of age having a significantly lower whelping rate than bitches younger than 6 years of age at the time of AI. Interestingly, when we analyzed age as a categorical variable in the multivariable model of whelping rate, there was no absolute “cut-off” point at which whelping rate declined with age. Instead, there was a linear decline in whelping rate beyond the age of first insemination. It is not surprising that age has an effect on whelping rate in the bitch as the decline in pregnancy rate associated with aging has been well documented in many species such as humans [39,40] and mares [41,42].

The mean litter size in our study (5.8 ± 3.1 pups) was similar to that reported in previous large-scale studies in which many breeds were represented (5.7 ± 0.1: [1]; 5.0 ± 2.9 pups: [2]; 5.4 ± 0.025 pups [5]). We found that litter size was significantly affected by the type of semen (fresh/frozen) inseminated, the size of the bitch and the age of the bitch at the time of AI. Predictably, litter size was larger in bitches inseminated with fresh semen compared to those inseminated with frozen-thawed semen. Linde-Forsberg et al. [38] reported similar findings after insemination of 527 bitches with both fresh and frozen-thawed semen. As with many other species, insemination with fresh semen involves the deposition of a significantly higher number of sperm, with longer viability (up to 7 days [43]) compared to insemination of low numbers of frozen-thawed sperm, with reduced longevity and viability due to capacitation-like changes associated with cryopreservation (12–24 h [35]). However, interestingly, sperm motility did not affect litter size. This was also reported in some previous canine studies [1–3]. One explanation for this finding is that sperm motility is only one subjectively assessed parameter of semen quality that does not give an insight into sperm membrane integrity, capacitation status and chromatin integrity, and the many other factors that affect sperm viability and fertility [30]. If more in vitro assessments to determine membrane, acrosome and DNA integrity were performed, it is possible that a correlation between semen quality and litter size may be seen.

Mean litter size increased with bitch size or body weight. This is a well-recognized finding [1,3,5]. Large bitches have greater uterine capacity to carry more fetuses than smaller bitches, therefore, lighter, smaller bitches have smaller litters compared to heavier, larger bitches.

Similar to whelping rate, a significant correlation was found between the age of the bitch at the time of AI and the resultant litter size. With each advancing year of age, litter size declined by an average of 1.3 pups per litter. When we analyzed age as a categorical variable in the multivariable model of litter size, we were not able to demonstrate an “absolute” age after which litter size declines. Instead, there was a linear decline in litter size beyond the age at which a bitch had her first litter. In contrast, previous studies reported a decline in litter size at 5 years of age in the Drever breed [4], and just before 5 years of age in bitches classified as having a medium body weight [3], and in large breeds, a decline in litter size was seen just before a bitch reached 6 years of age [1]. Similar to other domestic species that live longer than their reproductive lifespan, and in humans, advancing age has a negative effect on reproductive performance in the bitch. This decline in fertility associated with increasing bitch age is most likely multi-factorial and associated with a decreased ovulation rate, reduced oocyte quality and degenerative age-related changes to the endometrium. Age-related degenerative changes of the endometrium resulting in a decreased capacity to support embryo and fetal development have been well documented in the mare [44,45]. In the bitch, degenerative uterine changes may reduce the ability to support the maturation and growth of multiple fetuses. Similar to aging women [39] and mares [42,46] a reduced ovulation rate may also be a contributing factor to smaller litter sizes in older bitches. In a recent study, we showed that AMH was a significant predictor of litter size and that AMH declined with advancing age [47]. AMH is an established biomarker of ovarian reserve in humans [48], cows [49], sheep [50] and goats [51]. Not only is the number of oocytes potentially reduced in older bitches, but the quality or developmental competence of their oocytes may also be impaired as seen in both the older mare [42,52] and human [39], thus resulting in a smaller litter size due to greater embryonic loss.

Similar to whelping rate, in our study, litter size was not affected by the time of insemination in relationship to LH 0 or the type of AI performed, as both these independent variables were confounded by semen type. Frozen-thawed semen was only deposited into the uterus and timing of AI with frozen semen was always optimal (i.e. 3–4 days after ovulation or LH +5 to LH +6). Interestingly, the number of inseminations performed per cycle also did not affect litter size in our study. Marseloo et al. [26] showed with ultrasonography that most bitches completed ovulation over a 24 h period and some in less than 12 h. Therefore, a well-timed single insemination with an appropriate number of frozen-thawed spermatozoa should produce the same result as two inseminations 24 h apart.
However, Thomassen et al. [1] reported that bitches inseminated once per estrous cycle with frozen-thawed semen whelped 0.8 less pups, on average, compared with bitches inseminated twice per estrous cycle. Linde Forsberg et al. [2] also found a positive relationship between the number of intravaginal inseminations per cycle with frozen-thawed semen and litter size. The difference between these studies and the current study, is that an entire dose of frozen-thawed semen was used at each AI, whereas only one dose of frozen semen was available for each AI in our study. Therefore, in those bitches that were inseminated twice in our study, the dose was split.

The definition and method of calculating gestation length in many studies has lacked consistency. This is particularly so when data for calculation and analysis of gestation length has been obtained from breed registries, in which natural matings are most commonly performed. In some studies, gestation length has been determined from the date of the last mating [4], the date of the last AI [1,2] or not defined at all [53]. The interval from mating to whelping is highly variable, thus determining what factors affect gestation length using this information is difficult. However, studies in which gestation length was calculated from either LH 0 [25,54] or ovulation [55,56], by using serial progesterone assays during the breeding period, allowed a more accurate determination of gestation length. The mean gestation length for all bitches in our study was 65 ± 19 days. This is similar to previous reports which have used the same definition to calculate gestation length [25]. We found that gestation length was significantly affected by breed, litter size and age of the bitch.

In similar studies in which the number of bitches representing each breed was high, significant differences in gestation length were observed between different breeds [54,56,57]. To our knowledge, this is the first report of gestation length in Greyhound bitches (68.0 ± 1.5 days from LH 0), which was significantly longer than any other breed. The most controversial breed in regards to gestation length is the GSD with both Mir et al. [56] and Okkens et al. [58] reporting a shorter gestation length compared to other large breeds in their respective studies. This is in contrast to our findings, as well as those of Eilts et al. [54] and Okkens et al. [57], in which GSD bitches had a similar gestation length to other breeds. This difference may be due to differences in the calculation of gestation length, i.e. from mating [58], the small sample size of GSD bitches (n = 18 [56]), or possibly due to genetic differences within the breed. It is possible that the apparent difference in gestation length between breeds may be related to physiological variation in the day of LH0 or ovulation, relative to the progesterone concentration reaching 2 ng/mL or 6 ng/mL respectively, between breeds as suggested by Mir et al. [56].

We also found that litter size affected gestation length, with larger litters having a shorter gestation length. For each additional pup born above the mean litter size, gestation length decreased by 0.08 days. This finding is supported by many previous studies that also found a negative correlation between litter size and gestation length [1,4,54,56,58]. Gavrilovic et al. [4] reported that each pup born above the mean litter size for the Drexer breed, resulted in a reduction in gestation length by 0.25 days. However, gestation length in that study was determined from the date of last mating to the registered birth date, creating greater variation in the gestation length calculation.

Finally, we found that for each additional year of age, gestation length increased by 0.11 days. This is in contrast to all previous studies which found no effect of bitch age at the time of AI on gestation length [4,54,56,57]. This effect may have been detected in our study due to the large number of whelping bitches, or because of the greater accuracy in the calculation of gestation length or due to the multivariable analysis of the dataset.

There are relatively few reports in the literature on the incidence of dystocia or the C-section rate of whelping bitches. The incidence of dystocia in the general canine population of whelping bitches of mixed breeds has been previously estimated to be approximately 5% [59–61]. More recently, Bergstrom et al. [62] analyzed data obtained from a Swedish animal insurance database that included approximately 200,000 whelping bitches of different breeds. They found the incidence of dystocia was 16%. Our study is the only other known study to report a dystocia rate based on a large population of bitches of different breeds. Dystocia occurred in 28% of bitches in our study. This rate was defined as bitches requiring either an emergency C-section or medical assistance to deliver pups.

Importantly, the incidence of dystocia varies greatly between breeds, with a high incidence previously reported in miniature and small breeds [63,64], Scottish Terriers [62] and breeds selected for large heads such as the brachycephalic and achondroplastic breeds, which result in dystocia due to cephalo-pelvic disproportion [59–61,65]. It is these breeds that have the highest rate of C-sections performed for delivery of their pups. In past reports, approximately 60% of bitches with dystocia required a C-section for delivery of the pups [63,66]. More recently, in the large-scale study of Bergstrom et al. [62], of the 16% of bitches that presented for dystocia, 64% of these underwent a C-section to deliver the pups. More than 50% of the bitches that whelped in our study had a C-section for delivery of their pups but this procedure was not always performed to relieve a dystocia. Similar to other studies, breed had a significant influence on the C-section rate, with 87% of the bitches of brachycephalic breeds having a C-section to deliver their pups. However, the main reason that these bitches underwent a C-section in our study was because the owners, and some veterinarians, chose to perform a timed, elective C-section rather than risk a likely dystocia occurring, and the associated loss of pups, and in some cases the bitch. This is the first report of an elective C-section rate in a large population of whelping bitches. Performance of an elective C-section accounted for 28% of all whelping outcomes in our study. This high C-section rate may also be due to the over-representation of brachycephalic breeds in our study, as these breeds not only have a higher incidence of whelping complications, but also have greater fertility issues than other breeds, thus requiring greater breeding management. This approach to managing whelping in brachycephalic breeds may be a reflection of changing attitudes to whelping management amongst breeders and veterinarians over the past 10 years in New Zealand, and possibly also other countries such as Australia and the USA, with elective C-sections being seen as a proactive, rather than reactive approach, compared to an emergency dystocia situation in which neonatal survival and bitch welfare can be compromised. This is a controversial area of veterinary medicine that raises ethical and welfare concerns for these breeds, especially in many European countries.

Litter size also had an effect on the C-section rate in our study. Bitches with litters of only 1 or 2 pups had a C-section rate of 83%, whereas bitches with litter sizes of 3 or more pups had a C-section rate of 43%. It has been well documented previously that single puppy litters, or pups from a small litter (3 pups or less) are often oversized, which may result in an obstructive dystocia [63,64]. It has also been theorized that single puppies fail to produce enough hormonal stimulation to initiate parturition [67], described as ‘the single-pup syndrome’ [68]. It is for these reasons that veterinarians and breeders advocate performing an elective C-section in single puppy litters, or in bitches with a small litter of 2 or 3 pups.

It has been suggested that age-related changes to the uterus are one of the many potential underlying causes for primary uterine inertia in the bitch, and therefore it is more likely that older bitches will require greater veterinary intervention at whelping [68]. Interestingly, the age of the bitch at the time of whelping did not
affect the C-section rate or the proportion of bitches requiring medical assistance in our study. Similarly, Gaudet [63] did not find an effect of bitch age on the dystocia or C-section rate. In a more recent study involving 530 whelpings, Mun nich and Kuchenmeister [64] found that primiparous older bitches (>6 years) had a greater risk of dystocia. In our population, the breeding of older bitches (greater than 6 years of age) was strongly discouraged, which may account for the lack of an age effect on the dystocia rate in our study.

There have been several conflicting reports in regards to seasonality and its effect on canine reproductive performance parameters and patterns [4,5,53,69,70]. We found no effect of season on any of the reproductive outcomes analyzed in our study. This may be due to the temperate climate and reduced seasonality in New Zealand compared to many Northern Hemisphere countries in which many of the canine reproductive studies have been performed. We also did not have breeds in our study that are known to be genetically seasonal, such as the Basenji dog and Drever breed [4]. Similar to our findings, a large-scale retrospective study of 10,810 litters born from 224 different breeds did not find an effect of season on the distribution of whelpings throughout the year [5]. In contrast, other studies found the distribution of whelpings was greater in the warmer months of the year [47,71]. These studies were all performed in the Northern Hemisphere. However, in all of these studies it was acknowledged that a significant contributing factor towards the uneven distribution of whelpings was human intervention, not necessarily a seasonal influence on cycling or other reproductive parameters.

This study has identified those factors which significantly affected whelping rates and litter sizes in a large population of bitches of various breeds inseminated with fresh or frozen-thawed semen. Whelping rate was determined by breed of the bitch, age of the bitch, semen type (fresh or frozen-thawed) and sperm motility. Litter size was influenced by size of the bitch, age of the bitch and semen type. Clarification of the factors affecting gestation length will facilitate accurate prediction of whelping in certain breeds for clinicians working in canine reproduction. This is particularly important if an elective C-section is to be performed, which in certain countries, such as New Zealand, is a common occurrence.

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