THE EFFECTS OF STANDARDISED PARTURITION CARE AND ANIMAL-BOUND FACTORS ON STILLBIRTH: A RETROSPECTIVE STUDY OF CANINE PARTURITIONS IN A HOMOGENEOUS POPULATION

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1. Abstract

Background: Veterinarians are routinely confronted with phone calls and inquiries regarding canine parturition. However, since few veterinarians use a standard specific parturition protocol, various actions are undertaken. Neonatal mortality, both during parturition and in the neonatal period, is a significant problem in the canine population. It has been previously reported that the overall incidence of stillbirth varies between 3.5% – 10.9%. Previous studies are either based upon a small number of dogs and/or diverse breeds. Possible causes of high stillbirth numbers and animal-bound factors have been described in the literature, but often it is not clear on what type of investigation this information is based or whether different parturition protocols have been used. The aim of this large-scale observational retrospective study was to evaluate a standardised parturition care and determine animal-bound factors that might influence the stillbirth rate in puppies in a homogenous canine population of mainly Labrador Retrievers.

Methods: Data was collected from a guide dog facility. A total of 2585 puppies from 331 parturitions - occurring from 1998 to 2019 - were included in this study. Qualitative analysis was carried out by conducting interviews with four experienced staff members of the guide dog facility. Quantitative data was analysed using Chi-square tests and logistic regression models.

Results: The experienced staff members of the guide dog facility use a short and structured parturition protocol which they do not always strictly follow. 142 (5.5%) of the 2585 puppies were stillborn. A borderline significance between maternal age and stillbirth was detected. Digital vaginal exploration, use of oxytocin, and manual obstetric assistance were significantly associated with increased stillbirth rates. Pups in posterior presentation at expulsion, or pups of which the placenta was expulsed immediately following delivery, were also significantly more likely to be stillborn. The last pup of a litter had the highest stillbirth rate. Stillbirth rate increased as inter-pup interval increased. No effect on stillbirth was found for maternal parity, litter size, caesarean section, time between first passage of foetal fluids via the vulva and first pup, presentation of the first pup, the pup's gender, or the pup's birthweight.

Conclusion: Univariable analyses revealed that stillbirth was significantly influenced by digital vaginal exploration, use of oxytocin, manual obstetric assistance, a pup's presentation, a pup's placenta attachment, a pup's place in the sequence of births, and/or inter-pup interval. This information is valuable to gaining a more detailed understanding of parturitions in dogs and to make informed decisions during a parturition. The results of this study can also be used to establish a better adapted parturition protocol. To reduce the number of stillbirths, the results of the present study indicate that: 1) good parturition care at individual puppy level is important; 2) the age of the bitch may be correlated with stillbirth rate; 3) more research is needed on oxytocin therapy in bitches; 4) extra close monitoring is required for pups born with posterior presentation, pups born with the placenta attached, and the final pup in a litter, as these puppies showed a higher risk on stillbirth; 5) the interval between the birth of a pup and a intervention needs to be reconsidered.

Keywords – Stillbirth, stillborn puppies, neonatal mortality, parturitions, standardised parturition care, animal-bound factors, guide dogs.

2. Introduction

Veterinarians are routinely confronted with phone calls and inquiries regarding canine parturition. However, since few veterinarians use a standard specific parturition protocol, various actions are taken. Besides, the timing of interventions during parturitions appears to be an important factor in neonatal survival (Darvelid and Linde-Forsberg 1994; Münnich and Küchenmeister 2009). There are surprisingly few empirical studies on the criteria for parturition intervention in relation to stillbirth and neonatal mortality (liveborn pups dying within the first six weeks after birth), and the effect of certain animal-bound factors on stillbirth and neonatal mortality. Neonatal mortality, both during parturition and in the neonatal period, is a significant problem in dogs and other polytous animal species such as the cat, with a stillbirth rate of 5.0% – 8.5% (Fournier et al. 2017; Musters et al. 2011). It has been previously reported that the overall incidence of stillbirth in dogs is probably 3.5% (Groppetti et al. 2015), 4.3% (Tønnessen et al. 2012), 5.9% (Cornelius et al. 2019), 6.1% (Linde-Forsberg and Persson 2007), 7.0% (Gill et al. 2002), 7.4% (Chastant-Maillard et al. 2017) and 10.9% (Indrebø et al. 2007). The incidence of stillbirth in Labrador retrievers is 6.0% (Cornelius et al. 2019) and 6.6% (Tønnessen et al. 2012). Previous studies are either based upon a small number of dogs and/or diverse breeds. The aim of this large-scale observational retrospective study was to evaluate a standardised parturition care and to determine animal-bound factors that might influence the stillbirth rate in puppies in a homogenous canine population of mainly Labrador Retrievers. More insight in these criteria and animal-bound factors might help to predict (and eventually prevent) possible parturitions problems, because limited treatment and preventative options are available during a parturition (Ettinger and Feldman 2010).

2.1 Normal parturition

The average length of a pregnancy was measured to be 61.4 days in dogs, starting at the day of mating with time (t) = 0, which was determined on the basis of the plasma progesterone pattern (Okkens et al. 2001). The mean duration of gestation in Labrador Retrievers was 60.9 days (Okkens et al. 2001). The length of a pregnancy is influenced by litter size. Small litters result in a longer pregnancy, whereas large litters will shorten pregnancy. The number of pups per litter ranged from 1 to 12 with a median of 9.0 pups in Labrador Retrievers (Okkens et al. 2001), but a mean of 6.1 and 7.9 pups in Groppetti et al. (2015) and Dolf et al. (2018), respectively.

The bitch may become restless, seeks seclusion, or becomes excessively attentive several days before parturition. She may also show nesting behaviour and start vomiting 12-24 hours before parturition (Freak 1975). The rectal temperatures of bitches fluctuate during the last week before parturition and usually drop below 37.6°C 12-24 hours before parturition (Schweizer and Meyers-Wallen 2000), but research has shown that this finding is not always reliable. In one study, the nadir temperature was already measured 48 hours before parturition in 24% of the dogs and a drop in temperature was not seen at all in 35% of the dogs (Copley 2002). To assess the drop in rectal temperature measurements, measurements should be taken two or three times a day starting a week before parturition (England and Heimendahl 2010a).

The parturition can be divided into three stages, of which the last two stages are repeated for each puppy. Stage I starts with mild uterine contractions and ends when the cervix is fully dilated. This stage typically lasts 6 to 12 hours but can last as long as 36 hours (Mosier 1986). Uterine contractions can be hard to recognise. However, unapparent uterine contractions increase in frequency and intensity towards the end of stage I (Mosier 1986; Van Der Weyden et al. 1981). Signs of stage I may be restlessness, panting, pacing, nesting, nervousness, anxiety, inappetence, shivering, nausea, vomiting, reclusiveness, and digging or scratching at the floor. However, some bitches show no such behavioural signs (Andrew et al. 2007). Stage II starts with full cervical dilation and ends when all puppies are born. This stage usually takes 3-6 hours and 12 hours is considered to be the maximum (Roberts 1986). The

bitch has strong abdominal contractions that accompany the uterine contractions. Normally, the allantochorionic membrane appears and breaks first, but sometimes it breaks during delivery of the puppies. The first puppy, covered by the amniotic membrane, is usually delivered within 4 hours after the onset of stage II (Johnston 1986; Wallace and Davidson 1995). The amniotic membrane will break by itself or the bitch will break it and lick the puppy. Puppies are delivered every 10 to 30 minutes on average (Freak 1962). 60% of puppies are born in anterior presentation and 40% in posterior presentation (Van Der Weyden et al. 1981). Posterior presentation may be associated with delayed delivery of the first puppy due to slower dilation of the cervix (Lopate 2012). Finally, in stage III the placentas are expelled, and the uterine horns are shortened. The placenta usually follows directly or within 15 minutes after the delivery of each puppy. However, up to three puppies can be born before the passage of their placentas occurs (Andrew et al. 2007).

2.2 Abnormal parturition

Another word for an abnormal parturition is dystocia. Dystocia is very common in dogs and occurs when they have difficulty with the normal vaginal delivery of a puppy. The average incidence of dystocia is likely below 5% (Gill et al. 2002). However, in a Swedish insurance database that contains data on 200.000 bitches of different breeds the incidence was 16% (Bergström et al. 2006). Dystocia can be caused by maternal factors (uterine inertia, pelvic canal anomalies, intrapartum compromise or behavioural issues), foetal factors (malposition, oversize, malpresentation, anatomical anomalies), or a combination of these factors (Darvelid and Forsberg 1994; Johnston 1986; Wallace and Davidson 1995). The most common cause of maternal dystocia is uterine inertia, which causes 70% – 90% of all maternal dystocia (Romagnoli et al. 2007). Primary uterine inertia is the failure to initiate stage II. The bitch is either due or overdue to last mating, she is clinically restless and shows signs of nesting. Sometimes vaginal discharge is present. Secondary uterine inertia refers to the failure to progress when the bitch is in stage II (Gill et al. 2002). The most common foetal factors that contribute to dystocia are absolute oversize and/or malpresentation (Linde-Forsberg and Eneroth 2000). Death of the foetus may also cause dystocia (1.1%), because of malpositioning or inadequate stimulation during a parturition (Darvelid and Linde-Forsberg 1994; Mosier 1986).

Criteria for an abnormal parturition are (Arthur et al. 1989; Bennet 1980; Drobatz 2005; England and Heimendahl 2010a; Ettinger and Feldman 2010):

- Temperature drops below 37.6°C for 12-24 hours without signs of labour
- It has been more than 2-4 hours since the first chorioallantois passed, but nothing else has happened since then
- (Green) vaginal discharge is present, but no puppy is born within 2-4 hours
- Uterine contractions are absent for more than 2 hours
- Weak and irregular uterine contractions for more than 2 hours without expulsion of a puppy
- Strong and regular uterine contractions for more than 20-30 minutes without expulsion of a puppy
- More than 4 hours have passed since the birth of the last puppy and there are still more remaining in the womb
- The bitch has been in stage II for more than 6-12 hours
- Signs of illness in the bitch

2.3 Management of abnormal parturition

The current literature on dystocia argues that dystocia must be diagnosed rapidly to prevent outcomes such as stillbirth. In addition, the actual cause of dystocia must be diagnosed so that the best possible therapeutic intervention can be made. Furthermore, it is argued that in bitches that suffer from obstructive dystocia or that have a history of active parturition with non-productive yet significant straining, the aim should be to remove the obstruction (England and Heimendahl 2010b). Puppies can be lodged within the birth canal as a result of foetal or maternal abnormalities. However, research

shows that manual interventions to resolve such problems are difficult in dogs due to the size of the pelvic canal. In large dog breeds, manual relief of an obstruction can sometimes be achieved through retropulsion, realignment, and traction techniques if the obstruction is an abnormal foetal position or posture (Bennet 1974). However, these manipulations have to be performed between periods of straining and should not interfere with the uterine contractions (England and Heimendahl 2010b). Furthermore, it is said that applying traction to the neck and foetal limbs should be avoided as these can damage easily, and that precautions should be taken when using obstetrical instruments, as the vaginal wall or the puppy can become traumatised (Münnich and Küchenmeister 2009). With regard to the use of obstretical instruments, England and Heimendahl (2010b) argue that these are best used only when it is clear that removal of this puppy will enable completion of the parturition.

When maternal and foetal obstructions have been ruled out and the bitch does not show regular abdominal contractions during stage II, straining can be stimulated by exercising the bitch. Examples of such intervention found in the literature are walking the dog around the house or by taking the dog out for a car ride. These methods are described by many authors, but studies on it are not available. Another technique that can be used to stimulate straining during an inefficient parturition is described by England and Heimendahl (2010a). England and Heimendahl (2010a) argue that it can be effective to 'feather' the dorsal vaginal wall. Feathering is accomplished by inserting two fingers into the vagina and pushing them against the dorsal vaginal wall. As a result, an episode of uterine contractions will be induced (Fergusons reflex) (England and Heimendahl 2010a). The Ferguson reflex results in an increase in circulating oxytocin and intensifies uterine contractions (Furguson 1941). Research suggests that lack of response to the Ferguson reflex may indicate uterine inertia, reveal signs that the bitch might not be in stage II yet, or is exhibiting voluntary inhibition because of stress and excitement. Proposed solutions are to place an anxious bitch in a quiet, dimly lit, and comfortable room with or without the owners. Veterinarians can also give low doses of tranquilliser when the bitch is excessively agitated to facilitate the labour process (Freak 1948).

In a different study, Freak argues that medical intervention can be performed when there is a failure of straining. Administration of oxytocin increases the frequency of uterine contractions, in which case a foetus should be delivered after no more than three doses of oxytocin administered at a 20- to 30-minutes interval (Freak 1962). However, the success rate of bitches with dystocia who were whelping after receiving medical management only varies from 20-55% (Gaudet 1985; Darvelid and Linde-Forsberg 1994; Münnich and Küchenmeister 2009). It becomes clear from the literature that the dystocia often cannot be corrected by manual or medical assistance, in which case it is necessary to perform a caesarean section. The frequency of unplanned caesarean sections has been around 10% in the Labrador Retriever breeding colony at Guiding Eyes for the Blind located in the United States (Dolf et al. 2018).

To sum up, the criteria found for performing a caesarean section are (Drobatz 2005; Ettinger and Feldman 2010):

- Primary and secondary uterine inertia unresponsive to medical intervention
- Obstructive abnormalities of the maternal pelvis or soft tissues of the birth canal
- Deficiency or excess of foetal fluids
- Foetal malposition or mal presentation that cannot be corrected
- Mismatch of foetal and maternal size
- Foetal death with putrefaction
- Consistent foetal heart rates < 150 beats per minute (indicating foetal distress)
- Signs of illness in the bitch

2.4 Stillbirth

Not many studies have been done on maternal, management, or pup factors that could affect the risk of stillbirths during a parturition. Parity and age of the bitch may be an important risk factor for dystocia. A survey on dystocia in Nigeria indicated an increased risk of dystocia in primiparous bitches (Oluwatoyin and Fayemi 2011). Another large study found that puppies from the first litter of a bitch were at greater risk to be stillborn (Tønnessen et al. 2012). Another study showed the increasing age of bitches older than four years resulted in a higher risk of dystocia in boxers (Linde-Forsberg and Persson 2007). Also, the age of a bitch was associated with an increased risk of stillborn puppies in Groppetti et al. (2015) and Tønnessen et al. (2012). Little data is available on risk factors for dystocia or stillbirths in popular large breed dogs commonly used as guide dogs, such as Labrador Retrievers and Golden Retrievers. Neither breeds were on the list of breeds for risk of dystocia and caesarean sections in the study of Bergström et al. (2006). According to Tønnessen et al. (2012) and Cornelius et al. (2019) the number of stillborn puppies increased with litter size.

Puppies born from parturitions that resulted in caesarean sections were 1.22 times more likely to be stillborn compared to pups born from normal parturitions (Cornelius et al. 2019). Digital manipulation, traction of puppies, or use of oxytocin increased the risk of stillbirth (Cornelius et al. 2019; Münnich and Küchenmeister 2009). Olsson et al. (2003) and Klarenbeek et al. (2007) effectively showed that peripheral plasma oxytocin concentrations are higher and more variable during the expulsive stage than during late pregnancy. However, relationships between the secretion pattern of oxytocin and the onset and progress of myometrial contractility and spacing of the delivery of consecutive pups remain to be investigated.

In literature there are some studies on the relationship between pup factors and their influence on stillbirth and neonatal mortality. Lower body weight of puppies is a risk factor for stillbirths (Indrebø et al. 2007; Cornelius et al. 2019), but large pups are also at increased risk of stillbirths (Cornelius et al. 2019) and caesarean sections (Dolf et al. 2018). However, Gropetti et al. (2015) did not find a difference in birth weight between stillborn or live born pups. This study found a mean birth weight of 405.1 grams in litters of 4-8 pups in Labrador Retrievers. The time between sequential expulsions of stillborn puppies had a longer mean compared to that of live puppies (102 versus 65 minutes) (Cornelius et al. 2019). Cornelius et al. (2019) also showed that the last puppy to be born was significantly more likely to be stillborn. In short, possible causes of high stillbirth numbers and animal-bound factors have been described in the literature, but often it is not clear on what type of investigation this information is based or whether different parturition protocols have been used.

2.5 Aim of the study

There appears to be a gap in the literature that studies criteria for parturition intervention in relation to stillbirth in a structured and standardised way. A large-scale study that includes all pups at risk would be a valuable addition to the literature. This study aimed to answer the following **research question**: ‘‘What is the influence of standardised parturition care and what is the influence of animal-bound factors on stillbirth in a homogeneous population mainly consisting of guide dogs, which were approximately the same size and type of dog?’’. In order to do so, this question was subdivided into four **sub-questions:**

- How is parturition care organised at the guide dog organisation?
    - Does the guide dog organisation use a protocol?
    - Which criteria are used to base decisions on regarding intervention during the parturition process?
    - To what extent are the criteria of use during the parturition process?
    - What difficulties are encountered with regard to using a parturition protocol?
- Which parameters related to the standardised parturition care are of influence on stillbirth?

- Which parameters related to the bitch are of influence on stillbirth?
- Which parameters related to the puppies are of influence on stillbirth?

The purpose of this study was to analyse the standardised parturition care, and to study the relationship between the animal-bound factors and the standardised parturition care used to combat stillbirth.

3. Materials and methods

3.1 Data collection

In order to answer the research questions, a retrospective observational study was performed. Data was collected from a guide dog facility located in Amstelveen, the Netherlands. The *Koninklijk Nederlands Geleidehonden Fonds* (KNGF) is a non-profit organisation that breeds and trains guide dogs. The data represents bitches that were subject to standard pregnancy guidance. Parturitions were managed by experienced staff of the KNGF in foster homes. The decision process for assisted whelping was guided by an established protocol described in Appendix 7.1. According to this protocol, digital vaginal exploration should be performed when bitches have made straining movements regularly and intensely for 45 minutes prior to whelping of the first puppy, or for 30 minutes prior to whelping any subsequent puppy without any externally visible progress. Finally, when the bitch has shown only non-intense, abdominal straining efforts for 1½ – 2 hours vaginal explorations should be performed as well. When there is no pup present in the birth canal, a second digital vaginal exploration should take place after 30 min. When at that time a puppy has advanced towards or entered the birth canal, the bitch should be left again for 30 minutes, after which, if no pup has been expelled, the bitch should be administered with oxytocin. However, if the pup has not advanced in caudal direction upon the second exploration, bitches should be treated with oxytocin without delay. This procedure should be repeated after 45 minutes if no pup has been expelled. 45 minutes thereafter, a course of oxytocin treatments should be started, given every 1½ – 2 hours until the birth of the next puppy.

The four members of the KNGF that supervised the parturitions recorded the conditions during a parturition, possible interventions and the clinical condition of the pup through standardised forms (Appendix 7.2). The forms were filled in immediately during the parturition and were accurately recorded. Thereafter, the forms were properly stored in folders that are kept in the KNGF's closet. The forms are used by the experienced staff of the KNGF to get an idea about the clinical history of a bitch when she expects another litter. Inclusion criteria consist of: completeness, readability and date. Some parturition forms were incomplete with regard to birth weight, expulsion times, presentation or placental attachments. However, these forms were included in the present study. The standardised forms are used by the KNGF since 1998. The forms dating back to the years before 1998 were still handwritten reports and were excluded from this study, because the data was not structured well. The forms where nothing else was stated except the number of puppies born were excluded as well.

3.2 Qualitative analysis

Qualitative analysis was carried out by conducting individual semi-structured interviews face-to-face with four experienced staff members of the KNGF in March 2019. The interviews were taken to gain insight into the standardised parturition care. The duration of the interviews varied between 30-60 minutes. The questionnaire used is included under Appendix 7.4. A summary of the interviews are included in the results of this study.

3.3 Statistical analysis

The data that resulted from the standardised forms were first entered in Microsoft Excel 2016 and then exported to RStudio 3.4.4. The collected data was compared using descriptive statistics. The correlation was tested individually between factors and the dependent variable stillbirth in two stages. The factors were first screened using a chi-square test in univariable analyses. Afterwards, the factors were tested for collinearity. *P*-value < 0.05 was considered the level of significance for all statistical tests. The data were expressed as mean ± SD or as median and range if data were not normally distributed.

3.4 Animals

Data from all parturitions in the period between May 1998 and January 2019 were included in this study. There were 140 bitches in total. The breed distribution of the bitches was 93 (66.4%) Labrador Retrievers, 22 (15.7%) Golden Retrievers, 11 (7.9%) Labrador Retrievers x Golden Retrievers, 8 (5.7%) German Shepherds, 4 (2.9%) German Shepherds x Labrador Retrievers, 1 (0.7%) Flatcoated Retriever and 1 (0.7%) German Shepherd x Labrador Retriever x Golden Retriever. The age was known for 121 of the bitches, giving a median age of 2.4 (range 1.6 to 5.1) years for bitches at the moment of their first litter. 87 bitches had more than one litter, with a maximum of four litters, in the study period and all are included in the study.

3.5 Definitions and classification of groups

This study defines each pup as a single observational unit. The pup is dichotomised as born alive or stillborn. Stillborn puppies were defined as those puppies that were observed to be dead immediately after expulsion. Factors available from the forms regarding parturition protocol were digital vaginal exploration, use of oxytocin, manual obstetric assistance, and caesarean sections. Digital vaginal exploration means feathering of the dorsal vaginal wall in order to facilitate the delivery of a pup. The data describes the use of oxytocin per dose, i.e. oxytocin administration before the delivery of a puppy. There can be multiple administrations per litter. When oxytocin injections were administered more than twice or digital vaginal exploration was performed more than twice for a pup, the number of pups yielded in these groups were too small to test for statistical significance. Therefore, these three groups were classified as: “No”, “1”, “2” and “> 2”. Manual obstetric assistance means that the bitch required physical assistance to deliver a pup. Factors in bitches were: age, reproductive history (‘primiparous’ for first parturition and ‘multiparous’ when bitches had already experienced a parturition before), and litter size. The data from the KNGF provides information on the time between first passage of foetal fluids via the vulva and delivery of the first pup, whether the placenta was expelled immediately after a pup or not and whether stillbirth took place. Furthermore, the data specifies gender, presentation at expulsion (anterior or posterior), birthweight, sequence, and inter-pup interval. Birth sequence was defined as the place a pup holds in the sequence of births in a litter: first, middle (between the first and last puppy) or last. The inter-pup interval was defined as the time between sequential expulsions. Birth weight was measured immediately after expulsion. The presentation during expulsion was noted by experienced staff of the KNGF or by people from the bitch’s foster home. To facilitate analysis of data, the factors “birth weight” and “time between first passage of foetal fluids via the vulva and birth of first pup” were classified into quartiles. The first quartile represents the lowest 25% of registered values, the second and third quartiles represent 25% of values below and above the median respectively, and the fourth quartile represents the highest 25% of registered values. As a result, birthweight is grouped as: 180-390, 390-490 and 490-670 grams. The groups of factor “time between first passage of foetal fluids via the vulva and birth of the first pup” are shown as: 0-35, 35-215 and 215-1125 minutes. Parity of the bitch, age of the bitch, litter size and inter-pup interval were classified based on literature. Parity of the bitch was classified into primiparous and multiparous. Bitches grouped by age were evaluated as a categorical variable with four levels based upon their age at parturition (the age of the bitch at time of parturition was obtained in years by subtracting the birthdate of the litter from the birthdate of the bitch), 1.6-2, 2-4, 4-6 and 6-8.5 years old. Litter size at birth was defined as the sum of live-born and stillborn puppies and were grouped as 1-5, 6-9 and 10-15 pups per litter. The groups of inter-pup interval were: 0-15, 15-30, 30-45, 45-60, 60-120, 120-180, 180-240, 240-982 minutes.

4. Results

4.1 Qualitative analysis

The questionnaire for the interviews can be found in Appendix 7.4. The protocol used by the staff members at KNGF was designed by Utrecht University (UU). The protocol was communicated to the employees during the incorporation of the KNGF. The KNGF has been using this protocol for around twenty years and during this period it has never been amended. Before the KNGF implemented the protocol designed by UU, a different protocol was used which was not written down in one specific document. Staff members of the KNGF mainly use the protocol today as a guideline. They do not strictly follow it during a birth, but instead rely on their experience. Moreover, information about the protocol is generally not shared with the foster homes, because it tends to give rise to questions and worries about parturition. Instead, staff members at the KNGF try to involve the foster parents during the process of parturition, to comfort and reassure the foster parents.

Of the four employees that were interviewed, two found the protocol to be clear enough, but the other two did not. The latter said the protocol should include more details and provide more specific information on parturitions. At the same time, the use of the protocol shows advantages. It provides a clear guideline and allows for better communication with the veterinarian in case of emergency. According to the experienced staff members of the KNGF there are no disadvantages, since the protocol is only used as a guideline. In case of abnormal parturitions, staff members rely on their experience and not on the protocol.

According to the protocol, when bitches have made straining movements regularly and intensely for 45 minutes prior to whelping of the first puppy or for 30 minutes prior to any subsequent puppy without any externally visible progress, digital vaginal exploration should be performed. When bitches have shown only non-intense, abdominal straining efforts for 1½ – 2 hours, digital vaginal exploration should also be performed. When there was no pup present in the birth canal, a second digital vaginal exploration should be take place after 30 min. The present study found that employees frequently act in accordance with the protocol. However, the members sometimes wait longer than the prescribed 30 minutes between two digital vaginal explorations, which is not in accordance with the protocol. In these instances, they would try interventions that are not in accordance with the protocol. These included walking the bitch, taking her out for a ride, massaging the bitch, and allowing the puppies to be fed by the bitch to stimulate the parturition. The staff would take the bitch outside when it showed signs of needing to defecate/urinate. The employees think the position the bitch needs to take in order to urinate or defecate helps to stimulate the straining movements. Two of the employees think taking the bitch out for a car ride stimulates parturition, but the other two employees do not believe in its effectiveness (they think it was a coincidence that a pup was born). All members of the experienced staff rely on massaging the bitch as an intervention method: they believe it stimulates the parturition. This also applies to allowing the puppies to be fed by the bitch. Some staff members start using these interventions significantly earlier than others. Manual obstetric assistance is also used as an intervention method. When a bitch shows a lot of straining movements without movement of the pup, staff members decide to give manual obstetric assistance when they can feel the presence of the pup in the vagina of the bitch. Manual obstetric assistance is in accordance with the protocol. Intervention is postponed because employees know from experience that not all the bitches have similar parturitions and that duration of parturition varies per bitch.

In instances where a puppy had advanced towards or had entered the birth canal during the second digital vaginal exploration, the bitch was left again for 30 minutes. If no pup had been expelled or if the pup had not advanced in caudal direction within these 30 minutes, the bitch would be administered with 2 I.U. oxytocin subcutaneous. This procedure would be repeated after 45 minutes if no pup had been expelled. After another 45 minutes, a course of 2 I.U. oxytocin treatments would be started, given

every 1½ – 2 hours until the birth of the next puppy. The present study found that staff members used digital vaginal explorations more than two times per puppy, which is not in accordance with the protocol. This method is preferred over the use of oxytocin by staff members of the KNGF, which explains its prevalence. Depending on the size of the bitch, all the employees would start with an amount of oxytocin between 0.2 and 0.3 milliliters, which is correspondence with the protocol. The maximum amount oxytocin given was 0.3 milliliters. Two doses of oxytocin would be given per pup. The present study also found an average duration of one hour between the first and the second dose. However, according to the protocol, the time between the first and the second dose should be 45 minutes. Staff members at the KNGF tended to wait longer and first resort to the use of the interventions mentioned above, because they prefer to administer the smallest amount of oxytocin possible. When after the second dose of oxytocin the bitch still has not given birth to a pup, staff members would call upon a veterinarian for advice. In these cases, the veterinarian either decided to inject the bitch with another dose of oxytocin or, when this would not help, to perform a caesarean section. The caesarean section is the last intervention method used to manage the parturition. Sometimes the decision was made to let the last pup die, because staff members prefer this to all the disadvantages of a caesarean section.

There are a few amendments that the KNGF staff members would like to make to the protocol. They would like a Dutch version of the protocol and the protocol should be more detailed. All the members tried to report the most important details of the parturition. However, it occasionally happened that they forgot to report certain things because of the chaos surrounding the parturition.

The staff uses a number of methods to decide whether a parturition has ended. First, they count the number of puppies born and compare this number to the number of puppies that was expected based on the ultrasounds. They also perform an abdominal palpation. If there still remains some uncertainty after performing these procedures, a final dose of oxytocin will be given as a last resort.

4.2 Descriptive statistics

The median length of gestation in 331 pregnancies was 61 (range 54 to 71) days. A total number of 2585 puppies were born: 1316 (51%) males, 1226 (47%) females. Of 43 (2%) puppies the gender was not documented. 307 (12%) puppies were born after various ways of intervention and 2278 (88%) were born without any intervention. None of the bitches died during parturitions.

142 (5.5%) of the 2585 puppies were stillborn. 59 (41%) were male, 52 (37%) were female and in 31 cases (22%) the gender was not documented. In two litters (0.6%) the entire litter died: a singleton and a litter of four puppies. 95 (29%) litters had a total of 142 stillborn puppies. The median stillborn puppies in a litter was one (range one to five). Nine mummified and five premature foetuses were born. Umbilical hernias were found in three pups. Seven pups had a cleft palate and three pups had other malformations such as open spina bifida and one pup had multiple congenital malformations of the head and legs. Five puppies were born with omphalocele (an abdominal wall defect resulting in viscera misplaced outside the abdomen). Two of them were operated. One litter had multiple puppies with congenital abnormalities.

The average size of the litters of 331 parturitions was 7.8 ± 2.3 puppies (range 1 to 15) (Fig 1).

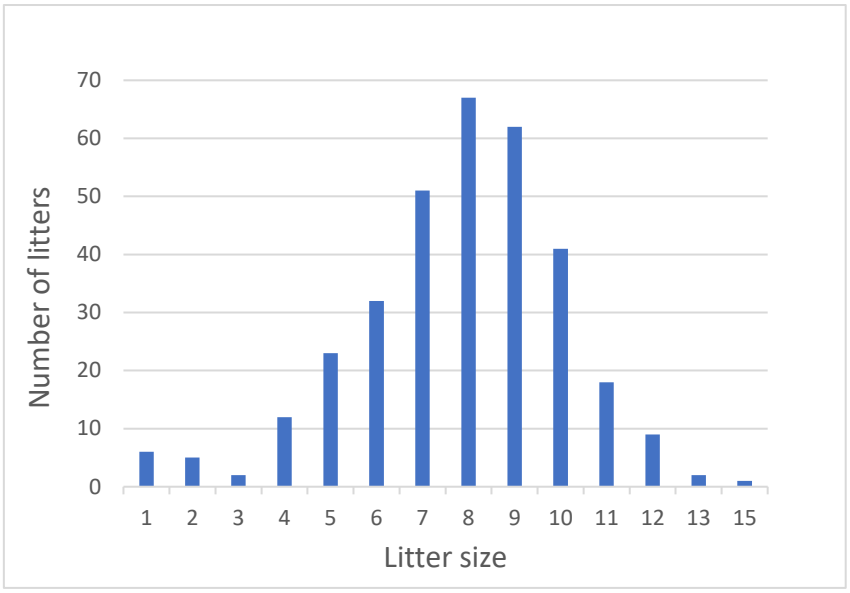

Figure 1. Distribution of the litter size of 331 parturitions.

The mean birthweight of 2450 puppies was 439 ± 73 grams (range of 180 to 670 grams) (Fig 2). All six puppies weighing less than 200 grams were stillborn. The heaviest pups were a stillborn female pup from a litter of 7 pups and a male pup from another litter of 7 pups weighing 670 and 650 gram, respectively.

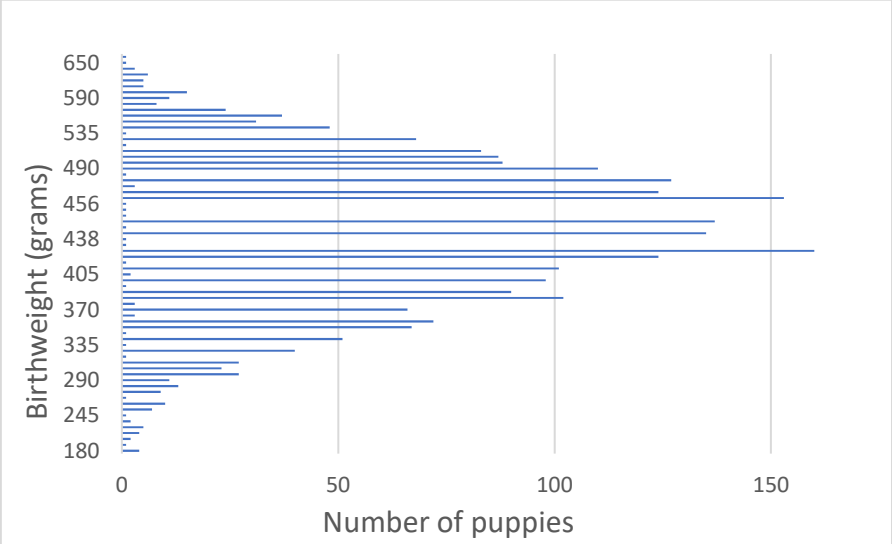

Figure 2. Distribution of birthweight of 2450 living and stillborn puppies.

The age was known for 121 of the bitches, giving the bitches a median age of 2.4 years (range 1.6 to 5.1) at the moment of their first nest. The median parity was one (range one to four) in 30 parturitions. The distribution of number of litters per parity of the bitch is shown in Fig. 3.

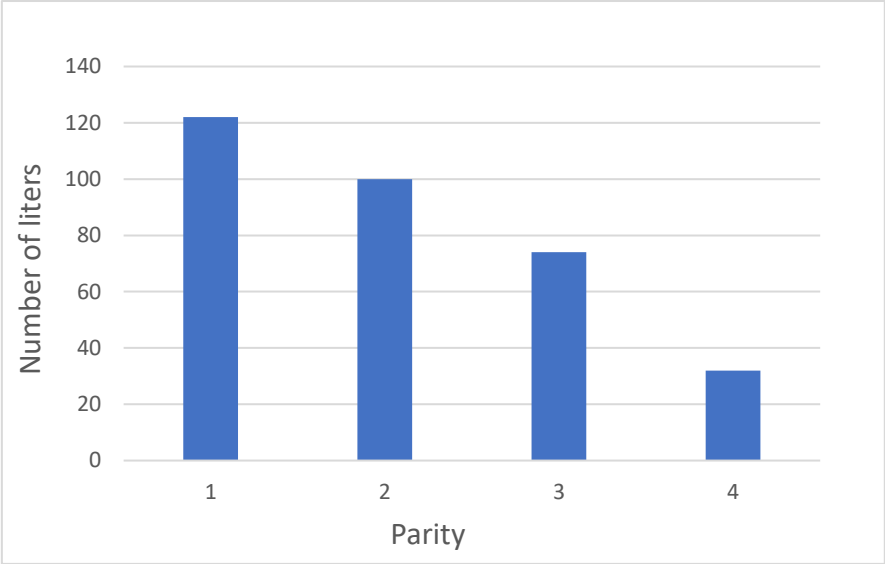

Figure 3. Distribution of number of litters per parity of the bitch.

4.3 Univariable analyses

4.3.1 Maternal factors

Parity of the bitch

A summary of the results of the maternal factors is shown in table 1. Parity of the bitch did not show any significant association with stillbirth (*p*-value 0.8). Also, the proportions were almost equal (Fig. 4).

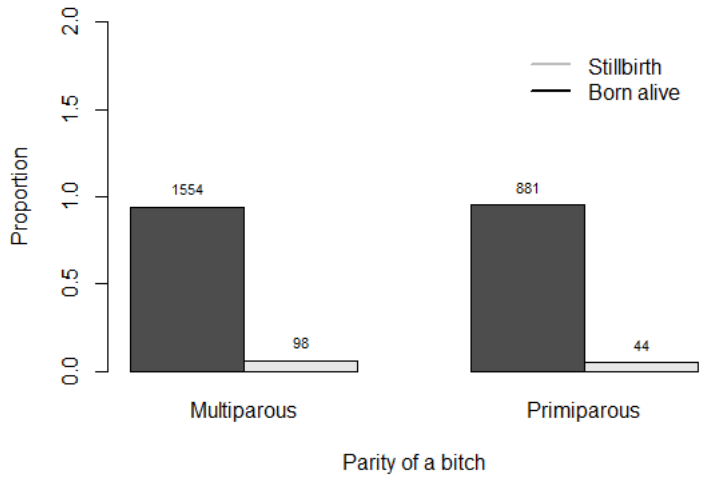

Figure 4. Diagram showing parity of a bitch versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Age of the bitch

Age of the bitch in years was not significantly associated with stillbirth. However, bitches of 6 to 8.5 years (n = 47) did show a borderline significance (*p*-value = 0.05). The proportions were almost equal (Fig. 5).

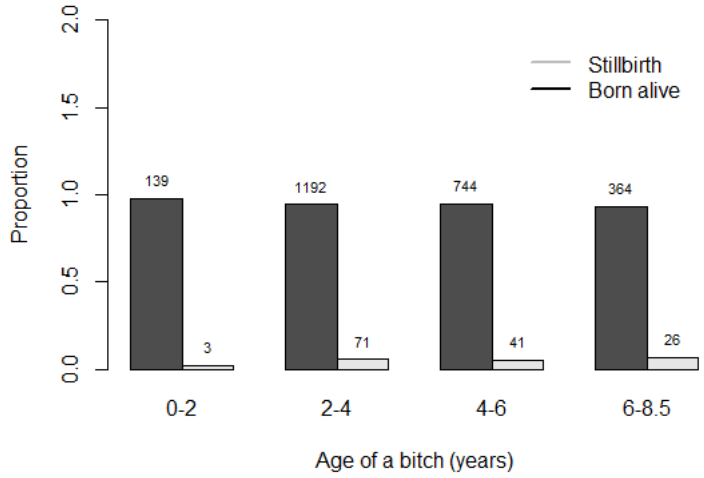

Figure 5. Diagram showing the bitches' age versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Litter size

The proportion of stillborn puppies was slightly larger in litter sizes of 10 to 15 puppies (Fig. 6) with a *p*-value of 0.07.

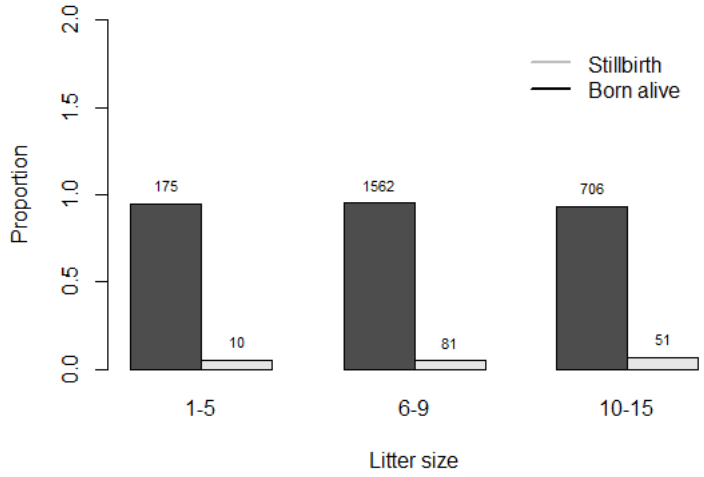

Figure 6. Diagram showing litter size versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Table 1. Bitch factors as risk factors for stillbirth. Variables have been screened by univariable analysis with *p*-values, odds ratios and 95% confidence intervals for stillborn and liveborn puppies. Ref, reference; Freq., frequencies; Prop., proportions.

Variable	Stillborn		Born alive		P-value	Odds ratio	Confidence interval
	Freq.	Prop.	Freq.	Prop.			
Parity of the bitch							
Multiparous	98	0.05	1554	0.95	Ref	1	Ref
Primiparous	44	0.06	881	0.94	0.21	0.8	0.6 – 1.1
Age of the bitch (years)							

1.6 – 2	3	0.02	139	0.98	Ref	1	Ref
2 – 4	71	0.06	1192	0.94	0.09	2.8	1.0 – 11.4
4 – 6	41	0.05	744	0.95	0.12	2.6	0.9 – 10.6
6 – 8.5	26	0.07	364	0.93	0.05	3.3	1.1 – 14.0
Litter size							
1 – 5	10	0.05	175	0.95	0.78	1.1	0.5 – 2.1
6 – 9	81	0.05	1562	0.95	Ref	1	Ref
10 – 15	51	0.07	706	0.93	0.07	1.4	1.0 – 2.0

4.3.2 Management factors

Digital vaginal exploration before the birth of a pup

The results of management factors are summarised in table 2. In 35 parturitions (10.6%), digital vaginal exploration was required for the delivery of a pup. Pups from parturitions that had digital vaginal exploration had an odds ratio of 4.8 (95% C.I. 2.8 – 7.9; *p*-value < 0.001) for being stillborn. The proportions are being shown in Fig. 7.

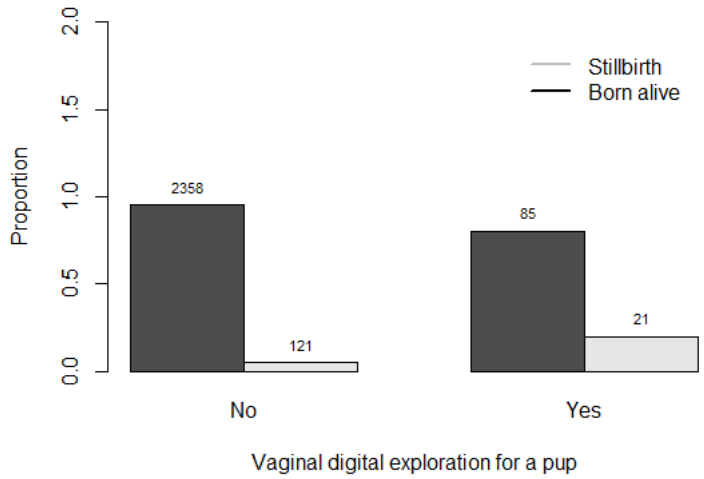

Figure 7. Diagram showing vaginal digital exploration versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

As the number of digital vaginal exploration increased, the risk of stillborn puppies increased as well (*p*-value < 0.001). Figure 8 shows how with each additional digital vaginal exploration, the risk of stillborn puppies increased.

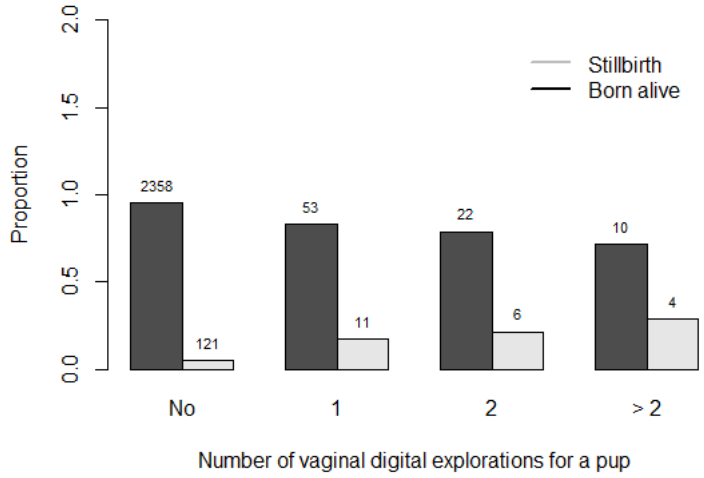

Figure 8. Diagram of number of vaginal digital explorations versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Use of oxytocin before the birth of a pup

Oxytocin was given during 16 (4.8%) parturitions, affecting 176 puppies. 16 of those 176 puppies ended up being born through a caesarean section. The median oxytocin quantity given at first and second administration were 0.2 and 0.3 international unit (IU), respectively (Fig. 9; Fig. 10).

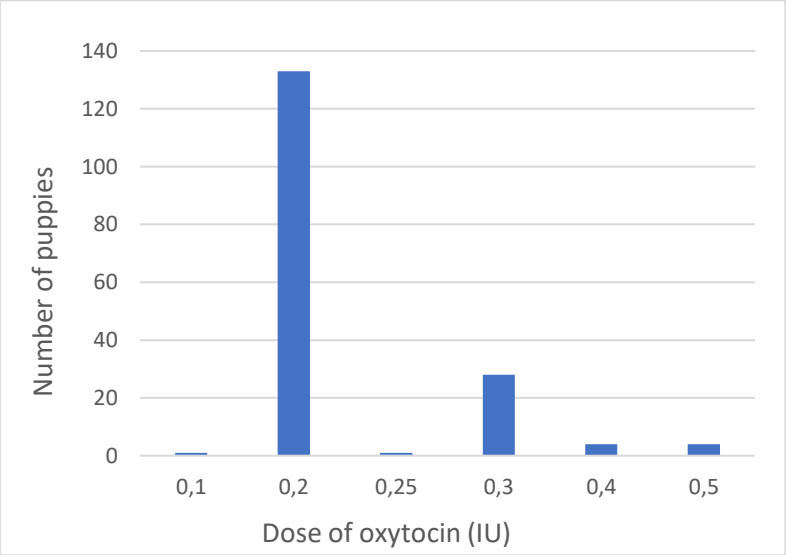

Figure 9. Distribution of quantities oxytocin given at first administration. IU, international unit.

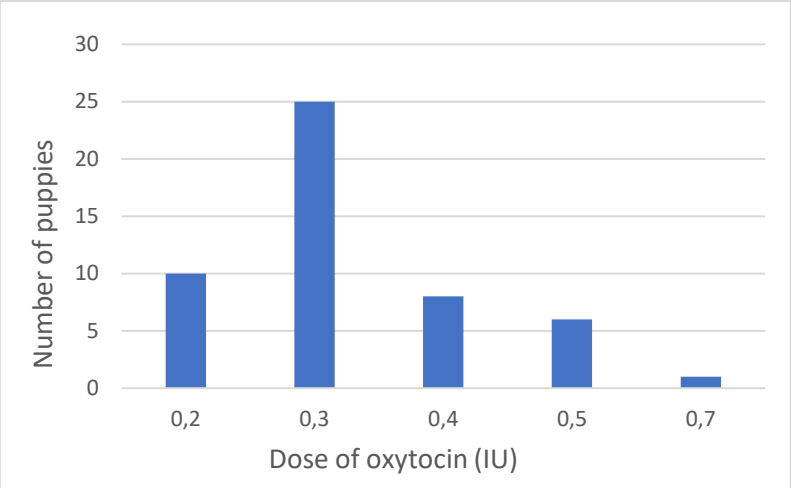

Figure 10. Distribution of quantities oxytocin given at second administration. IU, international unit.

Use of oxytocin in the period directly before birth of a puppy led to a significantly higher risk of stillbirths compared to births without oxytocin administration (*p*-value < 0.001). Figure 11 shows a large proportion of stillbirths in the group of puppies born after the bitch was administered oxytocin.

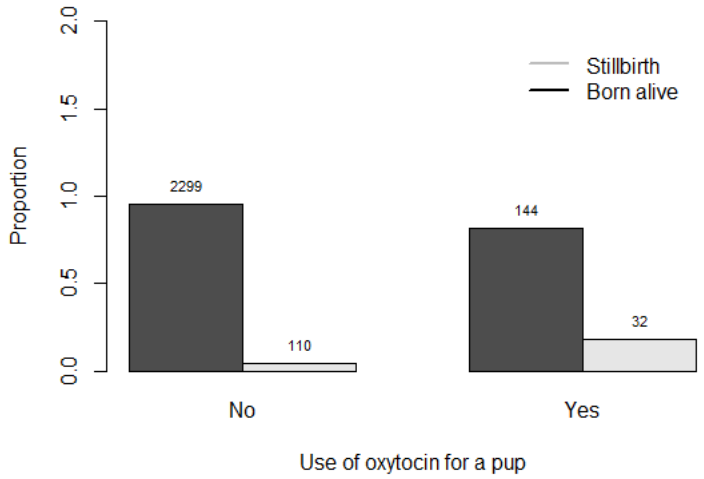

Figure 11. Diagram showing oxytocin use versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Moreover, a higher number of oxytocin administrations increased the risk of stillborn puppies significantly with 15%, 21% and 32% for first, second, or more oxytocin uses respectively (*p*-value < 0.001). This phenomenon is visualised in figure 12.

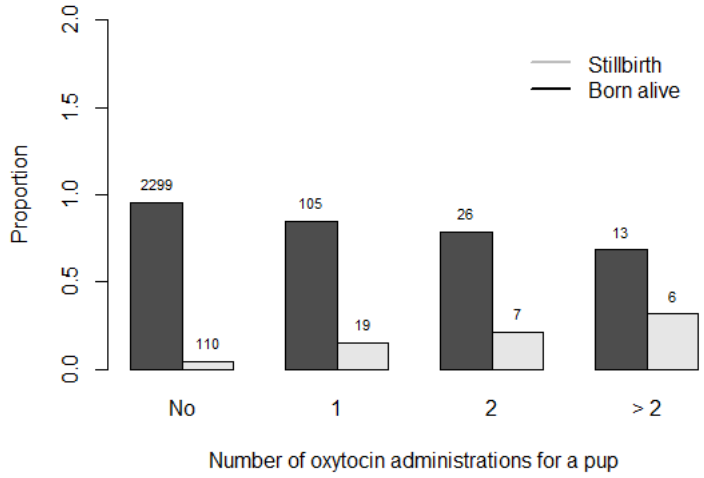

Figure 12. Diagram showing number of oxytocin administrations versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Manual obstetric assistance

In 33 out of the 331 parturitions, the bitches received manual obstetric assistance (number of puppies = 80). Four pups were born with the aid of uterine forceps. The proportion of stillborn puppies was larger in the group of puppies on which manual obstetric assistance was performed (Fig. 13) with a *p*-value of < 0.001.

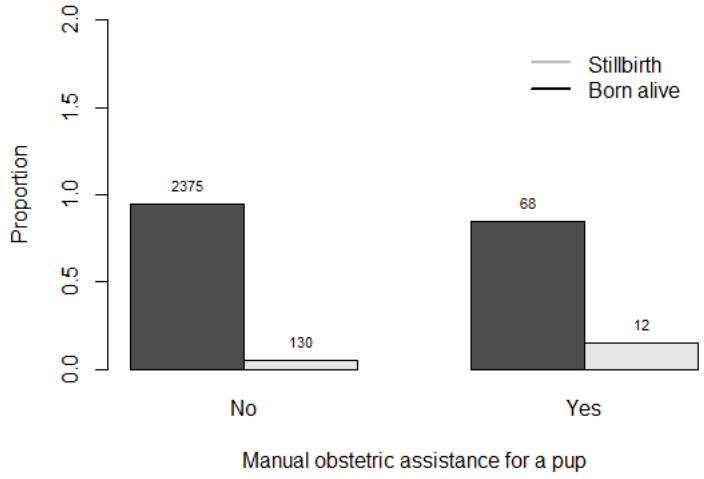

Figure 13. Diagram showing manual obstetric assistance versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Caesarean section

A total of 95 puppies were delivered via caesarean section, corresponding to 28 (8.5%) of all parturitions in the study. Two of the caesarean sections were elective caesarean sections for a singleton pup. To evaluate the effect of caesarean section on stillbirth, elective caesarean sections were excluded from this study. There is no significant association between caesarean sections and

stillbirths (*p*-value = 0.09). The proportion of stillborn puppies is smaller in the group of pups which were born through a caesarean section (Fig. 14).

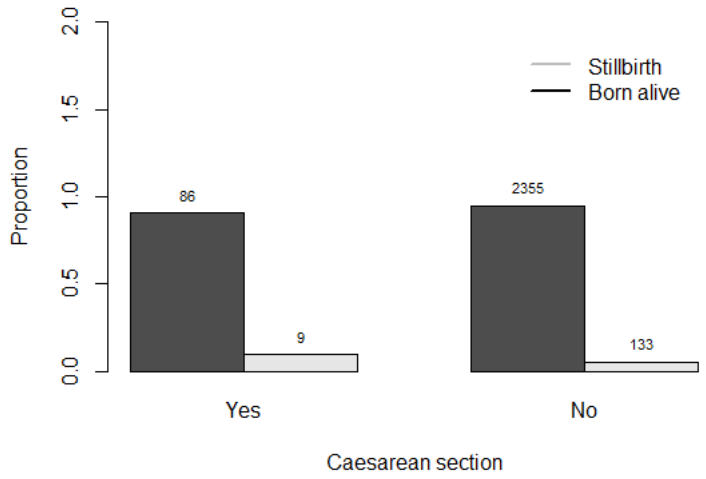

Figure 14. Diagram showing caesarean section versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Table 2. Interventions as risk factors for stillbirth. Variables screened by univariable analysis with *p*-values, odds ratios and 95% confidence intervals for stillborn and liveborn puppies. Ref, reference; Freq., frequencies; Prop., proportions.

Variable	Stillborn		Born alive		P-value	Odds ratio	Confidence interval
	Freq.	Prop.	Freq.	Prop.			
Digital vaginal exploration before the birth of a pup							
No	121	0.05	2385	0.95	Ref	1	Ref
Yes	21	0.20	85	0.80	< 0.001	4.8	2.8 – 7.9
Number of times digital vaginal exploration before the birth of a pup							
0	121	0.05	2358	0.95	Ref	1	Ref
1	11	0.17	53	0.83	< 0.001	4.0	2.0 – 7.7
2	6	0.21	22	0.79	< 0.001	5.3	1.9 – 12.6
> 2	4	0.29	10	0.71	< 0.001	7.8	2.1 – 23.7
Use of oxytocin before the birth of a pup							
No	110	0.05	2299	0.95	Ref	1	Ref
Yes	32	0.18	144	0.82	< 0.001	4.6	3.0 – 7.1
Number of oxytocin administrations before the birth of a pup							
0	110	0.05	2299	0.95	Ref	1	Ref
1	19	0.15	105	0.85	< 0.001	3.8	2.2 – 6.3
2	7	0.21	26	0.79	< 0.001	5.6	2.2 – 12.6
> 2	6	0.32	13	0.68	< 0.001	9.7	3.3 – 24.9
Manual obstetric assistance							
No	130	0.05	2375	0.95	Ref	1	Ref

Yes	12	0.15	68	0.85	< 0.001	3.2	1.6 – 5.9
Caesarean section							
Yes	9	0.09	86	0.91	Ref	1	Ref
No	133	0.05	2355	0.95	0.09	0.5	0.3 – 1.2

4.3.3 Pup factors

Time between first passage of foetal fluids via the vulva and the first pup

A summary of the results of the pup factors is shown in table 3 (see page 24), starting with evaluating the effect of the amount of time between the first passage of foetal fluids via the vulva and the birth of the first pup on the number of stillbirths. Caesarean sections were excluded from this evaluation. The time between first passage of foetal fluids via the vulva and the birth of the first pup was reported for 243 out of 331 parturitions. Eight of these pups (3.4%) were stillborn. The median time was 105 (range 0 to 1125) minutes. 50% occurred within 35 minutes after the first passage of foetal fluids via the vulva, 90% within 85 minutes and in 95% within 100 minutes. 11 (5%) of the 238 births occurred after more than 8.4 hours. One of these pups was stillborn. The time between breaking of the allantois and birth of the first puppy does not lead to a higher risk of stillbirths (*p*-value > 0.25). The proportion of stillborn puppies was slightly larger in the group of puppies born between 216 and 1200 minutes (Fig. 15).

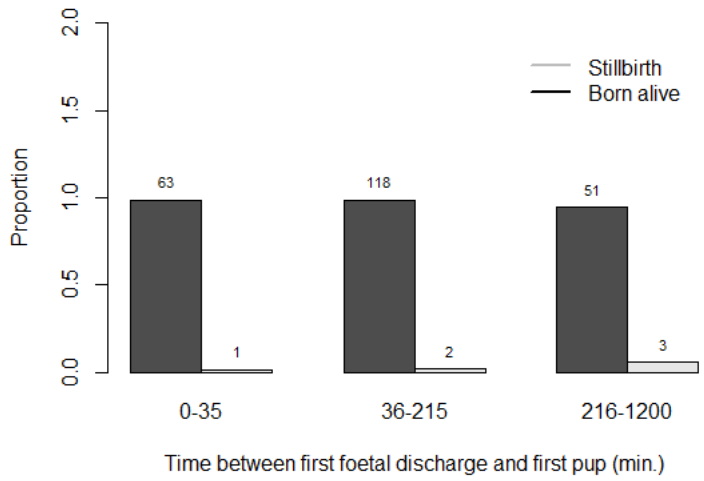

Figure 15. Diagram showing the time between the first passage of foetal fluids via the vulva versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies. Min., minutes.

Presentation at expulsion of the first pup

The percentage of stillborn puppies in relation to the presentation at expulsion of the first puppy could be calculated for 261 litters. In eight of 261 parturitions in which the presentation of the pups was evenly distributed (Fig. 16), the first puppy was stillborn. The relationship between presentation at expulsion of the first pup and stillbirth is not significant (*p*-value = 0.8).

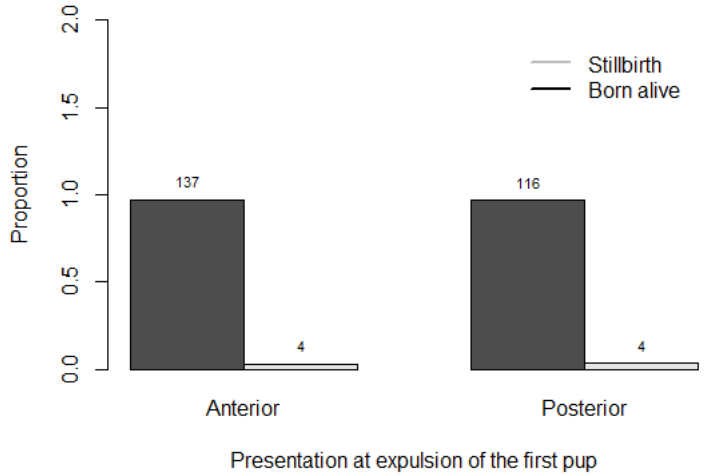

Figure 16. Diagram showing the presentation at expulsion of the first pup versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Presentation at expulsion of a pup

47.8% of the 2585 puppies were born in anterior presentation and 35.7% in posterior presentation. The presentation at expulsion was not recorded for 427 puppies (16.5%). The presentation at expulsion of a pup was significantly associated with stillbirths (*p*-value = 0.02). There was a higher proportion of stillbirths in groups of pups born with posterior presentation (Fig. 17).

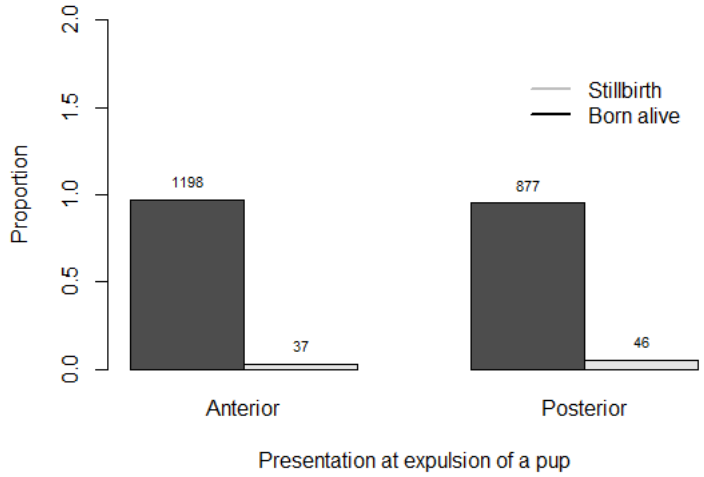

Figure 17. Diagram showing presentation at expulsion versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Pup born within an intact foetal membrane(s)

The ratio of instances where the placenta detached instantly versus instances where the placenta remained attached to the puppy was 1822:576 (76%:24%). Upon examining the relationship between the presence of the placenta of a pup and stillbirth, pups born with a placenta showed a higher proportion of stillbirths (4.0%) compared to pups born without a placenta (2.0%) (Fig. 18) with a *p*-value of 0.03.

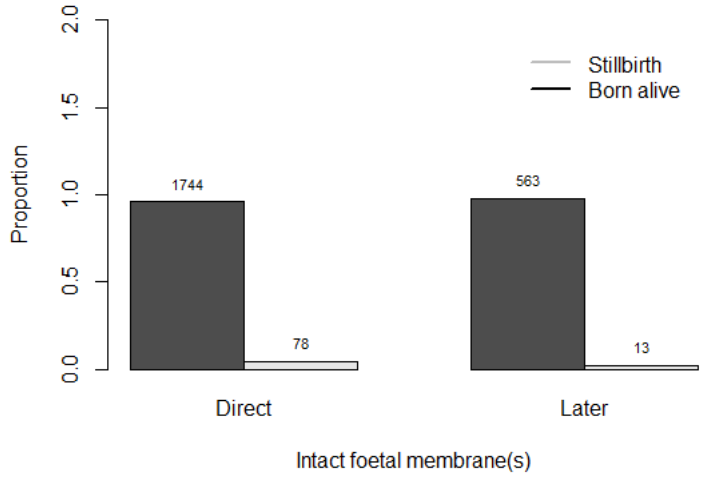

Figure 18. Diagram showing the moment of placenta expulsion versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Gender of a pup

Out of 2585 puppies in total, there were more male pups (n = 1316, 50.9%) than female ones (n = 1226, 47.4%). The gender of the puppy was not recorded for 43 puppies (1.7%). The gender of the puppy was not significantly associated with stillbirths (*p*-value = 0.09). The proportions were almost equal (Fig. 19).

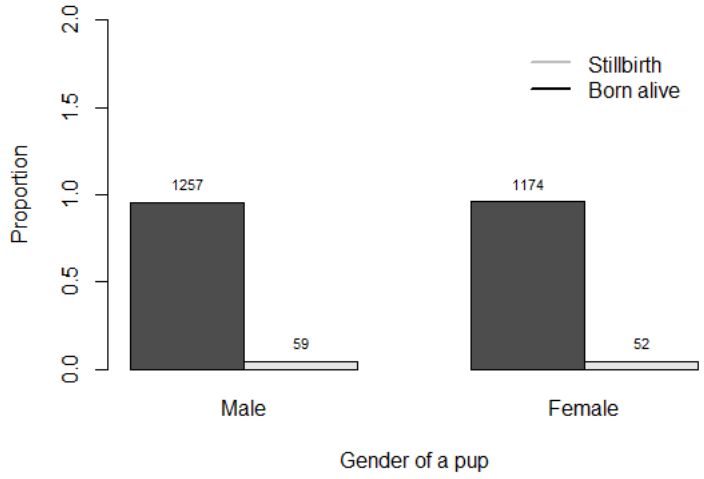

Figure 19. Diagram showing gender versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Birthweight of a pup

Pups with lower birthweight (< 390 grams) were not more likely to be stillborn (*p*-value = 0.06), neither were heavy pups (*p*-value = 0.22). The proportions of stillbirth were almost equal (Fig. 20).

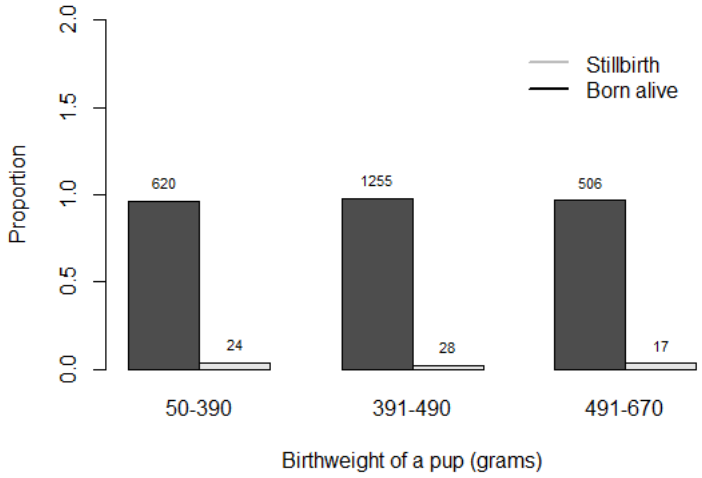

Figure 20. Diagram showing birthweight versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Birth sequence of pups

Litters consisting of one single pup were excluded. The highest stillbirth odds ratio (4.1) was found in puppies born last (*p*-value < 0.001). The proportion of stillbirth in this group is higher compared to the other groups (Fig. 21).

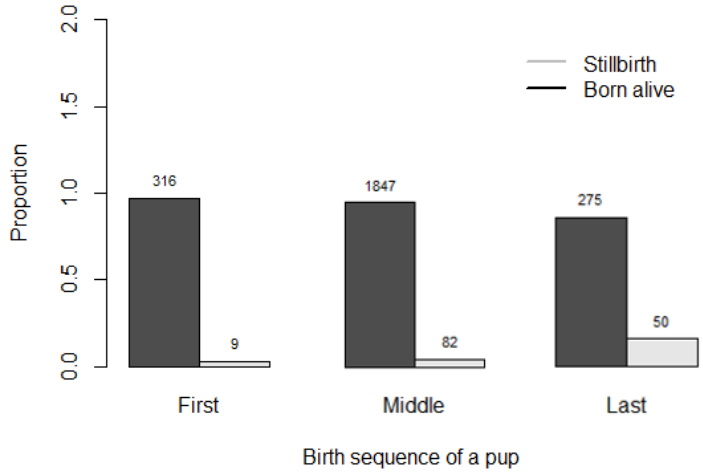

Figure 21. Diagram showing the birth sequence of pups versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies.

Inter-pup interval

In the evaluation of the effect of inter-pup interval on stillbirths, caesarean sections were excluded. Median and range of different inter-pup intervals per litter size is shown in Appendix 7.3. The risk of stillbirth increased when the interval time between pups was over 45 minutes (*p*-value < 0.001). This phenomenon is visualised in figure 22. There is an odds ratio of 20.5 in puppies with an inter-pup interval time over 4 hours. The relationship between stillbirth and inter-pup interval was not significant between 181-240 minutes.

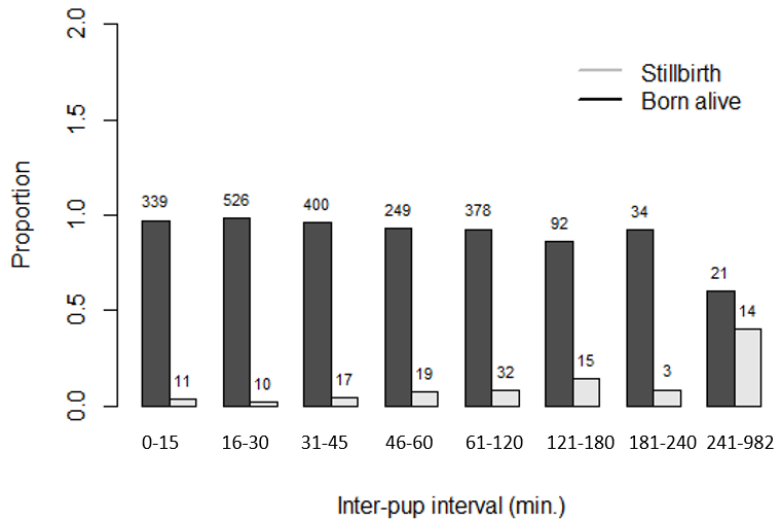

Figure 22. Diagram showing the inter-pup interval versus pups being born alive or stillborn. The numbers in the diagram represent the number of puppies. Min., minutes.

Table 3. Pup factors as risk factors for stillbirth. Variables screened by univariable analysis with *p*-values, odds ratios and 95% confidence intervals for stillborn and liveborn puppies. Ref, reference; Freq., frequencies; Prop., proportions.

Variable	Stillborn		Born alive		P-value	Odds ratio	Confidence interval
	Freq.	Prop.	Freq.	Prop.			
Time between first passage of foetal fluids via the vulva and first pup (minutes)							
0 – 35	1	0.02	63	0.98	Ref	1	Ref
35 – 215	2	0.02	118	0.98	0.96	1.1	0.1 – 23.2
215 – 1125	3	0.06	51	0.94	0.26	3.7	0.5 – 76.2
Presentation at expulsion of the first pup							
Anterior	4	0.03	138	0.97	Ref	1	Ref
Posterior	4	0.03	116	0.97	0.8	1.2	0.3 – 5.1
Presentation at expulsion of a pup							
Anterior	37	0.03	1198	0.97	Ref	1	Ref
Posterior	46	0.05	877	0.95	0.02	1.7	1.1 – 2.7
Pup born within an intact foetal membrane(s)							
Yes	78	0.04	1744	0.96	Ref	1	Ref
No	13	0.02	563	0.98	0.03	0.5	0.3 – 0.9
Gender of a pup							
Male	59	0.04	1257	0.96	Ref	1	Ref
Female	52	0.04	1174	0.96	0.77	0.9	0.6 – 1.4
Birthweight of a pup (grams)							
180 – 390	21	0.04	533	0.96	0.06	1.7	1.0 – 3.0
390 – 490	31	0.02	1342	0.98	Ref	1	Ref
490 – 670	17	0.03	506	0.97	0.22	1.5	0.8 – 2.6
Birth sequence of pups							

First	9	0.03	316	0.97	0.21	0.6	0.3 – 1.2
Middle	82	0.04	1847	0.96	Ref	1	Ref
Last	50	0.15	275	0.85	< 0.001	4.1	2.8 – 5.9
Inter-pup interval (minutes)							
0 – 15	11	0.03	339	0.97	Ref	1	Ref
15 – 30	10	0.02	526	0.98	0.23	0.6	0.2 – 1.4
30 – 45	17	0.04	400	0.96	0.49	1.3	0.6 – 2.9
45 – 60	19	0.07	249	0.93	0.03	2.4	1.1 – 5.2
60 – 120	32	0.08	378	0.92	< 0.001	2.6	1.3 – 5.5
120 – 180	15	0.14	92	0.86	< 0.001	5.0	2.3 – 11.6
180 – 240	3	0.08	34	0.92	0.14	2.7	0.6 – 9.2
240 – 982	14	0.40	21	0.60	< 0.001	20.5	8.4 – 52.0

5. Discussion and conclusion

In the present study, the overall occurrence of stillbirth was 5.5%. The variation in the instances of stillbirth (3.5% – 10.9%) reported by different studies is probably due to the number of animals, variety of breeds, study designs, the mode of arriving at the incidence and the geographical area from where the study was reported. For example, Tønnessen et al. (2012) did not include litters of which none of the pups survived, which may be an important confounder, especially in small litters. Research done by Bergström et al. (2006) is based on insurance datasets. These datasets can give insightful information on some related risk factors. However, the number of parturitions is not well captured in such datasets and they only include pups that were alive at the time of registration. Other studies utilised surveys (Gropetti et al. 2015; Indrebø et al. 2007; Linde-Forsberg and Persson 2007). As a result, some of the conclusions might not be entirely representative. For example, questions could have been misunderstood or the outcome of a parturition might have influenced the motivation to answer the questions in one way or another. Indrebø et al. (2007) included all pups at risk and the high incidence of stillbirths (10.9%) therefore may be due to more complete registrations of stillborn puppies, as the breeders signed a contract. Another factor could be the higher average litter size (7.6) compared to other studies: 7.0, 6.6 and 5.4 respectively (Groppetti et al. 2015; Linde-Forsberg and Persson 2007; Tønnessen et al. 2012). It is interesting to note that certain breeds are favoured among canine owners in certain countries; therefore a higher frequency of stillbirths recorded in these breeds may be due to the overall higher presence of dogs of these breeds in the study area, rather than due to breed predisposition.

5.1 Qualitative analysis

The purpose of the present study is to analyse the standardised parturition care and decision criteria used through qualitative analysis. The interviews were semi-structured. Semi-structured interviews allow the interviewer to ask follow-up questions when there are uncertainties or when more explanation is needed. Semi-structured interviews thus ideally result in a detailed conversations in which with a lot of information is given. The interviews were taken individually, so that employees were not influenced by the other employees of the KNGF. The number of interviews is low, because there were only four experienced staff members in total. Some interventions discussed during the interviews were not included in the statistical analysis, because a lot of data was missing or was not recorded. More information is necessary on when these interventions were performed as well: for example, the duration of and the reason for a walk with a bitch outside in order to stimulate parturition.

5.2 Univariable analyses

5.2.1 Maternal factors

Stillbirth risk increased with 2.35 times in pups from litters with dystocia compared to pups born in litters born without dystocia (Cornelius et al. 2019). Gill et al. (2002) found that foetal asphyxia was a significant cause of stillbirth and concluded that half of the number of stillborn puppies was likely caused by dystocia. Several studies have documented a significant effect of the parity of the bitch on the incidence of dystocia (Darvelid and Linde-Forsberg 1994; Linde-Forsberg and Persson 2007; Oluwatoyin and Fayemi 2011). Tønnessen et al. (2012) found a significant effect of parity on stillborn puppies. The results of the present study are in contradiction with those studies. However, the questionnaires used in Linde-Forsberg and Persson 2007 did not request information on previous litters and there were too few bitches giving birth to more than one litter during the studied period by Linde-Forsberg and Persson (2007). Besides, Cornelius et al. (2019), who were the first to include a large dataset and a control population of litters without dystocia events or stillbirths, found no relationship between parity and stillbirth (Darvelid and Linde-Forsberg 1994; Oluwatoyin and Fayemi 2011). Gill et al. (2002) found that stillbirths between parity one and four were relatively constant, but stillbirth rates increased after the fourth parity. This could explain why parity was not a significant

variable in the present study, because the bitches of the KNGF generally give birth to four litters during their lifespan. Another reason could be that the present study did not evaluate parity as a continuous variable, but only as a categorical variable. A second parturition is possibly easier due to an experienced bitch and a faster delivery, resulting in lower stillbirth rates compared to a first parturition. In addition, the experienced staff of the KNGF had knowledge of the bitch's history, allowing them to respond more swiftly to minimise the risk of stillbirth.

The frequency of stillborn puppies in the present study was found to be highest in bitches aged 6 - 8.5 years, but it was not significant. Groppetti et al. (2015) and Gill et al. (2002) also did not find a significant relation between the age of bitches and stillbirths. Tønnessen et al. (2012) showed that the risk on stillbirth increased by 22% for each one year increase in the bitch's age. The different study populations may explain this divergence in the relationship between stillbirth and maternal age. While stillbirth rates increase with maternal age, older bitches with good reproductive histories may still be bred from – 44 bitches were over six years of age in the present study. This could have influenced the results of the present study. Besides, stillbirth may have been influenced by the performance of caesarean sections, assistance of people or veterinarians during the parturition, and selection of the bitches. The same factors have most likely influenced the present study, but probably in a different way. However, older primiparous bitches (> six years of age) have a higher risk of having stillbirths compared to young primiparous bitches, because they have more uterine disorders and prolonged parturitions (Münnich and Küchenmeister 2009). This reinforces the recommendation not to breed from bitches over the age of 6 for the first time. The bitches of the KNGF are bred on a regular basis and the age at any given parity would not differ greatly among bitches. None one of the bitches were primiparous in the group aged 6-8.5 years and none one of the bitches were multiparous in the group aged 0-2 years. However, this was not evaluated in separate models in the current study.

In the current study, litter size was not correlated with stillbirths. This is not in agreement with other studies (Cornelius et al. 2019; Indrebø et al. 2007; Tønnessen et al. 2012). A litter size of 11 or greater was at increased risk of stillbirth (Cornelius et al. 2019; Tønnessen et al. 2012). A large litter may overstretch the uterus, reducing its ability to contract during parturition (Bennet 1974), and increase the likelihood of exhaustion (Münnich and Küchenmeister 2009). Only singleton and twin pregnancies were predisposed to dystocia and thus stillbirth, because of insufficient uterine stimulation and large pup size (Darvelid and Linde-Forsberg 1994). The results of Gill et al. (2002) indicated that perinatal mortality rates (the combined loss of puppies due to stillbirths and those liveborn puppies dying in the first seven days after birth) was highest at litter sizes of one and two. On the score of Gill et al. (2002) it is recommended to perform an elective caesarean section for a singleton pup in a large breed, such as the Labrador Retriever. After singleton and twin pregnancies, litter size was not an important predictor of the proportion of stillbirths within a litter in Gill et al. (2002). Also, Gropetti et al. (2015) did not record a significant relation between litter size and stillbirth. This may be due to the large range of breeds and sizes of animals included in these two studies, but this does not apply to the current study. The use of groups of litter size instead of litter size as a continuous variable could explain this.

5.2.2 Management factors

According to protocol, the time between two digital vaginal explorations should be 30 minutes. However, the staff of the KNGF generally performed more digital vaginal explorations and waited longer than 30 minutes between digital vaginal manipulations. Digital vaginal explorations were significantly associated with stillbirths. However, it is also possible that many puppies have been saved by vaginal digital manipulation as it may stimulate the Ferguson reflex. The Ferguson reflex results in an increase of circulating oxytocin and intensifies uterine contractions by massaging the dorsal vaginal wall. The members of the KNGF performs it correctly. Digital vaginal exploration is done only in case of an abnormal parturition. A puppy in this situation already has a higher risk of being stillborn. Besides, a pup could have died already while digital vaginal exploration is being carried out.

The present study showed that treatment with oxytocin was associated with stillbirth. Also, as the number of oxytocin administrations increased, the risk on stillborn pups increased as well. Indrebø et al. (2007) suggest that multiple oxytocin injections to a bitch with a lot of unborn puppies result in poor prognosis for the remaining puppies, though they do not provide any statistical analysis to support their claim (Indreb et al. 2007). For example, they report of an incidence where there was one bitch who received numerous oxytocin injections and had four stillborn puppies in a litter with a total of 12 puppies. Two remaining dead pups were removed via a caesarean section 24 hours after the birth of the first puppy (Indrebø et al. 2007). In humans, the foetal lactate level is an objective indicator of foetal distress, because high levels of lactate documents the use of secondary oxygenation pathways due to hypoxic events occurring during parturition (Armstrong et al. 2006; Blickstein and Green 2007). In Groppetti et al. (2010), distressed puppies had higher lactate levels compared to lower values in vigorous puppies. Foetal distress can be a consequence of dystocia and the hypoxic-ischemic effect of the uterine contractions on placental vessels. Besides, low Apgar scores were associated with high lactate concentrations. The Apgar score is used to evaluate the viability of new-born puppies, using behavioural parameters such as heart rate, respiratory effort, reflex irritability, motility, and colour of mucous membranes. Higher lactate values and low Apgar scores were detected in pups which had received oxytocin (Groppetti et al. 2010). Employees of the KNGF generally gave two doses of oxytocin. According to Münnich and Küchenmeister (2009), medical management fails when a total of 2-3 doses of oxytocin do not result in a puppy being born despite strong uterine contractions. In these cases, a caesarean section is necessary. Literature shows a widespread belief that oxytocin may induce premature placental separation. This idea seems to have originated from a statement made by Freak (1962). This study claimed that “oxytocin will speed up separation of foetal membranes and where it is being used late in the parturition it is wise to be prepared for assisted delivery without delay if natural delivery is not complete within 15 or 20 minutes of the time of injection”. This statement echoes throughout the literature and many studies advise a caesarean section if 2-3 doses of oxytocin at a 20-30 minutes interval fail to cause expulsion of a puppy (Gaudet 1985; Indrebø et al. 2007; Lopate 2012; Münnich and Küchenmeister 2009). However, placental detachment through oxytocin and dose-response studies are not available. This is a sign that more research is needed on medical therapy in bitches.

The present study found a relation between manual obstetric assistance and stillbirth. Münnich and Küchenmeister (2009) found more hypoxic and dead puppies in litters with moderate or severe traction of puppies compared to litters with slight traction. However, most puppies were already dead when extraction was needed and the expulsion duration in litters with severe extraction was higher. The duration of expulsion may have been a determining factor in stillbirth rates. In Münnich and Küchenmeister (2009), extraction of a puppy was indicated in cases where malpresentation or malposition of a foetus could be corrected, with only slight relative oversize, and in parturitions of bitches with uterine inertia, which did not respond to medical treatment while the puppy was within reach. These results correspond with the results of the present study. 105 puppies received manual obstetric assistance in the present study. In contrast with the statistical results, it is reasonable to believe that many stillborn puppies could have been saved if manual obstetric assistance had been given. Following the observation by Münnich and Küchenmeister (2009), many pups from the present study may have been already dead when manual delivery was carried out. Death of the pup was then likely the reason why manual extraction was necessary and not the result of manual extraction.

There is no association between caesarean sections and stillbirths in the present study. Veronesi et al. (2009) also found no significant differences in the type of delivery of puppies that were stillborn versus puppies that were born alive. However, this contrasts with the observations made by Cornelius et al. (2019). In this study, puppies born from litters where a caesarean section was performed were three times more likely to be stillborn compared to puppies from litters that were born via normal parturitions. Failed medical management showed stillbirths rates of over 12%, which is higher than the

stillbirth rates in parturitions where a caesarean section was performed without trying medical management first (Cornelius et al. 2019). It is likely that higher stillbirth rates were due to the severe dystocia that required the caesarean section in the first place, rather than the surgical procedure itself.

The staff of the KNGF decided to perform a caesarean section if the interventions were not successful. These interventions included: digital vaginal exploration, oxytocin administration, massaging the bitch, walking the bitch, taking the bitch out for a car ride, and letting the puppies drink milk from the bitch. Many of these interventions were taken based on the experience of the KNGF staff and are not included in the protocol. The vitality of the pups during a caesarean section would not be influenced when well-established anaesthetic and neonatal resuscitation protocols were used. However, anaesthetic drugs did influence the surgical procedure. The use of isoflurane and avoidance of ketamine and thiopental results in a higher neonatal survival rate (Moon-Massat and Erb 2002). Indrebø et al. (2007) suggests that the deaths of five puppies in a litter of seven were probably caused by a caesarean section performed too late. The study also advises to perform a caesarean section if the uterine contractions are still poor after a couple of injections of oxytocin and when several unborn puppies are still in the uterus (Indrebø et al. 2007).

5.2.3 Pup factors

Wallace and Davidson (1995) suggested that stage II starts when the amniotic sac is passed through the vagina and protrudes as a water bag between the lips of the vulva, which occurs shortly after the rupture of the allantochorionic membrane. The first puppy is usually born within four hours after the onset of stage II (Wallace and Davidson 1995). Mosier (1986) reported that it happens within 20-30 minutes. The present study showed an average time less than four hours between the first passage of foetal fluids via the vulva and the birth of the first pup, even when caesarean sections were performed. 44 parturitions and three caesarean sections exceeded the four hours. Nevertheless, the present study showed no positive relation between stillbirth and the time between the first passage of foetal fluids via the vulva and expulsion of the first pup. According to research, an abnormal parturition occurs when after a time period of more than 2-4 hours after expulsion of the first chorioallantois nothing else further occurs (Drobatz 2005; England and Heimendahl 2010a; Ettinger and Feldman 2010). However, studies on it are not provided. Linde-Forsberg and Persson (2007) found an average time between breaking of the allantois and birth of the first puppy of 1.9 hours in all parturitions (n = 96) and an average time of 1.5 hours in the group of bitches not in need of veterinary treatment (n = 72), with a range of 0 minutes to 12 hours. Unfortunately, the percentage of stillborn puppies in relation to this variable was not calculated in this study. The current study is the first to evaluate the time between the first passage of foetal fluids via the vulva and birth of the first pup. The study shows there can be a longer period of time before the first puppy is born without the risk of it being stillborn, but every parturition is different and parturition care in these cases remains important.

In the last 14 days of gestation, canine foetuses can change presentation. Puppies can be born in anterior (60%) or posterior (40%) presentation (Van Der Weyden et al. 1981). However, it is likely that this statement has also been echoed in the literature. Many authors consider posterior presentation to be a normal variation in dogs (Drobatz 2005; England and Heimendahl 2010a; Ettinger and Feldman 2010). In contrast to anterior presentation, posterior presentation accounted for more stillbirths in the present study. Groppetti et al. (2010) found higher lactate levels in pups that were in posterior presentation, but without statistical evidence. However, the Apgar score was significantly related to pup presentation, with lower Apgar scores in puppies with posterior presentation. A hypothesis could be that expulsion is more difficult because the puppy is being pushed forward while the foetal chest is distended instead of compressed by the pressure of its abdominal organs, or the elbows get caught more easily around the pelvic brim (Ettinger and Feldman 2010). In Gill et al. (2002), posterior presentation was related to higher neonatal mortality due to foetal asphyxia. However, the breed group analysis of the study showed that presentation at expulsion was a significant predictor of

neonatal mortality in the toy breeds only. It is suggested that in toy breeds with relatively large puppies, the canine foetuses are prevented from changing presentation because there is not enough space. As a result, more puppies were born in posterior presentation in toy breeds (Gill et al. 2002). When the first pup is in posterior presentation, an inadequate Ferguson's reflex and mechanical dilatation of the cervix may result in an abnormal parturition (Ettinger and Feldman 2010; Lopate 2012). This is a statement made by many authors, but studies on it are not available. The present study found no correlation between stillbirth and the presentation of the first puppy.

There are no reports on the influence of the direct presence of the pup's placenta during expulsion on the incidence of stillbirths. Gill et al. (2002) found that the odds ratio of neonatal mortality due to foetal asphyxia in toy breeds decreased when the pup was born with the placenta attached. In contrast, the present study found an increase in stillborn puppies when the puppies were born with the placenta attached. A higher stillbirth rate would be expected for puppies whose placenta had already detached from the endometrium than for puppies whose placenta was still attached. Immediate expulsion of the placenta could indicate that the placenta was already detached intra-uterine. The physiology of canine placental detachment is not yet well understood (Romagnoli et al. 2004). Canine placental detachment may occur even in the presence of high progesterone concentrations. This suggests that the placenta may be predetermined to last for about 45-50 days after its formation (Irons et al. 1997). If this is true it might indicate that the placentas of the first puppies had already reached their final maturation while the placentas of the final puppies had not, implying an asynchronous foetal development in studies in which a long inter-pup interval was found. An example of this is mentioned in a case report by Romagnoli et al. (2004), in which an inter-pup interval of 34 hours was described.

It was observed that gender had no influence on stillborn rates in the present study. Three other studies investigated the relation between gender and stillbirth as well and found no relation (Cornelius et al. 2019; Gill et al. 2002; Groppetti et al. 2015).

In the present study, all the pups with a birthweight < 200 gram were stillborn. In the present data set one puppy weighing only 50 grams was included, which could be considered an extreme value. This was a stillborn puppy and only the skull was recognisable. Plotting the leverage against the normalised residual squared clearly showed that it did have a large residual and that it had great impact on the estimates of the regression coefficient. Firstly, low birthweight is related to the proportion of stillborn puppies, but showed no further significant effect on stillbirth when this puppy was excluded from the data. In contrast, three other studies found a relationship between low birthweight and stillbirth (Cornelius et al. 2019; Gill et al. 2002; Tønnessen et al. 2012). For the purpose of this study, the low birthweight group contained pups with a birthweight lower than 390 grams. Gill et al. (2002) assume that pups below one standard deviation lower than the mean birthweight were growth retarded. However, compared to Gill et al. (2002), the present study takes pups into consideration that had an even lower birth weight. It is plausible that even a normal parturition, in itself a hypoxic stimulus, is a more stressful event in these fetuses because they have limited reserves. In human medicine the term Intra-Uterine Growth Retardation (IUGR) is used to describe the clinical entity 'low birth weight for gestational age'. There are 14% more stillbirths in infants with a birth weight less than the third percentile for gestational age (Renfield 1975). Further research is necessary to evaluate the extent of growth retardation and its contribution to stillbirths in dogs. Low birth weight did not affect mortality at birth in Groppetti et al. (2015). Contribution of stillborn pups with malformations involving an increase in birth weight (i.e. anasarca) can justify this finding (Groppetti et al. 2015). Besides, it is also plausible that small puppies pass more easily through the birth canal. In Cornelius et al. (2019) and Gill et al. (2002), the heavier puppies were at an elevated risk of being stillborn, which may be due to the increased risk of obstruction during parturition (Dolf et al 2018). This phenomenon was not seen in Groppetti et al. (2015) and in the present study. It is plausible that a heavier puppy is a greater physical challenge to the bitch than a lighter puppy. The influence of the experienced staff of the KNGF has to

be taken into account, because they may rescue a heavier puppy more rapidly based on experience or make the decision to perform a caesarean section. In Dolf et al. (2018), the weight of the heaviest puppy had an impact on the probability of the occurrence of a caesarean section in Labrador Retrievers. However, in the present study, only 26 (5.0%) pups out of a total of 523 heavier pups were born via a caesarean section. All these pups had a birth weight over 490 grams. The relationship between birth weight and caesarean section should be further investigated, but this is beyond the scope of this report.

The sequence in which the puppies were born showed a relation with the occurrence of stillbirth in the present study. The risk of stillbirth was highest for the last-born pup in a litter. This is in agreement with Cornelius et al. (2019). Gill et al. (2002) found the highest perinatal mortality for last-born puppies at each litter size in the large breed group. An explanation for the high mortality rates in last-born pups would be prolonged exposure to intermittent periods of anoxia, which occur during each uterine contraction. When foetal asphyxia was the cause of stillbirth, an increase in the number of stillborn puppies with each successive puppy in the litter was expected. This has been studied in Gill et al. (2002), but not in the present study. However, an increased mortality rate did not occur in Gill et al. (2002) and therefore other factors must also be taken into account.

The risk of stillbirth increased when the inter-pup interval was over 45 minutes in the present study. The *p*-value is not significant for the pups with an inter-pup interval between 180-240 minutes, probably because the number of stillbirths was smaller in this group. The optimal threshold for inter-pup interval to detect stillbirths in Cornelius et al. (2019) was 62 minutes. In Gill et al. (2002), the average inter-pup interval was less than 60 minutes and there was an increase in stillbirths when the inter-pup interval was longer than 90 minutes. Also, only stillborn puppies were born when the inter-pup interval was above 4 hours. All three measurements were lower than the 2-4 hours typically accepted as normal (Drobatz 2005; England and Heimendahl 2010a; Ettinger and Feldman 2010). It is important to remember that even though the risk of stillborn puppies increases with prolonged inter-pup interval, 60% of puppies with an inter-pup interval above four hours were born alive in the current study. However, in the study of Münnich and Küchenmeister (2009), the neonatal mortality rates were significantly lower when detection of obstetrical problems and obstetrical aid started within the first six hours after the beginning of stage II. Long inter-pup intervals are frequently observed and intervals of 34 hours have been reported. A possible explanation for this might be that uterine contractions occur mainly around the fetus that is most proximal to the cervix. For this reason, the placentas of the remaining fetuses remain undisturbed for some time (Romagnoli et al. 2004). Pups born after a prolonged vaginal delivery displayed statistically higher lactate values and lower Apgar scores at birth (Groppetti et al. 2010).

The present study has analysed stillbirth at individual puppy level, where the outcome variable is the proportion of stillborn pups in total. Tønnessen et al. (2012) used an outcome with the presence or absence of puppy loss in each litter. This measurement is probably influenced by the number of pups per litter. The rate of stillbirths will be lower in a large study like Tønnessen et al. (2012) when a small number of litters with a high rate of stillborn puppies will have less influence on the prevalence of stillborn puppies in the entire population. The amount of variation present at breed level was only 9% for stillbirths and therefore more than 90% of the variation was found at individual litter level in Tønnessen et al. (2012). In another study, genetic factors contributed 14% to the total variation in overall neonatal mortality, while litter and puppy factors explained 17% and 69% respectively (van der Beek et al. 1999). The practical implication of this result is that interventions aimed at reducing neonatal mortality should focus on the individual puppy rather than the litter itself.

5.3 Strengths and weaknesses

Guide dog facilities, such as the KNGF, are an important data source, because they offer data on the defined genetic backgrounds of the dogs. These dogs are generally healthy and part of a homogeneous population. Besides, the parturitions were supervised and therefore histories of both the parturition and the clinical condition of the pup at birth were available, thanks to exquisite record keeping through forms. The forms were filled in immediately during the parturition. The large and comprehensive dataset with complete control denominator of parturitions without dystocia and pups born alive and stillborn is unique. The quality of subjects was sufficient, which allows accurate identification of odds ratios that are associated with canine stillbirth presented in this study. Thus, the results should be reliable and representative for guide dogs in the Netherlands, and possibly also for similar populations in other countries.

This study has potential limitations. The primary limitation is that the results are specific to the population of dogs taken from one guide dog facility. Weaknesses include the use of a small breed variety, which results in poor generalisability of the results for each dog. The KNGF influenced the age and frequency at which bitches were bred and intervention methods by the staff or veterinarians had a great influence on parturitions. Instances of caesarean sections were included in most of the data, because otherwise there would be considerably less data on oxytocin use (as oxytocin is always injected in parturitions with non-obstructive dystocia as a last resort before making the decision to perform a caesarean section). This information should be considered when looking at the results. Caesarean sections did not have the same effect on the number of stillbirths as oxytocin use. Out of the interviews it became clear that the decision to perform a caesarean section would be made when the number of pups left in the uterus was still relatively high, whereas when there was only a single pup left in litter sizes above two puppies, multiple oxytocin injections would be given.

For some variables, the proportion of missing data was very high, especially for those relating to digital vaginal exploration, manual obstetric assistance, reasons for oxytocin use, and interventions not in accordance with the protocol that were used to stimulate the parturition. There can be several reasons for this. The questionnaires filled in at the time of parturition, which were analysed for the purpose of this study, sometimes lacked information: e.g. some questions were left unanswered or answers were marked with a dash, which meaning is unknown. Moreover, the specific times at which doses of oxytocin were injected were often unknown. The same goes for the specific dosages. From the interviews held with staff members of the KNGF it became clear that the administration of these details has not been given top priority lately. Because the parturitions took place in the foster homes, sometimes the experienced staff of the KNGF arrived too late or mistakes were made in distinguishing between foetal fluids and urine via the vulva. However, this could also occur in an actual veterinary practice, and therefore it is a good representation of reality. There were also instances in which discharge of amniotic fluids was reported twice, but only the first record was included in the calculations made in this study.

The online database Tommy was used for data collection, although the data reported by Tommy was not always faithfully represented. Data found in Tommy sometimes differed from the data collected by the KNGF. This is due to the fact that incidences of stillborn pups were initially not reported, because the registration of stillborn puppies was considered to be of no interest to the internal management of KNGF (as these puppies will not be trained). Sometimes stillborn pups were included, even though they were not mentioned in the report itself. Also, when a pup was born before 12 AM, Tommy sometimes listed the next day as the parturition date. In some cases, four litters were mentioned for a bitch, but only three reports were included.

Moreover, there is a black box of data that was not included in this study, because it contained too little information. Reports included in this study date back to 1998. However, some reports written in

1998 were missing, because these were still handwritten reports. For example, in some cases, only reports of a bitch's second litter are included, and the reports on the first litter are missing. This study did not consider the relation between year and oxytocin use, even though the data shows that in the past, oxytocin was given more frequently as well as in higher dosages.

The present study is a retrospective study without a clear control group containing abnormal parturitions that were not interfered with, but that would not be ethical. Another limitation of retrospective studies is that they are longitudinal and include unsystematically gathered data, acquired at different times.

5.4 Recommendations and conclusion

Neonatal mortality can occur in utero, during parturition, after birth, in the first weeks of life, or after weaning. The number of dead puppies is highest during parturition, immediately after birth, and in the first days of life. Stillbirth rates were 7.4%, accounting for 54.8% of the mortality (from date of birth to date of sale) in Chastant-Maillard et al. (2017). One important factor in neonatal mortality after parturition is birthweight, because lighter pups show higher neonatal mortality rates and lower Apgar scores than heavier pups (Groppetti et al. 2010). Pups with an Apgar score between 4-6 compared to puppies with Apgar score between 7-10 had a higher risk of dying (Veronesi et al. 2009). Perhaps the use of Apgar evaluations may result in a lower percentage of neonatal mortality rates. The Apgar evaluation requires just a few minutes and does not interfere with maternal grooming in puppies. There are many other predisposing factors to neonatal mortality such as dystocia, type and timing of intervention during parturition, in-breeding, malformations and genetic defects, diseases and vaccinations of the mother, environmental conditions, and infectious agents (Münnich 2008). It is important to know which of these factors influence stillbirth and neonatal mortality. In the present study, no pathological analyses of dead pups were performed so the causes of stillbirth are unknown. A follow up study, which includes neonatal mortality, is necessary because the present study only includes stillbirths. Also, further research is needed on the risk of neonatal mortality if a litter contains stillborn puppies, because neonatal mortality doubled in litters with stillborn puppies in Tønnessen et al. (2012).

The results indicate that numerous factors can contribute to stillbirth, although this is not conclusively proven through a multivariate analysis. The results of this study can serve as a basis for further research. The results of this study can also be used to establish a better adapted parturition protocol. The use of an evidence-based parturition protocol will facilitate decision making regarding adequate interference if the parturition is considered to be abnormal and will most likely result in a reduction of stillbirth and neonatal mortality.

In conclusion, this large-scale retrospective observational study revealed several important findings with regard to stillbirth in guide dogs. A low stillbirth rate of 5.5% was found. Qualitative analysis showed that the experienced staff members of the guide dog facility use a short and structured parturition protocol which they do not always strictly follow. Statistical univariable analyses showed that digital vaginal exploration, use of oxytocin, manual obstetric assistance, presentation, placenta attachment, the pup's place in the sequence of births, and inter-pup interval were all correlated with an increased risk of stillbirth. Stillbirth was not influenced by maternal age, maternal parity, litter size, caesarean section, time between first passage of foetal fluids via the vulva and first pup, presentation of the first pup, gender, and birthweight. The results of this study provide evidence on the influence of certain factors. This information is valuable to gaining a more detailed understanding on parturitions in dogs and to make informed decisions during a parturition. To reduce the number of stillbirths, the results of the present study indicate that: 1) good parturition care at individual puppy level is important; 2) the age of the bitch may be correlated with stillbirth rate; 3) more research is needed on oxytocin therapy in bitches; 4) extra close monitoring is required for pups born with posterior

presentation, pups born with the placenta attached, and the final pup in a litter, as these puppies showed a higher risk on stillbirth; 5) the interval between the birth of a pup and a intervention needs to be reconsidered.

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7. Appendix

Appendix 7.1 – Parturition protocol of the KNGF

When bitches had made straining movements regularly and intensely for 45 minutes prior to whelping of the first puppy or for 30 minutes prior to any subsequent puppy without any externally visible progress, digital vaginal exploration would be performed. When bitches had shown only non-intense, abdominal straining efforts for 1½ – 2 hours, digital vaginal exploration would also be performed. During vaginal exploration, additional straining was evoked by massaging the dorsal vaginal wall. When a puppy could be felt inside the birth canal, gentle manual traction would be exerted on the pup to aid the birth. When there was no pup present in the birth canal, a second digital vaginal exploration would be take place after 30 min. When at that time a puppy had advanced towards or had entered the birth canal, the bitch was left again for 30 minutes, after which, if no pup had been expelled, the bitch would be administered with 2 I.U. oxytocin sc (Intervet Nederland B.V., Boxmeer, the Netherlands). However, if the pup had not advanced in caudal direction upon the second exploration, bitches were treated with 2 I.U. oxytocin sc without delay. This procedure would be repeated after 45 minutes if no pup had been expelled. Forty-five min thereafter, a course of 2 I.U. oxytocin treatments would be started, given every 1½ – 2 hours until the birth of the next puppy.

Appendix 7.2 – Parturition form of the KNGF

KNGF GELEIDE HONDEN

10 VERSLAG BEVALLING

Teef	:	.....
Reu	:	.....
Fokgastgezin	:	.....
Nest nummer (v. deze teef)	:	.....
Nestletter	:	.....
Dekdatum	:	.....
Hoeveelste dag v. loopsheid	:	.....
Aantal dekkingen	:	.....
Koppeling	:	ja / nee

Teef onrustig dag	:	.....
Hijgen	:	ja / nee dag .....
Graven	:	dag .....
Likken	:	.....
Vaker defaeceren	:	.....
Vaker urineren	:	.....
Niet willen eten	:	.....
Ontsluitingsweeën	:	.....
Bijzonder gedrag	:	.....
Eerste perswee	:	.....
Verlies vruchtwater	:	.....

Temp. staat laatste 3 dagen voor bevalling:

.....	.....	.....
.....	.....	.....

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Bevalling en nestverzorging

KNGF GELEIDE HONDEN

....

Verloop voortekenen : .....
.....
.....
.....

Bijzonderheden : .....
.....
.....
.....
.....
.....
.....

KNGF GELEIDE HONDEN

10.1 Checklist bevalling

Onderwerp	Ja / nee	datum	Opmerkingen
Eerste Perswee-Tijd			
Pups geboren: Nummer:			
• Tijd			
• Geslacht			
• Ligging			
• Gewicht			
• Kenmerken			
• Kleur bandje: Plaats nagellak:			
Geboorte placenta			
Teef placenta opgegeten			
Teef zelf vlies verwijderd + navelstreng doorgebeten			
Complicaties: ja/nee, welke			
Tijd verstreken sinds geboorte laatste pup:			
Komst dierenarts gewenst: Reden:			
Opmerkingen:			

Appendix 7.3 – Table inter-pup interval

Litter size	Lit (N)	CS (N)	SB (N)	M + R 1-2	M + R 2-3	M + R 3-4	M + R 4-5	M + R 5-6	M + R 6-7	M + R 7-8	M + R 8-9	M + R 9-10	M + R 10-11	M + R 11-12	M + R 12-13	M + R 13-14	M + R 14-15
2	5	2	0	03:05													
				01:39													
				03:55													
				N = 6													
3	2	0	0	04:12	01:37												
				00:20	00:50												
				08:05	02:25												
				N = 6	N = 6												
4	12	2	5	0:55	01:00	01:00											
				00:02	00:10	00:15											
				02:50	06:15	04:05											
				N = 11	N = 11	N = 10											
5	23	1	4	00:50	01:00	01:00	01:04										
				00:10	00:10	00:16	00:10										
				03:02	05:15	03:45	05:45										
				N = 23	N = 23	N = 23	N = 22										
6	32	2	12	00:55	00:40	00:55	00:40	00:55									
				00:10	00:10	00:10	00:10	00:10									
				05:33	02:50	04:10	04:15	16:22									
				N = 31	N = 31	N = 31	N = 31	N = 30									
7	51	1	13	00:40	00:37	00:30	00:50	00:50	01:02								
				00:07	00:05	00:02	00:06	00:10	00:07								
				03:10	04:05	02:20	03:00	05:05	06:10								
				N = 51	N = 51	N = 51	N = 50	N = 50	N = 50								
8	67	5	27	00:40	00:35	00:35	00:35	00:40	00:38	00:50							
				00:05	00:05	00:10	00:02	00:11	00:05	00:10							
				05:00	02:11	02:08	02:15	03:45	06:00	04:35							
				N = 66	N = 66	N = 66	N = 65	N = 64	N = 63	N = 62							
9	62	6	34	00:33	00:30	00:32	00:30	00:35	00:40	00:40	01:13						
				00:05	00:02	00:08	00:05	00:05	00:10	00:03	00:13						
				03:05	02:31	05:42	03:05	02:20	02:40	10:00	11:31						
				N = 61	N = 60	N = 60	N = 59	N = 59	N = 59	N = 59	N = 56						
10	41	4	30	00:35	00:25	00:22	00:35	00:30	00:35	00:41	00:45	00:48					
				00:05	00:07	00:05	00:01	00:05	00:10	00:10	00:08	00:13					
				02:05	02:30	02:05	05:05	02:00	02:38	02:55	05:15	05:20					
				N = 40	N = 40	N = 39	N = 39	N = 39	N = 39	N = 37	N = 37	N = 37					
11	18	1	8	00:35	00:24	00:25	00:33	00:32	00:38	00:50	00:25	00:56	00:35				
				00:10	00:05	00:05	00:10	00:10	00:05	00:05	00:05	00:05	00:10				
				01:10	01:40	01:40	01:55	01:55	01:50	03:05	03:50	02:40	08:20				
				N = 18	N = 18	N = 18	N = 18	N = 18	N = 18	N = 18	N = 17	N = 17	N = 17				
12	9	2	8	00:35	00:35	00:20	00:37	00:25	00:20	00:40	00:43	00:49	00:35	01:15			
				00:07	00:15	00:10	00:05	00:08	00:10	00:05	00:03	00:01	00:15	00:20			
				02:30	02:08	00:35	02:08	01:20	01:40	01:52	00:55	02:05	01:25	01:45			
				N = 9	N = 9	N = 9	N = 9	N = 9	N = 8	N = 8	N = 8	N = 8	N = 8	N = 7			
13	2	1	2	00:30	00:32	00:17	00:15	00:26	0:33	00:29	00:51	00:45	00:15	00:45	01:20		
				00:25	00:23	00:05	00:09	00:22	00:22	00:08	00:45	00:40	00:15	00:45	01:20		
				00:35	00:40	00:29	00:20	00:30	00:43	00:50	00:57	00:50	00:15	00:45	01:20		
				N = 2	N = 2	N = 2	N = 2	N = 2	N = 2	N = 2	N = 2	N = 2	N = 2	N = 1	N = 1		
15	1	0	3	00:10	00:15	00:05	00:10	00:40	00:25	00:10	00:30	00:30	00:20	00:45	00:50	01:30	00:40
				00:10	00:15	00:05	00:10	00:40	00:25	00:10	00:30	00:30	00:20	00:45	00:50	01:30	00:40
				00:10	00:15	00:05	00:10	00:40	00:25	00:10	00:30	00:30	00:20	00:45	00:50	01:30	00:40
				N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1	N = 1

Figure 4. Median and range of different inter-pup intervals per litter size (hours + minutes) of 331 parturitions. Lit (N), number of litters; CS (N), number of caesarean sections; SB (N), number of stillborn puppies; M, median; R, range; N, number of puppies.

Appendix 7.4 – Questionnaire for the interviews

1. Wie heeft het protocol gemaakt?
2. Hoe wordt het protocol kenbaar gemaakt aan personeel?
3. Vinden er veranderingen in plaats?
4. Op basis waarvan en door wie wordt die veranderingen doorgevoerd?
5. Wat vindt u globaal van het protocol?
6. Hoelang gebruikt u dit protocol?
7. Heeft u ook eens andere protocollen gebruikt?
    a. Zo ja, welke vind u beter
    b. Zo ja, waarom vind u de één beter
    c. Zo ja, waarom is er overgestapt naar een ander protocol
8. Gebruikt u dit protocol bij **iedere** bevalling?
    a. Zo nee, waarom
9. Neemt u het protocol met de gezinnen door?
    a. Zo ja, waarom
    b. Zo ja, geeft het ook genoeg houvast voor het gezin
    c. Zo ja, geeft het u steun tegenover het gezin (bijv. waarom nu wachten)
    d. Zo nee, waarom niet
10. Het protocol:
    a. Is het duidelijk voor u?
    b. Merkt u moeilijkheden met het protocol?
        i. Zo ja, wanneer
        ii. Zo ja, met wat
        iii. Zo ja, waarom
    c. Wat vind u van de criteria / interventies (globaal)?
        i. Geeft het voldoende houvast
        ii. Geeft het een veilig gevoel
        iii. Zijn ze logisch
        iv. Zijn ze toepasbaar
        v. Zijn ze duidelijk
    d. Wat vind u van de interventies (specifiek):
        i. Toucheren:
            1. Wanneer toucheert u?
            2. Houdt u de tijden aan die in het protocol staan?
                a. Bij de eerste pup?
                b. Bij een andere pup?
                c. Tussen 1^{e} en 2^{e} keer toucheren?
            3. Waarom vaker toucheren dan 2 keer?
                a. Reden?
                b. Onderling afspraken?
                c. Gevoel?
                d. In welk tijdbestek?
        ii. Oxytocine:
            1. Wanneer gebruikt u oxytocine?
            2. Houdt u de tijden aan die in het protocol staan?
                a. Zo nee, waarom niet?
            3. Wat is de gebruikte dosering?
            4. Waarom voor de zekerheid?
            5. Waarom zonder eerst toucheren?
            6. Heeft u een idee waarom de reden niet wordt genoteerd?

7. Waarom > 0.2 ml?
8. Is er een maximaal aan aantal keer?

iii. Manueel helpen:
1. Reden?
2. Onderling afspraken?
3. Gevoel?

iv. Beweging:
1. Reden?
2. Onderling afspraken?
3. Gevoel?
4. In welk tijdbestek?

v. Auto rijden:
1. Reden?
2. Onderling afspraken?
3. Gevoel?
4. In welk tijdbestek?

vi. Massage:
1. Definitie (iedereen hetzelfde)?
2. Reden?
3. Onderling afspraken?
4. Gevoel?
5. In welk tijdbestek?

vii. Aanleggen:
1. Reden?
2. Onderling afspraken?
3. Gevoel?
4. In welk tijdbestek?

viii. Keizersnede:
1. Reden?
   a. Na een aantal keer oxytocine?
   b. Na bepaalde een tussenpuptijd?
2. Onderling afspraken?
3. Gevoel?

e. Houdt u zich **altijd** aan het protocol?
   i. Zo nee, wanneer en waarom niet
   ii. Zo nee, wat doet u dan
   iii. Zo ja, altijd

11. Wat zou u willen aanpassen aan het protocol / Wat mist u in het protocol?
12. Schrijft u alles op?
    a. Tijden en hoeveelheden bij handelingen:
       i. Reden onbekend is het dan volgens het protocol?
    b. Wanneer u afwijkt van het protocol
13. Op basis waarvan bepaalt u dat de partus is afgelopen?

8. List of references

Andrew G, Jeff D, Kathleen EG. (2007). Compendium on Canine Dystocia: Medical and Surgical Management. West Vet Emergency and Specialty Center. Boise, Idaho.

Armstrong L, Stenson B, Blickstein I, Green T. (2006). Effect of delayed sampling on umbilical cord arterial and venous lactate and blood gases in clamped and unclamped vessels. Archives of Disease in Childhood-Fetal and Neonatal Edition **91**(5):342-345

Arthur GH, Noakes DE, Pearson H. (1989). Dystocia and other disorders associated with parturition. In: Veterinary Reproduction and Obstetrics (6th ed.). London, Ohio: Bailliere Tindall. 182-230 p.

Bennet D. (1974). Canine dystocia - a review of the literature. Journal of Small Animal Practice **15**(2):101-117

Bennett D. (1980). Normal and abnormal parturition. In: Current therapy in theriogenology: diagnosis, treatment and prevention of reproductive disease in animals (1st ed.). Philadelphia, Pennsylvania: Saunders. 595-606 p.

Bergström A, Nødtvedt A, Lagerstedt AS, Egenvall A. (2006). Incidence and breed predilection for dystocia and risk factors for caesarean section in a Swedish population of insured dogs. Veterinary Surgery **35**(8):786-791

Blickstein I and Green T. (2007). Umbilical cord blood gases. Clinics in perinatology **34**(3):451-459

Chastant-Maillard S, Guillemot C, Feugier A, Mariani C, Grellet A, Mila H. (2017). Reproductive performance and pre-weaning mortality: Preliminary analysis of 27,221 purebred female dogs and 204,537 puppies in France. Reproduction in Domestic Animals **52**(2):158-162

Copley K. (2002). Comparison of traditional methods for evaluating parturition in the bitch versus using external fetal and uterine monitoring. In: Proceedings of the Society of Theriogenology Annual Conference. Colorado Springs, Colorado. 375-382 p.

Cornelius AJ, Moxon R, Russenberger J, Havlena B, Cheong SH. (2019). Identifying risk factors for canine dystocia and stillbirths. Theriogenology **128**:201-206

Darvelid AW and Linde-Forsberg C. (1994). Dystocia in the bitch: A retrospective study of 182 cases. Journal of Small Animal Practice **35**:402-407

Dolf G, Gaillard C, Russenberger J, Moseley L, Schelling C. (2018). Factors contributing to the decision to perform a cesarean section in Labrador retrievers. BMC Veterinary Research **14**(1):57

Drobatz KJ. (2005). Reproductive emergencies. In: Veterinary Clinics of North America: Small Animal Practice (2nd ed.). Philadelphia, Pennsylvania: Saunders. 397-420 p.

England GCW and Heimendahl A. (2010a). Pregnancy diagnosis, normal pregnancy and parturition in the bitch. In: BSAVA manual of canine and feline reproduction and neonatology (2nd ed.). Quedgeley, England: British Small Animal Veterinary Assocation. 89-97 p.

England GCW and Heimendahl A. (2010b). Pregnancy diagnosis, normal pregnancy and parturition in the bitch. In: BSAVA manual of canine and feline reproduction and neonatology (2nd ed.). Quedgeley, England: British Small Animal Veterinary Assocation. 121-134 p.

Ettinger SJ and Feldman EC. (2010). Abnormalities in Canine Pregnancy, Parturition, and the Periparturient Period. In: Textbook of veterinary internal medicine: disease of the dog and the cat (7th ed.). St. Louis, Missouri: Saunders. 1890-1901 p.

Fournier A, Masson M, Corbière F, Mila H, Mariani C, Grellet A, Chastant-Maillard S. (2017). Epidemiological analysis of reproductive performances and kitten mortality rates in 5,303 purebred queens of 45 different breeds and 28,065 kittens in France. Reproduction in Domestic Animals **52**(2):153-157

Freak MJ. (1948). The whelping bitch. Veterinary Record **60**:295-301

Freak MJ. (1962). Abnormal conditions associated with pregnancy and parturition in the bitch. Veterinary Record **74**:1323-1339

Freak MJ. (1975). Practitioner's-breeder's approach to canine parturition. Veterinary Record **96**:303-308

Furguson JKW. (1941). Surgery gynecology obstetrics **73**:359

Gaudet AD. (1985). Retrospective study of 128 cases of canine dystocia. Journal of the American Animal Hospital Association **21**:813-818

Gill MA. (2002). Perinatal and late neonatal mortality in the dog. Doctoral dissertation. Sydney, Australia: University of Sydney

Groppetti D, Pecile A, Del Carro AP, Copley K, Minero M, Cremonesi F. (2010). Evaluation of newborn canine viability by means of umbilical vein lactate measurement, apgar score and uterine tocodynamometry. Theriogenology **74**(7):1187-1196

Groppetti D, Ravasio G, Bronzo V, Pecile A. (2015). The role of birth weight on litter size and mortality within 24h of life in purebred dogs: what aspects are involved? Animal Reproduction Science **163**:112-119

Indrebø A, Trangerud L, Moe L. (2007). Canine neonatal mortality in four large breeds. Acta Veterinaria Scandinavica **49**:2

Irons PC, Nóthling JO, Volkman DH. (1997). Failure of luteolysis leads to prolonged gestation in a bitch: a case report. Theriogenology **48**:353-359

Johnston SD. (1986). Parturition and Dystocia in the bitch. In: Current Therapy in Theriogenology (1st ed.). Philadelphia, Pennsylvania: Saunders. 500-501 p.

Johnston SD, Kustritz MVR, Olson PNS. (2001). Disorders of the canine uterus and uterine tubes (oviducts). In: Canine and Feline Theriogenology (1st ed.). Philadelphia, Pennsylvania: Saunders. 206–224 p.

Klarenbeek M, Okkens AC, Kooistra HS, Mol JA, Bevers MM, Taverne MAM. (2007). Plasma oxytocin concentrations during late pregnancy and parturition in the dog. Theriogenology **68**:1169-1176

Linde-Forsberg C and Persson G. (2007). A survey of dystocia in the Boxer breed. Acta Veterinaria Scandinavica **49**:8

Linde-Forsberg C and Eneroth A. (2000). Abnormalities in pregnancy, parturition and the periparturient period. In: Textbook of Veterinary Internal Medicine (5th ed.). Philadelphia, Pennsylvania: Saunders. 1527-1539 p.

Lopate C. (2012). Reproductive Physiology of Canine Pregnancy and Parturition and Conditions of the Periparturient Period. In: Management of pregnant and neonatal dogs, cats, and exotic pets (1st ed.). Ames, Iowa: Wiley-Blackwell. 25-41 p.

Moon-Massat PF and Erb HN. (2002). Perioperative factors associated with puppy vigor after delivery by cesarean section. Journal of the American Animal Hospital Association **38**(1):90-96

Burke TJ. (1986). Normal and abnormal parturition. In: Small Animal Reproduction and Fertility. A clinical approach to diagnosis and treatment (1st ed.). Philadelphia, Pennsylvania: Lea & Febiger. 335-345 p.

Musters J, de Gier J, Kooistra HS, Okkens AC. (2011). Questionnaire-based survey of parturition in the queen. Theriogenology **75**(9):1596-1601

Münnich A. (2008). The pathological newborn in small animals: the neonate is not a small adult. Veterinary Research Communications **32**:81-85

Münnich A and Küchenmeister U. (2009). Dystocia in numbers – Evidenced-based parameters for intervention in the dog: Causes for dystocia and treatment recommendations. Reproduction in Domestic Animals **44**(2):141-147

Okkens AC, Teunissen JM, Van Osch W, Van Den Brom WE, Dieleman SJ, Kooistra HS. (2001). Influence of litter size and breed on the duration of gestation in dogs. Journal of Reproduction and Fertility Supplement **57**:193-197

Olsson K, Bergström A, Kindahl H, Lagerstedt AS. (2003). Increased plasma concentrations of vasopressin, oxytocin, cortisol and the prostaglandin $F_2\alpha$ metabolite during labour in the dog. Acta Physiologica Scandinavica **179**:281-287

Oluwatoyin AO, Fayemi OE. (2011). A survey on cases of dystocia in bitch at southwestern Nigeria. Global Veterinaria **6**:97-100

Renfield ML. (1975). The small for date infant. In: Neonatology: Pathophysiology and Management of the Newborn (1st ed.). Philadelphia, Pennsylvania: Lippincott Co. 189-199 p.

Romagnoli S. (2007). How I Treat Non-obstructive Dystocia in the Bitch and Queen. In: Proceedings of the Southern European Veterinary Conference & Congreso Nacional AVEPA. Barcelona, Spain.

Romagnoli S, Souza FF, Rota A, Vannozzi I. (2004). Prolonged interval between parturition of normal live pups in a bitch. Journal of Small Animal Practice **45**:249-253

Roberts SJ. (1986). Parturition. In: Veterinary Obstetrics and Genital diseases (2nd ed.). Ithica, New York: Cornell University. 251-255 p.

Schweizer CM and Meyers-Wallen VN. (2000). Medical management of dystocia and indications for cesarean section in the bitch. In: Kirk's Current Veterinary Therapy XIII Small Animal Practice (1st ed.). Philadelphia, Pennsylvania: Saunders. 933-939 p.

Tønnessen R, Borge KS, Nødtvedt A, Indrebø A. (2012). Canine perinatal mortality: a cohort study of 224 breeds. Theriogenology **77**:1788-1801

Van der Beek S, Nielen AL, Schukken YH, Brascamp EW. (1999). Evaluation of genetic, common-litter, and within-litter effects on preweaning mortality in a birth cohort of puppies. American Journal of Veterinary Research **60**:1106-1110

Van der Weyden GC, Taverne MAM, Okkens AC, Fontijne P. (1981). The intra-uterine position of canine foetuses and their sequence of expulsion at birth. Journal of Small Animal Practice **22**(8):503-510

Veronesi MC, Panzani S, Faustini M, Rota A. (2009). An Apgar scoring system for routine assessment of newborn puppy viability and short-term survival prognosis. Theriogenology **72**(3):401-407

Wallace MS and Davidson AP. (1995). Abnormalities in pregnancy parturition and periparturient period. In: Veterinary Internal Medicine (4th ed.). Philadelphia, Pennsylvania: Saunders. 1614-1624 p.