Ovulation timing in the bitch: Conception rate and influencing factors in 1401 estrus cycles

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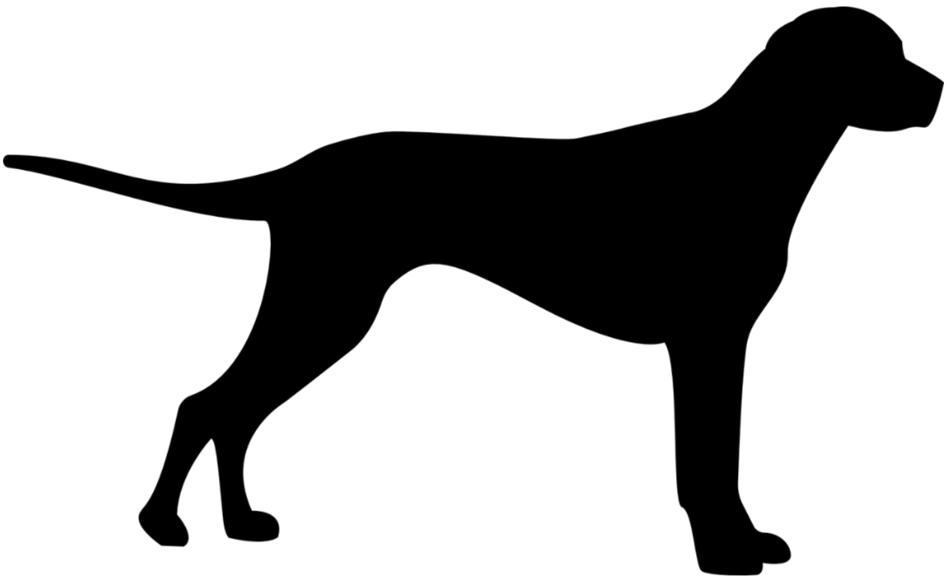

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Abstract
Introduction
The optimal date for breeding bitches is relatively difficult to determine due to challenges posed by the estrus cycle. Ovulation timing is a service provided by the university clinic in Utrecht for breeders to advise them on the optimal breeding date. The service consists of clinical visits during which bitches are examined for behavioral and physical signs associated with estrus. Also, a blood sample is taken for measurement of plasma progesterone concentration. Based on these findings the date of ovulation and the optimal date for breeding are determined.
Objectives
The objective of this study was to determine the conception rate and influencing factors when performing ovulation timing. A secondary goal was describing the population presented for ovulation timing in the university clinic.
Methods
Bitches who were presented for ovulation timing at the Utrecht university clinic from January 2003 to January 2021 were selected for this study. Cases with no registered progesterone concentrations and cases where no conception could not be interpreted were excluded. Available variables were univariably and multivariable analyzed for significance.
Results
In total there were 1401 cases included. Bitches with a known fertility problem (n=267) showed a significantly ($p < .001$) reduced chance of conception. Nullipara had a significantly ($p=.008$) lower conception rate with an 0.69 OR for conception compared to multipara. The odds ratio for conception for autumn (n=326), spring (n=309) and winter (n=392) was 0.58, 0.72 and 0.71, respectively, compared to summer (n=374). Bitches bred using AI (n=163) had a significantly ($p < .001$) lower conception rate compared to naturally mated bitches (n=824), 53.5% and 84.8%, respectively.
Conclusion
The overall conception rate when performing ovulation timing in bitches was 79.7%. The five variables in the multivariable model that significantly influenced conception rate were: ‘known fertility problem’, ‘nulliparity’, ‘method of breeding’, ‘season’ and ‘breed size’.

Introduction
Ovulation timing
During estrus, bitches are receptive to mounting for a longer period than the duration of the fertile window[1]. In an uncontrolled setting, for example in free ranging dogs, a bitch would mate multiple times during estrus[2]. With multiple matings the chance of a correctly timed mating is high. However, the current practice of dog breeding is to perform one or two matings during an estrus cycle in the bitch. Ovulation timing is performed to achieve a high probability of conception when only one or two matings are possible.

The reproduction unit of the university clinic in Utrecht has offered ovulation timing for the last four decades. For this service no referral is required. The goal is to determine the fertile window in the bitch. This is most needed in cases in which the fertile window is reduced, for example when using frozen sperm[3]. During the first appointment dogs are physically examined to evaluate their general health. Thereafter, behavioral and physical signs associated with proestrus and estrus are examined, followed by a gynecological examination that includes vaginoscopy and cytology. Lastly, a blood sample is taken for measurement of plasma progesterone concentration. On average a dog will need to be seen three times, with 48–72 hour intervals, to best determine the date of ovulation and consequently the optimal period for mating or artificial insemination. The reproduction unit recommends having the bitch naturally mated once two days after ovulation. Artificial insemination is regarded as inferior to natural mating and is only used in specific circumstances. This is in according to Dutch law, which states that unnatural reproduction should be avoided in companion animals[4]. For artificial insemination with frozen-thawed sperm the advice is to inseminate three days after ovulation. Gestation check is included in the ovulation timing service and preferably takes place somewhere between days 26 and 32 after mating. During gestation check abdominal palpation for embryonic vesicles and an ultrasound are performed. Usually, the ultrasound is performed by the clinician unless results of abdominal palpation are unclear and the owner agrees with the extra costs associated with a radiologist performing the ultrasound.

Reproductive cycle of the domestic dog

Female domestic dogs are monoestrus and typically non-seasonal spontaneous ovulators. Most bitches have an inter-estrus interval of 6-7 months, but this can vary from 5 to 12 months. Inter-estrus intervals may be very regular, although variability within animals is not uncommon. The first estrus occurs between 6–14 months of age, mainly depending on the size of the breed[5]. Four phases can be distinguished in the canine reproductive cycle: proestrus, estrus, metestrus and anestrus.

Proestrus

The proestrus phase lasts on average 9 days and starts with vulval hemorrhagic discharge. An increased plasma estradiol concentration progressively induces vulval swelling, hemorrhagic discharge, vaginal epithelial proliferation, edematous vaginal epithelium and being attractive for male dogs. The transition from the proestrus phase to the estrus phase can be recognized behaviorally with the female showing receptive behavior towards males or hormonally with the LH surge[5].

Estrus

The estrus phase lasts between 5-10 days[5]. During the estrus phase the plasma estradiol concentration declines while the plasma progesterone concentration increases[6]. At the start of the estrus phase the vaginal mucosa shows a wrinkly aspect on endoscopic examination[1]. Ovulation occurs between 24-48 hours after the LH surge[7].

Metestrus (diestrus)

The metestrus phase starts when the behavioral signs of the estrus phase disappear. This phase in non-pregnant bitches lasts between 49-79 days[8]. During the metestrus corpora lutea produce progesterone resulting in an increased plasma progesterone concentration. The end of the metestrus phase is marked by the decline of the plasma progesterone concentration to 1 or 2 ng/ml[5].

Anestrus

The anestrus phase lasts on average 5 (range 3–10) months[5] and during this phase there is a limited folliculogenesis that does not lead to ovulation in the ovaries[9,10].

Fertile window in the bitch

After ovulation canine oocytes still need to resume meiosis, which takes 48-72 hours to complete. Hereafter, the fertile oocytes remain viable for 2-3 days[11]. This means that the fertile window in the bitch starts between 72-120 hours after the LH surge. LH surge is concomitant with the more easily measurable increasing serum P4 concentration. Generally, survival rates of fresh sperm vary between 3.5-6 days after mating[12]. However, motile sperm has been detected in the female reproductive tract 11 days after mating[13,14]. For mating to be successful an open cervix is required. The opening of the cervix occurs 2.6 (±2.8) days before the LH surge and closes 6.7 (±1.4) days after the LH surge[15]. Research on AI with frozen-thawed sperm showed that the optimum date of insemination is 2 to 5 days after ovulation[16].

Reproductive performance of the bitch

The two most important parameters of reproductive performance in the bitch are whelping rate and litter size. Literature shows significant breed differences in reproductive performance[17]. Greyhounds, for example, have demonstrated a significantly higher whelping rate compared to breed averages when performing AI with frozen-thawed or fresh sperm[3,18]. Also, the age of the bitch influences her fertility;[3] older bitches, over six years of age, showed a significantly lower whelping rate with smaller litters.[17] Additionally, the litter size is significantly dependent on the size of the breed, with larger breeds having bigger litters[19]. Quality of sperm is an important factor independent of the fertility of the bitch. Frozen thawed sperm, when compared to fresh sperm, has greatly reduced pregnancy rates and litter sizes[3]. Suboptimal timing of mating or insemination also results in a reduced reproductive performance[17,20]. When timed too early the condition of sperm will decline while stored in the female reproductive tract. When natural mating is timed too late, the cervix may be closed, and when AI is timed too late, oocytes may not be viable anymore. The method of sperm deposition can also significantly influence the reproductive performance. Reported reproductive performance of natural mating is 78.6%-87.8%[19,21]. AI with fresh semen produces an 80% pregnancy rate, while frozen-thawed sperm yields a 68-75% pregnancy rate[3,17,22]. When sperm is deposited in the vagina, pregnancy rates are significantly reduced compared to intrauterine insemination[17].

Reduced fertility

A high coefficient of inbreeding may contribute to reduced fertility in domestic dogs[23]. Chu and colleagues found a significant correlation between the degree of inbreeding and litter size and a suggestive negative correlation between inbreeding and conception rate[24]. Domestic dog breeds often have a relatively high degree of inbreeding due to the popular sire effect: when a male dog wins a competition or award many breeders want his offspring, this creates artificial bottlenecks[25]. This effect results in a high coefficient of homozygosity when genetic analysis of the dog's genome is performed[26].

Aim of the study

The focus of this study is to determine the conception rate and identify factors that significantly influence conception rate when performing ovulation timing. To our knowledge there is no published study describing reproductive performance of naturally mated bitches when performing a standardized clinical ovulation timing protocol. A secondary goal was describing the population presented for ovulation timing in the university clinic.

Materials and Method

Study population

Bitches who were presented for ovulation timing at the Utrecht university clinic from January 2003 to January 2021 were selected for this study. Details about the bitch and clinical findings during appointments were registered in a digital fill-in form. The information was entered by students rotating in the clinics and veterinary specialists working in the reproduction unit. Additionally, a form was used in which the follow-up information on the outcome of pregnancy could be logged. The owner was provided with a letter containing questions regarding the outcome of parturition, which they could return by post. When a bitch was presented for ovulation timing for more than one estrous period over the course of the study each occasion was treated as a separate case. Cases with no registered progesterone concentrations were excluded, as they had not received ovulation timing advice. Cases for which conception could not be interpreted based on the registered information were also excluded.

Data analysis

Using a query, data were retrieved from the practice management software (PMS system) Vetware (Agfa-Gevaert N.V., Belgium, version 430-0190) on February 23, 2021 and presented in excel. Each case received a unique identifier (UID) based on patient-number and application date. For each case an interpretation for conception was made based on abdominal palpation and ultrasound using predetermined norms (

Table **1**). The ultrasound results were regarded as more important than abdominal palpation descriptions. This means that if no embryonic vesicles were palpated but on ultrasound live embryos were found this was interpretated as conception. Cases where conception was diagnosed with palpation and ultrasound was performed but no results were described the ultrasound was interpreted as supporting the abdominal palpation result. The form with follow-up information (pups born) was used to determine conception for cases with no interpretation. This was possible for 24 cases. Additionally, for 41 cases information about the method of breeding was retrieved from the follow-up form as this information was missing in the ovulation timing digital fill-in form. The excel sheet was exported to RStudio (RStudio, PBC, Boston, version 1.4.1103) for analysis.

Table 1: *The descriptions of the guidelines used for the interpretation of conception.*

Description	Interpretation
Ultrasound clearly stating gestation	Conception
Ultrasound clearly stating no gestation	No conception
Abdominal palpation describes embryonic vesicles + ultrasound performed but no description or no ultrasound performed	Conception
Abdominal palpation describes no embryonic vesicles + ultrasound performed but no description or no ultrasound performed	No conception
Unclear abdominal palpation described + ultrasound performed but no description or no ultrasound performed	No interpretation

Variables available for analysis were: ‘known general health problem’, ‘known fertility problem’, ‘parity’, ‘season’, ‘mating or AI’, ‘number of breedings’, ‘year of application’, ‘AI method’, ‘copulatory tie’, ‘FCI breed-group’, ‘breed size’ and ‘age’ (***Error! Reference source not found.***). Associations between conception rate and the variables were tested in two stages. First, all the selected variables were independently tested using a chi square test. A fisher exact test was used in place of the chi square test if the expected value of the chi square test was below six. secondly, the odds ratios with 95% confidence interval were calculated. Finally, the variables were tested using a multivariable logistic regression. In the multivariable test the variables - mating or AI, copulatory tie and AI method - are combined as ‘method of breeding’, resulting in 8 categories (*Table 3*). Variables included in the first model were: age category, year of application, known health problem, known fertility problem, nulliparity, method of breeding, season, breed size and number of breedings. The variables with a lower Akaike information criterion (AIC) than the overall model were dropped. Potential confounders were identified by major (>15%) changes in the coefficients of the model when dropping a variable from the model.

***Table 2**: The variables used in this study and their descriptions with the categorial outcomes, number of cases and the number of not available (NA)*

Variables	Description	Categories	Number	NA
Known general health problem	During the anamnesis owners could specify if the bitch has any problems with her general health	Yes / No	1286	115
Known fertility problem	During the anamnesis owners could specify if the bitch has had problems with fertility in the past	Yes / No	1279	122
Parity	Parity of the bitch	Nullipara / Multipara	1260	141
Season	Season in which the first appointment took place based on the week numbers. With, start spring at week 13, start summer at week 26, start autumn at week 39 and start winter at week 52	Spring / Summer / Autumn / Winter	1401	0
Mating or AI	Method used for breeding the bitch	Natural mating / Natural mating and AI / AI	1025	376
Number of breedings	Number of matings or inseminations	1 / >1	1088	313
Copulatory tie	The owner's observation if a copulatory tie took place during natural mating	Coupling / No coupling	673	728
AI method	Method of sperm deposition when performing AI in the bitch	Intrauterine / Intravaginal / Unknown	164	1237
FCI breed-group	The breed of the bitch categorized in breed groups according to Fédération Cynologique Internationale's (FCI) guidelines. If a breed group had insufficient numbers for analysis, cases were added to 'other'. Also, crossbreeds and not officially recognized breeds were added to 'other'	Retrievers, Flushing Dogs and Water Dogs/ Companion and Toy Dogs/ Sheepdogs and Cattledogs/ Pinscher, Schnauzer, Molossoid, Swiss Mountain and Cattledogs/ Spitz and primitive types/ Terriers/ Pointing Dogs/ Other	1401	0
Age	The age of the bitch at first appointment as continuous number	Continuous number	1397	4
Age category	The age of the bitch at first appointment divided in 4 categories	<3/ 3-5/ 5-6/ >6	1397	4
Year of application	The year of the first appointment divided in 3 categories	2003-2008/ 2009-2014/ 2015-2021	1401	0
Breed size	Size of the breed divided in categories based on average adult body weight. The classification as described by the American kennel club was used. Giant breed range from 35-55+ kg, large from 25-40 kg, medium from 15-30 kg, small from 3-15 kg and toy from 1-4 kg. The toy and small group were merged because the toy group only contained ten bitches	Giant / Large / Medium / Small	1323	78

Table 3: The variable 'method of breeding' with description, outcome categories, number of cases and number of not available (NA).

Variable	Description	Categories	Number	NA
Method of breeding	The variables: mating or AI, coupling and AI method are combined to create 8 categories	Mating with coupling/ Mating with no coupling/ Mating with unknown coupling/ Mating and AI/ AI intrauterine/ AI intravaginal/ AI unknown/ No information	1401	0

Results

The query in Vetware resulted in 2198 cases. For 752 cases no gestation could be interpreted, either because there was no documentation available (n=667) or the assessment was inadequately described (n=85), resulting in exclusion. Another 45 cases were excluded for containing no progesterone concentrations. A total of 1401 cases met the inclusion criteria.

An individual bitch could represent multiple cases. In total 883 unique bitches were included in the analysis. On average a bitch represented 1.59 cases, with a maximum of 6 cases (one bitch). 11 bitches represent 5 cases, 40 bitches represent 4 cases, 83 bitches represent 3 cases, 181 bitches represent 2 cases and 566 bitches represent 1 case. The mean age of bitches submitted for ovulation timing was 4.1 years (range 1.1-9.2). Nullipara (n=660) had a mean age of 3.1 ± 1.1 (range 1.1-7.8) years and multipara (n=600) had a mean age of 5.2 ± 1.4 (range 1.6-9.2) years (*Figure 1*).

In total 152 different breeds and crossbreeds were included. The ten breeds with most cases were Bearded Collie (27), Bernese Mountain Dog (29), Dobermann (60), Drentsche Partridge Dog (33), German Shepherd (67), Golden Retriever (184), Labradoodle (27), Labrador Retriever (281), Newfoundland (34), Saarloos Wolfhound (26). Method of breeding was significantly ($p < .001$) different between FCI breed groups (*Figure 2*). Breed size categories and method of breeding was not significantly ($p=.14$) different (*Figure 3*).

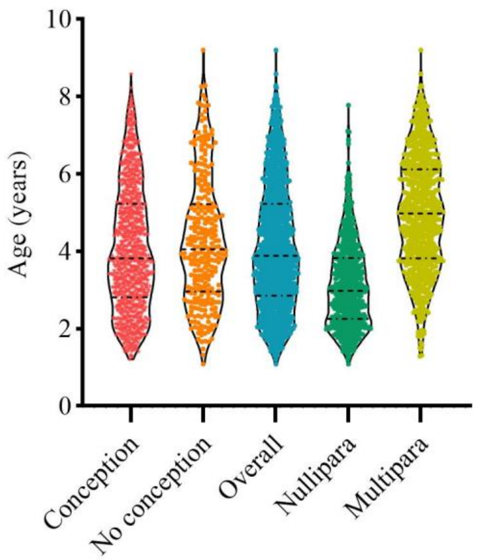

Figure 1: *The age distribution in years of 'cases with conception', 'cases without conception', 'all included cases', 'all nullipara' and 'all multipara'.*

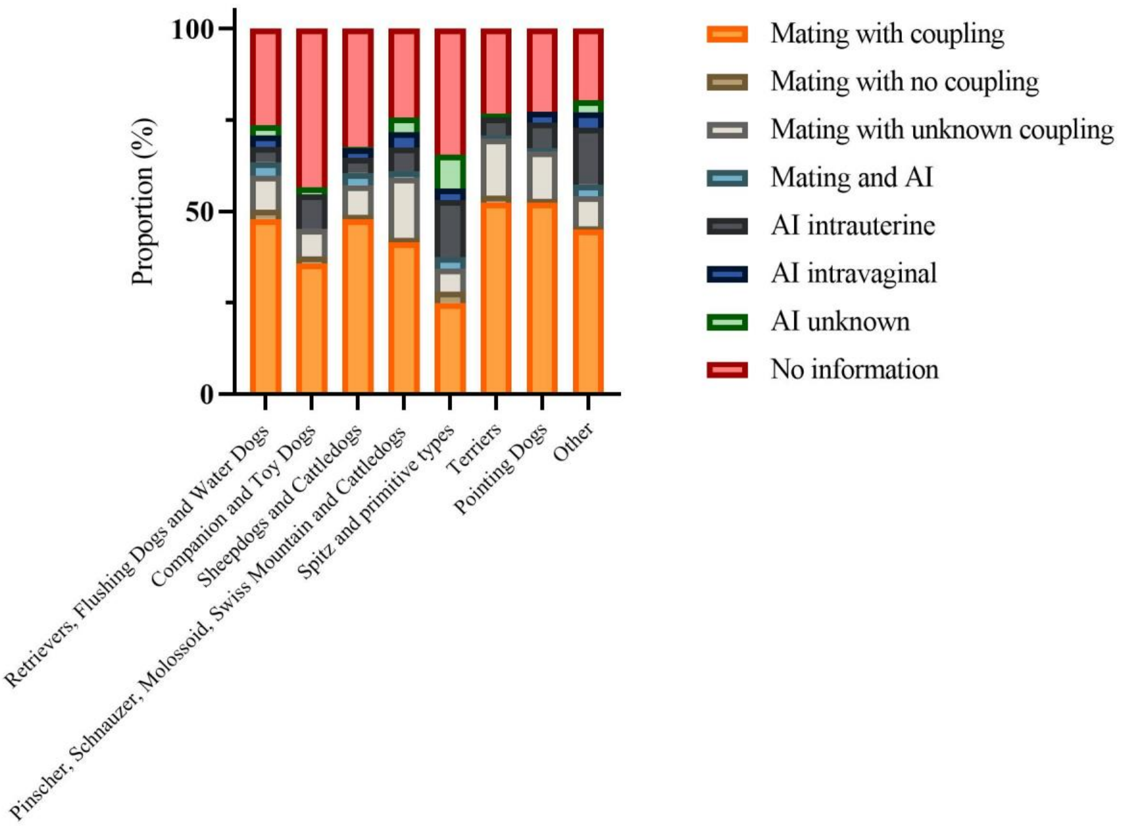

Figure 2: *The factor 'method of breeding' distribution in percentages for the FCI breed-groups 'Retrievers, Flushing Dogs and Water Dogs', 'Companion and Toy Dogs', 'Sheepdogs and Cattledogs', 'Pinscher, Schnauzer, Molossoid, Swiss Mountain and Cattledogs', 'Spitz and primitive types', 'Terriers', 'Pointing Dogs' and 'Other'.*

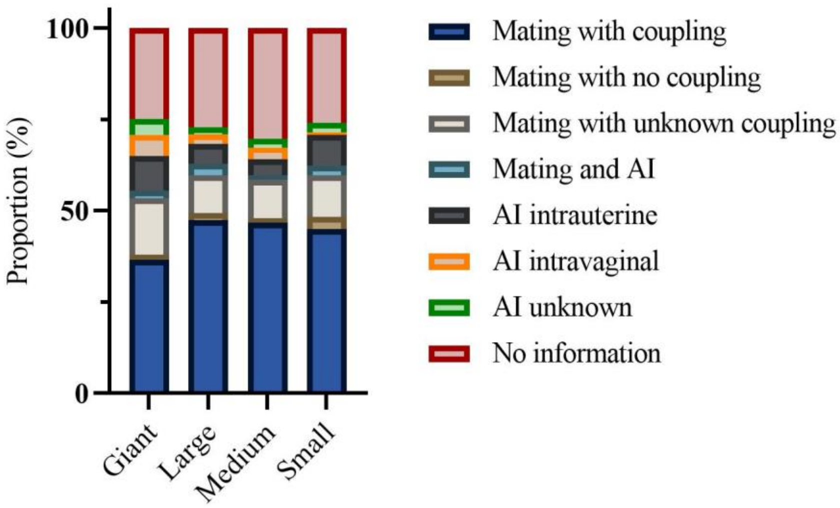

Figure 3: *The factor 'method of breeding' distribution in percentages for the breed size groups 'giant', 'large', 'medium' and 'small'.*

Conception rates

The overall conception rate when performing ovulation timing in bitches was 79.7%. Bitches with no known fertility problem had a conception rate of 82.8%. Naturally mated bitches with a copulatory tie with no known fertility problem had an 88.5% conception rate and when no copulatory tie was observed a 55.6% conception rate. AI in bitches with no known fertility problem resulted in a conception rate of 58.4%. Nullipara had a significant (p=.008) lower conception rate compared to multipara, 76.7% and 82.7%, respectively. (*Figure 4*).

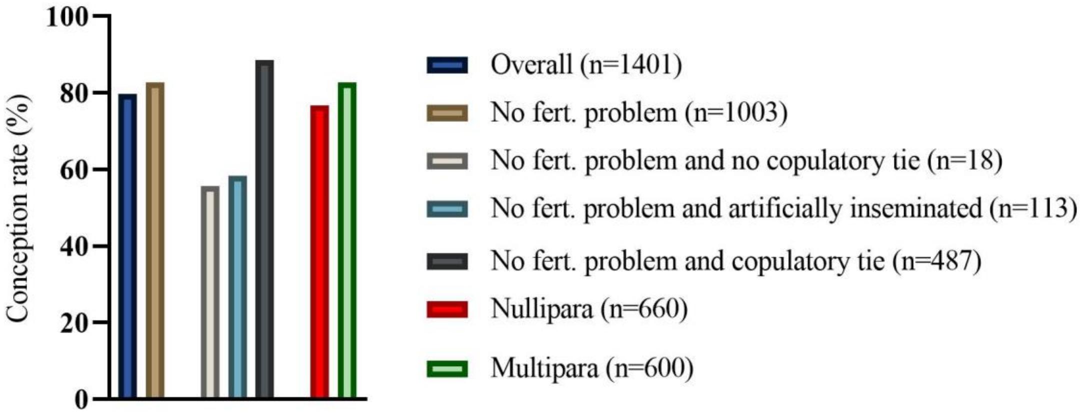

Figure 4: *The conception rates in percentages of 'all cases', 'cases with no fertility problem', 'cases with no fertility problem and no copulatory tie during mating', 'cases with no fertility problem and artificially inseminated', 'cases with no fertility problem and a copulatory tie during mating', 'cases with nullipara' and 'cases with multipara'.*

Univariable analysis

An overview of the results is shown in

Table **4**.

Bitches with a known fertility problem (n=267) showed a significantly ($p < .001$) reduced chance of conception. The odds ratio of conception for bitches with a known fertility problem was 0.45 compared to bitches with no known fertility problem. The owners of 145 bitches had reported a general health problem during anamnesis, but there was no significant (p=.88) difference in conception rate found compared to healthy bitches. Nullipara had a significantly (p=.008) lower conception rate with an 0.69 OR for conception compared to multipara.

The season in which the first appointment took place was significantly (p=.037) correlated to conception rate. The odds ratio for conception for autumn (n=326), spring (n=309) and winter (n=392) was 0.58, 0.72 and 0.71, respectively, compared to summer (n=374). There was no significant correlation between the year of application and conception rate (p=.65). In the category 2003-2008 there were significantly ($p < .001$) more bitches with 'mating with unknown coupling'. The 2003-2008 category consisted for 23.0% of naturally mated bitches with unknown copulatory tie compared to 2009-2014 and 2015-2021 with 4.8% and 2.6%, respectively. This shows that in the earlier years of study less information was entered into the fill-in form.

There was no significant correlation found between age and conception rate when testing age as a continuous variable (p= .059) or as a categorial variable (p=.25). Breed had a significant ($p < .001$) correlation with conception rate, with Terriers having the highest conception rate (87.4%) and Spitz and primitive types the lowest (46.9%).

Bitches bred using AI (n=163) had a significantly ($p < .001$) lower conception rate compared to naturally mated bitches (n=824), 53.5% and 84.8%, respectively. The odds ratio for conception for AI and 'mating and AI' were 0.21 and 0.44, respectively, compared to naturally mated bitches. For naturally mated bitches, occurrence of a copulatory tie during mating was significantly ($p < .001$) correlated with conception. Bitches mated with copulatory tie (n=648) showed an 86.3% conception rate and bitches mated with no copulatory tie (n=25) a 56% conception rate. Bitches without a copulatory tie during mating had a 0.20 odds ratio for conception compared to bitches with a copulatory tie. The method of AI was not significantly (p=.47) correlated with conception. Intrauterine (n=88) and intravaginal (n=41) insemination had a conception rate of 54.5% and 48.8%, respectively. In 38 cases bitches were both naturally bred and artificially inseminated. For these cases the occurrence of copulatory tie and method of insemination were not analyzed.

Table 4: *Variables tested with an univariable analysis with the number of cases, conception rates, odds ratios, 95% confidence intervals and p-values. Age was also tested univariably with number, mean, standard deviation (SD) and p-value.*

Variable	Categories	Number	Conception % (n)	No Conception % (n)	Odds ratio	95% CI	p-value
Known general health problem	Yes	145	80.7 (117)	19.3 (28)	1.06	0.69-1.67	0.88
	No	1141	79.8 (910)	20.2 (231)	1	ref	
Known fertility problem	Yes	276	68.1 (188)	31.9 (88)	0.45	0.33-0.60	< 0.001
	No	1003	82.8 (830)	17.2 (173)	1	ref	
Parity	Nullipara	660	76.7 (506)	23.3 (154)	0.69	0.52-0.91	0.008
	Multipara	600	82.7 (496)	17.3 (104)	1	ref	
Season	Spring	309	79.3 (245)	20.7 (64)	0.72	0.48-1.06	< 0.001
	Summer	374	84.2 (315)	15.8 (59)	1	ref	
	Autumn	326	75.5 (246)	24.5 (80)	0.58	0.39-0.84	
	Winter	392	79.1 (310)	20.9 (82)	0.71	0.49-1.02	
Breeding method	Natural mating	824	85.0 (700)	15.0 (124)	1	ref	< 0.001
	Natural mating and AI	38	71.1 (27)	28.9 (11)	0.43	0.22-0.94	
	AI	163	55.2 (90)	44.8 (73)	0.22	0.15-0.31	
Number of breeding	> 1	569	81.5 (464)	18.5 (105)	1	ref	0.20
	1	519	78.4 (407)	21.6 (112)	0.82	0.61-1.11	
AI method	Intra-uterine	88	54.5 (48)	45.5 (40)	1	ref	0.47
	Intravaginal	41	48.8 (20)	51.5 (21)	0.79	0.38-1.67	
	Unknown	35	62.9 (22)	36.1 (13)	1.41	0.64-3.21	
Copulatory tie	Mating with coupling	648	86.3 (559)	13.7 (89)	1	ref	< 0.001
	Mating without coupling	25	56 (14)	44 (11)	0.2	0.09-0.47	
FCI breed-group	Retrievers, Flushing Dogs and Water Dogs	509	84.5 (430)	15.5 (79)	1	ref	< 0.001
	Companion and Toy Dogs	53	81.1 (43)	18.9 (10)	0.79	0.4-1.72	
	Sheepdogs and Cattledogs	238	82.4 (196)	17.6 (42)	0.86	0.57-1.3	
	Pinscher, Schnauzer, Molossoid, Swiss Mountain and Cattledogs	243	67.5 (164)	32.5 (79)	0.38	0.27-0.55	
	Spitz and primitive types	32	46.9 (15)	53.1 (17)	0.16	0.08-0.34	
	Terriers	103	87.4 (90)	12.6 (13)	1.27	0.7-2.48	
	Pointing Dogs	101	79.2 (80)	20.8 (21)	0.7	0.41-1.22	
	Other	122	80.3 (98)	19.7 (24)	0.75	0.46-1.27	
Age category	< 3	402	81.6 (328)	18.4 (74)	1	ref	0.25
	3-5	595	78.5 (467)	21.5 (128)	0.82	0.6-1.13	
	5-6	188	83 (156)	17 (32)	1.1	0.7-1.75	
	> 6	216	76.4 (165)	23.6 (51)	0.73	0.49-1.1	
Method of breeding	Mating with coupling	648	86.3 (559)	13.7 (89)	1	ref	< 0.001
	Mating with no coupling	25	56 (14)	44 (11)	0.2	0.09-0.47	
	mating with unknown coupling	151	84.1 (127)	15.9 (24)	0.84	0.52-1.4	
	Mating and AI	38	71.1 (27)	28.9 (11)	0.39	0.19-0.85	
	AI intrauterine	88	54.5 (48)	45.5 (40)	0.19	0.12-0.31	
	AI intravaginal	41	48.8 (20)	51.2 (21)	0.15	0.08-0.29	
	AI unknown	35	62.9 (22)	37.1 (13)	0.27	0.13-0.57	
	No information	375	79.7 (299)	20.3 (76)	0.63	0.45-0.88	
Year of application	2003-2008	513	80.5 (413)	19.5 (100)	1	ref	0.65
	2009-2014	462	80.1 (370)	19.9 (92)	0.97	0.71-1.34	
	2015-2021	426	78.2 (333)	21.8 (93)	0.87	0.63-1.19	
Breed size	Giant	137	59.1 (81)	40.9 (56)	0.32	0.22-0.47	< 0.001
	Large	787	81.8 (644)	18.2 (143)	1	ref	
	Medium	248	81 (201)	19 (47)	0.95	0.66-1.38	
	Small	151	81.5 (123)	18.5 (28)	0.98	0.63-1.55	
Variable	**Categories**	**Number**	**Mean**		**SD**		**p-value**
Age	Yes	1116	4.08		1.6		0.08
	No	285	4.27		1.64		

Multivariable analysis

Nine variables were initially offered to the first multivariable model to analyze factors influencing conception rate when performing ovulation timing. ‘Age category’, ‘year of application’, ‘known health problem’ and ‘number of breedings’ were not retained in the final multivariable model. The five variables that remained in the final model were: ‘known fertility problem’, ‘nulliparity’, ‘method of breeding’, ‘season’ and ‘breed size’ (

Table 5).

Bitches with a known fertility problem had an odds ratio of 0.42 (95%CI 0.30-0.60) of conception when compared to bitches with no known fertility problem. Nullipara had a lower chance of conception compared to multipara (OR 0.69, 95%CI 0.50-0.95). Intrauterine artificially inseminated bitches had the lowest odds ratios in the model. Intrauterine insemination and intravaginal insemination had odds ratios of 0.15 (95%CI 0.08-0.26) and 0.18 (95%CI 0.08-0.39) of conception, respectively, when compared to natural breeding with copulatory tie. The odds ratio of conception for naturally mated bitches without copulatory tie was 0.21 (95%CI 0.08-0.57) compared to naturally mated bitches with copulatory tie. Bitches with the first appointment in summer showed the highest odds of conception compared to other seasons. Autumn, spring and winter had an odds ratio of 0.46 (95%CI 0.29-0.71), 0.54 (95%CI 0.34-0.87) and 0.53 (95%CI 0.34-0.82), respectively, compared to summer. Lastly, Giant breeds had significantly lower odds of conception, with an odds ratio of 0.39 (95%CI 0.24-0.61) compared to large dogs.

Confounding was found between age category and parity. In the univariable analysis age category was not found to be significantly correlated with conception rate. However, parity was found to be significantly correlated with conception rate. The mean age of nullipara was significantly ($p < .001$) lower than the age of multipara. This means that the effects of age and parity are mixed in this model and cannot be interpreted separately.
Also confounding between breed size and age category was recognized. This is caused by the age difference between the breed size groups. The division of the age categories was significantly ($p=.001$) different between breed size categories (

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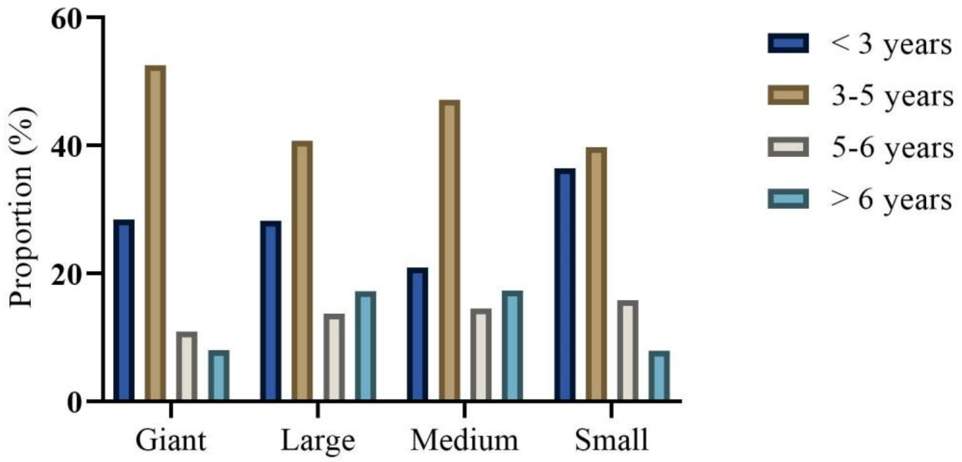

Figure 5 *The age category distribution in percentages for the breed size groups 'giant', 'large', 'medium' and 'small'.*

Table 5: The final multivariable model of the variables associated with conception rate showing the odds ratios (OR) and the 95% confidence intervals (95%CI).

Variable	Categories	OR	95%CI
Known fertility problem	Yes	0.42	0.30-0.60
	No	ref	ref
Parity	Nullipara	0.69	0.50-0.95
	Multipara	ref	ref
Method of breeding	Mating with coupling	ref	ref
	Mating with no coupling	0.21	0.08-0.57
	Mating with unknown coupling	0.87	0.51-1.57
	Mating and AI	0.41	0.18-1.00
	AI Intrauterine	0.15	0.08-0.26
	AI Intravaginal	0.18	0.08-0.39
	AI unknown	0.20	0.09-0.45
	unknown	0.68	0.46-1.00
Season	summer	ref	ref
	Autumn	0.44	0.28-0.70
	Spring	0.51	0.31-0.81
	Winter	0.53	0.34-0.82
Breed size	Giant	0.39	0.24-0.61
	Large	1.00	ref
	Medium	1.04	0.69-1.60
	Small	1.10	0.66-1.86

Discussion

This study found an overall conception rate of 79.7% when performing ovulation timing. In the population with no known fertility problem the conception rate was 82.8%. This corresponds with previous studies, where in naturally mated bitches a conception rate of 78.6%[21] and 87.8%[19] was reported. These studies retrieved their data from the Swedish Kennel Club (SKK) registry and the reproduction management software used by the breeders in France. Both studies did not describe a standardized method for the timing of the matings. However, it is likely that the breeders used some form of ovulation timing or performed multiple matings during one estrus cycle. In contrast, our study performs ovulation timing in a standardized clinical protocol. Our study population most likely contains a bigger proportion of bitches with a known fertility problem than the previous studies. Yet, in our study population a similar conception rate is accomplished.

The conception rate for artificially inseminated bitches (58.4%) was significantly ($p < .001$) lower compared to naturally mated bitches. This is much lower than previous studies, where a conception rate of 68%[22], 74%[3] and 73.1%[17] was described. The method of artificial insemination in our study population was less controlled compared to the previous studies. Our study has no information on the insemination protocol and expertise level. Some artificial inseminations were performed by veterinarians of the reproduction unit in the university clinic Utrecht. Other artificial insemination were performed by independent veterinarians and breeders (intravaginal AI). All inseminations in the previous studies were performed by highly experienced personnel with a clearly described protocol. In these protocols type of semen, the handling of the semen, the method of insemination and the material used are clearly described.

This might explain the lower conception rate for inseminated bitches in our study compared to previous studies.

The method of breeding was the factor with the most influence on conception rate, with intrauterine artificially inseminated bitches having the lowest odds ratio (0.15) in the multivariable model. This is in contrast with literature, which describes a lower conception rate for intravaginal AI compared to intrauterine AI[17]. In our study no information about sperm quality was available. Sperm quality is likely a confounder for method of AI in our study. As it is common practice to inseminate fresh sperm intravaginally and frozen thawed sperm intrauterinally. This means that there is an unreported factor influencing the conception rate of AI. This may explain why there was no significant (p = 0.47) difference between the AI methods found. This study found a conception rate of 54.5% for intrauterine AI and a 48.8% for intravaginal AI. This differs from a previous study which found a much lower conception rate for intravaginal insemination (10.0%) compared to intrauterine insemination (75%) when insemination was performed with frozen-thawed sperm. This supports our hypothesis that breeders use fresh semen to inseminate intravaginally[17].

The conception rate of naturally mated bitches was 85.0%, but when no copulatory tie was observed conception rate was reduced to 56.0%. Only 3.9% (25/673) of the cases with naturally mated bitches had no copulatory tie during any mating. This variable has only a small effect on the total conception rate in our study population. However, it is clinically a very useful factor, as breeders can perform a second mating when no copulatory tie in the first attempt is observed. In 21 cases a mating with coupling was performed after a mating without a copulatory tie. In 11 cases AI was performed after a mating without a copulatory tie. This observation fits the advice given by the reproduction unit of the university clinic to perform a second mating when the first was without a copulatory tie.

There were big differences found between FCI breed groups, with ‘Spitz and primitive types’ having an odds ratio of 0.16 (95%CI 0.08-0.34) compared to ‘Retrievers, Flushing Dogs and Water Dogs’. This reduced conception rate is likely influenced by the method of breeding. The method of breeding was not evenly divided between the FCI breed groups. The group ‘Spitz and primitive types’ had a significantly ($p < .001$) smaller proportion of naturally mated bitches with copulatory tie and a bigger proportion of artificially inseminated bitches than other breeds groups. The FCI breed-group ‘Pinscher, Schnauzer, Molossoid, Swiss Mountain and Cattledogs’ also showed a significantly decreased odds of conception (OR=0.38, 95CI: 0.27-0.55) compared to other breed groups. This correlation is more clearly understood when looking at breed size. The ‘Pinscher, Schnauzer, Molossoid, Swiss Mountain and Cattledogs’ category consisted for 51.0% of giant size breeds.

Giant breeds had significantly ($p < .001$) lower odds (OR: 0.32) of conception than the other breed sizes. Giant breeds had a conception rate of 59.1% compared to the other breed sizes, which had a conception rate of around 81%. The ‘method of breeding’ was, in contrast to ‘FCI breed-group’, not significantly (p=.14) different between the breed size categories. This means that the variable ‘breed size’ provides a more useful breed division than the FCI breed-groups. However, the age categories were significantly (p=.001) different between breed size categories. Smaller breeds mature at a younger age then the larger breeds do[5]. This might relate to the observation in our study that small breeds were more likely to be bred before the age of 3 years compared to bigger size categories. Both small breeds and giant breeds were less likely to be bred when older than 6 years of age compared to medium and large breeds. For small breeds this might be caused by reaching the maximum number of litters allowed by the breed club's norm before bitches reach the sixth years of age. For giant breeds this might be explained by their shorter life expectancy[27,28]. Giant bitches age faster compared to smaller sized bitches[29]. This faster process of aging might explain the small proportion of giant sized bitches in the older than six age category. Contradictory to our findings, a previous study reported no correlation between the weight of the bitch and whelping rate[3].

Bitches with a known fertility problem had a significantly ($p < .001$) reduced chance (OR:0.69) of conception compared to bitches with no known fertility problem; conception rates were 68.1% and 82.8%, respectively. There was no predetermined definition of 'known fertility problem'. During the first appointment students conducted an anamnesis with the owner. From this dialogue the owners' interpretation of fertility problems was most likely leading. Owners may interpret 'fertility problem' differently. For example, a difficult parturition or difficult to notice heat may both be interpreted by owners as fertility problems during the anamnesis. Because of the unclear definition it is difficult to compare the category 'known fertility problem' with previous studies or the clinical setting.

Nullipara bitches had a significantly ($p = .008$) lower conception rate compared to multipara bitches; conception rates were 76.7% and 82.8%, respectively. Nullipara bitches were also significantly ($p < .001$) younger than multipara bitches. This means that age may be a confounder for this effect. Another possible explanation for this difference may be that multipara bitches have proven to be fertile, making them more likely to succeed again. Also breeders may be more likely to breed a successful bitch again as previous results were positive. In contradiction, a previous study found no difference in conception rate between nullipara an multipara[22]. In this previous study all bitches were artificially inseminated with frozen–thawed semen. This is different from our study where most bitches were naturally mated. Possibly the success rate of a first time mating is lower in bitches compared to a mating with more experienced bitches.

Age as a continuous factor was not found to be significantly ($p=.08$) correlated to conception rate. When looking at the age of the bitch, when divided in categories, there is also no significant ($p=.25$) difference found. It is worth to note that many pedigree clubs have restrictions on the breeding of older bitches. The maximum age for breeding was eight years, which in 2020 was increased to nine years[30]. Which means that this study design, with breeders complying with the rules, is not optimal to demonstrate a correlation between age and conception rate.

The season in which the first appointment took place was significantly ($p=.037$) correlated with conception rate, with summer having the highest (84.2%) conception rate and autumn (75%) the lowest. The odds ratios in the multivariable model were lower than the odds ratios in the univariable model, resulting in significantly higher odds for conception in summer compared to all other seasons. A previous study found no seasonal influence on whelping rate[3]. This previous study was performed in New Zealand and the author noted that there is a reduced seasonality in New Zealand compared to many Northern Hemisphere countries. Another study found that more mating took place during winter compared to other seasons, however no conception rate was reported[21]. In our study we also found that most cases took place in winter ($n=392$), however the difference with summer ($n=374$) was small. The difference in cases between all the seasons was significantly ($p=.004$) different, with 309 cases in spring and 326 cases in autumn.

The number of breedings showed no significant (p=.20) correlation with conception rate, this is consistent with previous papers[3,17]. The year of application had no significant (p=.65) influence on the conception rate. Conception rate was very constant over the years. This was expected as there have been no big changes in the procedures of ovulation timing. This also means the fertility of the population presented for ovulation timing has not changed significantly. This may be an indication that the coefficient of inbreeding has not increased during the study period. Contradictory, there was a significant ($p < .001$) difference in the number of cases with a fertility problems between the 'year of application' categories. There were 119 cases in '2003-2008', 98 in '2009-2014' and 59 in 2015-2021. Although the number of cases with a known fertility problem reduced in the more recent years there was no increase in conception rate observed.

'Known general health problem' had no significant correlation with conception rate. Bitches with a known general health problem had slightly higher odds of conception (OR:1.06) than bitches with no known health problem. There was no predetermined definition for 'known general health problem'. Students and/or owners had to interpret this variable during anamnesis, which results in a poorly defined factor. Also, it is common practice not to breed bitches that have serious health problems. If serious health problems were found during the clinical visits there would be strongly advised against breeding the bitch.

Conclusion

The results of this study report an overall conception rate of 79.7% when performing ovulation timing in bitches. In the univariable analysis 'known fertility problem', 'parity', 'season', 'method of breeding', 'FCI breed-group' and 'breed size' were significantly correlated with conception rate. In the multivariable analysis known 'fertility problem', 'parity', 'method of breeding', 'season' and 'breed size' were significantly correlated with conception rate.

Future research

This research retrospectively analyzed data from a practice management software. This resulted in a high number of cases. However, because the digital fill-in form was not created with the objective of analyzing inserted data, retrieving this data proved difficult. Information was often missing or misplaced in free text columns. This study provides a basis for further research, in which definitions should be more clearly defined. This study also shows the value of a practice management software. With a well-designed fill-in form large scale datasets can be used for research and improvement of patient care.

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