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Embryonic resorption rates at canine pregnancy diagnoses: A retrospective evaluation

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ABSTRACT

Pregnancy diagnosis in canines is generally performed during embryonic phase, between 19 and 35 days of gestation. At this stage embryonic resorptions can be observed, which, according to the literature, affects 11–26% of conceptuses and 5–43% of pregnancies. Resorption has been hypothesized as a physiological event in uterine overcrowding, however other factors may be involved, such as infectious or non-infectious diseases. This study aimed to retrospectively evaluate the incidence of embryo resorption at ultrasonographic pregnancy diagnosis in different dog breeds, and to identify the main factors determining the occurrence of the resorption sites. 95 pregnancy diagnoses were performed 21–30 days post-ovulation by ultrasound examination on 74 different animals. Breed, weight, and age of the bitches were recorded, and the reproductive anamnesis was collected from their medical records. The overall pregnancy rate was 91.6%. In 48.3% of pregnancies (42/87), at least one resorption site was visible, and embryonic resorption rate was 14.2% (61 resorption sites/431 total structures). Binary logistic regression showed a significant effect of age ($P < 0.001$), but not the size of the litter ($P = 0.357$), nor the size of the mother ($P = 0.281$) or any previous reproductive problems ($P = 0.077$). Age was significantly higher in pregnancies with resorptions than in normal ones (60.88 ± 18.24 and 40.27 ± 15.74 months, respectively, $P < 0.001$). The embryonic resorption rate was in line with previous findings, while the incidence of affected pregnancies was higher. Although resorptions may occur physiologically in pregnancies with large litters, a relationship between embryo resorption and litter size was not identified in our sample group, while aging increased the resorption rates. This, together with the occurrence of repeated embryonic resorptions in some bitches included in the study, suggests how resorptions could also be the result of pathological events. The underlying mechanisms and other factors that may be involved need further clarification.

1. Introduction

Today, most dog breeders and owners request ultrasonographic pregnancy diagnoses, to better manage the bitch during gestation and parturition. Usually, the pregnancy diagnosis is performed at approximately 25 days of gestation, when it is possible to identify embryonic vesicles, the embryo proper and its heartbeat [1–7]. At this gestational age, it is thus possible to evaluate the embryo viability and detect potential pregnancy abnormalities, such as resorption sites [8,9].

In the embryonic phase of pregnancy, between day 19 and 35 [7,10], resorption sites are defined as "death of the embryo and resorption of the fluid component of the pregnancy" [8]. Resorptions can be due to various conditions, such as infectious diseases, for example Brucella canis [9,11] or canine herpesvirus [12,13], which induce embryonic and foetal death in dogs [14,15]. Non-infectious diseases can also lead to pregnancy loss, such as hypoluteoidism [16–18], uterine pathologies [19,20], hypothyroidism [18,21], aged gametes, chromosomal, developmental, and embryonic/fetal defects [15,18], maternal environment stress [15], nutritional deficiencies [15], and drugs and toxic agents [5,18].

When no signs of disease are found and resorptions still occur and do not involve all the conceptuses, these are considered as spontaneous resorptions [8,22]. In fact, when spontaneous embryonic resorptions occur, symptoms are rare [8]. One hypothesis is that spontaneous resorption could be physiological in pregnancies

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with large litters [23–25]. Uterine overcrowding may lead to inter-embryonal inhibition, causing the loss of some conceptuses involved, as already described in other animal species [26].

Resorptions have been investigated by two methods: ultrasound examination of the reproductive tract and direct observation of surgically removed organs (ovaries and uteruses). Ultrasound examination enables the appearance and evolution of resorption sites to be evaluated, while surgery permits their direct visualization [22,25,27–31]. In both cases, the incidence of resorption in the pregnancy is described as the percentage of conceptuses affected. Post-surgery, when organs are directly observed, the total number of conceptuses, counted as placental scars and embryonic/foetal structures still present, is related to the number of corpora lutea observed in the ovaries [27,29–32]. The incidence of resorption varies from 11% to 25.9% of the conceptuses observed [24,27,29,30,32–35]. The percentage of pregnancies affected by resorption varies from 4.8% to 42.9% [22,24,25,28,30,31,35].

The aims of this study were: 1) to retrospectively evaluate the incidence of embryo resorption at ultrasonographic pregnancy diagnosis in bitches, and 2) to identify the main factors determining the presence of the forementioned resorption sites.

2. Material and methods

Ethical statement

This study was evaluated by Ethics Committee of Pisa University (protocol number 36/2022).

2.1. Animals

This retrospective study included 74 privately owned bitches of different breeds presented at the Veterinary Teaching Hospital (VTH) "Mario Modenato" between January 2020 and June 2022 for 95 pregnancy diagnoses. Only pregnancy diagnoses performed by ultrasound examination between days 21 and 30 post-ovulation were included. Breed, weight, and age of the animals were recorded. The reproductive anamnesis was collected either from the dogs' medical records of our VTH or by owners' interviews. It was not always possible to obtain and verify other clinics medical records. Bitches were allocated to different size groups according to their body weight and breed: Large (L, >35 kg), Medium (M, 10–34.9 kg), Small (S, 5–9.9 kg), Toy (XS, <5 kg). Pregnancies of dogs with a history of pyometra, mucometra, previous embryo resorption, fetal death or abortions were included in the reproductive problem group. Failure to conceive was not considered as a reproductive problem as other factors (male, management of mating/AI) could have determined the failure. Bitches were also considered pregnant when only dead conceptuses or resorption sites were present.

2.2. Ultrasound examination and data collection

For ultrasound examination, a Toshiba Aplio 400 (Canon Medical Systems Europe B.V., Zoetermeer, The Netherlands), was employed, equipped with a linear (7–14 MHz, Toshiba PLT-1204BT) and a microconvex (4,2–10 MHz, Toshiba PVT-712BT 11MC4) probe. The ultrasonographic study was conducted with the same criteria for every dog. The animals were placed in lateral recumbency without clipping the hair, and putting abundant alcohol solution and ultrasound gel on their abdomen.

The body of the uterus was first identified and examined. The uterine horns were then evaluated beginning with the left one, each evaluated from the base to the ovary, in order not to miss any tract of the organ. Recumbency was then changed, and the right uterine horn was examined, again from the base to the ovary. If the dam allowed it, she was placed in dorsal recumbency and the uterine body and both horns were checked again. For the largest breeds, only the microconvex probe was used, while for the other dogs, the uterus and ovaries were visualized with the linear probe first and then with the microconvex probe.

At the time of diagnoses, the number of conceptuses was counted, and their viability assessed. All structures were visualized by B-mode and Doppler ultrasonography to assess the presence of a heartbeat. Resorption sites were identified as previously described, e.g., localized uterine horn enlargements with or without fluid or vesicles without a viable embryo [8,22,24]. Total number of structures was considered as the entirety of viable embryos and resorption sites.

In general, the owners were instructed to have a further pregnancy evaluation of their dog at around day 45 of the pregnancy. However, when resorptions were detected, the advice was to return for a second ultrasound evaluation one week later.

The number of puppies born was recorded either by the clinicians during delivery (natural or C-section) or by contacting the owners after whelping.

2.3. Statistical analysis

The difference in pregnancy rates between the size groups was evaluated by Chi-squared test. The data distribution was assessed by the Andersen-Darling normality test and the statistical analyses chosen accordingly. The median number of structures for each size group was also calculated, and pregnancies were divided into two groups: large litter size (above the median number of structures for the size group) and the other litters (below or equal to the median).

A binary logistic regression was used to model the determinants of resorption whose factors were the size group and age of the dam, litter size (large or not), and whether or not there were reproductive problems in the anamnesis. Only animals with known size, age, litter size and reproductive history were included in this statistical model. The factor with a P-value <0.05 was further analysed with Student's t-test. Values were presented as means ± standard deviation. Differences were considered statistically significant when $P < 0.05$. Analyses were performed with the statistical software Minitab 16.1.

3. Results

The study included 74 bitches and 95 pregnancies. A total of 18 bitches (out of 74) were evaluated for two or three different pregnancies during the examined period. The bitches belonged to 27 different breeds, as showed in Table 1.

The mean age was 50.18 ± 19.61 months (range: 18–99 months), and sizes were distributed as follows: L: n = 12, M: n = 20, S: n = 40, XS: n = 23.

The overall pregnancy rate was 91.6% (87/95). Negative diagnoses were reported in one, two, four and one bitches sized L, M, S and XS, respectively. There was no statistical difference in pregnancy rates between size groups ($P > 0.05$). Eight of the pregnant bitches had no viable embryos at the time of diagnosis. Negative diagnoses were excluded from further statistical analyses.

Overall, embryonic resorption was observed in 48.3% (42/87) of pregnancies. Twelve dogs were included for two pregnancy diagnoses during the study period: six showed embryo resorptions at both diagnoses, two showed resorption only once, and four bitches showed only normal, viable embryos. Three bitches were included for three pregnancy diagnoses: two of these showed resorption sites at each pregnancy, while one never showed resorption sites. Thus, both at first and at second diagnoses 9/15 (60.0%) of pregnancies showed resorption sites.

Table 1
List of breeds included in the study, with corresponding size class and number of subjects.

Breed	Size class	Number of animals included
Toy Poodle	XS	10
Pomeranian	XS	6
Griffon Bruxellois	XS	1
Russian Toy	XS	1
Jack Russell Terrier	S	15
Zwergschnauzer	S	6
Miniature Poodle	S	4
Bouledogue Français	S	1
Fox Terrier	S	1
West Highland White Terrier	S	1
Standard Schnauzer	M	4
English Cocker Spaniel	M	2
Lagotto Romagnolo	M	2
Weimaraner	M	2
Welsh Corgi	M	2
American Staffordshire	M	1
Border Collie	M	1
English Pointer	M	1
Malinois	M	1
Rough Collie	M	1
Shar Pei	M	1
Whippet	M	1
Rhodesian Ridgeback	L	3
Bernese Mountain dog	L	2
Labrador Retriever	L	2
Golden Retriever	L	1
Great Pyrenees	L	1

At pregnancy diagnoses, 431 conceptuses were counted, 370 of which were normally developed and healthy embryos, while 61 were resorption sites at different stages. The incidence of embryonic resorption was thus 14.2%.

The different ultrasonographic patterns of resorption sites observed were: localized uterine horn enlargement with hypoechoic centre; rounded, crenulated or collapsed vesicle without embryonic mass and filled with anechoic, hypoechoic, or hyperechoic fluid; rounded, crenulated or collapsed vesicle containing a dead embryo, and fluids with different echogenicity. The sites were found along the uterine horns, from the base to the apical part (Fig. 1).

As for three animals in group M, the reproductive history was unknown, only 84 pregnancies were included in the binary logistic regression. The model used to evaluate the relationship between the presence/absence of resorption (response) and age, size group, litter size (above or below the median number of the size group) and previous reproductive problems of the dam (continuous and categorical predictors) were statistically significant ($P = 0.001$), with an R^2 index of 30.14. For size groups, litter size and previous reproductive problems, a non-significant relationship with the response was found ($P = 0.357$, $P = 0.281$ and $P = 0.077$, respectively). In contrast, the age of the dam showed a significant relationship with the response ($P = 0.001$).

The mean age of the animals with an embryo resorption at pregnancy diagnosis was significantly higher than that of animals without a resorption (60.88 ± 18.24 and 40.27 ± 15.74 months, respectively) ($P < 0.001$). Table 2 shows the mean age of the bitches with or not with resorption sites in the different size groups.

Table 3 reports the median number of structures (entirety of viable embryos and resorptions, if present) at the time of evaluation and the proportion of pregnancies in which resorptions were present according to size group and litter size.

The anamnesis showed the following previous reproductive problems in 22 cases: resorptions in more than one pregnancy (11), fetal deaths (n = 1), pyometra (n = 4), pyometra and resorptions (n = 3), mucometra and resorptions (n = 3). Of the remaining 65 pregnancies evaluated, reproductive problems were either not present (n = 62), or not recorded (n = 3). In the binary logistic regression, the relationship between previous reproductive problems and the presence of resorption sites was not statistically significant (Table 4; $P = 0.77$).

Of the 87 pregnancies, 68 were monitored further by ultrasound (68/87, 78.2%). Of the remaining 19 pregnancies, eight of the dams had no resorption sites visualized at pregnancy diagnosis, and six only had non-viable embryos at pregnancy diagnosis. The remaining five were not brought for a second ultrasound and had both resorption sites and viable embryos at pregnancy diagnosis. Two bitches with only non-viable embryos at pregnancy diagnosis were examined again and the diagnosis was confirmed.

Of the remaining 66 monitored pregnancies, 63 continued without complications related to the resorptions, such as symptoms of general illness or vulvar discharge. Three pregnancies, however, showed more embryonic/fetal deaths at subsequent ultrasound evaluation after the first one. One was an 8-year-old miniature Poodle, with 3 viable embryos and 2 resorption sites at day 26, which had lost another 2 conceptuses by the end of the pregnancy, had losses visualized at days 31 and 37, with the last viable puppy delivered by planned C-section at term. The second pregnancy was of a 4-year-old Jack Russell Terrier with 2 viable embryos and 1 resorption site at day 24, in which 1 more conceptus was lost one week later. The owners chose to terminate the pregnancy to avoid a planned c-section related to the single puppy syndrome, therefore aglepristone (Alizin, Virbac S.r.l.) was administered. The same bitch, evaluated at the following pregnancy, had 8 structures, one of which was a resorption, at day 23. Thereafter, a fetal death was observed at day 45, while 59 days post-ovulation, the female was presented due to a green vulvar discharge, and the ultrasound examination revealed one more fetal death. Five puppies were born healthy.

Among the pregnancies included in this study, along with the presence of resorption sites, two animals developed a pyometra, and one a mucometra. In these cases, no viable embryos were present.

Information on the number of puppies delivered was obtained for 79 gestations (79/87, 90.1%, 343 puppies). In 61/79 pregnancies (77.2%), the number of embryos observed corresponded to the number of puppies born. In the remaining pregnancies, puppies were either underestimated (13/18, 72.2%, 17 more puppies than expected) or overestimated (5/18, 27.8%, 6 fewer puppies than expected).

4. Discussion

Resorption sites are an important issue in canine reproduction since they affect litter size and occasionally the whole pregnancy. Although spontaneous resorptions may not represent a danger for the other conceptuses, they reduce the litter size and increase economic losses for breeders. The morphological aspects of embryonic resorptions are well described in the literature, however the main causes remain unclear or unknown. The present study is characterized by the fact that the dogs included were privately owned, belonging to different breeds and were presented to a veterinary facility for pregnancy diagnoses and monitoring. This in contrast with several other studies which included single breeds and dogs belonging to a single or a small number of facilities [23–25,28].

In the present study, the bitches with resorption sites at pregnancy diagnosis showed no clinical signs of general illness, or vulvar discharge. Of the 68 pregnancies further evaluated, most continued uneventfully, as already described in other studies

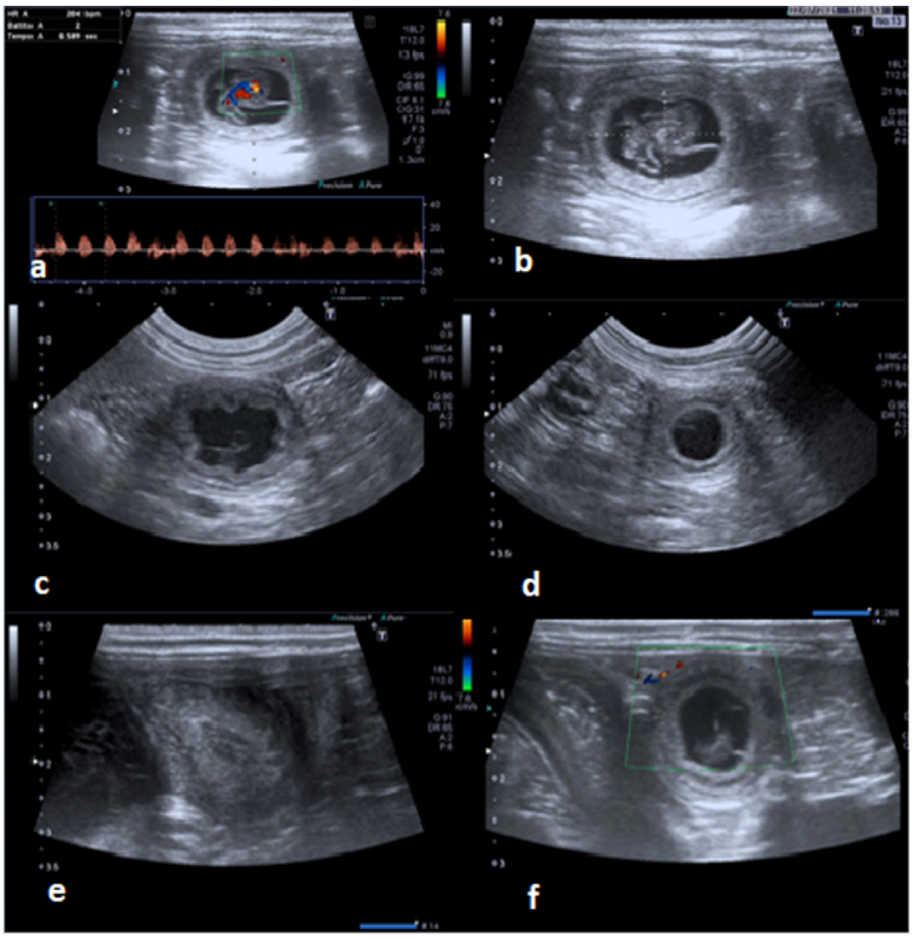

Fig. 1. Ultrasonographic images of the different patterns observed. (a) and (b) show a viable and healthy embryo, with a visible heartbeat and development in line with the gestational age (both images 29 days post-ovulation). (c), (d), (e) and (f) are resorption sites: (c) and (d) are crenulated (25 days post-ovulation) and round (25 days post-ovulation) empty vesicles respectively, without the embryonic mass, (e) is a localized uterine horn enlargement with hypoechoic centre (29 days post-ovulation), (f) is a vesicle containing a dead embryo, with the Doppler-mode showing no heart activity (25 days post-ovulation).

[22,25,28,33]. These findings sustain the fact that the largest proportion of resorptions found can be considered spontaneous, as previously hypothesized [8,22]. In contrast the results suggest that the three bitches showing resorption sites at pregnancy diagnoses followed by embryonic/fetal deaths later in the pregnancy had pathological pregnancies, which altered the viability of other conceptuses. The causes of the latter are however unknown since no further investigations were carried out.

The ultrasonographic patterns of resorption sites observed in this study are the same as those described in the literature and can be considered as embryonic resorptions with different timings [8,24]. The incidence of resorption sites in the sample considered was 14.2%. This is comparable to those previously reported in the literature [24,27,29,30,32–34]. The percentage of pregnancies affected by the condition was 48.3%, which is much higher than the data described in other studies (4.8%–12.3%) using the same method of evaluation (ultrasound examination) [22,25,28,35]. The highest incidence was observed by England and Russo [24], where 6 out of 20 dogs examined exhibited resorption sites (6/20, 30%).

Several reasons could explain this difference. One is that the most recent study, to our knowledge, is from 2010. Since then, ultrasound technology has greatly improved, and the images obtained today are almost incomparable with those of only few years ago. The precision in detecting all the structures in the uterus may thus have increased, especially for resorptions sites.

Another possible reason for the differences encountered could be the different populations included in the studies. In the present study, 26 breeds were included with different sizes, different ages,

Table 2
Mean age of the bitches with known parameters showing or not showing resorption sites at the time of pregnancy diagnosis, divided by size.

	Pregnancies presenting resorption sites	Age of the bitches in months (mean ± s.d.)	
		Pregnancies without resorptions	Pregnancies with resorptions
L	7/11 (63.6%)	49.00 ± 15.12	57.86 ± 15.71
M	6/15 (40.0%)	49.89 ± 18.00	64.50 ± 21.80
S	19/36 (52.8%)	36.00 ± 13.05	57.47 ± 15.64
XS	8/22 (36.4%)	36.79 ± 15.26	68.88 ± 23.42
overall	40/84 (47.6%)	40.27 ± 15.74^{A}	60.88 ± 18.24^{B}

Within line, between columns A≠B: $P < 0.05$.

Table 3
Median number of structures for size group and pregnancies above or equal/below this value.

Size group	Median number of structures	Resorptions in pregnancies with a number of structures above median	Resorptions in pregnancies with a number of structures equal or below median
L	6	3/5 (60%)	4/6 (80%)
M	6	1/5 (20%)	5/10 (50%)
S	5	9/17 (53%)	10/19 (53%)
XS	3	2/7 (29%)	6/15 (40%)
overall		15/34 (44.1%)[A]	25/50 (50.0%)[A]

Within line, between columns A≠B: P < 0.05.

and the animals were privately owned. The dogs lived in different environments and housing conditions (e.g., indoors, outdoors, indoors-outdoors), but also had different diets, vaccination status, and pesticide protocols. England et al. in all their studies [22,24,26] only included Labrador Retrievers housed in the same conditions, while Sendang et al. [25] only included Kangal Shepherd Dogs, again housed in the same conditions. These study groups were thus more homogeneous and were exposed to the same conditions (environmental pathogens, nutrients, weather, etc …). All these factors could thus affect the differences in resorptions rates observed.

Lastly, in the present study all the ultrasound examinations were performed at the same Veterinary Teaching Hospital. This facility is considered a reference centre for small animal reproduction, and dogs are referred for the most disparate reproductive issues, some of which were included in the study group. England et al. [22] reported that the incidence of resorption sites was not higher in bitches with previous reproductive problems, in particular failure to remain pregnant.

A statistically significant effect of reproductive problems on resorption was not identified in this study. However, 18 of the 22 bitches that had reproductive issues in their history showed resorption sites and three of them had a uterine pathology at the time of the ultrasound examination. These data, together with the three cases of other embryonic/fetal deaths during pregnancy monitoring, suggest that there are non-physiological factors behind many of these resorptions. As litter size was not associated with the presence of resorptions, the hypothesis that uterine overcrowding can be considered a major cause of this phenomena is not supported. However, possible physiological regulatory mechanisms underlying embryonic resorption in dogs cannot yet be ruled out.

The binary logistic regression analysis detected a significant relationship between the aging of the dam and the presence of resorptions. In the mare, the correlation between age and embryonic loss is well known [36]. Moreover, in Woods [36], aging was the only factor that correlated with the incidence of embryonic loss. In the bitch, in contrast, two studies on resorptions and pregnancy loss showed a significant inverse relationship between age and resorption, with animals younger than 4 years old being the most affected [30,37]. Nevertheless, aging in the bitch notoriously affects fecundity, litter size, conception rates and fetal death [38,39]. All these parameters are affected by the phenomenon of resorption. Our findings are aligned with the general decline in fertility observed in ageing female dogs.

A non-significant relationship was observed between the litter size at pregnancy diagnosis and the presence of resorptions. As reported previously, other authors have hypothesized that uterine overcrowding is a possible explanation for spontaneous embryo loss [23–25]. In these studies, the mean number of structures evaluated during pregnancy diagnosis/monitoring was higher in bitches affected by resorption than in bitches not affected. In the present study, no differences could be detected in the proportion of resorption in the two groups of litter size considered (above or equal/below to the median number of conceptuses for size group). A possible explanation of the lack of differences could be that some embryos or vesicles were resorbed before they could be detected, thus the litter size appeared smaller, or that there were non-diagnosed oocyte defects or fertilization failures among the reproductive problems. In the dog, research on genetic alterations leading to resorption/abortion/still-birth are scarce, but cases of still-birth and abortion with the presence of genetic abnormalities were reported [18]. Recent studies in mare's pregnancy pointed out the importance of genetic variants in the first two months of gestation, which are most likely to contribute to lethality of embryos [40,41]. Those alterations, i.e., aneuploidy and translocation, were already reported in humans and addressed as potential factors reducing the litter size in dogs [18]. Fertilization failure was demonstrated to happen in around 20% of conceptuses (29 unfertilized oocytes and 120 viable embryos retrieved) in a study on 23 ovariohysterectomized pregnant bitches [42]. In the same study, the rate of embryo loss increased with time until adhesion and implantation and they concluded that is possible that a greater mortality rate occurred in bitches until implantation, similar to what happens in other prolific species, such as pigs and rabbits [42].

Finally, the relationship between dam size and presence of resorption was non-significant, as described by Ortega-Pacheco et al. [30], but contrasting results were reported by Sharma et al. [37]. For the latter, there was a higher prevalence in medium and large size breeds, although in the conclusions, the authors reported that those size breeds were overrepresented in the experimental group.

Despite the overall significant statistical model in our study, the predictors used explained only 30% of the variability of the data, thus giving a low predictivity. This means that other predictors may exist beside those evaluated in this study, and further studies are therefore needed to better understand this phenomenon.

The present study has some limitations. Ultrasonographic evaluation alone may not record the real resorption rates. The diagnosis was made as early as 21 days post-ovulation, however, during the very first phase of pregnancy, some conceptuses can develop and resorb, without leaving any signs [43,44]. Today, pregnancy diagnosis can be performed even at 17–20 days post LH-peak [1,2,4,8,45], and it would be interesting to check for resorption signs from this time onwards.

Table 4
Proportion of reproductive problems in pregnancies with resorptions.

	No previous reproductive problems	Previous reproductive problems
Pregnancies with resorption at ultrasound diagnosis	22/62 (35.5%)	18/22 (81.8%)

Conception and resorption rates in the present study were estimated by counting the number of conceptuses, however in the literature they have been estimated either by the same method or by counting the number of corpora lutea [27,29–31]. In a clinical setting, it is not possible to evaluate the number of corpora lutea by ovariectomy/ovariohysterectomy, but only by ultrasound, leading to an estimate that is not without errors [46,47] but has good accuracy and repeatability [47]. The evaluation of corpora lutea also needs to be considered, in future ultrasonographic studies. It is also true, however, that multi-ovular follicles exist in bitches, and one of the most complete reviews on the topic shows that 12.6% of follicles can produce more viable oocytes that can lead to a conceptus and that 2.35% of the conceptuses exceed the number of follicles ovulated [48]. The real number of possible conceptuses that could develop is thus hard to determine, even counting the number of follicles ovulated/corpora lutea.

5. Conclusions

We believe that this is the first large retrospective study to evaluate resorption rates in a group of privately owned animals, belonging to different breeds, sizes, reproductive backgrounds, and ages. It shows that single embryo resorptions are frequent and usually uneventful for the bitch and the remaining conceptuses. As resorption increases with age and is often present in bitches with previous reproductive problems, possible underlying pathological factors need to be further investigated in the attempt to reduce the incidence of the phenomenon.

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CRediT authorship contribution statement

Petra Lascialfari: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Matteo Tesi:** Methodology, Investigation. **Cristiana Manetti:** Investigation, Writing – original draft. **Diana Fanelli:** Formal analysis, Writing – review & editing. **Alessandra Rota:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

None.

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