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RESEARCH ARTICLE

Factors Associated With Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility

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The ability to safely and effectively manage reproduction is central to the success of AZA captive-breeding programs. Although the AZA Wildlife Contraception Center routinely monitors contraceptive safety, there have been no studies that compare the effects of contraceptive use to separation of males from females, the other option for preventing reproduction. We used retrospective medical records and pathology reports submitted by AZA and related facilities for the seven AZA-managed canid species to assess rates of uterine pathology relative to female reproductive life histories. Our results showed that the prevalence of both pyometra and endometrial hyperplasia (EH) was associated not only with treatment with the two most common contraceptives (Suprelorin® and MGA implants) but also with the number of years barren (i.e., not producing a litter and not contracepted). Rates of pyometra and EH were especially high in African painted dogs and red wolves, but lowest in swift and fennec foxes. The number of years producing a litter had a low association, suggesting it could be protective against uterine pathology. A more recently developed Suprelorin® protocol using Ovaban® to prevent the initial stimulation phase, followed by implant removal when reversal is desired, may be a safer contraceptive option. These results concerning the relationship between reproductive management and uterine health have important implications for AZA-managed programs, since the unsustainability of many captive populations may be due at least in part to infertility. Managing a female's reproductive lifespan to optimize or maintain fertility will require a reconsideration of how breeding recommendations are formulated. Zoo Biol. 33:8–19, 2014.

Keywords: captive breeding; contraception; infertility; uterine pathology; foxes; wolves

INTRODUCTION

Contraception is central to reproductive management of many AZA-managed species, yet finding methods that are effective, safe, and reversible for the wide range of taxa needing fertility control remains a challenge. The two contraceptives most commonly used in female mammals, deslorelin (Suprelorin®, Peptech Animal Health, Macquarie Park, Australia) and MGA (melengestrol acetate, Wildlife Pharmaceuticals, Ft. Collins, CO) implants are effective in most species. However, in the 1990's MGA implants and other progestin-based contraceptives were shown to carry risk of uterine and mammary pathology in felids [Munson, 2006] and likely in other carnivores as well [Chittick et al., 2001; Moresco et al., 2009]. GnRH agonists such as Suprelorin® were not in wide use at the time of the Moresco canid study, so were not included in that analysis.

Suprelorin®, a GnRH agonist that had been developed and tested in domestic dogs and cats [Trigg et al., 2001; Rubion et al., 2006] became available to the zoo community

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around 2000. Because its mechanism of action is down-regulation of the pituitary–gonadal axis, in the absence of estrogen and progesterone [Herbert and Trigg, 2005], uterine and mammary tissue stimulation should not occur. Thus, Suprelorin® was considered a safe alternative for wild carnivores.

The AZA Wildlife Contraception Center (WCC) distributes an annual survey on contraceptive use in zoos and aquaria and encourages those institutions to contact the WCC when questions or problems arise regarding the use of contraceptive products. Early in 2009 the WCC received several reports of pyometra, a potentially life-threatening uterine infection, in African painted dogs (*Lycaon pictus*) and red wolves (*Canis rufus*), but it was unclear whether the infections might be associated with contraceptive use or merely reflect a tendency in those species. For example, Moresco et al. [2009] found a higher likelihood of pathology of the uterine endometrium (including pyometra) in painted dogs than in other wild canids, regardless of prior treatment with progestins. This species difference may be similar to the higher risk of pyometra seen in some domestic dog breeds [Smith, 2006; Hagman et al., 2011].

If undetected or untreated, pyometra can result in death. The usual treatment is surgical removal of the uterus and ovaries, which prevents future reproduction even though it may spare the life of the mother. The typical procedure of removing the ovaries also precludes the option of future gamete rescue. Regardless, the female is removed from the breeding population, which can have profound effects on the success and sustainability of the program, particularly if the female is genetically valuable. Even with medical treatment instead of surgery, future fertility may be compromised [Meyers-Wallen et al., 1986]. Given the serious implications of this condition for female health and fertility, it is critical that we better understand the factors affecting its occurrence.

The most prevalent uterine lesion found in zoo canids in an earlier study [Moresco et al., 2009] was endometrial hyperplasia (EH). There is some controversy about the association between EH and pyometra in domestic dogs, the model available for understanding the condition in wild canids [De Bosschere et al., 2001]. The traditional dogma is that EH is a pre-condition for pyometra [Verstegen et al., 2008], yet not all cases of EH proceed to pyometra and pyometra sometimes develops in the absence of EH. Particularly during the luteal phase of the reproductive cycle, when progesterone is elevated, a variety of physical, biological, and chemical substances have been shown to cause uterine endometrial irritation which can develop into pyometra in domestic dogs [De Bosschere et al., 2001].

The endocrine milieu that favors development of both EH and pyometra is elevated progesterone, further enhanced by estrogen priming [Teunissen, 1952], the natural pattern of hormone secretion during estrus and diestrus (following ovulation) in canids. Canids are unusual in that ovulation is followed by a very protracted, 2-month diestrous phase with elevated progesterone, a period equivalent to gestation [Asa, 1999]. This is in contrast to most other mammalian species that typically have a diestrous period of only about 2 weeks. Thus, the canid uterine endometrium is primed by estrogen then exposed to a long period of progesterone stimulation creating conditions favorable to supporting and nourishing embryos, conditions also ideal for pathogen growth. Even in the absence of infection, repeated non-conceptive cycles favor development of EH.

Thus, understanding and diagnosing EH in canids is important, because even when it does not lead to pyometra, it can be implicated in infertility. Changes in the integrity of the uterine endometrium associated with EH can compromise implantation and pregnancy maintenance. The primary risk factor for EH and pyometra is exposure to progesterone during natural cycles or treatment with synthetic progestins, for example MGA implants for contraception. Treatment with Suprelorin® has been assumed to be equivalent to one natural cycle, because as a hormone agonist, it can first stimulate estrus and ovulation before achieving down-regulation. Risk should be lower when the stimulation phase is prevented by 2 weeks treatment with the oral synthetic progestin megestrol acetate (Ovaban®: Schering-Plough Animal Health, Union, NJ) [Wright et al., 2001] and lowest following pregnancy and birth, since parturition is followed by more complete endometrial remodeling than non-pregnant cycles.

To assess possible risk factors, the WCC undertook a retrospective survey and analysis of the prevalence of both endometrial hyperplasia (EH) and pyometra in the seven AZA-managed canid species: African painted dogs; fennec (*Vulpes zerda*) and swift (*V. velox*) foxes; Mexican (*C. lupus baileyi*), red and maned (*Chrysocyon brachyurus*) wolves; and bush dogs (*Speothos venaticus*). We compared the reproductive and contraceptive histories of females to documentation of EH or pyometra in medical records and pathology reports contributed by participating institutions. We predicted that the risk of EH and pyometra would be higher in females treated with MGA implants or experiencing more non-conceptive cycles (i.e., separated from males rather than contracepted), moderate in females treated with Suprelorin® alone, and lower in females producing more litters or treated with Suprelorin® plus Ovaban®.

METHODS

Data Collection

We requested medical records and pathology reports for female African painted dogs, bush dogs, fennec foxes, maned wolves, Mexican gray wolves, red wolves and swift foxes held in North American zoos. Three species (painted dog, bush dog, and maned wolf) have only International Studbooks, but only animals housed in North American zoos were included. For North America, in general, non-AZA zoos were excluded unless the coordinator of the respective AZA Species Survival Plans® (SSP) deemed them to be important holding institutions that had records of sufficient quality. We

set the date range for the records based on estimations of the numbers of animals and likely quality of data. That is, the various canid species became numerous in AZA institutions at different times, SSPs were established on different dates, and SSP status was expected to be associated with better record-keeping. Other factors affecting the thoroughness of record-keeping and potential availability of records, which could vary by institution and by species, were when or whether there was at least one full-time veterinarian on staff and the time when medical animal record keeping system (MedARKS) started being used. We asked the respective SSP coordinators to consider these factors and recommend start dates, that is, the earliest records we would request. Start dates for records differed among species due to differences in their respective SSPs (Table 1); end date was close of the request period, December 15, 2010.

Generating Reproductive Histories

Reproductive histories were created for each female using a variety of sources. Data on birth dates, litters born, and inter-birth intervals were obtained from single population animal record keeping system (SPARKS). The analyses included only a female's reproductive lifespan, that is, the years she would have been exposed to reproductive hormones and/or contraceptives. Reproductive lifespan was calculated by subtracting age of puberty for the species from the female's age at death, age at ovariohysterectomy (OVH) or age on date of last medical record for females still living. Females that died or were ovariohysterectomized before puberty were excluded from the analysis. Age of puberty was calculated from the species' studbooks (Table 1).

Because the possible number of reproductive cycles per year or number of litters per year might affect the condition of the uterine endometrium, we used studbook data to calculate inter-birth intervals for each species. We looked for the shortest interval between births for each species, ignoring outliers (e.g., intervals less than gestation plus estrus for the species), with the assumption that females in good health could cycle (ovulate) at those intervals. We then used that number to calculate the mean number of cycles a female of that species could have per year if she did not conceive and produce a litter, which would approximate the number of estrous and luteal phases her uterus would experience (Table 1). Since several of the species are strict seasonal breeders constrained to only one ovulation or litter per year, this approach allowed us to characterize the reproductive data by year (for seasonal breeders) or by a fraction of a year (for non-seasonal breeders) for analysis.

The number of years a female did not produce a litter and was not contracepted is not reported in any database. That information is critical to a comparison of risk factors, since other than contraception, the management alternative for avoiding production of a litter is separation of females from males. Thus, it is important to establish the relative health risks of a female remaining barren but not treated with a contraceptive. To generate those data we added the number of years each female produced a litter to the number of years she was treated with a contraceptive, then we subtracted that sum from her reproductive lifespan.

Contraception information was gathered from the AZA Wildlife Contraception Center Database. Contraceptive analysis only included MGA and Suprelorin® implants; although some individuals had been treated with Lupron Depot® (leuprolide acetate: TAP Pharmaceuticals, Chicago, IL) or with Depo-Provera® (medroxyprogesterone acetate: Pfizer, New York, NY), there were too few cases for statistical analysis. However, the two different protocols for Suprelorin® treatment were analyzed separately: Suprelorin® only or Suprelorin® plus Ovaban® (megestrol acetate). Giving megestrol acetate (MA) for 1 week before and 1 week after Suprelorin® implant insertion counters the stimulation phase to prevent estrus and ovulation, which would then be followed by approximately 2 months of elevated progesterone. Thus, the hormonal milieu accompanying the first 3 months of treatment differs considerably between the two protocols, which could have different effects on the uterine endometrium.

Based on calculation of average reversal times for MGA implant use in canids recorded in the WCC database, when implants were not removed, mean duration of efficacy was approximately 3 years. Thus, if MGA implants were not removed, duration of efficacy was assumed to be 3 years; otherwise, time of implant removal was used as the end time. For Suprelorin®, with or without megestrol acetate, duration of efficacy was designated as 6 months for the formulation designated as a 6-month implant and as 12 months for the formulation designated 12-months. Although this may

TABLE 1. Date ranges for records and parameters comprising reproductive histories, by species

Species	Record start date	Record end date	Age at puberty (month)	Interbirth interval (month)	Mean no. of cycles/year
Painted dog	1/1/1990	12/15/2010	22	8	1.5
Bush dog	1/1/1960	12/15/2010	10	7	1.7
Fennec fox	1/1/1995	12/15/2010	10	7.5	1.6
Maned wolf	1/1/1984	12/15/2010	22	12	1
Mexican wolf	1/1/1984	12/15/2010	22	12	1
Red wolf	1/1/1984	12/15/2010	22	12	1
Swift fox	1/1/1984	12/15/2010	10	12	1

underestimate the duration of suppression, especially for non-seasonally breeding species, there have been insufficient data on Suprelorin® reversals reported for canids for quantification. When successive Suprelorin® implants, without megestrol acetate, were placed within 6 or 12 months for those formulations, respectively, we assumed there was continuous down-regulation and no additional stimulation phase.

Thus, the variables that comprised each female's reproductive lifespan were: number of years producing a litter, number of years treated with a contraceptive (MGA, Suprelorin®, or Suprelorin® plus megestrol acetate, as separate variables), and number of years not producing a litter and not contracepted (i.e., number of barren years).

Extraction of Data From Medical Records and Pathology Reports

Designation of pyometra was standardized based on clinical observations, bacterial culture, and pathological findings. Clinical signs of open pyometra included vulvar discharge with possible additional signs of lethargy, depression, anorexia, polyuria, polydipsia, vomiting, or diarrhea [Renton et al., 1993; Pretzer, 2008]. Clinical signs of closed pyometra were expected to be more marked, with possible systemic illness due to bacterial toxins in the intraluminal fluid of the uterus [Pretzer, 2008]. Evidence of enlarged uterus by radiograph or ultrasound or a pus-filled uterus following OVH or during necropsy provided further diagnostic confirmation. The bacterium most frequently isolated in canine pyometra is *Escherichia coli* [De Bosschere et al., 2001], although it was considered confirmation rather than requisite for definitive diagnosis. All animals were cross-referenced to pathology results from the Wildlife Contraception Center-Health Surveillance Program database [established by L. Munson and maintained by A. Moresco].

Statistical analysis was based on pyometra diagnoses of Yes, No, or Suspected, based on clinical, bacterial culture, and gross histopathological findings. We did not consider gross inspection alone to be adequate for a positive diagnosis of pyometra (Yes); cytologic or histologic confirmation was necessary. We assigned cases with clinical signs (e.g., fever, discharge from vulva) and gross observation of the uterus suggestive of pyometra but without histopathology results as Pyometra Suspected.

Designation of EH was also standardized. Because EH can be a chronic, subclinical condition [De Bosschere et al., 2001] that is difficult to diagnose without histopathology, the diagnosis of EH was based solely on histological data in reports from necropsy or post-OVH.

Statistical Analyses

All data were compiled in an Access relational database (Microsoft 2007) to allow integration of the variables. The development of uterine endometrial pathology, either pyometra or endometrial hyperplasia, is the result of complex interactions among factors such as the individual animal's physiology, exposure to either endogenous (natural reproductive cycles) or exogenous hormones (various contraceptive treatments), and its reproductive history. This complexity is represented in the way the data were collected and the database assembled. In recent years there has been tremendous criticism of the application of classical inferential statistics to this type of data that were not collected to test specific hypotheses [Anderson et al., 2000; Anderson and Burnham, 2002; Whittingham et al., 2006]. To assess the relative contribution of each factor, we used a multi-model inference approach [Anderson and Burnham, 2002; Burnham et al., 2011]. Using an information-theoretical method allows multiple hypotheses to be assessed with the application of a parsimony contrast [Whittingham et al., 2006; Burnham et al., 2011].

We used a generalized linear model (GLM) framework in the R statistical environment (v. 2.14.2, R Development Core Team 2012) with a multi-model selection step function. Those models with the lowest Akeike's information criterion (AIC) are considered the most parsimonious [Link and Barker, 2006]. This approach compares multiple models by estimating the difference between the information criterion and the data: ΔAIC [Anderson and Burnham, 2002]. Models with ΔAIC between 0 and 2 have the most evidentiary support. Only those models with a ΔAIC less than 2 were retained for further evidentiary analysis, that is, model parameter weight: w_i [Anderson and Burnham, 2002]. We performed multi-model selection analysis for each pyometra and for EH, first using the data for all species combined, then on a species-by-species basis (Table 1). The analysis was run twice for pyometra, first with Pyometra Suspected cases included with Pyometra Yes, then with Pyometra Suspected cases included with Pyometra No. There were too few cases to permit analysis of Pyometra Suspected as a separate factor. For species that did not have enough positive pyometra or EH cases for analysis, descriptive statistics are provided.

A contingency test (G-test) was used to assess the prevalence of pyometra in each species relative to the population, which combined data for all the species. The parallel assessment for endometrial hyperplasia used an adjusted G-test [Sokal and Rohlf, 1981]. An Aspin–Welch unequal variance t-test (NCSS, Kaysville, Utah) was used to compare the number of pyometra-positive and -negative females for age at first reproduction, for number of litters, and for number of reproductive years with no litters and no contraception.

RESULTS

The results for pyometra represent 775 records for the seven canid species. Records were requested from 1,027 females but received for only 871. Reasons for missing records were (1) the institution did not respond, or (2) the female's records had been lost. We excluded females that

TABLE 2. Results of GLM multimodel analyses for factors associated with pyometra or endometrial hyperplasia (EH)

	Model	ΔAIC	w_i
Pyometra[a]			
All species	MGA + Suprelorin®	0	0.56
	MGA + Suprelorin® + barren	2.4	0.31
Painted dog	Litters + MGA + Suprelorin®	0.3	0.22
Red wolf	Suprelorin® + barren	0	0.80
Mexican wolf	MGA	0	0.77
	MGA + Suprelorin®	1.4	0.12
Pyometra[b]			
All species	Barren + MGA	0.4	0.49
Endometrial hyperplasia			
All species	Litters + barren + MGA	0	0.27
Painted dog	MGA	0	0.31
	MGA + barren	1.13	0.08
Red wolf	Barren	0	0.15

[a]Pyometra Suspected added to Pyometra positive diagnosis.
[b]Pyometra Suspected added to Pyometra negative diagnosis.

died before or very soon after the time of puberty, so had not experienced a reproductive cycle. Only 290 pathology reports included histology results for the uterine endometrium following death or surgical removal of the uterus, which was necessary to diagnose endometrial hyperplasia.

Pyometra

A general linear model (GLM) with a multimodel inference approach was used to evaluate factors associated with pyometra. The first analysis combined suspected and positive cases of pyometra. We combined and analyzed data for six of the seven species (Table 2; there were too few cases of pyometra in swift foxes to meet the criteria for analysis). The results suggest that the strongest support was for the model that retained MGA implants and Suprelorin®, with some support also for number of years barren. Still, MGA and Suprelorin® had greater support than when number of years barren was added to the multimodel inference. Examination of the weights (w_i) for all species shows that 87% of the variance can be explained by these factors. The GLM was run again but with suspected cases of pyometra added to those negative for pyometra. The results indicate the strongest support for the model retained years barren and MGA implants, explaining 49% of the variance (Table 2). All subsequent results presented are for analyses where suspected pyometra was classified as Yes.

There were sufficient data for GLM analyses of painted dogs, red wolves, and Mexican wolves (Table 2). For painted dogs, the strongest support was for number of years treated with MGA implants or Suprelorin®; number of years producing a litter had a negative value, indicating a negative association with pyometra. For red wolves, years barren or treated with Suprelorin® had the strongest support, which accounted for 80% of the variance. For Mexican wolves, years treated with MGA implants or Suprelorin® had strongest support, accounting for 89% of the variance.

Rates of pyometra for all animals, regardless of reproductive history, ranged from a high of 23.5% to a low of 1.6% (Table 3 and Fig. 1a). The rate was significantly higher in painted dogs ($G = 244.6$, $P < 0.0001$) than that of the population, but significantly lower in swift ($G = 128.0$, $P < 0.0001$) and fennec foxes ($G = 73.3$, $P < 0.0001$), respectively. Mean age at birth of first litter (for those females that had given birth at least once) was significantly different between pyometra positive and negative cases only for bush dogs (Fig. 2a). Although the difference appears significant for swift foxes, only one female was positive for pyometra.

In the dataset analyzed for pyometra, most females treated with a contraceptive received only one type of product ($n = 139$), but 19 (13.7%) received more than one type (Table 4). Of the 65 total number of cases (all species) positive for pyometra, 31 females (47.7%) had been treated with a contraceptive at least once, but 34 (52.3%) had never been treated with any contraceptive product. Of the reports negative for pyometra, 127 out of 710 females (17.9%) had been treated with contraceptives. For females treated with contraceptives, the duration of treatment ranged from about 6 months to almost 6 years for MGA implants, from about

TABLE 3. Relative rates of pyometra and endometrial hyperplasia (EH) by species[a]

Species	Incidence of pyometra[b] (%)	Incidence of EH (%)
Painted dog	23.5	40
Bush dog	9.4	15
Fennec fox	2.7	9.1
Maned wolf	5.6	100
Mexican wolf	3.9	37.5
Red wolf	11.1	75
Swift fox	1.6	0
Total	8.4	38.1

[a]Combines data for all reproductive life history variables.
[b]Includes suspected and confirmed cases.

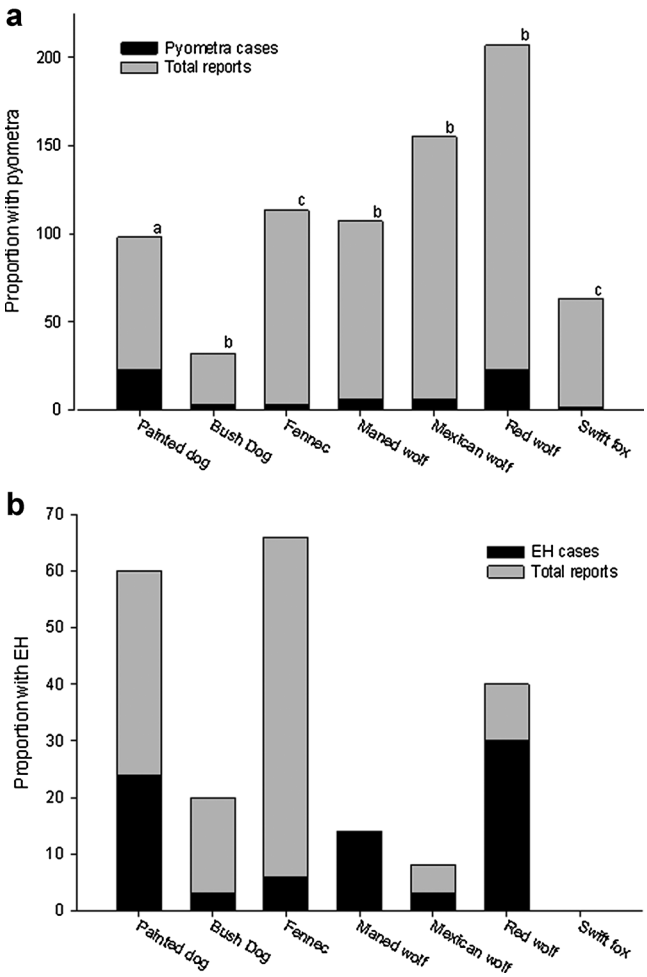

Fig. 1. (**a**) and (**b**) Comparison of the prevalence by species of (a) pyometra and (b) endometrial hyperplasia (EH); bars with different superscripts are significantly different.

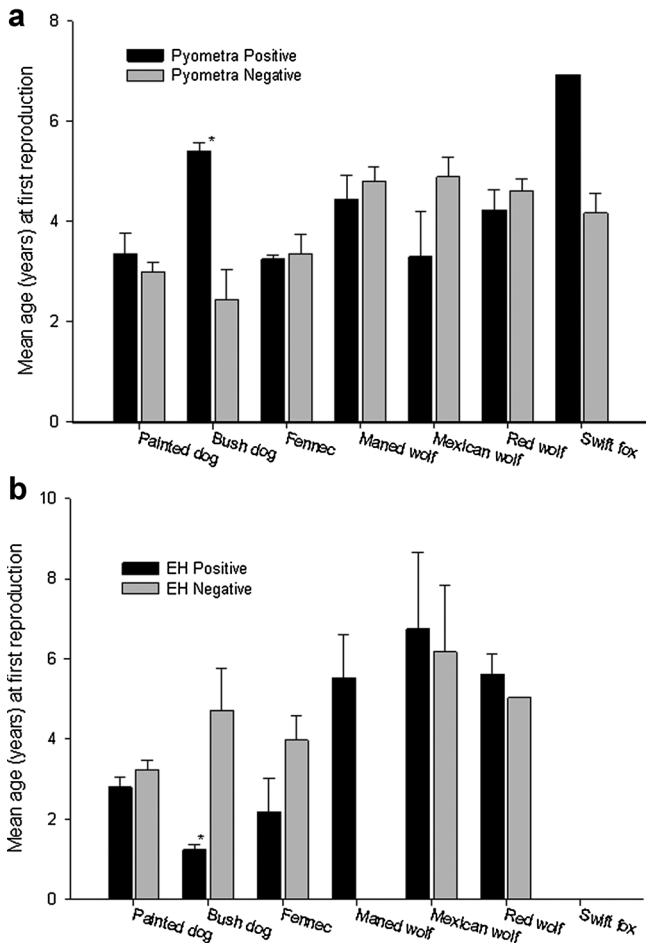

Fig. 2. (**a**) and (**b**) Comparison by species of the mean age at first reproduction among females diagnosed with (a) pyometra or (b) endometrial hyperplasia (EH); *Significant difference between positive and negative cases for that species.

6 months to 2 years for Suprelorin®, and from about 6 months to 3 years for Suprelorin® plus MA.

There was an insufficient number of cases of females treated with Suprelorin® plus MA for statistical analysis of its relationship with pyometra, but the association appears to be low. Of cases positive for pyometra (see Table 4) only five female painted dogs had received this combination and four of those had also been treated previously with either MGA implants or Suprelorin® only. Thus, only one female that was positive had been treated solely with Suprelorin® plus MA. In contrast, 23 females negative for pyometra had been treated with the Suprelorin® plus MA combination. A higher percentage of positive cases were associated with MGA or Suprelorin®-only treatment than Suprelorin® plus MA: 16.4% of females treated only with MGA implants and 18.6% of females only treated with Suprelorin® alone, compared to 7.7% of females only treated with Suprelorin® plus MA.

To evaluate the rate of pyometra associated solely with reproductive cycles, we compared the subset of females that had never been treated with a contraceptive and had never produced a litter, that is, females that we assumed had experienced repeated ovulatory cycles throughout their lives post-puberty. The rates of pyometra in these females and their relative ages (as number of reproductive years post-puberty) at the time of diagnosis are presented in Table 5. The number of cases varied by species, but species differences in pyometra rates in this sub-group of females were apparent. There were only two species (painted dogs and red wolves) with more than one female positive for pyometra in this data subset. Painted dogs had the highest rate (18.6%), followed by red wolves (10.4%), while fennec foxes and Mexican wolves had the lowest rates (Table 5). For the two species with enough cases for statistical comparison, there was no difference for pyometra-positive or -negative painted dogs in this data subset on mean number of reproductive years, but pyometra-positive red wolves were significantly older (more reproductive years) than those that were negative ($P < 0.0001$). Although there was an insufficient number of positive cases for the other species, it is interesting to note that the minimum number of reproductive years at diagnosis was above 8 reproductive years for all but painted dogs, red wolves, and bush dogs, where minima were 2.3, 3.1, and 3.7 years, respectively. That

TABLE 4. Comparison of the number of positive and negative cases of pyometra in females treated with contraceptives

	Total *N*	MGA implants (only/ever)[a]	Suprelorin® (only/ever)	Suprelorin® + MA (only/ever)	More than one contraceptive
Painted dog					
Pyometra Yes[b]	23	5/7	2/4	1/5	4
Pyometra No	75	12/13	2/5	3/7	4
Bush dog					
Pyometra Yes	3	0	0	0	0
Pyometra No	29	0	0/1	1/2	1
Fennec fox					
Pyometra Yes	3	0	0	0	0
Pyometra No	110	1/6	4/6	2/5	5
Maned wolf					
Pyometra Yes	6	1/1	0	0	0
Pyometra No	101	6/7	4/6	0/3	3
Mexican wolf					
Pyometra Yes	6	0/2	0/2	0	0
Pyometra No	149	2/2	12/13	3/3	1
Red wolf					
Pyometra Yes	23	4/4	6/6	0	0
Pyometra No	184	29/30	12/13	3/3	1
Swift fox					
Pyometra Yes	1	0	0	0	0
Pyometra No	62	1/1	1/1	0	0
Totals					
Pyometra Yes	65	10/14	8/12	1/5	4
Pyometra No	710	51/59	35/45	12/23	15

[a]Only = female was only treated with this type of contraceptive and no other; Ever = female was ever treated with this type of contraceptive, so may have also been treated with another.
[b]Includes suspected and confirmed cases.

is, not only was the rate of pyometra high for painted dogs and red wolves in this data subset, but they appeared likely to be diagnosed with pyometra at a relatively young age.

Endometrial Hyperplasia

Fewer records were available for analysis of EH than for pyometra, which limited the options for statistical analyses. As for pyometra, a GLM with a multimodel inference approach was used to evaluate factors associated with EH. We combined and analyzed data for six of the seven species (there were no cases of EH reported for swift foxes). The results suggest that the strongest support was for the model that retained number of years producing a litter, treated with MGA implants and barren (Table 2). However, this accounted for only 27% of the variance, indicating the

TABLE 5. Comparison of positive and negative cases of pyometra among females never treated with a contraceptive and never producing a litter

	Number of negative pyometra cases	Percent of negative cases (%)	Mean (±SE) reproductive years[a] (range)[b]	Number of positive pyometra cases	Percent of positive cases (%)	Mean (±SE) reproductive years[a] (range)[c]
Painted dog	35	81.4	5.5 ± 0.5 (1.5–10.3)	8	18.6	5.5 ± 0.8 (2.3–9.5)
Bush dog	16	94.1	5.0 ± 0.6 (0.7–10.8)	1	5.9	3.7
Fennec fox	67	98.5	7.8 ± 0.5 (0.8–14.7)	1	1.5	8.3
Maned wolf	47	97.9	5.9 ± 0.5 (0.7–13.73)	1	2.1	12.6
Mexican wolf	91	98.9	5.0 ± 0.3 (0.7–14.8)	1	1.1	9.2
Red wolf	69	89.6	3.8 ± 0.4* (0.8–12.9)	8	10.4	9.8 ± 0.7* (3.1–13.8)
Swift fox	33	100	5.6 ± 0.6 (1.1–12)	0	NA	NA

[a]Number of reproductive years = number of years beyond puberty.
[b]Range in number of reproductive years of females in medical records not diagnosed with pyometra.
[c]Range in number of reproductive years of females in medical records diagnosed with pyometra.
*Significantly different ($P < 0.0001$).

association was weak overall. At the species level, there only were sufficient data for analyses for painted dogs and red wolves. For painted dogs, the strongest support was for MGA implants followed by number of years barren; these accounted for 39% of the variance. For red wolves the strongest support was for years barren, but this accounted for only 15% of the variance.

Rates of EH ranged from a high of 100% (maned wolves) to 9% (fennec foxes; Table 3). No histology results were submitted for swift foxes. These differences in prevalence were significantly greater for red wolves ($G = 0.0001$, $P < 0.05$) and significantly lower for fennec foxes ($G = 10.69$, $P < 0.05$) and bush dogs ($G = 10.58$, $P < 0.05$). Although the prevalence for maned wolves was 100%, they could not be included in the contingency test because the number for negative cases of EH was zero.

In the subset of data analyzed for EH, most females treated with a contraceptive received only one type of product ($n = 53$), but 10 received more than one type (Table 6). Of the 80 total cases positive for EH, 32 females (40%) had been treated with a contraceptive at least once, but 48 (60%) had never been treated with any contraceptive product. Of the records negative for EH, 31 out of 130 females (23.8%) had been treated with contraceptives.

Age at first reproduction between females positive and negative for EH differed significantly ($P < 0.05$) only for bush dogs (Fig. 2b). Statistical comparison was not possible for maned and red wolves. Only EH-positive reports were submitted for maned wolves, and their mean age was within the range reported for the other three species (5.5 years). Only one red wolf negative for EH had produced a litter.

There was an insufficient number of cases of females treated with Suprelorin® plus MA for statistical analysis of its relationship with EH, but as with pyometra the association appears to be low. Of cases positive for EH (see Table 4) only five females (four painted dogs and 1 bush dog) had received this combination, and two of the painted dogs had also been treated with either MGA implants or Suprelorin® only.

DISCUSSION

The pattern emerging from our various analyses is that the prevalence of uterine pathology was higher for females that spent more years not reproducing. Although the ranking of risk factors varied somewhat among species, the factors that were consistently high were years contracepted with either MGA or Suprelorin® implants or years barren (i.e., not producing a litter and not contracepted). In contrast, years producing a litter were generally not associated with uterine pathology or even found to be protective.

Analysis of the full dataset for all seven species using GLM multimodel inference showed that the prevalence of pyometra (combining positive and suspected categories of

TABLE 6. Comparison of the number of positive and negative cases of endometrial hyperplasia in females treated with a contraceptive versus those never treated[a]

	Total *N*	MGA implants (only/ever)[a]	Suprelorin® (only/ever)	Suprelorin® + MA (only/ever)	More than one contraceptive
Painted dog					
EH Yes	24	7/8	1/2	2/4	2
EH No	36	4/4	3/5	0/2	2
Bush dog					
EH Yes	3	0	0	1/1	0
EH No	17	0	0/1	0/1	1
Fennec fox					
EH Yes	6	0/1	0/1	0	1
EH No	60	1/4	3/4	2/4	3
Maned wolf					
EH Yes	14	1/1	0	0	0
EH No	—	—	—	—	—
Mexican wolf					
EH Yes	3	0	0	0	0
EH No	5	1/1	0	0	0
Red wolf					
EH Yes	30	9/10	3/4	0	1
EH No	10	3/3	2/2	0	0
Swift fox					
EH Yes	—	—	—	—	—
EH No	2	0	0	0	0
Totals					
EH Yes	80	17/20	4/7	3/5	4
EH No	210	9/12	8/12	2/7	6

[a]Only = female was only treated with this type of contraceptive and no other; Ever = female was ever treated with this type of contraceptive, so may have also been treated with another.

pyometra) was highest for years treated with MGA implants and next highest for years treated with Suprelorin® implants, the two contraceptive products used most commonly in these species. Results of GLM multimodel inference at the species level revealed some differences among species but considerable overlap as well. For painted dogs and Mexican wolves, highest incidence of pyometra was associated with years treated with MGA implants, but for red wolves the highest incidence was associated with the number of years barren (i.e., not producing a litter and not contracepted), followed by years treated with Suprelorin®. These species differences may have been influenced by the number of years each species was exposed to the specific contraceptive product or years spent barren.

When we repeated the GLM analysis with the full dataset, using the more conservative approach of considering suspected (not confirmed) cases of pyometra to be negative, the highest incidence of confirmed pyometra was associated with years barren and years treated with MGA implants. The analysis using this definition for pyometra diagnosis differed from the other only in ranking the number of barren years higher than Suprelorin® years. The overall results showing the association of barren years with uterine pathology have especially important implications for reproductive management. Although contraception may be viewed as an artificial and therefore more risky approach, in fact, preventing a female from reproducing by separation from males can have a similar outcome as the use of some contraceptives.

In the GLM multimodel analysis of cases of endometrial hyperplasia, number of years barren and years treated with MGA implants were again the factors showing the strongest association with uterine pathology. This trend continued with painted dogs and red wolves that had sufficient uterine histology data for analysis at the species level. However, the number of years producing a litter appearing as a factor in the combined-species model seems anomalous, since it did not appear as an important factor in any other models for EH or pyometra (Table 2) and in fact was negative in one, indicating a negative association or even a protective effect. In this model, support for litter years was not very strong, likely due to the small dataset. This limitation could be addressed by more zoos submitting reproductive tracts to the Reproductive Health Surveillance Program associated with the AZA Wildlife Contraception Center (www.stlzoo.org/contraception).

The similarity of results for pyometra and EH is not surprising, since EH is often considered a condition that can predispose to pyometra, and EH can develop following inflammation [Verstegen et al., 2008]. In the normal reproductive cycle, after estrogen priming during the estrous phase, progesterone production throughout the luteal phase following ovulation prepares the uterine endometrium to nourish developing embryos. That environment is also favorable to the growth of bacteria, if they are able to gain access, as occurs with pyometra. Melengestrol acetate, the active ingredient in MGA implants, is a synthetic progestin with effects similar to progesterone. Thus, treatment with MGA can stimulate endometrial growth and produce conditions that can favor development of EH and pyometra.

We expected Suprelorin® (deslorelin, a GnRH agonist) to be a safer contraceptive option, since the prolonged period of uterine stimulation that accompanies MGA implant treatment (2 or more years per implant) is avoided. However, as an agonist, Suprelorin® first stimulates the reproductive axis, which can result in ovulation followed by prolonged progesterone production during the subsequent luteal phase. Nevertheless, we expected the approximately 2-month induced luteal phase to be roughly equivalent to a normal luteal phase, not adding to risk above that of a typical cycle. More importantly, 2 months is a considerably shorter period of progesterone exposure than the continuous release of progestin from an MGA implant. Thus, we were surprised to find that Suprelorin® treatment was associated with both pyometra and EH at about the same level as MGA implants. This might be explained by results of studies of domestic dogs, which have shown that estrogen priming followed by progesterone had a more profound effect on the uterus than either estrogen or progesterone alone [Teunissen, 1952]. Therefore, a cycle stimulated by Suprelorin®, or even natural cycles, with estrogen priming preceding 2 months of elevated progesterone, might have as much if not more effect on the endometrium than long-term progestin treatment initiated (e.g., MGA implants) during anestrus, when estrogen would be very low.

Understanding the specific effects of contraceptives is complicated by several factors. Because these were retrospective analyses, the variables were neither controlled nor equal within or across species. In addition, some individuals were treated only once while others were treated for several years, and some females were treated with more than one contraceptive in succession. Animals were of different ages, with different reproductive histories, for example, number and frequency of litters produced, and age of first reproduction. In addition, there was no way to determine whether non-reproductive females were having normal ovulatory cycles. Despite these caveats, the accumulated data do show that the two most commonly used contraceptive treatments were associated with increased risk of uterine pathology. However, it is important to note that uterine pathology occurred even in females never treated with a contraceptive.

The risk of pyometra increases with age in domestic dogs [Fukuda, 2001]. However, age (number of reproductive years in this analysis) was not statistically associated with uterine pathology except in red wolf females never treated with contraceptives, that is, those positive for pyometra were significantly older. Statistical analyses were often limited by the small number of cases, but in a comparison of nulliparous females that were never treated with contraceptives, most animals had experienced many years of reproductive cycles when pyometra was diagnosed (Table 5). Exceptions were seen in a few painted dogs and red wolves as well as one bush dog, adding to the pattern of painted dogs and red wolves, in

particular, not only appearing to be more vulnerable to the development of uterine pathology but perhaps at younger ages. These females had undergone only natural reproductive cycles, affected just by endogenous hormones, with no exposure to contraceptives. These results are comparable to those reported for domestic dogs, that is, an increased prevalence of pyometra in females that undergo repeated natural cycles [Niskanen and Thrusfield, 1998].

In each type of analysis, there was a trend for higher rates of uterine pathology in painted dogs and red wolves. The prevalence of both pyometra and EH was significantly higher in these two species relative to the population of canids analyzed, regardless of reproductive history. In particular, this outcome for painted dogs is consistent with the report by Moresco et al. [2009], suggesting a species susceptibility. In contrast, swift and fennec foxes had the lowest rates of pyometra and EH, but there was only one histology report submitted for swift foxes by which to judge EH.

One approach to assessing possible reasons for these species differences is to compare their natural reproductive patterns. For example, the number of days of annual progesterone exposure is a function of the number of cycles per year and the relative length of the luteal phase. In canids, the luteal phase is roughly equivalent to the length of gestation. For the two fox species gestation is about 52 days [Asa, 1999; Asa and Valdespino, 2003], whereas in red wolves it is about 62 days and in painted dogs about 72 days [Newell-Fugate et al., 2012]. Thus, the red wolf and painted dog would be exposed to progesterone about 10 and 20 days more per cycle, respectively, than the foxes. However, although the swift fox ovulates only once annually, the fennec can have more than one cycle per year [Valdespino et al., 2002], which would increase its exposure to endogenous progesterone. The painted dog has both a relatively long luteal phase and can also, in some instances, have a second cycle in 1 year [Frame et al., 1979], perhaps exposing it to more progesterone annually than the other canid species. However, those cases usually involve a second cycle when the current litter is lost, which means the prior cycle was not a barren cycle but a pregnancy, which should have been protective against uterine pathology. The case is best for the swift fox, whose low rate of uterine pathology may be related to its relatively short luteal phase and single annual ovulation. Regardless of number of cycles per year, data for all canids studied to date suggest that they are monestrous, with an anestrous period intervening between cycles. That period of anestrous is considerably longer in species that ovulate less frequently. However, the important consideration for all canids, compared to other mammalian species, is that they have relatively long periods of proestrus and estrus when estrogen is being secreted, and long luteal phases with elevated progesterone.

Regardless of the possible explanations, uterine pathology, particularly in painted dogs and red wolves, presents a very real risk to their health and their fertility. Not only can EH predispose a female to life-threatening pyometra but it can render her infertile due to alterations in the uterine endometrium that prevent or reduce the ability of the female to maintain a pregnancy. The most effective way to prevent EH may be by allowing females to regularly undergo pregnancy and parturition.

This protective effect of pregnancy has also been reported for domestic dogs [Hagman et al., 2011]. Although progesterone is also elevated during gestation, the process of parturition and subsequent endometrial remodeling can return the endometrium to a baseline state. In addition, parturition is accompanied by increased prostaglandin (that stimulates uterine contractions) and an abrupt pre-partum decline in progesterone. In contrast, after the luteal phase of a non-pregnant cycle ends, progesterone concentrations gradually decrease across days or even weeks [Concannon et al., 1977].

Our results suggest that the number of years a female produced a litter was associated with lower risk of EH or pyometra, although the results for EH were not as clear, perhaps due to the limited number of cases submitted. Also, the number of years pregnant was not consistent across individuals or species, so we cannot predict how frequently a female should be allowed to reproduce. Nevertheless, spacing pregnancies should be preferable to clustering them, separated by long periods of contraception or separation from males. Interrupting non-reproductive periods with pregnancy and parturition could help maintain uterine health. Current reproductive management often involves delaying first reproduction in young females or breeding annually till a female is genetically well-represented then recommending against reproduction for prolonged periods. Those extended periods without reproduction are likely to be increasing the risks of uterine pathology in those females.

Although separating males from females may present social and logistical problems, managers sometimes consider that option to carry lower risk to the female's health than contraceptive treatment. Our results reveal that the two contraceptives used most in canids (MGA implants and Suprelorin®) carry a roughly equivalent risk to uterine health compared to separation. Thus, avoiding contraception to achieve reproductive management does not avoid the risk of uterine pathology. In fact, a variation of the Suprelorin® treatment protocol may be associated with lower risk.

There was an insufficient number of cases of the more recently introduced Suprelorin® contraceptive protocol (Suprelorin® plus 2 weeks of megestrol acetate pills (Ovaban®) to prevent the stimulation phase) to include in the GLM or prevalence analyses. That is especially unfortunate, because this protocol avoids exposure to the endogenous progesterone which would follow stimulated ovulation. Thus, we predict that it should pose a lower risk of uterine pathology than the other methods or than natural cycles. Without the stimulation phase and with down-regulation of the pituitary gonadotrophs and the resulting absence of the gonadal steroids that can induce EH, this protocol should be a safer alternative. Megestrol acetate, of

course, is itself a synthetic progestin. However, this 2-week treatment presents a much lower risk for two reasons. First, 2 weeks is considerably less exposure than 2 or more months. Second and perhaps more importantly, if treatment is initiated when a female is not in proestrus or estrus, there is no estrogen priming, which should reduce the availability of progestin receptors and the ability of progestin to act. Although reversibility of Suprelorin® is not yet well-established, WCC studies are underway to define the parameters of reproductive suppression following Suprelorin® treatment.

A possible additional concern is that some reports to the WCC have shown or suggested considerable variability in hormone concentrations and patterns among individuals during the reversal phase. For example, progesterone concentrations may be higher during the first luteal phase during recovery, which might exacerbate any underlying uterine pathology. Conjecture is that as levels of deslorelin (the active ingredient of Suprelorin®) fall below the threshold required to maintain pituitary down-regulation, it may be temporarily stimulatory until levels decline sufficiently to have no effect. To counter this possibility, the WCC has worked to develop implant insertion protocols that allow implant removal, to hasten reversal and eliminate any recovery phase stimulation. A link to a video illustrating these insertion techniques can be found on the American Association of Zoo Veterinarians (AAZV) webpage. The WCC (in collaboration with Christensen et al. [2012]) has developed a transcervical biopsy technique to aid in diagnosing EH, with a hope to test treatments that might reverse the condition and restore fertility.

Although canids might be the most likely taxon to develop pyometra, uterine pathology in other species can take many forms, including EH. However, there has been no systematic study of the prevalence of uterine pathology in other taxa except perhaps felids [Munson, 2006]. Those analyses focused primarily on contraceptive use compared to non-contracepted reproductive tissues, without factoring in years separated from males versus years producing litters. There have been no other studies that compared treatment with contraception to pregnancies and to separation of genders that result in barren cycles. Yet there have been occasional reports published for other species on uterine pathology related to non-reproductive years, for example, nulliparity, in a variety of species [Hildebrandt et al., 2000; Agnew et al., 2004; Hermes et al., 2004; Napier et al., 2009; Crosier et al., 2011]. Given the challenge of sustainability facing zoos, it is critical that further research be focused on assessing reproductive health and fertility and on addressing problems identified so that fertility can be maintained or restored. Reproduction is a process which requires the coordination and sequential action of many hormones and physiological processes. Uterine health is only one of the potential endpoints that can be measured. More extensive monitoring and study of more of the endpoints is central to determining to what extent reproductive failures are due to infertility or other factors such as behavioral incompatibility.

CONCLUSIONS

1. This study found that uterine pathology (endometrial hyperplasia and pyometra) was associated with number of years contracepted with two particular contraceptives (MGA implants and Suprelorin® alone) and with number of years barren (not producing a litter and not contracepted).
2. Years producing a litter was associated with a lower incidence of uterine pathology.
3. Suprelorin® plus Ovaban® may protect the uterine endometrium by minimizing exposure to progesterone.
4. African painted dogs and red wolves seemed more susceptible to both types of uterine pathology, whereas swift and fennec foxes seemed least susceptible.
5. Uterine pathology not only threatens a female's health (i.e., pyometra) but also her fertility (endometrial hyperplasia).
6. AZA-managed programs should consider a female's lifetime reproductive recommendations in order to maintain her fertility.

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