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Estimated impact of multiple conceptuses per follicle on fecundity in the bitch

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ABSTRACT

Multiple conceptuses from one follicle due to multi-ovular follicles or monozygotic twins inflate the ratio between the numbers of conceptuses and corpora lutea and possibly the effect ascribed to experimental treatment. The aim of this study was to estimate the impact of multiple conceptuses per follicle on fecundity in the bitch. The numbers of conceptuses and corpora lutea of 105 bitches originated from 7 published experimental studies and of 132 from ovariohysterectomies done in a private practice and a welfare organisation. Ninety five bitches, producing 597 conceptuses, had at least as many conceptuses as corpora lutea; 83 had as many, 10 had one more and 2 had 2 more. Bootstrap resampling on these 95 bitches let us to conclude that 12.6% of bitches are expected to produce more oocytes that are each capable of yielding a conceptus than the number of follicles that ovulate and that 2.35% of conceptuses are expected to be in excess of the number of follicles that ovulate. Applying the results of the current study to 2 earlier studies showed that multiple conceptuses from one follicle may, at maximum, have increased the effect ascribed to prostatic fluid by 0.022 and 0.024 conceptuses per corpus luteum, which is minor in relation to the magnitude of the ascribed effects of 0.34 and 0.22 conceptuses per corpus luteum. The number of conceptuses relative to the number of corpora lutea provides a valid and precise measurement of fertility in the bitch.

1. Introduction

Due to the variation in ovulation rate among bitches [1–3], the relationship between the number of conceptuses and the number of corpora lutea (the latter being used as a proxy for ovulation rate) may provide a better measurement of fertility in an experimental setting than the mere number of conceptuses [2]. Follicles containing more than one oocyte, known as multi-ovular follicles (MOFs) occur in bitches [4–10]. The possibility of MOFs bring into question the validity of using the number of corpora lutea as an indication of ovulation rate and, hence, using the relationship between the number of conceptuses and the number of corpora lutea to measure fertility in bitches.

Ovariohysterectomy of the pregnant bitch enables the researcher to precisely and accurately determine the number of corpora lutea and post-implantation conceptuses [2,3,11–13]. The number of post-implantation conceptuses relative to the number of corpora lutea (implantation ratio) reflects the net outcome of the number of follicles that ovulated, the number of oocytes that were fertilised and the number of early embryos that died [14]. The higher the implantation ratio in a bitch is, the smaller the effects of fertilisation failure and embryonic death will be thereon. Thus the detracting effects of fertilisation failure and embryonic death will be smallest in bitches with implantation ratios of 100% or higher, compared to bitches with lower implantation ratios.

MOFs may have up to 17 oocytes although 2 or 3 are more common [4]. MOFs are more common in mongrels than in purebred bitches [9], and their prevalence decreases with age of the bitch and with size of the follicle [4,5]. As many as 7% [10] to 40% [9], of pre-antral follicles have been found to be MOFs. In contrast, only 1% of pre-antral follicles in young anoestrous bitches (1–2) and none in older bitches (7–11) contained more than one oocyte [4]. MOFs may ovulate [7]; a view being supported by reports indicating that the number of oocytes or embryos flushed from bitches was higher than the number of corpora lutea present [8,15]. It is unknown how many of the oocytes released by ovulation from MOFs are able to

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mature and be fertilised [16] as the oocytes within a MOF can be at different developmental stages, and can be viable or degenerate [6]. Research suggests that only one oocyte of good quality is contained within a MOF [9,10].

As early as 1973 Anderson and Simpson [1] showed that, in 6 of 22 litters the number of conceptuses exceeded the number of corpora lutea by one. Anderson and Simpson's finding raises the question to what extent multiple conceptuses derived from a follicle (MCFAF) may have caused spurious outcomes of past experiments in which groups of bitches were exposed to different treatments aimed at affecting fertility. Two past studies done at this institution [3,11] serve as examples. In the first Nöthling [11] inseminated 10 bitches vaginally with frozen-thawed semen to which prostatic fluid was added after thawing (Group T) and another 10 with similar semen to which no fluid was added (Group C). Group T had 93 corpora lutea and yielded 52 conceptuses whereas Group C had 107 corpora lutea and yielded 24 conceptuses. Nöthling concluded that the addition of prostatic fluid improved the fertility of vaginally inseminated frozen-thawed dog semen. In the second study Nöthling et al. [3] inseminated 12 bitches vaginally with frozen-thawed semen to which prostatic fluid was added after thawing (Group P) and another 12 with similar semen to which albumin-free Talp was added (Group Ta). Group P had 126 corpora lutea and yielded 76 conceptuses whereas Group Ta had 117 corpora lutea and yielded 45 conceptuses. Nöthling et al. [3] concluded that the addition of prostatic fluid yielded higher fertility than the addition of albumin-free Talp. The question arises to what extent the effect of prostatic fluid may have been inflated due to the occurrence of MCFAF.

The aims of Experiment 1 of the current study were (1) to estimate the overall probability of a bitch having more than one conceptus derived from a smaller number of follicles (such as 2 conceptuses derived from one follicle), (2) to estimate the overall frequency of more than one conceptus being derived from a smaller number of follicles, (3) estimate the probability of more than one conceptus derived from a smaller number of follicles in bitches with different known numbers of conceptuses and (4) estimate the probability that different numbers of bitches in a group consisting of a specified number of bitches would have at least 2 conceptuses derived from one follicle.

The aim of Experiment 2 was to determine the maximum extent to which MCFAF could have inflated the effect ascribed to prostatic fluid by Nöthling [11] and Nöthling et al. [3].

2. Materials and methods

2.1. Experiment 1

The current study was approved by the Animal Use and Care Committee of the University of Pretoria, South Africa (Project number V059/11).

We comprehensively reviewed the literature on the topic (Section 2.1.1) and collected data from bitches undergoing routine ovariohysterectomy during pregnancy or immediately after caesarean section at a private practice and a welfare organisation (Section 2.1.2) to identify bitches with more conceptuses than corpora lutea.

2.1.1. Collection of data from the literature

A comprehensive literature search was conducted using the search engine of the virtual library of the Faculty of Veterinary Science, University of Pretoria, South Africa (www.library.up.ac.za/vet/virtlib.htm) and the databases PubMed, Science Direct, Scopus and CAB to identify literature related to the topic 'fertility in the bitch'. The search terms "fertility AND bitch", "ovulation rate AND bitch", "fertilisation rate AND bitch", "implantation rate AND bitch", "early embryonic death AND bitch", "foetal death AND bitch", "multi-ovular follicles AND bitch", "insemination AND bitch", "corpus luteum AND bitch", "ovulation AND bitch", "fertilisation AND bitch" and others to include all publications addressing the topic of fertility in the bitch. A systematic review of citations in the retrieved papers was also carried out.

Only those publications that met the criteria below were used to determine the prevalence of bitches having more conceptuses than corpora lutea:

1. The number of conceptuses and the number of corpora lutea had to be recorded for each bitch.
2. The uterus was removed for inspection and the ovaries were removed and inspected or sliced to accurately count the number of corpora lutea.
3. The time at which the reproductive organs were removed was stated.

The numbers of conceptuses and corpora lutea for each bitch were recorded as Subset A.

2.1.2. Collection of data from bitches that underwent ovariohysterectomy at a private practice and at a welfare facility

The reproductive organs used in the present study were collected at a private small animal practice (Rant-en-Dal Veterinary Clinic, Krugersdorp, South Africa), as well as an animal welfare organisation (Society for Prevention of Cruelty against Animals (SPCA), Howick, South Africa), from bitches that were destined for ovariohysterectomy (and pregnancy was incidental), or immediately after caesarean section in bitches from which owners no longer wished to breed.

Anaesthesia and surgery were done according to generally approved and recommended methods. As premedication 7 μg/kg of medetomidine HCl (Zoetis Animal Health, Sandton, South Africa) was administered intravenously. Anaesthesia was induced by intravenously administered propofol (Propofol® 1%, Fresenius Kabi AG, Bad Homburg v.d.H., Germany) at a dose of 1–2 mg/kg and maintained with sevoflurane (1–2%) (Safeline Pharmaceuticals, Northcliff, South Africa) in oxygen.

Death of the embryos or foetuses during ovariohysterectomy in pregnant bitches was humane as it occurred within a few minutes after the blood supply to the uterus was ligated, which happened while they were fully anaesthetised. During surgery, care was taken not to damage the ovaries or the structures they contained by crushing or pinching them with instruments or rough handling. After excision the ovaries were placed in properly labelled bottles with 10% formalin. After its removal the uterus of pregnant bitches was opened and the number of post-implantation conceptuses recorded. In bitches that underwent caesarean section the number of puppies and dead foetuses was recorded.

The same person dissected all ovaries and counted the corpora lutea. Formalin-fixed ovaries were each cut into slices 1–2 mm thick. Care was taken to assess whether any corpus luteum seen on the second and subsequent slices formed part of a new structure or whether it was part of a corpus luteum counted in previous slices. The number of corpora lutea in the ovaries of each bitch was recorded.

The numbers of conceptuses and corpora lutea collected from bitches that underwent ovariohysterectomy at the private practice and welfare organisation were recorded as Subset B.

2.1.3. Data analysis

Those 95 bitches that each had at least as many conceptuses as corpora lutea were used for bootstrap resampling (with the number