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A model for cystic endometrial hyperplasia/pyometra complex in the bitch

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Abstract

The objective of this study was to develop a reliable model for the study of the cystic endometrial hyperplasia and pyometra complex (CEH/P) in the bitch. Greyhound bitches ($n = 15$) were ovariectomised and allocated into three groups (Group 1, $n = 5$; Group 2, $n = 5$; Group 3, $n = 10$, including 5 used from Group 1). Simulated proestrus, estrus and diestrus were induced by treatment with estradiol benzoate and megestrol acetate. The duration of cervical opening during estrus was determined by the intra-vaginal infusion of radio-opaque medium and subsequent radiography of the uterus (Group 1). One milliliter of a culture of *Escherichia coli* (with five uro-pathogenic virulence factors as identified by PCR: pap, sfa, hlyA, cnf1 and fim) was inoculated intra-vaginally daily throughout the simulated estrus (Group 2). One milliliter of the culture ($n = 6$) or sterile Luria–Bertani broth ($n = 4$) was introduced directly into the uterus on simulated diestrus Days 8 or 12 (Group 3). Necropsies were performed 12 and 7–14 days after the inoculation (Groups 2 and 3). The cervix remained open throughout the duration of simulated estrus (5–6 days) in four out of five bitches, and for a shorter duration (3 days of a 6-day estrus period) in one bitch (Group 1). CEH/P was induced by inoculation of bacteria into the uterus (10/10 bitches) but not into the vagina (0/5 bitches), ($P = 0.003$). A model for the study of CEH/P has been validated.
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1. Introduction

The cystic endometrial hyperplasia/pyometra complex (CEH/P) is an acute or chronic, poly-systemic, diestrual disorder of the adult, ovary-intact bitch characterised by hyperplasia of the endometrium and infiltration of inflammatory cells, which may be present in all layers of the uterus [1–3]. CEH/P results from an infection, commonly *Escherichia coli* (*E. coli*), ascending from the vagina [4].

In previous studies, CEH was induced in intact bitches by the administration of high doses of estrogen and progesterone for prolonged intervals, by uterine biopsies, uterine scarification, and uterine irritants such as silk suture material [5–7]. Furthermore, CEH/P can be induced in ovary-intact bitches by the intra-uterine inoculation of *E. coli* during diestrus, with or without ligation of cervix [5,8].

A model for CEH using ovariectomised bitches treated with a uterine irritant (silk suture) and estradiol benzoate and megestrol acetate has been described [9,10]. However, no model for CEH/P involving inoculation of bacteria into the vagina or the uterus of ovariectomised bitches has been established. The objective of this study was to validate such a model in order to facilitate further studies of the pathogenesis, treatment and control of this disease. A preliminary study was undertaken to determine whether *E. coli*, present in an inoculum, would enter the uterine lumen

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after being infused intra-vaginally in bitches during a simulated estrus.

2. Materials and methods

The study comprised three experiments. The objectives were to determine: (i) the duration of cervical patency in ovariectomised bitches during simulated estrus; (ii) the effect of intra-vaginal inoculation of *E. coli* on the occurrence of CEH/P; (iii) the effect of intra-uterine inoculation of *E. coli* on the occurrence of CEH/P.

2.1. Animals and treatments

Mature, ovary-intact greyhound bitches ($n = 15$) aged 2–3 years, and in anestrus (determined by physical examination of external genitalia, vaginal cytology and plasma progesterone concentrations) were ovariectomised. The bitches were allocated into three groups: Group 1, $n = 5$; Group 2, $n = 5$; Group 3, $n = 10$ (including five bitches from Group 1). Ovariectomies were performed under standard anesthesia protocols. Anesthesia was induced using propofol (Rapinovet; Schering-Plough, North Ryde, Australia) and maintained using isoflurane (Isoflurane; Advanced Anaesthesia Specialists, North Ryde, Australia). Two weeks after ovariectomy, simulated stages of the estrous cycle were induced by treating the bitches with estradiol benzoate (Mesalin; Intervet, Bendigo East, Australia; 0.6–4.8 μg/kg im, twice daily for 13 days), followed by megestrol acetate (Ovarid; Jurox Pty Ltd., Rutherford, Australia; 2.0 mg/kg orally, once a day, until euthanasia) [10].

2.2. Vaginal cytology, stages of the cycle

Vaginal smears were taken before ovariectomy (to confirm anestrus) and during the period of treatment with estradiol benzoate and megestrol acetate (to determine the simulated stages of the estrous cycle). The smears were taken every 3 days, from Days 1–7 of treatment, then every 2 days from Day 8 of treatment until Day 4 of estrus, and then daily from Day 5 of estrus until Day 1 of diestrus. The vaginal smears were stained with Diff-Quick (Laboratory Aids, Narrabeen, Australia) and superficial cell indices (SCI) determined [11]. Anestrus was when SCI was 0% and plasma progesterone concentrations were <0.5 ng/mL. Estrus was the period when the SCI was >90% and the bitches showed positive postural reflexes (marked tail flagging, vulva lifting in response to perineal stroking). Day 1 of diestrus (Di1) was the day when SCI decreased by at least 20% [12].

2.3. Plasma progesterone assay

Blood samples were collected by jugular venipuncture before ovariectomy. Plasma progesterone concentrations were determined in a commercially available coated tube radioimmunoassay (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA) [13]. All samples were measured over three assays; the sensitivities were 0.03, 0.03 and 0.07 ng/mL, respectively.

2.4. Preparation of E. coli inoculum

A strain of *Escherichia coli* (*E. coli*) isolated from a naturally occurring case of pyometra and possessing genes for five uro-pathogenic virulence factors (pap, sfa, hlyA, cnf1 and fim) as determined by polymerase chain reaction was used [14]. A loop full of *E. coli* seed culture was used to inoculate 10 mL of Luria–Bertani broth and the culture was incubated in a shaking incubator at 37 °C at 200 rpm for 22 h. The concentration of bacteria in the culture was determined using a standard plate count method and compared to the optical density of 10^{-1} and 10^{-2} dilutions of the culture at 595 nm (A595). The A595 measurements were used to estimate the number of bacteria in the culture immediately prior to inoculation.

2.5. Necropsy

The bitches were euthanased by intravenous injection of pentobarbitone sodium (Lethabarb; Virbac, Peakhurst, Australia). A ventral midline incision was made, a clamp was placed on the anterior vagina and the genital tract was removed.

2.6. Uterine microbiology

Samples were collected from the uterus for microbiological culture. When there was no accumulation of fluid in the uterus, 5 mL of saline was flushed through the uterine horns and collected from the body of the uterus. The uterine contents were serially diluted (1–9 mL) up to 10^{-7} with saline, and 100 μL of each dilution was inoculated on to Luria–Bertani agar plates. After 24 h of incubation, the number of colonies on each plate was counted to determine the concentration of *E. coli* in the uterine contents.

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