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Interstrain Differences in the Development of Pyometra after Estrogen Treatment of Rats

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This case report describes the unanticipated development of pyometra in Brown Norway rats after treatment with estrogen. Sprague Dawley and Brown Norway rats were ovariectomized and randomly assigned to treatment groups (subcutaneous implantation of either a capsule containing 20 mg 17β-estradiol or an empty capsule, as a control). After irradiation of only the right eye, the rats were followed for several months in an attempt to determine the effects of estrogen on radiation cataractogenesis and investigate potential strain differences in this phenomenon. However, all Brown Norway rats that received estradiol treatment developed pyometra, whereas none the Sprague Dawley or control Brown Norway rats did. This case demonstrates the potential adverse effects of exogenous estrogen therapy, which are strain-specific in the rat. Caution should be taken when designing estrogen-related experiments involving Brown Norway rats and other potentially sensitive strains.

Pyometra, an acute or chronic infection of the uterus with accumulation of pus in the uterine lumen, is a relatively common condition in dogs, cats, cows, and mares.[8] In rats, pyometra is a well-documented sequela of a genital tract infection with *Mycoplasma pulmonis*[17] but has not been reported to occur spontaneously in SPF rat colonies. Here we describe pyometra in estrogen-treated ovariectomized Brown Norway rats that were part of a cataractogenesis study.

Materials and Methods

All experiments were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee, and all rats housed in AALAC-accredited facilities. Fifteen 6-wk-old female Hsd:Sprague Dawley SD and 15 6-wk-old female BN/RijHsd (Brown Norway) rats (Harlan Sprague Dawley, Indianapolis, IN) were conventionally housed in 1 room in a large multispecies animal facility. The rats were housed in same-strain pairs in conventional open-top opaque polycarbonate shoebox cages (Lab Products, Seaford, DE) with pelleted paper bedding (Harlan Teklad, Indianapolis, IN). No cages were placed on the top shelf of the stainless steel shelving unit. The rats were fed ad libitum (Teklad 7001, Harlan Teklad) and received acidified water in water bottles. The room was maintained on a 12:12-h light:dark cycle (lights on at 0700) at 20 °C and at least 40% relative humidity. Cages were changed weekly. Soiled cages were washed in a tunnel washer with a final rinse temperature of 82.2 °C. All personnel wore exam gloves, dust masks, and lab coats or disposable gowns when handling the rats.

The health status of the colony was determined by using indirect sentinels tested on a quarterly basis. Two 4-wk-old Hsd:Sprague Dawley SD rats were provided for every 100 cages of rats or per animal room. After 12 wk of exposure to soiled bedding, sentinel rats were euthanized by CO_2 asphyxiation and bled by cardiocentesis. Serum was sent to the Research Animal Diagnostic Laboratory at the University of Missouri and tested for rat coronavirus, Sendai virus, pneumonia virus of mice, parvovirus NS1, rat parvovirus, Kilham rat virus, Toolan H1 virus, *Mycoplasma pulmonis*, and Theiler murine encephalomyelitis virus. Parasite checks were performed inhouse and consisted of anal tape tests, direct exam of cecal contents, and pelage exams. The facility was negative for rat pathogens and parasites.

Surgery. After an acclimation period of 7 d, rats (approximate age, 49 d) were ovariectomized and assigned randomly to control (OVX control) or estradiol-implant (OVX E2) groups. Rats were weighed at the time of surgery and again at irradiation. Animals were anesthetized with ketamine, acepromazine, and atropine prior to surgery and irradiation treatments. Surgeries were performed 1 wk prior to irradiation. The surgery site was prepped, and sterile technique was followed. Each rat was ovariectomized and then an empty capsule or capsule containing 20 mg crystalline 17β-estradiol (Sigma–Aldrich, St Louis, MO) was implanted subcutaneously middorsum. Each estradiol capsule provided a continuous course of physiologic levels of estrogen (about 2 μg/d) throughout the period of observation. Capsules (length, 1 cm) were fashioned from tubing (inner diameter, 0.062 in.; outer diameter, 0.125 in.; Veterinary Silicone Silastic Tubing Dow Corning, Konigsber Instruments, Pasadena, CA) and sealed (Silicone Type A Medical Adhesive, Dow Corning).

Irradiation. The right eyes of anesthetized 49- to 56-d-old rats were gamma-irradiated with 0 or 5 Gy ^{60}Co as described previously.[3] Rats were anesthetized about 10 min prior to irradiation. The radiation dose was confined to the right orbit; the contralateral eye received less than 2% of the total dose to the target eye.

Cataract scoring. Cataract examinations were performed each month. Rats were placed in an acrylic induction chamber (SurgiVet, Waukesha, WI), and a 4% isoflurane–96% oxygen mixture was delivered from a vaporizer into the chamber. Anesthetized rats were removed from the chamber and held by an assistant while an ophthalmologist used a hand-held slit lamp to examine the lenses of the rat. The rats recovered quickly and were returned to their home cages.

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Case Report

At 51 d after capsule implantation, 1 Brown Norway rat in the OVX E2 group was found dead; the rat had a bloated abdomen but otherwise appeared normal (Figure 1). Necropsy revealed that the uterus was enlarged markedly, with yellow to white discoloration and hyperemia of the serosal vasculature (Figure 2). On incision, the uterine lumen was noted to be distended with a thick, white, caseous material. A sample was taken for culture and tissues saved in 10% buffered formalin for histopathology. Culture revealed abundant growth of *Escherichia coli*. Tissues were processed by standard methods (embedded in paraffin, sliced at 3 to 5 µm, and stained with hematoxylin and eosin). Histologic examination was consistent with pyometra. The endometrium was ulcerated extensively and covered with a layer of necrotic tissue, macrophages, and degenerate heterophils that extended deep to the myometrium. Moderate infiltrates of lymphocytes and plasma cells were present throughout the myometrial and adventitial layers and extended into the cervical and vaginal musculature (Figure 3). No other significant gross abnormalities were identified at necropsy.

Over the next 100 d, 7 more rats were either found dead or euthanized due to poor condition (thin body condition with a bloated abdomen). By 255 d after implantation, 100% of the rats in the estradiol-implanted group were noted to have bloated abdomens and were found dead or euthanized for poor condition (that is pale, hunched posture, poor haircoat). Severe pyometra was confirmed on necropsy. One additional rat was cultured, and again *E. coli* grew abundantly.

Thirteen of 15 Brown Norway from the control group were euthanized according to experimental schedule (at 22 mo after irradiation) after sufficient cataract data were collected. Necropsies were performed, and none of these rats had any evidence of pyometra. The remaining 2 rats were euthanized early due to masses on their mandibles (not evaluated by histopathology, Table 1). No other morbidities were noted in this group during the period of observation.

Concomitantly, the OVX E2 group of Sprague Dawley rats was monitored closely. When they were found dead or euthanized for health reasons or experimental schedule (Table 1), a necropsy was performed. Internal organs were examined, and no Sprague Dawley rats had lesions suggestive of pyometra.

Figure 1. Typical swollen abdomen of Brown Norway rats with pyometra.

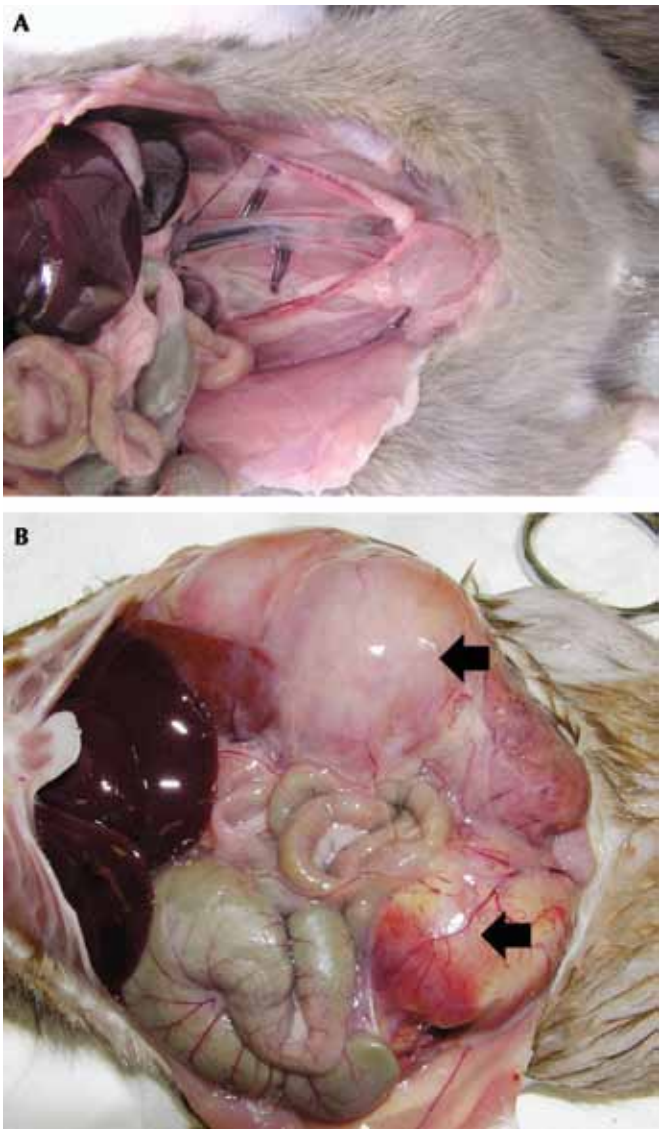

Figure 2. (A) Normal uterus from a control Brown Norway rat. (B) An estrogen-treated Brown Norway rat with markedly distended uterine horns (arrowheads).

Discussion

Estrogen supplementation of ovariectomized rats is a widely used animal model in biomedical research for a multitude of disciplines. Many of these experiments use the Sprague Dawley strain of rat because it is a widely available outbred strain of rat. However, other stocks and strains of rats have been used, including 'Wistar rats' and inbred Brown Norway rats. Studies involving animals indicated as 'Wistar rats' seem to have used outbred Wistar rats; by convention Wistar-based inbred lines are designated by using uppercase Roman letters[1] and include WKY (Wistar Kyoto) and WF (Wistar Furth). Advantages of inbred strains include genetic stability, isogenicity, homozygosity, international availability, the ability to confirm the strain identity of animals, phenotypic uniformity between animals of the same strain, and the individuality of 1 inbred strain compared with others.[1] However, inbreeding may perpetuate deleterious traits, such as increased susceptibility to disease. In particular, Brown Norway rats have T cells polarized to Th2 reactions,[4] making these rats more likely to mount antibody responses (more effective against parasitic and extracellular bacteria) than cell-mediated reactions (better suited for intracellular infections). They are also prone to developing Th2-mediated autoimmune diseases, such as heavy metal-induced glomerulopathy and myasthenia gravis.[4,17] The anomalous immune system of the Brown Norway rat may have contributed to the development of pyometra in the present case report.

Strain differences in susceptibility to pyometra likely depend on a complex interplay between genes and hormones. Some authors have described spontaneous inflammatory pelvic

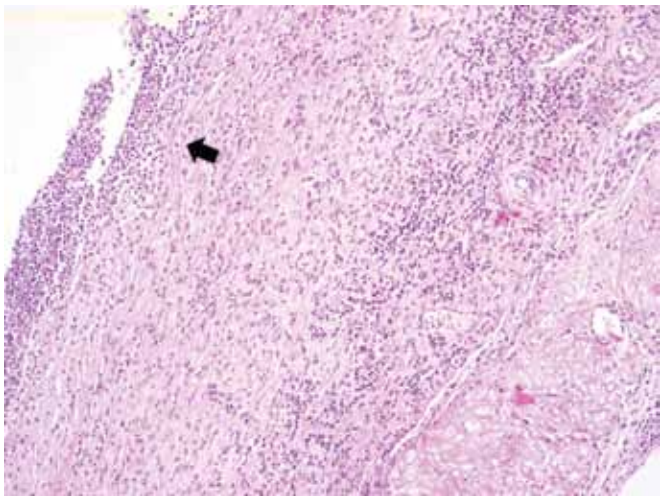

Figure 3. The uterine endometrium (arrow) is ulcerated and covered with a layer of necrotic cellular debris and inflammatory cells. Inflammatory infiltrates are evident throughout the myometrium and adventitial layers. Hematoxylin and eosin stain; magnification, ×100.

Table 1. Death or euthanasia of rats (n = 15 per group) after implantation of 17-β-estradiol

No. of days after implantation	No. of rats		
	OVX E2 Brown Norway	OVX control Brown Norway	OVX E2 Sprague Dawley
1–85	1[a], 4[b]	0	0
86–170	7[b]	0	0
171–255	3[b]	0	0
256–340	0	0	0
341–425	0	0	3, 1[c]
426–510	0	0	3, 8[d]
511–595	0	0	0
596–680	0	2[e]	0
681–765	0	13[f]	0

Rats were found dead if not otherwise indicated.
[a]Euthanized after irradiation (rat moved during irradiation and was irradiated beyond shielded area).
[b]Euthanized or found dead due to pyometra
[c]Euthanized due to mammary tumor
[d]Euthanized due to mammary tumor, dyspnea, or pituitary mass
[e]Euthanized due to mandibular masses
[f]Euthanized at the end of the study.

disease in intact Wistar rats treated with estrogen,[15] whereas others used diethylstilbestrol and 17β-estradiol (respectively) to induce pyometra in Brown Norway rats and Brown Norway crosses to identify the genes responsible for the susceptibility to pyometra.[6,14] Whereas others have observed a high incidence of pyometra in ovary-intact Sprague Dawley rats treated with estrogen,[16] we found no effect of estrogen in ovariectomized Sprague Dawley rats. This difference supports the general opinion that estrogen increases susceptibility to pyometra by enhancing the stimulatory effects of progesterone on the uterus.[13] Progesterone promotes endometrial growth and secretory activity while decreasing myometrial activity, causing cystic endometrial hyperplasia and accumulation of uterine secretions.[9] In addition, progesterone inhibits the immune response to bacterial infection. However, bacteria inoculated into the uterus of Wistar rats grew more rapidly in animals that received estrogen treatment for 7 d than in those treated with progesterone or left untreated.[11] In our study, both Brown Norway and Sprague Dawley rats were ovariectomized and treated with estrogen only or placebo; therefore, estrogen enhanced the incidence of pyometra in Brown Norway rats in the absence of exogenous progesterone. Perhaps estrogen itself alters the expression of pyometra-susceptibility genes in Brown Norway rats, whereas both estrogen and progesterone are required for the expression of these genes in Sprague Dawley rats.

Time and dosage factors likely play a role in the incidence of pyometra associated with estrogen treatment. In 1 model of estrogen-induced pyometra,[6] Brown Norway rats treated with 17β-estradiol (27.5 mg capsule implant) manifested a 100% incidence of pyometra 20 wk (but not 12 wk) later. In contrast, the 17β-estradiol dose that we used was 30% lower and led a 100% incidence of pyometra after a shorter period of time (the first case occurred at 7 wk after implantation).

Many species have shown adverse effects of estrogen therapy. Nude mice treated with estrogen can develop perianal ulceration, *Staphylococcus*-induced urolithiasis,[5] hydronephrosis, and death.[10] In utero exposure of rat pups to diethylstilbestrol (a synthetic nonsteroidal estrogen) results in retarded epididymal development in adult male rats,[21] and neonatal mice exposed to this agent develop uterine adenocarcinoma.[12] In addition, diethylstilbestrol has been used to induce pituitary adenoma and thymic atrophy models in rats.[2,7,19] Administration of exogenous estrogen during diestrus in the event of mismating greatly increases the risk of pyometra in the bitch.[9] Estradiol can be used as a chemotherapeutic agent in treating prostate neoplasia of dogs. However, the treatment benefit must be weighed against the risk of development of estrogen-induced life-threatening bone marrow toxicity, which can lead to aplastic anemia and thrombocytopenia.[9,18] In cases of spontaneous pyometra, normal vaginal flora are the most common causes of infection. In rats, *Mycoplasma pulmonis* is isolated frequently in cases of pyometra,[17] as are *Staphylococcus aureus*, *Proteus mirabilis*, and *Morganella morganii*.[6] In the present report, *E. coli* was the inciting agent.

We conclude that rodent models for steroid hormone research should be considered carefully. Although others have supplemented Brown Norway rats with estrogen successfully,[20] our data suggest that other strains may be more suitable for estrogen-related studies. Sensitivity to estrogen appears to vary among rats strains, and dosages appropriate for some strains may need to be adjusted for others. Sprague Dawley rats seem to be relatively resistant to developing side effects associated with estrogen therapy, whereas Wistar and Brown Norway rats appear to be more sensitive. Additional studies may be required to establish safe dosages of estrogen for other strains and stocks of rats.

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