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High Mobility Group Box-1 (HMGB-1) as a Potential Regulator of Inflammation and Determinant of Scar Formation in Fetal Wounds
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Previous studies have shown that a different type of healing occurs in embryonic skin during the first two trimesters of development. A mouse model of fetal wound healing was used for the present studies (Fig 1). Before murine embryonic day 15 (E15), wounds exhibit a unique pattern of healing leading to regeneration. Loss of the ability to heal without scar formation is seen with increasing age of the embryonic skin. Fetal wounds generated at late stages of development (embryonic day 18, E18) heal with inflammation and scar formation. Currently, reasons for differences in wound-induced inflammation in early and late embryonic skin are not entirely understood. We hypothesize that differences in expression or signaling of HMGB-1, a non-histone DNA binding protein that acts as a DAMP (damage-associated molecular pattern), controls the inflammatory response in fetal wounds. Cellular injury results in the release of HMGB-1 from the cell, where it stimulates inflammation by binding to RAGE (receptor of advanced glycation end products) or toll-like receptors present on inflammatory cells. The purpose of this study is to characterize HMGB-1 in fetal repair. To meet this end, immunohistochemical staining of wounded skin from the scarless (E15) and scar-forming (E18) time periods were analyzed. Ongoing studies are being conducted to analyze in vivo exogenous HMGB-1 treatment of E15 wounded skin and the downstream effects on healing.

Unilateral Cholesterol Granuloma in a Male C57BL/6 Mouse in a Colony with a High Incidence of Perineal Swellings
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The incidence of perineal swellings among male C57BL/6 mice greater than 8 months of age was anecdotally reported to exceed 43% in a breeding colony with Pthrp (parathyroid hormone-related protein) genetic mutations. Elevated incidences of bulbourethral (Cowper’s)
gland abnormalities ranging from 6.7% to 83.8% have previously been reported in several strains of mice. At the time of clinical presentation, mice appeared clinically normal with the exception of uni- or bilateral perineal swellings. Affected males had markedly diminished reproductive performance and had not sired litters since the onset of clinical signs. Low fecundity rates have been similarly documented in CFW/R1 male mice with perineal swellings. To further characterize a suspected bulbourethral gland cyst or abscessation of a local structure and possible cause of low fertility, a mouse was submitted for pathologic evaluation. Gross examination revealed bilateral dilation of the saccular portion of the bulbourethral gland measuring 1.4 x 0.95 x 0.8 cm on the right and 0.95 x 0.8 x 0.7 cm on the left. The left sac was clear and mostly transparent while the right sac was dark red and appeared to contain whitish-tan to black luminal structures approximately 0.15 cm in diameter. Microscopically, the right contained multifocal luminal cholesterol granulomas associated with hemorrhage and hemosiderin accumulation. While cystic dilation of the bulbourethral glands is a common and reported lesion in some strains, the unilateral presence of intraluminal cholesterol granulomas and hemorrhage has not previously been reported in this species or this anatomical location to our knowledge. In the veterinary literature, case reports are limited to horses with cholesterol granulomas in fourth and/or lateral ventricles of the brain and one report of a cat with a uterine cholesterol granuloma. Human literature cites that cholesterol granulomas most often occur in the temporal bone. Cholesterol granulomas are foreign-body reactions to the presence of cholesterol crystals. In this case with prominent hemorrhage in the ipsilateral gland, the formation of cholesterol crystals is likely associated with lipid components of red blood cell membranes.

Strain Specific Effects of Ketamine-Xylazine-Acepromazine, Ketamine-Xylazine, and Isoflurane Anesthesia in BALB/c and C57BL/6 Strains of Mice
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The mouse is the most frequently used animal model for biomedical research. To meet research needs, a surgical plane of anesthesia is commonly induced, yet information is limited regarding strain specific variability in physiologic responses. Anesthetic protocol selection is often based on availability of equipment, invasiveness and duration of surgical manipulation, and comfort level of the investigator. Rarely is an anesthetic protocol selected based on the strain of the mouse used, yet published reports indicate strain specific differences such as alveolar anesthetic concentration, blood pressure, and heart rate. The purpose of this study was to identify strain specific responses of mice subjected to differing anesthetic protocols with the aim of providing guidance in selecting the most appropriate anesthetic protocol and reduce the overall number of animals by decreasing mortality due to anesthetic complications. C57BL/6 and BALB/c mice (n=10 for each anesthetic protocol) were induced to a surgical plane of anesthesia using three protocols: inhalant isoflurane (ISO), injectable ketamine-xylazine (KX), and injectable ketamine-xylazine-acepromazine (KXA). Depth of anesthetic plane and respiratory rate were
monitored at ten minute intervals. An electrocardiogram was obtained on a subset of animals. Complete blood counts and chemistry panels were analyzed at baseline and post-anesthesia. Strain-specific differences in physiologic parameters and spinal reflexes were not observed between C57BL/6 and BALB/C strains. Inhalant isoflurane provided an easily controlled surgical plane for both strains and a statistically significant shorter induction and recovery rate when compared to KXA and KX. If an injectable protocol is desired, a dose of 150/20/3 mg/kg KXA effectively produced a surgical plane of anesthesia in both strains. We were unable to determine a dose of KX that consistently provided 20 minutes of surgical anesthesia without significant mortality. While significant differences existed between anesthetic protocols, more sensitive monitoring equipment may be required to reveal differences amongst strains.

UVB Exposure and Topical Estrogen Effects on the Development of Skin Cancer in a Pre- and Post-Menopausal Mouse Model

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Topical or systemic estrogen and its effects on the skin have been studied in post-menopausal women but data is lacking concerning usage of topical estrogenic compounds by younger, pre-menopausal women. It is hypothesized that topical estrogen application to previously UVB exposed skin accelerates skin carcinogenesis. The specifics aims of this study are to determine the effects of topical estrogen on Ultraviolet light-B (UVB) induced skin tumor development and progression using Skh-1 hairless mice. Seventy female mice were divided into two groups and one group was irradiated three times weekly with 2240J/m2 for 10 weeks to model human UVB exposure from childhood through early adulthood. Mice then received either ovariectomy (post-menopausal) or sham surgery (pre-menopausal) and were treated topically with 10nmol 17β-estradiol or vehicle control three times weekly for 15 weeks with no further UVB irradiation. Tumor numbers and size were measured weekly during the 15 week treatment period. Neither unirradiated mice receiving topical estrogen or vehicle control developed tumors during the course of the study. Topical treatment with estrogen following 10 weeks of UVB exposure in intact mice induced an increased tumor burden compared to UVB exposed mice receiving vehicle control. Ovariectomized mice had increased tumor burden regardless of topical treatments when compared to UV exposed intact mice. Studies are ongoing to determine potential mechanisms behind these observations. These data indicate that the application of exogenous estrogen to previously UVB exposed skin can potentially initiate an increase skin tumor development in premenopausal women. These findings have negative implications for the use of lotions and creams containing estrogenic compounds on sun exposed sites by young women.
Management of a Rabbit Model of Osteomyelitis Using Probiotics and Objective Tracking Measurements to Maintain Normal Gastrointestinal Function

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Therapeutic and experimental use of antibiotics in rabbits can be challenging due to the sensitivity of essential microorganisms in the cecum required for breakdown of indigestible fiber. Any disruption of the normal intestinal flora can potentially lead to fatal microbial dysbiosis. Significant gastrointestinal (GI) distress, indicated by varying degrees of diarrhea, was observed in a New Zealand white rabbit model of osteomyelitis following antibiotic administration. Per standard operating procedures, rabbits were singly housed and provided with automatic water. Rabbits received a pelleted diet, supplemental hay, and dietary enrichment. Pain management for osteomyelitis was provided via opioid analgesia, which may have contributed to some inappetence. In order to maintain normal GI flora and prevent diarrhea, prophylactic treatment included nutritional supplements such as probiotic oral gel mixed with flavored Greek yogurt, acidophilus tablets, and commercial critical care diet. Presentation of the supplements ranged from free choice to syringe-feeding depending on the degree of weight loss. Initiating prophylactic treatment of probiotics resulted in a significant decrease in the incidence of diarrhea over 5 trials. However, the frequency and severity of diarrhea was greatly dependent on the antibiotic used in each trial. In order to provide a consistent and objective assessment of lameness, food intake, fecal output and consistency, visual guides were created and used as training tools for the research staff. Over time, we developed a successful method in which to manage the colony using nutritional support and probiotics to maintain healthy microorganisms in the intestinal tract as well as an objective and clear assessment of clinical signs. In conclusion, rabbits can maintain normal GI function with antibiotic administration when probiotics in a palatable form are made available.

P83 The Impact of Bedding Type on Cage Change out Frequency

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The frequency of cage changes varies among institutions as a result of several considerations: animal stress, allergen exposure to personnel, experimental interference, and costs associated with bedding and sterilization procedures. The goal of this study was to evaluate the effectiveness of a new sanitized corn cob bedding material as compared with standard corn cob in a ventilated mouse rack system. Intracage ammonia levels, bacterial growth and absorptive capacity of bedding were measured for cages of female C57BL/6 mice under standard and autoclaved conditions on static and ventilated racks in a barrier facility. Intracage ammonia concentration was measured daily, and cages were removed when measurements were equal to or greater than 25 ppm. Quantity of bacterial growth and bacterial species in bedding were determined at the time of cage removal. Bedding absorptive capacity and bacterial load were also evaluated in all conditions without the addition of mice. Cages with nonautoclaved
sanitized corncob bedding took significantly longer to reach ammonia concentrations of 25 ppm than standard corncob. Autoclaved sanitized corncob bedding did not differ significantly from nonautoclaved standard corncob in length of time required to measure 25 ppm ammonia. All nonautoclaved sanitized corncob cages remained in the study a minimum of 3 wk. No significant differences were noted on bacterial load at the conclusion of mouse housing. Standard corncob was significantly more absorbent than sanitized corncob bedding, and autoclaved sanitized corncob bedding was significantly more absorbent than autoclaved corncob. This study demonstrated that mouse cages with nonautoclaved sanitized corncob bedding on ventilated racks may be used with a cage change interval of 3 wk.