# Estrogen induces severe urinary tract defects – a strain dependent difference in nude vs NOG female mice

Scand-LAS

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- In collaboration with Taconic Biosciences

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# Introduction to breast cancer and *in vivo* tumor models

- High incidence of breast cancer worldwide, need for new treatments
- Breast cancers are estrogen responsive or non-responsive
- Growth of breast cancer can be modeled by inoculation of human breast cancer cells to immunodeficient mice
- In these xenograft studies, external estrogen supplementation is needed to support the estrogen sensitive tumor growth

# Introduction to estrogen-caused adverse effects

Toxicol Pathol. 2009 Feb;37(2):227-34. doi: 10.1177/0192623308329281. Epub 2009 Jan 29.

**Urinary retention and cystitis associated with subcutaneous estradiol pellets in female nude mice.**

Pearse G[1], Frith J, Randall KJ, Klinowska T.

- In addition to supporting tumor growth, estrogen supplementation is known to affect other estrogen-sensitive tissues and induce adverse effects
  - Urinary tract function and infections, compromised survival
- Due to severity of these adverse effects, mice are often sacrificed prematurely
  - Early termination of the experiments

# Major findings by Pearse et al.,2009

Toxicol Pathol. 2009 Feb;37(2):227-34. doi: 10.1177/0192623308329281. Epub 2009 Jan 29.

**Urinary retention and cystitis associated with subcutaneous estradiol pellets in female nude mice.**

Pearse G[1], Frith J, Randall KJ, Klinowska T.

TABLE 1.—Summary of the animals examined microscopically.

| Group examined | Estradiol pellets (mg) | No. and sex of animals | Day of sacrifice (postimplantation) | Cystitis |
|---|---|---|---|---|
| Animals treated with anticancer agent | 0.5 mg twenty-one–day release | 31 (F) | 21–40 | 25/31 |
| Untreated controls | 0.5 mg twenty-one–day release | 14 (F) | 21–40 | 7/14 |
| Males | 0.5 mg twenty-one–day release | 6 (M) | 31 | 0/6 |
| Low-dose females | 0.36 mg sixty-day release | 10 (F) | 60 | 0/10 |
| Cycling females | Nonpelleted | 6 (F) | – | 0/6 |

- "Macroscopically, bladders were variably distended with clear, pale yellow urine, or white, amorphous, sand-like material."
- "Bladder walls were notably thickened in some cases."
- "In total, thirty-three (33) of forty-five (45) animals had macroscopic and/or microscopic abnormalities of the urinary bladder."
- "Intraluminal bacterial colonies of Gram-positive coco bacilli were seen in a few cases."

# Aims of the study

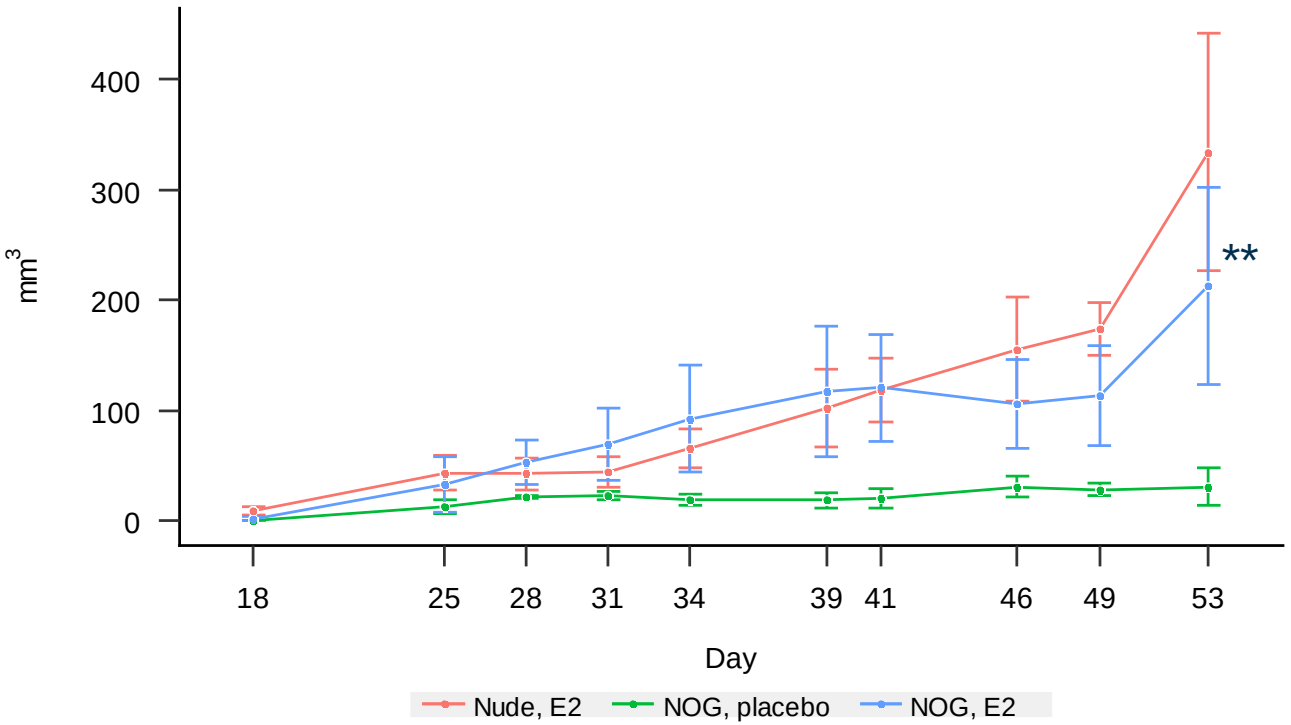
- To compare tumor growth and estrogen-caused adverse effects in female athymic nude and CIEA NOG mice in an orthotopic estrogen sensitive breast cancer xenograft model

- To achieve a model for long term cancer studies with estrogen supplementation without compromising animal health

# Study setup

- 5-6 weeks old female athymic nude (Hsd: Athymic nude) and CIEA NOG (NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac, provided by Taconic Biosciences) mice, n=8 in the study groups
- Soy-free diet (Teklad 2916) and tap water *ad libitum*
- Implantation of estrogen-releasing (5 µg/day) or placebo MedRods (PreclinApps) 1 week before cancer cell inoculation
- Inoculation of 5 x $10^6$ BT-474 (ER, PR, HER2 positive) human breast cancer cells orthotopically into mammary fat pad
- Tumor growth (with caliper in three dimensions (volume = L*W*H*π/6)), body weight and clinical condition recording twice a week
- Sacrifice at 8 weeks post inoculation

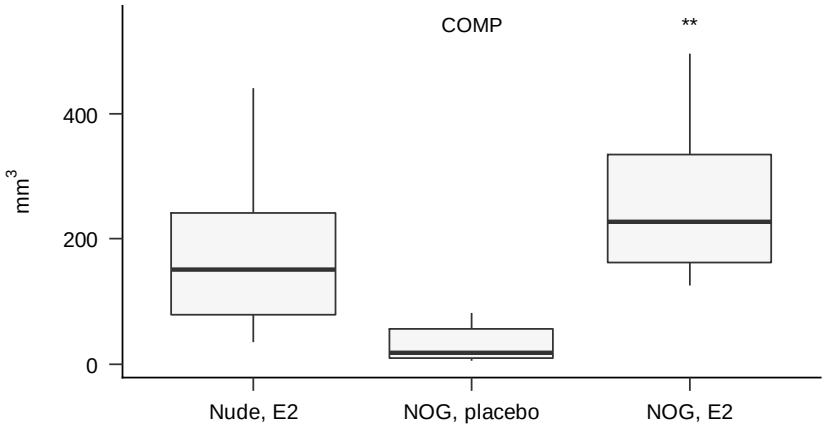
# Orthotopic tumor growth



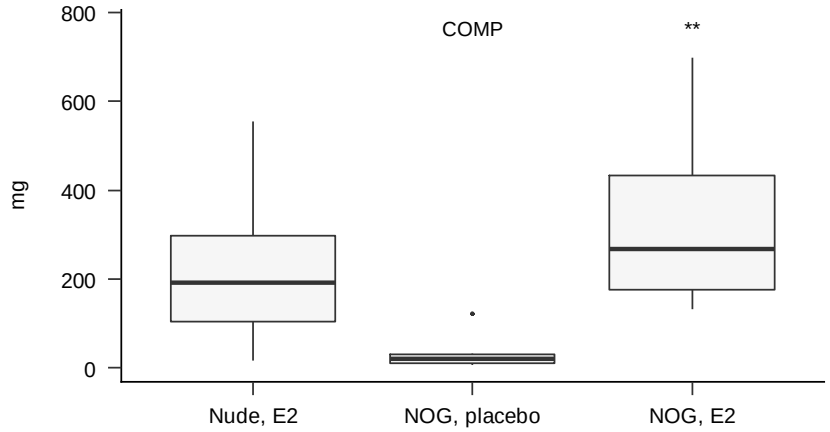

- E2 supported the BT-474 tumor growth
- Only minor tumor growth was observed in the absence of E2
- Tumor growth in nude and NOG mice was comparable
- Tumor take in nude mice was 100% and in NOG mice 65%

# Orthotopic tumor growth

## *Ex vivo* tumor volume

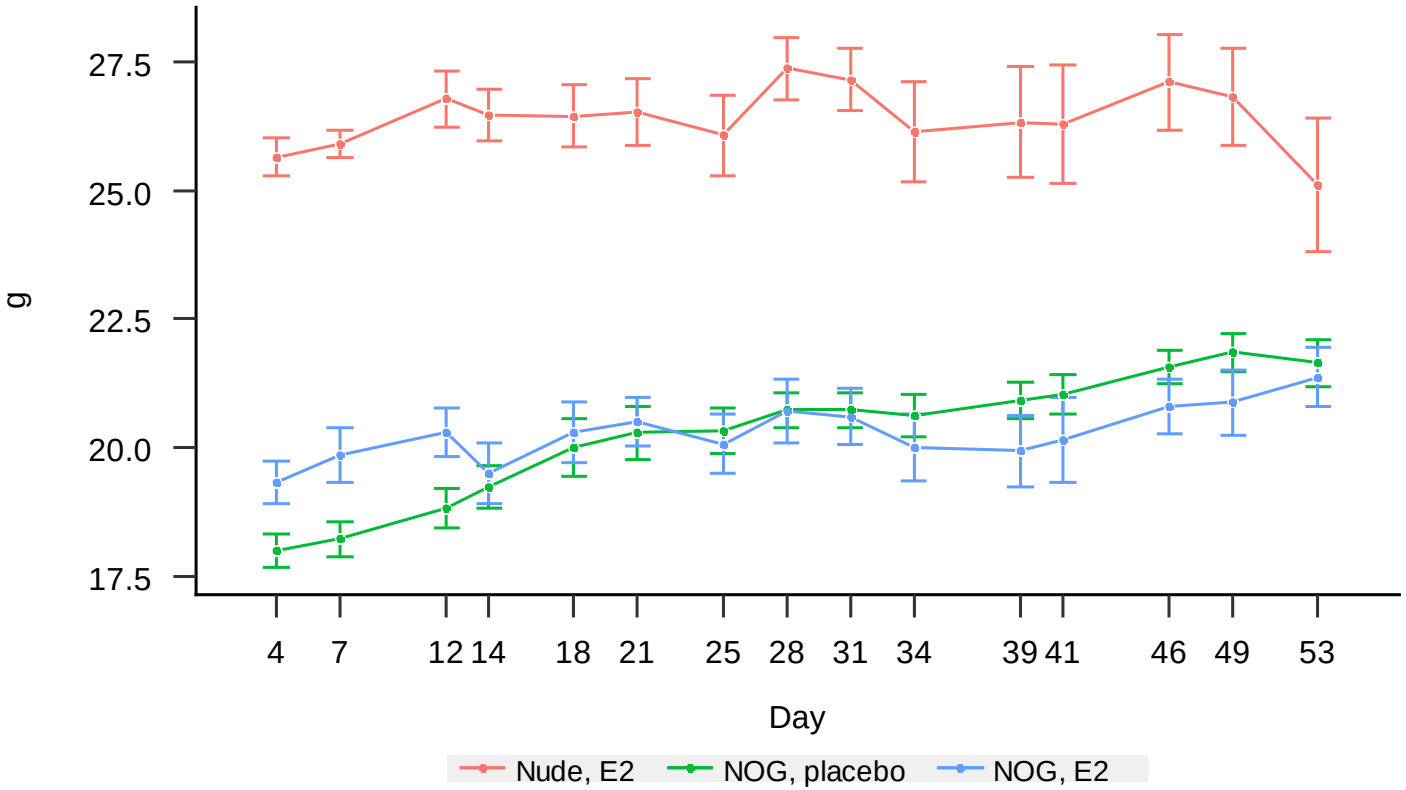



## *Ex vivo* tumor weight




- In NOG mice, estrogen supplementation increased tumor size and weight measured at endpoint ($p<0.01$)

# Weight development during the study



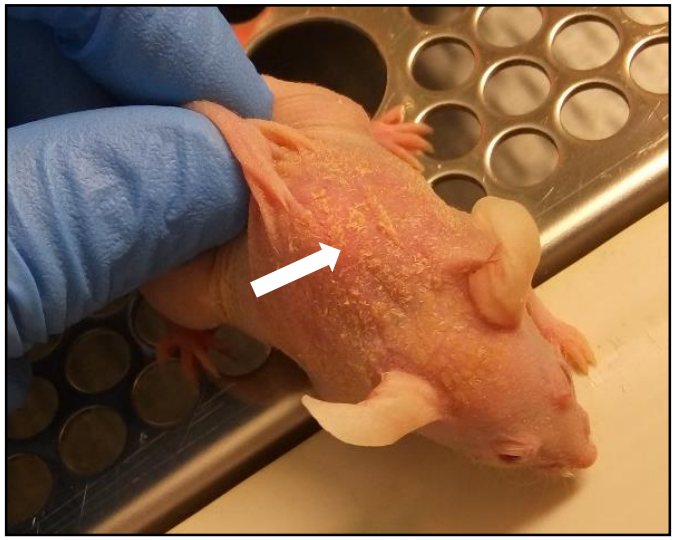

- Body weight development consistent with all study groups

# Clinical condition of the mice

- No changes in clinical condition in NOG mice with E2 supplement
- Nude mice exhibited severe E2 related adverse effects

# Types of estrogen adverse effects in nude mice

1. Rash



- Rash was observed in a subset of mice
- Typically located in the upper or lower back of the mice

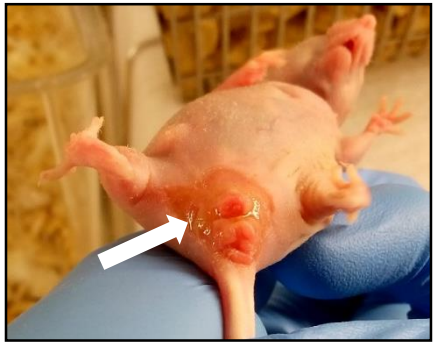
# Types of estrogen adverse effects in nude mice

2. Wounds


- Wounds in the front and hind limbs
- Approximately in half of the mice receiving estrogen

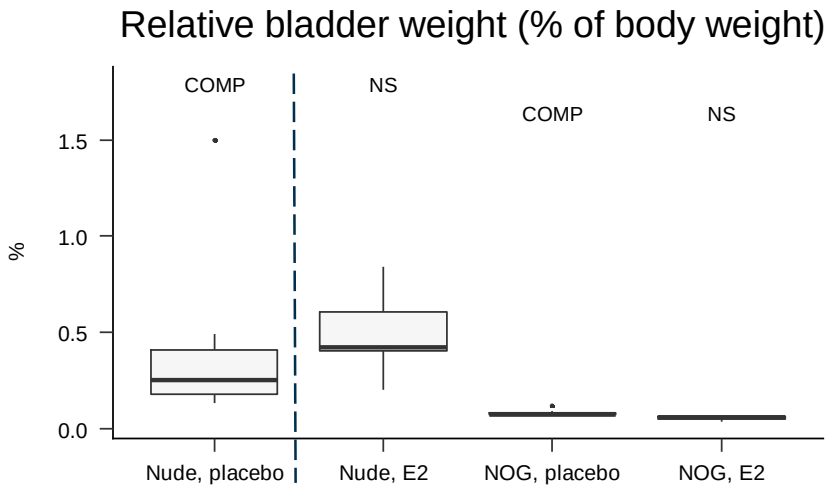
# Types of estrogen adverse effects in nude mice

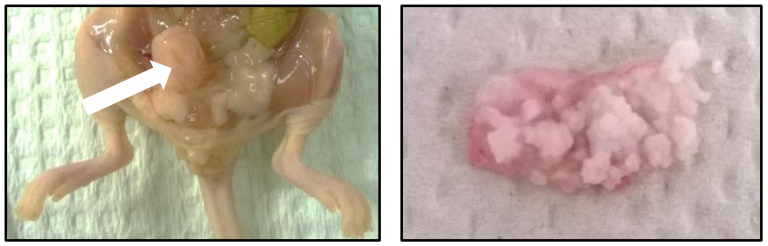
3. Secretion, irritation and skin retrieval



- Most severe changes in urogenital area
- Secretion, irritation and finally skin retrieval was observed
- Approximately in half of the mice receiving estrogen

# Bladder dysfunction and formation of bladder stones



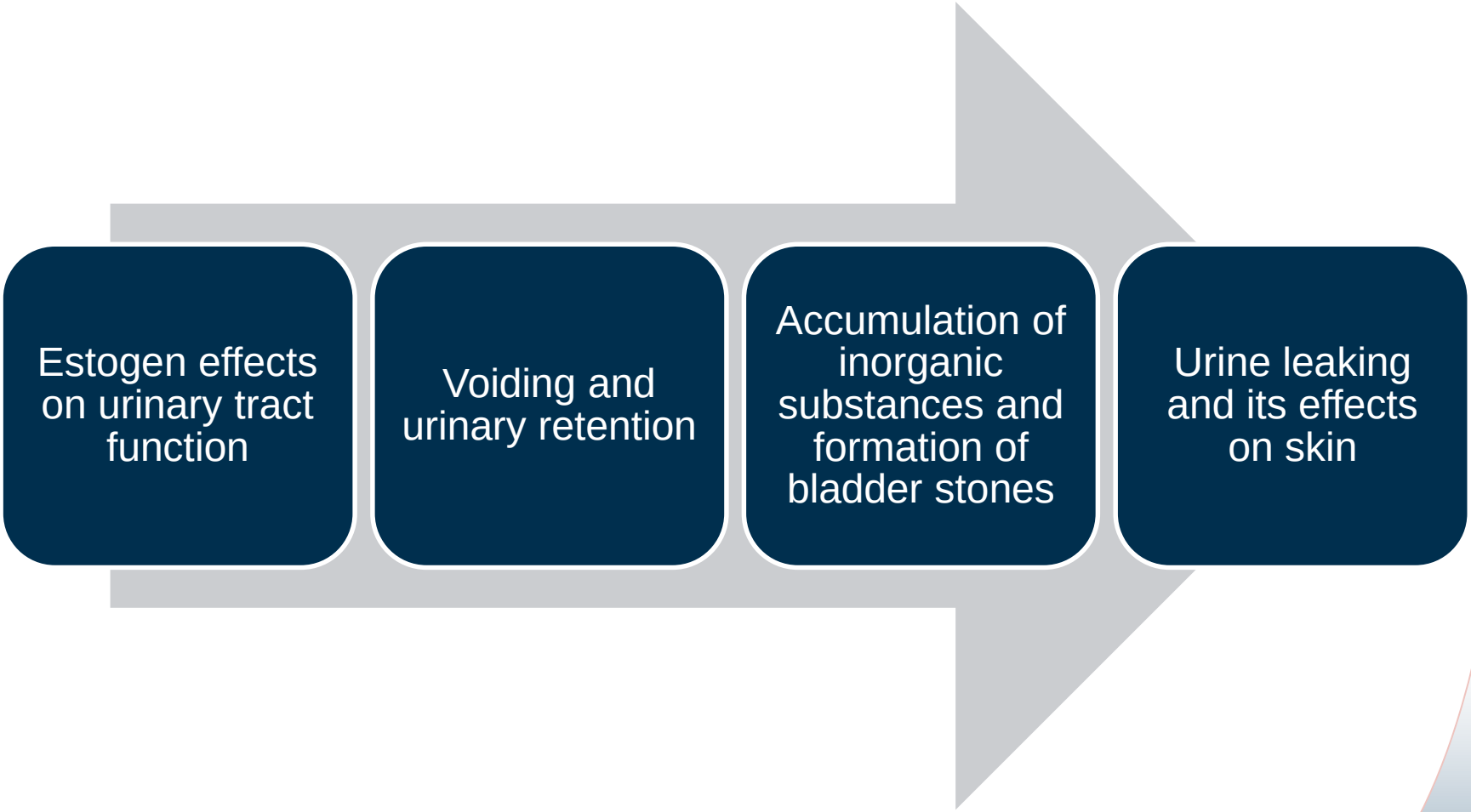
Example of enlarged bladder and the bladder stones inside.

- Over 60% of the nude mice had bladder stones
- Increased bladder weight
- Bladder function was compromised and the mice also exhibited voiding problems

# Bladder stone analysis

- The bladder stones (size from 1x1 to 7x4 mm) were collected at sacrifice and analysed by infrared spectroscopy (IDEXX BioResearch, Germany)
- Chemical composition of the stones: magnesium ammoniumphosphate hexahydrate = struvite stones
- The formation of struvite stones is linked to infection in many animals with voiding problems
  - Becknell et al., PlosONE, 2015 and Urology, 2013

# Hypothesis for adverse effect formation



# Summary

- No E2 related adverse effects were seen in NOG mice
- Altogether 62,5% of nude mice exhibited E2 related adverse effects
  - Formation of bladder stones did not correlate with the appearance of skin lesion
- Due to severity of these effects 50% of the nude mice had to be prematurely sacrificed before the intended end of the study
  - First symptoms already one week from implantation
  - Earliest sacrifice at 4 weeks

| Study group | Skin changes | Bladder stones | Kidney defects | Other | Early sacrifice |
|---|---|---|---|---|---|
| **Nude, E2** | 4/8; 50% | 5/8; 62,5% | 1/8; 12,5% | 3/8; 37,5% | 4/8; 50% |
| **NOG, E2** | 0/6; 0% | 0/6; 0% | 0/6; 0% | 0/6; 0% | 0/6; 0% |

# Conclusions

- Nude mice exhibited severe estrogen-related adverse effects in the urinary tract while no adverse effects were observed in NOG mice
- In studies where estrogen supplementation is required, the choice of mouse strain should be carefully considered
- Careful monitoring of animal health during the study is essential

# Clinical Assessment (Collins DE et al., 2017)

Comp Med. 2017 Feb 1;67(1):11-21.

## Clinical Assessment of Urinary Tract Damage during Sustained-Release Estrogen Supplementation in Mice.

Collins DE[1], Mulka KR[2], Hoenerhoff MJ[3], Taichman RS[4], Villano JS[5].

⊕ Author information

**Abstract**

Estrogen supplementation is a key component of numerous mouse research models but can adversely affect the urinary system. The goal of this study was to develop a clinical scoring system and identify biomarkers of occult urinary tract lesions prior to the development of systemic illness in mice. Ovariectomized or sham-surgery SCID mice were implanted subcutaneously with a placebo pellet or one containing sustained-release estradiol (0.18 mg 60-d release 17β-estradiol). Mice were assessed twice weekly for 4 to 6 wk by using a clinical scoring system that included body condition, general activity, posture, hair coat, hydration, abdominal distension, urine staining of coat and skin, and ability to urinate. Samples were collected weekly for urinalysis, BUN, creatinine, and serum estradiol levels. Terminal samples were analyzed for histopathologic lesions. Compared with placebo controls, estradiolsupplemented mice had higher serum estradiol levels at weeks 2 and 3; significant differences in total clinical scores by the 3-wk time point; and in body condition, general activity, posture, hair coat, and urine staining scores by the 6-wk terminal time point. Urinary tract lesions included hydronephrosis, pyelonephritis, cystitis, and urolithiasis. All mice with urolithiasis had crystalluria, and 5 of the 6 mice with pyelonephritis or hydroureter had dilute urine (that is, specific gravity less than 1.030). However, these findings were not specific to mice with lesions. A total clinical score of 3.5 (maximum, 24) identified estradiol-supplemented mice with 83% specificity and 50% sensitivity, but no single clinical parameter, biomarker, or the total clinical score accurately predicted occult urinary tract lesions. Considering the lesions we observed, prudence is warranted when using pelleted sustained-release estradiol in mice, and important parameters to monitor for animal health include urine staining, body condition score, urine sediment, and urine specific gravity.

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