Estrogen-Related Effects in Breast Cancer Xenograft Models

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Pharmatest Services
Who We Are

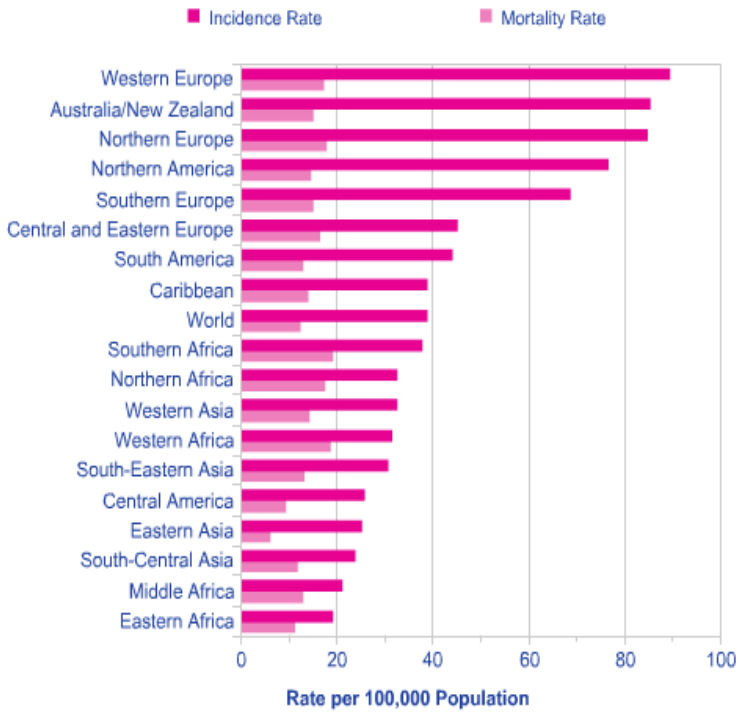
- CRO specialized in preclinical efficacy services in the areas of oncology and skeletal diseases
- Special expertise in bone metastasis models in oncology
- *In vitro* assays, *in vivo* models, *ex vivo* bone analysis services
- High quality services on global customer base
- Mission: To offer clinically predictive preclinical models in order to decrease currently high failure rates in clinical trials

Outline of the webinar

- Introduction to preclinical breast cancer models and estrogen supplementation
- Aim of the study
- Results
  - Estrogen and breast cancer tumor growth
  - Estrogen-related adverse effects
- Conclusions
- Clinical assessment
- Acknowledgements

Introduction to breast cancer and HR+ subtypes

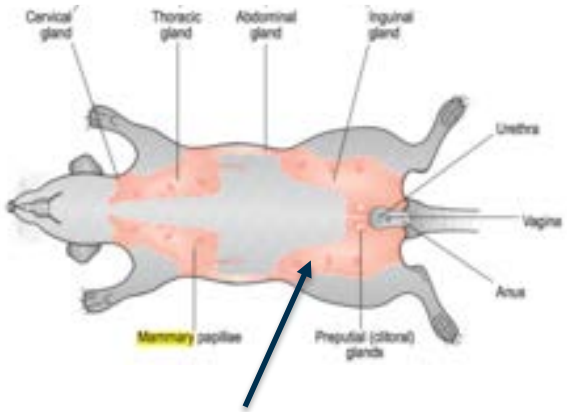
- High incidence of breast cancer worldwide
- 70-80% of breast cancers are hormone receptor positive (HR+)
  - Estrogen and progesterone receptor positive (ER+, PR+)
  - Can be treated with anti-hormone treatments or aromatase inhibitors



https://www.intechopen.com/breast-cancer-awareness-month.html

Introduction to *in vivo* animal models of breast cancer and estradiol (E2) supplements

- Growth of breast cancer can be modeled by inoculation of human breast cancer cells to immunodeficient mice
  - Subcutaneous model → E2 needed
  - Orthotopic model → E2 needed
  - Bone metastatic model → No E2 needed
- In these xenograft studies, external estrogen supplementation is needed to support the estrogen sensitive tumor growth
  - ER+, PR+, HER2+ (i.e. BT-474) → E2 needed
  - ER+, PR+, HER2- (i.e. MCF-7) → E2 needed
  - ER-, PR-, HER2- (i.e. MDA-MB-231) → No E2 needed
- Other indications of estrogen supplements for example in studies of reproductive health and osteogenesis



Tumor cell inoculation in orthotopic model

Image modified from:
https://healtheappointments.com/rodent-anatomy-essays/

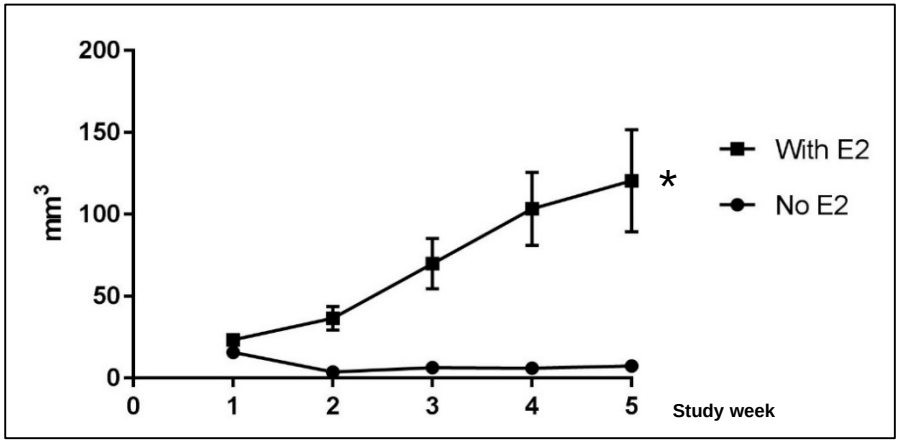
Estrogen supplements used in preclinical studies

- In xenograft studies, most commonly used estrogen products include 17β-estradiol (E2) as active substance
- Different methods for E2 administration include oral administration, injections, pellets, and osmotic pumps
- The doses from 0.18 mg to 1.7 mg result in serum levels of 700 to 900 pg/ml in mice
  - Much higher than the average circulating estrogen levels in mice
  - Corresponds to mid-cycle levels in women

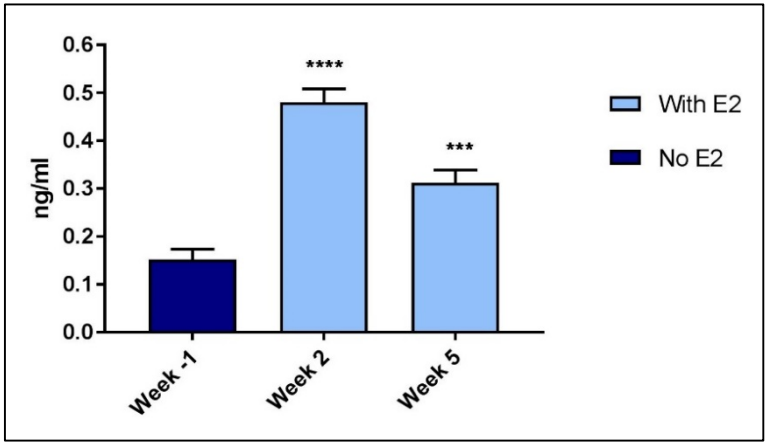
Serum E2 levels in mice in orthotopic MCF-7 tumor growth model

Minisymposium, AACR Annual Meeting 2017, Bernoulli J. et al, Pharmatest Services

Tumor growth



Serum E2



- E2 5 µg/day (MedRod™, a novel substance delivery system, PreclinApps)

Introduction to estrogen-related 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

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

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

Other literature examples of estrogen-related effects

- **Low dose, low cost estradiol pellets can support MCF-7 tumour growth in nude mice without bladder symptoms.** Dall et al. 2015, Journal of Cancer.
- **Hydronephrosis and urine retention in estrogen-implanted athymic nude mice**. Gakhar et al. 2009, Vet Pathol.
- **Low dose estrogen supplementation reduces mortality of mice in estrogen-dependent human tumor xenograft model**. Kang et al. 2009, Biol. Pharm. Bull.
- **Safe and effective method for chronic 17B-estradiol administration to mice**. Levin-Allerhand et al. 2003, American association for Laboratory Animal Science.
- **Clinical assessment of urinary tract damage during sustained-release estrogen supplementation in mice**. Collins DE et al. 2017, Comp. Med.

HOW CAN WE (OR CAN WE) MODEL BREAST CANCER WITHOUT THESE SYMPTOMS?

Are these symptoms related to certain mouse strains?

Mouse strains with estrogen-related adverse effects

- Many mouse strains are known and published to develop estrogen-related adverse effects
  - Nude mice
    - Athymic
    - NMRI
    - Balb/c
  - NOD.Scid mice
  - Also some immunocompetent strains e.g. C57BL/6J

- Adverse effects may also be partly dose dependent and/or dependent on how estradiol is released

Aims of the study

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

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

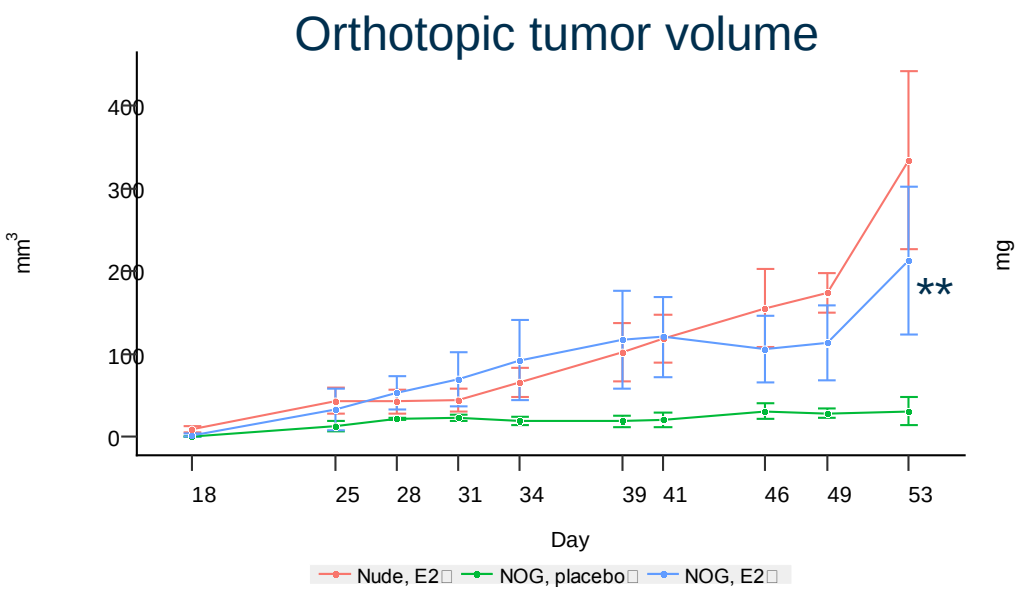
Study setup

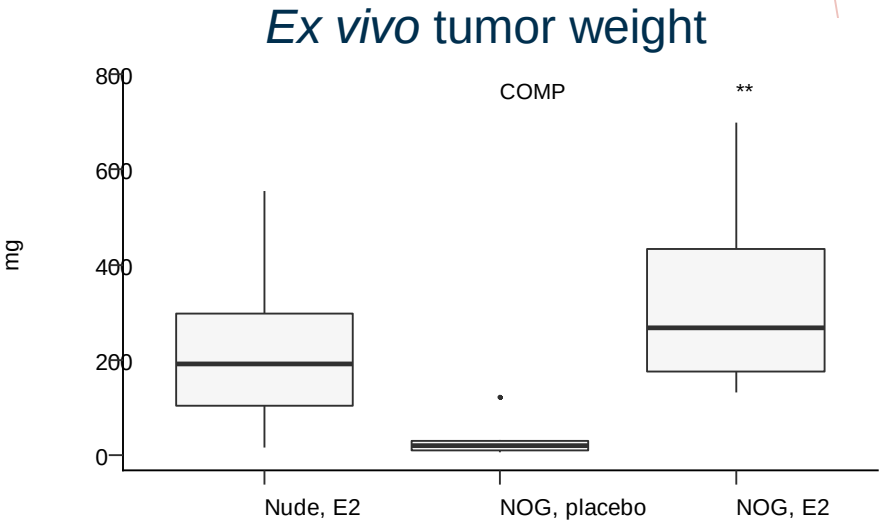
- 5-6 weeks old female athymic nude (Hsd: Athymic nude) and CIEA NOG (NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac, Taconic Biosciences) mice, n=8 in the study groups
- Soy-free diet (Teklad 2916) and autoclaved water *ad libitum*
- Implantation of estrogen-releasing (5 µg/day) or placebo MedRods (PreclinApps) 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 = π/6*Lenght*Width*Height), body weight and clinical condition recording twice a week
- Sacrifice at 8 weeks post inoculation

RESULTS I

Tumor growth and immunohistochemical characterization

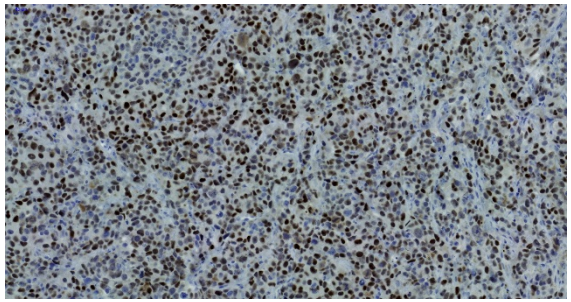
Orthotopic tumor growth



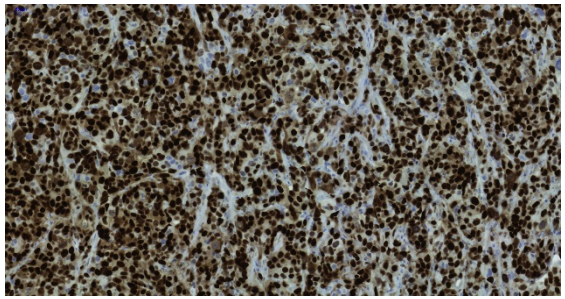


- E2 supported the BT-474 tumor growth
- Only minor tumor growth was observed in the absence of E2
- Tumor growth in nude and NOG mice was comparable

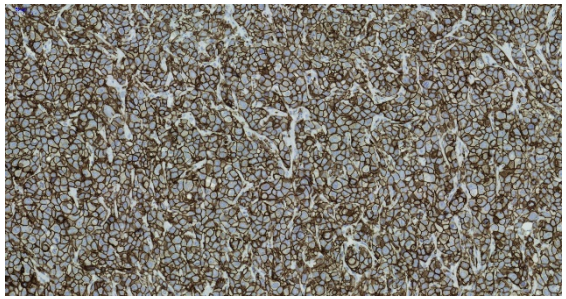
HR and HER2 status in the tumors



ER+



PR+



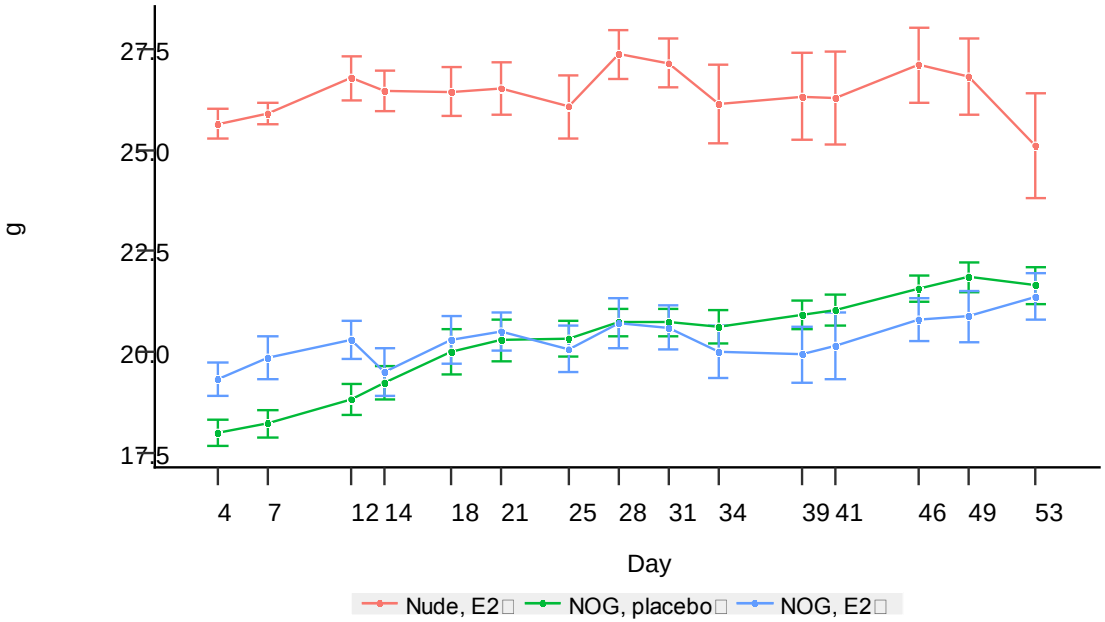
HER2+

- BT-474 tumors express ER, PR and HER2
- The expression profile was similar in NOG and nude mice

RESULTS II

Clinical condition and adverse effects in mice

Body weight development during the study



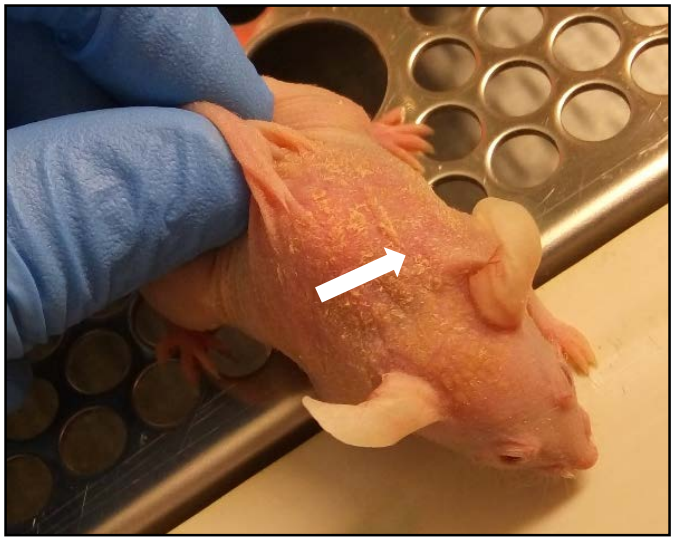
- Body weight development consistent in NOG mice
- Trend towards decreased weight in nude mice towards the end of the study

Clinical condition of the mice

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

Types of estrogen-related 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

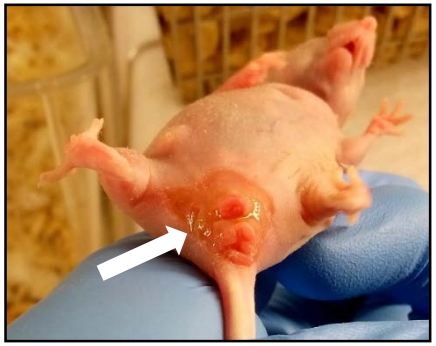
Types of estrogen-related adverse effects in nude mice

2. Wounds

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

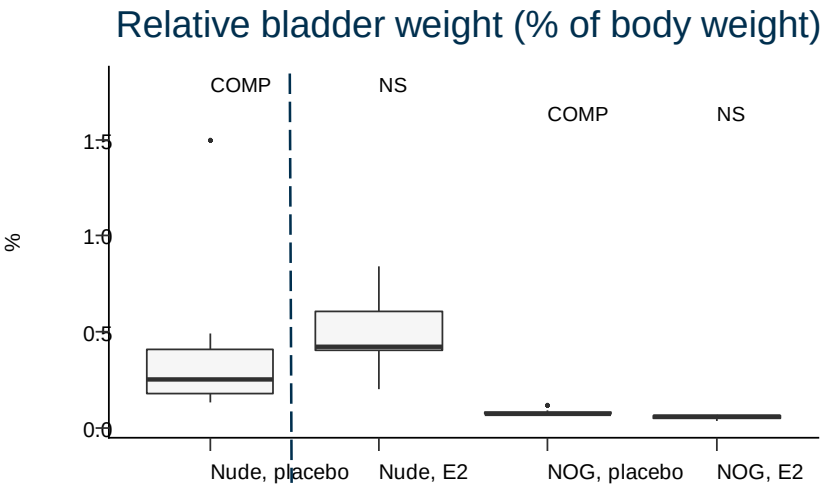
Types of estrogen-related adverse effects in nude mice

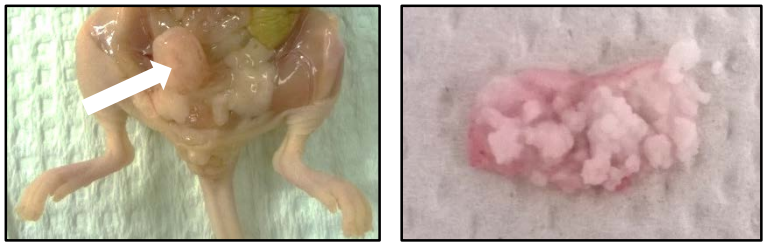
3. Secretion, irritation and skin retrieval



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

Bladder dysfunction and formation of bladder stones





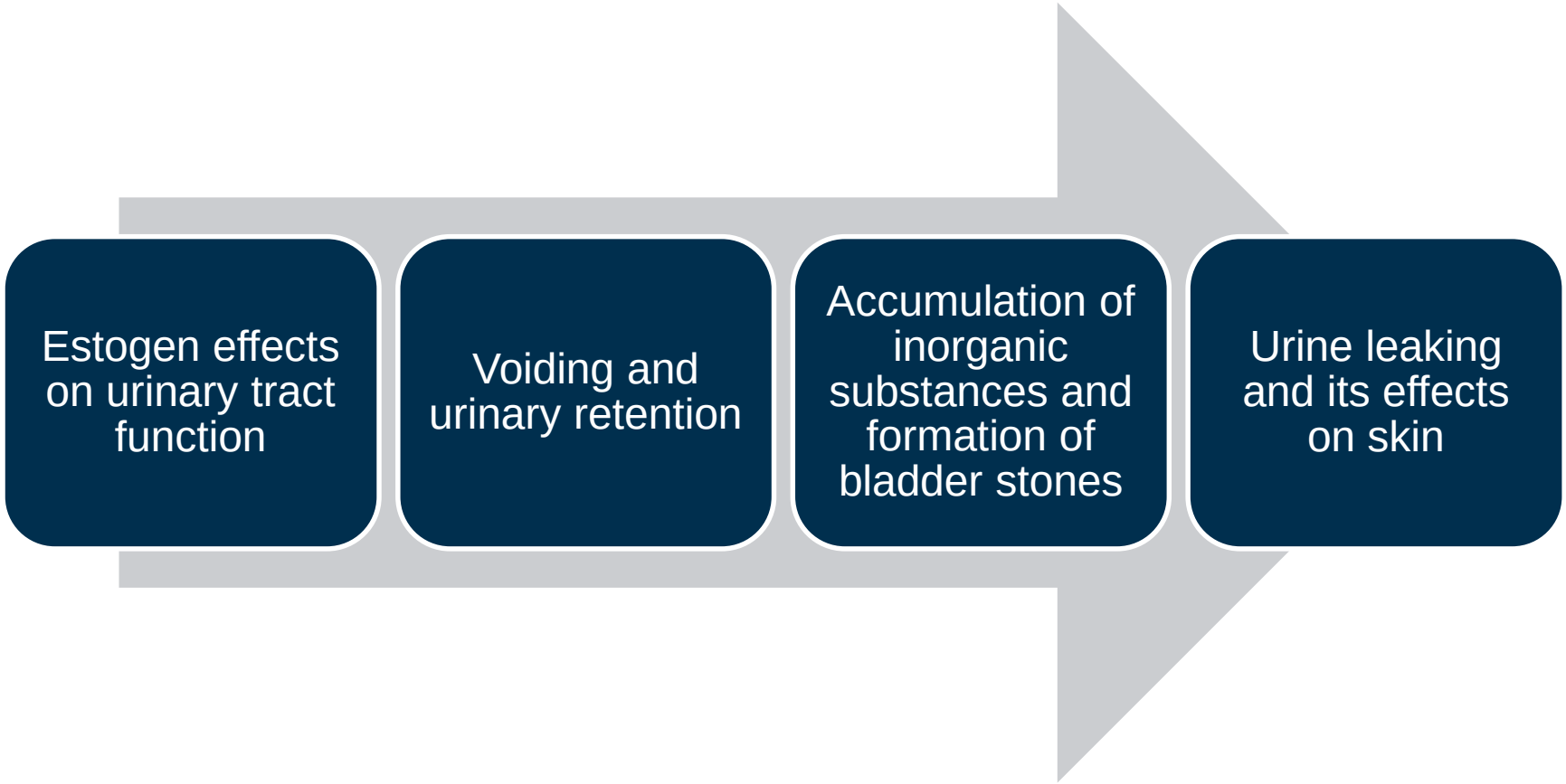
Example of enlarged bladder and the bladder stones inside.

- Over 60% of the nude mice developed 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 ammonium phosphate 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 in mice



Summary I

- Estrogen sensitive breast cancer cell lines do not grow or grow poorly in nude mice without estrogen supplement
- With estrogen supplementation the tumors grow but the mice may exhibit estrogen-related adverse effects

Summary II

- No estrogen-related adverse effects were seen in NOG mice
- Altogether 62,5% of nude mice exhibited estrogen-related adverse effects
  - Formation of bladder stones did not correlate with the appearance of skin lesions
- 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 estrogen implantation
  - First 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 estrogen-related adverse effects related to the function of 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 considered
- It is possible that high doses of estrogen induce adverse effects in NOG mice but administration method has no effect
- Careful monitoring of animal health during the study is recommended. Clinical scoring system was recently introduced by Collins DE et al. 2017, Comp. Med

Clinical Assessment

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.

PMID: 28222835 PMCID: PMC5310620 [Available on 2017-08-01]

Summary from clinical assessment

Collins DE et al., 2017, Comp Med

- “When used as a predictive test, the clinical score discriminated between estradiol-supplemented and placebo groups at a cut-off of a total clinical score of greater than 3.5.”
- “This value was predictive of mice receiving estradiol supplementation at 50% sensitivity and 83.33% specificity.”
- “However, no such cut-off point for the prediction of urinary tract lesions could be determined on the basis of the clinical scoring system, indicating a lack of correlation between clinical scores and pathology scores”

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- Pharmatest personnel

More information

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