

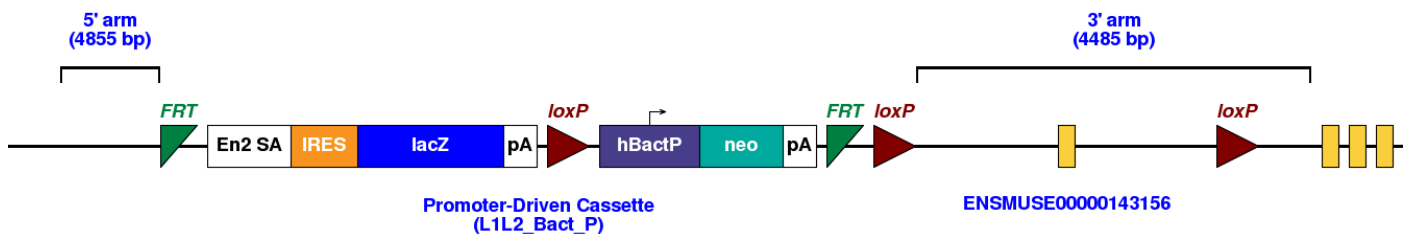
Knockout mouse lines presenting with welfare issues affecting their survival (abnormal survival [MP:0010769]) are processed through a bespoke sub-pipeline known as the “sick mouse procedure” (SMP) to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify phenotypic abnormalities arising from the targeted allele.

## *Ptpn2*<sup>tm1a(EUCOMM)Wtsi</sup>

Protein tyrosine phosphatase, non-receptor type 2

Genetic Background: C57BL/6N;C57BL/6NTac

ES Cell Clone: EPD0445\_4\_E01



### Affected genotypes

Homozygous (*Ptpn2*<sup>tm1a(EUCOMM)Wtsi</sup>).

#### Alternative breeding strategy

Following initial welfare observations, wild-type x heterozygous mating strategy was employed to complete phenotyping work in standard pipeline using heterozygous mice only.

Heterozygous mice showed no significant phenotypic findings on the primary screen.

### Welfare observations

Homozygous mice exhibit:

- Small body size = 100% (8/8)
- Eye defects - increased incidence of partial or complete closure and increased incidence of corneal opacity. = 100% (6/6)
- Decreased survivor rate, all homozygous mice do not survive to sexual maturity. = 100% (8/8)

**Homozygous Viability:**

All genotyped mice from het x het intercross considered. When at least 28 mice are available, viability at p14 is calculated. [ $>13\%$  = *Homozygous viable*;  $>0\%$  and  $<13\%$  = *Sub-viable*;  $0\%$  = *Lethal*]

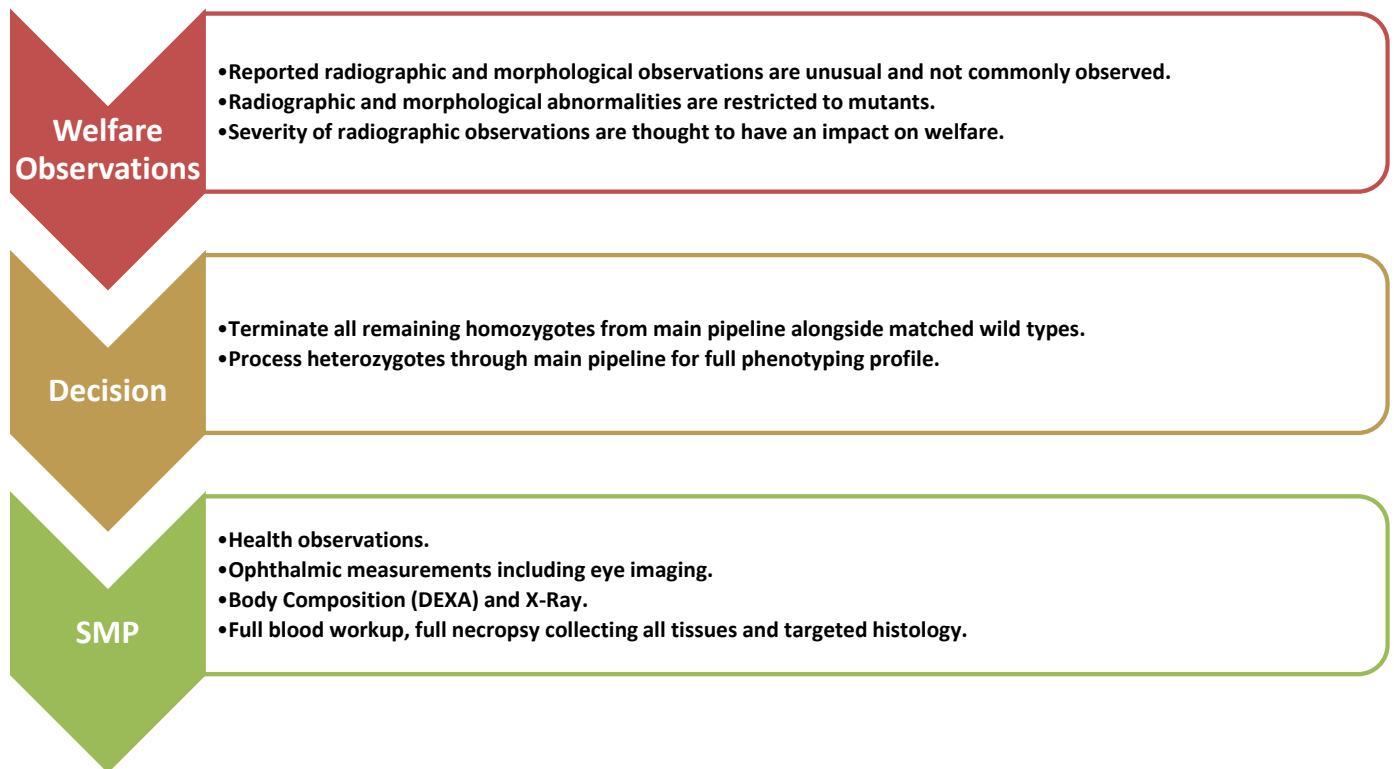
- **Viable** : 16 Homs / 82 Total = 19.51 %

**Sick Mouse Procedure (SMP)**

Initial welfare observations were reported when the first homozygotes were born during the breeding and expansion stage. Homozygotes were still viable when issued to the phenotyping pipelines (4 weeks) but severity progressed from 6 weeks of age.

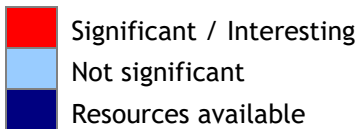
Welfare observations in homozygotes described above progressed to moderate severity and SMP (see schematic below) was initiated on mice between 4 and 5 weeks of age. 5 male and 3 female homozygotes were processed alongside 4 male and 3 female matched wild-types. No further homozygotes were phenotyped due to the aforementioned alternative breeding strategy employed to reduce further welfare implications.

**Schematic Outline of Bespoke SMP Pipeline**



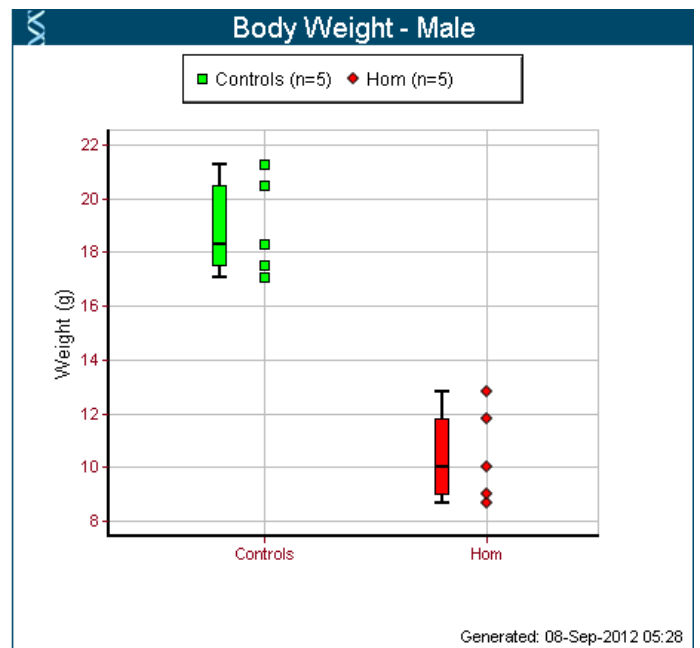
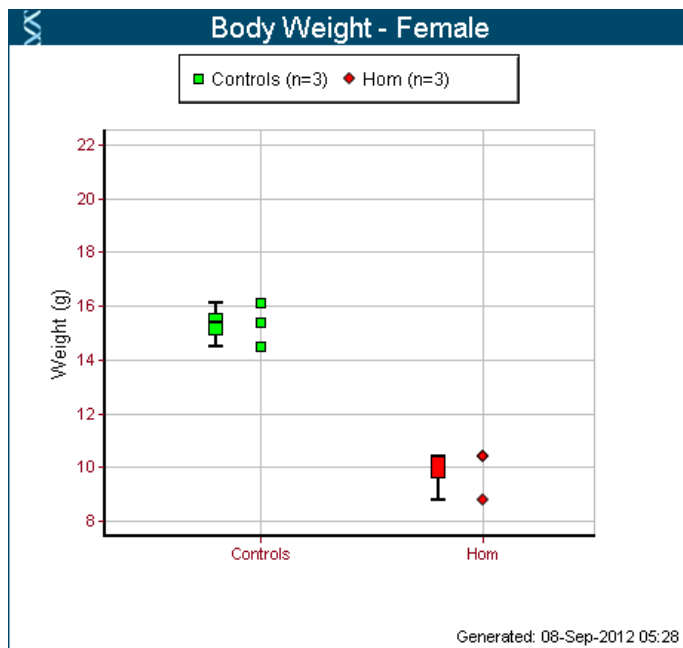
## Phenotyping Heat Map

Colony Prefix	Allele Name	Genotype	Weight Curves	Ophthalmic Measurements	Body Composition (DEXA)	X-ray Imaging	Plasma Chemistry	Haematology (CBC)	Peripheral Blood Leukocytes	Tissue Biobank
MCTP	Ptpn2tm1a(EUCOMM)Wtsi	Homozygous								

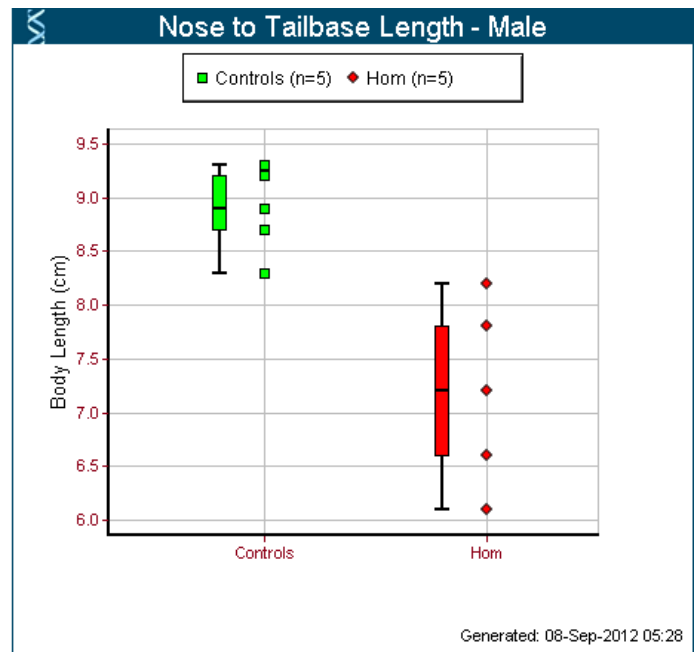
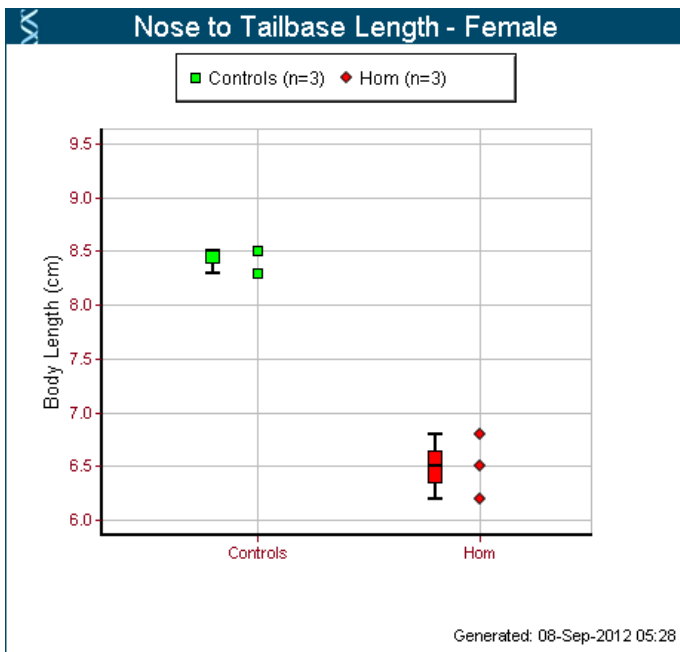


## Phenotyping data of interest (significant changes)

### In life phenotyping

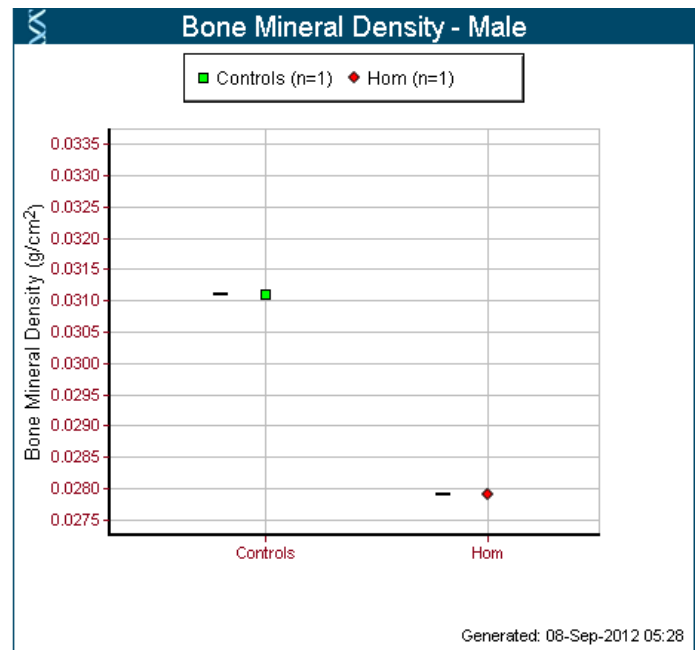
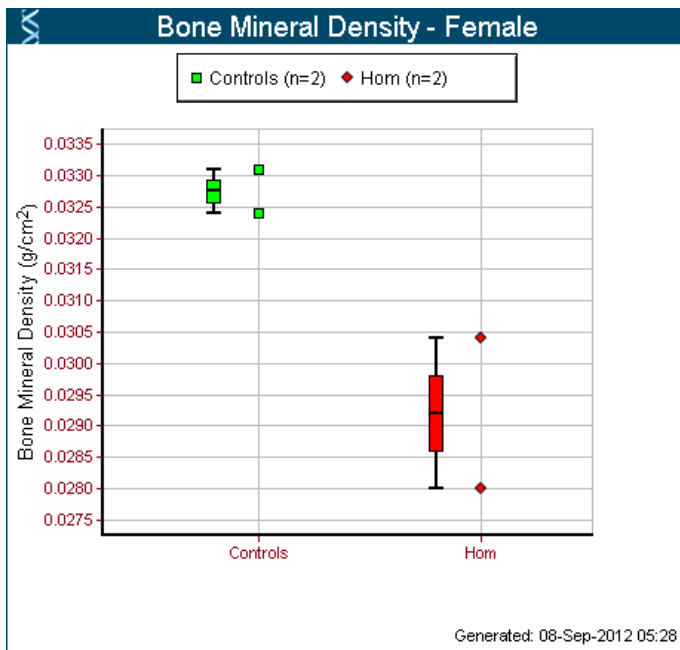


**Males and Females – Decreased Body Weight [MP:0001262]**

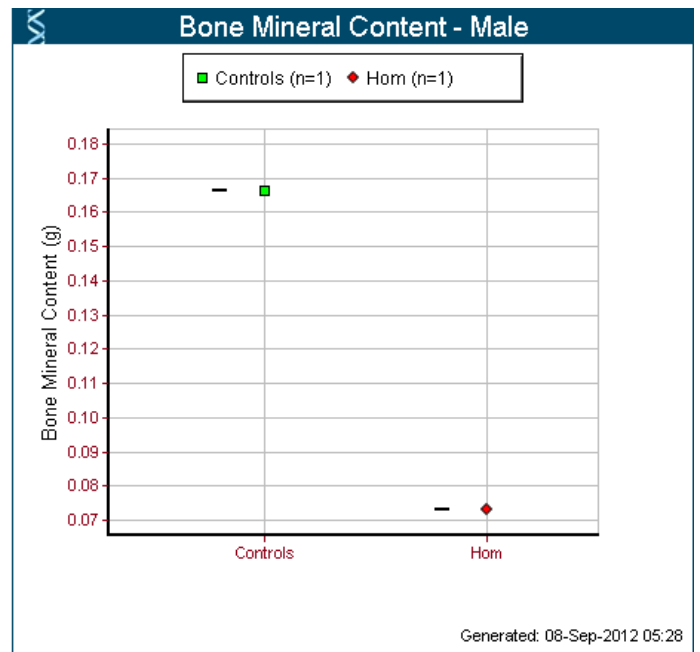
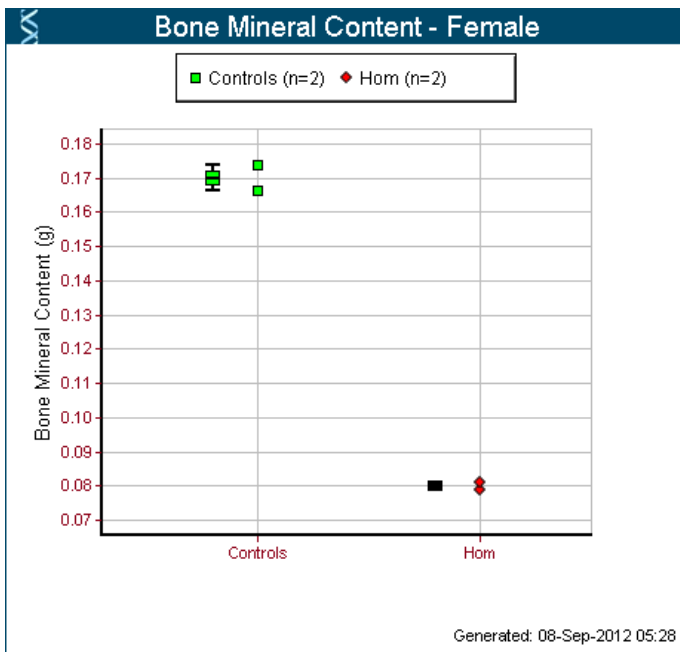


**Males and Females – Decreased Body Length [MP:0001258]**

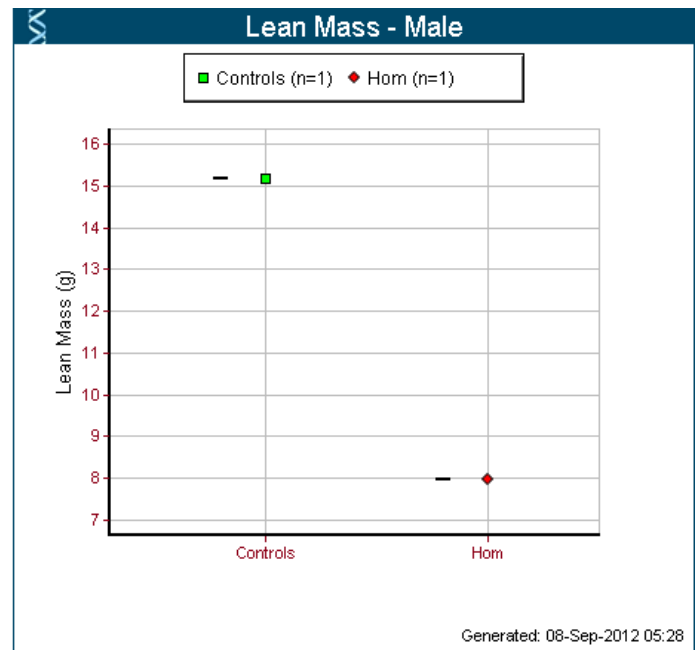
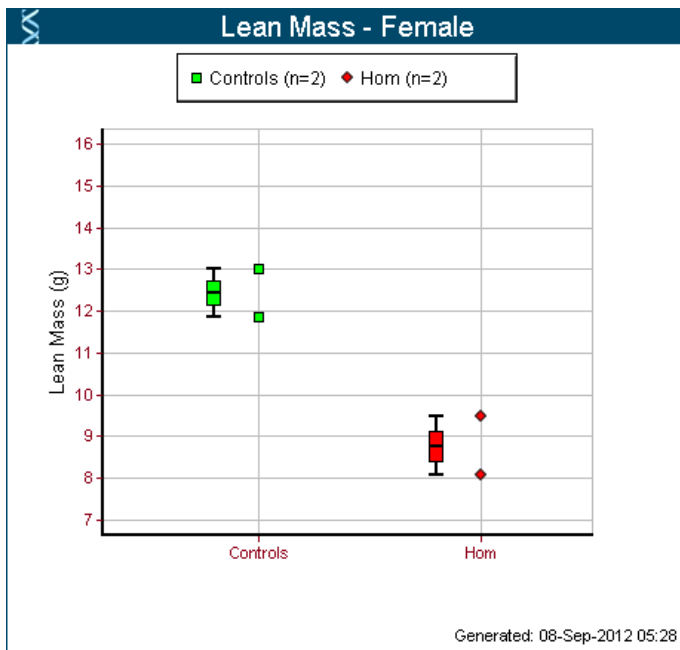
**Body Composition (DEXA) and X-RAY**



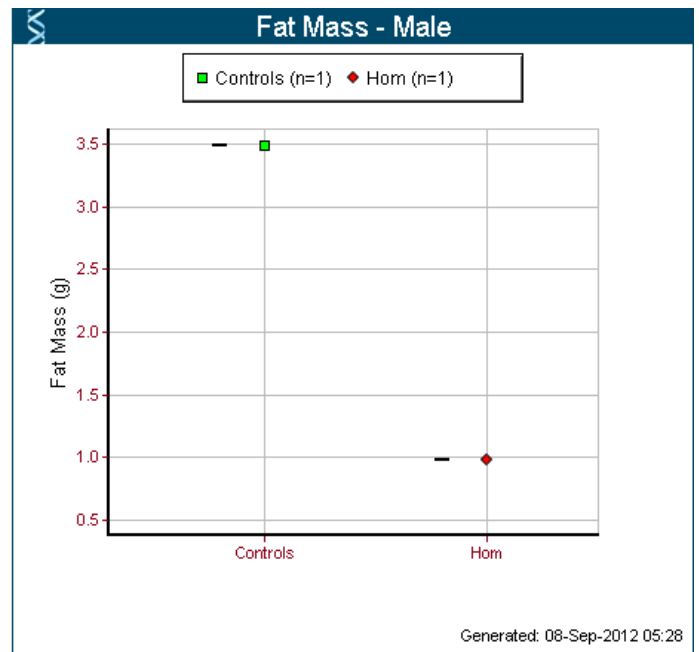
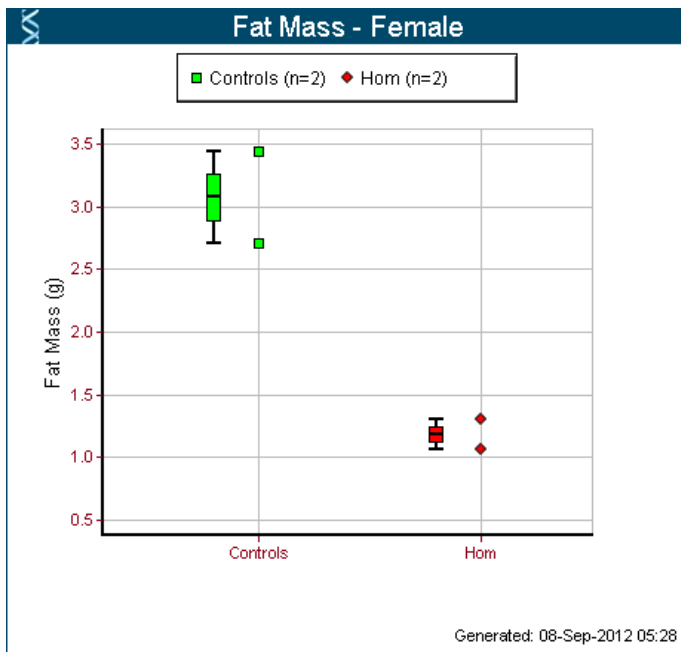
**Males and Females – Decreased Bone Mineral Density [MP:0000063]**



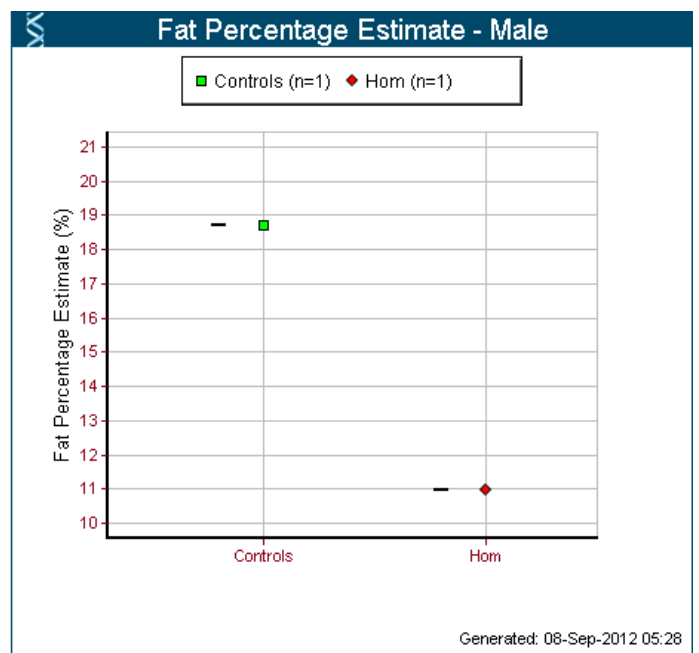
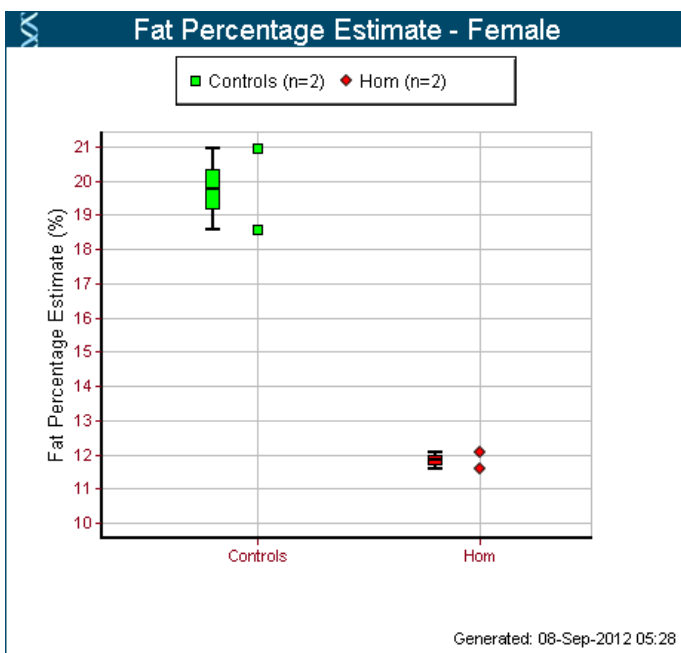
**Males and Females – Decreased Bone Mineral Content [MP:0010124]**



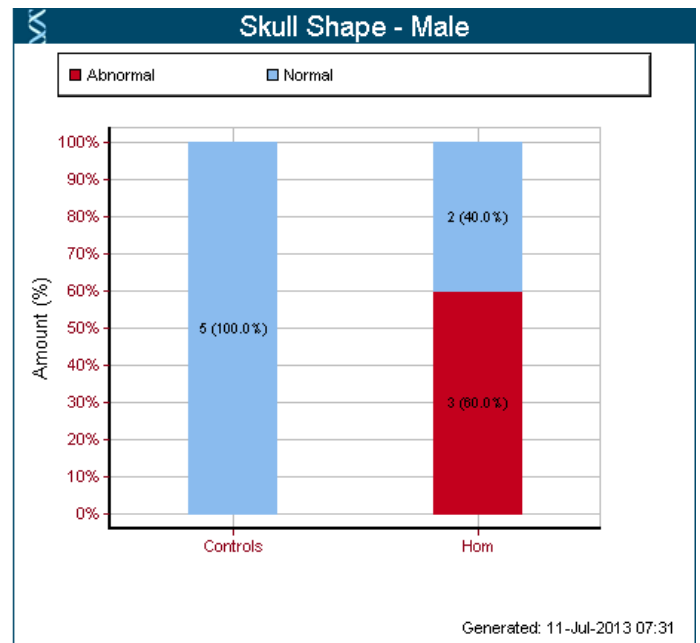
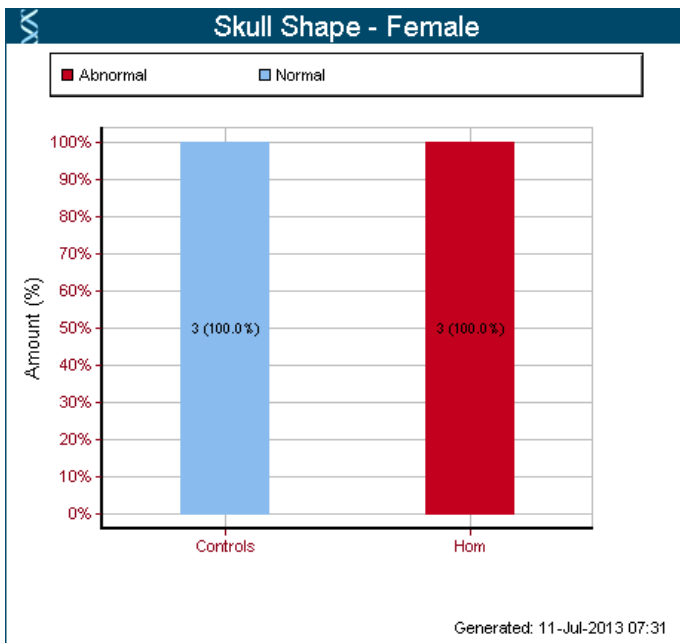
**Males and Females – Decreased Lean Body Mass [MP:0003961]**



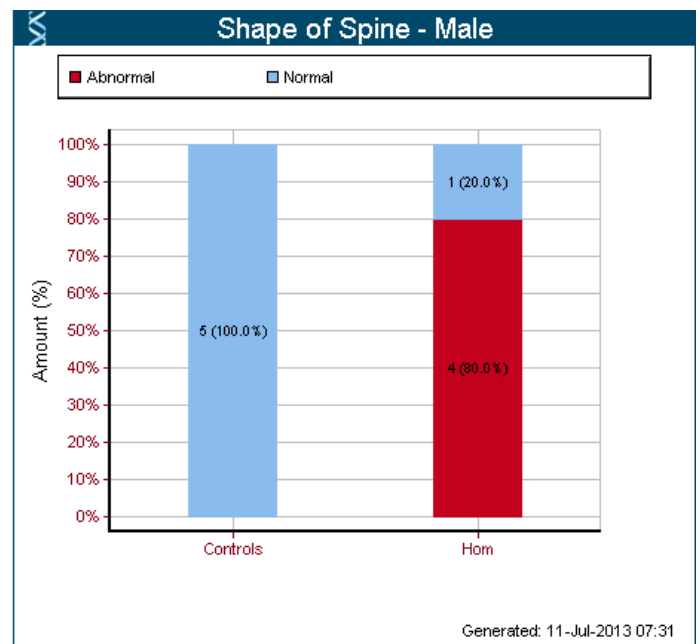
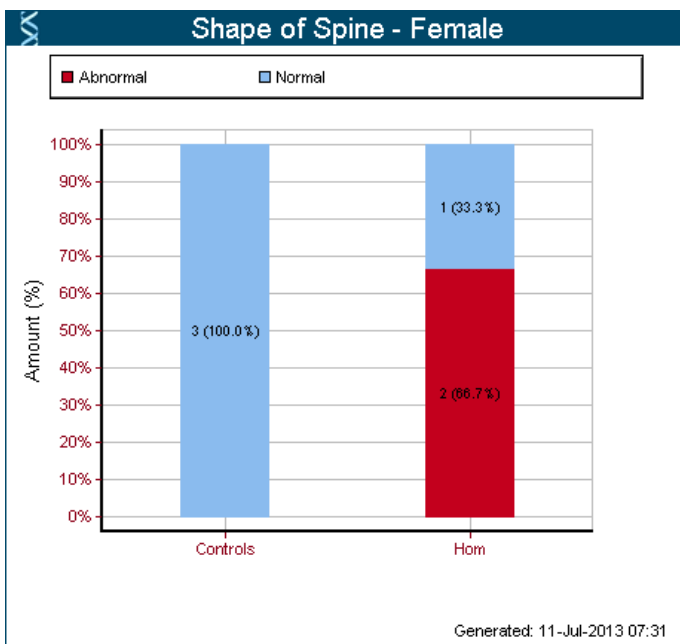
**Males and Females – Decreased Total Body Fat Amount [MP:0010025]**



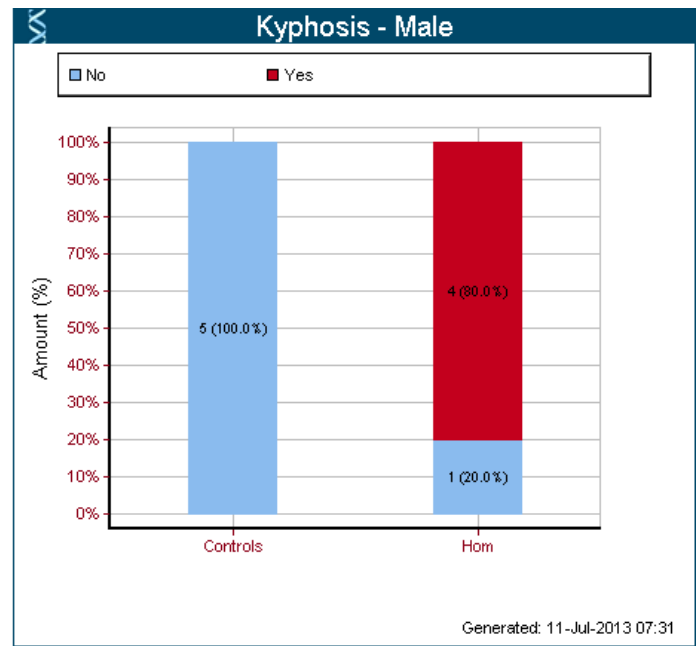
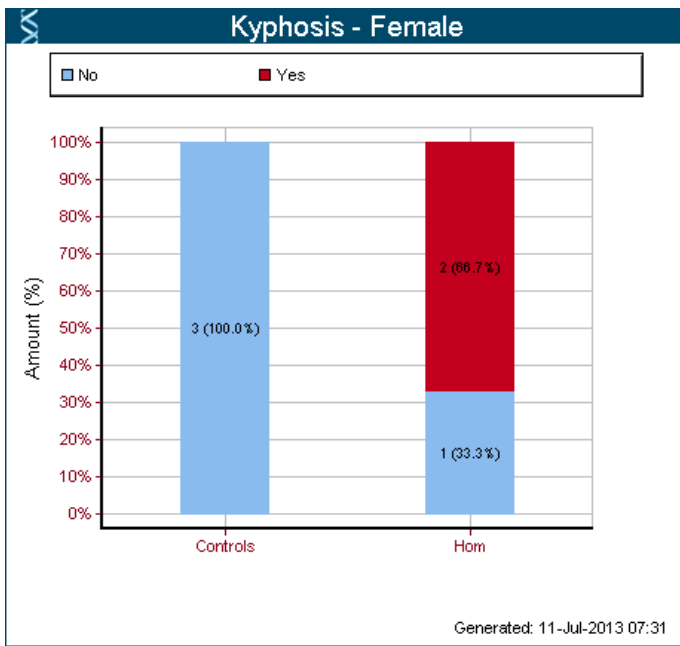
**Males and Females – Decreased Percent Body Fat [MP:0005459]**



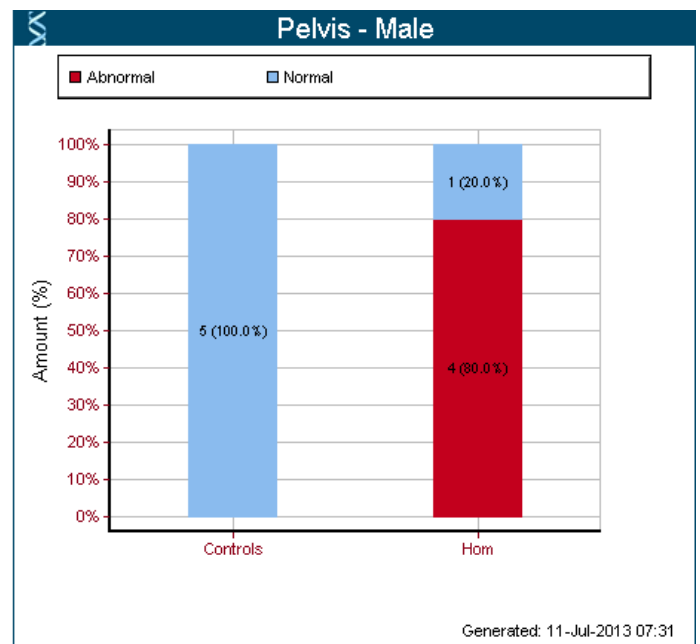
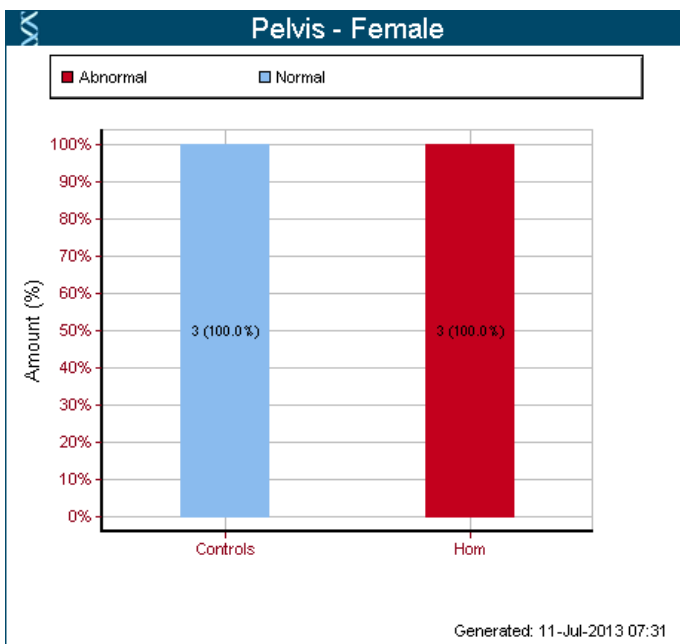
**Males and Females –Abnormal Cranium Morphology [MP:0000438]**



**Males and Females –Abnormal Spine Curvature [MP:0004174]**

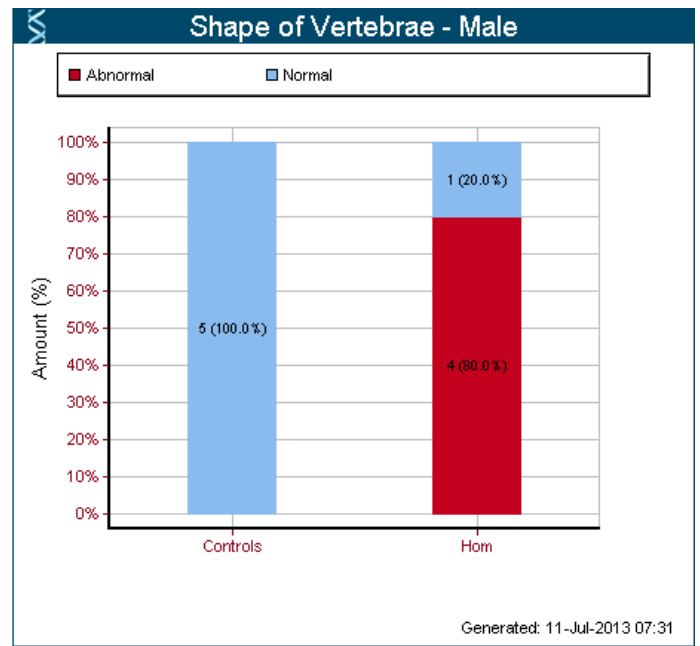
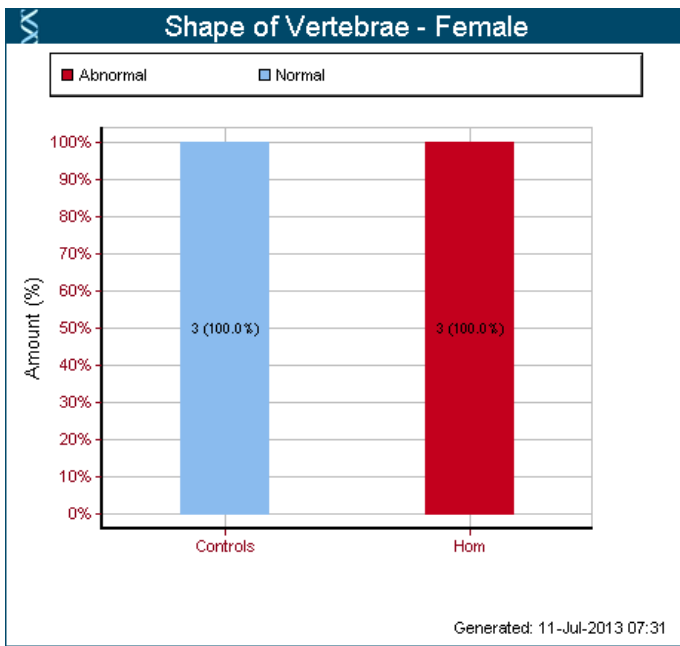


**Males and Females – Kyphosis [MP:0000160]**

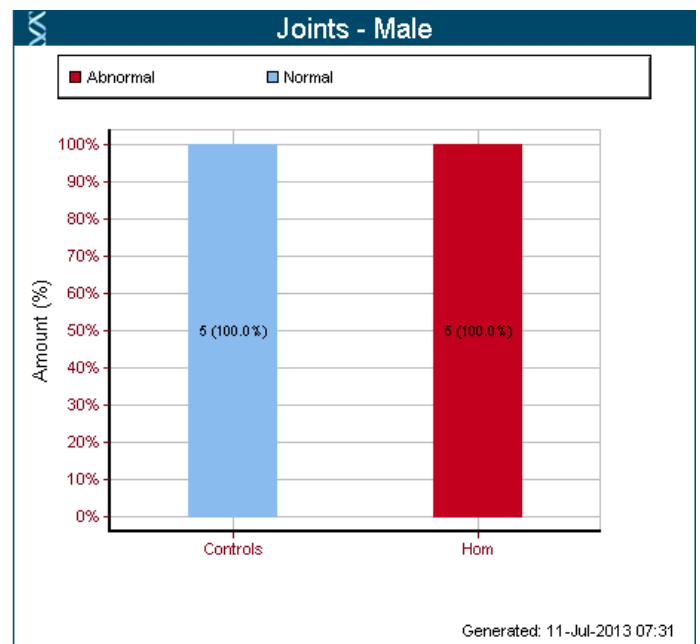
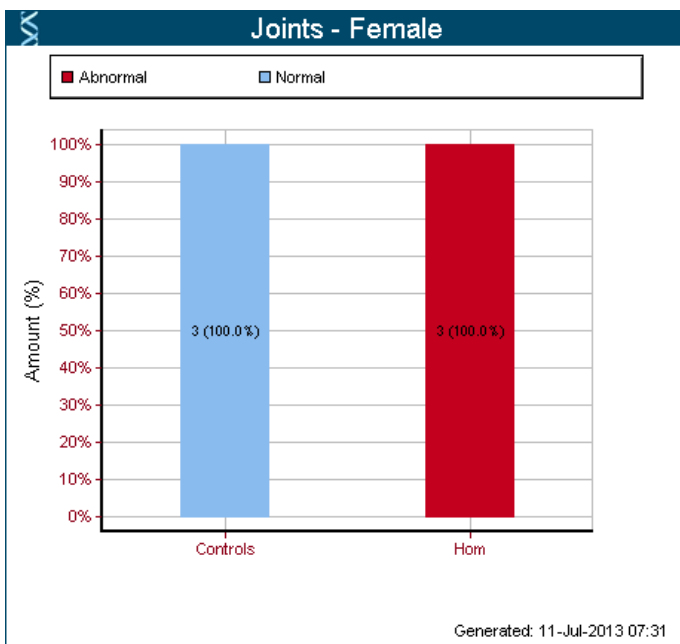


**Males and Females – Abnormal Pelvic Girdle Bone Morphology [MP:0004509]**



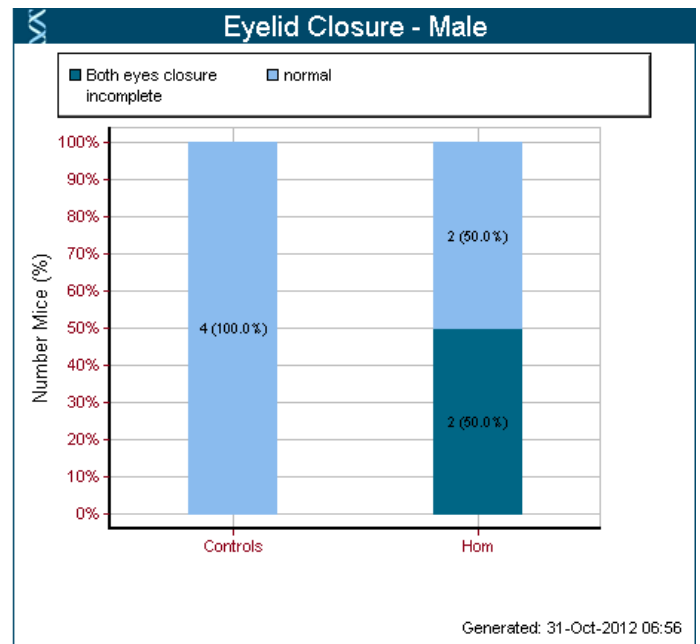
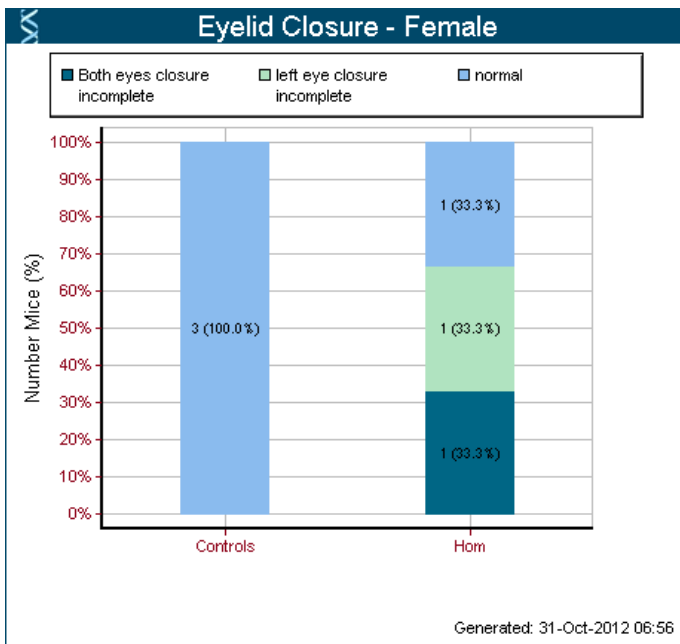


**Males and Females – Abnormal Vertebrae Morphology [MP:0000137]**

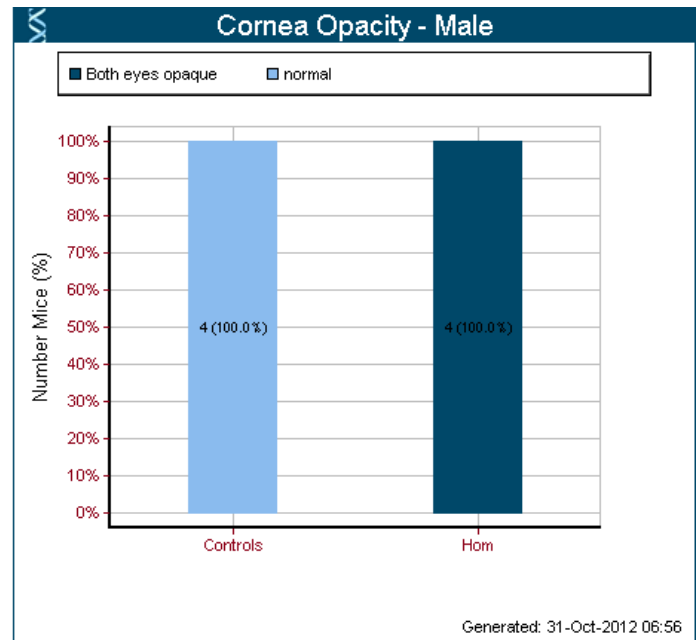
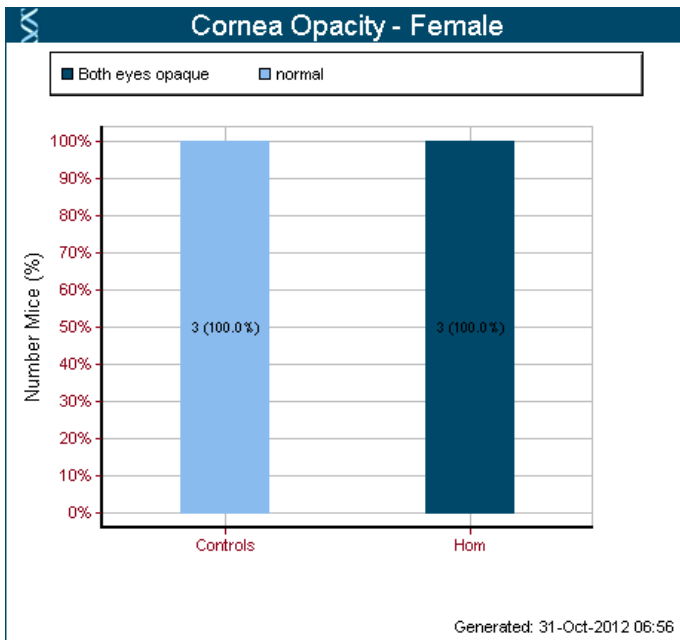


**Males and Females – Abnormal Joint Morphology [MP:0002932]**

Ophthalmic Measurements

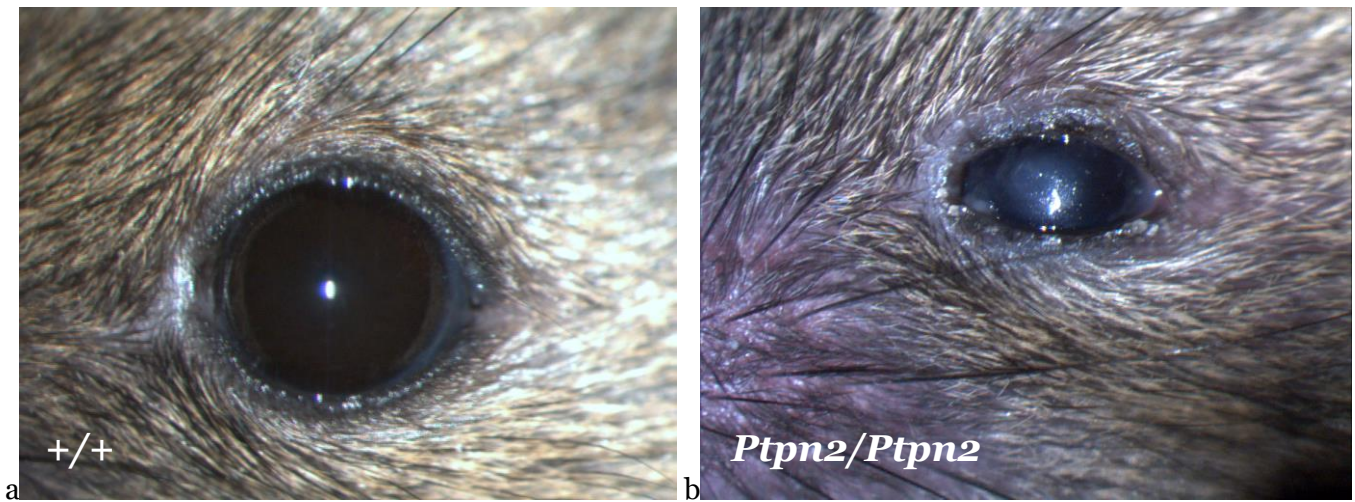


**Males and Females –Narrow Eye Opening [MP:0005287]**



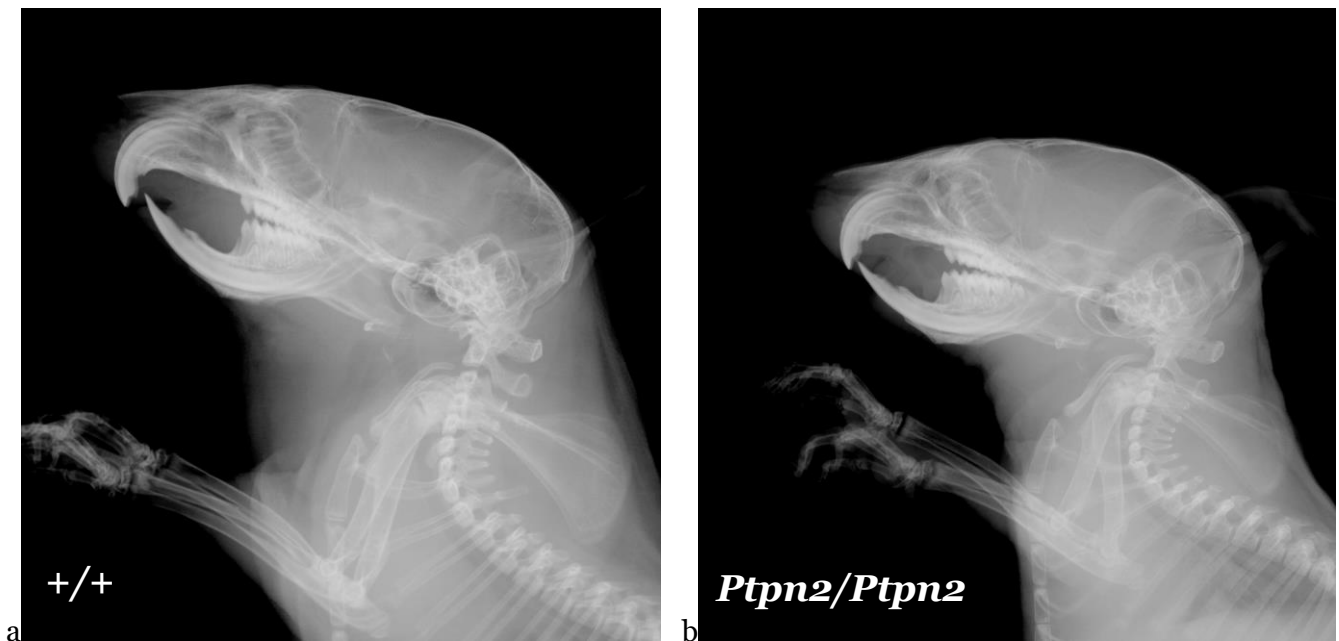
**Males and Females – Corneal Opacity [MP:0001314]**

Ophthalmic Images

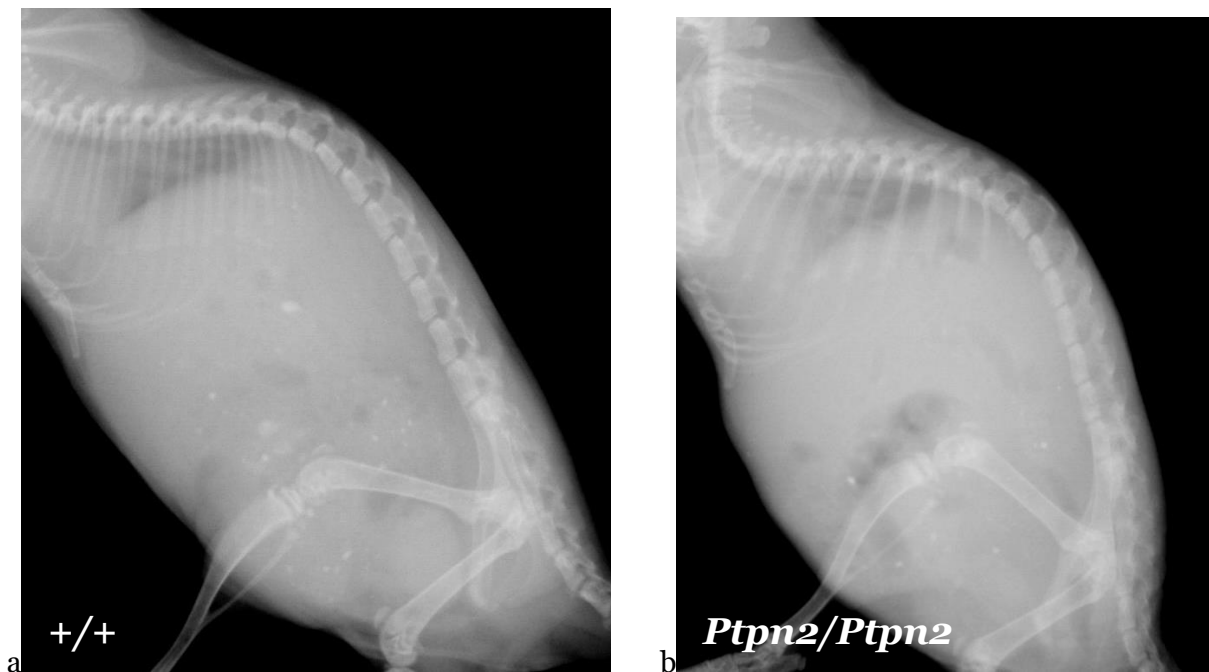


**Figure 1.** Lateral view of wild-type (a) and homozygous (b) mice showing eye defects. Abnormal Eyelid Morphology [MP:0001340] and Corneal Opacity [MP:0001314] in homozygotes.

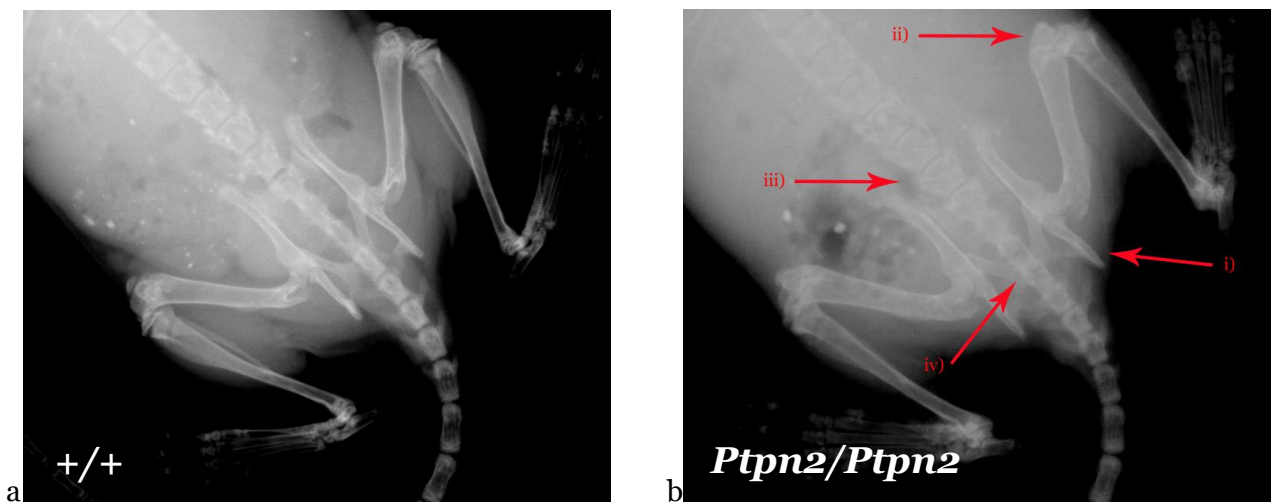
X-Ray Images



**Figure 2.** Lateral view of wild-type (a) and homozygous (b) skulls showing abnormal shape. Abnormal Cranium Morphology [MP:0000438] in homozygotes.

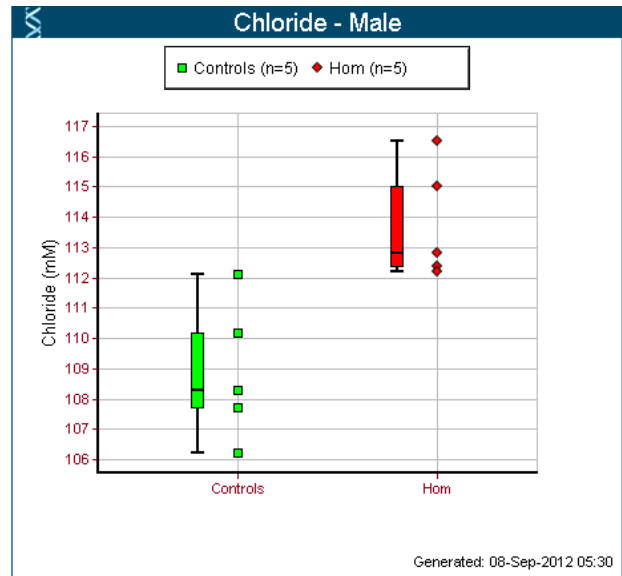
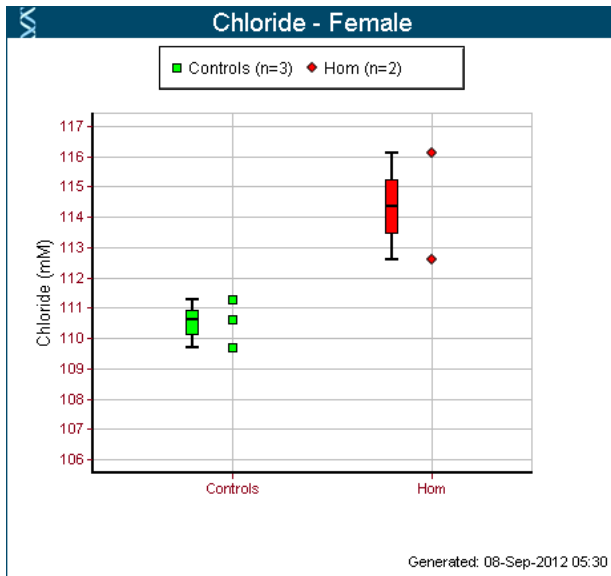


**Figure 3.** Lateral view of wild-type (a) and homozygous (b) showing spine defects. Abnormal Spine Curvature [MP:0004174] and Kyphosis [MP:0000160] in homozygotes.

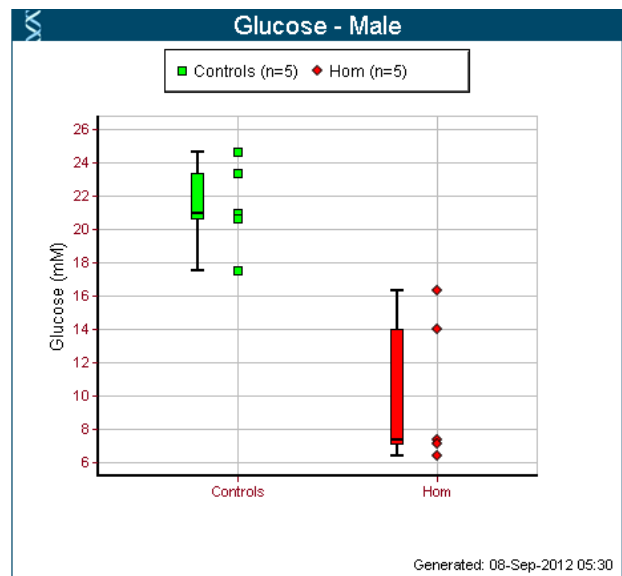
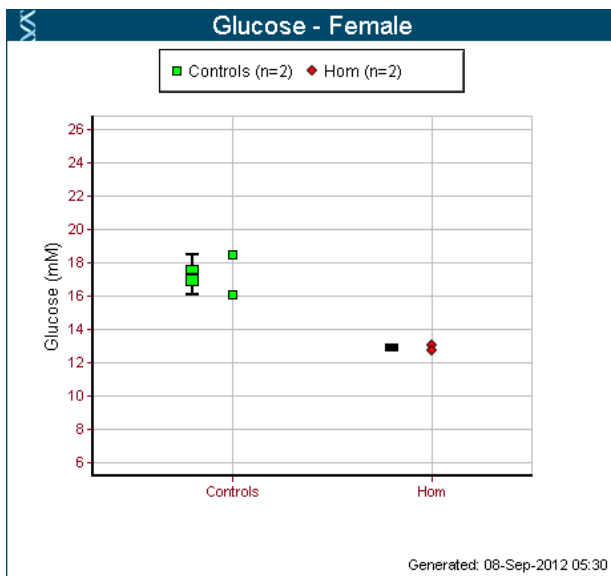


**Figure 4.** Dorsal ventral view of wild-type (a) and homozygous (b) showing short pelvis (i), abnormal morphology of stifle (ii), short lumbar (iii) and sacral (iv) vertebrae. Abnormal Pelvic Girdle Bone Morphology [MP:0004509], Abnormal Joint Morphology [MP:0002932] and Abnormal Vertebrae Morphology [MP:0000137] in homozygotes.

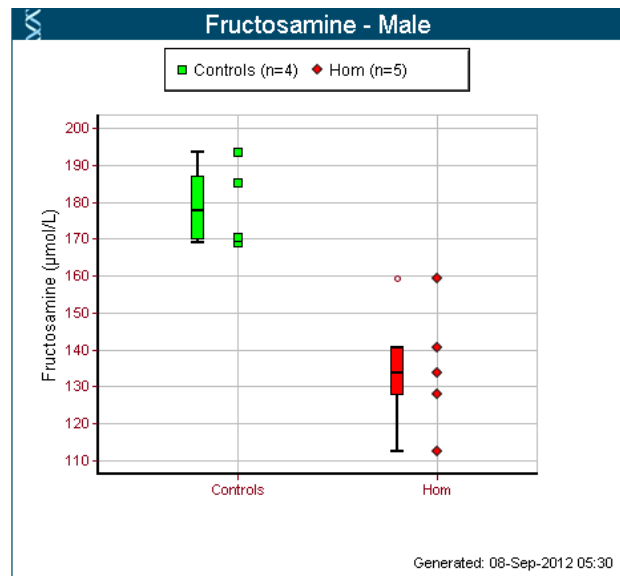
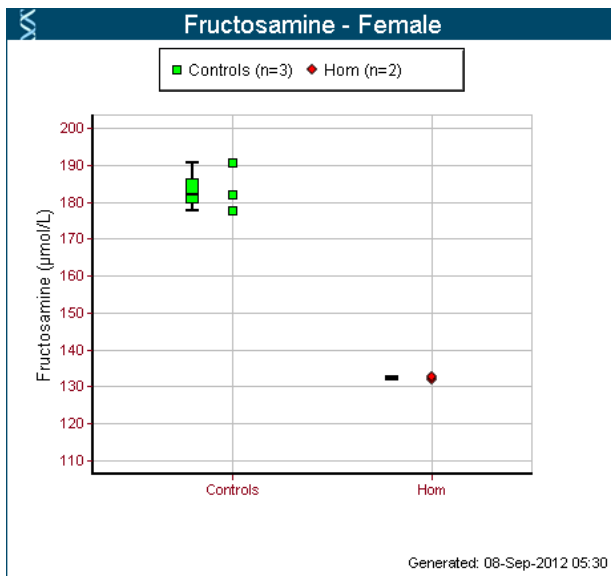
*Ex vivo phenotyping*



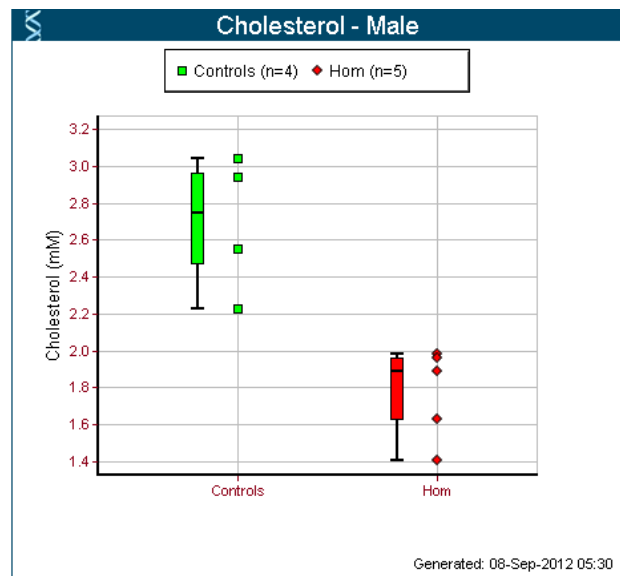
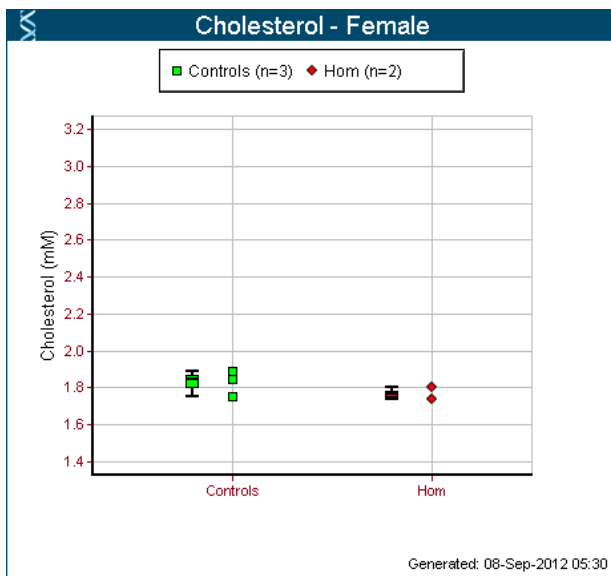
**Males and Females - Increased Circulating Chloride Level [MP:0003019]**



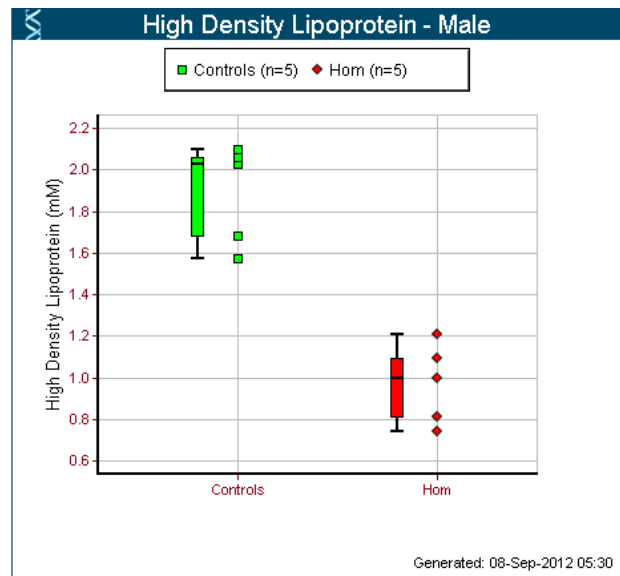
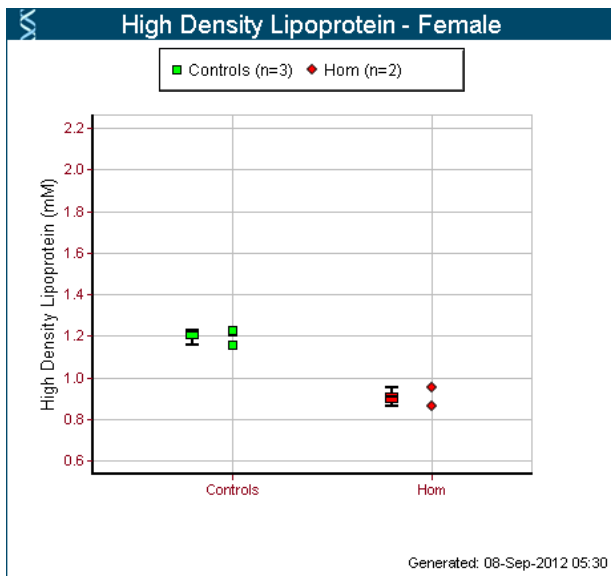
**Males and Females - Decreased circulating glucose level [MP:0005560]**



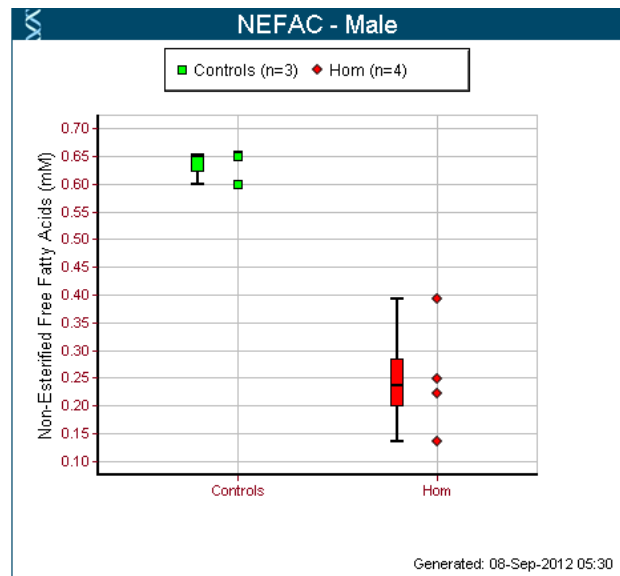
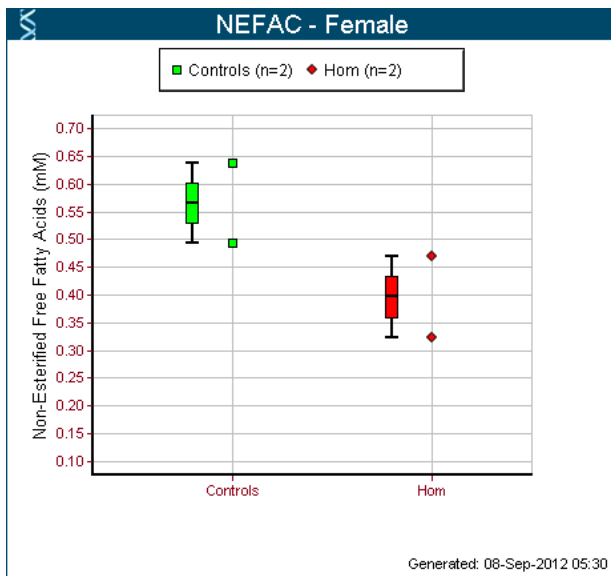
**Males and Females - Decreased Circulating Fructosamine level [MP:0010088]**



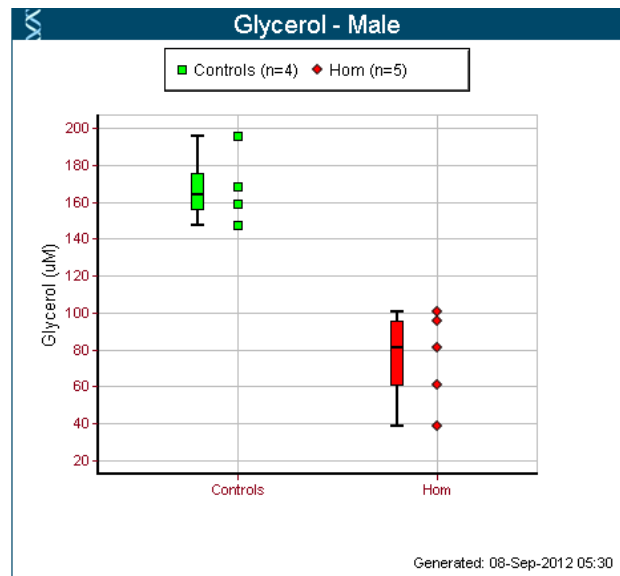
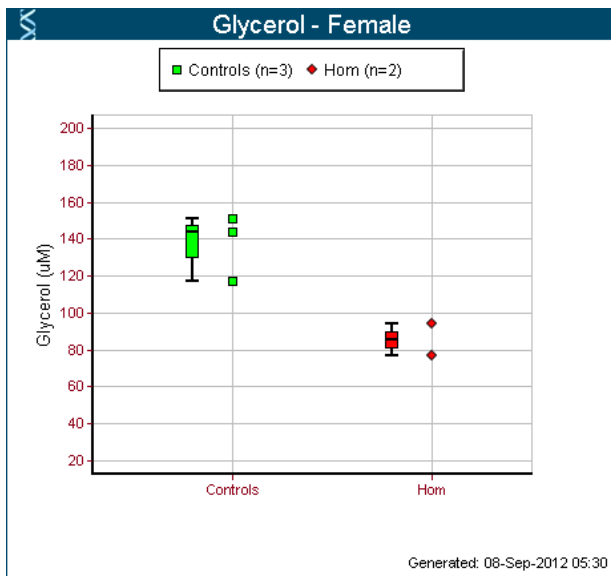
**Males only - Decreased Circulating Cholesterol Level [MP:0005179]**



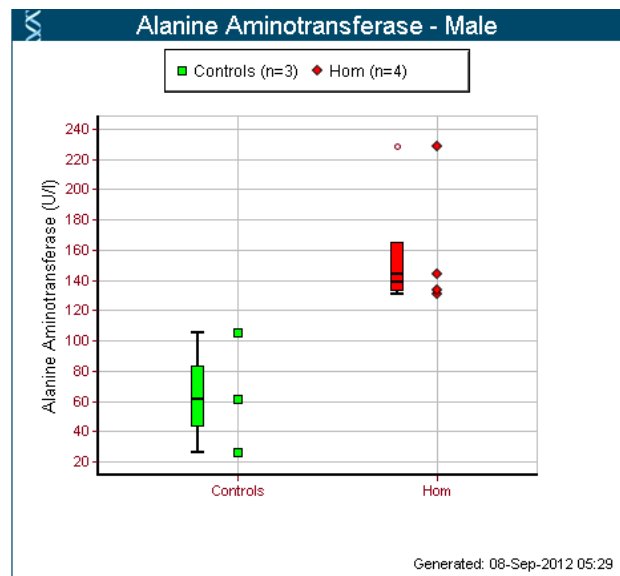
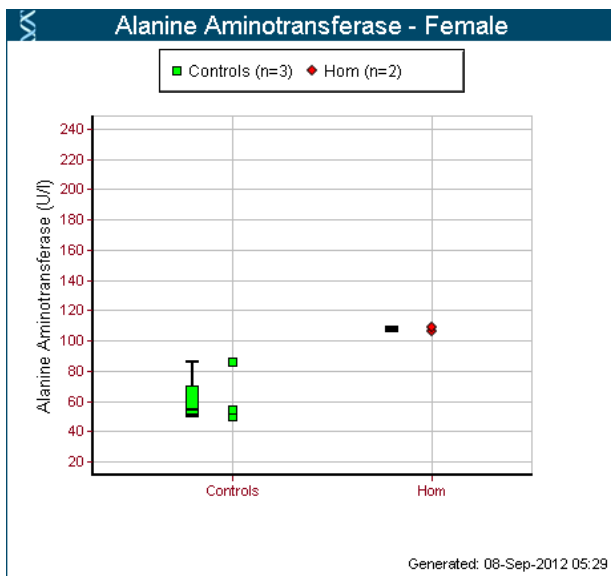
**Males only - Decreased Circulating HDL Cholesterol Level [MP:0000186]**



**Males and Females - Decreased Circulating Free Fatty Acid Level [MP:0002702]**

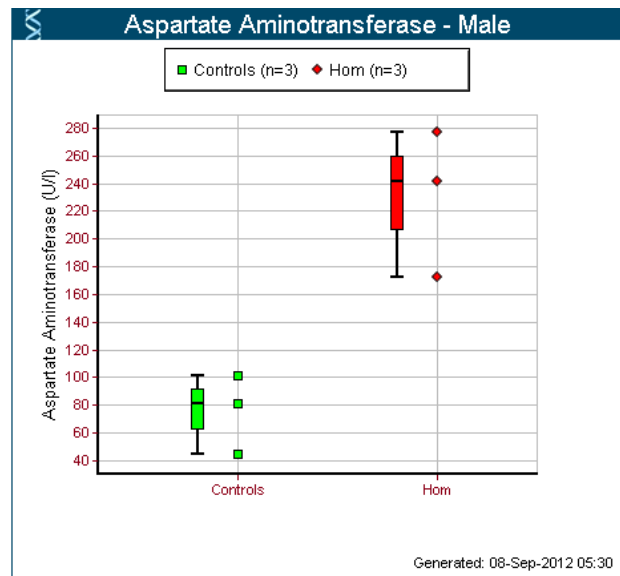
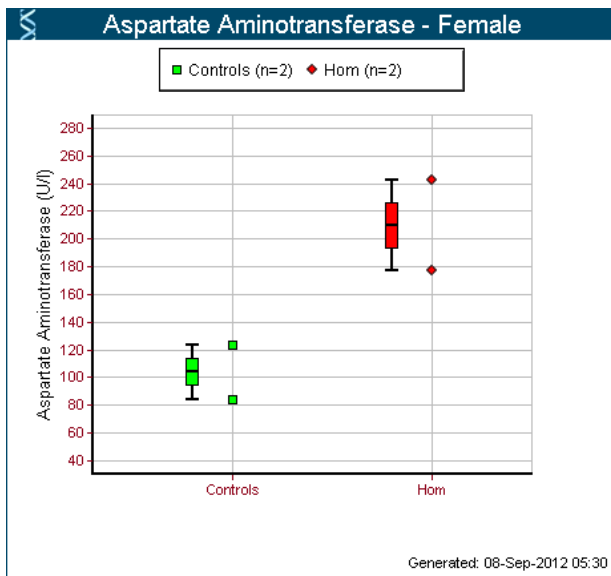


**Males and Females - Decreased Circulating Glycerol Level [MP:0003442]**

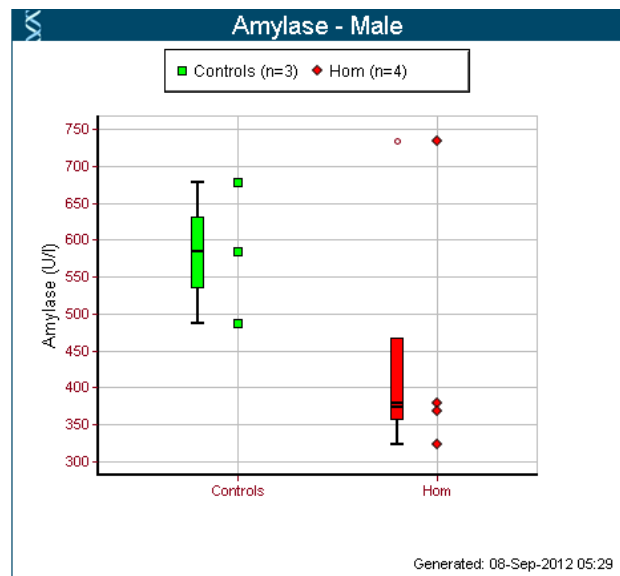
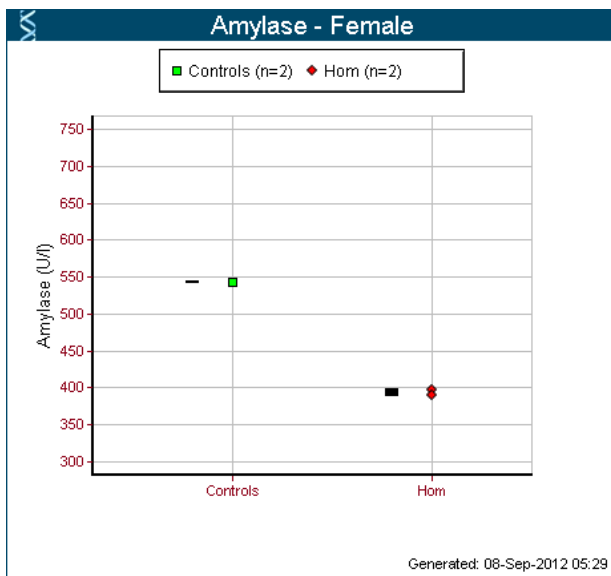


**Males and Females - Increased Circulating Alanine Transaminase Level [MP:0002941]**

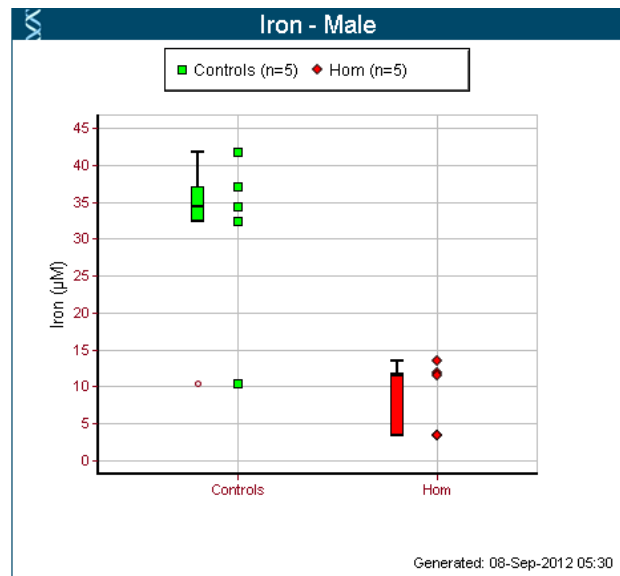
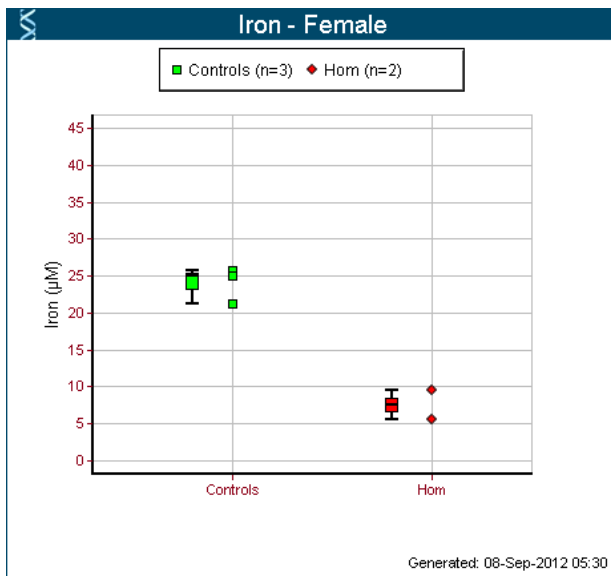




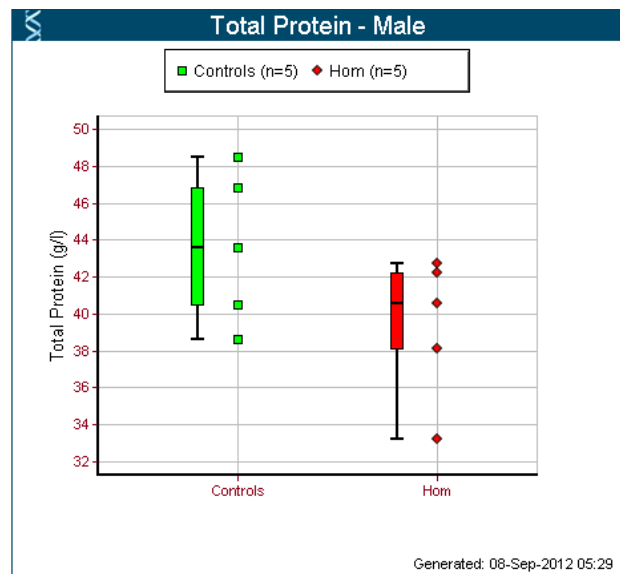
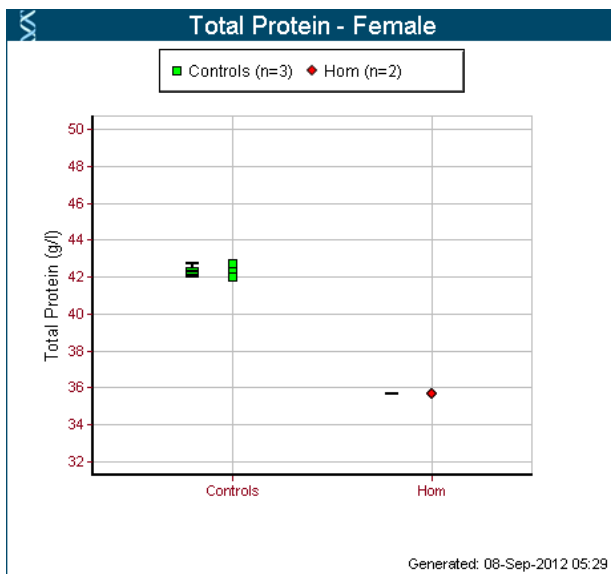
**Males and Females - Increased Circulating Aspartate Transaminase Level [MP:0005343]**



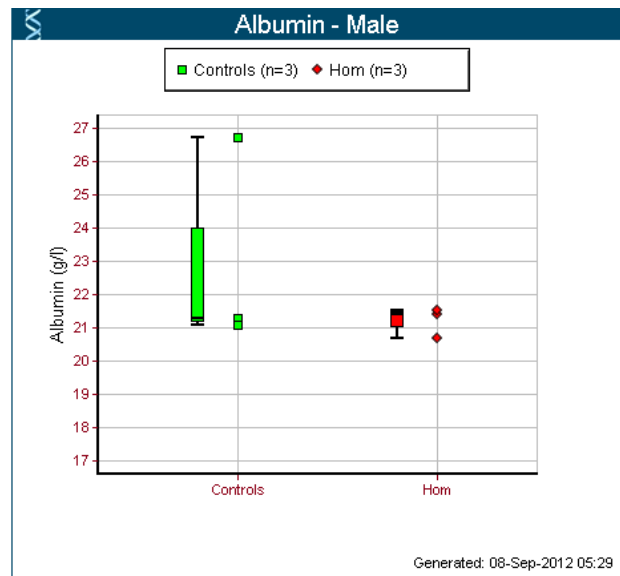
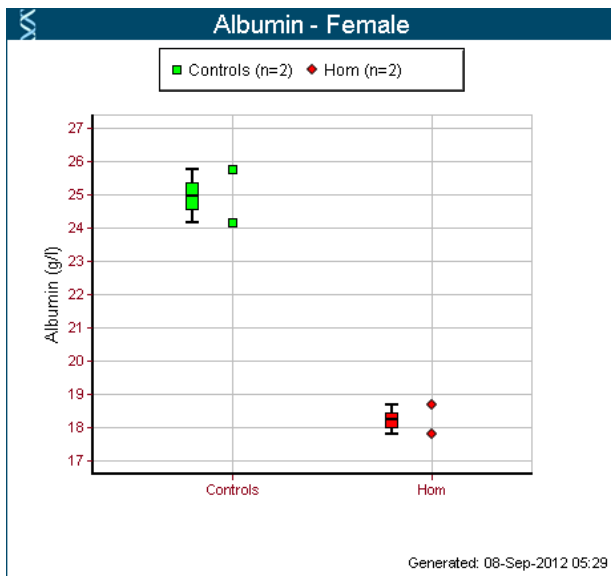
**Males and Females - Decreased Circulating Amylase Level [MP:0008805]**



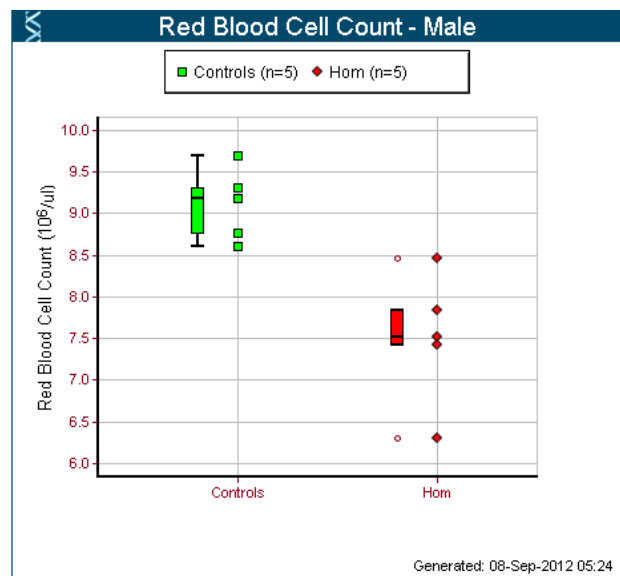
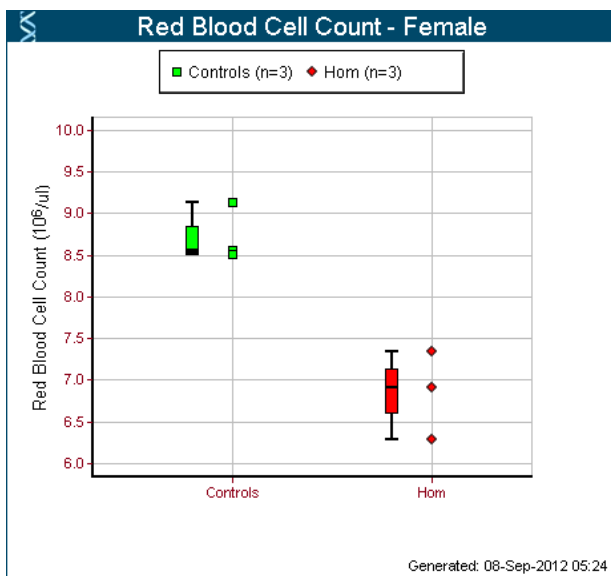
**Males and Females - Hypoferremia [MP:0004151]**



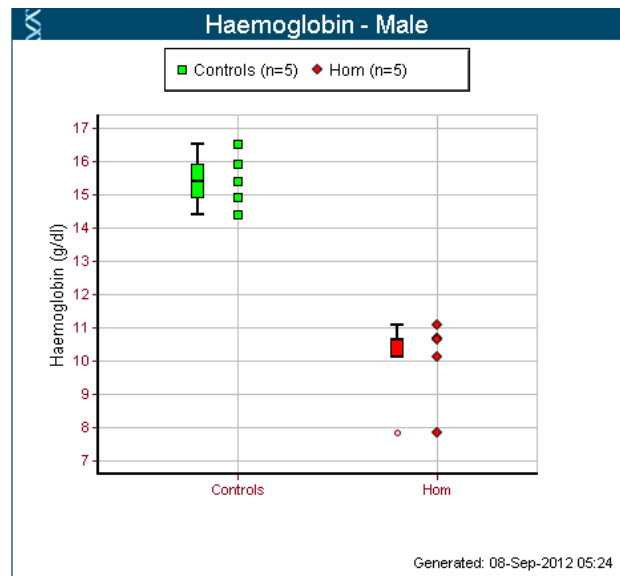
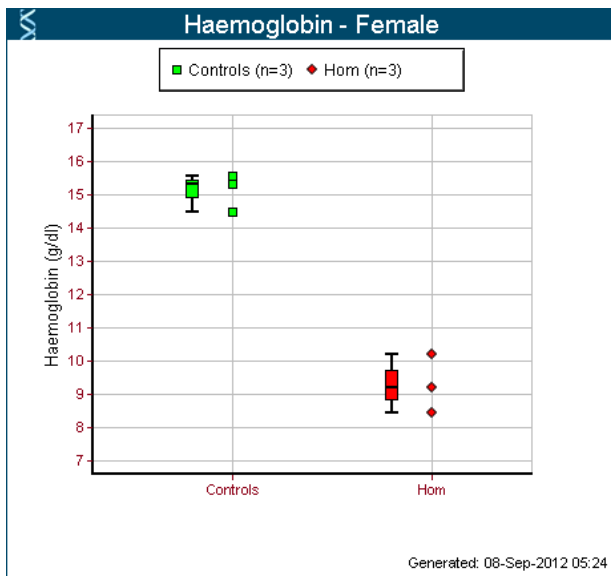
**Females only - Decreased Circulating Total Protein Level [MP:0005567]**



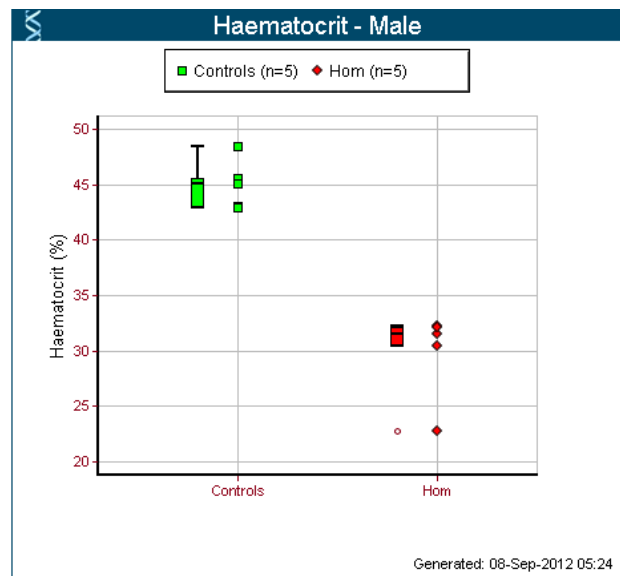
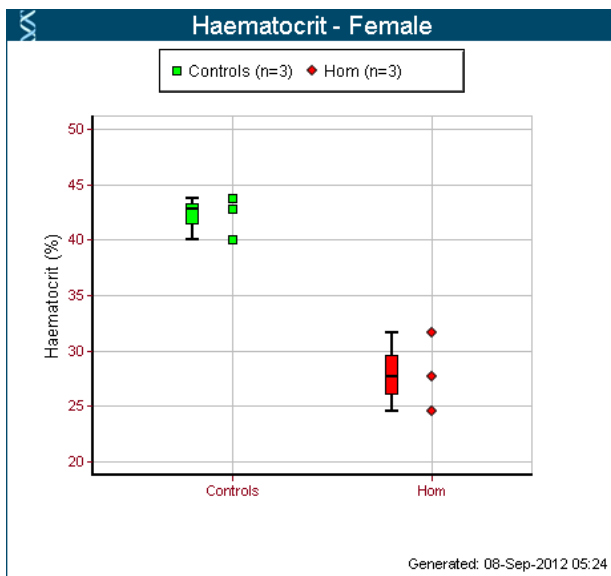
**Females only - Hypoalbuminemia**  
 [MP:0005419]



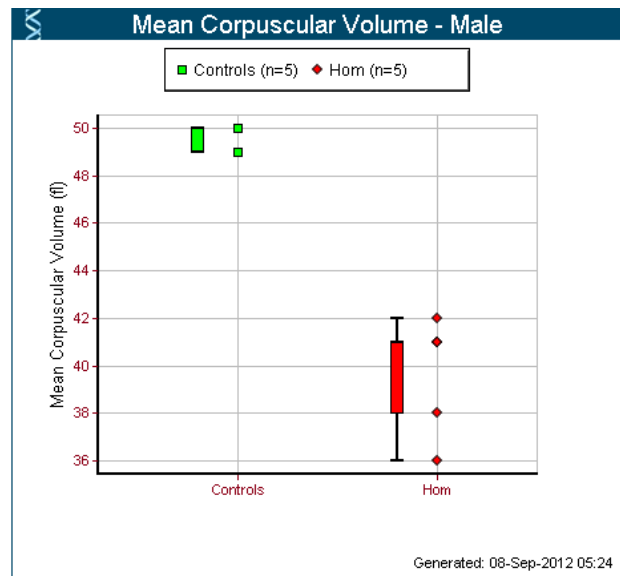
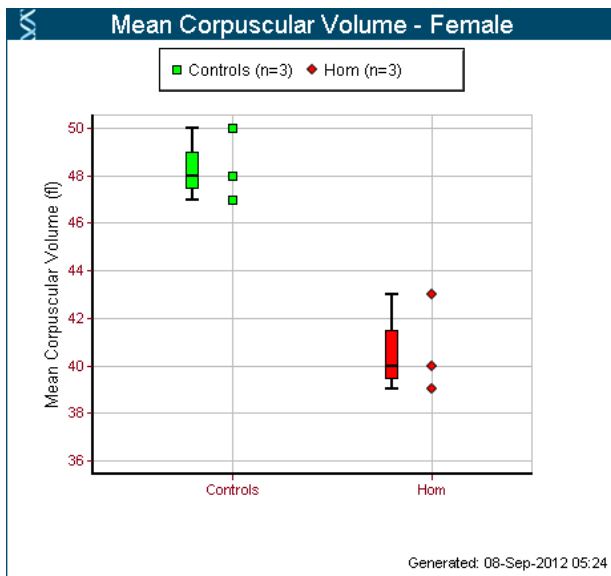
**Males and Females - Decreased Erythrocyte Cell Number** [MP:0002875]



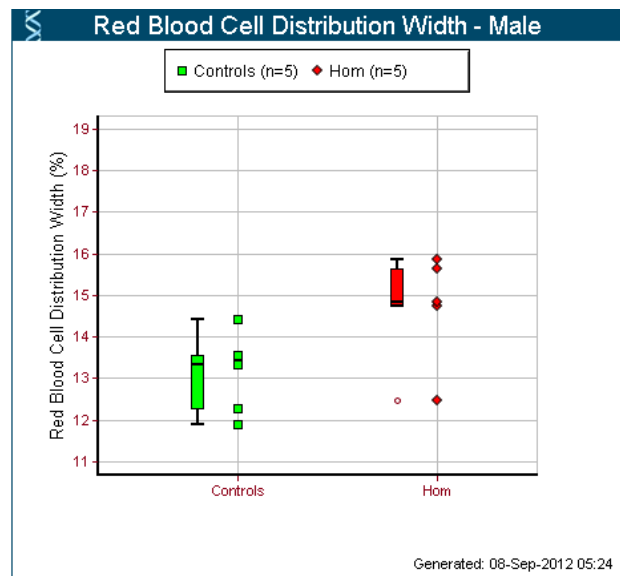
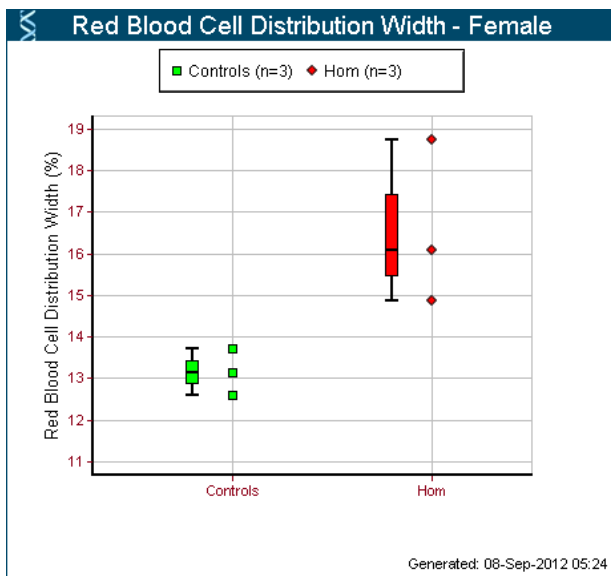
**Males and Females - Decreased Haemoglobin Content [MP:0002874]**



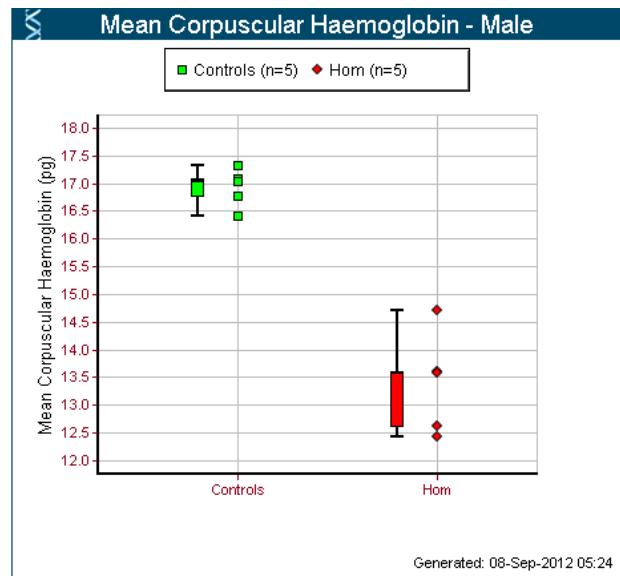
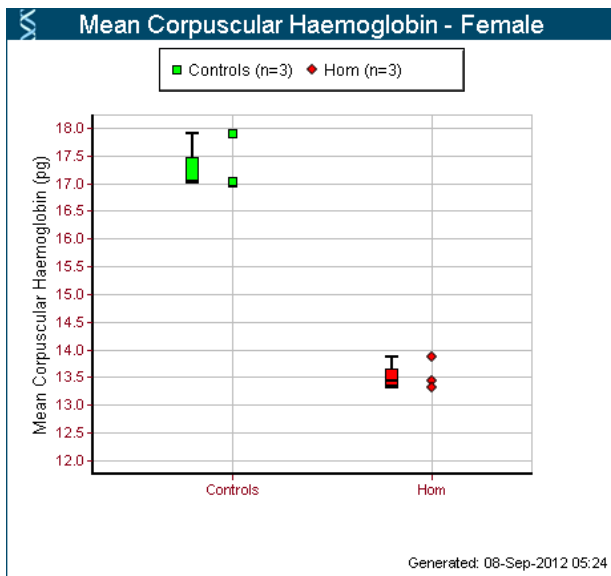
**Males and Females - Decreased Haematocrit [MP:0000208]**



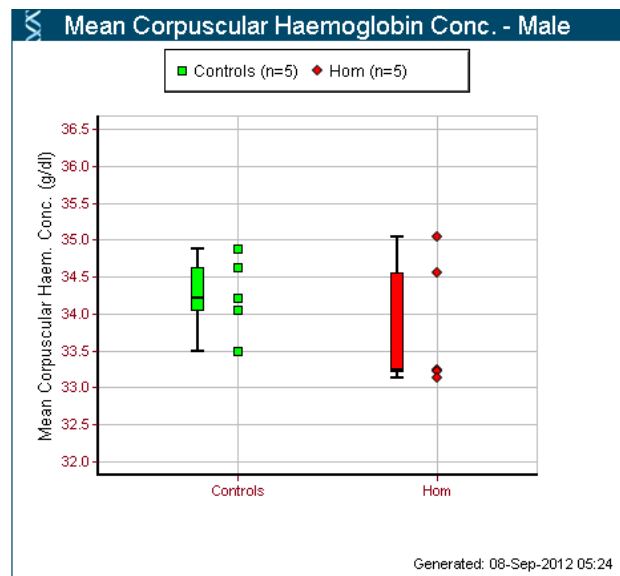
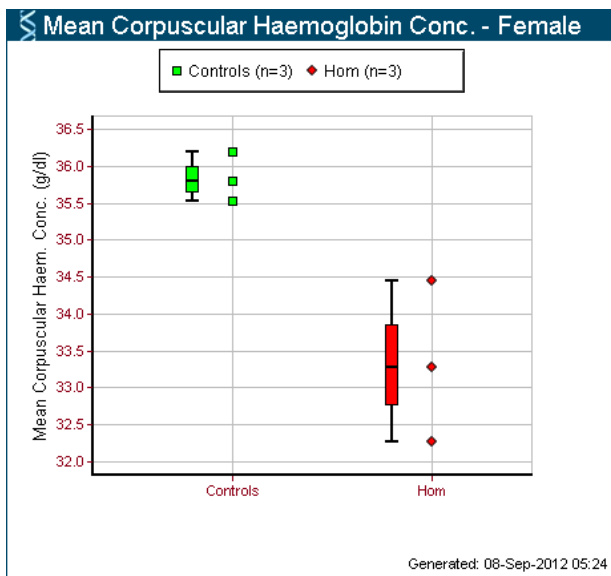
**Males and Females - Decreased Mean Corpuscular Volume [MP:0002591]**



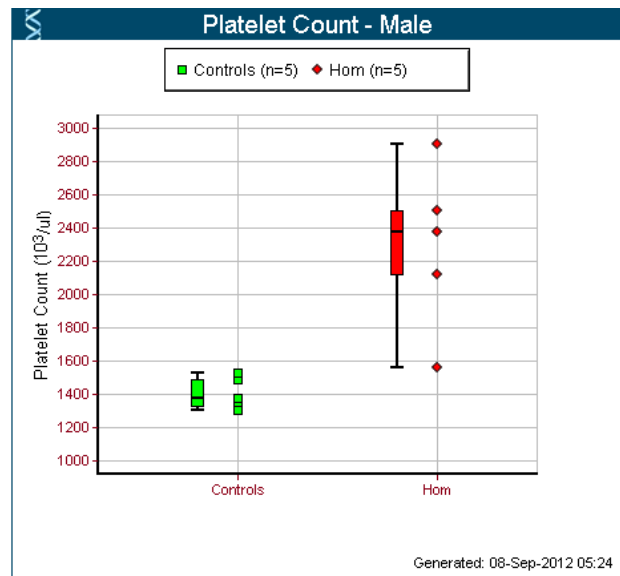
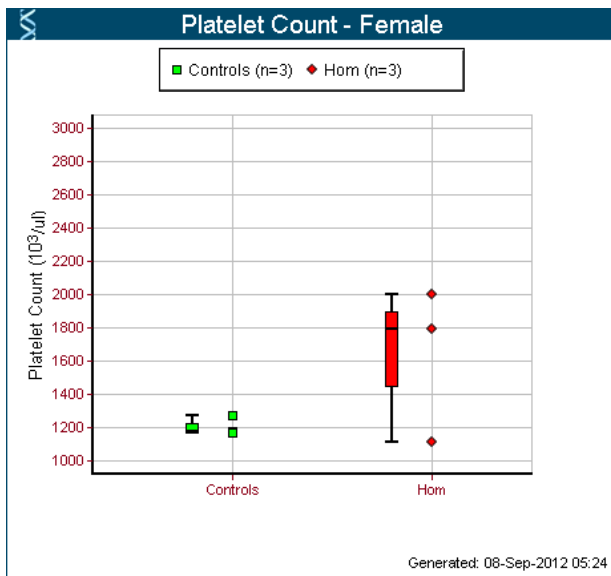
**Males and Females - Increased Red Blood Cell Distribution Width [MP:0010067]**



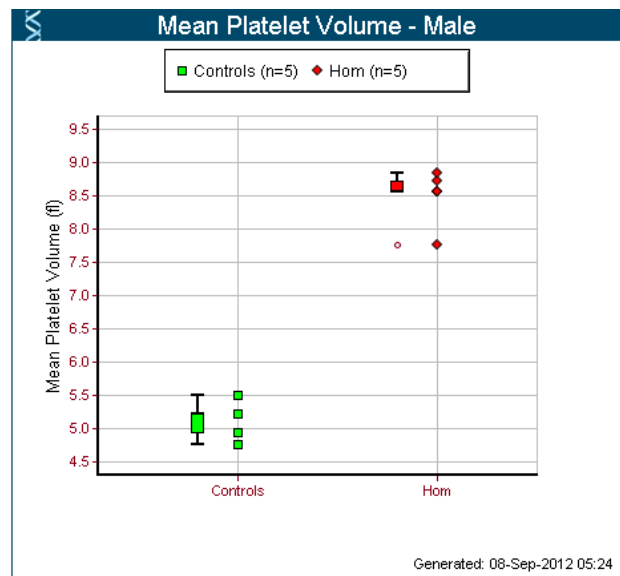
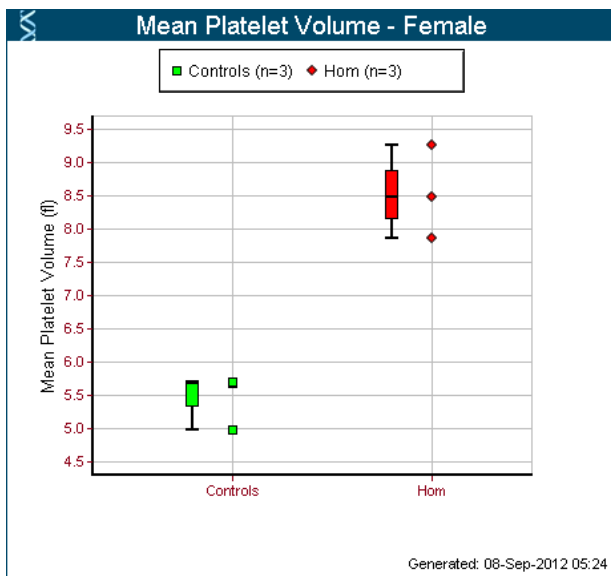
**Males and Females - Decreased Mean Corpuscular Haemoglobin [MP:0005562]**



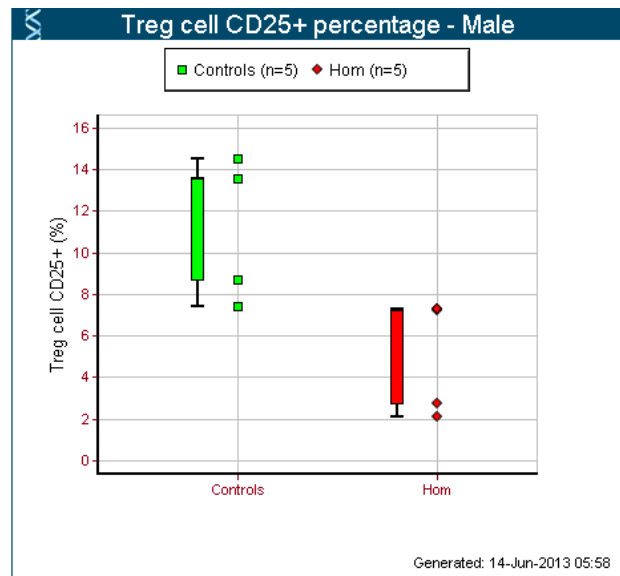
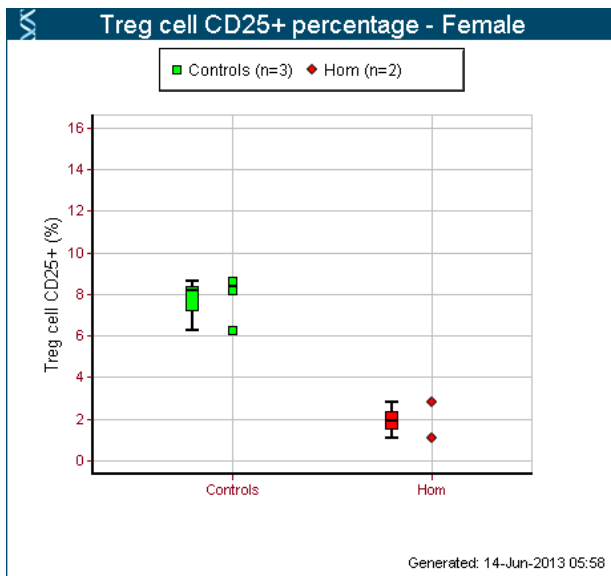
**Females only - Decreased Mean Corpuscular Haemoglobin Concentration [MP:0005642]**



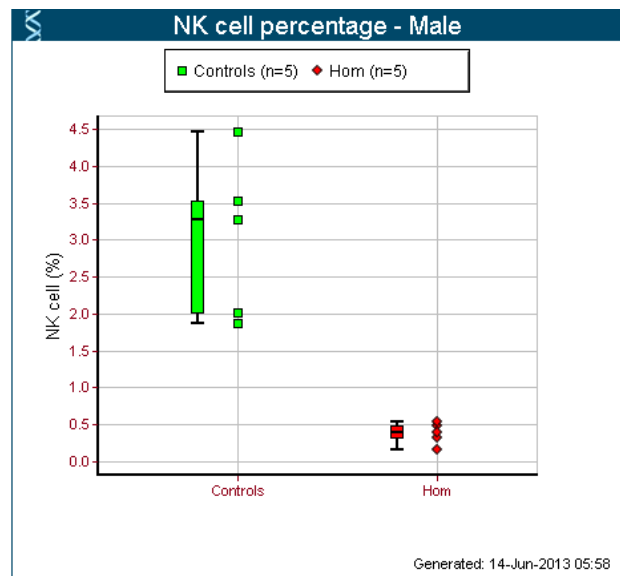
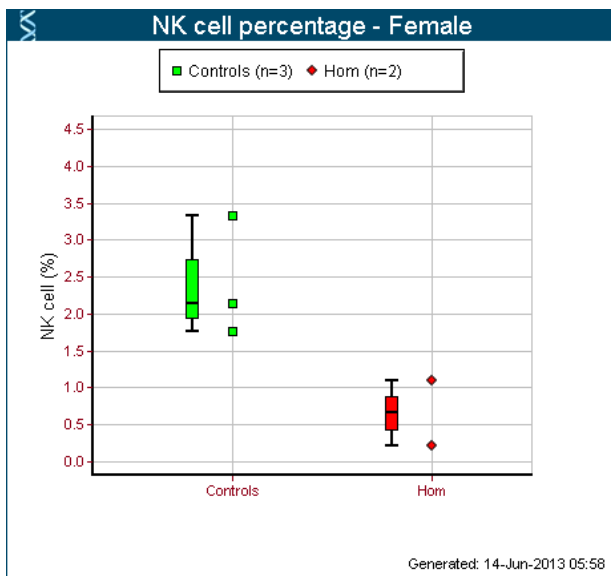
**Males and Females - Increased Platelet Cell Number [MP:0005505]**



**Males and Females - Increased Mean Platelet Volume [MP:0002599]**

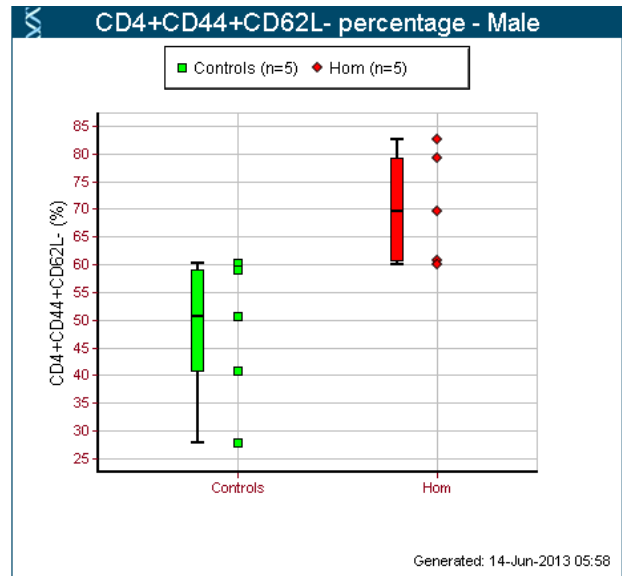
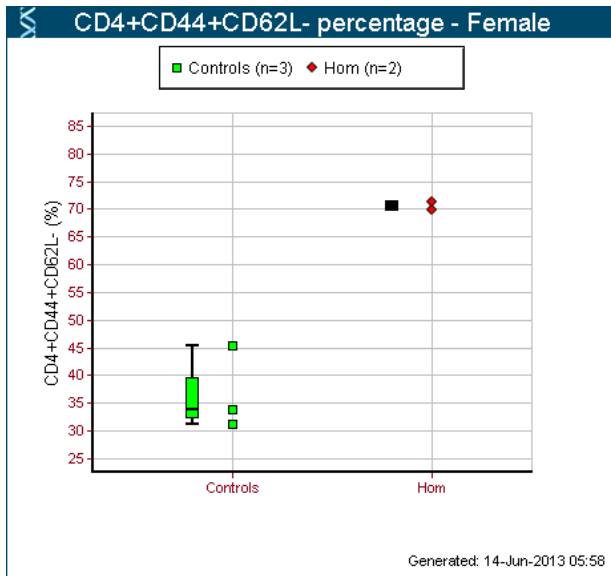


**Males and Females - Decreased Regulatory T Cell Number [MP:0010169]**

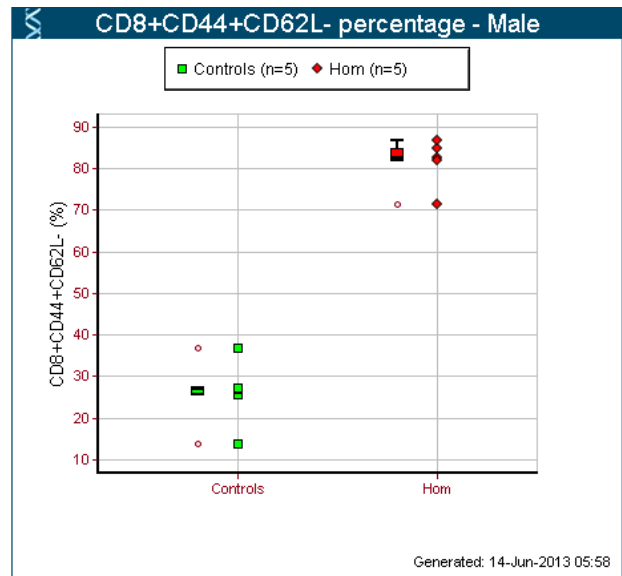
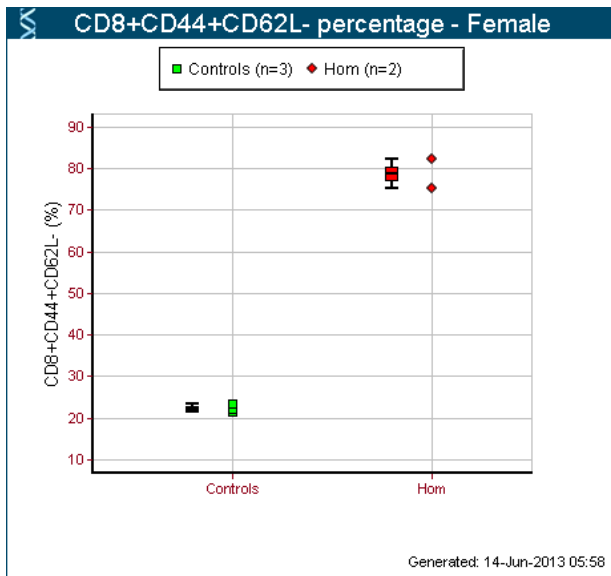


**Males and Females - Decreased NK Cell Number [MP:0008045]**

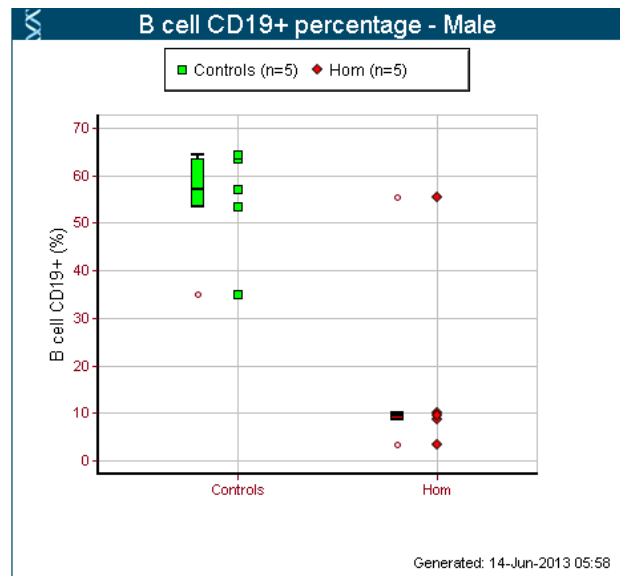
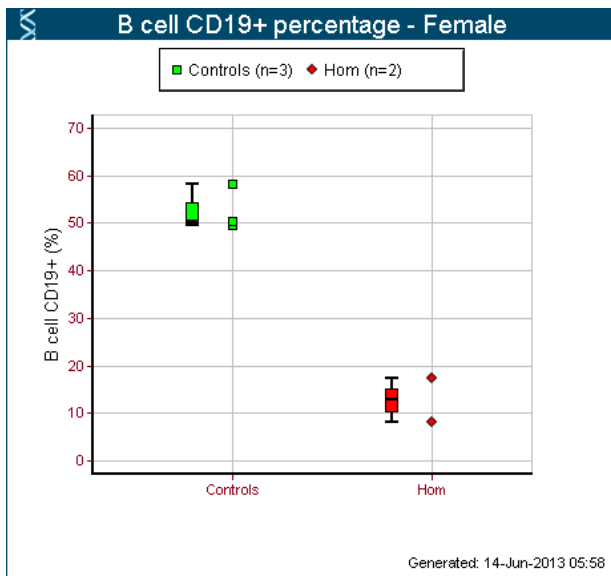




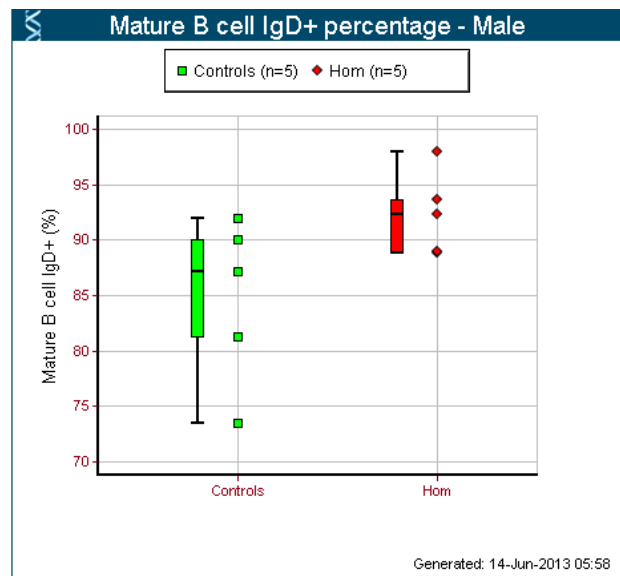
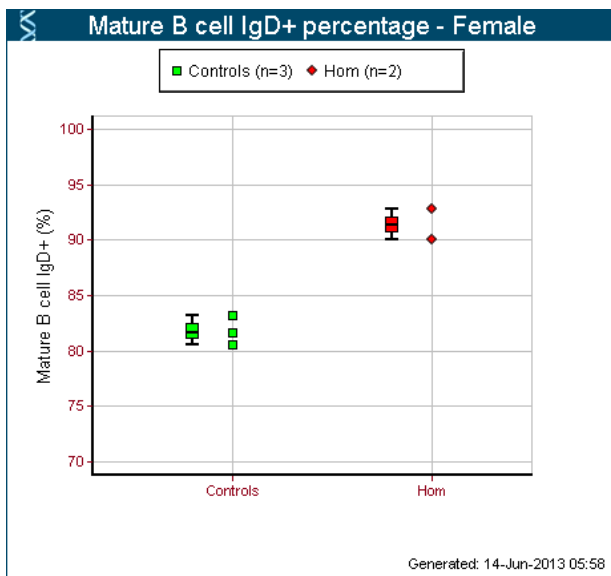
**Males and Females - Increased CD4-positive, Alpha-beta Memory T Cell Number [MP:0010835]**



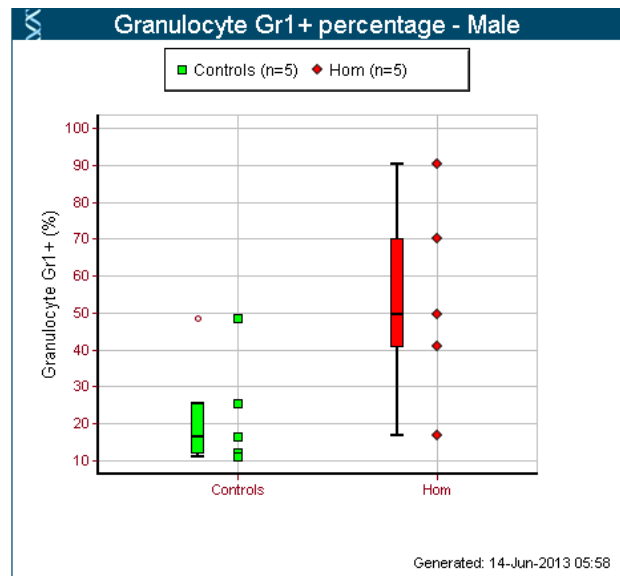
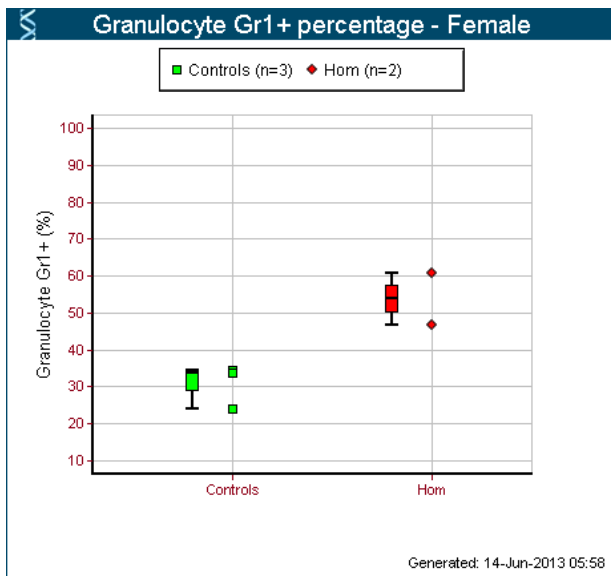
**Males and Females - Increased CD8-positive, Alpha-beta Memory T Cell Number [MP:0010838]**



**Males and Females - Decreased B Cell Number [MP:0005017]**



**Males and Females - Increased mature B cell number [MP:0008210]**



**Males and Females - Increased Granulocyte Number [MP:0000322]**

*Necropsy observations*

Macroscopy observations: spleen enlarged [MP:0000691].