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Reproductive senescence, fertility and reproductive tumour profile in ageing female Han Wistar rats

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ABSTRACT

A study using vehicle administration in 104 female rats investigated reproductive aging in Han Wistar rats as a useful tool to interprete carcinogenicity studies where hormonal patterns are perturbated. From 16 weeks of age oestrous cycles were monitored every 6 weeks to investigate reproductive ageing. A subset of 20 females was used to assess fertility at 21 months of age. The animals were necropsied after 106–107 weeks on study and female reproductive organs, mammary glands and pituitary glands were examined for hyperplasias and/or tumours.

The majority of rats had regular oestrous cycles up to 6 months of age. After this age, there was a rapid decline in the number of rats with regular oestrous cycles and an increase in irregular cycles and cycles in persistent di-oestrus with an occasional pro-oestrus. By the end of the study, the majority of animals were acyclic and the few remaining cyclic animals had irregular cycles. In the fertility assessment, 19/20 animals mated but only four animals became pregnant. These pregnant animals had normal numbers of corpora lutea of pregnancy but had high pre-implantation losses and could not sustain a viable pregnancy.

65 animals (62.5%) showed adenomas and/or pituitary hyperplasia in the pituitary gland at necropsy. The pituitary tumours were likely to be prolactin secreting that give rise to pseudopregnancy and mammary tumours, demonstrated by the fact that 43/65 (66%) of the affected animals had histopathological signs of these conditions. Multiple corpora lutea were found in 61% of all animals at time of termination. Only one uterine tumour was seen in this study probably due to lack of persistent oestrus seen in these animals.

1. Introduction

A study using vehicle administration in 104 female Han Wistar rats, which was of an equivalent duration to a carcinogenicity study, was initiated as a useful tool to interprete such studies where hormonal patterns are perturbated. This study investigated reproductive ageing in female Han Wistar rats since there are little data available in female Han Wistar rats compared with data available for Sprague-Dawley rats.

Fertility was assessed in a subset of females, which required their early removal from the study. The rest of the animals completed the study as planned, except that vehicle dosing was terminated after 78 weeks. The female reproductive organs and pituitary glands were examined for tumours between 106 and 107 weeks.

The detected neoplasms in the female reproductive organs were compared with published data to evaluate the incidences in Han Wistar and Sprague-Dawley rats. Moreover, the presence of these tumours in the pituitary glands was used to establish their effect on hormonal axes and development of tumours in the reproductive organs of aging females.

2. Materials and methods

2.1. Animals

Female Wistar Hannover (substrain RccHan:WIST) rats, supplied by Harlan UK Ltd. (Bicester, UK), were housed in groups of 4 animals in plastic tinted cages. The room was maintained between 19 and 23 °C, 40–70% relative humidity and had a light cycle of 12 h artificial light and 12 h darkness. At the start of the study, the female rats were approximately 6 weeks old. Water from the site drinking water supply was provided in water bottles and pelleted R&M1 (E) SQC diet (Special Diet Services Ltd., England)

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was freely available. The study was conducted in accordance with the relevant UK Animal Welfare Laws at UK AstraZeneca laboratories.

2.2. Vehicle control

The vehicle control used was water containing 0.5% (w/v) hydroxypropyl methylcellulose and 0.1% (w/v) polysorbate 80, which is a standard vehicle used in this laboratory for toxicology studies.

2.3. Experimental procedures

One hundred and four female rats were administered the vehicle by oral gavage once daily for 78 weeks. Surviving animals were then kept on study, undosed, for a further 26 weeks.

All females had vaginal smears taken for stage of oestrous cycle from approximately 16 weeks of age, for 2 weeks during every 6 week period (screened for 2 weeks, 4 week break, then repeat cycle). Screening continued for each individual animal until persistent di-oestrus had been recorded in 2 recording periods.

During Week 79 when the animals were 21 months old, twenty animals were selected for assessment of fertility. Animals that appeared to have ovulated at least once during the last oestrous cycle recording period were selected. The females were paired with male Wistar Hannover rats approximately 10–11 weeks of age. The pairing period for each individual pair of animals was a maximum of 28 nights. Females were screened each morning until evidence of mating (sperm in vaginal smear or vaginal plug *in situ*) was seen (designated Day 0 of gestation) or until the end of the pairing period.

Mated females were killed for scheduled necropsy by administration of halothane between on days 12 and 14 of gestation (Weeks 80–84). Non-mated females were killed for necropsy at the end of the pairing period. The pregnancy status of each female was assessed. Where no implantations were apparent on external uterine examination, the uterus was stained with 2% (v/v) ammonium sulphide solution. For pregnant animals, the number of corpora lutea in each ovary was determined and type and position of each intrauterine implantation was assessed.

Animals killed for welfare reasons and surviving animals were euthanased by administration of halothane. Surviving animals were euthanased between weeks 106 and 107.

Necropsy was performed on mated females, premature decedents and surviving animals. After a thorough gross examination, the pituitary gland, ovaries with oviducts, uterus, cervix, vagina and the mammary gland were sampled and fixed in 4% (v/v) buffered formaldehyde, processed to wax blocks, sectioned at 6 μm, stained with haematoxylin and eosin (HE) and examined by light microscopy.

2.4. Data analysis

Oestrous cycle data was summarised per animal per recording period as follows:

Regular pattern and duration: Cycles lasting 4 or 5 days with regular pattern *e.g.* O,M,D, (D) P; O,D,D, (D), P; O, D, D, D (D);

where

O = Oestrus—cornified epithelial cells only.

M = Met-oestrus—remnants of cornified epithelial cells, leukocytes and nucleated epithelial cells (appear banana shaped when viewed side on).

D = Di-oestrus—predominantly leukocytes but other cells may be present.

P = Pro-oestrus—clumps of nucleated epithelial cells only.

Cycles with minor abnormalities: cycles generally of regular pattern and duration except for single occurrences of cycle of 6 days; cycle of 3 days; cycle with pro-oestrus before di-oestrus or cycle with two days of oestrus; met-oestrus or pro-oestrus (extended O, M or P).

Cycles with significant abnormalities: cycles with multiple minor abnormalities ±cycles >6 days and/or cycles <3 days.

Cycles with persistent di-oestrus and occasional pro-oestrus or extended pro-oestrus but without oestrus or met-oestrus.

Acyclic: no evidence of cycling and in di-oestrus continuously.

Tumours associated with the pituitary gland, the female reproduction tract and the mammary gland were diagnosed/characterized according to the goReni nomenclature. The incidence of tumours was plotted against 32 carcinogenicity studies using either Sprague-Dawley or Han Wistar rats (Gikinis and Clifford, 2011; Gikinis and Clifford, 2013; Table 2).

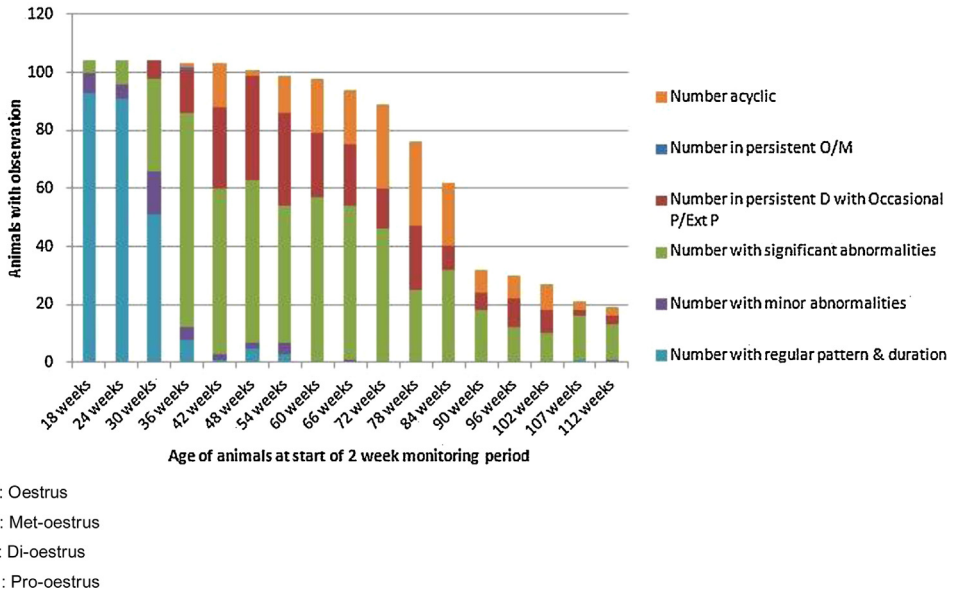

Fig. 1. Oestrous cycle classification.