# Causes and incidence of failure to litter in control rats from extended one-generation reproductive toxicity studies

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## Introduction and aim

Studies on reproductive toxicity are necessary under the European Union program on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). The Extended One-Generation Reproductive Toxicity Study (OECD 2018, Test No. 443) tests for effects of pre- and post-natal chemical exposure. Dosing covers mating, fertility, pregnancy, lactation and development of offspring up to 13 weeks of age.

Failure to litter (FL) is defined as observed mating with no offspring produced.

Diagnostic criteria and reporting are not well defined in the context of multi-generation developmental and reproductive toxicology (DART) studies. The study pathologist must interpret pathology findings and treatment relatedness of FL (Figure 1).

We retrospectively analysed the incidence and causes of FL in control rats to aid interpretation.

## Materials and methods

Forty-two OECD 443 studies conducted between 2018 and 2022 at Labcorp Early Development Laboratories Limited, Huntingdon/Eye, UK, were examined. They included 30 oral gavage and 12 dietary studies, with Sprague Dawley® and RccHan®:WIST rats in 30 and 12 studies, respectively. Clinical, macroscopic and microscopic findings, estrous cycle, sperm assessment and reproductive performance data were analysed for each control pair that failed to litter.

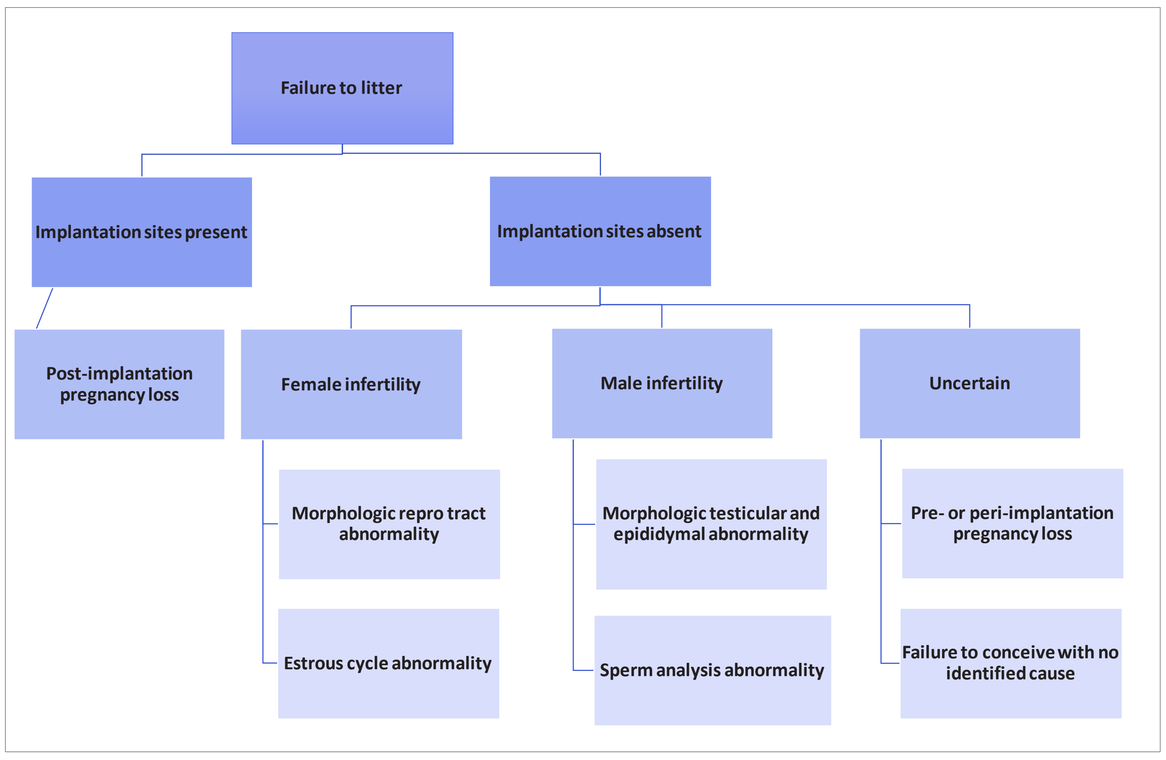


**Figure 1. Interpreting failure to litter.**

## Results

- FL animals were observed in 24/42 (57.1%) studies, showing no association with dosing procedure.
- The total incidence of FL was 42/1030 (4.1%), with a higher incidence in the RccHan®:WIST [16/299 (5.3%)] than Sprague Dawley® [26/731 (3.6%)] strain.
- The causes and incidence of FL are depicted in Figure 2.
- Male infertility accounted for 13/42 (31%) of cases and was associated with tubular degeneration/atrophy of the testes [11/42 (26.2%)] and with low progressive sperm motility [2/42 (4.8%)].
- Post-implantation loss of embryos/foetuses occurred in 5/42 (12%) females. Pyo/hydrometra was seen in 3/42 (7.1%) cases.
- Findings with uncertain significance included pseudopregnancy [2/42], females acyclic prior to mating [2/42], luteal cysts [1/42] and epididymal adipose necrosis [1/42].
- There were no findings that accounted for FL in 15/42 (35.7%) cases.

## Discussion and conclusions

Tubular degeneration/atrophy (Figure 3) accounted for most of FL in control pairs and may be seen as a low incidence, background finding in male rodents.

Severely decreased progressive sperm motility was considered the most likely cause of FL in two pairs and was not associated with any histologic changes in the male reproductive tissues.

Several cases of FL were due to post-implantation loss which is likely related to maternal factors. The cases presented under this term in this study did not show any altered reproductive parameter or morphology. Post-implantation loss is identified by the presence of regressing implantation sites in the uterine horns (Figure 4).

Findings including females acyclic prior to mating, pseudopregnancy, luteal cysts and focal moderate adipose tissue necrosis of the epididymis were attributed uncertain significance because they are occasionally seen in successfully conceiving animals as well as FL pairs.

Pseudopregnancy in rodents, a phase when progesterone is secreted in excess of estrogen with a concomitant prolactin surge, is histologically characterised by corpora lutea hypertrophy in the ovary, increased mucification of the mucosa of the cervix and vagina, atrophy of the uterus with a folded endometrium and lobuloalveolar hyperplasia of the mammary gland (Figure 5). In the rat pseudopregnancy can occur due to reproductive senescence (repetitive pseudopregnancy) and after sterile mating/vaginocervical stimulation.

The cause of FL could not be identified in half of the cases, which did not present with any observations in the clinical or reproductive parameters.



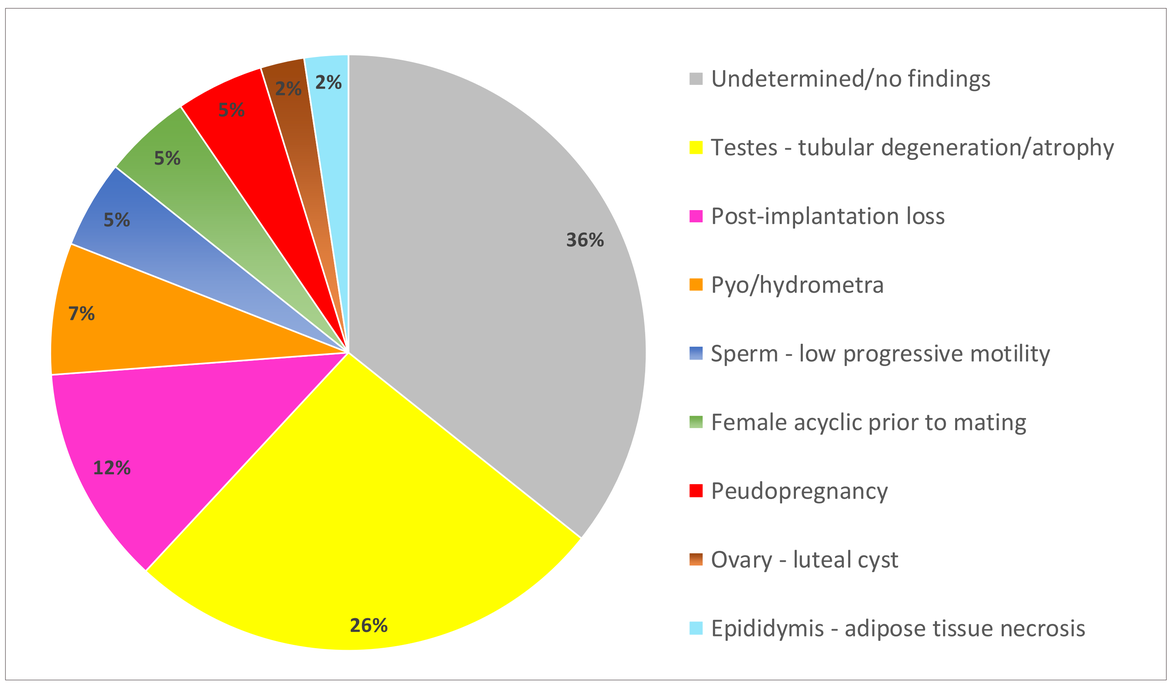


**Figure 2. Causes of failure to litter.**

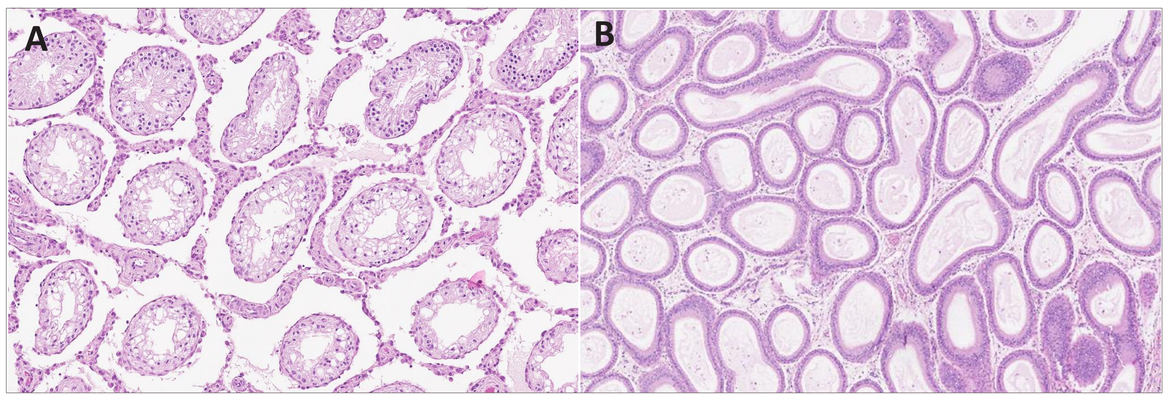


**Figure 3. Control male SD rat on an OECD 443 study. This pair failed to litter due to male infertility.** In the testes, severe diffuse degeneration/atrophy of the seminiferous tubules showing no spermatogenic cells was present **(A)**, and the epididymal convoluted tubule was devoid of spermatozoa **(B)**.

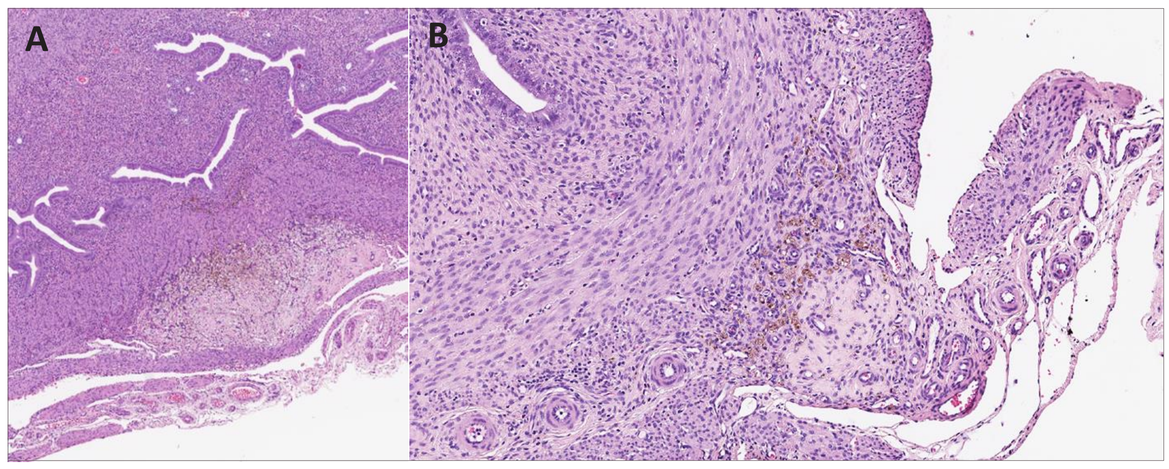


**Figure 4. Two control female SD rats on an OECD 443 study. These females failed to litter due to post-implantation loss.** Longitudinal section of the uterine horn: an implantation site consisting of a nodular infiltrate of brown pigment-laden cells admixed with hyaline extracellular material can be seen on the mesometrial aspect of the uterine wall between circular and longitudinal layers of the myometrium **(A)**. Transversal section of the uterine horn: an implantation site in advanced stage of regression consisting of fewer pigment-laden cells admixed with hyaline extracellular material on the mesometrial aspect of the uterine wall **(B)**.

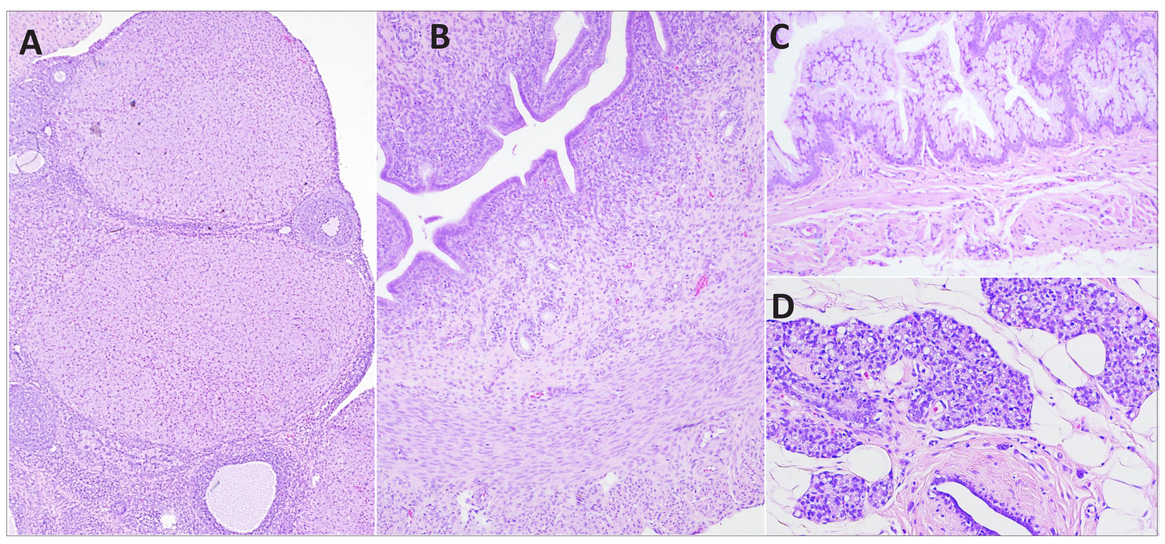


**Figure 5. Control female SD rat on an OECD 443 study. This female failed to litter and exhibited pseudopregnancy.** The female showed enlarged eosinophilic corpora lutea (hypertrophy) of the ovary **(A)**, atrophy of the uterus with a folded endometrium **(B)**, marked, diffuse mucification of the epithelium of the vaginal mucosa **(C)**, and slight lobuloalveolar hyperplasia of the mammary gland **(D)**.

## References

OECD (2018), Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

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