

CONFOUNDING OF REPRODUCTION ENDPOINTS BY UNDERNUTRITION: A CASE-STUDY

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Conflict of Interest: Penman Consulting provide consultancy services to a Consortium of the manufacturers of the substances used as examples in this poster, and this article reflects the positions developed in the course of that work.

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Introduction

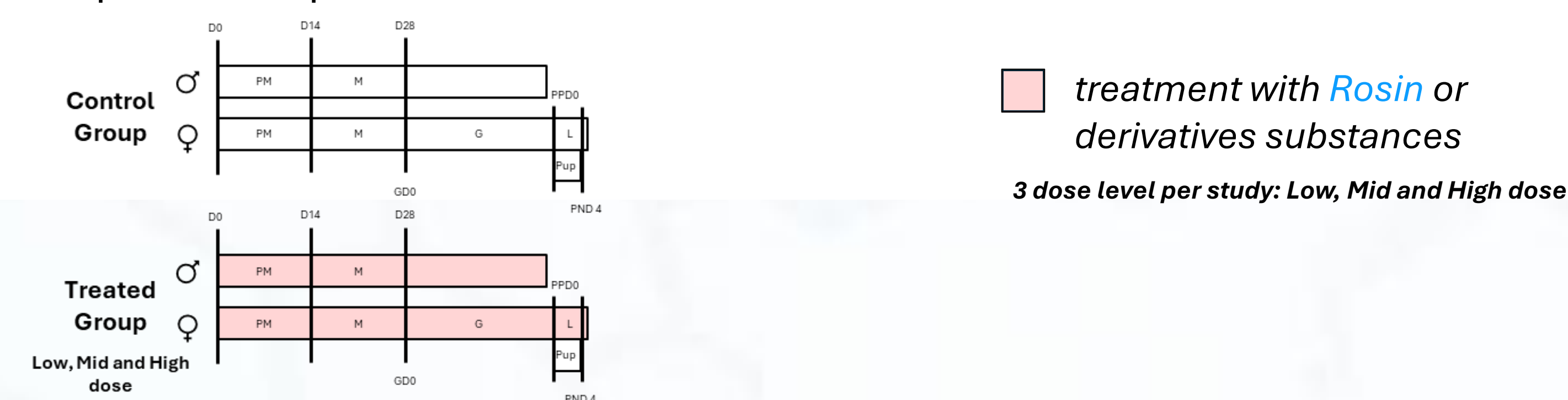
It is well-understood that undernutrition lowers fertility of female rodents and retards the development of their offspring. However, the extent to which this phenomenon confounds the interpretation of reproductive findings in regulatory toxicology studies remains controversial. It is believed that a misinterpretation of regulatory study data and so an over-use of precautionary principle could have dramatic consequences for the industry, especially in the hazard-based assessment approach currently imposed to the chemical industry.

Here, we present a case study (taken from an ongoing regulatory assessment under EU REACH) exemplifying this problem and providing some answers.

Methods

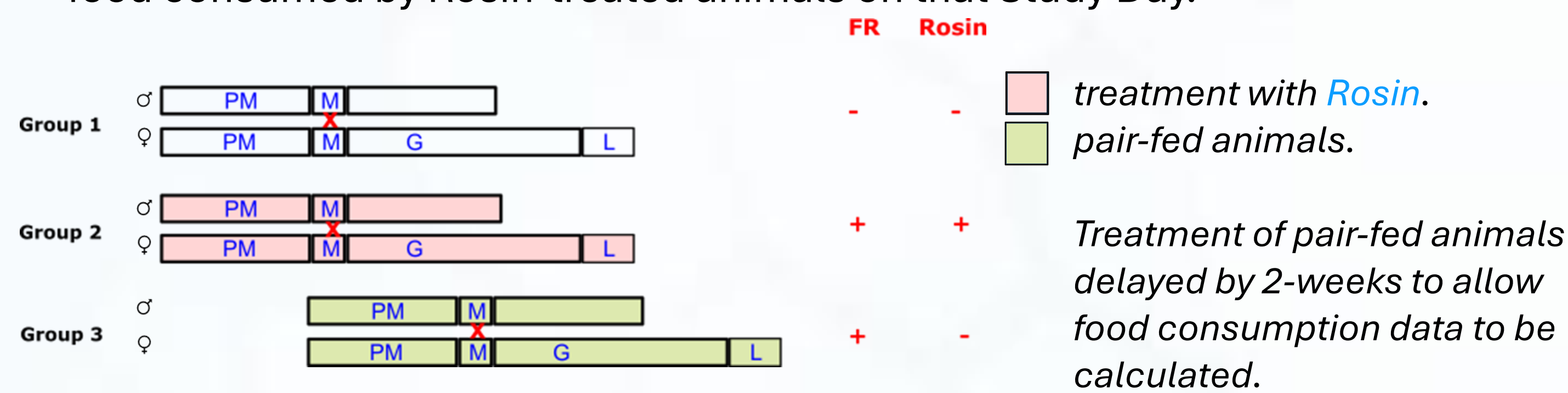
STUDIES A - Reproduction / Developmental Toxicity Screening Test

- GLP-compliant OECD 422 test guideline studies conducted for a set of 15 related **Rosin** (CASRN 8050-09-7) and derivatives substances.
- Han-Wistar rats administered **test substance in the diet**.
- Extensive Multiple Linear Regression analyses were conducted across these regulatory studies to reveal relationships between undernutrition and reproductive parameters.



STUDY B – Pair fed Study

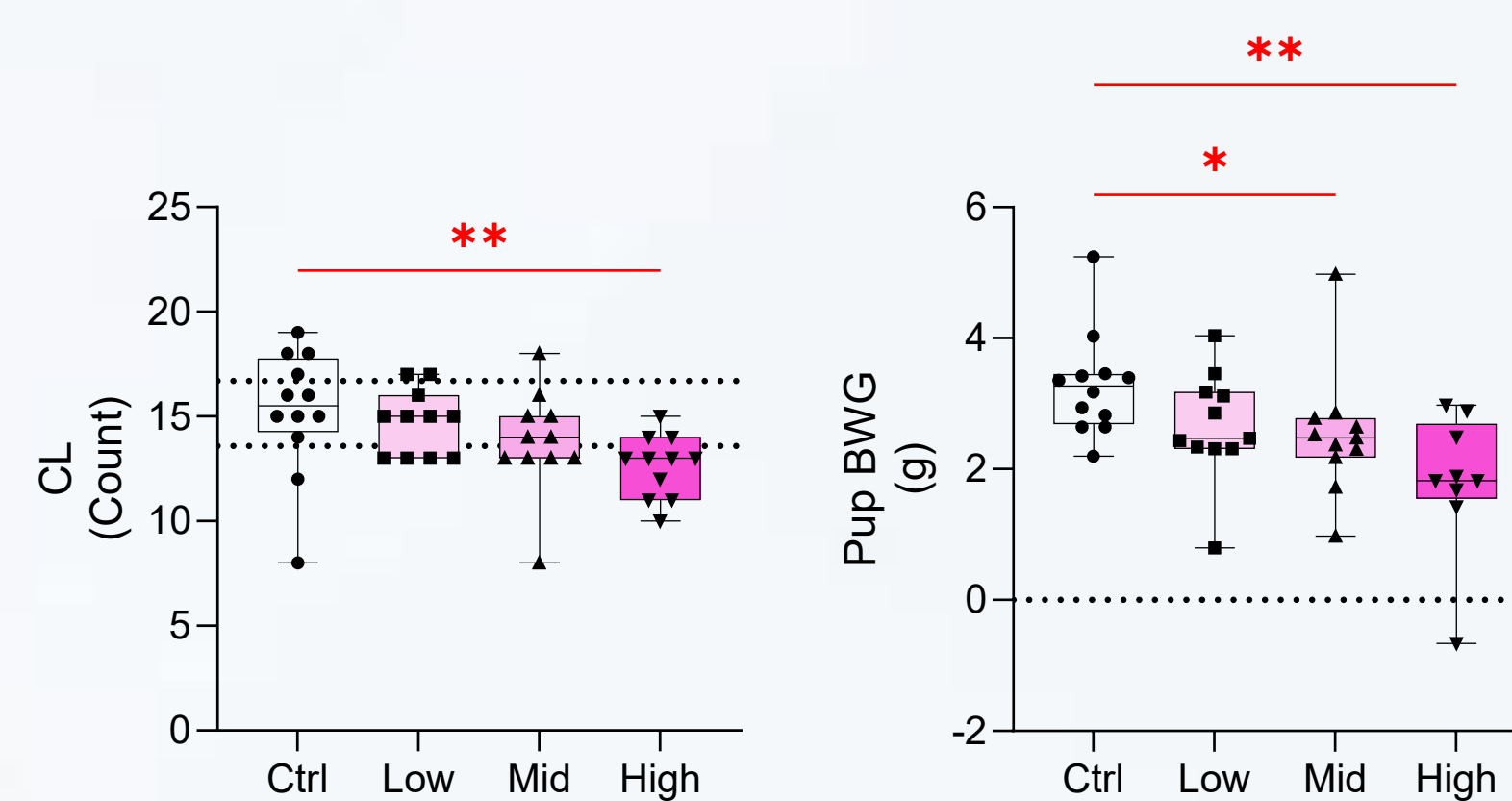
- To investigate **whether undernutrition caused** the fertility and developmental effects seen with the substances, a bespoke pair-feeding OECD 421 study (Han-Wistar) was conducted with **Rosin**.
- Pair-fed animals received daily amounts of food equal to the mean amount of food consumed by Rosin-treated animals on that Study Day.



Results (Studies A)– Effects on Ovulation and Offspring Growth

In **Rosin** OECD 422:

- Mild reduction in mean **Corpora Lutea (CL)** counts with several substances.
- Retarded Pup Growth (LD1 to LD4) with several substances



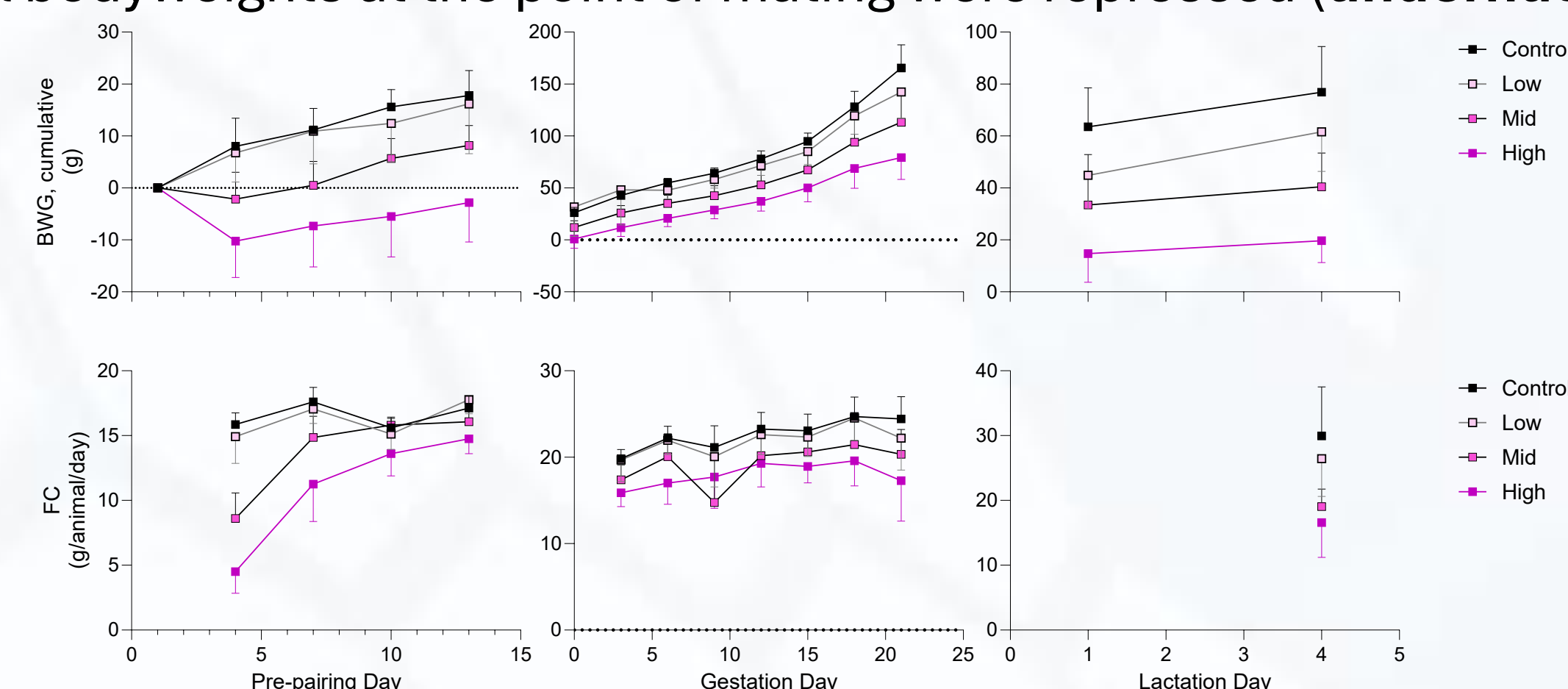
Figures shows data from female animals exposed to dietary **Rosin**.

*: $p < 0.05$; **: $p < 0.01$

Results (Studies A)– Undernutrition due to Reduced Food Intake

In **Rosin** OECD 422:

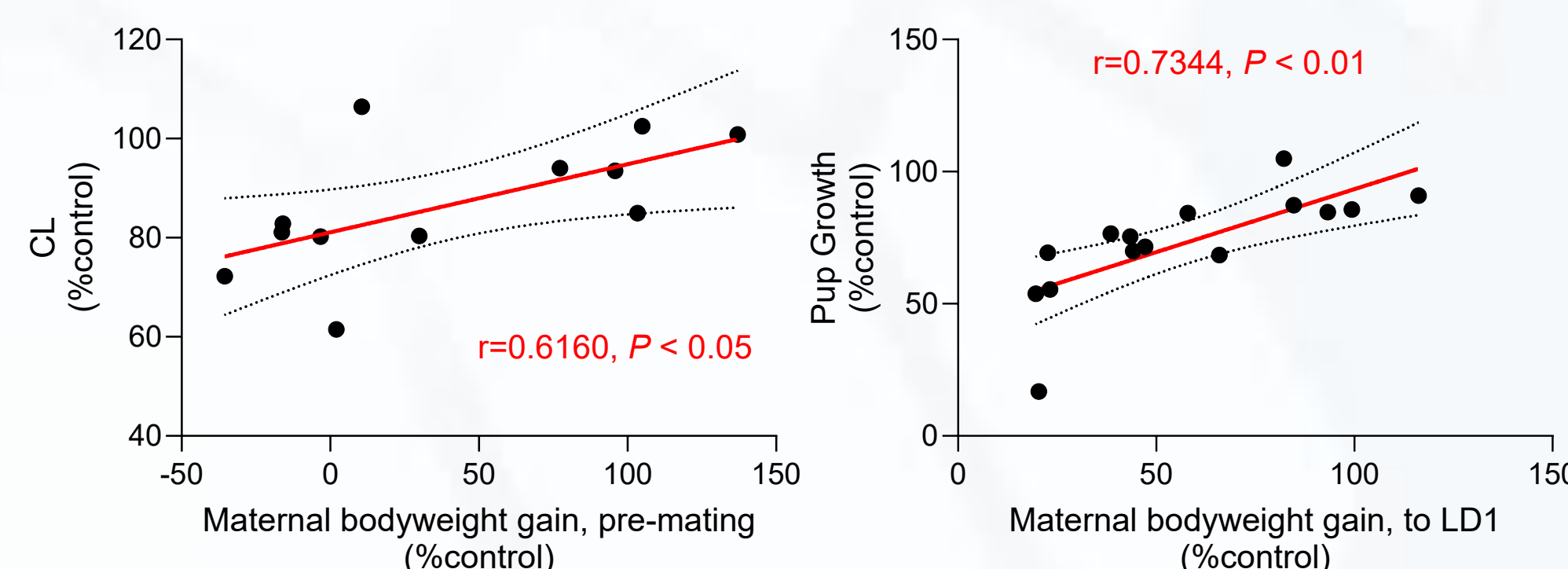
- Immediately upon treatment start, self-imposed transient reduction in **Food Consumption (FC)** that never fully recovers leading to lower **Bodyweight Gain (BWG)**
- Animal bodyweights at the point of mating were repressed (**undernutrition**)



Figures shows data from female animals exposed to dietary **Rosin**.

Results (Studies A)– Correlation of Effects with Undernutrition

Across all **Rosin** and derivatives substances OECD 422 studies:

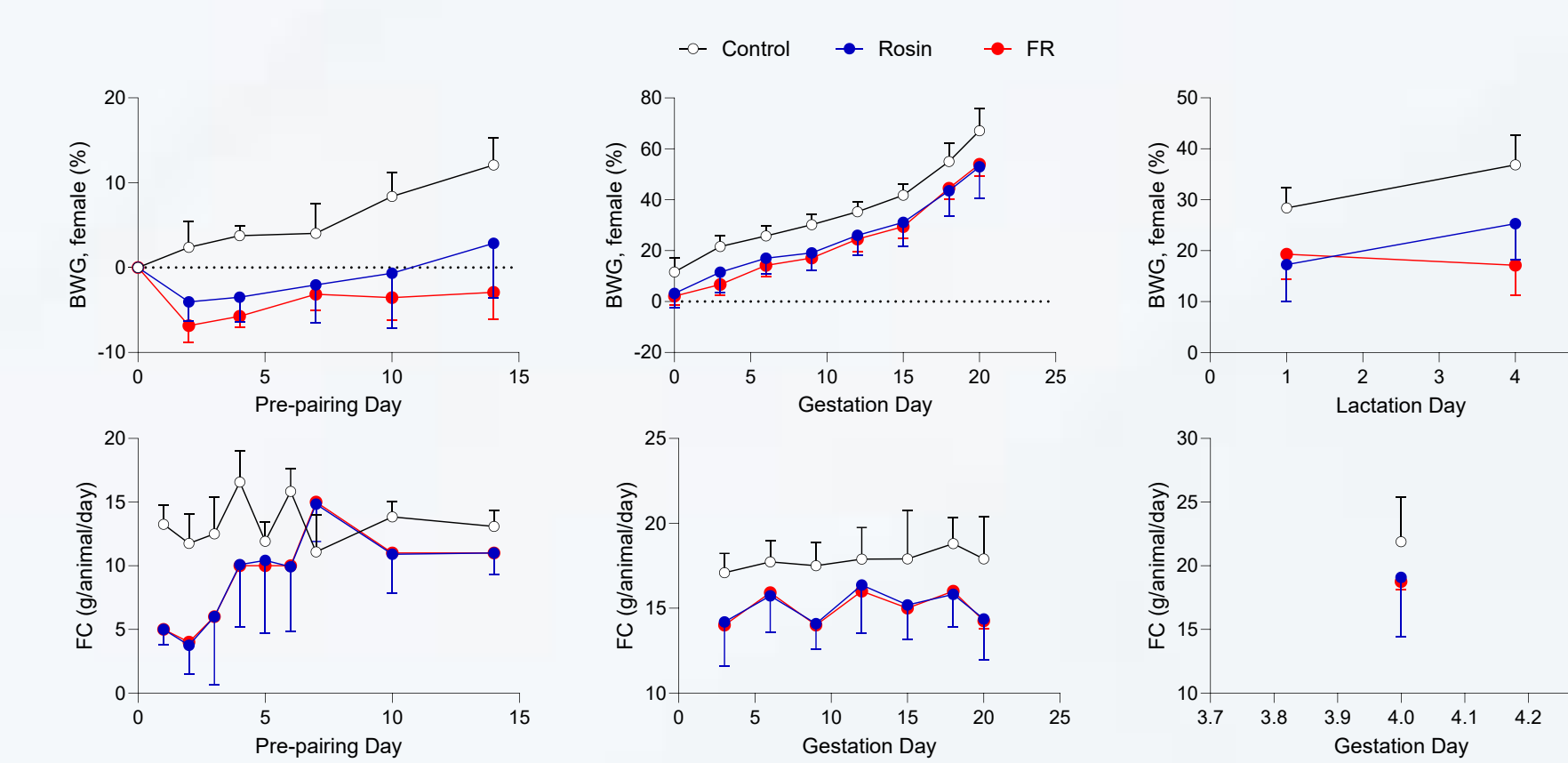


Figures shows data from female animals exposed to dietary **Rosin** or derivatives or their offspring

For each parameter, the mean value in the High-dose group was expressed as a percentage of mean value in concurrent Control group

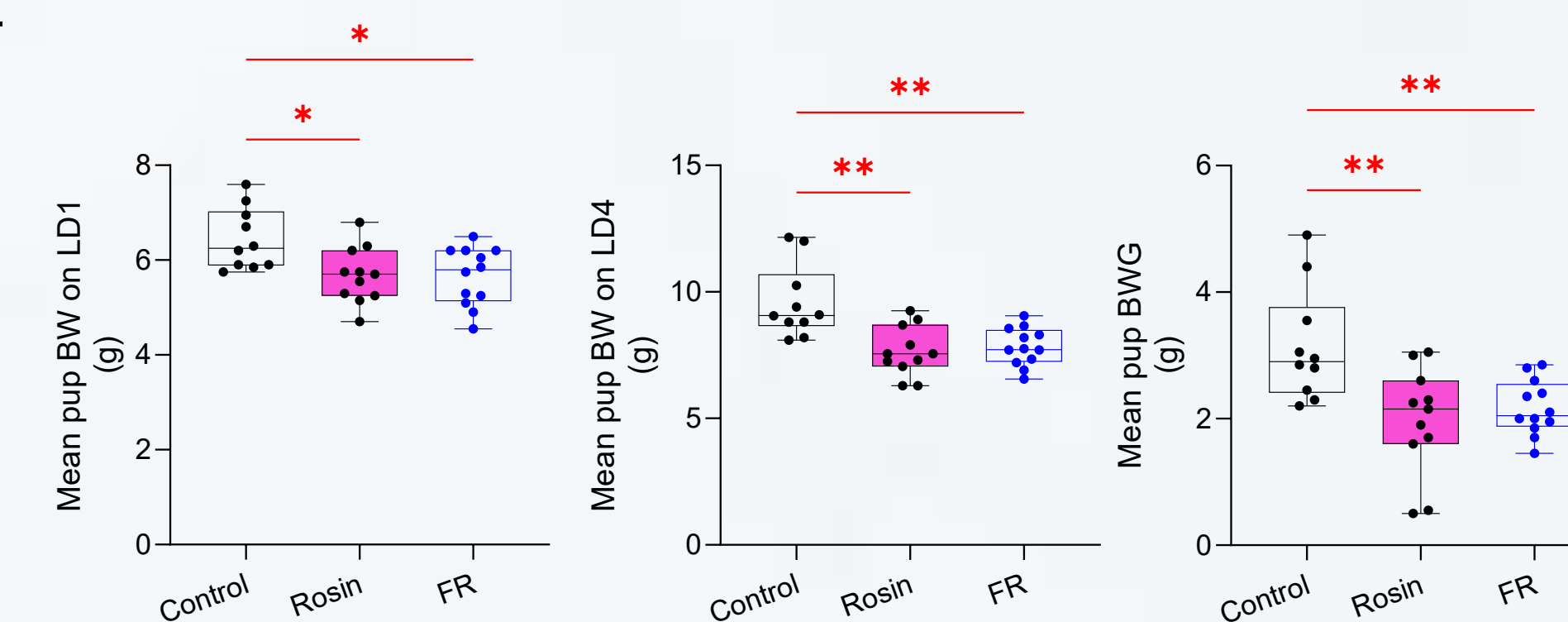
Results (Study B: pair-fed study)– FC and BWG Trajectories

- ‘Engineered’ restricted FC trajectory in **pair-fed (FR)** group is a good match to self-imposed restricted FC trajectory in female rats exposed to dietary **Rosin**
- Similar for cumulative BWG trajectory



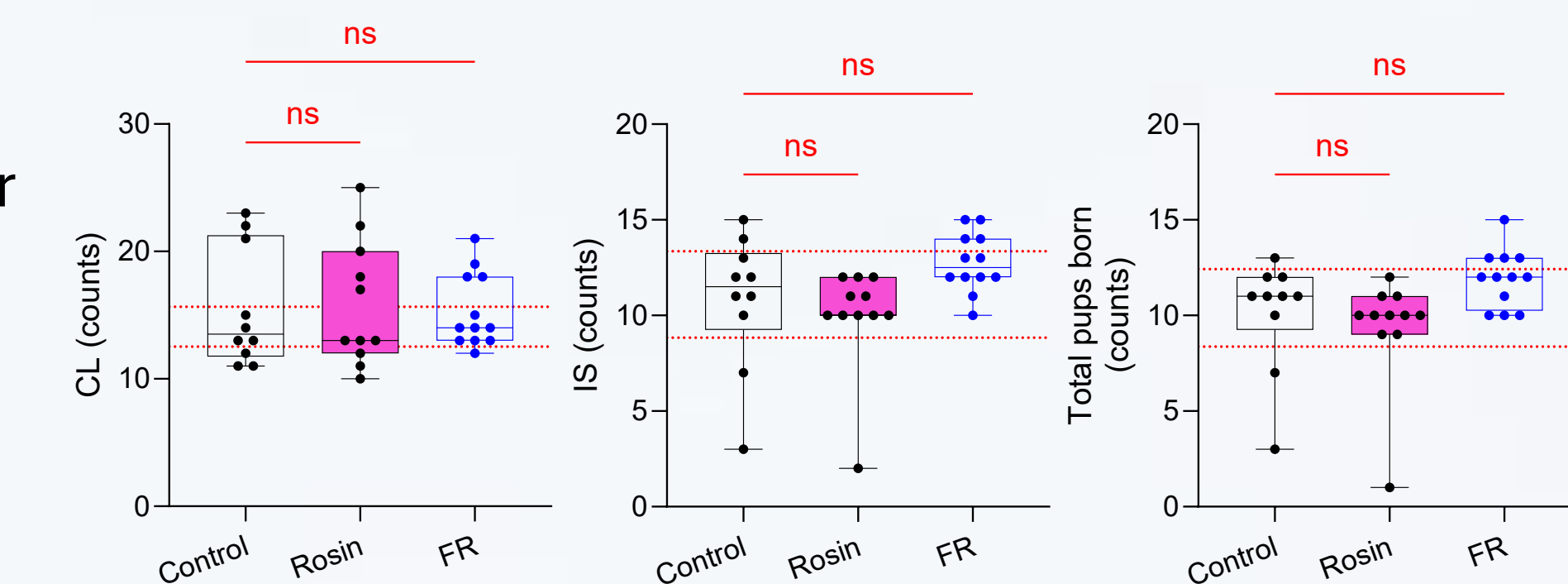
Results (Study B)– Undernutrition Causes Retarded Offspring Growth

- Litter mean BWG feed-restricted females' offspring biologically and statistically indistinguishable from the offspring of **Rosin**-treated females.
- Data support hypothesis that retarded offspring growth is caused solely by undernutrition of dams



Results (Study B)– Rosin Fails to Reduce CL counts

- Study could not address cause of lower ovulation as **Rosin** failed to lower fertility under conditions required for pair-feeding.



ns: not significant

Dotted lines: Historical control data

Discussion & Conclusion

- The impaired development of rat offspring seen in dams exposed to dietary **Rosin** and derivatives substances is a **non-specific secondary consequence of maternal undernutrition**.
- Further work is required to establish whether the fertility effects of these substances too are caused by undernutrition.
- More generally, the data presented here may be of considerable general interest because they clearly demonstrate that **undernourishment at levels not uncommonly seen in toxicology studies affect reproduction in a common regulatory study design**.