

Development and validation of a method for precise dating of female puberty in laboratory rodents: The puberty ovarian maturation score (Pub-Score)

Francisco Gaytan^{1,2,3}, Concepción Morales⁴, Silvia Leon^{1,2,3}, Violeta Heras^{1,2,3}, Alexia Barroso^{1,2,3}, Maria S. Avendaño^{1,2,3}, Maria J. Vazquez^{1,2,3}, Juan M. Castellano^{1,2,3}, Juan Roa^{1,2,3} and Manuel Tena-Sempere^{1,2,3,5}

¹Department of Cell Biology, Physiology and Immunology, University of Córdoba; ²Instituto Maimónides de Investigación Biomédica de Córdoba & Hospital Universitario Reina Sofía (IMIBIC/ HURS); ³CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III; and ⁴Department of Pathology, University of Córdoba, 14004 Córdoba, Spain; and ⁵FiDiPro Program, Department of Physiology, University of Turku, FIN-20520 Turku, Finland

Short Title: Dating puberty in rodents with Pub-Score

Key Words: Puberty, rat, mouse, ovary, cycle, follicle, corpus luteum, dating method

Corresponding authors: Francisco Gaytan (bc1galuf@uco.es)

Manuel Tena-Sempere (fi1tesem@uco.es)
Department of Cell Biology, Physiology & Immunology
Faculty of Medicine, University of Córdoba
Avda. Menéndez Pidal s/n. 14004 Córdoba, SPAIN
FAX: +34-957-218288

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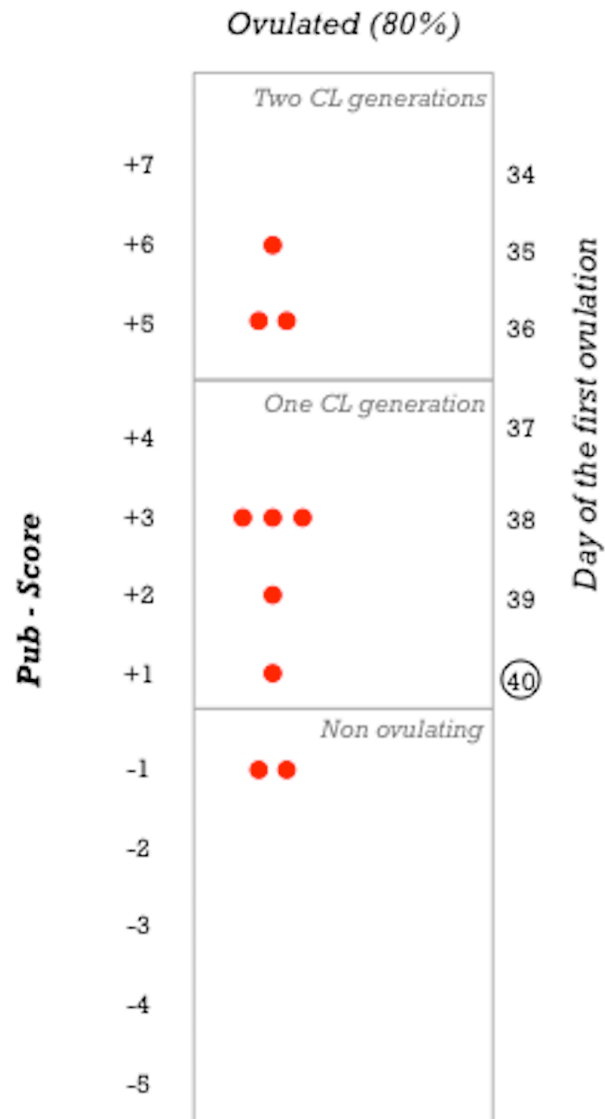
Author Contributions: F.G. and C.M. conducted the histological examinations and primarily defined the scoring method; S.L., V.H., A.B., M.S.A., M.J.V. & J.M.C. participated in the collection of samples and pubertal indices within the course of different experimental studies and discussed the data; J.R. participated in sample collection, discussed the data and prepared the figures; M.T.-S. evaluated and discussed all the data. F.G. & M.T.-S. defined the final scoring method and jointly wrote the paper. All authors revised the manuscript.

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Supplemental Figures

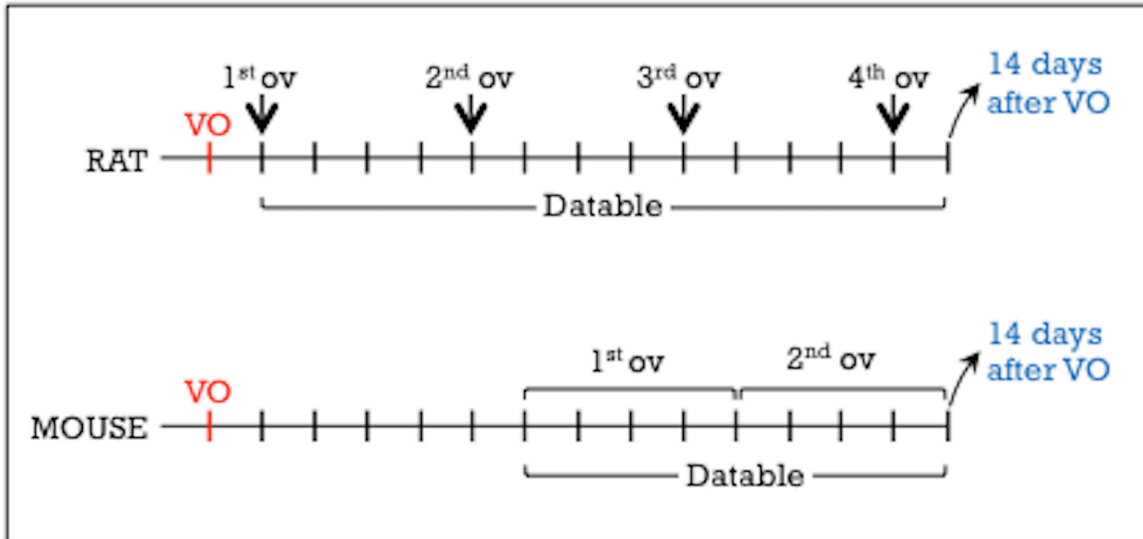
35 Supplemental Figure S1: Scatterplot presentation of Pub-score data

An illustrative example of Pub-score dating of female puberty is presented. A group of 10 mice, sampled for histological analysis of the ovaries at PND-40, were subjected to dating following the Pub-score method.



40 **Supplemental Figure S2:** *Time window for Pub-score dating of puberty in rodents*

Scheme depicting the time-window for accurate retrospective timing of female puberty, hallmarked by the occurrence of first ovulation, since the age of VO (as external sign of puberty) in rats and mice (*upper and lower panels, respectively*).



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