



HAL
open science

The mammalian ovary: Concerns about the evaluation of prenatal environmental exposures

L.L. Lecante, P. Lelandais, Séverine Mazaud-Guittot, P.A. Fowler

► To cite this version:

L.L. Lecante, P. Lelandais, Séverine Mazaud-Guittot, P.A. Fowler. The mammalian ovary: Concerns about the evaluation of prenatal environmental exposures. *Current Opinion in Endocrine and Metabolic Research*, 2021, 18, pp.171-177. 10.1016/j.coemr.2021.03.012 . hal-03268776

HAL Id: hal-03268776

<https://hal.science/hal-03268776v1>

Submitted on 30 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **The mammalian ovary: concerns about evaluation of prenatal environmental**
2 **exposures.**

3

4 Laetitia L. Lecante ¹, Pauline Lelandais ², Séverine Mazaud-Guittot ², Paul A. Fowler ^{1*}

5

6 ¹ Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD,
7 UK.

8 ² Inserm, EHESP, Irset (Institut de Recherche en Santé, Environnement et Travail) UMR_S
9 1085, University Rennes, 35000, Rennes, France.

10

11 * corresponding author: p.a.fowler@abdn.ac.uk

12

13 **Abstract**

14

15 The number and volume of processed natural or synthetic chemical toxicants introduced on
16 the market has soared over the past decades. Possible human environmental exposures to
17 potentially adverse compounds have, therefore, increased, as has awareness regarding their
18 potential hazard for reproduction. Concomitantly, numbers of couples seeking assisted
19 reproduction has climbed sharply. Toxicant risk assessment represents a concern at both
20 individual and population and socio-economic levels. Here, we review current methods used
21 to assess impacts of prenatal environmental exposures on mammalian ovary development
22 and female reproductive function. We highlight technical challenges that need to be
23 overcome in a regulatory context and the necessity for the development of guidelines and
24 policies to better characterise potentially deleterious substances for the female reproductive
25 function.

26

27 **Key words**

28 Endocrine disrupting compounds, ovary, development, fetus, adverse outcome

29

30 **Abbreviations**

31 AOP: Adverse outcome pathway; DOHaD: Developmental Origins of Health and Disease;
32 EDC: Endocrine disrupting compound; MIE: molecular initiating event; ODS: Ovarian
33 Dysgenesis Syndrome; POI: Premature Ovarian insufficiency; PCOS: Polycystic Ovarian
34 Syndrome; QSAR: Quantitative structure-activity relationship; REACH: Registration,
35 Evaluation, Authorisation and restriction of Chemicals; TDS: Testicular Dysgenesis
36 Syndrome. PCW: post-conceptional weeks; dpc: days post-conception, dpp: days post-
37 partum.

38

39 **Introduction**

40

41 Since the 19th century, there have been significant increases in production of environmental
42 pollutants and toxicants and, simultaneously, escalation in numbers of confirmed and
43 potential toxicants to which we are exposed [1]. Nevertheless, concerns about environmental
44 pollutants have been slow to emerge although that has improved over the past 40 years.

45 This raised awareness has led authorities to direct more resource to research on risk
46 assessment of exposure to such molecules, with endocrine disrupting compounds (EDCs) of
47 particular concern [2]. The European Commission adopted a common law concerning the
48 Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) in 2006 [3].

49 This is even more crucial because, in addition to synthetic molecules, some naturally
50 occurring substances are also potentially harmful for human health. Evidence that some sub-
51 populations are more vulnerable to such exposures, especially during pregnancy, is
52 supported by growing numbers of studies on the fetal exposome [4], with short- and long-
53 lasting impacts on organs. Long-term effects of dysregulated development on adult function
54 were first hypothesised for the association of low birth weight and chronic noncommunicable
55 diseases in adulthood [5,6]. This gave rise to the concept of developmental origins of health
56 and disease (DOHaD or “Barker Hypothesis”) linking *in utero* exposures during critical
57 developmental “windows” with post-natal disease. Increasing frequencies of male
58 reproductive disorders over the past 70 years are considered symptomatic of a common
59 underlying entity, testicular dysgenesis syndrome (TDS) [7]. Although less clear, partly due
60 to the delay between *in utero* environmental exposures and observed adverse outcomes, a
61 similar trend is proposed regarding female reproductive impairments. Symptoms like
62 premature ovarian failure, delayed menarche and Mayer-Rokitansky-Küster-Hauser (MRKH)
63 syndrome, are gathered under an ovarian dysgenesis syndrome (ODS) umbrella [8].

64

65 The mammalian germ cell lineage arises early during fetal life from an extra-embryonic
66 territory and migrates into the presumptive gonadal territory at the surface of the
67 mesonephros. This coincides with the proliferation and differentiation of somatic cells into
68 the nascent bipotential gonad. In the ovary, the crucial element of differentiation is the
69 genesis of a stock of germ cells, by rounds of exponential proliferation, and their
70 commitment into meiosis before their arrest at the diplotene stage of prophase I.

71 Subsequently, germ cells are enclosed into a finite number of functional units, the primordial
72 follicles. Female reproductive lifespan is determined by this ovarian “stockpile” (reserve) [9].

73 Importantly, although ovarian genesis is based on a sequence of morphogenetic processes
74 starting during fetal life that is common to mammals, stage duration and overlap differ greatly

75 between species. One major difference is the endocrine environment around follicle
76 formation, which is characterised by a sudden decrease in estrogen levels in rodents, but
77 high levels of estrogens for species like humans. If the prenatal exposure window is
78 associated with the establishment of the germ cell reserve, the somatic cells are similarly
79 undergoing active differentiation into either the epithelial or interstitial cell lineages. Unlike
80 the adult ovary, whose endocrine relationships within the pituitary-hypothalamic-gonadal axis
81 are well characterised, the endocrine properties of the fetal ovary are less well known in
82 most species.

83

84 In this review, we update on the study of prenatal chemical toxicant exposure effects on
85 early steps of ovarian differentiation. We identify the technical blocks in current experimental
86 strategies in order to highlight research challenges, especially in a regulatory context.

87 Indeed, the endpoints currently used to unravel these questions remain mainly highly
88 focused on the germ cell lineage and on toxicity without questioning their potential endocrine
89 disrupting activity. These questions are crucial to identify pollutants or pollutant categories
90 that exert the most adverse endocrine disrupting effects on the developing ovary. This is
91 essential to categorise for regulators those EDCs for which *in utero* exposure presents risks
92 for female fertility.

93

94 **Methodological approaches**

95

96 Epidemiological studies first highlighted plausible associations between *in utero* exposures
97 and female reproductive alterations, such as for diethylstilbestrol [10]. Nevertheless, the
98 delay between exposure and adverse outcomes, and additional exposures after birth, makes
99 the establishment of cause-effect links very complex. To overcome confounding factors, *in*
100 *vivo* animal studies conducted under controlled conditions and *in vitro/ex vivo* studies were
101 used to address possible long- and short- term effects, respectively. A common limitation of
102 rodent studies is due to the experimental constraints on exposure routes which do not
103 necessarily correspond to the environmental exposure of the mother or differ in the routes of
104 exposure.

105

106 *In vitro/ex vivo* studies allow dissection of short-term effects at cellular and molecular levels.
107 However, while some cell lines can be considered as proxies for the human germ cell
108 lineage, such as embryonic stem cells [11], there is a lack of validated cell lines for the
109 human fetal ovarian somatic cell lineages. Therefore, organotypic cultures of fetal organs in
110 rodents or humans have been used for decades to address direct and short-term effects of
111 chemicals [12]. However, *ex vivo* cultures isolate the ovary from the other organs of the

112 body, not only those from the reproductive axis, but also organs involved in the absorption,
113 distribution, metabolism, and elimination of chemicals. These processes, determining factors
114 in real life, are, therefore, not necessarily investigated. To the best of our knowledge, multi-
115 organ cultures have not yet been used for fetal ovary studies. This is a method gap since the
116 approach could rebuild the systemic complexity of ovarian function [13], and take into
117 account the relationships between organs, including the hypothalamo-pituitary ovarian axis
118 (active in second trimester human fetuses). This approach would also be highly relevant to
119 take into account the bio-transformation of chemicals by both placenta and fetal liver, and
120 the endocrine relationships between organs, including the adrenals.

121
122 Unlike large mammals and humans, who are exposed to a wide range of environmental
123 pollutants, longitudinal studies are possible in small animal models bred in highly controlled
124 environments. Tight control of the environment is possible with human multi-organ chip
125 microfluidic culture technology [14], yet very long-term experiments are currently
126 unachievable. While fetal exposome studies are crucial to understand the complexity of
127 exposures, there remain issues around the more relevant dose to study: classic toxicological
128 studies with doses ranging from low to unrealistically high doses vs environmentally relevant
129 doses to identify realistic targets. The question of the optimum dose/s to test comes when
130 addressing chemical effects from suspected EDCs. Exposomics also opens new avenues in
131 experimental strategy design, shifting research in the field from exposure to single
132 component to “real-life” mixtures. However, to achieve environmental realism, choosing the
133 most relevant mixtures, and concentrations of each component, remains a major challenge.
134 Adding to the complexity is the unfortunate fact that experimental designs of cocktails may
135 induce complex responses within which the role/s, if any, of each single environmental
136 pollutant is almost impossible to assess. From a broader point of view, the choice of
137 exposure/s and relevant pathophysiological endpoints, do not meet regulatory requirements.
138 Regulatory tests are required to pinpoint highly sensitive biomarkers of exposure and future
139 adverse outcomes. Overall, this raises the importance of cross-sectional studies to address
140 the potential repercussions of fetal environmental exposures on ovarian development and
141 future function.

142
143 **Classification of environmental pollutant according to their effects: the Adverse**
144 **Outcome Pathway (AOP) challenge**

145
146 At the cellular level, several morphogenetic consequences of endocrine disruption have
147 been described, including alteration of: (i) cell determination and differentiation, (ii)
148 development and growth of the organ, (iii) cell, and more specifically germ cell proliferation.

149 Many human-made or natural chemicals displaying estrogenic properties have been studied
150 in animal models. However, the mechanisms of action on the ovary are often hypothesised
151 rather than demonstrated. Transcriptional studies following prenatal exposure to estrogenic
152 compounds have begun to unravel their mechanisms of action [15-19]. Endocrine disruption
153 is a first-line readout of exogenous compound effects on the fetal testis. This is poorly
154 investigated in the ovary where alterations in prenatal endocrine activity are rarely studied
155 [20]. While this is understandable in rodents, the steroidogenic capabilities of the human
156 fetal ovary are well known [21].

157

158 At the subcellular level, exogenous compounds can trigger effects such as the formation of
159 reactive oxygen species, alterations of lipid and protein structures, and DNA damage
160 [22,23]. The classical readout of such damage is DNA quality, including both its integrity and
161 epigenetic alterations (defined as inheritable modifications without alterations of the genetic
162 sequence). Epigenetic regulation is crucial in physiological processes and several studies
163 show xenobiotic-induced epigenetic alterations in laboratory models [24,25]. Importantly,
164 epigenetic marks play roles in regulating expression of crucial genes at specific timepoints of
165 development. For the germ cell lineage this is vital since these modifications could be
166 transmitted to the next generation, leading to intergenerational or even transgenerational
167 effects. While epigenetic effects rely on several mechanisms, such as DNA methylation,
168 histone modifications and interfering non-coding RNA, DNA methylation alterations by
169 environmental exposures remains the most studied [26]. Germ cell meiotic commitment and
170 progression are key morphological events that are sensitive to xenobiotics, especially
171 estrogenic compounds (e.g. BPA)* [27,28]. Discrepancies between studies may be
172 explained by differences in the routes of exposure and by interspecies difference in patterns
173 of expression of estrogen receptor variants [29-31]. The challenge of epigenetic alterations
174 in the fetal ovary is the interference by xenobiotics with methylation status or physiological
175 demethylation/remethylation processes that takes place during fetal development [32]. This
176 is also true for DNA damage and repair [33,34].

177

178 The germ cell lineage, a vector of long-term effects on subsequent generations, is often in
179 the crosshairs of these studies, the somatic cell lineage being mostly left in the shade. The
180 lack of interest in the study of epigenetic alterations on the somatic lineage is unfortunate
181 considering the tight relationship between somatic and germ cell lineages, and therefore the
182 possible indirect effects of the alteration of one cell lineage on events such as primordial
183 follicle formation. Overall, the single cell 'omics revolution has, unfortunately, had limited
184 contact with fetal ovarian toxicological studies.

185

186 Classification of pollutants according to their molecular mechanisms of action is extremely
187 delicate and requires large-scale studies at the transcript and/or the protein levels. These
188 studies are essential for AOP description. The principle of AOP is to define a series of
189 events initiated by a molecular initiating event (MIE) that ultimately leads to adverse effects
190 in the function of a given organ and can be induced by multiple exogenous compounds.
191 Surprisingly, to date, very few AOPs describe the ovary [35] and the best established in
192 shown in Fig. 1 (e.g. diisobutyl phthalate). One reason is that mechanisms of action of
193 xenobiotics on the fetal ovary are poorly understood. Animal models have provided many
194 correlations between fetal exposures to toxicants and long-term adverse effects on the
195 ovary, such as early decrease of the follicle reserve. Indeed, premature ovarian insufficiency
196 (POI, e.g. phthalates, polychlorinated biphenyls and organochlorine pesticides) [36],
197 polycystic ovarian syndrome (PCOS, e.g. perfluorooctanoic acid), infertility (e.g. phthalates,
198 organophosphate pesticides), delayed or precocious puberty are commonly investigated in
199 rodents in relation to fetal exposures [37-39], unlike ovarian cancer and endometriosis (e.g.
200 polybrominated diphenyl ethers).

201

202 **Classification of environmental pollutant according to their chemistry: the QSAR** 203 **challenge**

204

205 Typically, xenobiotics triggering an impact on the ovary are categorised as either estrogenic
206 or antiandrogenic, and these properties were used to design experimental strategies for
207 mixtures [40]. *In silico* quantitative structure-activity relationship (QSAR) models were
208 recently introduced as a way to identify chemicals most likely to be harmful based on their
209 similarities [41-44] with chemical structures of characterised compounds. While well
210 accepted that estrogenic compounds display various estrogenic potencies (e.g. Bisphenol A)
211 [45], this is not always taken into account for compounds with other activities. In addition, the
212 question of the dose is crucial because one compound can display multiple characteristics,
213 and properties can vary along concentration ranges. Classification of chemicals according to
214 their chemical family, their structure, or their known targets, is hazardous when challenging
215 their expected effect on a complex organ such as the developing ovary. A further
216 complication is that receptor endowments can vary between different cell types and stages
217 of development. All these parameters must be taken into account in study design and
218 interpretation. So far no ovarian chemical risk has been identified directly from QSAR.

219

220 **Classification of environmental pollutant according to windows of sensitivity**

221

222 In rodents, four specific time windows of heightened ovary sensitivity to disruption by
223 exogenous insults have been identified: (i) gonadal sex determination (e.g. paracetamol,
224 tamoxifen), (ii) meiotic division (e.g. bisphenol A, atrazine), (iii) follicle assembly (e.g.
225 polycyclic aromatic hydrocarbons, genistein) and (iv) the first wave of follicle recruitment
226 (e.g. benzo[a]pyrene, Di (2-ethylhexyl) phthalate) [46]. While almost synchronous for all
227 germ cells in small laboratory rodents, morphogenetic processes of ovarian differentiation
228 overlap in larger mammals and humans. Asynchronicity of processes make the precise
229 identification of sensitive windows difficult and they may more likely correspond to
230 morphogenetic events rather than developmental time periods (e.g. humans, sheep,
231 monkey). Follicles form in rodent models, like mice and rats, shortly after birth when the
232 circulating levels of estrogens drop to nadir, while in humans, and large mammal models,
233 they assemble in the womb in a high (human) or relatively high (sheep) estrogenic endocrine
234 environment (Fig. 2). Therefore, exposure to estrogenic compounds via the mother do not
235 necessarily target identical processes. A critical challenge, especially in the case of
236 endocrine disruption, is to determine whether a given pollutant will have similar effects on
237 specific process in different species. This has to be taken into account in the choice of the
238 appropriate time window/s according to the suspected process/es targeted by the chemical/s
239 of interest.

240

241 Discussion

242

243 The weight of evidence that female reproductive disorders partly result from deleterious
244 environmental exposures, such as to EDCs, during prenatal life has been building. These
245 effects represent challenges, not only in terms of technical approaches, but also in data
246 interpretation. Nevertheless, if a decrease in the germ cell “stockpile” may lead to reduced
247 future fertility, we have to be cautious regarding this assertion. Indeed, depending on the
248 severity of the depletion of germ cells, compensatory mechanisms cannot be excluded
249 [47,48]. An additional difficulty is the range of key factors subsequently affecting fertility, for
250 instance, the quality as well as number of oocytes and follicles are key components of
251 female reproductive function. The complexity of studying female reproductive function comes
252 from the fact that it does not rely only on ovarian function and germ cell/follicle reserves, but
253 also on relationships between ovarian cell types and their functions (e.g. steroidogenesis)
254 and with organs such as the hypothalamo-pituitary-ovary axis, liver and adrenal gland, as
255 well as with the placenta in fetal life.

256

257 An additional dimension of the challenges of studying exposure of the fetal ovary to EDCs is
258 the complexity of mechanisms involved: will the EDC be toxic for the germ cell lineage, alter

259 fetal endocrine function, disturb somatic cell lineage programming, dysregulate formation of
260 follicles and generate epigenetic alterations? Thus, endocrine disruption could be a direct
261 result of exposure, or an indirect, long-lasting, adverse effect on the endocrine cell lineage.
262 Fetal ovarian cultures present difficulties beyond the small number of endpoints currently in
263 the experimental toolbox. These include relatively small organ size and the limited number of
264 conditions that can be addressed simultaneously, which, together with the current outdated
265 toxicological guidelines, are relatively insensitive to detect disturbances that might lead to
266 long-term effects. These will limit the number of fetal ovarian studies of *in utero* exposure to
267 pollutants, including EDCs, published, especially in the human, exacerbating the problem by
268 adding publication bias. A consequence is the potential misinterpretation of the level of risk a
269 particular toxicant might pose to the female fetus and her future fertility.

270

271 **Footnote to go after and close to line 185**

272 *For ease of reading, (e.g. text) is used to provide examples of endocrine disruptors
273 associated with the process under discussion.

274

275 **Acknowledgements**

276 This work was supported by the European Union's Horizon 2020 FREIA project (grant
277 agreement No. 825100). The authors declare no conflicts of interest regarding this study.

278

279 **References**

- 280 * [1] R. Vermeulen, E. L. Schymanski, A.-L. Barabási, and G. W. Miller, **The exposome**
281 **and health: Where chemistry meets biology**, *Science*, vol. 367, no. 6476, pp. 392–
282 396, 2020, doi: 10.1126/science.aay3164.
- 283 [2] Endocrine Society position statement on endocrine-disrupting chemicals.
284 [https://www.endocrine.org/~media/endosociety/files/advocacy-and-outreach/position-](https://www.endocrine.org/~media/endosociety/files/advocacy-and-outreach/position-statements/all/endocrinedisruptingchemicalspositionstatement.pdf?la=en)
285 [statements/all/endocrinedisruptingchemicalspositionstatement.pdf?la=en](https://www.endocrine.org/~media/endosociety/files/advocacy-and-outreach/position-statements/all/endocrinedisruptingchemicalspositionstatement.pdf?la=en) (accessed
286 Feb. 10, 2021).
- 287 [3] REACH Legislation - ECHA. <https://echa.europa.eu/regulations/reach/legislation>
288 (accessed Feb. 12, 2021).
- 289 ** [4] J. E. Rager *et al.*, **Review of the environmental prenatal exposome and its**
290 **relationship to maternal and fetal health** *Reproductive Toxicology*, vol. 98. Elsevier
291 Inc., pp. 1–12, 2020, doi: 10.1016/j.reprotox.2020.02.004.
- 292 [5] D. J. P. Barker and C. Osmond, Infant mortality, childhood nutrition, and ischaemic
293 heart disease in England and Wales., *Lancet*, vol. 327, no. 8489, pp. 1077–1081,
294 1986, doi: 10.1016/S0140-6736(86)91340-1.
- 295 [6] B. M. Brenner, D. L. Garcia, and S. Anderson, Glomeruli and blood pressure: less of

- 296 one, more the other?, *Am. J. Hypertens.*, vol. 1, no. 4 Pt 1, pp. 335–347, 1988, doi:
297 10.1093/ajh/1.4.335.
- 298 [7] N. E. Skakkebaek, E. Rajpert-De Meyts, and K. M. Main, Testicular dysgenesis
299 syndrome: An increasingly common developmental disorder with environmental
300 aspects, *Human Reproduction*, vol. 16, no. 5. Oxford University Press, pp. 972–978,
301 2001, doi: 10.1093/humrep/16.5.972.
- 302 [8] G. M. Buck Louis, C. D. Lynch, and M. A. Cooney, Environmental influences on
303 female fecundity and fertility, *Seminars in Reproductive Medicine*, vol. 24, no. 3.
304 *Semin Reprod Med*, pp. 147–155, 2006, doi: 10.1055/s-2006-944421.
- 305 [9] P. Smith, D. Wilhelm, and R. J. Rodgers, Development of mammalian ovary, *Journal*
306 *of Endocrinology*, vol. 221, no. 3. BioScientifica Ltd., pp. 145–161, 2014, doi:
307 10.1530/JOE-14-0062.
- 308 [10] S. H. Swan, Intrauterine exposure to diethylstilbestrol: Long-term effects in humans,
309 *APMIS*, vol. 108, no. 12, pp. 793–804, 2000, doi: 10.1111/j.1600-
310 0463.2000.tb00001.x.
- 311 [11] J. B. Holm *et al.*, Intrauterine exposure to Paracetamol and Aniline impairs female
312 reproductive development by reducing follicle reserves and fertility, *Toxicol. Sci.*, vol.
313 150, no. 1, pp. 178–189, 2016, doi: 10.1093/toxsci/kfv332.
- 314 [12] A. J. Childs and R. A. Anderson, Experimental approaches to the study of human
315 primordial germ cells, *Methods Mol. Biol.*, vol. 825, pp. 199–210, 2012, doi:
316 10.1007/978-1-61779-436-0_15.
- 317 * [13] S. Xiao *et al.*, **A microfluidic culture model of the human reproductive tract and**
318 **28-day menstrual cycle**, *Nat. Commun.*, vol. 8, no. 14584, 2017, doi:
319 10.1038/ncomms14584.
- 320 [14] D. E. Ingber, Is it Time for Reviewer 3 to Request Human Organ Chip Experiments
321 Instead of Animal Validation Studies?, *Adv. Sci.*, vol. 7, no. 22, p. 2002030, 2020, doi:
322 10.1002/advs.202002030.
- 323 [15] M. A. Brieno-Enriquez *et al.*, Gene expression is altered after bisphenol A exposure in
324 human fetal oocytes in vitro, *Mol. Hum. Reprod.*, vol. 18, no. 4, pp. 171–183, 2012,
325 doi: 10.1093/molehr/gar074.
- 326 [16] S. H. Liew, M. A. Sarraj, A. E. Drummond, and J. K. Findlay, Estrogen-dependent
327 gene expression in the mouse ovary, *PLoS One*, vol. 6, no. 2, p. e14672, 2011, doi:
328 10.1371/journal.pone.0014672.
- 329 [17] J. M. Naciff *et al.*, Gene expression profile induced by 17alpha-ethynyl estradiol,
330 bisphenol A, and genistein in the developing female reproductive system of the rat,
331 *Toxicol. Sci.*, vol. 68, no. 1, pp. 184–199, 2002, doi: 10.1093/toxsci/68.1.184.
- 332 * [18] Q. Li *et al.*, **Transcriptome analysis reveals that cyclophosphamide induces**

- 333 **premature ovarian failure by blocking cholesterol biosynthesis pathway**, *Life*
334 *Sci.*, vol. 239, no. 116999, 2019, doi: 10.1016/j.lfs.2019.116999.
- 335 [19] A. M. Zama and M. Uzumcu, Targeted Genome-Wide Methylation and Gene
336 Expression Analyses Reveal Signaling Pathways Involved in Ovarian Dysfunction
337 after Developmental EDC Exposure in Rats¹, *Biol. Reprod.*, vol. 88, no. 2, pp. 52–53,
338 2013, doi: 10.1095/biolreprod.112.104802.
- 339 [20] P. A. Fowler *et al.*, In utero exposure to cigarette smoke dysregulates human fetal
340 ovarian developmental signalling, *Hum. Reprod.*, vol. 29, no. 7, pp. 1471–1489, 2014,
341 doi: 10.1093/humrep/deu117.
- 342 [21] E. A. Wilson and M. J. Jawad, The effect of trophic agents on fetal ovarian
343 steroidogenesis in organ culture, *Fertil. Steril.*, vol. 32, no. 1, pp. 73–79, 1979, doi:
344 10.1016/s0015-0282(16)44119-1.
- 345 [22] J. B. Samuel, J. A. Stanley, R. A. Princess, P. Shanthi, and M. S. Sebastian,
346 Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative
347 stress and impaired steroid hormone levels, *J. Med. Toxicol.*, vol. 7, no. 3, pp. 195–
348 204, 2011, doi: 10.1007/s13181-011-0143-9.
- 349 [23] U. Luderer, Ovarian toxicity from reactive oxygen species, in *Vitamins and Hormones*,
350 vol. 94, Academic Press Inc., 2014, pp. 99–127.
- 351 [24] G. Cruz, W. Foster, A. Paredes, K. D. Yi, and M. Uzumcu, Long-term effects of early-
352 life exposure to environmental oestrogens on ovarian function: role of epigenetics, *J.*
353 *Neuroendocrinol.*, vol. 26, no. 9, pp. 613–624, 2014, doi: 10.1111/jne.12181.
- 354 [25] R. Chianese *et al.*, Bisphenol A in Reproduction: Epigenetic Effects, *Curr. Med.*
355 *Chem.*, vol. 25, no. 6, pp. 748–770, 2018, doi: 0.2174/0929867324666171009121001.
- 356 [26] A. M. Zama and M. Uzumcu, Epigenetic effects of endocrine-disrupting chemicals on
357 female reproduction: an ovarian perspective, *Front. Neuroendocrinol.*, vol. 31, no. 4,
358 pp. 420–439, 2010, doi: 10.1016/j.yfrne.2010.06.003.
- 359 [27] A. Ziv-Gal and J. A. Flaws, Evidence for bisphenol A-induced female infertility: a
360 review (2007–2016), *Fertil. Steril.*, vol. 106, no. 4, pp. 827–856, 2016, doi:
361 10.1016/j.fertnstert.2016.06.027.
- 362 [28] Y. Hu *et al.*, The association between the environmental endocrine disruptor
363 bisphenol A and polycystic ovary syndrome: a systematic review and meta-analysis,
364 *Gynecol. Endocrinol.*, vol. 34, no. 5, pp. 370–377, 2018, doi:
365 10.1080/09513590.2017.1405931.
- 366 [29] M. Uzumcu and R. Zachow, Developmental exposure to environmental endocrine
367 disruptors: Consequences within the ovary and on female reproductive function,
368 *Reprod. Toxicol.*, vol. 23, no. 3, pp. 337–352, 2007, doi:
369 10.1016/j.reprotox.2006.10.006.

- 370 * [30] S. Rattan and J. A. Flaws, **The epigenetic impacts of endocrine disruptors on**
371 **female reproduction across generations**, *Biol. Reprod.*, vol. 101, no. 3, pp. 635–
372 644, 2019, doi: 10.1093/biolre/iox081.
- 373 [31] M. Uzumcu, A. M. Zama, and E. Oruc, Epigenetic mechanisms in the actions of
374 endocrine-disrupting chemicals: gonadal effects and role in female reproduction,
375 *Reprod. Domest. Anim.*, vol. 47, no. Suppl 4(0 4), pp. 338–347, 2012, doi:
376 10.1111/j.1439-0531.2012.02096.x.
- 377 [32] Y. Zeng and T. Chen, DNA Methylation Reprogramming during Mammalian
378 Development, *Genes (Basel)*., vol. 10, no. 4, p. 257, 2019, doi:
379 10.3390/genes10040257.
- 380 ** [33] J. Gebel, M. Tuppi, N. Sanger, B. Schumacher, and V. Dotsch, **DNA Damaged**
381 **Induced Cell Death in Oocytes**, *Molecules*, vol. 25, no. 23, p. 5714, 2020, doi:
382 10.3390/molecules25235714.
- 383 [34] A. M. Bothun *et al.*, Quantitative proteomic profiling of the human ovary from early to
384 mid-gestation reveals protein expression dynamics of oogenesis and folliculogenesis,
385 *Stem Cells Dev.*, vol. 27, no. 11, pp. 723–735, 2018, doi: 10.1089/scd.2018.0002.
- 386 [35] H. K. L. Johansson *et al.*, **Putative adverse outcome pathways for female**
387 **reproductive disorders to improve testing and regulation of chemicals**, *Arch.*
388 *Toxicol.*, vol. 94, no. 10, pp. 3359–3379, 2020, doi: 10.1007/s00204-020-02834-y.
- 389 [36] P. Vabre *et al.*, Environmental pollutants, a possible etiology for premature ovarian
390 insufficiency: A narrative review of animal and human data, *Environ. Heal. A Glob.*
391 *Access Sci. Source*, vol. 16, no. 1, p. 37, 2017, doi: 10.1186/s12940-017-0242-4.
- 392 [37] L. Das, S. Parbin, N. Pradhan, C. Kausar, and S. K. Patra, Epigenetics of reproductive
393 infertility, *Frontiers in Bioscience - Scholar edition*, vol. 9. Frontiers in Bioscience, pp.
394 509–535, 2017, doi: 10.2741/s497.
- 395 [38] D. A. Dumesic, M. O. Goodarzi, G. D. Chazenbalk, and D. H. Abbott, Intrauterine
396 environment and polycystic ovary syndrome, *Semin. Reprod. Med.*, vol. 32, no. 3, pp.
397 159–165, 2014, doi: 10.1055/s-0034-1371087.
- 398 [39] P. Filippou and R. Homburg, Is foetal hyperexposure to androgens a cause of
399 PCOS?, *Hum. Reprod. Update*, vol. 23, no. 4, pp. 421–432, 2017, doi:
400 10.1093/humupd/dmx013.
- 401 [40] H. K. L. Johansson *et al.*, Perinatal exposure to mixtures of endocrine disrupting
402 chemicals reduces female rat follicle reserves and accelerates reproductive aging,
403 *Reprod. Toxicol.*, vol. 61, pp. 186–194, 2016, doi: 10.1016/j.reprotox.2016.03.045.
- 404 [41] G. E. Jensen, J. R. Niemela, E. B. Wedebye, and N. G. Nikolov, QSAR models for
405 reproductive toxicity and endocrine disruption in regulatory use - a preliminary
406 investigation, *SAR QSAR Environ. Res.*, vol. 19, no. 7–8, pp. 631–641, 2008, doi:

- 407 10.1080/10629360802550473.
- 408 [42] M. Novič and M. Vračko, QSAR models for reproductive toxicity and endocrine
409 disruption activity, *Molecules*, vol. 15, no. 3, pp. 1987–1999, 2010, doi:
410 10.3390/molecules15031987.
- 411 [43] L. Zhang *et al.*, Identification of putative estrogen receptor-mediated endocrine
412 disrupting chemicals using QSAR- and structure-based virtual screening approaches,
413 *Toxicol. Appl. Pharmacol.*, vol. 272, no. 1, pp. 67–76, 2013, doi:
414 10.1016/j.taap.2013.04.032.
- 415 [44] N. Andersson *et al.*, Guidance for the identification of endocrine disruptors in the
416 context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, *EFSA J.*, vol. 16,
417 no. 6, p. e5311, 2018, doi: 10.2903/j.efsa.2018.5311.
- 418 [45] G. G. Kuiper *et al.*, Comparison of the ligand binding specificity and transcript tissue
419 distribution of estrogen receptors alpha and beta, *Endocrinology*, vol. 138, no. 3, pp.
420 863–870, 1997, doi: 10.1210/endo.138.3.4979 Abstract.
- 421 [46] H. K. L. Johansson, T. Svingen, P. A. Fowler, A. M. Vinggaard, and J. Boberg,
422 Environmental influences on ovarian dysgenesis - developmental windows sensitive
423 to chemical exposures, *Nat. Rev. Endocrinol.*, vol. 13, no. 7, pp. 400–414, 2017, doi:
424 10.1038/nrendo.2017.36.
- 425 [47] A. N. Hirshfield, Follicular recruitment in long-term hemicastrate rats, *Biol. Reprod.*,
426 vol. 27, no. 1, pp. 48–53, 1982, doi: 10.1095/biolreprod27.1.48.
- 427 [48] S. Mazaud *et al.*, Establishment of the reproductive function and transient fertility of
428 female rats lacking primordial follicle stock after fetal gamma-irradiation,
429 *Endocrinology*, vol. 143, no. 12, pp. 4775–4787, 2002, doi: 10.1210/en.2002-220464.
430

431 **Annotations**

432

433 **[1]** This review highlights not only the complexity of the chemical exposome but also the
434 challenges in assessing its effects in an integrative way.

435 **[4]** In this review, the authors provide an overview of the prenatal chemical exposome. They
436 pinpoint the knowledge gaps that are still to be overcome in order to better characterise the
437 exposome and thus link it with fetal outcomes.

438 **[13]** The integrated microfluidic platform set up by the authors supports, not only follicle
439 maturation and differentiation, but also dynamic hormonal secretion through an extended
440 period of time. This model represents significant technical progress that can be used for the
441 integrative understanding of the female reproductive physiology and also as a more
442 representative model for pharmacology and toxicology studies.

443 **[18]** This study provides a mechanistic insights into the understanding of premature ovarian
444 failure following cyclophosphamide exposure and demonstrate that it occurs through
445 alteration of the steroid biosynthesis pathway.

446 **[30]** The authors review the epigenetic effects of several endocrine disruptors on different
447 components of the reproductive system. These could explain the multigenerational, and
448 even transgenerational effects, that are observed following exposures.

449 **[33]** From a molecular point of view, the authors review the mechanisms underlying the DNA
450 repair system which is such crucial for the oocyte quality. It provides a clear overview on the
451 possible targets that need to be investigated when addressing xenobiotic effects,
452 especially when they are known to be associated with multigenerational effects.

453 **[35]** Suggesting new avenues to investigate causes of female reproductive disorders, this
454 review pinpoints the urgent need for an intensified work towards characterising female
455 reproductive adverse outcomes pathways. Such pathways are an essential basis of robust
456 policy formulation.

457

458 **Figure legends**

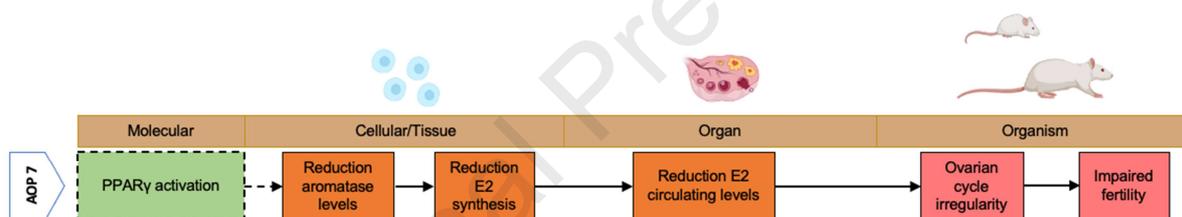
459

460 **Figure 1 : AOP7 from the AOPwiki was mainly established from rodent data and**
 461 **epidemiological studies, illustrating the importance of cross-sectional studies for the**
 462 **development of such AOPs.**

463 This well established AOP presents the peroxisome proliferator activated receptor gamma
 464 (PPAR γ) activation as the molecular event that leads to ovarian cycle irregularity and
 465 impaired fertility in adult females, and describes the key events leading to this adverse
 466 outcome. The ovarian cycle irregularity that ultimately causes impaired fertility following the
 467 initiating PPAR γ activation, is dependent upon a reduction in aromatase levels that lead to
 468 lowered circulating estradiol (E2) levels. Adapted from the AOPwiki at [https://aopwiki](https://aopwiki.org/aops/7)
 469 [ki.org/aops/7](https://aopwiki.org/aops/7).

470 Newly formulated putative AOPs for endocrine disruption of the ovary are given in reference
 471 [35] as part of the FREIA project outputs (<http://freiaproject.eu/wp/>).

472



473

474

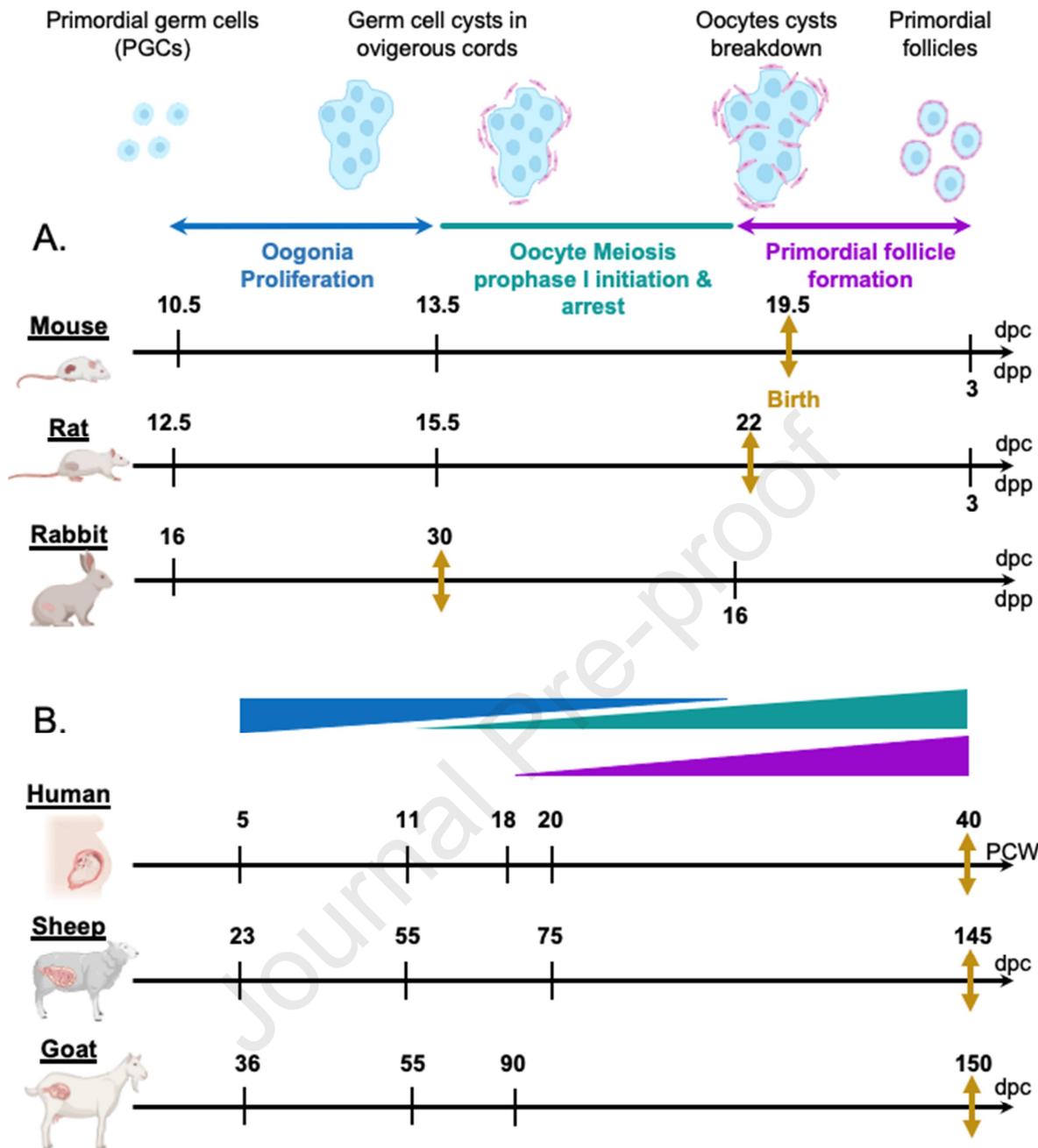
475 **Figure 2: Comparison of the sequence of morphogenetic events occurring during fetal**
476 **and/or neonatal development in the ovaries of different mammalian species.**

477 Representative timelines of germ cells cyst formation by rounds of incomplete mitosis,
478 meiotic onset and arrest in diplotene stage of prophase I and primordial follicle formation in
479 rodents (mouse and rat), rabbit (A), and human, sheep and goat (B).

480 Although the overall sequence of morphogenetic events culminating in the formation of
481 primordial follicles is similar in mammals, timing of specific events differs between species.
482 Indeed, after differentiating in an extra-embryonic territory (5 dpc in mouse, 9 dpc in rabbit),
483 primordial germ cells settle the genital ridge at about 10.5 dpc in mice, 12.5 dpc in rat, 5 DW
484 in humans, 23 dpc in sheep and before 36 dpc in goat. By 16 dpc, most germ cells have
485 entered the gonad in rabbit. During their migration and after they enter the gonad, germ cells
486 undergo a series of incomplete mitotic divisions allowing germ cells cysts formation. As a
487 sign of ovarian differentiation, germ cells then cease mitosis to enter in a synchronous
488 manner in meiosis I at 13.5 dpc in mouse, 16.5 dpc in rat and around birth in rabbit. Finally,
489 after meiosis, oocytes cysts breakdown to form primordial follicle pool, from shortly before
490 birth in mouse, after birth in rat and from 16 dpp in rabbit (Fig 1A). While rodents and rabbit
491 germ cells enter synchronously in the key steps of their differentiation, in human, sheep and
492 goat several germ cells population coexist at a given timepoint. Indeed, while some germ
493 cells keep proliferating until 20 DW in humans and 90 dpc in sheep, others enter meiosis as
494 early as 11 DW and 55 dpc in humans and goat, respectively. In sheep, meiosis I onset
495 occurs at 55 dpc and these cells are increasingly prevalent by 75 dpc. The first primordial
496 follicles are observed at mid gestation (around 16 DW) in human, 75 dpc in sheep and 90
497 dpc in goat. Besides, while in humans mainly primordial and anecdotic primary follicles can
498 be found in the ovary at birth, in sheep and goat, folliculogenesis occurs during fetal life. (in
499 sheep, first primary and first antral follicles observed at 100 dpc and 135 dpc, respectively)
500 PCW: post-conceptual weeks; dpc: days post-conception, dpp: days post-partum.

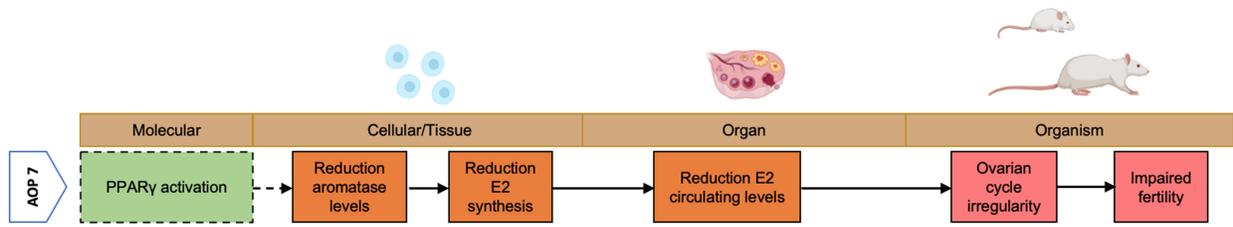
501 (Drawings were edited from BioRender.com)

502

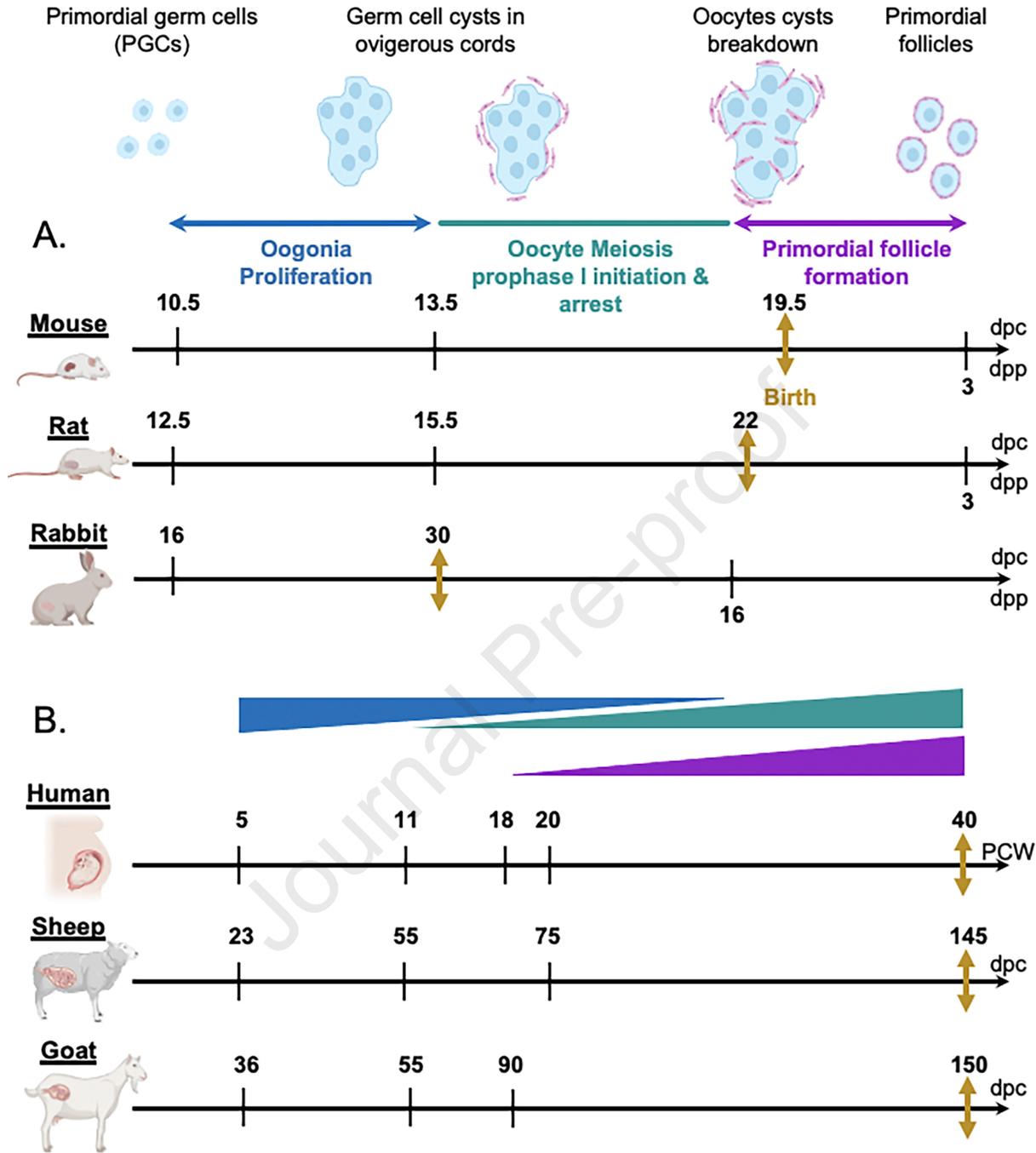


503

504



Journal Pre-proof



Declaration of interests

The mammalian ovary: concerns about evaluation of prenatal environmental exposures.

Laetitia L. Lecante ¹,

Pauline Lelandais ²,

Séverine Mazaud-Guittot S. ²,

Fowler P. A. ^{1*}

¹ Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.

² Inserm, EHESP, Irset (Institut de Recherche en Santé, Environnement et Travail) UMR_S 1085, University Rennes, 35000, Rennes, France.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: