THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Chronic fetal hypoxia disrupts the peri-conceptual environment in next-generation adult female rats

Citation for published version:
Aiken, CE, Tarry-adkins, JL, Spiroski, AM, Nuzzo, AM, Ashmore, TJ, Rolfo, A, Sutherland, MJ, Camm, EJ, Giussani, DA & Ozanne, SE 2019, 'Chronic fetal hypoxia disrupts the peri-conceptual environment in next-generation adult female rats', *Journal of Physiology*. https://doi.org/10.1113/JP277431

Digital Object Identifier (DOI):
10.1113/JP277431

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Journal of Physiology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 04. Dec. 2020

Chronic fetal hypoxia disrupts the peri-conceptual environment in next-generation adult female rats

Aiken C.E.[1,2*], Tarry-Adkins, J.L.[1], Spiroski A.M.[3], Nuzzo A.M.[4], Ashmore T.J[1]., Rolfo A.[4], Sutherland M.J.[3], Camm E.J.[3], Giussani D.A.[3†] & Ozanne, S.E. [1†]

[1]University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-MRC Institute of Metabolic Science, Level 4, Box 289, Addenbrooke's Treatment Centre, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, UK.

[2] University Department of Obstetrics and Gynaecology, University of Cambridge, Cambridge CB2 2SW, UK

[3]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3EG, UK

4Dipartimento di Scienze Chirurgiche, Universita degli Studi di Torino, 10124 Turin, Italy

*Corresponding author
Email: cema2@cam.ac.uk
Phone:+44 (0) 1223 336784

†These authors contributed equally.

Running title: Chronic fetal hypoxia exposure accelerates oviductal ageing
Table of contents category: Reproduction and developmental
Keywords: Developmental programming; oviducts; hypoxia; infertility; reproductive ageing

.

Key points:

- Exposure to chronic hypoxia during gestation influences long-term health and development, including reproductive capacity, across generations.

- If the peri-conceptual environment, in the developing oviduct, is affected by gestational hypoxia, then this could have implications for later fertility and the health of future generations.

- In this study, we show that the oviducts of female rats who were exposed to chronic hypoxia whilst *in utero*, have reduced telomere length, decreased mitochondrial DNA biogenesis, and increased oxidative stress

- Our results show that exposure to chronic gestational hypoxia leads to accelerated ageing of the oviduct in early adulthood, and help us understand how exposure to hypoxia during development could influence reproductive health across generations.

Abstract (245):

Exposure to chronic hypoxia during fetal development has important effects on immediate and long-term outcomes in offspring. Adverse impacts in adult offspring include impairment of cardiovascular function, metabolic derangement, and accelerated ovarian ageing. However, it is not known whether other aspects of the female reproductive system may be similarly affected. In this study, we examine the impact of chronic gestational hypoxia on the developing oviduct. Wistar rat dams were randomized to either normoxia (21%) or hypoxia (13%) from day 6 post-mating until delivery. Post-delivery female offspring were maintained in normoxia until 4 months of age. Oviductal gene expression was assayed at the RNA (q-rtPCR) and protein (Western blotting) levels. Oviductal telomere length was assayed using Southern blotting. Oviductal telomere length was reduced in the gestational hypoxia-exposed animals compared to the normoxic controls ($p<0.01$). This was associated with a specific post-transcriptional reduction in the KU70 subunit of DNA-pk in the gestational hypoxia-exposed group ($p<0.05$). Gestational hypoxia-exposed oviducts also showed evidence of decreased mitochondrial DNA biogenesis; reduced mtDNA copy number ($p<0.05$), and reduced gene expression of *Tfam* ($p<0.05$) and *Pgc1α* ($p<0.05$). In the hypoxia-exposed oviducts there was up-regulation of mitochondrial-specific antioxidant defense enzymes (MnSOD; $p<0.01$). Exposure to chronic gestational hypoxia leads to accelerated ageing of the oviduct in adulthood. The

74 oviduct plays a central role in early development as the site of gamete transport,
75 syngamy, and early development, hence accelerated ageing of the oviductal
76 environment could have important implications for fertility and the health of future
77 generations.
78
79

80 **Introduction**

81 Many human fetuses are exposed to chronic gestational hypoxia, either via factors
82 intrinsic to the pregnancy, for example impaired utero-placental blood flow (Kuzmina
83 *et al.*, 2005), or factors arising from the maternal environment, for example pregnancy
84 at high altitude (Ducsay, 1998; Postigo *et al.*, 2009; Giussani *et al.*, 2016). The
85 immediate effects of gestational hypoxia have been characterized in both human
86 pregnancies and animal models, and include adverse outcomes such as IUGR, low birth
87 weight and stillbirth (Giussani *et al.*, 2001; Keyes *et al.*, 2003; Richter *et al.*, 2012;
88 Gonzalez-Candia *et al.*, 2016). The long-term outcomes for the adult offspring of
89 chronic gestational hypoxia are generally less well understood, but some aspects, for
90 example the increased risk of later cardiovascular dysfunction, have been well
91 described in animal models (Giussani *et al.*, 2012; Giussani & Davidge, 2013).
92 Furthermore, there is evidence from animal models that exposure to chronic gestational
93 hypoxia can adversely impact brain development (Phillips *et al.*, 2017), renal ageing
94 (Gonzalez-Rodriguez *et al.*, 2013), and insulin resistance (Camm *et al.*, 2011).
95

96 The link between exposure to various suboptimal intrauterine environments and
97 subsequent impairment of reproductive function has been demonstrated in a number of
98 animal models (Aiken *et al.*, 2013; Aiken *et al.*, 2016). These studies have mainly been
99 performed in rodents, and have focused primarily on alterations to maternal diet (Chan
100 *et al.*, 2015b). It has been shown that accelerated ageing of the somatic ovarian tissue,
101 with a concomitant decrease in ovarian reserve in early-mid reproductive life, is a
102 consequence of a maternal low protein diet (Aiken *et al.*, 2013), obesogenic maternal
103 diet (Aiken *et al.*, 2016), and maternal caloric restriction (Bernal *et al.*, 2010) in various
104 rodent models.
105

106 The primary outcome of most studies that have demonstrated a link between the early
107 life environment and impairment of female fertility has been ovarian reserve (Chan *et*
108 *al.*, 2015b; Ho *et al.*, 2017). As a key determinant of future reproductive potential
109 (Depmann *et al.*, 2015; Pelosi *et al.*, 2015), ovarian reserve is a useful and specific
110 marker of fertility potential, but reproduction depends on a wide range of factors
111 beyond the availability of gametes. In the female, successful pregnancy depends not
112 only on a viable oocyte, but also on a suitable reproductive tract environment. The
113 oviduct has several vital roles in successful reproduction, including gamete transport

114 (Wang & Larina, 2018), syngamy (Parada-Bustamante *et al.*, 2016), and early
115 embryonic development (Robertson *et al.*, 2015). Oviductal problems are a major cause
116 of infertility in human populations, accounting for approximately 25-35% of all female
117 infertility (Practice Committee of the American Society for Reproductive, 2015). Such
118 problems can range from complete blockage of the oviduct, which impairs gamete
119 transport and prevents conception, to sub-clinical oviductal damage, for example
120 through smoking, which alters the tubal epithelium and increases the risk of ectopic
121 pregnancy (Horne *et al.*, 2014; Nio-Kobayashi *et al.*, 2016). Impact on the oviductal
122 environment of the adult offspring is thus an important consideration in investigating
123 the effect of developmental programming on female reproductive potential.
124
125 A limited number of studies have previously reported on the impact of an adverse
126 intrauterine environment on the developing oviduct. Wister rat offspring exposed to a
127 maternal low-protein diet during gestation, followed by postnatal catch-up growth,
128 showed evidence of reduced telomere length and increased oxidative stress in the
129 oviduct in early adulthood (Aiken *et al.*, 2013). We hypothesise that exposure to chronic
130 gestational hypoxia may also adversely affect the oviduct, and hence the peri-
131 conceptual environment, in a similar way.
132
133 Using an established model of hypoxic pregnancy in rats, we investigated the impact
134 of exposure to a 40% reduction in environmental oxygen (13% versus 21% ambient
135 oxygen from day 6 of pregnancy) on the oviduct of the adult female offspring. A
136 reduction in the environmental oxygen tension by 40% reflects the difference in oxygen
137 availability between pregnancies occurring at sea level compared to 3500-4000m
138 altitude (Postigo *et al.*, 2009). Hence our rat model of gestational hypoxia is highly
139 relevant to human pregnancy at these altitudes, where it is estimated that ~40,000 babies
140 are born each year in Bolivia alone (Roost *et al.*, 2009). The aim of this study was
141 therefore to evaluate whether there is evidence of accelerated ageing in the oviducts of
142 young adult female rats exposed to chronic gestational hypoxia.
143
144 **Materials and Methods**
145 **Ethical approval**
146 All animal experiments were approved by the University of Cambridge Animal Welfare
147 and Ethical Review Board. All animal experiments were conducted in accordance with

148 the British Animals (Scientific Procedures) Act (1986) and were compliant with EU
149 Directive 2010/63/EU. Animals underwent euthanasia by CO_2 inhalation and cervical
150 dislocation.
151
152 *Study design*
153 Wistar rat dams at 10-12 weeks of age (Charles River Ltd., Margate, UK; wild-type
154 RRID: RGD_13508588) were housed in individually ventilated cages (21% oxygen,
155 70-80 air changes/hour) under standard conditions, with a regular 12-hour light/dark
156 cycle. All animals were fed a standard laboratory chow diet (20% protein) and fed *ad*
157 *libitum* with free access to water. After initial acclimatization (10 days) they were mated
158 with fertile male Wistar rats, and pregnancy confirmed through the observation of a
159 vaginal plug. The day of the plug was designated day 0 of pregnancy (full term 21-22
160 days). Upon confirmation of pregnancy, dams were weighed and housed individually.
161 On day 6 of pregnancy, dams were randomly divided into two groups; control (21%)
162 and hypoxic (13%) pregnancy (n=8 per group). Pregnant rats assigned to the hypoxia
163 group were placed inside a chamber that could hold 9 rat cages, which combined a PVC
164 isolator with a nitrogen generator, as previously described (Giussani *et al.*, 2012;
165 Herrera *et al.*, 2012). The hypoxia model did not alter maternal food intake or
166 gestational length. Pregnancies undergoing hypoxia were maintained at a constant
167 inspired fraction of oxygen of 13% from day 6 to 20 of gestation. All dams delivered
168 under normoxic conditions, and normoxia (21%) was maintained for all animals during
169 lactation, weaning and thereafter. Following determination of birth weight, litters were
170 culled to 4 males and 4 females to standardise nutritional access and maternal
171 care (Herrera *et al.*, 2012). All pups were suckled by their own mothers. At four months
172 of age, adult female pups underwent euthanasia by CO_2 inhalation and cervical
173 dislocation. At postmortem, the reproductive tract tissues were harvested immediately
174 after dissection. The oviducts were snap-frozen in liquid nitrogen until used for
175 analysis. No sample was refrozen after the initial thaw.
176
177 *Telomere length analysis*
178 High-molecular weight DNA was extracted using the DNeasy Blood and Tissue kit
179 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quantity
180 and purity was determined using a Nanodrop spectrophotometer (Nanodrop
181 Technologies (Thermo Fisher, Scientific, Hemel Hempstead, UK). Agarose gels were

182 run to ensure all DNA samples were of high-molecular weight. DNA (1.2µg) was
183 digested with *Hinf*I and *Rsa*I restriction enzymes for 2h at 37°C. The restricted samples
184 were quenched with 5x SDS loading buffer (Roche Diagnostics, Mannheim, Germany)
185 and loaded onto agarose gels containing SYBR safe stain (Invitrogen, Paisley, Scotland,
186 UK). After pulsed field gel electrophoresis, the gels were checked for non-specific
187 degradation of an undigested DNA control and complete digestion of the enzyme-
188 restricted DNA by visualizing the stained gels under UV light (Syngene, Cambridge,
189 UK). The separated DNA fragments were transferred to nylon membrane (Roche
190 Diagnostics, Mannheim, Germany) by Southern blotting, and telomeric repeat length
191 was determined using a commercial method of chemiluminescent detection as
192 described previously (Tarry-Adkins *et al.*, 2006). Molecular weight markers on each
193 gel were a mid-range pulsed-field gel marker (New England Biolabs, Ipswich, MA,
194 USA) and dioxygenin (DIG; low range) molecular-weight marker (Roche Diagnostics,
195 Mannheim, Germany). Standard undigested and digested genomic samples of DNA
196 from a 4-month control animal were also included on each gel to verify digestion
197 efficiency. Telomere signals were analyzed using Adobe Photoshop (Adobe Systems
198 Inc. San Jose, CA, USA) and Alpha Ease Software (Alpha Innotech, San Leandro, CA,
199 USA). Telomere length was measured as described previously (Tarry-Adkins *et al.*,
200 2006).

201

202 *Gene expression analysis*

203 An initial panel of 38 candidate genes was developed to test which molecular pathways
204 might be altered in the somatic oviduct following exposure to chronic gestational
205 hypoxia. These genes were chosen based on (i) previous work on the effects of
206 developmental programming on ovarian, para-ovarian adipose tissue, and oviductal
207 gene expression (Aiken *et al.*, 2015; Aiken *et al.*, 2016; Tarry-Adkins *et al.*, 2018) (ii)
208 knowledge of programming mechanisms in other organ systems in the same gestational
209 hypoxia rat model (Camm *et al.*, 2010; Giussani *et al.*, 2012; Herrera *et al.*, 2012) and
210 (iii) relevant literature review. RNA was extracted from snap-frozen oviducts using a
211 miRNeasy mini kit (Qiagen, Hilden, Germany) following manufacturers' instructions,
212 with the addition of a DNaseI digestion step to ensure no genomic DNA contamination.
213 RNA quantification was performed using a NanoDrop spectrophotometer (Nanodrop
214 Technologies, Wilmington, DE, USA). RNA (1 µg) was used to synthesize cDNA using
215 oligo-dT primers and M-MLV reverse transcriptase (Promega, Madison, Wisconsin,

216 USA). Gene expression was determined using custom designed primers (Sigma, Poole,
217 Dorset, UK) and SYBR Green reagents (Applied Biosystems, Warrington, UK) as
218 previously described (Tarry-Adkins *et al.*, 2009). Primer sequences are in
219 supplementary table 1. Quantification of gene expression was performed using a Step
220 One Plus RT-PCR machine (Applied Biosystems, Warrington, UK). Equal efficiency
221 of the reverse transcription of RNA from all groups was confirmed through
222 quantification of expression of the house-keeping gene *ppia*, the expression of which
223 did not differ between groups.
224
225 *Protein quantification*
226 Due to the extremely small amount of tissue available, limited protein quantification
227 was performed. Genes were selected for protein expression analysis on the basis of (i)
228 RNA quantification results and (ii) rationale from previous studies in the same model.
229 Protein was extracted from whole tissue lysates of snap-frozen oviducts, as described
230 previously (Tarry-Adkins *et al.*, 2015; Tarry-Adkins *et al.*, 2018). Protein (20μg) was
231 loaded onto 10%, 12% or 15% polyacrylamide gels, dependent upon the molecular
232 weight of the protein to be measured. The samples were electrophoresed and transferred
233 to polyvinylidene fluoride membranes. Detection steps used the following primary
234 antibodies; P53 (R & D Systems; cat no: MAB1355, 1:1000, RRID:AB_357649),
235 $P16^{INK}$ (Abcam, Cambridge, UK; cat no: Ab189034, 1:1000, RRID:AB_2737282),
236 OGG1 (Novus Biologicals; cat no: NB100-106,1:1000, RRID:AB_10104097), MRE11
237 (ProteinTech, Cambridge, UK, cat no: 10744-1-AP, 1:1000, RRID:AB2145118),
238 KU70 (ProteinTech, Cambridge, UK, cat no: 10723-1-AP, 1:1000, RRID:AB_), KU80
239 (Novus, cat no: NB100-508, 1:1000, RRID:AB_2218756), Total Ox Phos rodent
240 antibody cocktail (Abcam, Cambridge, UK, cat no: Ab110413, 1:5000,
241 RRID:AB_2629281), HIF1α (Abcam: cat no: Ab51608, 1:1000, RRID:AB_880418),
242 $GP91^{phox}$ (ProteinTech, Cambridge, UK; cat no: 19013-1-AP, RRID:AB_1342287),
243 $P47^{phox}$ (ProteinTech, Cambridge, UK; cat. no: 15551-1-AP, 1:1000,
244 RRID:AB_11182937), XO (Santa-Cruz, Wimbledon, Middlesex, UK; cat. no: SC-
245 20991, 1:200, RRID:AB_2214858), HMOX1 (ProteinTech, Cambridge, UK, cat no:
246 20960-1-AP, 1:1000, RRID:AB_10732601), Catalase (Abcam, Cambridge, UK, cat.
247 no.: Ab1877-10, 1:10000, RRID:AB_187710), MnSOD (Upstate, Watford, UK; cat.
248 no.: 06-984, RRID:AB_310325), CuZnSOD (ProteinTech, Cambridge, UK; cat. no.:
249 10269-1-AP, 1:1000, RRID:AB_2193750). Anti-rabbit secondary antibodies (Cell

250 Signaling Technology, Danvers, MA, USA, 1:2000) were utilised for all primary
251 antibodies except P53, which required an anti-mouse secondary antibody (Cell
252 Signaling Technology (Danvers, MA, USA), 1:2000). Equal protein loading was
253 confirmed by staining electrophoresed gels with Coomassie Blue (Bio-Rad, Hemel
254 Hempstead, Herts, UK) to visualize total protein. To ensure that the chemiluminescent
255 signal changed in a linear manner, the ratio between loading controls (100% and 50%
256 pooled sample) was confirmed for each detected protein.

257

258 *Statistical Analysis*

259 All data were initially analyzed using a 2-way ANOVA with gestational
260 hypoxia/normoxia as the independent variable. Raw p values were transformed to take
261 account of the false discovery rates. Maternal environmental effects were compared
262 between groups using 2-tailed Student's T tests. Data are represented as means ± SEM.
263 Where p values are reported, an alpha level <0.05 was considered statistically
264 significant. All data analysis was conducted using the R statistical software package
265 version 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria). In all cases,
266 n refers to the number of litters, and n=7-8 for all groups. The adequacy of the sample
267 size was determined via a power calculation based on the effect sizes for somatic
268 ovarian expression for ageing-related genes a previous rodent developmental
269 programming model (Aiken *et al.*, 2016) using an alpha level of 0.05 to give power of
270 0.8. Sample analysis was performed using project codes to blind the investigators to the
271 experimental groups.

272

273

274 **Results**

275 There was no impact of chronic gestational hypoxia on either maternal food intake
276 during gestation (normoxia 79±2g/kg/day^{-1} v. hypoxia 70±3g/kg/day^{-1}) or length of
277 gestation (normoxia 20±1 days v. hypoxia 20±1 days).

278

279 *Maintenance of oviductal telomere length*

280 At 4 months of age, there were significantly more very short (1.3-4.2kB, $p<0.001$)
281 telomeres in the oviducts of gestational hypoxia-exposed adult females compared to the
282 normoxic group (Figure 1A). There were no significant differences between the

hypoxia and normoxia-exposed groups in the proportion of telomeres that were short (4.2-8.6kB), long (8.6-45.5kB) or very long (45.5-145kB).

Cell-cycle markers of ageing

Alongside the increase in very short telomeres observed in hypoxia-exposed tissues, there was an increase in cell-cycle markers that increase with cellular ageing. Gene expression of *p21* was significantly increased in the hypoxia-exposed group compared to the controls ($p<0.04$). There was also a trend towards increased *p53* expression ($p=0.09$), but this did not reach statistical significance (Table 1). At the protein expression level, there was no significant difference in P16ink levels between groups, but there was a significant increase in P53 ($p<0.05$; Table 2)

DNA damage repair mechanisms

Gene expression of *Ogg1* was elevated in the hypoxia-exposed group compared to the normoxic group (1294±135 v. 1710±132 units; $p<0.05$) (Table 1). At the protein level, the elevation of OGG1 in the hypoxia-exposed group was of borderline significance ($p=0.08$; Table 2). By contrast *Mre11* expression was decreased by more than 50% in the hypoxia-exposed group compared to the controls (723±119 v. 307±79, $p<0.05$) (Table 1), however there was no difference in MRE11 protein expression between the experimental groups (Table 2). There was a trend towards an overall reduction in the catalytic subunit of the DNA protein kinase (*DNA pkcs)* that is required for double-stranded break repair and telomere maintenance ($p<0.1$; Table 1), but no differences in the expression of either of the components of the binding subunit, *Ku70 or Ku80* (Figure 1B). However, at the protein level, there was a significant deficit of KU70 in the oviducts of animals exposed to gestational hypoxia ($p<0.05$), with no difference in KU80 levels (Figure 1C).

There was no significant difference between hypoxia-exposed and normoxic groups in expression of any other DNA damage sensing or early repair mechanisms that were included in the candidate genetic screen; *Neil1*, *Nthl1* or *Xrcc1* (Table 1).

Mitochondrial Biogenesis

Mitochondrial DNA (mtDNA) copy number was reduced in hypoxia-exposed animals compared to controls ($p<0.05$, Figure 2A). The expression of *Tfam* was significantly reduced in oviducts of animals exposed to gestational hypoxia compared to normoxic

317 controls ($p<0.05$; Figure 2B). *Pgc1α* also showed reduced expression in the hypoxia-
318 exposed group ($p<0.05$; Figure 2C). There was no difference between groups in
319 expression of *Nrf2* or *Lonp1* (Table 1). Hence, there is evidence that mtDNA biogenesis
320 may be impaired in the oviduct after exposure to chronic gestational hypoxia.
321
322 We further investigated the gene expression of components of the mitochondrial
323 respiratory complex. There was significant reduction in gene expression of complex I
324 ($p<0.01$) and complex IV ($p<0.05$) in the hypoxia-exposed group compared to the
325 normoxia group. There was also a significant reduction in the gene expression of citrate
326 synthase (*Cs*) ($p<0.05$; Table 1). There was no difference in the expression levels of
327 complex II, complex III or cytochrome C (*Cycs*). However, there was no significant
328 difference in protein expression between the hypoxia-exposed and normoxia-exposed
329 groups in any of the tested mitochondrial respiratory components (Table 2).
330
331 *Oxidative stress and anti-oxidant defense capacity*
332 There was no direct evidence of increased oxidative stress markers in any of the
333 pathways tested in the oviducts at either the gene expression or protein levels (*Hif1α,*
334 *Gp91phox, P22phox, P47phox, Xo, Gpx1, Hmox1)* (Table 1 and Table 2).
335
336 In terms of antioxidant defense capacity, there was no significant difference in gene
337 expression of *Catalase*, *Cuzusod* or *Ecsod* in the hypoxia-exposed compared to the
338 normoxia group. However there was an increase in *MnSOD* expression at both the RNA
339 and protein level (Figure 2 D&E), which is in keeping with the suggestion that
340 mitochondrial biogenesis may be suboptimal in the gestational oviduct. MnSOD is the
341 specific mitochondrial isoform of the powerful superoxide dismutase group of anti-
342 oxidants. Increased expression of MnSOD may thus indicate a successful attempt to
343 buffer the impact of excess free radical generation resulting from impaired
344 mitochondrial biogenesis.
345
346 *Lipid peroxidation*
347 There was a significant increase in the gene expression of *Alox12* (a key component of
348 the lipoxygenase pathway) in the hypoxia-exposed group compared to the controls
349 ($p<0.05$; Table 2). There was no difference in the gene expression levels of *Alox15*
350 between the hypoxia-exposed and control groups.

351

352 **Discussion**

353 We show evidence of accelerated ageing in the oviducts of female offspring in early-
354 mid adulthood, following exposure to chronic gestational hypoxia. Accelerated ageing
355 is demonstrated at a cellular level by decreased telomere length and increased
356 expression of markers of cellular ageing, in particular *p21* and *p53*. The observed
357 decrease in oviductal telomere length was accompanied by a specific post-
358 transcriptional reduction in KU70, which is a key functional sub-unit of the DNA-
359 activated protein kinase required for telomere length maintenance (Jette & Lees-Miller,
360 2015). The observed up-regulation of *Ogg1* in the oviducts of the hypoxia-exposed
361 animals is in keeping with an increase in oxidative DNA damage. *Ogg1* excises 7,8-
362 dihydro-8-oxoguanine (8-oxoG) from damaged DNA, which limits the impact of
363 ubiquitous oxidative damage accumulated during normal ageing (Radicella *et al.*,
364 1997). Hence the observed increase in *Ogg1* suggests a greater exposure to oxidative
365 DNA damage in the oviducts following gestational hypoxia.

366

367 There was also clear evidence that mitochondrial biogenesis is reduced in the oviduct
368 following exposure to chronic gestational hypoxia. In particular, the key regulatory
369 genes controlling mitochondrial biogenesis (*Tfam* and *Pgc1α*) were both down-
370 regulated in the hypoxia-exposed group compared to the controls. *Tfam* is the master
371 regulator of mitochondrial biogenesis via gene expression from the mitochondrial
372 genome (Picca & Lezza, 2015) and *Pgc1α* regulates mitochondrial biogenesis via
373 nuclear gene expression (Picca & Lezza, 2015). Alongside the observed reduction in
374 mtDNA copy number, there is thus evidence that both key mechanisms regulating
375 mitochondrial biogenesis are impaired following exposure to gestational hypoxia.
376 Evidence of a mitochondrial deficit is particularly interesting as oviductal function
377 depends on ciliary motility and coordinated smooth muscle contraction (Halbert *et al.*,
378 1976; Bylander *et al.*, 2013; Zhao *et al.*, 2015). Both of these processes are dependent
379 on normal mitochondrial function and ATP production (Dirksen & Zeira, 1981; Lydrup
380 & Hellstrand, 1986), in particular in the ciliated cells of the oviduct epithelium.
381 Oviductal ultra-structure, including mitochondria in the ciliated epithelial cells, appears
382 to be established mainly during late fetal life (Kenngott *et al.*, 2008; Zhao *et al.*, 2015),
383 which correlates with the timing of exposure to a chronic hypoxic environment in our
384 study.

385

386 There is remarkably little published evidence regarding oviductal phenotype in other
387 developmental programming models, despite the plethora of studies that have examined
388 ovarian reserve (Bernal *et al.*, 2010; Aiken *et al.*, 2013; Chan *et al.*, 2015a; Aiken *et*
389 *al.*, 2016). However, at least one previous study has examined the impact of a maternal
390 low protein diet on mtDNA copy number and telomere length in the oviduct (Aiken *et*
391 *al.*, 2013). In keeping with our findings here, oviductal telomere length was shown to
392 be particularly sensitive to the early life environment, more so than the somatic ovarian
393 tissue (Aiken *et al.*, 2013), an effect that was magnified with increasing age (Aiken *et*
394 *al.*, 2013). In the current study, we observe the same highly significant reduction in
395 telomere length in young animals near the start of reproductive life. An important point
396 for future development of this work is to test directly whether oviductal shortening in
397 response to gestational hypoxia is magnified later in reproductive life. Interestingly, in
398 response to a maternal low protein diet, oviductal mtDNA copy number was increased
399 compared to the controls, which contrasts with our finding here. This suggests that
400 reduced mitochondrial biogenesis is a specific effect of gestational hypoxia rather than
401 a generic impact of early life stress on the oviduct. The relatively small number of
402 proteins in the developing oviduct affected by exposure to gestational hypoxia also
403 points towards a highly specific impact on cellular ageing within the oviduct, rather
404 than ubiquitous tissue damage caused by the adverse early life environment. We also
405 did not observe ubiquitous up-regulation of markers of oxidative stress in the oviducts
406 (*Hif1α, Gp91phox, P22phox, P47phox, Xo, Gpx1, Hmox1)*, which are normally highly
407 sensitive to generic tissue damage adding further evidence that the effect reported is
408 highly specific.

409

410 In keeping with the strong evidence of reduced mitochondrial biogenesis in the
411 hypoxia-exposed oviducts, we also observed an increase in mitochondrial-specific
412 antioxidant defense. MnSOD was up-regulated in the hypoxia-exposed group
413 compared to the controls, indicating that there may be an increase in reactive oxygen
414 species produced. Mitochondria are the major intracellular source of reactive oxygen
415 species, but there was no direct evidence of an increase in any of the oxidative stress
416 markers that were assayed in this study. However this may become apparent as the
417 animals age.

418

419 Oviducts are a relatively homogeneous tissue, with very low levels of telomerase
420 expression (Lee *et al.*, 2001). This is a significant advantage for our study, which
421 provides novel insight into this relatively under-studied yet crucial part of the female
422 reproductive system. A limitation of the study is the inherently tiny amount of tissue
423 available from each experimental animal (average oviductal weight ≤5mg (Sen &
424 Talwar, 1973)). This meant that the assays performed on protein, RNA and DNA had
425 to be strictly prioritised rather than testing all potential genes and proteins of interest.
426 The extremely small mass of the tissue also meant that we were unable assign tissue for
427 histological examination, or cell-type specific analysis. These are important aims for
428 future work. In particular, future work should focus on whether the muscularis or the
429 epithelium or both are affected by the phenotype described. Either could plausibly have
430 a significant influence on oviductal function and future fertility. Accelerated ageing in
431 the muscularis could affect efficient transport of gametes or conceptus, thus influencing
432 the future risk of ectopic pregnancy. Accelerated ageing in the epithelium could
433 influence the composition of the oviductal fluid, and hence the culture medium for the
434 early embryo. Assessing oviductal function *in vivo*, including assessing fertility
435 outcomes, would help to verify the implications of our results and refine our
436 understanding of the phenotype. This should form the basis of future programmes of
437 work.
438
439 Oviduct-related infertility is a key cause of female sub-fertility, accounting for ~30%
440 of cases (Kawwass *et al.*, 2013), and increases with advancing maternal age
441 (Maheshwari *et al.*, 2008). Our work suggests that there may be a developmentally
442 programmed component to the acceleration in cellular ageing and hence oviductal
443 dysfunction observed in women ≥ 35 years (Maheshwari *et al.*, 2008). The age of the
444 animals studied here equates to early in reproductive life, and hence the observed
445 evidence of cellular ageing in the oviducts is even more striking. Aside from infertility,
446 ageing of the oviducts is a significant risk factor predisposing to tubal ectopic
447 pregnancy (Nybo Andersen *et al.*, 2000), which can be a fatal complication of oviductal
448 dysfunction (Farquhar, 2005). The risk of ectopic pregnancy increases sharply with
449 maternal age from 1.4% of all pregnancies in women aged 21 years, to 6.9% of
450 pregnancies in women above the age of 44 (Nybo Andersen *et al.*, 2000). The active
451 role of the oviductal epithelium in the pathogenesis of ectopic pregnancy is becoming
452 increasingly clear (reviewed in (Horne & Critchley, 2012)) as is the requirement for

normal regulation of smooth muscle contractility (Shaw *et al.*, 2010). Hence, our finding that adult females who have been exposed to chronic gestational hypoxia show accelerated ageing and dysregulated mitochondrial biogenesis in the oviducts may have potential clinical significance not only for patients with difficulty conceiving but also in understanding risk factors for ectopic pregnancy.

Conclusion

Large numbers of pregnancies world-wide are exposed to chronic gestational hypoxia, either through pregnancy at high altitude or through utero-placental insufficiency (Ducsay, 1998; Kuzmina *et al.*, 2005; Postigo *et al.*, 2009; Giussani *et al.*, 2016). The recognition of the adverse impact of lower than normal oxygenation during pregnancy on ageing of the oviducts, with attendant consequences for gamete and embryo transport in potential next generation mothers, is an important area for further research and exploration.

Additional information

Competing interests

The authors have no competing interests to declare.

Author contributions

CEA conceptualised the study, analysed and interpreted the data, and drafted the manuscript. JTA, AMS, AMN, TJA, AR, MJS and EJC acquired and analysed the data, and critically revised the manuscript. DAG and SEO conceptualised and designed the study, and drafted the manuscript. All of the authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

CEA was supported by a grant from the Addenbrooke's Charitable Trust (ACT; RG94137) and by an Issac Newton Trust/Wellcome Trust ISSF/ University of Cambridge Joint Research Grant. SEO is supported by the MRC (MC_UU_12012/4). DAG is supported by The British Heart Foundation (PG/14/5/30546).

487 **References**
488

489 Aiken CE, Tarry-Adkins JL & Ozanne SE. (2013). Suboptimal nutrition in utero
490 causes DNA damage and accelerated aging of the female reproductive
491 tract. *FASEB J* **27,** 3959-3965.
492

493 Aiken CE, Tarry-Adkins JL & Ozanne SE. (2015). Transgenerational
494 Developmental Programming of Ovarian Reserve. *Sci Rep* **5,** 16175.
495

496 Aiken CE, Tarry-Adkins JL, Penfold NC, Dearden L & Ozanne SE. (2016).
497 Decreased ovarian reserve, dysregulation of mitochondrial biogenesis,
498 and increased lipid peroxidation in female mouse offspring exposed to an
499 obesogenic maternal diet. *FASEB J* **30,** 1548-1556.
500

501 Bernal AB, Vickers MH, Hampton MB, Poynton RA & Sloboda DM. (2010).
502 Maternal undernutrition significantly impacts ovarian follicle number and
503 increases ovarian oxidative stress in adult rat offspring. *PLoS One* **5,**
504 e15558.
505

506 Bylander A, Lind K, Goksor M, Billig H & Larsson DG. (2013). The classical
507 progesterone receptor mediates the rapid reduction of fallopian tube
508 ciliary beat frequency by progesterone. *Reprod Biol Endocrinol* **11,** 33.
509

510 Camm EJ, Hansell JA, Kane AD, Herrera EA, Lewis C, Wong S, Morrell NW &
511 Giussani DA. (2010). Partial contributions of developmental hypoxia and
512 undernutrition to prenatal alterations in somatic growth and
513 cardiovascular structure and function. *Am J Obstet Gynecol* **203,** 495 e424-
514 434.
515

516 Camm EJ, Martin-Gronert MS, Wright NL, Hansell JA, Ozanne SE & Giussani DA.
517 (2011). Prenatal hypoxia independent of undernutrition promotes
518 molecular markers of insulin resistance in adult offspring. *FASEB J* **25,**
519 420-427.
520

521 Chan KA, Bernal AB, Vickers MH, Gohir W, Petrik JJ & Sloboda DM. (2015a). Early
522 life exposure to undernutrition induces ER stress, apoptosis, and reduced
523 vascularization in ovaries of adult rat offspring. *Biol Reprod* **92,** 110.
524

525 Chan KA, Tsoulis MW & Sloboda DM. (2015b). Early-life nutritional effects on the
526 female reproductive system. *J Endocrinol* **224,** R45-62.
527

528 Depmann M, Faddy MJ, van der Schouw YT, Peeters PH, Broer SL, Kelsey TW,
529 Nelson SM & Broekmans FJ. (2015). The Relationship Between Variation
530 in Size of the Primordial Follicle Pool and Age at Natural Menopause. *J Clin*
531 *Endocrinol Metab* **100,** E845-851.
532

533 Dirksen ER & Zeira M. (1981). Microtubule sliding in cilia of the rabbit trachea
534 and oviduct. *Cell Motil* **1,** 247-260.
535

536 Ducsay CA. (1998). Fetal and maternal adaptations to chronic hypoxia:
537 prevention of premature labor in response to chronic stress. *Comp*
538 *Biochem Physiol A Mol Integr Physiol* **119,** 675-681.
539
540 Farquhar CM. (2005). Ectopic pregnancy. *Lancet* **366,** 583-591.
541
542 Giussani DA, Bennet L, Sferruzzi-Perri AN, Vaughan OR & Fowden AL. (2016).
543 Hypoxia, fetal and neonatal physiology: 100 years on from Sir Joseph
544 Barcroft. *J Physiol* **594,** 1105-1111.
545
546 Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE, Gottschalk R, Blake EZ,
547 Horder KA, Thakor AS, Hansell JA, Kane AD, Wooding FB, Cross CM &
548 Herrera EA. (2012). Developmental programming of cardiovascular
549 dysfunction by prenatal hypoxia and oxidative stress. *PLoS One* **7,** e31017.
550
551 Giussani DA & Davidge ST. (2013). Developmental programming of
552 cardiovascular disease by prenatal hypoxia. *J Dev Orig Health Dis* **4,** 328-
553 337.
554
555 Giussani DA, Phillips PS, Anstee S & Barker DJ. (2001). Effects of altitude versus
556 economic status on birth weight and body shape at birth. *Pediatr Res* **49,**
557 490-494.
558
559 Gonzalez-Candia A, Veliz M, Araya C, Quezada S, Ebensperger G, Seron-Ferre M,
560 Reyes RV, Llanos AJ & Herrera EA. (2016). Potential adverse effects of
561 antenatal melatonin as a treatment for intrauterine growth restriction:
562 findings in pregnant sheep. *Am J Obstet Gynecol* **215,** 245 e241-247.
563
564 Gonzalez-Rodriguez P, Jr., Tong W, Xue Q, Li Y, Hu S & Zhang L. (2013). Fetal
565 hypoxia results in programming of aberrant angiotensin ii receptor
566 expression patterns and kidney development. *Int J Med Sci* **10,** 532-538.
567
568 Halbert SA, Tam PY & Blandau RJ. (1976). Egg transport in the rabbit oviduct: the
569 roles of cilia and muscle. *Science* **191,** 1052-1053.
570
571 Herrera EA, Camm EJ, Cross CM, Mullender JL, Wooding FB & Giussani DA.
572 (2012). Morphological and functional alterations in the aorta of the
573 chronically hypoxic fetal rat. *J Vasc Res* **49,** 50-58.
574
575 Ho SM, Cheong A, Adgent MA, Veevers J, Suen AA, Tam NNC, Leung YK, Jefferson
576 WN & Williams CJ. (2017). Environmental factors, epigenetics, and
577 developmental origin of reproductive disorders. *Reprod Toxicol* **68,** 85-
578 104.
579
580 Horne AW, Brown JK, Nio-Kobayashi J, Abidin HB, Adin ZE, Boswell L, Burgess S,
581 Lee KF & Duncan WC. (2014). The association between smoking and
582 ectopic pregnancy: why nicotine is BAD for your fallopian tube. *PLoS One*
583 **9,** e89400.
584

585 Horne AW & Critchley HO. (2012). Mechanisms of disease: the endocrinology of
586 ectopic pregnancy. *Expert Rev Mol Med* **14,** e7.
587
588 Jette N & Lees-Miller SP. (2015). The DNA-dependent protein kinase: A
589 multifunctional protein kinase with roles in DNA double strand break
590 repair and mitosis. *Prog Biophys Mol Biol* **117,** 194-205.
591
592 Kawwass JF, Crawford S, Kissin DM, Session DR, Boulet S & Jamieson DJ. (2013).
593 Tubal factor infertility and perinatal risk after assisted reproductive
594 technology. *Obstet Gynecol* **121,** 1263-1271.
595
596 Kenngott RA, Neumuller C & Sinowatz F. (2008). Prenatal differentiation of
597 bovine oviductal epithelium: an electron microscopic study. *Anat Histol*
598 *Embryol* **37,** 418-426.
599
600 Keyes LE, Armaza JF, Niermeyer S, Vargas E, Young DA & Moore LG. (2003).
601 Intrauterine growth restriction, preeclampsia, and intrauterine mortality
602 at high altitude in Bolivia. *Pediatr Res* **54,** 20-25.
603
604 Kuzmina IY, Hubina-Vakulik GI & Burton GJ. (2005). Placental morphometry and
605 Doppler flow velocimetry in cases of chronic human fetal hypoxia. *Eur J*
606 *Obstet Gynecol Reprod Biol* **120,** 139-145.
607
608 Lee YL, Lee KF, Xu JS, Wang YL, Tsao SW & Yeung WS. (2001). Establishment and
609 characterization of an immortalized human oviductal cell line. *Mol Reprod*
610 *Dev* **59,** 400-409.
611
612 Lydrup ML & Hellstrand P. (1986). Rate of oxidative and glycolytic metabolism in
613 the guinea-pig oviduct in relation to contractility and hormonal cycle.
614 *Acta Physiol Scand* **128,** 525-533.
615
616 Maheshwari A, Hamilton M & Bhattacharya S. (2008). Effect of female age on the
617 diagnostic categories of infertility. *Hum Reprod* **23,** 538-542.
618
619 Nio-Kobayashi J, Abidin HB, Brown JK, Iwanaga T, Horne AW & Duncan WC.
620 (2016). Cigarette smoking alters sialylation in the Fallopian tube of
621 women, with implications for the pathogenesis of ectopic pregnancy. *Mol*
622 *Reprod Dev* **83,** 1083-1091.
623
624 Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J & Melbye M. (2000).
625 Maternal age and fetal loss: population based register linkage study. *BMJ*
626 **320,** 1708-1712.
627
628 Parada-Bustamante A, Orostica ML, Reuquen P, Zuniga LM, Cardenas H &
629 Orihuela PA. (2016). The role of mating in oviduct biology. *Mol Reprod*
630 *Dev* **83,** 875-883.
631

632 Pelosi E, Simonsick E, Forabosco A, Garcia-Ortiz JE & Schlessinger D. (2015).
633 Dynamics of the ovarian reserve and impact of genetic and
634 epidemiological factors on age of menopause. *Biol Reprod* **92,** 130.
635
636 Phillips TJ, Scott H, Menassa DA, Bignell AL, Sood A, Morton JS, Akagi T, Azuma K,
637 Rogers MF, Gilmore CE, Inman GJ, Grant S, Chung Y, Aljunaidy MM, Cooke
638 CL, Steinkraus BR, Pocklington A, Logan A, Collett GP, Kemp H, Holmans
639 PA, Murphy MP, Fulga TA, Coney AM, Akashi M, Davidge ST & Case CP.
640 (2017). Treating the placenta to prevent adverse effects of gestational
641 hypoxia on fetal brain development. *Sci Rep* **7,** 9079.
642
643 Picca A & Lezza AM. (2015). Regulation of mitochondrial biogenesis through
644 TFAM-mitochondrial DNA interactions: Useful insights from aging and
645 calorie restriction studies. *Mitochondrion* **25,** 67-75.
646
647 Postigo L, Heredia G, Illsley NP, Torricos T, Dolan C, Echalar L, Tellez W,
648 Maldonado I, Brimacombe M, Balanza E, Vargas E & Zamudio S. (2009).
649 Where the O2 goes to: preservation of human fetal oxygen delivery and
650 consumption at high altitude. *J Physiol* **587,** 693-708.
651
652 Practice Committee of the American Society for Reproductive M. (2015). Role of
653 tubal surgery in the era of assisted reproductive technology: a committee
654 opinion. *Fertil Steril* **103,** e37-43.
655
656 Radicella JP, Dherin C, Desmaze C, Fox MS & Boiteux S. (1997). Cloning and
657 characterization of hOGG1, a human homolog of the OGG1 gene of
658 Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **94,** 8010-8015.
659
660 Richter HG, Camm EJ, Modi BN, Naeem F, Cross CM, Cindrova-Davies T, Spasic-
661 Boskovic O, Dunster C, Mudway IS, Kelly FJ, Burton GJ, Poston L &
662 Giussani DA. (2012). Ascorbate prevents placental oxidative stress and
663 enhances birth weight in hypoxic pregnancy in rats. *J Physiol* **590,** 1377-
664 1387.
665
666 Robertson SA, Chin PY, Schjenken JE & Thompson JG. (2015). Female tract
667 cytokines and developmental programming in embryos. *Adv Exp Med Biol*
668 **843,** 173-213.
669
670 Roost M, Altamirano VC, Liljestrand J & Essen B. (2009). Priorities in emergency
671 obstetric care in Bolivia--maternal mortality and near-miss morbidity in
672 metropolitan La Paz. *BJOG* **116,** 1210-1217.
673
674 Sen KK & Talwar GP. (1973). Similarities and differences in the binding of
675 oestradiol-17beta to rat oviduct and uterus. *J Reprod Fertil* **35,** 369-372.
676
677 Shaw JL, Denison FC, Evans J, Durno K, Williams AR, Entrican G, Critchley HO,
678 Jabbour HN & Horne AW. (2010). Evidence of prokineticin dysregulation
679 in fallopian tube from women with ectopic pregnancy. *Fertil Steril* **94,**
680 1601-1608 e1601.

681
682 Tarry-Adkins JL, Aiken CE, Ashmore TJ & Ozanne SE. (2018). Insulin-signalling
683 dysregulation and inflammation is programmed trans-generationally in a
684 female rat model of poor maternal nutrition. *Sci Rep* **8,** 4014.
685
686 Tarry-Adkins JL, Chen JH, Smith NS, Jones RH, Cherif H & Ozanne SE. (2009). Poor
687 maternal nutrition followed by accelerated postnatal growth leads to
688 telomere shortening and increased markers of cell senescence in rat
689 islets. *FASEB J* **23,** 1521-1528.
690
691 Tarry-Adkins JL, Fernandez-Twinn DS, Madsen R, Chen JH, Carpenter A,
692 Hargreaves IP, McConnell JM & Ozanne SE. (2015). Coenzyme Q10
693 Prevents Insulin Signaling Dysregulation and Inflammation Prior to
694 Development of Insulin Resistance in Male Offspring of a Rat Model of
695 Poor Maternal Nutrition and Accelerated Postnatal Growth. *Endocrinology*
696 **156,** 3528-3537.
697
698 Tarry-Adkins JL, Ozanne SE, Norden A, Cherif H & Hales CN. (2006). Lower
699 antioxidant capacity and elevated p53 and p21 may be a link between
700 gender disparity in renal telomere shortening, albuminuria, and
701 longevity. *Am J Physiol Renal Physiol* **290,** F509-516.
702
703 Wang S & Larina IV. (2018). In vivo three-dimensional tracking of sperm
704 behaviors in the mouse oviduct. *Development* **145.**
705
706 Zhao W, Zhu Q, Yan M, Li C, Yuan J, Qin G & Zhang J. (2015). Levonorgestrel
707 decreases cilia beat frequency of human fallopian tubes and rat oviducts
708 without changing morphological structure. *Clin Exp Pharmacol Physiol* **42,**
709 171-178.
710
711

712

713

Gene	Normoxia	Hypoxia	
Ppia	32234 ±2363	28269±3394	NS
P53	10775±1237	13417±1332	0.09
P21	5188±1053	9292±1374	0.04
Alox12	3120±744	7714±2089	0.05
Alox15	925±225	854±147	NS
Ogg1	1294±135	1710±132	0.03
Neil1	769± 63	730±117	NS
Nth1	1505±27	1329±151	NS
Xrrc1	2675±375	2175±372	NS
Nrf2	11560±1704	7555±893	NS
Dna pkcs	2134±323	1421±192	0.1
Mre11	723±119	307±79	0.04
Ku70	2380±397	1533±389	NS
Ku80	8743±1410	5709±1219	NS
Bax	2093±199	1750±329	NS
Bcl2	4036±530	2599±293	0.05
BaxBcl2	0.41±0.02	0.5±0.08	NS
Tfam	6447±844	3866±632	0.04
Pgc1a	1806±121	903±236	0.01
Cs	18621±2551	9627±156	0.02
Lonp1	7518±874	7262±1035	NS
Cycs	27321±4613	15812±4446	0.08
Complex I	26745±721	22123±2086	0.01
Complex II	19112±3730	14311±1389	NS
Complex III	27555±4854	18414±1721	NS
Complex IV	46402±4883	33668±1533	0.05
Hif	8172±791	8276±628	NS
Gp91phox	6191±1727	6904±1023	NS
P22phox	5128±1081	7298±1030	NS
P47phox	1887±136	2620±631	NS
Xo	19493±2381	15989±1793	NS
Gpx1	67342±11501	34576±8409	NS
Hmox1	3492±202	3720±255	NS
Catalase	12593±1716	13651±280	NS
Nfkβ	6419±476	6073±307	NS
Mnsod	9286±2005	15399±577	0.04
Cuznsod	171954±8398	160528±13018	NS
Ecsod	35354±3730	23778±3163	NS

714
715 **Table 1** Effect of gestational hypoxia compared to normoxia on gene expression in the
716 oviducts of adult female rats. All reported p values have been adjusted to take account
717 of multiple hypothesis testing. n=7-8 for all groups (n refers to the number of litters)

718
719

Protein	Normoxia	Hypoxia	
P53	100±17	158±19	0.05*
$P16^{INK}$	100±30	100±24	NS
OGG1	100±22	137±13	0.08
MRE11	100±30	77±22	NS
KU70	100±10	58±12	0.03*
KU80	100±18	115±18	NS
Complex I	100±36	142±56	NS
Complex II	100±29	150±38	NS
Complex III	100±15	96±18	NS
Complex IV	100±22	137±31	NS
Complex V	100±2	108±6	NS
CS	100±13	110±16	NS
HIF1α	100±12	124±15	NS
$GP91^{phox}$	100±27	97±15	NS
$P47^{phox}$	100±24	119±4	NS
XO	100±10	92±11	NS
HMOX1	100±44	37±11	NS
CATALASE	100±10	125±23	NS
MnSOD	100±9	156±10	<0.01**
CuZnSOD	100±30	94±23	NS

Table 2 Effect of gestational hypoxia compared to normoxia on protein expression in the oviducts of adult female rats. All reported p values have been adjusted to take account of multiple hypothesis testing. *$p<0.05$, **$p<0.01$. n=7-8 for all groups (n refers to the number of litters)

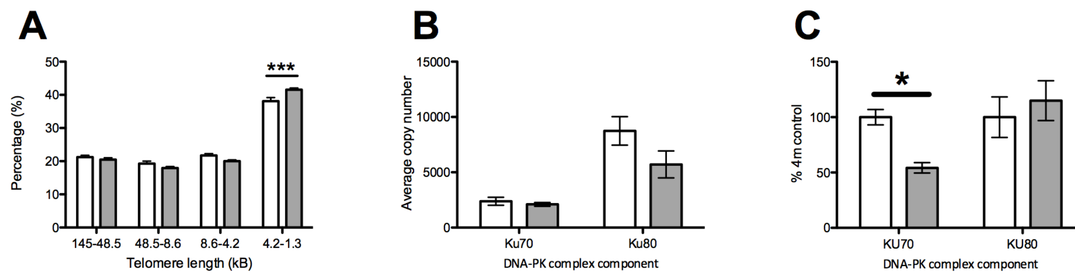
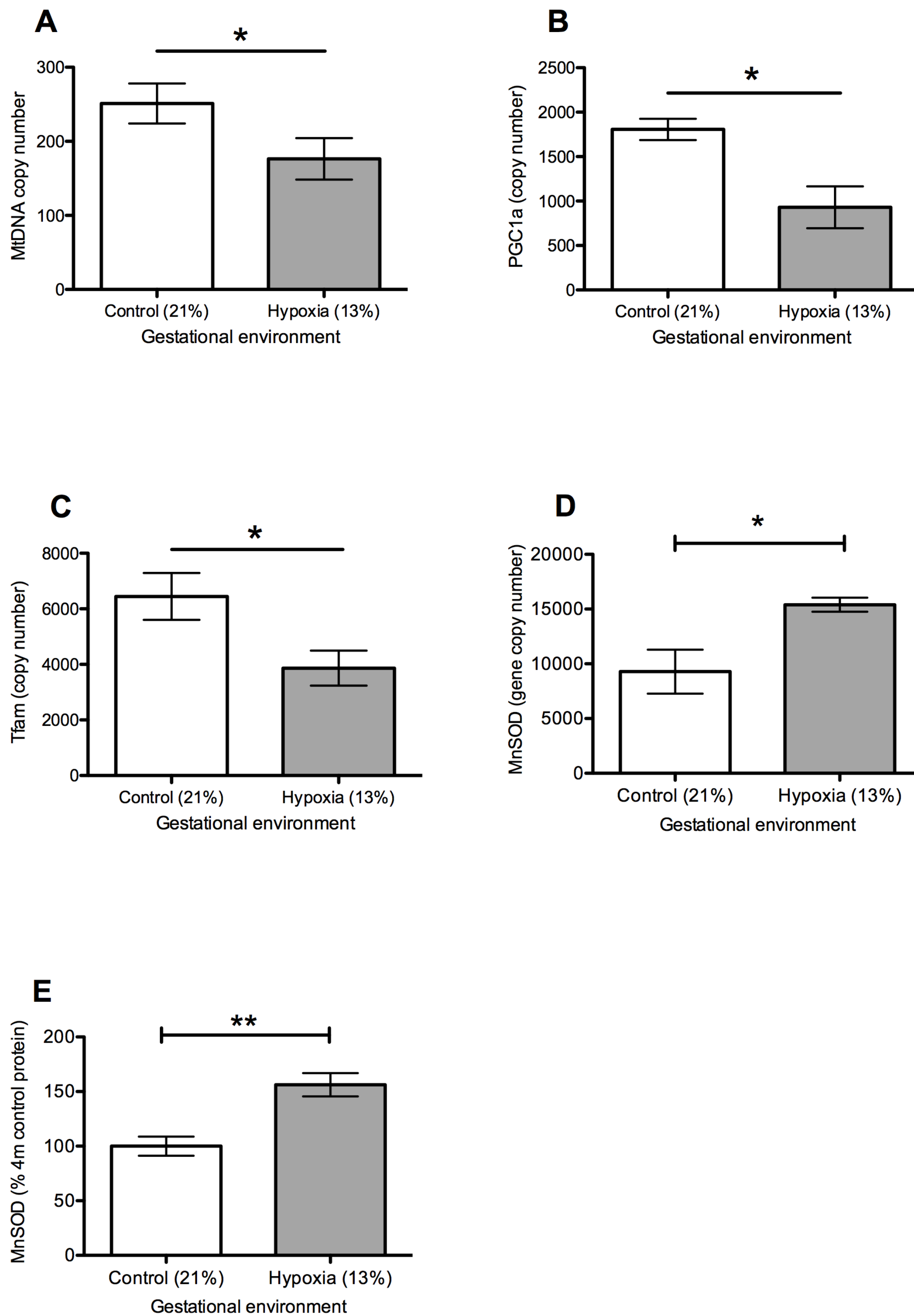


Figure 1 A) Oviductal telomere length in adult female rats exposed to gestational hypoxia compared to normoxia. **B)** Effect of gestational hypoxia compared to normoxia on gene expression of components (*Ku70* and *Ku80*) of the DNA-activated protein kinase (DNA-PK) in the oviducts. **C)** Effect of gestational hypoxia compared to normoxia on protein expression of KU70 and KU80. Data shown as mean ± SEM. Open bars: normoxia (21% oxygen) during gestation, grey bars: hypoxia (13% oxygen) during gestation. *$p<0.05$, ***$p<0.001$. n=7-8 for all groups (n refers to the number of litters)



738
739 **Figure 2** Effect of gestational hypoxia compared to normoxia on expression of
740 mitochondrial biogenesis regulators and mitochondrial anti-oxidant defense in the
741 oviducts. Data shown as mean ± SEM. Open bars: normoxia (21% oxygen) during
742 gestation, grey bars: hypoxia (13% oxygen) during gestation. **A)** MtDNA copy number,
743 **B)** *Tfam* gene expression, **C)** *Pgc1α* gene expression, **D)** *MnSOD* gene expression, **E)**

744 MnSOD protein expression. *$p<0.05$, **$p<0.01$. $n=7$-8 for all groups (n refers to the
745 number of litters)
746