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Early experiences matter: a review of the effects of prenatal environment on offspring characteristics in poultry

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Early experiences matter: a review of the effects of prenatal environment on offspring characteristics in poultry

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Review

1 Title Page

2 PRENATAL EFFECTS ON POULTRY OFFSPRING

3 **Early experiences matter: a review of the effects of prenatal environment on**
4 **offspring characteristics in poultry**

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6

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23

24 **ABSTRACT**

25

26 Early life experiences can be important in determining offspring phenotypes and may
27 influence interaction with the environment and hence health, welfare and productivity.

28 The prenatal environment of poultry can be divided into the pre-lay environment and
29 the egg storage/incubation environment, both of which can affect offspring outcomes.

30 The ability to separate maternal and egg/incubation effects makes birds well suited to
31 this type of research. There are many factors including feeding and nutrition,

32 environmental conditions, husbandry practices, housing system, social environment,

33 infectious environment and maternal health status that can influence both the health

34 and performance and behavior and cognition of the offspring. There are some aspects

35 of the environments that can be changed to produce beneficial effects in the offspring,

36 like addition of certain additives to feed or short changes in incubation temperatures,

37 while other aspects should be avoided to reduce negative effects, such as

38 unpredictable feeding and lighting regimens. Measures of offspring characteristics

39 may prove to be a useful method of assessing parent stock welfare if known stressors

40 result in predictable offspring outcomes. This has the advantage of assessing the

41 parent environment without interfering with the animals and possibly affecting their

42 responses and could lead to improved welfare for the animals.

43 **Key words:** early experiences, prenatal environment, posthatch performance, welfare

44

45

46

INTRODUCTION

47 The early life (prenatal and neonatal) period has been found to be extremely important
48 in shaping animal phenotypes throughout life. Examples of how variation in
49 experience at the very start of life can impact upon lifetime biology have been seen
50 across a wide range of different animal groups, including mammals, fish and birds
51 (reviewed by Rutherford et al., 2012). Similarly the human fetal environment has
52 been shown to have lasting effects on offspring phenotype with correlations being
53 found between low birth weight and later ill health (Barker, 2004). For farmed species
54 it is becoming increasingly apparent that the early life environment may be critical in
55 determining how well individual animals cope with their postnatal environment and
56 has consequential effects on farm performance and individual animal health and
57 welfare. In this paper, we will use the definition of welfare which refers to an
58 animal's state in relation to its environment and its ability to cope in that environment.
59 Animals that fail to cope or have difficulty coping in an environment are said to have
60 poor welfare (Broom 1991). As prenatal experiences determine how well an animal
61 fits with its postnatal environment, prenatal experiences can affect welfare.

62

63 In the case of avian species, both pre-lay and egg storage/incubation (referred
64 to subsequently as 'incubation') periods can influence the post-hatch development and
65 responses of individual birds. Factors in the prenatal environment have been shown to
66 have lasting effects on how the offspring respond to their environment (reviewed by
67 Lickliter, 2005; Henriksen et al., 2011). It has been demonstrated that in addition to
68 genetic factors a range of maternal and early life environmental factors can influence
69 development, physiology and behavior of the progeny (e.g. Lickliter, 2005). For
70 example, prenatal exposure to androgens can influence growth and activity levels in

71 the chicks (e.g. Eising et al., 2006) and incubator-hatched quail chicks respond
72 selectively to the maternal calls of their own species and show attention biases that
73 help lead them to the proximity of their mother (e.g. Heaton et al., 1978). This
74 phenomenon has begun to receive more attention in the past few decades and has led
75 to a variety of studies of the effects of prenatal environment on poultry.

76

77 Avian species prenatal environments differ from that of mammals and can be
78 divided into two phases: pre-lay and incubation. Consequently the maternal
79 environment and the incubation/egg environment can have independent and lasting
80 effects on the offspring (Mousseau and Fox, 1998; Lay and Wilson, 2002; Henriksen
81 et al., 2011). This characteristic makes birds useful in the study of early life
82 environment as influences directly from the mother (e.g. nutrition levels or antibody
83 deposition) and the environment (e.g. temperature, day length) can be separated
84 (Lickliter, 2005). The short generation intervals of some avian species such as
85 chickens and quail also make them ideally suited for research. As a consequence,
86 there are a number of studies designed to manipulate the pre-lay or incubating
87 environment to assess subsequent effects on progeny. Thus the purpose of this paper
88 is to review how stressors applied to the maternal parent and embryo affect the **health**
89 **and** welfare of the offspring. There have been studies comparing the genetic
90 contributions of the paternal parent on, e.g. offspring body weight (Dottavio et al.,
91 2005) or immunity (Li et al., 2008), but the effects of stressors applied to the sire on
92 offspring outcomes has received little attention and will not be covered here. Due to
93 the disparate nature of literature relating to this topic, and to ensure a transparent and
94 repeatable selection process, a systematic review (SR) approach was adopted (Arnott
95 et al., 2012).

96

97 SR use scientific review methods to minimize systematic and random errors
98 (Cook et al., 1997) that may occur in a more traditional narrative review where the
99 author may have pre-conceived opinions and biases regarding a topic (Sargeant et al.,
100 2006). Specific search strategies and inclusion criteria are used in SR, eliminating
101 selection bias and subjecting all studies to critical appraisal. Generally, SR use a
102 quantitative analysis approach, such as meta-analysis. However, as with the Arnott et
103 al. (2012) review, this was considered unsuitable due to the broad nature of the review
104 topic, and therefore a systematic, transparent search strategy that could be repeated by
105 others and a protocol to identify suitable studies to include was developed. Once these
106 studies were identified, a narrative approach was used due to the broad review
107 questions that examined many outcomes and a relatively small number of appropriate
108 studies to examine the results and formulate reasonable conclusions.

109

110

MATERIALS AND METHODS

111 *Searches*

112 The online database 'ISI Web of Knowledge', which searches simultaneously ISI
113 Web of Science (1970-present), MEDLINE (1950-present) and BIOSIS Previews
114 (1969-present), was used to examine the literature. The search terms were
115 combinations of words relating to prenatal and incubation stress. The final search
116 string is given below (asterisks indicate where the electronic database looked for
117 words beginning with stated letters, for example chicken* was used to find references
118 relating to the words 'chicken' and 'chickens');

119 (prenatal or maternal or perinatal or gestation*) and (stress or programm* or
120 nutrition* or effect*) and (poultry or chicken* or chick* or hen or bird* or avian)

121

122 Additionally detailed searches of several relevant journals was conducted (e.g.
123 *Applied Animal Behavior Science, Poultry Science, Animal Welfare, British Poultry*
124 *Science*) and any articles deemed relevant but missed in the database search were
125 included. This was all conducted as per the methods described in Arnott et al. (2012).

126

127 ***Search Results***

128 The initial search was conducted in Web of Knowledge on 07 Dec 2009, with updates
129 added after a repeated search using similar terms on 09 July 2011 and 16 Oct 2013.
130 After removal of duplicates, the search yielded 3165 references in both English and
131 non-English languages. The references and any available abstracts were imported into
132 Reference Manager (Thomson Reuters, New York, USA) bibliographic database for
133 manipulation and were then classified as outlined below.

134

135 ***Classification of Results***

136 A general overview of the result classifications will be presented here as the
137 procedures and categories have been used in another publication (please refer to
138 Arnott et al. (2012) for a detailed explanation).

139

140 Initially the references were screened for relevance and any that were clearly
141 irrelevant, or were review articles were removed (2830 papers removed which were
142 irrelevant, leaving 335). The remaining papers were examined in more detail and
143 classified according to whether they contained maternal outcomes only (i.e. with no

144 progeny measures) or whether they investigated aspects of maternal nutrition or
145 applied some form of stressor to the hen prior to ovulation or the egg during
146 incubation and then investigated offspring outcomes. Stressor here refers to any form
147 of non-nutritional challenge with impacts on hen biology.

148

149 As an aim of this review was to identify pre-hatch hazards that affect offspring
150 **health and** welfare, studies were classified according to the early life treatment
151 applied. Early life hazards were classified under the following headings: Feeding
152 Method and Nutrition, Environmental Parameters, Artificial Challenges, Husbandry,
153 Housing System, Social Environment, Infectious Environment and Maternal Health
154 Status.

155

156 *Quality Assessment*

157 A quality assessment of the studies was made as recommended by Sargeant et al.,
158 (2006), using the following protocol which was largely adopted from the REFLECT
159 statement (O'Connor et al., 2010). Any studies not meeting these standards were
160 discarded (for full details see Arnott et al., 2012). The criteria used included:

- 161 1) Randomization: individuals randomly allocated to treatment groups
- 162 2) Treatment intervention: experimental treatment intervention clearly described
- 163 3) Control: use of a suitable control group
- 164 4) Sample size: use of a sample size of five experimental units or more (as
165 recommended by Festings and Altmann, 2002)
- 166 5) Statistical Methods: use of appropriate and clear statistical methods
- 167 6) Avoidance of data repetition: multiple publications reporting on same data

168 7) Exclusion of conference abstracts/proceedings

169 After quality assessment, one reference was identified as a conference proceeding
170 and 5 references failed to pass sample size, statistical methods and control criteria and
171 were removed. In total, 108 articles proceeded to the final data extraction process of
172 the systematic review.

173

174 RESULTS

175 *Feeding Method and Nutrition*

176 The nutritional status of the mother and of the egg during incubation can affect the
177 health and growth rate of the embryo and in some cases may have lasting effects later
178 in the animal's life (Couch and Ferguson, 1975; McCellan and Novak, 2001).

179

180 *Effects on Offspring Health and Performance.* The majority of studies
181 conducted in poultry were designed to test the effects of supplementing a basal feed
182 (designed to meet the nutritional requirements of the animal) on offspring
183 performance and health (28 out of 34 papers that made up the Feeding Method and
184 Nutrition hazard category, a subset of the total 108 papers). For example, carotenoids
185 are thought to confer many benefits including prevention of some infections (Chew
186 and Park, 2004), regulation of cell proliferation and differentiation (Livny et al., 2002)
187 and enhanced antioxidant capacity (Stahl and Sies, 2003). In hens, carotenoids are
188 deposited into the egg yolk and give the yolk its characteristic yellow-orange color
189 (Surai, 2002) but they also provide other benefits to the chick: Feeding broiler
190 breeders carotenoid-supplemented feed led to an increased concentration of
191 carotenoid in the egg yolks (Karadas et al., 2005), improved hatching rates and

192 enhanced the antibacterial activity of the egg (Cucco et al., 2007), while the immune
193 response in the hatchlings was significantly improved. (Haq et al., 1996).

194

195 Supplementing maternal diets with vitamin E (150 and 450 p.p.m.) two weeks
196 prior to an immune challenge (2 mL intramuscular injection of *Brucella abortus* and
197 Freund's incomplete adjuvant in a 1:1 ratio) increased antibody levels in the plasma
198 of chicks at 2 and 7 days of age; however offspring from hens fed 90, 300 and 900
199 p.p.m. vitamin E did not exhibit any response. This inconsistent relationship between
200 vitamin E and antibody transfer has also been found in other species (Jackson et al.,
201 1977). Additionally, vitamin E, as well as **some** minerals, can have beneficial effects
202 on reproductive performance. Taiwan native breeder hens given 40-120 mg/kg
203 vitamin E and broiler breeders given 0.5 mg/kg Se in their basal diet had increased
204 fertility and hatchability of offspring (Tsai et al., 2008) and decreased embryonic
205 mortality (Pappas et al., 2006). However, some supplements, like high concentrations
206 of vitamin D₃ (cholecalciferol) (2500-5000 µg/kg) or added fats (sunflower or fish
207 oil), resulted in decreased fertility and hatchability compared to low (24 µg/kg) or
208 unsupplemented concentrations (Ameenuddin et al., 1986; Pappas et al., 2006;
209 Bozkurt et al., 2008). Similarly high concentrations of Zinc (150 µg/g) in laying hen
210 diets may cause marginal immunosuppression in the chicks but does seem to affect
211 their growth (Stahl et al., 1989).

212

213 Vitamin D₃ given to broiler breeders can however improve leg health in the
214 offspring by decreasing the incidence of tibial dyschondroplasia and decreasing
215 rickets scores, while body weights and feed intake increase with increasing
216 supplementation (D₃: up to 4000 IU/kg) (Atencio et al., 2005a,b; Driver et al., 2006).

217 D₃ supplementation can also lead to faster morphological gut maturity, and as a result
218 function, in the offspring when the hens are provided 4000 IU/kg compared to a
219 control diet of 1000 IU/kg (Ding et al., 2011). Suboptimal nutrition of the hen and/or
220 the developing embryo can also, unsurprisingly, affect the offspring. Malnutrition
221 during incubation (achieved by removing quantities of albumen from the egg) can
222 lead to raised corticosterone levels, brain sparing through the diversion of well
223 oxygenated blood to the brain at the possible expense of other organs (measured by
224 calculating the mean brain to body weight ratio), low hatch rates in the chicks
225 (Rodricks et al., 2004) and decreased post hatch body weights up to 7 days of age
226 (Everaert et al., 2013). However, during the laying phase (18 to 55 weeks of age) hens
227 hatched from eggs that had partial albumen removal had higher body weights than
228 control and sham removal birds but they also had reduced egg weights, laying rates,
229 egg mass and increased number of second grade eggs (Willems et al., 2013).
230 Restricting Dwarf broiler breeders to 90% of what they would eat *ad libitum* resulted
231 in decreased egg production, reduced hatchability and decreased egg and chick
232 weights compared to *ad libitum* fed birds (Triyuwanta and Nys, 1992); however when
233 these birds were fed *ad libitum*, they had reduced fertility rates compared to restricted
234 birds (Triyuwanta and Nys, 1990). A more severe feed restriction (or complete feed
235 withdrawal) can also be used in forced moulting to ‘reset’ the reproductive system of
236 birds and results in heavier eggs with better hatchability than the eggs being produced
237 before the moult (Tona et al., 2002); although there is hen mortality and decreased
238 immune function associated with this procedure (Patwardhan and King, 2011).

239

240 ***Effects on Offspring Behavior and Cognition.*** Maternal nutrition can affect
241 the behavior and cognition of the offspring. For example, laying partridges fed feed

242 supplemented with higher concentrations of n-3 fatty acids (4.40 g/kg) had chicks that
243 performed better in a memory retention task at one day of age than chicks from hens
244 receiving a lower concentration of n-3 fatty acid (0.48 g/kg) (Fronte et al., 2008).
245 Also, the method of feeding itself can have affects on the offspring. Laying hens that
246 were exposed to an unpredictable food restriction schedule had chicks with longer
247 tonic immobility durations, which is thought to be an indicator of fear, than *ad libitum*
248 fed birds (Janczak et al., 2007a).

249

250 Thus there is good evidence that additional supplementation of the hen's feed
251 above the recommended NRC standards with some vitamins or minerals can result in
252 improved growth, development and/or learning in the offspring. Restricting nutrients
253 has mixed results on the offspring – hatched chicks may be smaller than those of *ad*
254 *libitum* fed birds but free feeding of some strains, like broiler breeders, can lead to
255 decreased fertility and hatchability. In addition, there are still a number of unresolved
256 welfare issues surrounding feed restriction of parent stocks (see Decuypere et al.,
257 2010).

258

259 ***Environmental Parameters***

260 There are a number of environmental or climatic parameters that could affect the
261 offspring, including the pre-lay environment of the hen and the environment that the
262 embryo is exposed to between ovulation and hatching.

263

264 ***Effects on Offspring Health and Performance.*** Incubation temperature or
265 fluctuations in temperature can have both positive and negative influences on the
266 hatched chicks. For example, increasing the incubation temperature from 37.8 to

267 39.5⁰C and the Relative Humidity (RH) from 56% to 65% for three hours a day
268 during the second week of incubation (days 8-10) increased chick hatchability
269 compared to standard environmental parameters or periodically increasing the
270 temperature and RH during the last week of incubation (days 16-18) (Collin et al.,
271 2007). Eggs exposed for short periods of time (3 h) to increased temperature (to 39.5
272 or 41⁰C) later in incubation still had chicks with increased hatchability compared to
273 standard incubation parameters (Yahav et al., 2004). Periodic exposure to cold
274 temperatures (30 minutes on day 18 and two 30 minute sessions on day 19 of
275 incubation) was also beneficial, resulting in chicks hatching with higher body weights
276 than those not exposed to cold stress (Shinder et al., 2009). Embryos that were cooled
277 for 6 h/day from days 15-18 also had enhanced thyroxine response to thyrotrophin
278 releasing hormone which may help the chick cope with later stressors (Decuyper et
279 al., 1988). However longer exposures to relative extremes of temperature (e.g.
280 temperature reductions to 36.9-38⁰C for 6 h/day for the first 8 days of incubation) led
281 to reductions in hatchability or chicks with decreased body weights (Byerly, 1938 in
282 Oppenheim and Levin, 1975; Yalcin and Siegel, 2003). Reduced temperatures
283 (36.7⁰C) early in incubation also led to broiler chicks with reduced relative shank and
284 femur weight and chicks with more leg problems than those incubated at standard
285 temperatures (Oviedo-Rondon et al., 2009). Additionally, longer exposure to higher
286 temperatures (38.9⁰C from day 7 of incubation onwards) led to chicks with a 26%
287 lower heart weights at hatch and higher overall levels of mortality and ascites-related
288 mortalities (4.1 and 3.8% higher respectively) than chicks from eggs incubated at the
289 standard temperature of 37.8⁰C (Molenaar et al 2011a). It is important to note that
290 some of the temperatures referred to above are invariably the so-called set
291 temperature (the temperature to which the incubator thermostat is set). Early on in

292 incubation the set temperature and embryo temperature will be similar if not identical
293 however, once past the mid-time point of incubation, metabolic heat output increases
294 dramatically, meaning that the temperature experienced by the embryo is invariably
295 greater than the set (or air) temperature. This does not alter however that there is an
296 interaction between the age of the embryo, the duration of exposure and the
297 temperature to which the embryo is exposed, with the optimum conditions varying
298 depending on the parameter selected.

299

300 In addition to incubation temperatures, the temperatures that the hens are
301 housed at also influences offspring characteristics. Hens kept during weeks 22-27 of
302 life under standard thermal conditions (21⁰C) or warmer but within the
303 thermotolerance range conditions (31⁰C) had similar laying rates. However, the hens
304 raised in warmer temperatures had lighter eggs and higher concentrations of yolk
305 progesterone, testosterone, and estradiol, and chicks hatched from these eggs had
306 higher morphological quality scores (based on measures described in Tona et al.,
307 2003) that varied from 0 (very bad) to 40 (very good) and made fewer distress calls
308 when exposed to a novel food. These findings may indicate a beneficial adaptive
309 responses as maternal environment can result in different offspring phenotypes which
310 may be more suited to particular environmental conditions (Bertin et al., 2013).

311

312 Factors such as the age of the parent flock will affect the offspring. Generally
313 younger birds have smaller eggs which result in lighter offspring, chick weights in
314 poultry species typically being approximately 70% of the total egg weight. For
315 example, broiler chicks hatched from young parents (27 or 32 weeks of age) had
316 lower body weights than chicks hatched from older parents (57, 42 or 65 weeks old)

317 (Nelson et al., 1992; Yalcin et al., 2008) and quail chicks from younger parents (11
318 weeks of age) were lighter than chicks from older parents (37 weeks of age) at hatch
319 (Guibert et al. 2012). Additionally, eggs from younger (24 weeks of age) hens had
320 higher yolk levels of antibody Immunoglobulin Y (IgY) than birds from 36 and 82
321 week old hens (Barua et al., 2000) which may have implications for disease control
322 programs. However, broiler breeders just coming into lay (about 23 weeks old) had
323 lower egg fertility and hatchability and higher embryo mortality, indicating that there
324 is a lower limit to the benefits of incubating eggs from young parent birds (Pappas et
325 al., 2006).

326

327 Egg storage times before incubation can also have effects on the subsequent
328 offspring. Fertilized eggs are typically stored for approximately seven days before
329 being incubated. Eggs stored for longer periods (18 days before incubation) had
330 delayed hatching and an increase in triiodothyronine (T3) most likely due to higher
331 concentrations of corticosterone between internal pipping and hatching and which
332 may indicate a more stressful hatching event compared to embryos hatching from
333 eggs stored for three days (Tona et al., 2003). Embryos stressed due to hypoxia (O₂
334 decreased below physiological concentrations) and anoxia (total depletion of O₂) also
335 resulted in increased plasma corticosterone levels in newly hatched chicks and had an
336 increased number of adverse conditions such as a delay in righting response (Rodricks
337 et al., 2008), impaired development of thermogenesis (Azzam et al., 2007) and lower
338 body weights for up to 10 days post hatch (Camm et al., 2001) compared to embryos
339 that did not experience hypoxia during incubation. Chicks exposed to high O₂ levels
340 (25%) during days 9-19 of incubation also had decreased residual yolk weight at
341 hatch and higher yolk-free weights and longer chick body lengths than chicks

342 incubated with lower (21%) O₂ concentrations (Lourens et al., 2007). Yolk-free body
343 mass at day 18 of incubation was also higher with higher O₂ levels; although
344 differences between high and normal O₂ concentration treatments disappeared by 48 h
345 after hatch (Molenaar et al., 2011b) Fertile eggs exposed for 24 hours to 6% CO₂ and
346 either 10 or 20% O₂ during the first 10 days of incubation had increased cardiac and
347 non-cardiac malformations, such as thickening of the inter-atrial septum
348 demonstrating that increased CO₂ levels can be teratogenic; however newly hatched
349 chicks were also 10% heavier than in the control groups (Haring et al., 1970).

350

351 Exposure to environmental toxins, which would be more prevalent among
352 wild or farmed birds with access to range or which could contaminate crops which are
353 made into feeds for farmed animals, usually had negative effects in the offspring.

354 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a toxin known to cause, among other
355 symptoms, wasting syndrome, carcinogenicity and immunotoxicity (Poland and
356 Knutson, 1982). It can get into the environment through combustion and incineration
357 and chemical and biological processes. Poultry were used as a model for wild turkeys
358 to determine the risks of exposure. Chicks from eggs injected with the toxin showed
359 suppressed B-cell proliferation and catalytic enzyme production (Peden-Adams et al.,
360 1998). One time exposure of Japanese quail embryos to
361 dichlorodiphenyldichloroethylene (DDE) resulted in female offspring with
362 accelerated puberty onset and male offspring with reduced reproductive performance
363 (e.g. mount attempts); however reproductive physiology in adults appeared to be
364 unaffected (Quinn et al., 2008). This means there may be immune and neurological
365 risks for the offspring of wild or farmed birds that come into contact with the external

366 environment or that are fed contaminated feedstuffs, especially if exposure occurs
367 when young.

368

369 *Effects on Offspring Behavior and Cognition.* Increasing incubation
370 temperature can also have positive behavioral effects: chicks from eggs exposed to 24
371 h of 40.6°C incubating temperature on day 16 showed less aggressive pecking to
372 conspecifics at 21 weeks of age than those from eggs incubated under standard
373 conditions; although it is not clear exactly what the mechanism behind this difference
374 in behaviour was (Lay and Wilson, 2002). However, chicks from eggs exposed to
375 hypoxic conditions (24 hours of 14% O₂) on day 10 or day 14 of incubation had
376 impaired short term memory but this was not consolidated into long term memory
377 impairment (Gibbs et al., 2009). Additionally, chicks from eggs that were incubated
378 with decreased O₂ levels during days 10-14 were less successful in an avoidance
379 discrimination task (exposed to 2 different color beads, one had an aversive taste the
380 other did not) than birds hatched from control eggs (Rodricks et al., 2004) and chicks
381 from eggs partially wrapped in a membrane during incubation also had reduced
382 success in an object avoidance test 60 and 120 minutes after being taught the task
383 (Camm et al., 2001).

384

385 Offspring from eggs exposed to 2 h of pulsing bright lights (750-1000 lux) on
386 day 18 of incubation, feather pecked more on days 7, 14 and 21 after hatch (Riedstra
387 and Groothuis, 2004). The authors hypothesized that this was due to light exposure
388 influencing lateralization of the brain therefore decreasing the ability of the birds to
389 discriminate between familiar and unfamiliar birds whom they would 'explore' by
390 pecking. Conversely, embryos incubated with a 12L:12D light schedule had better

391 retention of a passive avoidance and discrimination task after hatch compared to dark
392 incubated only chicks (Sui and Rose, 1997). Occasional exposure to sounds in the
393 incubation environment (15 min/hour from day 10 to hatching), using both natural,
394 species-specific noises and artificial noises (sitar music) increased hippocampal
395 function (Chaudhury et al., 2009) which may facilitate learning and memory and
396 influenced postnatal auditory preferences (Jain et al., 2004); however, only high
397 decibel music (110 dB) positively modulated spatial orientation, learning and memory
398 when compared with noise (non patterned or rhythmic) of the same decibel levels
399 (Sanyal et al., 2013). Maternal age can also influence offspring characteristics: chicks
400 from 37 week old Japanese quail were less reactive when encountering a novel
401 environment but they were more stressed by social isolation than chicks from 11 week
402 old quail (Guibert et al., 2012).

403

404 Stress can also affect the offspring through inherited epigenetic modifications
405 and this process may have been favored by domestication: White Leghorn and Red
406 Junglefowl hens undergoing stress in the form of unpredictable light:dark rhythms
407 showed decreased ability to perform a spatial learning task; however, this effect was
408 greater in the White Leghorn hens. These White Leghorns also had offspring with
409 reduced spatial learning abilities, that were more competitive and that grew faster than
410 offspring from non-stressed hens, whereas offspring from the Red Junglefowl hens
411 exposed to the same stressors did not exhibit this reduction in spatial learning
412 (Lindqvist et al., 2007).

413

414 Thus small environmental manipulations, such as short increases or decreases
415 in incubation temperature can have beneficial effects on the offspring while young to

416 middle aged parent flocks tend to have more successful offspring than very young or
417 old parent flocks. Irrespective of the age of the parent bird, storage times that exceed
418 7 days (e.g. 18 vs 7 days storage), varied lighting schedules, restricted gas exchange
419 across the egg shell and exposure to toxins may lead to detrimental effects on the
420 offspring and should be avoided when possible.

421

422 *Artificial Parameters*

423 Artificially manipulating the concentration of stress hormones in the individual or
424 their offspring can give an insight into the potential effects of any related stressor.

425

426 *Effects on Offspring Health and Performance.* Corticosterone is the most
427 frequently studied stress hormone in poultry but other hormones (such as
428 noradrenaline) or lines of birds selected for low or high stress levels are also studied.
429 For example, quail selected for high plasma corticosterone laid eggs with higher yolk
430 corticosterone (Hayward et al., 2005) and had lower egg fertility (Schmidt et al.,
431 2009b) than birds selected for low plasma corticosterone. The offspring from ‘high
432 stress’ quail had greater size differences between their left and right tibia and middle
433 toe lengths, indicating that developmental instabilities like increased stress can lead to
434 greater fluctuating asymmetry in the offspring (Satterlee et al., 2008). Raised
435 corticosterone levels in hens (achieved by corticosterone implants or injections) also
436 decreased growth rates and increased mortality in their chicks compared to hens with
437 non-functional implants or injected with saline (Hayward and Wingfield, 2004;
438 Hayward et al., 2006; Satterlee et al., 2007; Wall and Cockram, 2010) and eggs with
439 increased corticosterone from, e.g. corticosterone injections, led to increased mortality
440 and decreased body weights in the chicks for up to 77 days post hatch (Eriksen et al.,

441 2003; Janczak et al., 2006, 2007b). Quail subjected to corticosterone injections *in ovo*
442 showed higher corticosterone concentrations when stressed at 64 days of age by being
443 placed individually in an opaque box than quail subjected to sham injections or post-
444 natal corticosterone injections, demonstrating the potentially longer-lasting effects of
445 early stressors (Marasco et al., 2013). Corticosterone can also be raised by subjecting
446 the birds to unpredictable stressors. Japanese quail hens were exposed to mild
447 stressors (unpredictability in the environment and sudden movements) three times a
448 day for twenty-four consecutive days and compared with hens in which no stressors
449 were applied. Stressed hens had heavier eggs, contained more albumen, hatched
450 earlier and were heavier at hatching than non-stressed hens' eggs (Guibert et al.,
451 2011). Even the **physiology** of the parent birds can affect corticosterone levels in the
452 offspring, with chicks hatched from a standard heavy broiler breeder line having
453 higher corticosterone levels than an experimental lighter broiler breeder line (Tona et
454 al., 2004).

455

456 Other hormones affect offspring development as well. So for example quail
457 eggs injected with testosterone had a lower hatchability and cell mediated immunity
458 than eggs not injected or injected with the carrier only (Daisley et al., 2005; Cucco et
459 al., 2008); although these offspring did have higher growth rates (Cucco et al., 2008).
460 However the negative effects of testosterone supplementation were found to be
461 reduced when chicks were supplemented with carotenoids in their feed (Cucco et al.,
462 2008). Additionally, eggs injected with noradrenaline to increase the basal
463 concentration produced chicks with raised levels of circulating corticosterone (Gibbs
464 et al., 2009).

465

466 ***Effects on Offspring Behavior and Cognition.*** Early exposure to stress
467 hormones can affect fear behavior. Hens selected for 'low stress' had offspring that
468 took longer to induce tonic immobility (TI: an indicator of stress or fear, Davis, 2008)
469 and chicks from eggs injected with testosterone had longer TI durations than chicks
470 from eggs that weren't injected with testosterone during incubation (Okuliarova et al.,
471 2007). Eggs injected with corticosterone before incubation resulted in: chicks that had
472 higher latencies in a human approach test and fewer chicks that would cross a barrier
473 to reach food (Janczak et al., 2006); chicks that had raised basal plasma corticosterone
474 during a beak trimming stress test (Lay and Wilson, 2002; Rodricks et al., 2006) and
475 chicks that performed more aggressive pecking to their conspecifics (Lay and Wilson,
476 2002). Chicks from quail exposed to unpredictable mild stressors, such as sudden
477 movements or noises, had higher reactivity when introduced to a novel environment
478 and they reacted more strongly to social isolation (Guibert et al., 2011). However,
479 quail chicks from eggs injected with testosterone had reduced numbers of total calls
480 and a greater latency to call in open field tests, took less time and had a shorter
481 latency to approach novel objects, and had lower fecal corticosterone after a 90
482 minute isolation test than offspring from control eggs (Daisley et al., 2005). Male
483 quail injected with testosterone *in ovo* showed increased food competitiveness and
484 had a more female-like phenotype than sham treated males, suggesting that maternal
485 androgens interact with sexual differentiation of the brain and behaviour and
486 development of secondary sex characteristics (Riedstra et al., 2013).

487

488 Corticosterone injections in eggs also influenced learning ability, with chicks
489 from eggs injected with larger doses (0.3 nmol/egg) performing worse in an object
490 discrimination task than those injected with very small doses (0.01 nmol/egg)

491 (Rodrick et al., 2006). Quail chicks injected with noradrenaline and exposed to a
492 maternal call during incubation did not show a preference for the call while chicks
493 only exposed to the call during incubation did (Markham et al., 2006). Similarly,
494 chicks injected with corticosterone during incubation and exposed to an imprinting
495 stimulus (another chick) did not prefer the stimulus while the control chicks did
496 (Nordgreen et al., 2006).

497

498 Thus, there is good evidence indicating that early life exposure to increased
499 stress levels can have a later effect on chick mortality, development, stress sensitivity
500 and behavior and this has implications for the management of captive breeder birds
501 and potentially also wild birds. Although stress levels were raised artificially in these
502 studies, other aspects of the production environment can lead to increased stress levels
503 (e.g. Parsons 1990) and this could affect production and bird welfare.

504

505 ***Husbandry Parameters***

506 There was only a small sample of studies that examined the effects of husbandry
507 procedures on the offspring.

508

509 ***Effects on Offspring Health and Performance.*** One study involved the
510 practice of forced molting, a method which is still practiced in many countries, like
511 America but is illegal in the UK, and can involve withdrawing food completely from
512 the hens until they have ceased laying for a few days resulting in a week or more
513 without food (Patwardhan et al., 2011). This practice is stressful for the hens and
514 leads to increased concentrations of antibodies, such as levels of IgY, in the yolks of
515 their eggs for at least 10 days after the hens began to re-lay which could have

516 beneficial effects for early immune function of fertilized eggs (Barua et al., 2001).
517 Even non-invasive procedures such as habituating quail to humans by increased
518 exposure to humans, gentle handling and food rewards and then exposing habituated
519 and non-habituated quail to human disturbances had significant effects. Eggs had
520 more yolk, lighter shells, increased progesterone, and decreased androgen levels and
521 the chicks hatched earlier and weighed more than non-habituated quail eggs (Bertin et
522 al., 2008).

523

524 ***Effects on Offspring Behavior and Cognition.*** Chicks from quail habituated
525 to humans had a greater fear response as measured by tonic immobility tests (reduced
526 number of inductions) and showed more fear behavior when exposed to the
527 experimenter and a novel environment. It was postulated that the habituation process
528 may have been stressful for the hens and may have led to higher stress hormone levels
529 being transferred to the eggs (Bertin et al., 2008). There were also social effects of the
530 habituation treatments as offspring from non-habituated quail spent less time near
531 conspecifics and males showed less crowing and courtship behavior toward females
532 than offspring from habituated quail (Bertin et al., 2009).

533

534 The egg quality, social, and sexual changes in the offspring have implications
535 not only for birds hatched in production systems but also when interacting with wild
536 species during conservation efforts.

537

538 ***Other Parameters***

539 Other parameters, such as social environment, housing conditions and the infectious
540 environment and maternal health status may influence factors in the offspring.

541

542 *Effects on Offspring Health and Performance.* For example, dominant hens
543 allocate more testosterone to male eggs than female eggs, while subordinate hens
544 allocate more testosterone to female eggs (Muller et al., 2002). Housing environment
545 can also influence the hormones allocated to eggs. Hens housed in floor pens laid
546 eggs with yolks higher in androstenedione and estradiol while caged hens had eggs
547 with higher concentrations of estradiol in the albumen (Janczak et al., 2009).

548

549 Vaccination of the hens before they begin to lay can lead to long lasting
550 maternal antibodies being passed into the egg and offspring. For example, pre-lay
551 hens vaccinated against Salmonella led to long lasting Salmonella specific antibodies
552 found in the egg yolk as Immunoglobulin G (IgG) and in the offspring as IgG in the
553 serum and IgA in the intestinal fluid. Chicks from vaccinated hens also had higher
554 antibody response when orally infected with Salmonella strains than non-vaccinated
555 hen offspring (Hassan and Curtis, 1996). Vaccinating adult birds and the use of chicks
556 from vaccinated hens is helping to control and reduce infections of the birds from
557 bacteria that can be found in a production environment.

558

559 *Effects on Offspring Behavior and Cognition.* Maternal androgens increase
560 competitiveness in the nestling and aggression and growth rate in the juvenile
561 (Schwabl, 1993, 1996b; Eising et al., 2001). These hormones may be a way for the
562 hen to signal the state of the environment to her offspring and subsequently affect
563 their development and phenotype. Also prenatal sensory experience has a role in the
564 lateralized postnatal visual discrimination in birds. During the hatching process, the
565 chick embryo has its left eye blocked by its body and yolk sac but the right eye is

566 exposed to light coming through the shell, leading to a 90% left side turning bias
567 found in domestic chicks (Rogers, 1991). However, when both eyes are prenatally
568 exposed to light or when the left eye is exposed and the right eye is blocked from
569 light, the turning bias disappears, while birds who experimentally had their right eye
570 exposed and left eye blocked from light prenatally still maintained the turning bias
571 (Casey and Karpinski, 1999). Thus treatment of incubating and hatching eggs can
572 have long lasting effects on the brain and behavior of avian offspring.

573

574

DISCUSSION

575 There are numerous factors that can have an influence on the hen and the incubating
576 egg that will result in lasting effects on the phenotype of the offspring. These can
577 range from the supplements provided in the feed (e.g. Atencio et al., 2005a,b) or even
578 the amount of feed provided (e.g. Triyuwanta and Nys, 1992), the temperature and
579 relative humidity during incubation (e.g. Collin et al., 2007), to age of parent flock
580 (e.g. Yalcin et al., 2008), levels of stress hormones in the hen (e.g. Schmidt et al.,
581 2009a,b), habituation of the parents to humans (e.g. Bertin et al., 2009) to the
582 dominance status of the hen (e.g. Muller et al., 2002). Some factors can results in
583 benefits to the offspring, such as supplementing vitamin D3 in broiler breeder feed
584 (Driver et al., 2006) while others may have detrimental effects, such as stress in the
585 parents leading to a reduction in memory and learning in the offspring (Rodricks et
586 al., 2006).

587

588 The presence of some factors, like exposure to environmental toxins (e.g.
589 Quinn et al., 2008), is unlikely to occur in current commercial poultry production

590 where the birds are housed although the trend to move towards extensive systems has
591 the potential to increase the risks (Schoeters and Hoogenboom, 2006). However there
592 is a risk that farmed poultry may be fed feed made with crops exposed to
593 environmental toxins (van Barneveld, 1999). Other factors, like restricted feeding in
594 broiler breeders, are standard practice (e.g. Triyuwanta and Nys, 1992). The most
595 prevalent result of the different hazards found in production systems is likely to be
596 increased stress leading to increased concentrations of circulating corticosterone.
597 Increased stress could be caused by parasitic infections, pathologies, feed deprivation,
598 social stress, exposure to novelty and noise and behavioral restrictions (e.g. Parsons,
599 1990), meaning that the standard housing of poultry could be contributing to raised
600 corticosterone levels compared to less intensively reared or wild fowl. This increased
601 stress could lead to offspring with decreased weights, increased mortality, learning
602 impairments and increased stress sensitivity (e.g. Hayward and Wingfield, 2004).
603 However more research is needed to determine how stressful current systems are in
604 terms of, for example, elevated concentrations of corticosterone. Flock age is an on-
605 going concern in poultry production, as breeder birds may be placed in systems pre-
606 lay and continue producing for well over a year. Older flocks have chicks with higher
607 body weights but they also have higher chick mortality (Yalcin et al., 2008) and lower
608 yolk antibodies (Barua et al., 2000) which can have implications for the total number
609 of viable offspring produced. Some deleterious effects are commonly encountered,
610 such as reduced porosity in eggs from young breeding birds whereas stressors such as
611 bright pulsing lights during incubation, would only occur in accident or emergency
612 situations and should not present a regular problem for poultry welfare.
613

614 However, some hazards, like periods of increased or decreased temperatures
615 during incubation, can be beneficial and may more closely mimic a hen brooding a
616 clutch of eggs who takes breaks from sitting (White and Kinney, 1974).
617 Supplementing poultry feed is a relatively easy way to provide additional protection
618 to the offspring, e.g. vitamin D₃ given to broiler breeders resulted in chicks with better
619 leg health (Atencio et al., 2005a,b) but dosages should be monitored to prevent any
620 negative effects, e.g. high levels of zinc being supplemented to hens acted as a mild
621 immunosuppressant in the chicks (Stahl et al., 1989). Although it should be noted that
622 the studies examining these effects have been fairly small scale experimental trials to
623 date. On-farm studies would be useful to confirm that the same positive effects occur
624 on a much larger scale and that changing the factors, such as incubation temperatures,
625 is practically and economically feasible.

626
627 In terms of poultry welfare measures, offspring condition could reflect the
628 suitability of the parent environment. For example, it has been suggested that
629 fluctuating asymmetry in chicks be used as a proxy measure for chronic stress in the
630 hens as asymmetries increase with increased stress (Satterlee et al., 2000). So hens in
631 one environment producing chicks with larger asymmetries than hens from another
632 environment may be more stressed and have poorer welfare. The differences in the
633 two environments can then be compared to attempt to improve conditions for the
634 more stressed hens. Other measures such as comparing chick mortality, growth rate,
635 leg conditions, etc. from hens being housed and managed in different ways could also
636 be used to assess suitability of the hen environment. Using offspring to assess parent
637 environment has the advantage that the animals in the environment under question are
638 not interfered with; however more research and validation studies using this method

639 compared to other generally accepted welfare measures are needed before offspring
640 measures become a widespread parent welfare measurement.

641

642 In conclusion, the maternal environment includes many factors such as
643 feeding method and nutrition, environmental parameters, artificial challenges,
644 husbandry, housing system, social environment, infectious environment and maternal
645 health status and these can all influence the development, physiology and behavior of
646 the offspring. As the conditions we keep commercial poultry in are quite artificial
647 compared to their wild ancestors, they have the potential to be stressful which could
648 negatively affect the offspring. The literature strongly suggests that maternal conditions
649 prior to lay, or conditions during incubation could have important effects on poultry health,
650 welfare and productivity. Consideration of how potential negative prenatal effects can be
651 avoided may benefit poultry production. Additionally, we also have the potential to
652 provide factors to the hen that will benefit the offspring and help improve their
653 welfare. Using the offspring to help assess suitability of parent environment is a
654 promising welfare assessment tool but it requires further study and validation against
655 current welfare measures before becoming a widespread method.

656

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Response to referees

Reviewer 1 (Highlights in manuscript in yellow)

So the strong point of the review is the broad approach, the weakness is the lack of in depth analyses. In general the paper is very well written and I think this broad approach is of interest to the reader who wants to become familiar with the topic.

Thank you for your comments. We agree with you that this review takes a broach approach to the effects of early life experiences. From going through the literature on this subject we noticed that there was a lack of general review papers which could be of use to someone wanting to begin researching this topic.

We felt a broad approach was the best starting place for a review and there is the potential for some areas, like the incubation temperature literature that a reviewer mentioned, to later be written up as their own in-depth review papers of specific aspects within early life effects. These papers would then be of interest to a more specific audience.

Line 196: vitamin E is not a mineral

Corrected

Line 245: Please check and if yes state that the supplementations were above requirements as set by e.g. NRC requirements. It's important to know if levels were supplemented in deficient diets or not.

Yes, these supplementation levels were above recommended standards. This has been clarified in text.

Line 273: With regard to long term exposure to high ambient temperatures it might be nice to add R. Molenaar et al (2011 Poultry Science 90:624-632). They show that long term exposure to high temperature during incubation leads to reduced heart weight after incubation and increased mortality due to ascites.

Thank you for recommending this paper. We have included it in our discussion (Lines 281-285)

Line 333: Effect of long term exposure to hyper- or hy-poxia on offspring are described by A. Lourens et al. (2007 Poultry Science 86:2194-2199) and R. Molenaar et al. (2011 Poultry Science 90:1257-1266) and may be of value to mention.

Again, thank you for recommending these references. We have included them in the manuscript (Lines:335-341)

Line 349:Farmed animal may be exposed to these substances via contaminated feed.
See also line 570

A good point. We have included this in the manuscript at Lines 348-9 and Lines 584-586.

Line 353-354: Is there a biological explanation why a one day (day 16) increase in incubator temperature leads to less aggression?

The authors were not sure what the mechanism behind this was - stating it could be due a variety of factors such as direct and/or indirect stress plus possible other unidentified factors. I have added text about the uncertainty of this result to the manuscript.

Line 397: What do you mean with 'long incubation times' ?

*Our mistake – this should read **long storage times before** incubation. This has been corrected and the example of 18 days vs 7 days storage has been given.*

Line 431-434: Can this difference between breeds really be attributed to weight or are they just line differences?

You are correct. The breeds are different in more than just weight. We've changed weight to differences in physiology of the breeds leading to differences in the offspring.

Reviewer: 2 (Highlights in the manuscript in green)

Comments to the Author

The title of the manuscript tackles the very important subject of trans-generational influences in poultry. This subject is, however very big, and the authors try to cover the entire field using a meta-analysis.

We agree that this is a very big subject. There are reviews into specific topics of prenatal effects on offspring characteristics but we felt it was valuable to provide a more broad review for those beginning to investigate this topic or those who wanted more general knowledge of how prenatal experiences can affect later chick development than to begin with a review into a specific group of factors or influences on prenatal development and ignore the rest of the factors.

Animal welfare is mentioned (lines 145 – 149) as a main rationale of the paper. There are, however, many sections in the manuscript which are not closely related to welfare.

Animal welfare has a number of definitions, one of which is the ability of an animal to cope with its environment (e.g. Broom 1991 J. Anim. Sci. 69:4167-4175). As prenatal environment can have an effect on offspring development, this will affect the ability of the offspring to cope with its environment. We have also included 'health' (which is part of welfare) and a definition of what we mean by welfare to help clarify this.

The selection procedure was carried out using a list of quality criteria. Unfortunately the articles retained do not represent the existing spectrum of scientific knowledge within the subjects and categories of the paper. In some fields the information provided are rather trivial (e.g. the effects of nutritional deficiencies (lines 245 – 251), or superficial (e.g. treatment of stress, influence of incubation temperature and atmosphere on development and adaptation).

It was not our intent to provide only the most important topics in the study of prenatal effects on offspring development but to show what a large array of factors can have effects on postnatal characteristics in the offspring. It would also not be possible to include the entire spectrum of scientific knowledge in this one paper without having it get excessively long and probably difficult to read. We felt that review papers on specific topics of postnatal factors could do the job of covering the whole range of scientific knowledge within a factor topic, while this paper is meant to be an introduction to a large topic.

The schematic selection of papers out of the huge amount of existing publications has obviously not retained those who may provide a general understanding of the subject matters.

The approach used will have resulted in some papers of the type referred to being excluded. We would defend this trade-off though on the grounds that we wanted to retain the focus of the paper and keep it to a manageable length. We would hope that those seeking a more general understanding would be encouraged by reading our paper to access the relevant papers.

An important number of citations cannot be retrieved in the reference list.

We have had colleagues who are experts in poultry nutrition, behaviour and physiology review the reference list and they felt that the important citations had been mentioned. Additionally, reviewer 1 suggested a few extra papers they felt would be useful to include, see below.

Lourens, A., H. van den Brand, M.J.W. Heetkamp, R. Meijerhof, and B. Kemp. 2007. Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. *Poult. Sci.* 86:2194-2199

Molenarr, R., R. Hulet, R. Meijerhof, C.M. Maatjens, B. Kemp, and H. van den Brand. 2011a. High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens. *Poult. Sci.* 90:624-632.

Molenaar, R., I. van den Anker, R. Meijerhof, B. Kemp, and H. van den Brand. 2011b. Effect of eggshell temperature and oxygen concentration during incubation on the developmental and physiological status of broiler hatchlings in the perinatal period. *Poult. Sci.* 90:1257-1266.

If this reviewer would like to suggest papers that they feel are essential then we would be happy to incorporate them. I'm sure you can appreciate that different individuals may have different opinions on what papers are most important so unless we greatly expanded the paper, it would be difficult to please everyone in this respect.

The whole field of trans-generational influences in poultry cannot be handled in a paper. It may be useful to restrict the review on more specified subjects and to provide more detailed information on this.

As mentioned above, we felt it was valuable to provide a broad review for those beginning to investigate this topic or those who wanted more general knowledge of how prenatal experiences can affect later chick development as there are currently no reviews of this sort available. There are some reviews of certain factors that affect postnatal development and if it was thought to be of interest to the readers of the journal to update them or write more specific reviews for factors that currently do not have review papers, this could be considered but we felt it more important to start with a broad review which could be of use to a larger audience.

*For example, if someone was interested in maternal hormonal influences on begging behaviour in the offspring, they could read Smiseth et al 2011 *Anim. Behav.* 81:507-517. Or if they were interested in transgenerational effects of innate immunity, they could find Berghof et al 2013 *Poult. Sci.* 92:2904-2913. But if a reader just wanted an overview of how prenatal environment can affect poultry offspring, there isn't a review paper that fulfils this need. Therefore we feel this is an important place to start and a broad review in this field could be of use to a much wider audience than a review on a specific aspect of prenatal effects.*