1	JUDGEMENT BIAS DURING PREGNANCY IN DOMESTIC PIGS
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13 Abstract

14 In humans and rats, changes in mood and affect are known to occur during pregnancy, however 15 it is unknown how gestation may influence mood in other non-human mammals. This study 16 assessed changes in pigs' judgment bias as a measure of affective state throughout gestation. 17 Pigs were trained to complete a spatial judgement bias task with reference to positive and 18 negative locations. We tested gilts before mating, and during early and late pregnancy, by 19 assessing their responses to ambiguous probe locations. Pigs responded increasingly negatively 20 to ambiguous probes as pregnancy progressed and there were consistent inter-individual 21 differences in baseline optimism. This suggests that the pigs' affective state may be altered 22 during gestation, although as a non-pregnant control group was not tested, an effect of learning 23 cannot be ruled out. These results suggest that judgement bias is altered during pregnancy in 24 domestic pigs, consequently raising novel welfare considerations for captive multiparous 25 species.

26

27 Keywords:

28 Pregnancy; Gestation; Cognitive bias; Affective state; Information processing; Pig.

29 Background

30 Research investigating the links between pregnancy, affect and cognition is most often carried 31 out with a human-centric focus with studies typically using case studies and cohorts. In 32 humans, changes in affective state during pregnancy are common and alterations in levels of 33 anxiety, depression and cognitive ability have been demonstrated in humans and rodents [1-34 3]. These changes are often linked to the large and rapid hormone fluctuations that occur 35 during the gestational period [4-5]. Where human subjects cannot be used, rodent models are 36 often employed to experimentally investigate how factors such as diet, enrichment or stress 37 can influence behaviour during pregnancy [6-8]. To infer anxiety and depressive-like 38 behaviours, lab-based behavioural tests, such as a forced swim or open-field test are often 39 used [9]. These studies are conducted under laboratory conditions and are generally aimed at 40 modelling human gestation, rather than investigating how gestation may impact on the rodent 41 itself. Results from both human and rodent studies are varied, however most show that

42 affective state is altered throughout gestation (for review see [2]) and it is clear that

- 43 pregnancy impacts maternal affective state.
- 44

45 Understanding an animals' affective state, or emotion and mood, is a key component of 46 animal welfare [10]. Affective state can influence and alter cognitive processes, such as 47 judgement, [11-12] which may then be used to infer and understand an animals' affective 48 state. Cognitive bias or judgement bias is the influence of affect on information processing, 49 with more content individuals likely to make positive assumptions about ambiguous stimuli 50 [13]. Judgement bias tests have been used to assess changes in affective state in a range of 51 species, including pigs, dogs, honeybees and European starlings [14-17]. Research typically 52 focuses on the impact of external stimuli on judgement bias; this is likely to act via alteration 53 to the internal, physiological environment ultimately resulting in changes in behaviour and 54 judgement bias [11; 18-19]. As such, we would expect internal stimuli, such as physiological 55 changes, would also impact judgement bias directly even in the absence of external 56 influences. Pregnancy is one of the biggest physiological changes a mammal may experience, 57 involving major hormonal and cognitive adjustments [20-21], yet little is known of how 58 information processing and affective state may change in relation to pregnancy in animals.

59

60 The domestic pig (Sus scrofa domesticus) has been used as a human model in a wide range of 61 medical research such as infectious disease [22], nutritional [23] and neurological studies [24]. 62 Pigs allow for longer lifespan studies and are more similar to humans than other laboratory 63 species, such as rodents [25-26]. More commonly, pigs are farmed around the globe for meat production. Modern intensive farming systems have been designed to produce food as quickly 64 65 and cost efficiently as possible, and research is continually ongoing to understand how animal 66 welfare can be optimised within these systems. Despite many studies on the behavioural and 67 welfare needs of sows during pregnancy [27-30], only one study used a specific judgement bias 68 task to assess affective state in gestating sows. This study focused on whether judgement bias 69 could be used as a welfare indicator in gestating sows, finding that group-housed, gestating

70 sows can learn a go/no-go judgement bias task and individual affective states can differ despite 71 experiencing the same management conditions [31]. However, this study did not investigated 72 how gestation itself influenced judgement bias. More recently another study showed that gestating gilts that were classified as 'friendly' visited an electronic sow feeder more often than 73 74 individuals that were classified as 'fearful' [32]. The authors hypothesised that this feeding behaviour may be similar to a judgement bias task and that the friendly individuals may have 75 76 been more optimistic. However, again this study did not investigate how gestation itself 77 influenced judgement bias.

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We investigated how gestation may alter judgement, and therefore affective state, in domestic pigs. We compared within- and between-individual affective state, as measured by a spatial judgement bias test, before mating, and during early and late pregnancy. We hypothesised that within-individual judgement bias would be more pessimistic during pregnancy than prior to mating, leading to an increase in latency to approach ambiguous cues throughout pregnancy. This is the first study to our knowledge to investigate the possible impact of gestation on judgement bias in domestic pigs.

86 Methods and materials

87 This work was carried out between July and October 2015 (replicate one) and between January
88 and July 2017 (replicate two) on a pig farm in the UK.

89 Animal housing and husbandry

20 gilts (primiparous female pigs; N=10 for each replicate) were selected based on age and time 91 until first mating. Using gilts allowed for training time before gestation, as there is limited time 92 between pregnancies once a sow has begun breeding. The average age of all 20 pigs on day one 93 of training was 241.7 (SE: 3.56) days. Replicate two contained one Duroc and three Landrace 94 pigs, the breed of all other individuals was Large White. Pigs were housed in pens of five or 95 six animals, each pen (4.67m x 5.35m) contained a sheltered sleeping area with straw bedding

96 (2.70 x 4.67m) and a run partially exposed to outdoor elements, such as wind and natural light 97 (2.65 x 4.67m). A standard lactating sow ration was fed once a day before mating and 98 throughout gestation; there was continuous access to water and natural lighting. During the 99 course of the study the animals remained within the same groups and pens to keep the external 100 environment as controlled as possible throughout. The study pigs were able to interact with 101 pigs in the pen next door via the gate and animals in the neighbouring pens may have been 102 moved/changed. Due to involvement in a separate study, replicate one pigs received 103 Regumate[®] (containing a steroidal progestin) orally with feed 23 days before planned estrus to allow for synchronised farrowing. As of June 2020, no previous research was found 104 105 investigating possible effects of Regumate[®] on affective state or behaviour of pigs. Due to this 106 research taking place on a working farm, it was not possible to test a non-pregnant control 107 group

108 Judgement bias

109 All pigs were habituated to the test arena in groups for two to three sessions, and then 110 individually to habituate the pigs to eating from the bowl which was placed in the centre of the 111 test arena. Following this, individuals were trained to associate the bowl location with a positive 112 (P) and a negative (N) outcome. When in the P location, the bowl contained a small amount of 113 chocolate raisins (replicate 1) or sugar-coated chocolates (replicate 2) and when it was in the N 114 location, the bowl contained unpalatable food (bitter tasting coffee beans) to discourage the 115 pigs from approaching this location. The pigs were trained to discriminate between these 116 reference locations by alternating P and N trials. Latency to reach the bowl was recorded using 117 video cameras and was then used as a metric to assess whether each individual had learned the 118 discrimination. Each trial was 30 seconds in duration. Correct responses were recorded when 119 the subject approached and touched their nose to the bowl during the positive (P) trials; during 120 negative (N) trials, a correct response was recorded when the individual did not approach the 121 bowl within 30 seconds. The location of P and N was counterbalanced across individuals. For 122 both replicates a criterion of 70% correct responses in the last 20 trials was required before

moving onto the testing phase. Per individual, forty-four training trials were conducted during replicate one and sixty-two for replicate two. Replicate two required more training trials due to the pigs being slower to differentiate between the positive and negative locations. Five pigs from replicate one failed to meet this criterion and were removed from the study. Two pigs from replicate 2 did not meet this criterion. The analysis represents only those 13 that met the learning criterion.

129

Each testing session comprised two sets of nine trials carried out on the same day, involving 130 131 five different bowl locations; the P and N reference locations and three intermediate ambiguous 132 probes: near positive (NP), middle (M) and near negative (NN). Only one bowl was in the arena 133 during each trial. The ambiguous probes placed in predetermined equidistant positions (0.74m) 134 and were not reinforced (i.e., they were left empty). They were presented in a pseudo-135 randomised order and interspersed among training trials. All 'during pregnancy' testing 136 sessions were preceded by five 'reminder' training trials the day before testing. Each pig was 137 tested three times: before gestation (1-2 weeks before mating); early gestation (4 weeks after 138 mating); and late gestation (10-11 weeks after mating). One pig in replicate two was not tested 139 before gestation and was only tested in the early and late test phases.

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141 Statistical analysis

142 All data were analysed in R version 3.4.1 using general linear mixed effects models with the 143 *lmer* function in the package *lme4* [33]. To test the effects of gestation time on cognitive bias, 144 the response variable was *time taken to approach* the presented probes; fixed explanatory 145 effects were *probe location*, coded as a continuous variable from positive (1) to negative (5) with ambiguous locations at points 2, 3 and 4; and gestation time coded as a factor with three 146 147 levels (pre, early and late gestation). Probe location squared was included as initial data 148 exploration suggested curvature in the fits. Interactions between gestation time and probe 149 location and probe location squared were also included.

To find the most appropriate structure for the random model, we compared eight models: two intercept only models and six combinations of random intercept and slope models such that random intercepts were fitted for each pig at each experimental timepoint (or for each pig independent of experimental replicate), with variation allowed between gestation times and the shape of the curve was allowed to vary between pigs (Table 1).

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157 The Akaike Information Criteria (AIC) values for all models were compared using the model.sel function in the MuMIn package [34]. In each case the residuals of the final minimal 158 159 model were visually assessed for deviations from normality. For the final models, predicted fits were produced using the *predict* function in base R. R² values for each model were calculated 160 using the *r.squaredGLMM* function in the *MuMIn* package [34]. For every model, the general 161 162 pattern of results was robust, with the different random models only affecting the predictions 163 very slightly. The best model is reported in the main text, and the results and corresponding 164 figures for the two models where AIC comparison had delta < 2 are reported as supplementary 165 information.

166 **Results**

167 Judgement Bias

The pigs' responses to ambiguous locations in the cognitive bias test changed throughout gestation (Tables 2, 3; Figure 1). Pigs consistently approached the positive probe quickly and the negative probe slowly (or not at all), getting generally slower during gestation (Figure 1). However, whilst the mean speed of approach was fairly linear between positive and negative pre- and early gestation (Figure 1a,b), by late gestation, pigs showed a shift towards pessimism, such that the positive probe continued to be approached quickly but ambiguous probes were approached more slowly (Figure 1c).

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All models retained all interactions and gave qualitatively similar results. The best model wasmodel 1, where the intercept was allowed to vary for each pig at each gestation time (Table 1).

178 However, the result for model 2, where the intercept was allowed to vary for each pig at each

179 gestation time, within each replicate, was equally well supported (delta AIC <2; Table 2, Figure

180 S1).

181

182 Table 1: Statistical model details. Random models with fixed slopes (models 1 and 2) or 183 slopes allowed to vary across probe location (models 3-8), with experimental replicate included

184 (models 2,4,6 and 8) or not (models 1,3,5 and 7).

Model	Random slope	Random intercept
1	1	Gestation time:Pig ID
2	1	Replicate/Gestation time:Pig ID
3	Location	Gestation time:Pig ID
4	Location	Replicate/Gestation time:Pig ID
5	Location ²	Gestation time:Pig ID
6	Location ²	Replicate/Gestation time:Pig ID
7	Location+Location ²	Gestation time:Pig ID
8	Location+Location ²	Replicate/Gestation time:Pig ID

185

186 **Table 2: Table of candidate LMERs.** Table of candidate LMERs explaining time to

187 approach the probe in relation to the interaction between the location of the presented probe

and the gestation time for pigs that reached the 70% learning criterion only (n=13). Each

189 model retained all fixed terms (Location*Gestation time+Location²*Gestation time) with only

190 the random model varying. *Model* corresponds to the random model listed in Table 1, $AIC_c =$

191 corrected Akaike Information Criteria values; ΔAIC_c = difference in AIC_c values between the

best model (lowest AIC_c) and the given model; w = Akaike weights; r^2 (F only) = r^2 for the

193 fixed model only, r^2 (F+R) r^2 for the fixed plus random model.

Model	df	AIC _c	ΔAIC_{c}	W	r ² (F only)	r ² (F + R)
1	12	215.2	0.00	0.580	0.751	0.805
2	11	216.9	1.72	0.245	0.748	0.806
3	16	219.7	4.50	0.061	0.751	0.805
5	13	219.8	4.57	0.059	0.751	0.805
7	13	221.2	6.01	0.029	0.750	0.818
4	22	222.2	7.02	0.017	0.738	0.810

6	16	224.1	8.89	0.007	0.740	0.817
8	16	227.5	12.35	0.001	0.729	0.833

194 195

196 Table 3: Results of the best supported statistical models. Minimum adequate linear mixed 197 effects model for the effects of probe location and gestation time on the time taken for pigs to 198 approach the probe under testing, for pigs that reached the 70% learning criteria only (n=13). 199 The results equate to the best supported random models.

	Model 1			Model 2		
Term	DF	F	Р	DF	F	Р
Location	1, 141	62.96	< 0.001	1, 141	62.96	< 0.001
Gestation time	2, 168	2.03	0.134	2, 167	2.04	0.133
Location ²	1, 141	9.57	0.002	1, 141	9.57	0.002
Location: Gestation time	2, 141	6.07	0.003	2, 141	6.07	0.003
Location ² : Gestation time	2, 141	6.16	0.003	2, 141	6.16	0.003

201 Figure 1: The time to approach each location at three stages of gestation. Log time taken

to approach each location for pigs at three different stages of the pig's 16-week gestational

203 period; a) pre-gestation, b) early gestation (5 weeks) and c) late gestation (10-11 weeks). The

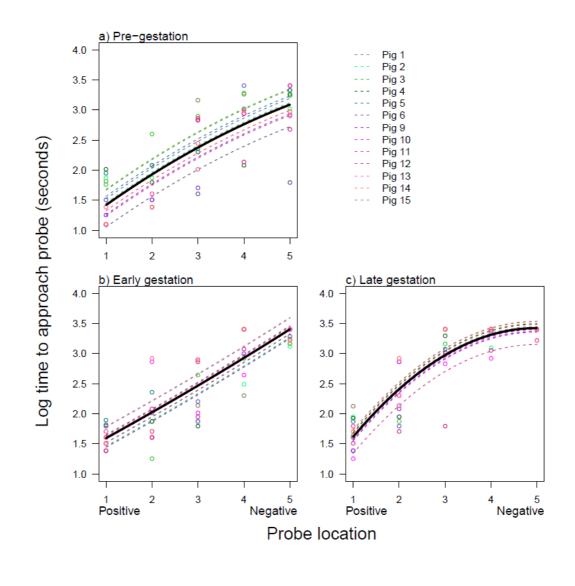
204 open circles are raw datapoints and the lines are model predictions from the minimal adequate

205 model fixed to the level of experimental replicate 1. Results from model 2 are shown, where

the intercept is allowed to vary for each pig at each gestation time, within each replicate. Pigs

207 1-5 are from replicate 1 and pigs 6-15 are from replicate 2.

208



211 Discussion

212 Commercially farmed breeding pigs often experience multiple consecutive pregnancies 213 throughout their lifespan. In livestock species judgement bias tasks have most commonly been 214 used to assess the impact of external factors on affective state. However, internal factors, such 215 as the large physiological changes as associated with pregnancy, also have the potential to 216 influence affective state and therefore judgement bias. The aim of this study was to assess 217 judgement bias in domestic pigs throughout gestation. It was hypothesised that the gilts would 218 be more pessimistic during pregnancy than prior to mating, as indicated by an increase in 219 latency to approach the ambiguous cues. Our results showed this to be the case, with the gilts taking longer to approach the ambiguous locations in the later stage of gestation than before 220 221 mating which indicates that judgement bias changed as gestation progressed. This was most 222 apparent at the middle and most ambiguous location (Figure 1) and suggests the pigs were more 223 pessimistic during the late gestational stage. Crucially, the latency to reach the positive location 224 did not vary markedly throughout gestation, showing that other changes, for example, increase 225 in weight, did not affect response latencies (Figure 1). Thus, these results show increased 226 pessimism during the late stage of pregnancy, despite the fact that the immediate external 227 environment remained constant. This infers that, alongside external factors, internally-driven 228 factors can also influence judgement bias and affective state in domestic pigs.

229

230 The possibility that pigs' judgement bias may change from a positive to a more negative state 231 during the late stage of gestation suggests that the pigs' welfare needs may change too. This 232 highlights the importance of considering the impact of large physiological changes, such as 233 pregnancy, on animal welfare. This study may have implications, not only for the welfare of 234 farmed animals that experience gestation, but also for research into affective state during 235 pregnancy in other captive multiparous mammalian species, including how this may impact 236 cumulatively across the life course on their health and welfare. For example, in humans, 237 multiparous women appear to be more at risk and have a different pattern of anxious or

depressive symptoms compared to primiparous women [35-36]. In humans, hormone fluctuations and other physiological changes throughout pregnancy are often correlated with changes in mood and affective state [4-5]. Pigs are frequently used as models for humans in medical and pharmaceutical studies [22-23; 39-40], so it is possible that a change in affective state during gestation may be caused by comparative mechanisms, however, further research is required to validate this.

244

245 Alongside this interesting result, there are some limitations to take into consideration. Previous 246 studies have shown that multiple testing time points can result in an increase in pessimistic 247 responses [37-38] and this increase in latencies during the later testing phases is similar to what 248 was found in this study. As it was not possible to test a non-pregnant control group, this effect 249 of learning cannot be ruled out. However, the effects of gestation represent a plausible driver 250 for the changes in affect we report as previous research in rodents and humans has shown that 251 mood and affective state can vary throughout gestation [1-3]. Future studies should consider 252 the role of learning by including a non-pregnant control group. There were also some 253 differences between replicates, such as one replicate receiving Regumate®, and different 254 rewards being used. Despite this, the effect of replicate on the data was marginal (Figure 1), 255 showing that the change in judgement bias over the course of pregnancy was robust and not 256 influenced by these differences between replicates.

257

In conclusion, this study shows that judgement bias in farmed domestic pigs may change with stage of gestation, inferring that internally driven stimuli can directly affect judgement bias without external influence. This study raises novel welfare considerations for captive multiparous species and provides a basis for future research into the effect of gestation on judgement bias in non-human animals.

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421 Acknowledgements

422 We would like to thank the farm staff for their help and cooperation.

423 Data accessibility

424 Data is available as supplementary material on Dryad (Doi to be confirmed)

425 Competing interests

- 426 The authors declare no competing interests
- 427

428 Author contributions:

- 429 EVB carried out data collection. LMC conceived of the study; SC carried out statistical
- 430 analysis; EVB, SC, AW, MF and LMC assisted with study design and coordination, drafting
- 431 the final manuscript and gave final approval for publication.

432 **Ethical statement:**

- 433 The University of Lincoln, College of Science Ethics Committee approved this study.
- 434 COSREC189, COSREC262.

435 Funding

- 436 EVB, SC and AW had no funding. LMC and MF were funded by the Biotechnology and
- 437 Biological Sciences Research Council grant BB/K002554/2

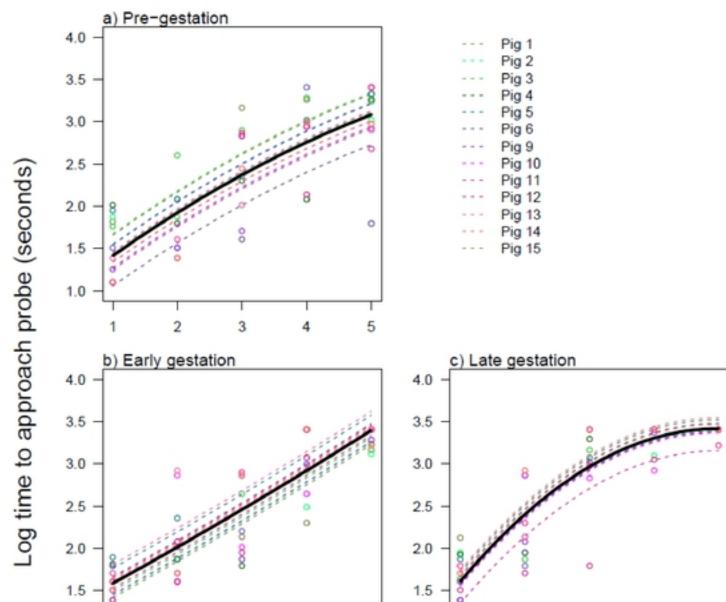
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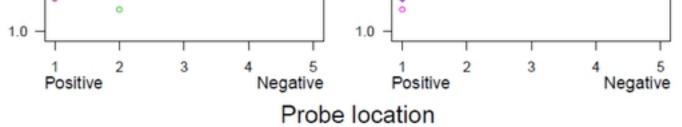
Supplementary Material

Figure S1: The latencies for all 13 pigs to approach each location during three stages of

gestation. Log time taken to approach each location for pigs at three different stages of the pig's 16week gestational period; a) pre-gestation, b) early gestation (5 weeks) and c) late gestation (11 weeks). The open circles are raw data points and the lines are model predictions from the minimal adequate model fixed to the level of experimental replicate 1. Results from model 1 are shown, where the intercept is allowed to vary for each pig at each gestation time. Only the 13 pigs that had >70%

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