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Human antenatal corticosteroid therapy independent of preterm birth programmes cardiac dysfunction and fibrosis in ovine adult offspring

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Keywords: Antenatal corticosteroids, Dexamethasone, Fetal programming, Cardiovascular disease.

Abstract

Antenatal corticosteroids (ACS) are widely administered to women at risk of preterm birth, yet their independent contribution to long-term cardiovascular risk remains unresolved. Using a term-born ovine model that separates ACS exposure from prematurity, we mapped cardiovascular responses from fetal life to adolescence following a clinically relevant dexamethasone regimen. ACS triggered acute fetal vasoconstriction and vascular remodelling, followed by the emergence of systolic and diastolic cardiac dysfunction by term. These effects persisted into adolescence, manifesting as impaired cardiac contractile function, restrictive ventricular filling and increased myocardial fibrosis, despite preserved resting blood pressure. By isolating the effects of ACS from those of preterm birth, here we show that fetal glucocorticoid exposure is a direct driver of adverse cardiovascular programming. These findings reveal a previously unresolved mechanism linking a global perinatal intervention to long-term cardiac remodelling, thereby having important implications for understanding cardiovascular risk in individuals exposed to ACS before birth.

INTRODUCTION

Antenatal corticosteroids (ACS) remain one of the most effective interventions for improving survival in threatened preterm birth, accelerating fetal lung maturation and reducing respiratory and neurological complications.¹ While the lifesaving effects of ACS in preterm infants are unequivocal, there has been concern for potentially avoidable adverse cardiovascular side effects of ACS in exposed infants, including the estimated 40% that go on to deliver at term.²

Evidence on long term cardiovascular outcomes in humans is limited and inconsistent. The Auckland steroid trial cohort showed no increase in cardiovascular mortality in mid-life, however, detailed cardiac phenotyping was not performed,^{3,4} and few data address individuals born at term after ACS exposure. Smaller studies have reported increased aortic stiffness,⁵ or no consistent differences in blood pressure or cardiac morphology (as reviewed by Sacco *et al.*⁶), but have been constrained by small sample sizes, methodological variability, and limited use of advanced cardiovascular assessments.

Preclinical studies in rodents consistently demonstrate that excess glucocorticoid exposure disrupts myocardial maturation, alters contractile gene expression, induces cardiomyocyte hypertrophy, and increases myocardial collagen deposition.⁷⁻¹¹ However, because rodents are born developmentally immature, with substantial cardiac and vascular maturation occurring postnatally, the translational relevance of these observations to human pregnancy is uncertain.¹² Therefore, there is a clear unmet need for studies designed to isolate potential direct adverse effects of ACS independent of prematurity on offspring cardiovascular outcomes in preclinical animal models of improved human translational relevance.

Owing to the close similarity between ovine and human fetal cardiovascular physiology, including comparable patterns of cardiac developmental milestones and haemodynamic transition at birth, the sheep represents an ideal model in which to define the independent cardiovascular effects of ACS.¹³ We reasoned that if ACS promote adverse cardiovascular outcomes in offspring independent of prematurity, then adverse effects should persist in adult offspring of ACS pregnancies, even when delivered at term. We show that human antenatal glucocorticoid therapy independent of preterm birth does not induce hypertension, but does programmes significant cardiovascular dysfunction, including cardiac and aortic fibrosis in ovine adult offspring.

RESULTS

Maternal ACS treatment at 0.8 of gestation has significant effects on fetal cardiovascular structure and function measured 48 hours following ACS exposure

Prior to the administration of dexamethasone (Dex) or saline, both groups of mothers had a similar blood gas and metabolic status (Extended data Table 1). However, following administration of Dex, the saturation of maternal haemoglobin with oxygen (% SatHb) decreased from 4 h after the first injection and for the duration of the study compared to Saline (Extended data Table 1). Additionally, Dex mothers had greater blood glucose concentrations at 6 and 8 h after each injection compared with saline mothers (Extended data Table 1).

Prior to maternal administration of Dex or saline, both groups of fetuses had a similar blood gas and metabolic status (Extended data Table 2). However, following administration of Dex, fetuses had an increased Hb concentration compared to saline fetuses at 2, 4 and 6 h post-1st injection and 4 h post-2nd injection. Additionally, Dex fetuses had a lower pH than saline fetuses 24 h after the 1st injection but had returned to the same pH as saline fetuses by 72 h (48 h after 2nd injection). Although a statistically lower pH than saline fetuses, whose pH ranged from 7.34-7.40 across this period, the lowest pH value in Dex fetuses was 7.30, and therefore not be considered acidotic or abnormal.

Prior to maternal administration of Dex or saline, both groups of fetuses displayed similar basal cardiovascular function (Fig 1a). However, following administration of Dex, fetal arterial blood pressure rose and remained higher than saline fetuses for the duration of the study (Fig 1a). This increase in fetal arterial blood pressure following Dex administration was associated with a fetal femoral arterial vasoconstriction. Femoral artery blood flow and femoral vascular conductance both decreased in the 12-24 h after the 1st injection of Dex compared to saline fetuses. The fall in fetal femoral vascular conductance was maintained for 30 h (Fig. 1a). Fetal heart rate increased in the 12-24 h after each maternal administration of Dex compared to saline (Fig. 1a).

Ewes and fetuses were humanely killed two days (48 h) after the second injection of either Dex or saline. Isolated femoral arteries from Dex fetuses had a greater wall thickness (Fig. 1b), but reduced elastin density (Fig. 1c) compared to saline fetuses. Additionally, Dex fetuses had an increased volume of smooth muscle actin (SMAD; Fig 1d) and nitrotyrosine staining (Fig. 1e) in their femoral arteries compared to saline fetuses. Combined, these data suggest that relative to maternal treatment with saline, maternal treatment with Dex leads to a sustained increase in fetal peripheral

58 vascular resistance and fetal arterial blood pressure. Further, fetal femoral vessels show greater
59 indices of stiffness and oxidative stress by the end of 3 days post-ACS treatment.

60 **Maternal ACS treatment at 0.8 of gestation has significant effects on cardiovascular structure**
61 **and function measured in the fetus at term**

62 Relative to maternal treatment with saline (controls), maternal treatment with Dex at 0.8 of
63 gestation resulted in impaired fetal left ventricle (LV) systolic function, shown by a reduced LV
64 developed pressure (LVDP; Fig 2a) in fetuses at term. Similarly, relative to control fetuses, Dex
65 fetuses had impaired diastolic function, shown by a greater residual pressure in the LV at the end of
66 diastole (LVEDP, Fig. 2b). These effects of maternal treatment with Dex on fetal LVDP and LVEDP
67 were independent of any effects of progesterone pre-treatment at 80 dGA, as maternal treatment with
68 progesterone alone did not affect these variables significantly (Fig. 2a and 2b). All fetuses had a
69 similar degree of fibrosis (Fig. 2c) and LV weight relative to body weight (Fig. 2d). Combined, these
70 data suggest that relative to maternal treatment with saline, maternal treatment with Dex at 0.8 of
71 gestation leads to impaired systolic and diastolic function, but this is not associated with cardiac
72 fibrosis or remodelling in the heart of the fetus at term.

73 **Maternal ACS treatment at 0.8 of gestation has significant effects on cardiovascular structure**
74 **and function in term-born adolescent lambs at 9-11 months of age.**

75 Lambs from mothers treated with saline or Dex at 0.8 of gestation were born spontaneously
76 at term at similar gestational ages and with similar weights (Fig. 3a and 3b). Lambs were then raised
77 until 9-11 months of age for experimentation. Whilst under anaesthesia, prior to surgical
78 instrumentation, adolescent lambs from mothers treated with Dex at 0.8 of gestation had lower LV
79 fractional shortening (Fig. 4a) and ejection fraction (Fig. 4b) compared to adolescent lambs from
80 mothers treated with saline at 0.8 of gestation. In addition, adolescent lambs from mothers treated
81 with Dex at 0.8 of gestation had lower aortic root maximum diameter in systole (Fig. 4c), an increased
82 maximum left atrial diameter (Fig. 4d) and a greater mitral E/A wave ratio owing to a fall in the A
83 wave peak velocity (Fig. 4e and 4f) compared to adolescent lambs from mothers treated with saline
84 at 0.8 of gestation. This was despite a similar body weight, heart rate, stroke volume and mitral
85 pressure gradient (Extended data Fig. 1). Combined, these echocardiography data suggest that relative

86 to maternal treatment with saline, maternal treatment with Dex at 0.8 of gestation leads to evidence
87 of aortic and left ventricular stiffness in the term-born adolescent lamb at 9-11 months of age.

88 Further investigation of *in vivo* cardiovascular function following full postoperative recovery
89 in the conscious animal, showed that term-born adolescent lambs born from mothers treated with
90 saline or Dex had similar basal arterial blood pressure and femoral blood flow (Fig. 5a and 5b).
91 However, relative to adolescent lambs born from mothers treated with saline, adolescent lambs born
92 from mothers treated with Dex had a greater increase in femoral vascular conductance in response to
93 treatment with the NO donor SNP (Fig. 5c & 5d). Further, when the production of NO was inhibited
94 by administration of the NO synthase inhibitor L-NAME, relative to adolescent lambs born from
95 mothers treated with saline, adolescent lambs born from mothers treated with Dex had a greater fall
96 in femoral artery blood flow (Fig 5e and 5f). When corresponding changes in heart rate were plotted
97 against changes in arterial blood pressure induced pharmacologically by intravenous treatment with
98 phenylephrine or SNP, relative to adolescent lambs born from mothers treated with saline, adolescent
99 lambs born from mothers treated with Dex showed an increase in the gain of their autonomic
100 baroreflex function curve (Fig. 5g). Furthermore, isolated cardiac function using the Langendorff
101 apparatus showed that relative to adolescent lambs born from mothers treated with saline, adolescent
102 lambs born from mothers treated with Dex had a greater ratio of the sympathetic to parasympathetic
103 control of heart rate (Fig 5h) and LVDP (Fig 5i), when stimulated with isoprenaline and carbachol.
104 Similarly to what was measured in the fetal heart, adolescent lambs born from mothers treated with
105 Dex had impaired systolic and diastolic LV function. This was demonstrated by a reduced LVDP
106 (Fig 5j) and maximal rate of LV contraction (dp/dt_{max} ; Fig. 5k), with greater residual pressure at the
107 end of diastole (LVEDP; Fig. 5l) and slower rate of LV maximal relaxation (dp/dt_{min} , Fig 5m).
108 Additionally, relative to adolescent lambs born from mothers treated with saline, adolescent lambs
109 born from mothers treated with Dex had a reduced coronary flow rate corrected for heart weight (Fig.
110 5n). Post mortem examination of isolated aortas showed that relative to adolescent lambs born from
111 mothers treated with saline, adolescent lambs born from mothers treated with Dex had an equivalent
112 aortic wall thickness and area (Fig. 6a and 6b), coupled with a reduced aortic lumen area (Fig 6c) and
113 increased ratio of wall:lumen area (Fig. 6d). Further, histological analysis revealed greater fibrosis in
114 both the aorta (Fig. 6e) and LV (Fig. 6f) in adolescent lambs born from mothers treated with Dex.
115 Combined, these data support that the cardiac systolic and diastolic dysfunction measured in fetal life

116 persists in adolescent lambs born at term from mothers treated with Dex at 0.8 of gestation, but this
117 is now associated with restrictive left ventricular filling at adulthood. However, this cardiac
118 dysfunction and restrictive left ventricular filling is not associated with systemic hypertension but
119 rather with indices of cardiac stiffening due to fibrosis. The data also support that adolescent lambs
120 born from mothers treated with Dex show compensatory indices of sympathetic hyper-reactivity in
121 the heart and greater NO sensitivity and NO bioavailability in the systemic circulation.

DISCUSSION

122 Our study identified significant short, medium, and long-term effects across the life course in
123 offspring cardiovascular structure and function in ACS-treated pregnancies in an ovine model of
124 improved human translational relevance. Short-term, maternal treatment with ACS at 0.8 of gestation
125 induced fetal peripheral vasoconstriction, fetal hypertension and led to oxidative stress and vascular
126 remodelling in the fetal femoral artery 48h after the end of exposure. By term, ACS exposed
127 pregnancies had developed fetal cardiac systolic and diastolic dysfunction, with no evidence of
128 cardiac fibrosis or remodelling. In adolescence, basal arterial pressure was similar between groups,
129 however ACS offspring born at term showed persisting impaired systolic function, impaired coronary
130 flow, reduced cardiac compliance, cardiac sympathetic dominance, increased cardiac and aortic
131 fibrosis, and greater reliance on nitric oxide to maintain blood pressure homeostasis (Fig. 7).
132 Importantly, persisting adverse effects of ACS on offspring cardiovascular structure and function at
133 adulthood were independent of being born preterm or having low birth weight. Therefore, we show
134 that human ACS therapy alone and independent of preterm birth or low birth weight triggers a fetal
135 origin of increased cardiovascular risk, programming cardiac dysfunction and fibrosis in ovine adult
136 offspring. Previous studies in rodents and sheep have identified that ACS treatment in more immature
137 fetuses results in programmed hypertension coupled with renal and cardiac pathology.^{10,14,15}
138 Importantly, ACS in the current study, which was administered at the clinically relevant 0.8 of
139 gestation, did not induce hypertension in adolescence, aligning with human data showing that exposed
140 men and women remain normotensive by middle age.³ However, our study suggests these men and
141 women may still harbour subclinical cardiac vulnerability independent of hypertension that needs
142 investigation. These data highlight that while ACS provides beneficial effects on infant lung
143 maturation and infant survival in human pregnancies at risk of preterm birth, current clinical treatment

144 regimens need further optimisation to maintain benefits but remove potential adverse long-term
145 cardiovascular side-effects in adult offspring.

146 *Short-term effects of ACS.* The late-gestation rise in fetal plasma cortisol, produced by the
147 developing adrenal gland, is a critical maturational signal that drives the transition of fetal tissues to
148 their postnatal phenotype, preparing the infant for independent oxygenation, endocrine production,
149 glucose and cardiovascular homeostasis.^{16,17} In resistance arteries, this endogenous rise in cortisol is
150 associated with an increase in resting wall tension and changes in constrictor and dilator capacity,
151 due to the maturation of intracellular pathways.¹⁸ Consistent with the fetal *in vivo* haemodynamic
152 changes measured in the current study a few days after onset of ACS treatment, it has been previously
153 reported that fetal treatment with cortisol, dexamethasone or betamethasone results in an increase in
154 fetal blood pressure associated with an increase in peripheral vascular resistance.¹⁹ Clinically, this
155 hypertensive effect can prove beneficial, as ACS exposure is associated with a lower requirement for
156 postnatal blood pressure support in extremely preterm infants.²⁰ However, in the current study,
157 isolation of the fetal femoral arteries 48 hours after the end of ACS treatment demonstrated increased
158 fetal femoral artery wall thickness, decreased elastin density, increased smooth muscle actin (SMAD)
159 and nitrotyrosine staining. These effects resemble structural and functional vascular remodelling
160 associated increased hypercontractility, oxidative stress and vascular stiffening. Indeed, previous
161 studies of isolated ovine femoral arteries following fetal dexamethasone infusion at 0.75 of gestation
162 show greater constrictor reactivity to ET-1.²¹ Additionally, glucocorticoid excess has been associated
163 with enhanced superoxide production in human umbilical vein endothelial cells, due to an increase in
164 reactive oxygen species production by the mitochondrial electron transport chain, NAD(P)H oxidase,
165 and xanthine oxidase, contributing to early vascular endothelial dysfunction.²²

166 *Medium-term effects of ACS.* Next, we wanted to determine whether the short-term effects
167 on the fetal cardiovascular system of ACS treatment at 0.8 gestation would be transient and resolve,
168 or whether they would be maintained and be reflected in persisting adverse cardiovascular outcomes
169 measured in fetuses near term. To achieve this, we developed an ovine model of human clinically
170 relevant ACS therapy, whilst allowing fetuses to continue gestation to term, by prior maternal
171 treatment with progesterone at 80 days of gestation. In sheep, glucocorticoid treatment during
172 pregnancy increases the placental oestrogen to progesterone ratio, unleashing the parturitional
173 cascade that leads to preterm birth.^{23,24} Therefore, treatment with progesterone early in ovine

174 pregnancy is an established method to maintain uterine quiescence and prevent preterm birth in
175 glucocorticoid-exposed pregnant ewes.²³ In the present study, maternal treatment with ACS at 0.8
176 gestation resulted in impaired systolic and diastolic left ventricular function in isolated fetal hearts 3
177 weeks later. Importantly, this effect was independent of progesterone treatment earlier in gestation,
178 as ovine pregnancies treated with progesterone alone did not show any significant effects on fetal
179 cardiac function measured near term. The cardiac dysfunction determined in ACS treated
180 pregnancies in fetuses near term was not related to a difference in collagen deposition or overt
181 structural remodelling between ACS exposed and control fetuses. Therefore, these data suggest an
182 impairment in both the contractility and relaxation ability of cardiomyocytes themselves. Studies in
183 the chicken embryo allow the direct effects on the developing heart of ACS to be isolated without
184 confounding effect of glucocorticoids on the mother and/or placenta, as may occur following maternal
185 ACS treatment in mammals. Such studies in chicken embryos support a direct effect of ACS on
186 cardiomyocytes as they also revealed impaired left ventricular systolic and diastolic function with
187 underlying mechanisms including a premature switch from cardiac tissue accretion to differentiation,
188 increased oxidative stress, and activated signalling of cellular senescence.^{25,26} Combined, these
189 studies raise concerns for the large proportion of fetuses exposed to ACS, including those who go to
190 be born at term,² for whom there is currently insufficient data on adult cardiac function.

191 *Long-term effects of ACS.* Having demonstrated sustained adverse effects of maternal
192 treatment with ACS at 0.8 of gestation on cardiac function in fetuses near term, next we focussed on
193 potential longer-term effects for the cardiovascular physiology of the adult offspring. To address this,
194 a separate cohort of control and ACS-treated ovine pregnancies were allowed to deliver
195 spontaneously, and the lambs were weaned and maintained on pasture at the Barcroft Centre
196 (University of Cambridge) until study at 9-11 months of age, equivalent to human adolescence.
197 Control and ACS-treated pregnancies delivered at similar term gestational age and body weight,
198 confirming that this model was successful and could be used to isolate the direct effects of ACS on
199 the cardiovascular physiology of the adult offspring independent of preterm birth and fetal growth
200 restriction.

201 At adulthood, offspring of control and ACS-treated pregnancies underwent echocardiography
202 to enhance the clinical translation of these studies. This non-invasive assessment showed a decrease
203 in fractional shortening and ejection fraction, an increase in the left atrial diameter, and an increase

204 in the mitral E/A wave ratio due to a fall in the A wave in adult offspring of ACS-treated pregnancies
205 compared to controls. ACS-exposed lambs had a left atrium diameter 120% greater than controls. In
206 humans, this increase would be classified as severe pathology, as increased left atrial size predicts
207 earlier mortality.²⁷ This cardiac phenotype in ACS exposed lambs represents significantly impaired
208 systolic function with restrictive left ventricular filling, resulting from increased afterload and/or
209 impaired compliance of the left ventricular free wall.²⁸

210 Following echocardiography, the lambs were surgically instrumented under general
211 anaesthesia with vascular catheters and flow probes to test *in vivo* cardiovascular function. At least
212 a week later, following full postsurgical recovery, cardiovascular monitoring in the conscious animal
213 showed that ACS-exposed lambs had normal arterial blood pressure, suggesting that the
214 echocardiographic diagnosis of restrictive left ventricular filling was not due to an increase in
215 afterload. Despite being normotensive at baseline, ACS-exposed lambs had enhanced sensitivity to
216 NO. This was demonstrated by a greater increase in femoral vascular conductance (femoral blood
217 flow/arterial pressure) in response to a 30-minute infusion of sodium nitroprusside, signifying greater
218 active NO-mediated vasodilatation in the peripheral vasculature in ACS-exposed adolescent lambs
219 compared to controls. Additionally, adolescent offspring exposed to maternal ACS were more
220 dependent on NO to maintain their peripheral vascular resistance and blood pressure normal, since
221 blocking endogenous production of NO synthase with L-NAME resulted in a greater peripheral
222 vasoconstriction, illustrated by a greater fall in femoral blood flow. Together, these data suggest that
223 ACS-exposed adolescent lambs may achieve normotension in adulthood due to upregulation in NO
224 signalling and sensitivity that aids to offset a peripheral constrictor phenotype programmed *in utero*.
225 Further *in vivo* examination of cardiovascular function revealed that adult offspring of ACS treated
226 pregnancies showed an enhanced gain of the cardiac baroreflex relative to controls. A greater cardiac
227 baroreflex gain represents an increase in cardiac sympathetic dominance in autonomic control. This
228 cardiac phenotype may develop to compensate for the weakened left ventricular systolic function to
229 maintain cardiac output. However, this is unsustainable and has been associated with increased risk
230 of eventual heart failure.²⁹

231 Next, to determine if the cardiac dysfunction measured in the isolated heart of fetuses near
232 term via the Langendorff preparation was permanent and thereby also reflected in adolescence, and
233 to match the *in vivo* echocardiography to *ex vivo* assessment of cardiac function in ACS-exposed

234 lambs, the hearts of adolescent offspring were also mounted onto an isolated Langendorff preparation
235 at the end of all procedures. This analysis showed impaired left ventricular developed pressure,
236 increased left ventricular end diastolic pressure, reduced coronary flow rate, an enhanced
237 chronotropic and inotropic responsiveness to the β_1 adrenergic isoprenaline, and a reduced
238 chronotropic and inotropic responsiveness to the muscarinic agonist carbachol in adolescent lambs
239 of ACS-exposed pregnancies compared to controls. Therefore, this *ex vivo* analysis of cardiac
240 function matched the systolic and diastolic dysfunction measured via echocardiography, and indices
241 of cardiac sympathetic dominance determine in ACS-exposed lambs, which were determined *in vivo*.
242 In addition, these data support that cardiac dysfunction originating *in utero* is sustained and persists
243 until young adulthood in offspring of ACS-treated pregnancies. Further, this cardiac phenotype in
244 ACS-treated adults was accompanied by increased collagen deposition in the LV and aorta and
245 reduced stretch of the aortic root in systole despite an equivalent stroke volume to controls. This
246 suggests that cardiac dysfunction in ACS-exposed adolescent offspring is due to cardiac fibrosis and
247 stiffening rather than triggered by systemic hypertension. This is consistent with findings reported
248 in human young adults born preterm, in whom ACS exposure was also associated with increased
249 aortic stiffness.⁵ In humans, whether preterm birth or ACS therapy is responsible for any underlying
250 cardiovascular dysfunction determined in ex-preterm adults is difficult to disentangle as prematurity
251 and ACS treatment go hand-in-hand. Our findings suggest that any hypertension in ex-preterm
252 humans is likely due to prematurity, as we did not find evidence of high blood pressure in ACS-
253 exposed adolescent lambs, which were born at term. Conversely, any cardiac dysfunction measured
254 in ex-preterm humans is likely due to ACS independent of prematurity, as we did find significant
255 systolic and diastolic dysfunction in ACS-exposed adolescent lambs, despite being born at term.

256 *Clinical considerations.* Given our experimental findings that ACS given at a clinically
257 relevant gestational age results in impaired cardiac function and increased cardiac fibrosis in
258 adolescent lambs, we sought to determine whether a comparable effect could be found in humans.
259 Specifically, we endeavoured to identify an epidemiological cohort that would allow assessment of
260 long-term cardiac outcomes following ACS exposure, particularly in individuals who were treated
261 for threatened preterm birth but who ultimately were born at term. Such a cohort would provide a
262 clinically relevant comparison between term-born infants exposed to ACS and term-born infants with
263 no such exposure, thereby isolating any effects of glucocorticoids from those of prematurity.

264 However, despite extensive review of available datasets and registries, we were unable to identify an
265 appropriate cohort in which ACS administration was systematically documented for pregnancies
266 complicated by threatened preterm labour and linked to postnatal or later-life cardiac function. Two
267 small studies have assessed cardiac function in children whose mothers received ACS during
268 pregnancy. The first focused on LV wall and internal diameters, which were not different between
269 ACS treated and not-treated children.³⁰ The second compared betamethasone exposed and non-
270 exposed preterm infants and found no difference in cardiac output, stroke volume, fractional
271 shortening or ejection fraction by 8 years of age, however there was no term-born reference group.³¹
272 The most comprehensive cardiac phenotyping currently available has been conducted as part of the
273 Cardiovascular Health Assessment of Preterm and TERM-born children (CHAPTER) Study by
274 Lewandowski and colleagues.³² In this study children who previously received echocardiography
275 scans shortly after birth and three months postpartum,^{33,34} were followed up between ages 3-12 years
276 for two additional echocardiography scans as well as detailed cardiovascular magnetic resonance
277 imaging between ages 6-9 years. However, the cohort lacked an appropriate number of term-born
278 fetuses that were exposed to ACS and those from a healthy pregnancy. The absence of suitable
279 clinical data highlights a significant gap of important knowledge. Given the widespread use of ACS
280 and the growing recognition that *in utero* exposure can have lasting cardiovascular consequences on
281 offspring health, there is a clear and pressing clinical need to establish prospective cohorts or leverage
282 improved clinical documentation to evaluate whether the cardiac effects observed in our sheep model
283 translate to humans. Addressing this gap is essential for informing evidence-based clinical practice
284 and optimising long-term cardiovascular health in ACS-exposed offspring.

285 *Conclusions and implications.* This study demonstrates that exposure to ACS alone can
286 program cardiac dysfunction and fibrosis in adolescent sheep offspring born at term, highlighting
287 effects relevant to humans. Notably, ACS in sheep did not induce hypertension in adulthood, aligning
288 with human data showing that exposed individuals remain normotensive by middle age, but they may
289 still harbour subclinical cardiac vulnerability. There is no question that ACS therapy for threatened
290 preterm labour should remain, as its life-saving effects for babies born preterm are uncontested.
291 However, our findings highlight that current treatment regimens need further refining to maintain
292 benefits whilst minimising adverse off-target effects. There is evidence that the clinical dose of ACS
293 is in excess of that required to stimulate the physiological maturation of fetal organs, as administering

294 a third of the clinical dose of ACS is sufficient for lung maturation in sheep.³⁵ Studies have also
295 reported that adverse effects of glucocorticoids on the cardiovascular system are secondary to
296 oxidative stress.³⁶ We have shown in several animal models of glucocorticoid exposure that
297 combined therapy of glucocorticoids with antioxidants maintains beneficial maturational effects on
298 the lung while minimising adverse side-effects on the developing cardiovascular system.⁹ Therefore,
299 lower doses or combined antioxidant and glucocorticoid therapy may be safer than current ACS
300 regimens to optimise infant outcomes after preterm birth, and this needs testing in human clinical
301 trials.

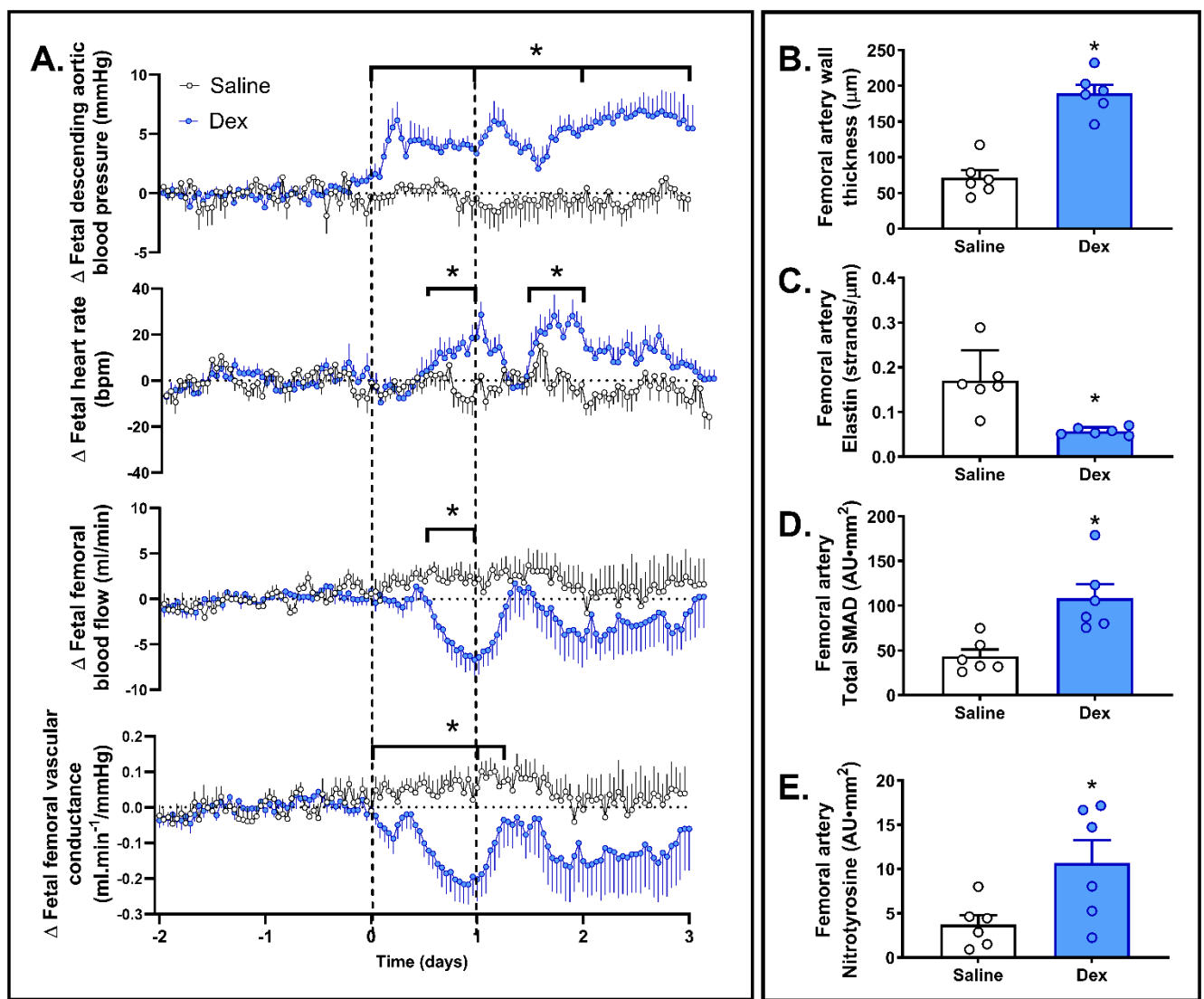


Figure 1. Values show the mean $\pm$ SEM change from baseline calculated every hour for arterial blood pressure, femoral blood flow, femoral vascular conductance and heart rate (A) and for the wall thickness, and levels of elastin, SMAD and nitrotyrosine staining (B-E) in femoral arteries in fetuses following maternal treatment at 0.8 of gestation with saline (white symbols) or Dex (blue symbols). 12mg of dexamethasone dissolved in saline (Dex) or the same volume of saline (Saline) was administered intramuscularly to the ewe on day 0 and day 1, represented by the two dotted lines. Relative to maternal treatment with saline, maternal treatment with Dex increased fetal descending aortic pressure (MAP) and fetal heart rate (FHR) and reduced fetal femoral blood flow (FBF) and femoral vascular conductance (FVC). Relative to maternal treatment with saline, maternal treatment with Dex increased wall thickness, decreased elastin density and increased smooth muscle actin (SMAD) and nitrotyrosine staining in fetal femoral arteries. Continuous fetal cardiovascular data shown in Fig.1A was averaged over one hour and displayed as a circle, with repeated measures ANOVA for the effect of treatment group, repeated for time, applied. For all cardiovascular parameters in Fig.1A. there was an interaction between treatment group and time, therefore repeated measures were performed for each day separately. If an interaction persisted, data was analysed over 12 or 6 hour (hr) blocks, with the largest time period without an interaction selected and represented by the horizontal bracket above (MAP, 24 hr; FHR, 12 hr; FBF, 12 hr; FVC, 24 hr then 6 h blocks).³⁷ Data in Fig. 1B-D were analysed statistically by the Student's *t* test for unpaired data. *, effect of treatment, $P < 0.05$.

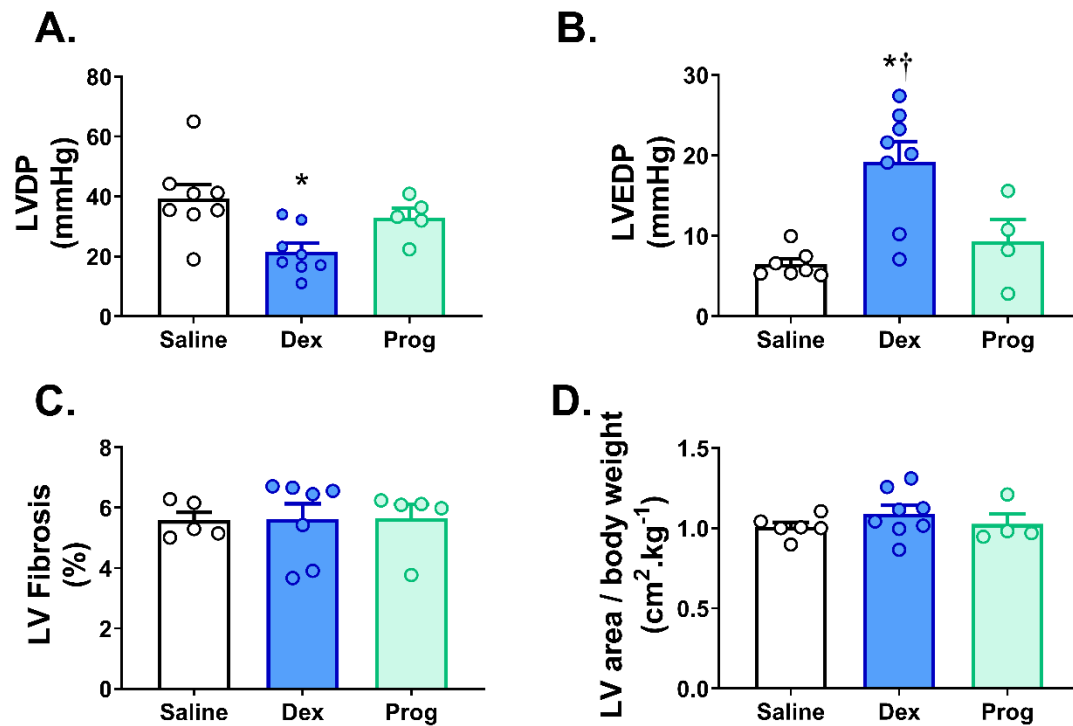


Figure 2. Values show the mean+SEM for left ventricular developed pressure (LVDP, A), left ventricular end diastolic pressure (LVEDP, B), LV fibrosis (C) and the LV area relative to the body weight (D) in term fetuses following maternal treatment with saline (white symbols), maternal treatment with dexamethasone (Dex, blue symbols) at 0.8 of gestation after progesterone treatment at 80 days of gestation, or maternal treatment with progesterone alone at 80 days of gestation (Prog, green symbols). Relative to maternal treatment with saline, maternal treatment with Dex impaired systolic (low LVDP) and diastolic (high LVEDP) function, without affecting LV fibrosis or LV wall area. The effects of maternal treatment with Dex on cardiac function in the term fetus were independent of any effects of progesterone pre-treatment at 80 dGA. Data were analysed statistically by One-Way ANOVA with Tukey's *post hoc* test. * vs Saline, † vs Progesterone alone, P<0.05.

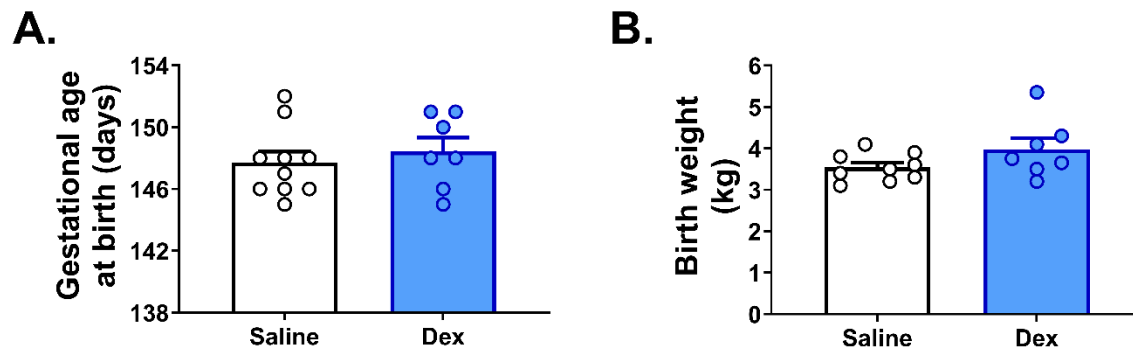


Figure 3. Values show the mean+SEM for gestational age (A) and weight (B) at birth in lambs following maternal treatment with saline at 0.8 of gestation (white symbols) or maternal treatment with dexamethasone at 0.8 of gestation after progesterone treatment at 80 days of gestation (Dex, blue symbols). Relative to maternal treatment with saline, maternal treatment with Dex did not affect gestational age or birth weight. Data were analysed statistically by the Student's *t* test for unpaired data.

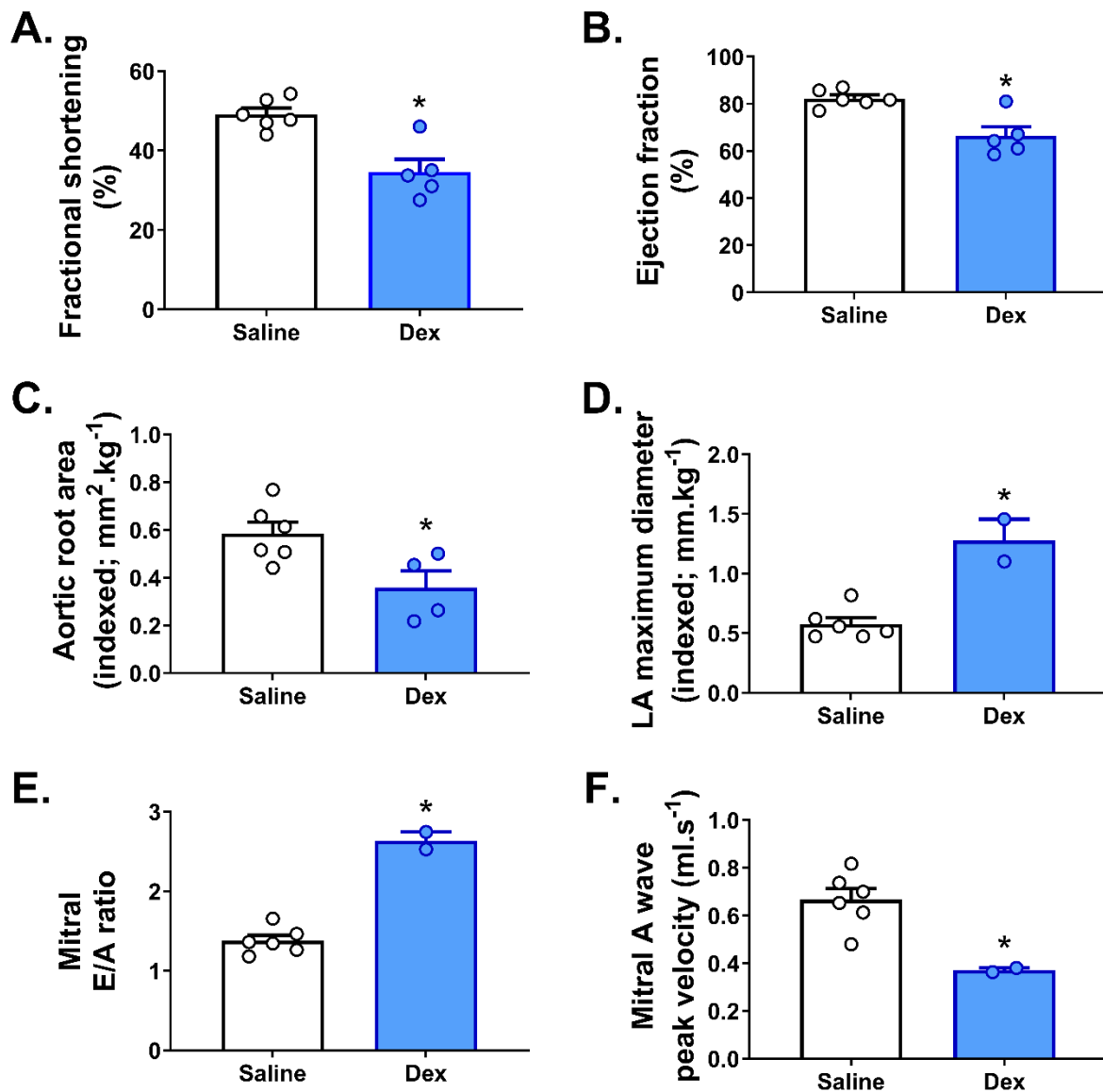


Figure 4. Values show the mean+SEM for fractional shortening (A), ejection fraction (B), the maximal aortic root diameter during systole (C), the left atrial (LA) maximum diameter (D), the mitral E/A wave ratio (E), and the mitral A wave peak velocity (F) in 9-11 month old adolescent lambs following maternal treatment with saline at 0.8 of gestation (white symbols) or maternal treatment with dexamethasone (dex, blue symbols) at 0.8 of gestation after progesterone treatment at 80 days of gestation. Relative to maternal treatment with saline, maternal treatment with Dex at 0.8 of gestation programmed greater indices of aortic stiffness (reduced aortic root diameter during systole), and a greater left atrial diameter likely due to decreased compliance of the left ventricle demonstrated by an increased mitral E/A wave ratio. Data were analysed statistically by the Student's *t* test for unpaired data; *P<0.05.

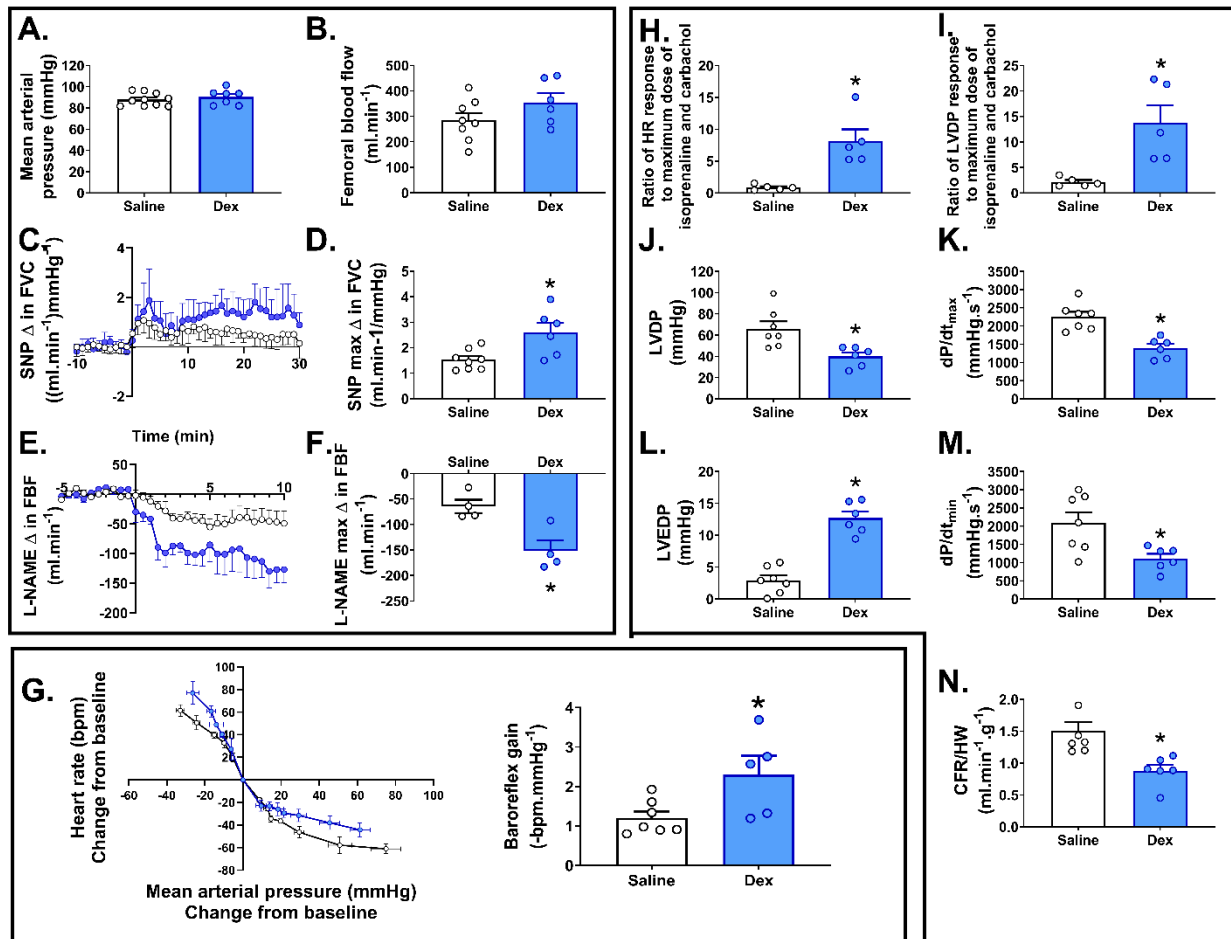


Figure 5. Values show the mean+SEM for basal mean arterial pressure (A) basal femoral blood flow (B) the change from baseline calculated every minute for mean femoral vascular conductance (FVC) following treatment with sodium nitroprusside (SNP, C), the maximal change from baseline for mean femoral vascular conductance (FVC) following treatment with SNP (D), the change from baseline calculated every min for mean femoral blood flow (FBF) following treatment with L-NAME (E), and the maximal change from baseline for mean FBF following treatment with L-NAME (F), the cardiac baroreflex function curve with calculation of the baroreflex gain (inset) determined through *in vivo* cardiovascular studies (G), the ratio of the heart rate (HR, H) and left ventricular developed pressure (LVDP; J) in responses to maximal doses of isoprenaline and carbachol determine, LVDP (J), the maximum first derivative of the LV pressure (dP/dt_{max}, K), the left ventricular end diastolic pressure (LVEDP, F), the minimum first derivative of the LV pressure (dP/dt_{min}, M) and the coronary blood flow relative to heart weight (CFR/HW, H) by the isolated Langendorff preparation in 9-11 month old adolescent lambs following maternal treatment with saline (white symbols) or maternal treatment with dexamethasone (Dex, blue symbols) at 0.8 of gestation after progesterone treatment at 80 days of gestation. Relative to maternal treatment with saline, maternal treatment with Dex at 0.8 of gestation programmed a greater dependence on NO to maintain basal blood pressure and blood flow, greater baroreflex gain and indices of cardiac sympathetic dominance in the adolescent lambs. Data were analysed statistically by the Student's *t* test for unpaired data, *P<0.05.

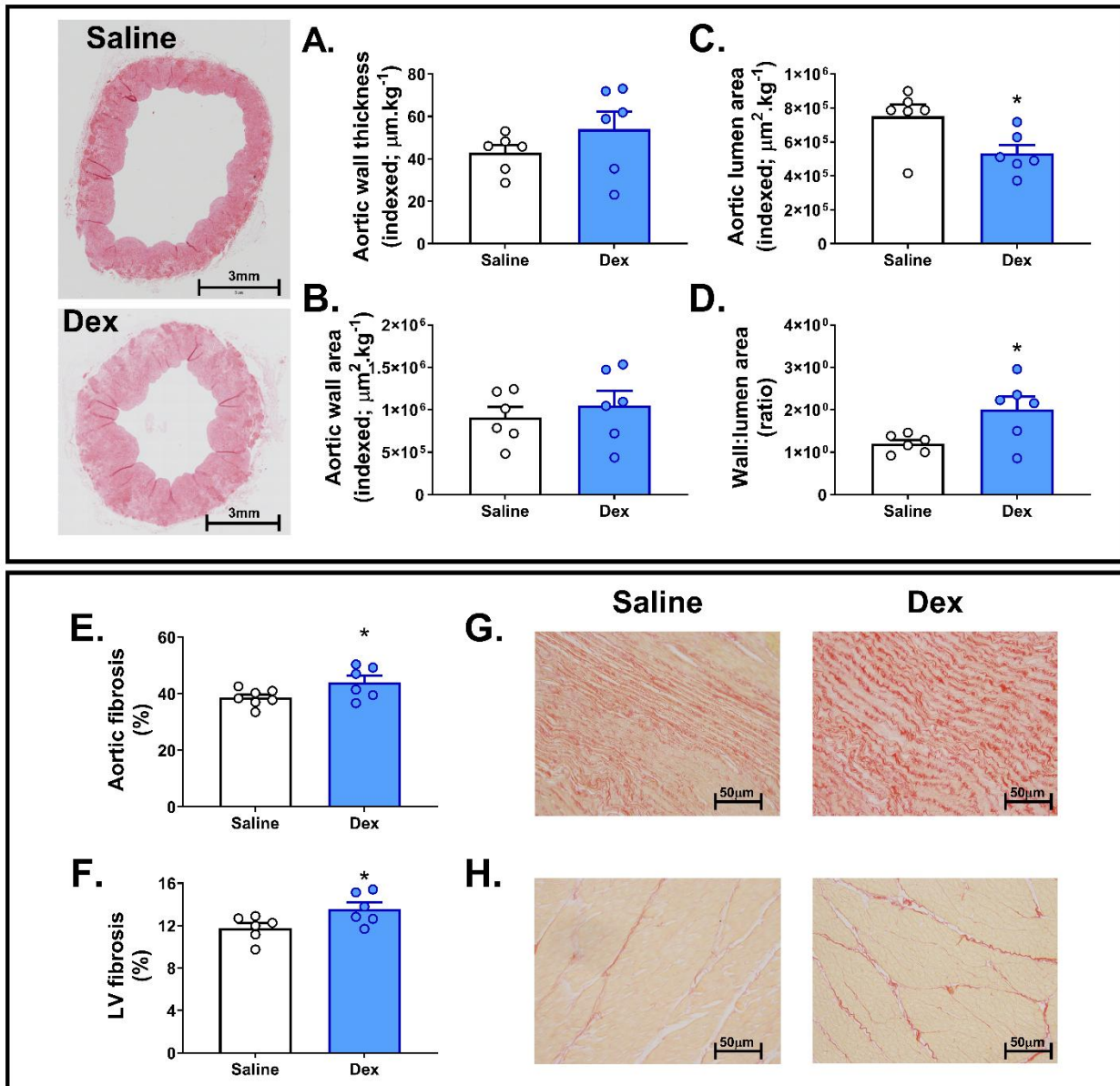


Figure 6. Values show the mean+SEM for aortic wall thickness (A), aortic wall area (B), aortic lumen area (C), the aortic wall:lumen area ratio (D), the percentage of aortic fibrosis (E) and left ventricular (LV) fibrosis (F) in 9-11 month old adolescent lambs following maternal treatment with saline at 0.8 of gestation (white symbols) or maternal treatment with dexamethasone (Dex, blue symbols) at 0.8 of gestation after progesterone treatment at 80 days of gestation. Panels G and H show examples of aortic wall remodelling. Panels I and J show examples of LV fibrosis. Relative to maternal treatment with saline, maternal treatment with Dex at 0.8 of gestation programmed greater indices of aortic wall remodelling, aortic wall fibrosis and LV wall fibrosis in the adolescent lambs. Data were analysed statistically by the Student's *t* test for unpaired data. *, $P < 0.05$.

Antenatal corticosteroids

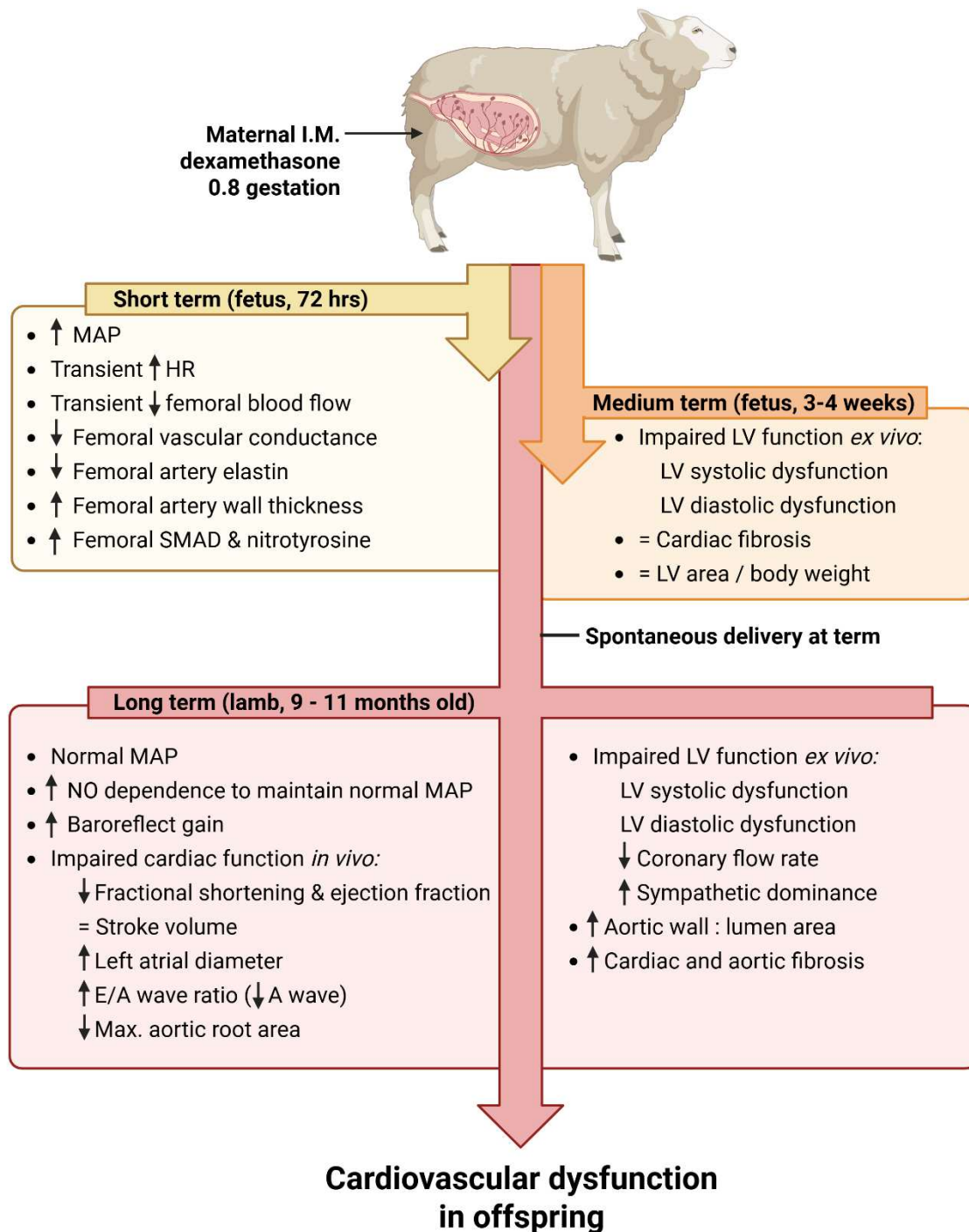


Figure 7. Maternal antenatal corticosteroid administration at 0.8 gestation in sheep result in short-, and medium- *in utero* changes and long-term changes in cardiovascular function in the offspring when born at term. *In utero* changes were assessed at 72 hours (short-term) or 3-4 weeks (medium term) after maternal ACS administration. Long-term changes were assessed 9-11 months after spontaneous birth at term. Intramuscular (I.M.), mean arterial pressure (MAP), heart rate (HR), smooth muscle actin (SMAD), left ventricle (LV), nitric oxide (NO), maximum (Max.).

ONLINE METHODS

Ethical approval

303 All animal procedures conform with the guidelines from Directive 2010/63/EU of the
304 European Parliament on the protection of animals used for scientific purposes. All procedures were
305 performed under the Home Office Project Licenses PL70/7645, PPL 80/1301, PPL80/2232 under the
306 UK animals Scientific Procedures Act 1986 following ethical review approval by the University of
307 Cambridge Animal Welfare and Ethical Review Board (AWERB). The experimental design was
308 conducted according to the ARRIVE guidelines for reporting on *in vivo* experiments in animals.³⁸

Experimental design

309 Using a human clinically relevant course of antenatal corticosteroid (ACS) therapy,³⁹ the
310 objectives of this study were to determine in sheep pregnancy the short, medium- and long-term direct
311 effects of maternal treatment with dexamethasone on the cardiovascular system of the offspring.
312 Three studies addressed these objectives:

313 Study 1. Short-term effects of maternal ACS treatment at 0.8 of gestation on fetal cardiovascular
314 structure and function measured 48 hours following exposure;

315 Study 2. Medium-term effects of maternal ACS treatment at 0.8 of gestation on fetal cardiovascular
316 structure and function measured in the fetus at term;

317 Study 3. Long-term effects of maternal ACS treatment at 0.8 of gestation on cardiovascular structure
318 and function in adolescent lambs at 9-11 months of age, born at term.

319 As in humans, dexamethasone readily crosses the placenta and is detectable in the fetal
320 circulation at pharmacologically relevant concentrations.⁴⁰⁻⁴² In sheep, administration of
321 glucocorticoids can induce preterm labour several days following treatment.¹⁶ To prevent this, ewes
322 randomly assigned to determine the medium- and long-term effects of dexamethasone on
323 cardiovascular structure and function in the offspring in Study 2 and Study 3, respectively, were also
324 given long-acting progesterone (150mg I.M.; Depo-provera; Medroxyprogesterone acetate; Pfizer) at
325 80 days of gestation (term is *ca.* 147 days). Pre-treatment with progesterone maintained pregnancy
326 until term in ACS exposed sheep. Therefore, this experimental design permitted the direct effects of
327 ACS independent of pre-term birth on offspring cardiovascular outcomes to be isolated. A group of

ewes treated with progesterone alone at 80 days of gestation controlled for any effects of this treatment on offspring cardiovascular function.

Surgical preparations

Pre-operative procedures. For all surgeries, food but not water was withdrawn for 24 h prior to theatre admission. General anaesthesia was induced in singleton pregnant ewes^{43,44} or lambs^{37,45} with alfaxalone (2-3 mg.kg⁻¹ I.V.; Alfaxan, Jurox) and maintained with isoflurane (1.5-2.0% in 4:1 O₂:N₂O) by use of a positive pressure ventilator (Datex-Ohmeda Ltd., Hatfield, Hertfordshire, UK). Oxygen saturation, end-tidal carbon dioxide and heart rate were monitored non-invasively throughout. Antibiotics (30 mg.kg⁻¹ I.M. procaine benzylpenicillin; Depocillin; Intervet UK Ltd., Milton Keynes, UK), analgesic (1.4 mg.kg⁻¹ S.C. carprofen; Rimadyl; Pfizer Ltd., Kent, UK) and Vitamin B12 (0.04 mg.kg⁻¹ I.M. Anivit B12; Animalcare Ltd., York, UK) were administered immediately before the start of surgery.

Operative procedures. For Study 1, 12 Welsh Mountain ewes carrying a singleton pregnancy were surgically instrumented at 117 ± 2 days gestation, as previously described in detail.^{43,44} In brief, midline abdominal and uterine incisions were made to exteriorise the fetal hind limbs. A catheter was inserted into a fetal femoral artery and advanced to the descending fetal aorta (i.d., 0.86 mm; o.d., 1.52 mm; Critchley Electrical Products, Kingsgrove, NSW, Australia). Another catheter was anchored onto the fetal hind limb for recording of the reference amniotic pressure. A Transonic flow probe (2-mm aperture; R-series, Transonic Systems Inc., Ithaca, NY, USA) was implanted around the contra-lateral fetal femoral artery. The uterine and abdominal incisions were closed in layers, following which catheters were inserted into the maternal femoral artery (Teflon; i.d. 1.0 mm, o.d. 1.6 mm, Altec, UK) and vein (PVC; i.d. 0.86 mm, o.d. 1.52 mm, Critchley Electrical Products, NSW, Australia).

For Studies 2 and 3, 44 Welsh Mountain ewes carrying a singleton pregnancy were surgically instrumented at 100 ± 1 days of gestation, as previously described in detail.^{37,46} Following a midline abdominal incision and uterotomy, the fetal hind limbs were exposed, and the fetal sex determined. The fetus was returned into the intrauterine cavity, and the uterine and maternal abdominal incisions were closed in layers. Next, catheters were inserted into the maternal femoral artery (Teflon; i.d. 1.0

355 mm, o.d. 1.6 mm, Altec, UK) and vein (PVC; i.d. 0.86 mm, o.d. 1.52 mm, Critchly Electrical
356 Products, NSW, Australia).

357 For Study 3, 23 Welsh Mountain singleton lambs born at term underwent surgery during
358 adolescence at 9-11 months of age, as previously described in detail ^{37,46}. In brief, catheters were
359 inserted into the femoral artery (Teflon; i.d. 1.0 mm, o.d. 1.6 mm, Altec, UK) and vein (PVC; i.d.
360 0.86 mm, o.d. 1.52 mm, Critchly Electrical Products, NSW, Australia). A Transonic flow probe (4
361 mm aperture; SB-series, Transonic Systems Inc., Ithaca, NY, USA) was implanted around the contra-
362 lateral femoral artery.

363 For all Studies 1-3, all catheters and flow probe leads were exteriorized together through a
364 small incision in the ewe or adolescent lamb's flank and housed in a pouch sutured to the skin. The
365 dead space of all catheters was filled with heparinized saline (100 I.U. heparin/ml in 0.9% NaCl) and
366 the catheter ends were closed with three-way taps. Inhalation anaesthesia was withdrawn, and the
367 ewe or adolescent lamb was extubated only after spontaneous breathing had returned.

368 *Postoperative procedures.* During recovery, pregnant ewes and adolescent lambs were housed in
369 individual floor pens with a 12 h:12 h light:dark cycle, hay and water *ad libitum* and were fed
370 concentrates twice daily (100 g sheep nuts no. 6; H & C Beart Ltd., Kings Lynn, UK). In study 1,
371 antibiotics were administered daily to the fetus through the femoral artery (300 mg ampicillin:
372 Penbritin, SmithKline-Beecham Animal Health, Surrey, UK) and amniotic catheters (300 mg
373 ampicillin). In the pregnant ewe or adolescent lamb, antibiotics were administered for 5 days (30
374 mg.kg⁻¹ I.M. of procaine benzylpenicillin; Depocillin, Intervet UK Ltd.). In the pregnant ewe or
375 adolescent lamb, analgesia was administered for three days post surgery (1.4 mg.kg⁻¹ S.C. carprofen;
376 Rimadyl; Pfizer Ltd.). To monitor wellbeing, daily arterial blood samples were taken from the
377 pregnant ewe, fetus and adolescent lamb. Blood samples were analysed for pH, HCO₃⁻ and acid base
378 excess concentrations and partial pressures of O₂ and CO₂. Blood samples were corrected for
379 temperature (ewe/lamb at 38°C and fetus at 39°C) and processed using an ABL5 analyser
380 (Radiometer Copenhagen, Crawley, UK). Haemoglobin concentration, O₂ saturation and haematocrit
381 values were measured using an ABL80 haemoximeter (Radiometer Copenhagen, Crawley, UK).
382 Blood glucose and lactate concentrations were measured on YSI blood analyser (YSI 2300 STAT
383 Plus, Yellow Springs Instruments, Ohio, USA). Catheters for pregnant ewes, fetuses and adolescent

384 lambs were flushed daily with heparinised saline (100 I.U. heparin/ml in 0.9% NaCl) to maintain
385 patency.

Experimental protocols

386 *Study 1. Short-term effects of maternal ACS treatment at 0.8 of gestation on fetal cardiovascular*
387 *structure and function measured 48 hours following exposure.*

388 *Fetal cardiovascular function.* Following surgery at 117 ± 2 days of gestation, pregnant ewes were
389 maintained in floor pens inside a laboratory at the Barcroft Centre, University of Cambridge. On the
390 fifth day of postoperative recovery, at 122 ± 2 days of gestation, pregnant ewes were transferred into
391 a metabolic crate within a laboratory for long-term cardiovascular recording over several days. The
392 fetal arterial and amniotic catheters were connected to sterile pressure transducers (COBE; Argon
393 Division, Maxim Medical, Athens, TX, USA) and the femoral Transonic flow probe was connected
394 to a flow meter (T206; Transonics Systems Inc., Ithaca, NY, USA). Calibrated mean fetal arterial
395 blood pressure (corrected for amniotic pressure), fetal femoral artery blood flow and fetal heart rate
396 (triggered via a tachometer from the pulsatility of either the arterial blood pressure or blood flow
397 signals) were recorded continually at 1 s intervals using a computerised Data Acquisition System.
398 After 48 h of baseline cardiovascular recording, ewes were randomly assigned to two experimental
399 groups. Six ewes received two injections of dexamethasone (12 mg IM dexamethasone sodium
400 phosphate; Merck Sharpe, Dohme Ltd., Herts, UK) in 2 mL saline solution (0.9% w/v NaCl) 24 h
401 apart (7 AM, 124 ± 1 and 125 ± 1 days of gestation). Six ewes received 2 injections of saline vehicle
402 solution (2 mL 0.9% w/v NaCl) 24 h apart (7 AM, 124 ± 1 and 125 ± 1 days of gestation) to serve as
403 sham controls. Two days later, at 127 ± 1 days of gestation, the continuous cardiovascular recording
404 ceased, and animals were humanely killed with an overdose of anaesthetic (200 ml.kg^{-1} sodium
405 pentobarbital I.V.; Pentoject; Animalcare Ltd., York, UK). At post mortem, a fetal femoral artery
406 was isolated and fixed in formalin for histological analysis.

407 *Fetal peripheral vascular oxidative stress and remodelling.* To determine any short-term effects of
408 maternal ACS treatment on indices of oxidative stress and remodelling in the peripheral vasculature,
409 fetal femoral arteries were processed for calculation of wall thickness and area, elastin strand density,
410 the expression of smooth muscle actin (SMAD) and nitrotyrosine (NT) staining. 7 μm paraffin
411 embedded sections were de-waxed with xylene and rehydrated in decreasing concentration of ethanol

412 (98%, 75%, then 50%) before being washed in water. Elastic Van Gieson staining was used to
413 identify elastin strands present within the vessel wall. The elastin strand density was calculated by
414 counting the number of strands within the wall, then dividing this by the wall thickness. To calculate
415 the density of SMAD and NT expression, samples were treated before incubation at 4 °C overnight
416 with 20 µl of murine anti-SMAD (Dako Ltd., Ely, UK) or anti-NT (Cayman, California, USA) IgG
417 antibody, or the same volume of phosphate-buffered saline (PBS) for controls. The following day,
418 antibodies were removed, the samples washed with PBS and then incubated with 20 µl of secondary
419 antibody (rabbit anti-mouse) for 30 minutes. Following a further PBS wash, samples were incubated
420 with 20 µl of a tertiary, goat anti-rabbit IgG4 antibody for 40 minutes. Samples were then washed
421 with PBS and distilled water, before incubation with 30 µl of an enhancing agent. This was removed,
422 the samples washed with water and allowed to dry before coverslips were applied. Staining density
423 was quantified and statistically analysed on a digital camera system (Leitz HM-Lux Microscope and
424 Euromax Eurocam with WinTV2000 software). Images were captured as a JPEG at 4x magnification
425 (exposure 2.5 ms, light intensity 2.5 lux). Using a stage micrometer (Watson) for calibration, 3
426 distinct 1 µm² fields were imaged. The pixel brightness for each field (scale 0-255 where 255
427 represents maximum brightness) was measured, and the mean of the three readings per sample taken.
428 To control for background brightness, this value was subtracted from the mean of the unstained
429 control of each sample. The resultant value was then multiplied by the wall area to give a
430 representative volume of either SMAD or NT.

431 ***Study 2. Medium-term effects of maternal ACS treatment at 0.8 of gestation on cardiovascular***
432 ***structure and function measured in the fetus at term.***

433 Following surgery at 100 ± 1 days of gestation, pregnant ewes were maintained in floor pens inside
434 a laboratory at the Barcroft Centre, University of Cambridge. Approximately two weeks following
435 surgery, the ewes were randomly assigned to three experimental groups. 8 ewes received 2 injections
436 of saline vehicle solution (2 mL 0.9% w/v NaCl) 24 h apart (115 and 116 days of gestation) to serve
437 as sham controls. 8 ewes pre-treated with progesterone at 80 days of gestation received two injections
438 of dexamethasone (12 mg IM dexamethasone sodium phosphate; Merck Sharpe, Dohme Ltd., Herts,
439 UK) in 2 mL saline solution (0.9% w/v NaCl) 24 h apart (115 and 116 days of gestation) to serve as
440 the ACS term group. 5 ewes pre-treated with progesterone at 80 days of gestation received 2 injections

441 of saline vehicle solution (2 mL 0.9% w/v NaCl) 24 h apart (115 and 116 days of gestation) to control
442 for any effects of progesterone only. Pregnancies were maintained until close to term (138 ± 2 days
443 of gestational age) and animals were humanely killed with an overdose of anaesthetic ($200 \text{ mL} \cdot \text{kg}^{-1}$
444 sodium pentobarbital I.V.; Pentoject; Animalcare Ltd., York, UK). At post mortem examination, the
445 fetal heart was isolated to determine effects on basal and stimulated cardiac function.

446 *Isolated Langendorff preparation in hearts of fetuses at term.* Fetal hearts were mounted onto the
447 Langendorff apparatus and perfused at a constant pressure of 40 mmHg. The ductus arteriosus was
448 ligated. A recirculating solution of Krebs–Henseleit bicarbonate buffer containing ($\text{mmol} \cdot \text{L}^{-1}$) 120
449 NaCl, 4.7 KCl, 1.2 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 KH_2PO_4 , 25 NaHCO_3 , 10 glucose, and 1.3 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was
450 filtered through a 5 μm cellulose nitrate filter (Millipore, Bedford, MA, USA) and gassed with
451 $\text{O}_2\text{:CO}_2$ (95:5) at 39.5°C . A small flexible non-elastic balloon was inserted into the left ventricle
452 through the left atrium. The balloon was filled with deionized water and attached to a rigid deionized
453 water-filled catheter connected to a calibrated pressure transducer (Argon Medical Devices, Frisco,
454 TX, USA). The balloon volume was set at 2.5 mL and differences in pressure for the set volume were
455 calculated.⁹ After an initial 15 min stabilization period, basal fetal heart rate (FHR), fetal left
456 ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP) were
457 recorded. Basal left ventricular developed pressure (LVDP) was calculated as $\text{LVSP} - \text{LVEDP}$.

458 *Cardiac remodelling in the fetuses at term.* To determine any medium-term effects of maternal
459 dexamethasone treatment at 0.8 of gestation on cardiovascular remodelling in the term fetus, hearts
460 were cut into 1 cm transverse slices at the end of the isolated Langendorff preparation. A mid cardiac
461 section, slice 5, (slice 1 assigned to the apex) was used to determine fetal LV wall thickness. A
462 transparent point counting grid was applied, with each point equivalent to 0.08 cm^2 . LV wall surface
463 area was normalised to body weight measured at post mortem.

464 ***Study 3. Long-term effects of maternal ACS treatment at 0.8 of gestation on cardiovascular***
465 ***structure and function in the adolescent lambs at 9-11 months of age, born at term.***

466 Following surgery at 100 ± 1 days of gestation, another cohort of pregnant ewes were maintained in
467 floor pens inside a laboratory at the Barcroft Centre, University of Cambridge. Approximately two
468 weeks following surgery, pregnant ewes were randomly assigned to two experimental groups. 7 ewes

469 received two injections of dexamethasone (12 mg IM dexamethasone sodium phosphate; Merck
470 Sharpe, Dohme Ltd., Herts, UK) in 2 mL saline solution (0.9% w/v NaCl) 24 h apart (115 and 116
471 days of gestation). 16 ewes received saline vehicle solution (0.9% w/v NaCl) to serve as sham
472 controls. Pregnancies were maintained until spontaneous vaginal birth at term. Newborn lambs were
473 maintained with their mothers at foot until weaning at 5 months of age. Following weaning, lambs
474 were taken to pasture within the paddocks of the Barcroft Centre and were maintained until 9-11
475 months of age. Newborn lambs were weighed within 12 h of birth and then daily for the first week of
476 life. Thereafter, body weight was recorded at 2 weeks of age, 30 days of age, and then at subsequent
477 30-day intervals until they were 9-11 months old. At this age, lambs underwent non-invasive and
478 invasive *in vivo* cardiovascular experiments.

479 *In vivo non-invasive assessment of cardiac function by echocardiography in the adolescent lamb.*
480 One investigator (CJS) performed echocardiography by using ultrasound (P10-4 Toshiba
481 PowerVision 7000) with a phased array 2.0 MHz probe (PSK-20CT). The echocardiographic
482 assessment in the adolescent lamb was conducted under general anaesthesia on the surgical table, just
483 prior to surgical instrumentation, *via* transabdominal ultrasound examination. M-mode ultrasound
484 was used for the assessment of LV fractional shortening (FS) and ejection fraction (EF). B-mode
485 imaging was performed for measurements of ventricular and atrial chambers, aortic (AV) and mitral
486 (MV) valve and aortic root dimensions, ventricular wall, and interventricular septum (IVS) thickness.
487 LV fractional shortening (FS) was calculated as the proportional difference between end-diastolic
488 (EDD) and end-systolic (ESD) diameter; $FS = (EDD - ESD) / EDD$. PW Doppler was used to obtain
489 Doppler signals from the inflow [early (E) and late diastolic (A) velocities and the E/A ratio] and
490 outflow tracts for evaluation of diastolic and systolic function, respectively, together with calculation
491 of LV stroke volume (SV) and cardiac output (CO). CO was calculated as $CO = [\text{outflow tract}$
492 $\text{dimension} \times VTI] \times \text{heart rate}$. The ejection fraction (EF) formula equals the amount of blood pumped
493 out of the ventricle with each contraction (stroke volume or SV) divided by the end-diastolic volume
494 (EDV), the total amount of blood in the ventricle, and expressed as a percentage, as $EF = (SV / EDV)$
495 $\times 100$. All measurements were performed in a single heartbeat according to standardized protocols
496 described for echocardiography.⁴⁷

497 *In vivo invasive cardiovascular function in the adolescent lamb.* After a minimum of 5 days of
498 postsurgical recovery, basal and stimulated *in vivo* cardiovascular function was determined in the 9-

499 11 months old lambs, as previously described.^{37,46} Briefly, lambs were moved into a metabolic crate
500 in the morning and the arterial catheter was connected to a sterile pressure transducer (Argon
501 Division, Maxxim Medical, Athens, Texas, USA) and the femoral flow probe to a Transonic flow
502 meter (T206; Transonics Systems Inc., Ithaca, NY, USA). Lambs were acclimatised to being in the
503 crate for 2-3 h before commencing recordings. Experiments were carried out at a similar time of day.
504 A custom-built Data Acquisition System (Maastricht - Programmable AcQuisition system, M-PAQ
505 and IDEEQ software, Maastricht Instruments, the Netherlands; 1000Hz sample rate) was used to
506 record arterial blood pressure and femoral blood flow continuously. Heart rate was calculated on-line
507 by the program using the femoral blood flow or systolic pulse as a trigger. Femoral vascular resistance
508 (FVR) and femoral vascular conductance (FVC) were calculated by applying Ohm's Law to the
509 circulation, using the following equations: $FVR = MAP/FBF$ and $FVC = FBF/MAP$, where MAP is
510 the mean arterial blood pressure and FBF is femoral blood flow.

511 *In vivo contribution of NO to cardiovascular function in the adolescent lamb.* To determine any
512 effects of maternal treatment with dexamethasone in programming alterations in NO signalling in the
513 adult offspring born at term, *in vivo* cardiovascular function was recorded when the lambs were
514 treated either with the nitric oxide (NO) donor, sodium nitroprusside (SNP), or the NO synthase
515 inhibitor NG-nitro-L-arginine methyl ester (L-NAME). Sodium nitroprusside (SNP; S-0501; Sigma
516 Chemicals; dissolved in heparinised saline) was infused into the femoral vein catheter at a
517 concentration of $2.5 \text{ mg.kg}^{-1}/\text{min}$ for 30 min. Cardiovascular data were recorded for 30 min prior to
518 and during SNP infusion. On a separate day, a bolus of L-NAME (100 mg.kg^{-1} ; Sigma Chemicals;
519 dissolved in 10 ml of heparinised saline) was infused into the femoral artery catheter. Cardiovascular
520 data were recorded for 30 min prior to the L-NAME bolus and until peak responses in MAP and FBF
521 were observed. To analyse changes in cardiovascular function before and after drug administration,
522 one-minute averages were calculated for at least 10 min before and after treatment.

523 *In vivo assessment of baroreflex function in the adolescent lamb.* On a separate day, to determine any
524 effects of maternal treatment with dexamethasone in programming alterations in cardiac baroreflex
525 function in the 9–11-month-old lambs, changes in heart rate and MAP were recorded in response to
526 bolus doses administered in randomised order of the alpha-adrenergic agonist phenylephrine (PE; 7
527 doses: 0.5, 2, 4, 8, 16, 32 and $64 \text{ } \mu\text{g.kg}^{-1}$, diluted in 1ml of dH₂O; L-phenylephrine; P-6126; Sigma
528 Chemicals, UK) or the vasodilator SNP (5 doses: 0.625, 1.25, 2.5, 5 and $10 \text{ } \mu\text{g.kg}^{-1}$, diluted in 1ml of

529 dH₂O; S-0501; Sigma Chemicals, UK). Bolus doses were administered via the femoral artery
530 catheter. Changes in heart rate were plotted against corresponding changes in arterial blood pressure
531 to plot a cardiac baroreflex function curve. The baroreflex gain was calculated by applying a logistic
532 sigmoidal fit to the data using established techniques.⁴⁸

533 *Isolated Langendorff preparation in the adolescent lamb.* At the end of *in vivo* cardiovascular
534 experiments, the adolescent lambs were given a lethal overdose of anaesthetic (200 ml.kg⁻¹ sodium
535 pentobarbital I.V.; Pentoject; Animalcare Ltd., York, UK) and the heart was isolated and mounted
536 onto the Langendorff apparatus. Basal cardiac function was determined as for the fetal hearts. The
537 only difference was that hearts of adolescent lambs were perfused at a constant pressure of 70 mmHg,
538 the recirculating solution of Krebs–Henseleit bicarbonate buffer was gassed with O₂:CO₂ (95:5) at
539 38°C, and the small flexible non-elastic balloon inserted into the left ventricle through the left atrium
540 was filled with 40 mL of deionized water before being attached to a calibrated pressure transducer
541 (Argon Medical Devices, Frisco, TX, USA). In addition, for hearts of adolescent lambs, the
542 maximum and minimum first derivatives of the LV pressure (dP/dtmax and dP/dtmin) were calculated
543 using an M-PAQ data acquisition system (Maastricht Programmable AcQuisition System, the
544 Netherlands). Coronary flow rate (CFR) was calculated gravitationally by timed collections of
545 perfusate. To determine changes in stimulated cardiac function, the cardiac responsiveness to
546 autonomic agonists were investigated using established procedures.⁹ Briefly, LV chronotropic and
547 inotropic responsiveness to the muscarinic agonist Carbachol (carbamylcholine chloride, Sigma-
548 Aldrich Co. Ltd, Poole, UK; range, 10⁻¹⁰ to 10⁻⁶ M) and the β₁ adrenoreceptor agonist Isoprenaline
549 ((-)-Isoprenaline (+)-bitartrate salt, Sigma-Aldrich Co. Ltd, Poole, UK; range, 10⁻¹¹ to 10⁻⁷ M) were
550 determined. Agonist doses were given sequentially but randomized for each heart with respect to
551 dose order. The heart was perfused in a non-recirculating way to avoid the accumulation of Carbachol
552 or Isoprenaline within the system. A recovery time (between 5 and 15 min) was introduced between
553 each bolus to allow variables to stabilize at baseline before administration of the next bolus dose.
554 Sympathetic dominance was calculated as the ratio of cardiac chronotropic or inotropic responses to
555 the maximum dose of Isoprenaline or Carbachol.⁹

556 *Cardiovascular remodelling in the adolescent lamb.* To determine any long-term effects of maternal
557 ACS treatment at 0.8 of gestation on cardiovascular remodelling measured in the adolescent lamb at
558 9-11 months of age, the aorta and LV were isolated at post mortem examination and prepared for

559 histological analysis. Aortic transverse sections of 0.5-1 cm were taken from the middle of descending
560 aorta samples. Sections were dehydrated in industrial methylated spirit (IMS), followed by alcohol
561 clearance in HistoClear (National Diagnostics). Sections were embedded in paraffin (Paraplast Plus,
562 Sigma) using a Leica Tissue Processor and Embedding Centre (TP1020 and EG1150, Leica
563 Biosystems), sectioned with a manual microtome (RM2235, Leica Biosystems) at 7 μ M thickness,
564 and transferred onto Superfrost Plus microscope slides (ThermoFisher Scientific). Aortic sections
565 were cleared using HistoClear, rehydrated in IMS followed by staining with Hematoxylin and Eosin
566 (H&E) to determine aortic structure. For each aortic section, a superimage at 10x magnification was
567 created for stereology analysis (inverted microscope, DMI6000B, Leica Biosystems and VIS
568 software, Visiopharm). Aortic medial wall thickness was determined by measuring the distance
569 between the aortic lumen to the start of the adventitia. The aortic intima was sufficiently thin (1-2
570 cell layers only) and did not vary in size between samples, so confounding effects on medial thickness
571 measurements by this layer were considered negligible. To control for irregularly shaped aortas due
572 to processing, wall thickness was measured from where isotropic uniform randomly distributed lines
573 in random orientation crossed the vessel lumen. The average of 8-14 measures was taken for each
574 section. Wall and lumen areas were determined using the point counting method. A grid of 16x9
575 points was displayed onto the image, and each point assigned as either wall or lumen. Two-
576 dimensional areas in μm^2 were obtained by multiplying the number of points by the area each point
577 represented. Data from three sections were used to calculate wall thickness, areas and ratios for each
578 aorta for each animal. In addition, hearts from adolescent lambs were cut into 1 cm transverse slices
579 and together with descending aortic sections, fixed with formalin. LV biopsies were taken from heart
580 slices 2-5 (slice 1 assigned to the apex) and embedded in paraffin. 7 μ M thick LV and aortic sections
581 were cleared using HistoClear and rehydrated in IMS and stained with Picrosirius Red solution
582 (Abcam) for 1 h. Sections were rinsed in acidified water, dehydrated in IMS, HistoClear and dipped
583 in xylene before mounting. Whole slide superimages containing three aortic sections or biopsies from
584 three different LV slices were taken at 1.6x magnification (inverted microscope, DMI6000B, Leica
585 Biosystems and VIS software, Visiopharm). Each aortic section or LV biopsy was considered a
586 region of interest (ROI). From each ROI, 10 images were taken from isotropic uniformly randomly
587 sampled locations at 25x magnification. For LV sections, images containing perivascular areas or
588 excessive tears in the tissue were excluded. For aortas, images containing lumen, adventitia or fold

589 artefacts were excluded. The percentage of fibrosis in each tissue was determined in a minimum of
590 7 images per animal by point counting using a 10x10 grid.

591 **Calculations and statistics**

592 All data are expressed as mean $\pm$ SEM. The dataset for each comparison was checked for normality
593 using the Shapiro-Wilk test, and all data sets were confirmed as normally distributed. Data were
594 compared using the Student's *t* test for unpaired data, one- or two-way ANOVA with repeated
595 measures, as appropriate. For all comparisons, significance was accepted when $P < 0.05$.

596

597 Reference legend

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746 **Extended Data Table 1.** Maternal arterial blood gas and metabolic status during the experimental protocol
747 in saline and dexamethasone treated animals.

Time (Day)	Time (Hours)	Group	pH _a	PCO ₂ (mmHg)	PO ₂ (mmHg)	SatHb (%)	[Hb] (g.dl ⁻¹)	Hct (%)	BG (mmol.l ⁻¹)	BL (mmol.l ⁻¹)
-2	-48	Saline	7.49±0.01	35.0±0.9	97.6±2.9	95.4±1.8	10.1±0.7	28±1	2.09±0.26	0.38±0.03
		Dex	7.47±0.01	34.3±0.4	105.4±5.1	92.1±1.7*	9.4±0.9	29±2	2.21±0.28	0.37±0.06
-1	-24	Saline	7.49±0.01	34.1±0.6	103.4±2.7	94.7±1.7	9.8±0.5	28±1	2.01±0.16	0.34±0.03
		Dex	7.49±0.01	34.0±0.3	101.7±3.9	93.7±1.3	9.4±0.7	30±3	2.26±0.22	0.42±0.11
0	-1	Saline	7.48±0.01	35.1±1.0	104.2±3.6	96.5±0.9	9.7±0.5	29±1	2.05±0.12	0.43±0.07
		Dex	7.49±0.01	35.7±1.0	98.1±2.6	93.6±1.4	9.4±0.8	29±2	2.16±0.05	0.34±0.05
	0	First injection								
	1	Saline	7.50±0.02	34.2±0.9	101.8±3.1	95.7±1.4	9.7±0.5	28±2	2.29±0.15	0.47±0.07
		Dex	7.48±0.01	36.8±0.7	103.0±7.8	92.7±1.1	9.1±0.7	26±2	2.09±0.07	0.32±0.01
	2	Saline	7.48±0.01	36.8±2.0	103.6±2.1	96.2±1.5	8.8±0.4	29±1	2.36±0.16	0.42±0.08
		Dex	7.48±0.01	35.5±0.6	105.2±8.7	93.5±0.7	8.9±0.7	26±2	2.37±0.09	0.40±0.18
	4	Saline	7.51±0.01	34.8±0.7	102.8±1.4	96.3±1.9	8.6±0.3	28±2	2.84±0.26	0.40±0.11
		Dex	7.48±0.01	35.2±0.9	101.8±2.3	92.0±0.8*	8.8±0.6	26±2	3.27±0.27	0.27±0.05
	6	Saline	7.49±0.01	35.6±1.7	98.4±2.3	97.0±1.2	9.1±0.2	27±1	2.76±0.21	0.40±0.08
		Dex	7.48±0.01	32.8±1.0	99.5±5.5	91.5±0.8*	9.2±0.8	27±2	3.76±0.36*	0.30±0.06
	8	Saline	7.48±0.01	34.4±0.8	97.4±4.7	95.7±1.7	9.5±0.3	28±1	2.61±0.10	0.40±0.08
		Dex	7.48±0.01	34.7±1.3	104.8±5.6	91.8±1.3*	8.6±0.8	26±2	3.41±0.28*	0.38±0.07
	10	Saline	7.47±0.02	37.0±1.7	100.8±3.2	96.6±0.8	9.0±0.2	27±1	2.77±0.17	0.55±0.16
		Dex	7.48±0.01	33.2±1.2	108.3±3.7	92.6±0.8*	9.2±1.1	26±2	3.24±0.20	0.51±0.10
	12	Saline	7.50±0.01	34.4±0.7	104.2±0.5	97.8±1.1	9.4±0.2	27±1	2.95±0.15	0.49±0.13
		Dex	7.48±0.01	34.0±0.8	103.0±4.3	92.2±1.0*	8.9±0.8	28±3	3.36±0.19	0.37±0.09
1	24	Saline	7.49±0.01	34.8±1.0	102.3±2.6	97.1±0.6	9.4±0.2	28±1	2.19±0.13	0.46±0.06
		Dex	7.52±0.01	35.7±0.8	100.3±2.3	93.4±1.3*	9.8±1.1	28±2	2.57±0.08	0.49±0.16
		Second injection								
	25	Saline	7.49±0.01	36.4±1.0	102.4±3.2	97.5±0.8	8.9±0.4	28±2	2.35±0.15	0.47±0.12
		Dex	7.51±0.01	36.0±0.5	109.5±4.9	93.1±1.0*	8.7±1.1	27±2	2.59±0.08	0.32±0.04
	26	Saline	7.49±0.01	35.0±1.3	112.4±3.4	97.5±1.0	8.7±0.3	28±1	2.40±0.11	0.44±0.14
		Dex	7.51±0.01	37.2±0.9	107.7±7.1	93.5±1.4*	9.0±0.8	27±3	2.76±0.17	0.38±0.05
	28	Saline	7.50±0.02	35.2±2.2	103.0±2.0	97.8±1.2	8.7±0.3	26±1	2.79±0.18	0.43±0.12
		Dex	7.50±0.01	36.0±0.4	100.3±2.9	92.8±1.2*	8.9±0.8	26±3	3.50±0.31	0.30±0.03
	30	Saline	7.52±0.01	34.0±1.4	106.0±3.0	97.5±1.2	8.7±0.2	27±1	2.80±0.12	0.47±0.12
		Dex	7.50±0.01	34.2±0.7	103.2±2.8	92.3±1.0*	9.4±0.9	27±3	3.47±0.22*	0.34±0.04
	32	Saline	7.52±0.01	35.0±1.8	102.8±6.1	97.7±0.7	8.9±0.6	26±1	2.83±0.16	0.47±0.12
		Dex	7.52±0.01	35.2±1.5	101.2±6.3	90.6±1.4*	8.7±0.7	25±2	3.44±0.18*	0.35±0.03
2	48	Saline	7.51±0.01	34.2±0.5	105.3±4.0	97.7±0.5	9.1±0.3	27±1	2.42±0.18	0.47±0.10
		Dex	7.52±0.01	34.3±0.7	107.6±4.4	93.5±1.7*	9.1±0.8	26±6	2.49±0.20	0.45±0.12
3	72	Saline	7.50±0.01	33.4±1.5	99.8±2.0	98.3±1.1	9.1±0.3	25±1	2.58±0.21	0.76±0.30
		Dex	7.49±0.02	35.8±0.6	103.0±4.9	94.0±1.5*	8.6±0.7	28±4	2.22±0.74	0.51±0.03

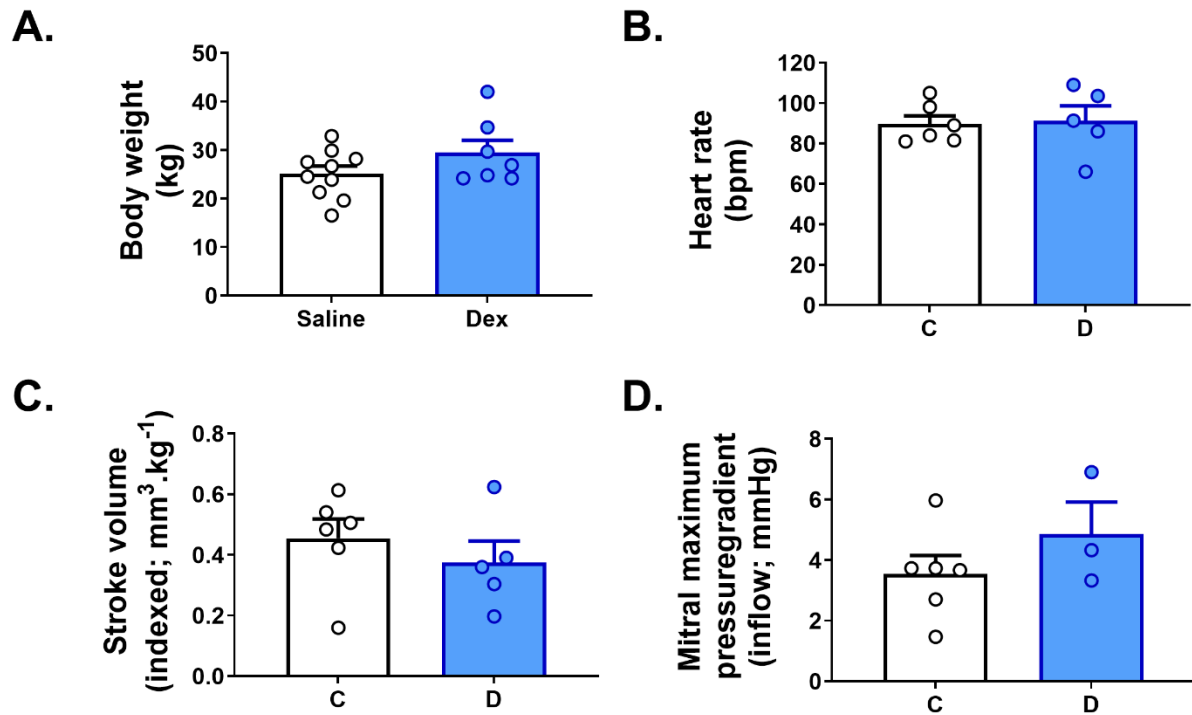
Values show the mean±SEM for the arterial pH (pH_a), the arterial partial pressure of carbon dioxide (PaCO₂); the arterial partial pressure of oxygen (PaO₂), the percentage saturation of haemoglobin with oxygen (Sat Hb)

Hb, the haemoglobin concentration ([Hb]); the haematocrit (Htc), the blood glucose concentration (BG) and the blood lactate concentration (BL) in the maternal arterial circulation following maternal treatment at 0.8 of gestation with saline or Dex. 12mg of dexamethasone dissolved in saline (Dex) or the same volume of saline (Saline) was administered intramuscularly to the ewe on day 0 and day 1. Data were analysed statistically by One-way ANOVA with repeated measures.

749 **Extended Data Table 2.** Fetal arterial blood gas and metabolic status during the experimental protocol in
750 saline and dexamethasone treated animals.

Time (Days)	Time (Hours)	Group	pH _a	PCO ₂ (mmHg)	PO ₂ (mmHg)	SatHb (%)	[Hb] (g.dl ⁻¹)	Hct (%)	BG (mmol.l ⁻¹) ¹⁾	BL (mmol.l ⁻¹)
-2	-48	Saline Dex	7.38±0.01	50.0±1.1	23.6±0.9	68.3±2.2	9.6±0.7	32±1	0.69±0.06	0.91±0.05
			7.36±0.01	50.1±1.4	25.9±2.3	62.6±4.0	10.6±0.4	31±1	0.77±0.08	0.73±0.03
-1	-24	Saline Dex	7.37±0.01	49.8±0.6	24.5±0.8	66.0±2.3	9.8±0.6	30±1	0.75±0.09	0.84±0.04
			7.36±0.01	51.7±1.5	25.7±1.7	62.0±2.5	10.3±0.4	31±2	0.77±0.05	0.78±0.08
0	-1	Saline Dex	7.37±0.01	50.8±1.0	24.2±1.2	68.2±2.5	9.4±0.8	29±2	0.08±0.07	1.37±0.31
			7.34±0.01	53.7±1.1	23.3±1.1	59.3±2.6	10.4±0.3	33±1	0.77±0.09	0.87±0.05
	0	First injection								
		1	Saline Dex	7.36±0.04	50.8±0.5	25.2±2.1	71.2±2.3	9.5±0.7	30±4	0.81±0.07
7.34±0.01	53.2±1.0			23.5±0.7	62.2±3.0	10.4±0.3	32±1	0.81±0.07	0.64±0.15	
	2	Saline Dex	7.38±0.03	52.2±1.1	24.0±1.9	71.0±2.7	9.5±0.7	30±4	0.87±0.13	1.12±0.15
			7.33±0.02	55.0±1.3	23.5±1.3	59.2±2.0	11.1±0.3*	34±1	0.76±0.08	0.87±0.07
	4	Saline Dex	7.36±0.01	51.8±1.0	24.2±2.2	67.8±1.5	9.5±0.7	30±4	0.95±0.13	1.08±0.11
			7.35±0.02	51.7±1.4	22.8±1.3	62.0±2.5	11.7±0.3* ^Δ	35±2	1.21±0.10	1.19±0.11
	6	Saline Dex	7.36±0.01	51.4±0.9	24.0±1.9	67.8±3.6	9.4±0.8	30±4	0.99±0.15	1.19±0.19
			7.34±0.01	52.3±1.0	24.3±1.6	61.7±2.9	11.5±0.4* ^Δ	37±1	1.41±0.21	1.33±0.11
	8	Saline Dex	7.40±0.03	49.6±1.0	23.6±2.2	65.8±2.5	9.8±0.8	29±3	0.89±0.08	1.16±0.14
			7.34±0.02	50.3±1.7	24.5±1.7	65.1±3.7	11.0±0.3	35±2	1.26±0.27	1.19±0.11
	10	Saline Dex	7.38±0.01	50.2±1.0	24.2±2.2	67.0±4.2	9.4±0.9	32±2	0.96±0.11	1.08±0.10
			7.33±0.01	52.8±2.1	23.2±1.2	66.4±4.0	10.9±0.3	35±2	1.13±0.17	1.00±0.05
	12	Saline Dex	7.38±0.03	52.4±0.9	23.8±2.4	64.5±4.5	9.6±0.7	31±2	1.03±0.14	1.09±0.07
			7.33±0.02	51.2±1.9	23.5±1.3	61.3±1.5	10.8±0.2	35±1	1.25±0.17	1.06±0.06
1	24	Saline Dex	7.37±0.02	51.9±0.9	24.4±1.1	67.8±2.9	9.2±0.7	30±1	0.76±0.06	1.15±0.13
			7.31±0.02*	53.5±1.5	24.9±1.5	63.6±2.4	10.6±0.4	32±2	1.13±0.14	0.93±0.07
	25	Second injection								
		Saline Dex	7.35±0.01	52.4±0.7	24.0±1.8	67.1±4.7	9.1±0.6	31±2	0.86±0.09	1.11±0.12
7.30±0.02*	55.0±2.1		24.7±1.4	61.5±3.5	10.4±0.3	32±2	0.99±0.10	0.99±0.09		
	26	Saline Dex	7.38±0.03	52.4±1.3	24.0±2.6	65.6±3.6	9.2±0.7	31±2	0.92±0.08	1.00±0.21
			7.30±0.02* ^Δ	55.4±1.9	24.5±2.1	56.5±2.6	10.4±0.6	32±2	1.01±0.13	1.16±0.12
	28	Saline Dex	7.39±0.03	51.8±1.0	23.6±1.6	65.8±3.7	9.1±0.6	31±2	0.98±0.12	1.09±0.08
			7.30±0.02* ^Δ	53.8±1.9	23.5±1.9	61.5±4.4	10.6±0.4*	33±3	1.21±0.18	1.19±0.13
	30	Saline Dex	7.39±0.03	50.8±1.2	24.0±2.3	67.0±3.8	9.1±0.5	31±2	0.92±0.09	1.09±0.08
			7.30±0.02*	52.0±1.9	25.2±2.0	64.8±3.2	10.4±0.4	32±2	1.18±0.22	1.19±0.13
	32	Saline Dex	7.40±0.04	50.0±0.9	23.2±1.5	66.2±1.7	8.8±0.5	30±2	0.97±0.01	1.14±0.04
			7.32±0.02*	49.2±2.9	24.0±1.6	60.6±3.9	10.2±0.4	33±2	1.25±0.16	1.08±0.18
2	48	Saline Dex	7.35±0.01	51.6±1.0	24.7±1.2	67.2±2.2	9.2±0.7	31±1	0.92±0.07	1.26±0.18
			7.32±0.02*	50.2±2.4	27.2±3.4	60.4±2.7	9.7±0.5	30±2	0.97±0.11	1.26±0.31
3	72	Saline Dex	7.34±0.01	52.8±0.9	21.5±1.2	62.4±4.0	9.3±0.7	29±2	0.96±0.09	1.54±0.24
			7.36±0.01	51.8±2.3	26.4±1.6	69.0±3.1	9.5±0.5	30±1	0.90±0.15	1.27±0.3

Values show the mean+SEM for the arterial pH (pHa), the arterial partial pressure of carbon dioxide (PaCO₂); the arterial partial pressure of oxygen (PaO₂), the percentage saturation of haemoglobin with oxygen (Sat Hb) Hb, the haemoglobin concentration ([Hb]); the haematocrit (Htc), the blood glucose concentration (BG) and the blood lactate concentration (BL) in the fetal arterial circulation following maternal treatment at 0.8 of gestation with saline or Dex. 12mg of dexamethasone dissolved in saline (Dex) or the same volume of saline (Saline) was administered intramuscularly to the ewe on day 0 and day 1. Data were analysed statistically by One-way ANOVA with repeated measures.



Extended Data Figure 1. Additional echocardiography data. Values show the mean+SEM for body weight (A) heart rate (B) stroke volume (C) and the mitral maximum pressure gradient (D) in 9-11 month old adolescent lambs following maternal treatment with saline at 0.8 of gestation (white symbols) or maternal treatment with dexamethasone at 0.8 of gestation after progesterone treatment at 80 days of gestation (Dex, blue symbols). Data were analysed statistically by the Student's *t* test for unpaired data. *, $P < 0.05$.