Estradiol-induced labor in periparturient ewes: Gene expression changes in maternal reproductive tissues and impact on lamb birth characteristics

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Our study explored the effects of administering estradiol (E2) on the timing of labor in periparturient ewes and the survival and development of their lambs. We focused on how E2 alters gene expression in critical reproductive tissues, including the myometrium, endometrium, cervix and maternal placenta, and its effects on lamb weight and vigor. Our findings indicated that E2 can quickly and effectively induce labor primarily by altering gene expression in the cervix, where E2 promotes inflammation and tissue remodeling necessary for birth. No adverse effects were observed on the offspring. These findings suggest that E2 could provide livestock producers with a reliable method for managing the timing of lambing without increasing the risk of lamb mortality.

Summary

We investigated the effects of exogenous estradiol (E2) on labor initiation in periparturient ewes and its effects on offspring development. Two experiments were conducted: Exp.1 assessed the effect of E2 on birth timing, lamb birth weight and lamb vigor, while Exp.2 examined changes in gene expression in maternal reproductive tissues (myometrium, endometrium, cervix and placenta). Multiparous ewes (139-142 days of gestation) were randomly assigned to receive Silastic® implants containing E2 (300 mg in Exp. 1, 200 mg in Exp. 2, E group) or empty implants (Control, C group). Implants were removed two days after parturition (Exp.1) or 26 hours after treatment (Exp.2). Maternal jugular blood samples were taken to measure E2 and progesterone (P4) concentrations, and tissue samples were collected for gene expression (RNA-Seq) analysis. Results indicated that E2 concentrations in maternal blood were similar between groups before treatment. However, E2 concentration was greater in both experiments in the E compared with the C group after treatment. No differences in P4 concentrations between the E and C groups were observed. In addition, E2 shortened labor (2.84±1.02 in E vs. 7.18±1.1 days in C, P = 0.01) without affecting lamb characteristics. Furthermore, E2 treatment affected gene expression across all the tissues tested ($P \leq 0.10$), with the most potent effects in the cervix, with 8,073 differentially expressed genes in the E compared with the C group. Our findings suggest that E2 induces labor by altering gene expression, particularly in the cervix, where it upregulates genes associated with inflammation and cervical ripening and downregulates genes linked to smooth muscle contraction and tissue integrity. Additionally, E2 had no adverse effects on the offspring, offering livestock producers a potential tool for managing the timing of lambing and improving reproductive outcomes.

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Introduction

Parturition is a complex and poorly understood process involving the development of the fetal organs, activation of myometrial contractions, cervical ripening and dilation, fetal membrane rupture, fetal delivery and placental expulsion (Jenkin and Young, 2004). These events should occur synchronously, and each of them is critical for successful delivery (Mesiano, 2001). Among the pathways involved and the various factors identified, the role of steroid hormones, specifically E2 and P4, has emerged as particularly significant in determining the timing of labor onset (Shuler et al., 2018). An increase in estrogenic actions has been

linked with regulating myometrial contractility and cervical ripening, whereas P4 promotes uterine relaxation and inhibits spontaneous contractions and the onset of labor (Pastore et al., 2012; Solano and Arck, 2020).

While most pregnancies end at term, 10% of neonatal losses in U.S. livestock are caused by preterm births (Wu et al., 2006), which frequently have unclear causes. This postpartum loss represents a significant burden on the investment in breeding, nutrition and veterinary care that goes into each birth. Furthermore, managing the timing of lambing is a critical aspect of sheep farming, directly affecting both the ewe's health and the lambs' survival. Traditionally, the unpredictable onset of labor poses challenges for livestock producers, making it difficult to optimize care and resources.

Hormonal interventions that safely and effectively induce labor could allow producers to schedule lambing more precisely. This ability to program delivery would improve animal health outcomes, streamline farm operations, enhance time management and reduce the workload during the busy lambing season. Although E2 is known to be crucial for labor, little is known about how it affects gene expression in reproductive tissues and affects the timing of delivery. Our study aimed to fill that gap by examining the alterations in gene expression of myometrial, endometrial, cervical and placental tissues after treatment with exogenous E2, as well as the impact on the timing of labor and the characteristics and viability of the offspring.

Experimental Procedures

Two experiments were conducted utilizing the same approach but with varying dosages to evaluate the effect of E2 on birth timing and offspring characteristics (Experiment 1) and gene expression (Experiment 2) on reproductive tissues (myometrium, endometrium, cervix, and placenta). Multiparous Rambouillet ewes between 139 and 142 days of gestation were randomly assigned to either the exogenous estradiol-treated group (E), which received six Silastic® implants containing 50 mg of E2 in Exp.1 (300 mg/ewe; n=12) and four implants in Exp.2 (200 mg/ewe; n=6); or the control (C) group, which received six empty Silastic® implants in Exp.1 (n=12) and four empty implants in Exp.2 (n=6). Implants were inserted subcutaneously in the axillary region of the ewe and removed two days after parturition (Exp.1) or at the time of slaughter (26 hours after treatment; Exp.2). Maternal jugular vein blood samples were collected one day before and at different time points after treatment in Exp.1 or at 26 hours after treatment in Exp.2 to measure circulating concentrations of E2 and P4 using immunoassay.

In Experiment 1, ewes were treated, and their time to delivery after treatment, live lamb birth weight and lamb vigor were recorded. Lamb vigor was assessed using a scale ranging from 0 to 4, where 0 represents extreme activity and vigor with the lamb standing on all four feet, 1 indicates high activity with the lamb standing on its back legs and knees, 2 represents moderate activity with the lamb active on its chest and holding its head up, 3 indicates weakness with the lamb lying flat but still able to hold its head up, and 4 indicates severe weakness with the lamb unable to lift its head and showing minimal movement. In Experiment 2, ewes were treated and slaughtered 26 h later for tissue collection; cross sections of the myometrium, endometrium, caruncles and cervix were collected for RNA-Seq analysis. After RNA-Seq data quality control and mapping, differentially expressed genes (DEGs) were identified using DESeq2. Statistical significance ($P < 0.05$ in Exp.1 and $P \leq 0.1$ and log2 fold change $|0.5$ in Exp.2) was assessed using the MIXED procedure of SAS for both experiments.

Results and Discussion

In Experiment 1, differences were observed in E2 concentrations in maternal blood at various time points, with the E group generally having higher concentrations than the C group, especially at +8 h and

Table 1. Maternal E2 and P4 levels before and after treatment, Exp.1 and Exp.2

		Exp.1					Exp.2
Hormone	**Treatment**	**Day -1**	**+8h**	**Day +1**	**Post-partum***	**Birth +2 d**	**+ 26h**
E2 (pg/ml)	**E**	12.18 ± 1.98	192.91 ± 17.32 A	108.15 ± 9.25 A	92.39 ± 16.06	22.86 ± 3.4 A	149.21 ± 55.93 A
	C	16.76 ± 2.25	10.97 ± 20.47 B	20.31 ± 10.93 B	63.54 ± 19.31	1.6 ± 3.43 B	30.61 ± 11.73 B
	p-value	0.38	<0.001	<0.001	0.26	<0.001	<0.01
P4 (ng/ml)	**E**	9.31 ± 1.43	9.1 ± 1.24	7.42 ± 1.2	0.94 ± 0.29	0.2 ± 0.05	6.50 ± 1.42
	C	8.84 ± 1.73	9.02 ± 1.46	9.6 ± 1.42	0.64 ± 0.32	0.2 ± 0.05	8.99 ± 1.42
	p-value	>0.8	> 0.96	>0.18	>0.48	=1	>0.24

*Postpartum samples were taken within 8 hours after parturition. The time from treatment to parturition varied between groups (see table 2). AB Indicates significant differences between E2 and C at a particular time point, $P<0.05$.

Day +1, which demonstrates the effectiveness of the E2 treatment. The blood concentrations of P4 did not differ between treatment groups, suggesting that elevated E2 alone can trigger labor even without changes in P4 concentrations, as the average delivery time after treatment was 2.84 ± 1.02 d (Table 2).

As shown in Table 2, in Exp.1 timing of parturition was influenced by treatment. The E group significantly reduced the days from treatment to delivery. (the average gestation length in this flock of Rambouillet ewes was 147 d). No differences were observed in lamb birth weight or vigor.

As shown in Fig. 1, E2 treatment affected gene expression in all the tissues tested (p-value ≤ 0.1 and log2 fold change |0.5|). The cervix exhibited the strongest effect, with 8,073 DEGs, including E2 upregulation of prostaglandin-endoperoxide synthase 2 (*PTGS-2*), prostaglandin E synthase 2 (*PTGES2*), estrogen receptor 2 (*ESR2*), and interleukin 6 (*IL-6*), and downregulation of interleukin 10 (*IL-10*), alpha-smooth muscle actin (*ACTA2*), myosin heavy chain 11 (*MYH11*), gap junction protein Alpha 1 (*GJA1*), and tissue inhibitors of metalloproteinases (*TIMPs*). The endometrium had 722 DEGs, including E2 upregulation of *OXTR* and *ESR1* and downregulation of *SULT1E1* and *TIMPs*. The caruncles had a more modest response, with 109 DEGs, while the myometrium was the least responsive to the treatment, with 90 DEGs, including E2 upregulation of *PTGS-2*.

Our findings suggest that E2 causes changes in gene expression in the maternal reproductive tissues, leading to parturition, but without a decline in circulating levels of P4 in maternal blood. These effects appear to be primarily driven by the alterations in the cervix, where E2 upregulated genes associated with inflammation and cervical ripening, and downregulated genes associated with smooth muscle contraction and tissue integrity.

Furthermore, E2-induced labor did not compromise offspring health, as the lamb birth weight or vigor did not change. These findings are significant for the livestock industry as they offer a potential tool for managing the timing of lambing, which can help reduce the risks associated with unpredictable labor. By controlling birth timing, producers can ensure that lambing occurs under optimal conditions, improving the health outcomes for both ewes and lambs.

Table 2. Experiment 1: Time to delivery, lamb birth weight and lamb vigor

Treatment	Days from Treatment to Delivery	Lamb Birth Weight (Kg)	Lamb Vigor (0-4 scale) *
E	2.84 ± 1.02 A	4.94 ± 0.20	0.3 ± 0.14
C	7.18 ± 1.11 B	4.99 ± 0.19	0.09 ± 0.13
p-value	< 0.01	> 0.84	>0.3

* Lamb vigor was assessed using a scale ranging from 0 to 4; see the text for an explanation.
AB indicates significant differences between E2 and C, p<0.05.

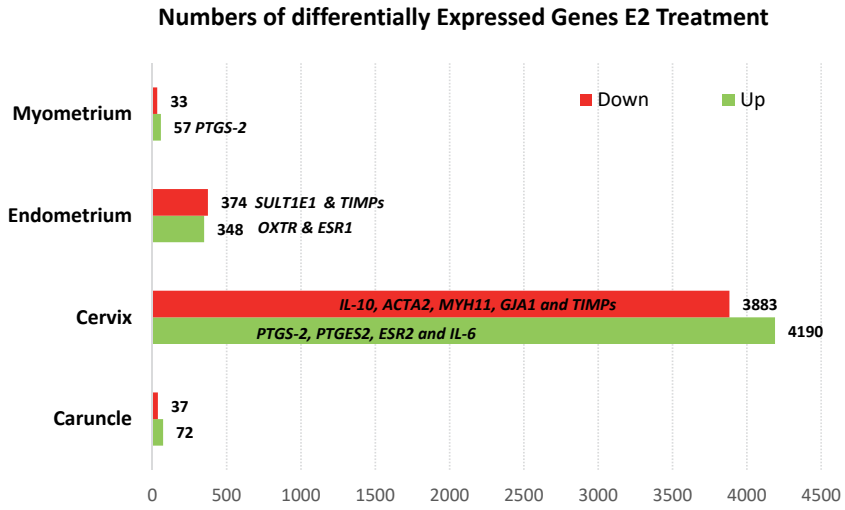

Figure 1. Exp.2: Differentially expressed genes by E2 treatment

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