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Maternal and fetal effects of isoxsuprine injection during late pregnancy in Tuj ewes assessed by doppler ultrasonography

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ABSTRACT: This study investigated the effects of isoxsuprine administration on uterine and umbilical artery hemodynamics during the last two weeks of pregnancy in Tuj ewes using Doppler ultrasonography and evaluated the associated biochemical changes in ewes and lambs. Twenty-one ewes at 136 days of gestation were assigned to three groups ($n = 7$ each): double-dose isoxsuprine (G1), single-dose isoxsuprine (G2), and control (G3). Ultrasonographic examinations were performed three times weekly until lambing. Uterine artery diameter (UTA-DM), pulse rate (UTA-PR), resistance index (UTA-RI), blood flow velocity (UTA-BFVe), blood flow volume (UTA-BFVo), umbilical artery blood flow velocity (UMA-BFVe), resistance index (UMA-RI), and pulse rate (UMA-PR) were measured. Blood samples collected after examinations were analyzed for progesterone, estrogen, cortisol, nitric oxide, and glucose concentrations. Lamb blood samples obtained at birth were evaluated for surfactant-associated protein B (SP-B) and glucose concentrations. UTA-BFVe, UTA-BFVo, and UMA-BFVe increased significantly on isoxsuprine injection days (days 136 and 143), with more pronounced increases observed in G1 and G2 compared to G3 ($p < 0.05$). Correspondingly, UTA-RI and UMA-RI decreased significantly on these injection days, with more pronounced reductions in G1 and G2 compared to G3 ($p \leqslant 0.05$). In addition, the weight of newborn lambs in G1 was higher than in G3 and SP-B concentration was higher in G1 and G2 ($p < 0.05$). In conclusion, prepartum isoxsuprine administration significantly affected both Doppler ultrasonographic and biochemical parameters in ewes, while also increasing SP-B concentrations and lamb birth weight.

KEYWORDS: doppler, ewes, isoxsuprine, umbilical artery, uterine artery

INTRODUCTION

In humans, various measures are taken to detect and prevent fetal hypoxia. These measures include continuous monitoring the uterus activities, placental function, and fetal perfusion during the prepartum period and pharmacological interventions aimed at enhancing uterine perfusion in pregnant women [1, 2]. These interventions are collectively referred to as intrauterine resuscitation. One such method involves the administration of tocolytic agents to manage severe uterine contractions which can lead to fetal growth restriction or chronic asphyxia [1]. The most commonly used tocolytic agent in veterinary obstetrics is isoxsuprine. Isoxsuprine, a β1- and β2-adrenomimetic drug, is used to relax the cow uterus thereby facilitating fetal repositioning or aiding in uterine relaxation during cesarean section [3]. Isoxsuprine relaxes the smooth musculature of the uterus and blood vessels and has a peripheral vasodilator effect. Through this mechanism, it reduces vascular resistance and increases blood flow, leading to measurable changes in Doppler ultrasonographic parameters such as decreased pulsatility index (PI) and resistance index (RI) in uterine and umbilical arteries [3].

In ewes, the clinical indication for isoxsuprine use is primarily associated with uterine relaxation and conditions requiring improved uterine perfusion, particularly during obstetric interventions such as dystocia, fetotomy, and cesarean section. Isoxuprine has been reported to be used to facilitate fetal manipulation, reduce excessive uterine contractions, and improve surgical handling of the uterus [4]. Additionally, preoperative isoxsuprine administration in ewes has been associated with reductions in Doppler indices (PI and RI) and increases in blood flow velocities, along with improved neonatal outcomes such as higher APGAR scores and better blood gas parameters, suggesting enhanced fetal oxygenation and viability [4]. Furthermore, isoxsuprine has been shown to facilitate cervical relaxation, reduce cervical transit time, and support its use in non-surgical reproductive techniques [5].

Alternative approaches such as β2-agonists (e.g., clenbuterol) and epidural anesthesia are also used in ewes delivery. Clenbuterol provides effective uterine relaxation due to its selective β2-adrenergic activity; however, it may be associated with systemic side effects including tachycardia, hypotension, and metabolic alterations [6, 7]. Epidural anesthesia is frequently used to reduce abdominal contractions and provide analgesia, but it does not directly enhance uterine blood flow and may prolong delivery by reducing expulsive efforts. Compared to these approaches, isoxsuprine may provide both uterine relaxation and improve uterine perfusion; however, due to its systemic β-adrenergic effects, it can induce transient maternal and fetal cardiovascular changes, and therefore its clinical use should be carefully evaluated [8].

The survival of premature infants after birth is critically dependent on the presence of adequate pul-

monary surfactant on the alveolar surface to prevent alveolar collapse. Both *in vivo* and *in vitro* studies on fetal lung development have shown that β-adrenergic agents enhance surfactant secretion [9]. The risk of respiratory distress syndrome (RDS) is significantly reduced in neonates born prematurely following in utero exposure to β-adrenergic drugs [10]. Isoxsuprine administration in the last days of pregnancy has increased the synthesis, storage, and secretion of surfactant in rabbit fetuses, thereby promoting fetal pulmonary maturation [9].

In ewes, the uterine artery blood flow velocity increases throughout pregnancy, providing more blood flow to the placenta and fetus. Monitoring alterations in flow indices during this process can assist in the evaluation of high-risk pregnancies. Changes in fetal circulation detected by Doppler ultrasonography (USG) can be quickly assessed by imaging the fetal heart/vessels or umbilical vessels [11]. In all domestic animal species, the survival rate of offspring born with an average birth weight is higher than those born below average weight range [12]. In pregnant women subjected to nutritional restriction, inadequate placental blood flow and fetal growth restriction occur [13]. In farm animals, the poor growth performance of newborn offspring has been linked to reduced uterine and placental blood flow, leading to significant economic losses in livestock production [14, 15].

Although the cardiovascular effects of isoxsuprine on ewes and their fetuses have been extensively documented, its impact on glucose metabolism remains less understood. Infants born to women treated with β-sympathomimetic agents are at risk of developing hypoglycemia [16]. Maternal injection of isoxsuprine in rats depletes maternal and fetal hepatic glycogen stores and leads to transient hyperglycemia and stimulates insulin production. The depletion of hepatic glycogen stores during the intrauterine period results in initial hyperglycemia, thereby increasing the risk of subsequent hypoglycemia in newborn rat pups [17].

This study aims to evaluate the hemodynamic values in ewes and their lambs by administering specific doses of isoxsuprine during the final 14 days of pregnancy in ewes and to investigate the relationship between selected endocrine changes. Previous studies on isoxsuprine in pregnant ewes are both limited and include studies conducted in the past using invasive methods. In this study, current methods were employed, and although the findings may provide useful preliminary insights, further pharmacokinetic and safety studies are required before considering clinical application.

MATERIALS AND METHODS

Ethical approval

This study protocol was approved by the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2022-40), Kars, Türkiye and was conducted in accordance with the national and institutional guidelines for the care and use of animals.

Animals

In the study, 29 Tuj ewes, which had previously given birth at least once, were clinically healthy, age of 3–4 years, body weight 40–50 kg, and a body condition score (BCS) of 3.0–3.5 (1 = Extremely thin, 5 = Obese), were used. Five fertile and healthy Tuj rams were utilized for estrus detection and mating. During the breeding season when the study was conducted, the ewes were allowed to graze in pastures and housed in a closed barn system in the evening. During the pregnancy period, which coincided with the winter season, the ewes were placed on an intensive feeding regimen and 0.5 kg ewes/day concentrated feed (16% crude protein, 2,700 kcal metabolizable energy) supplemented to their routine diet. Water was provided *ad libitum* throughout the study period. The study was conducted at Kafkas University Faculty of Veterinary, Prof. Dr. Ali Rıza AKSOY Education, Research, and Application Farm in Kars province.

Estrus synchronization protocol and pregnancy diagnosis

The study was initiated during the breeding season (September). For estrus synchronization, a medroxyprogesterone acetate-containing sponge (60 mg, Esponjavet®, Hipra, Spain) was inserted intravaginally on day 0, followed by an injection of 400 IU equine chorionic gonadotropin (Oviser®, Hipra) and d-cloprostenol (12.5 mg, Enzaprost-T, Ceva, Türkiye) on day 5. The sponges were removed on day 7, and the ram was introduced for mating. Estrus detection was conducted four times daily for half an hour for four days.

Pregnancy diagnosis was conducted using transrectal USG (5–7.5 MHz, Draminski iScan, Draminski, Poland) at 30 ± 3 days after the ewes were mated. USG examinations were performed transrectally while the ewes were in a standing position. Before the examination, the ewes were restrained, and any feces present in the rectum were cleaned. Pregnancy was confirmed by visualizing the fetus. The pregnancy examination was repeated on day 60 to confirm. Of the 29 ewes included in the study, 21 were confirmed to be pregnant, and the study proceeded with these ewes.

Experiment design

Ewes were allocated into three groups ($n = 7$ each) balanced for age, BCS, and parity. Beginning 14 days prior to the expected lambing date, Doppler USG (Esaote Biomedica®, Genova, Italy) of the uterine and umbilical arteries, along with blood sampling was performed three times per week on the specified days (Monday, Wednesday, and Friday) until parturition.

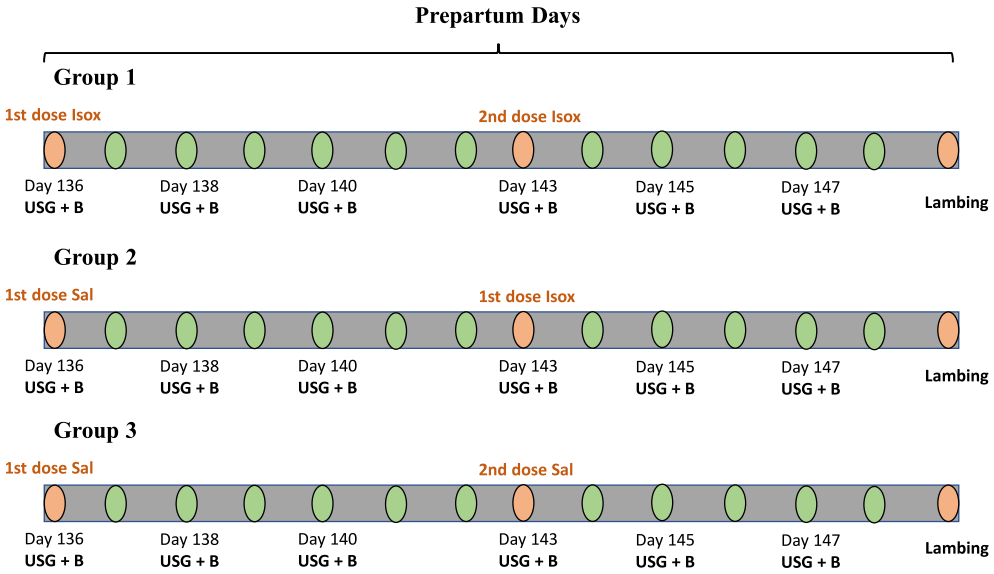

Fig. 1 Sampling days of Groups 1, 2, and 3. Isox: Isoxsuprine; Sal: Saline; USG: Ultrasonography; B: Blood sample.

Group 1 (Double Dose, G1): The ewes received an intramuscular injection of 0.6 mg/kg of isoxsuprine [18] (1 ml contains 10 mg of isoxsuprine HCl, Utelax®, Verano, Türkiye) on days 136 and 143 of gestation. Group 2 (Single Dose, G2): In contrast to Group 1, 2.5 ml saline was administered on day 136, followed by isoxsuprine (2.5 ml) on day 143. Group 3 (Control, G3): No isoxsuprine was injected to the ewes in this group. Saline (2.5 ml) was intramuscular injected on designated days (days 136 and 143). Following the first injection, Doppler USG and blood sampling were conducted three times per week on the specified days for all groups (Fig. 1).

Uterine and umbilical artery

Doppler USG images were utilized for the analyses of uterine and umbilical artery blood flow. A 5–10 MHz (automatic) multifrequency linear probe was employed to visualize the uterine artery (UTA), while a convex probe was used to image the umbilical artery (UMA). Imaging was performed 30 min following the isoxsuprine injection. Three images displaying consistent Doppler waveforms were selected, and the average values were derived from these images (Fig. 2). The PixelFlux (Chameleon® Software, Münster, Germany) software was utilized to calculate the resistance index (RI, unitless), pulse rate (PR, beats/min), blood flow velocity (BFVe, cm/s), and blood flow volume (BFVo, ml/min). Three distinct B-mode images of the artery were obtained in the transverse (cross-sectional) plane to determine the vessel diameter (DM, mm). The diameter was calculated as the mean of two perpendicular measurements (vertical and horizontal) taken as the inner-to-inner distance of the vessel wall. All measurements were performed by a single operator.

Collection and processing of blood samples

Blood samples were collected on days 136, 138, 140, 143, 145, and 147 of gestation. Thirty minutes after the isoxsuprine injection, blood samples were drawn from the jugular vein using a holder (BD Vacutainer®, Becton, Dickinson and Company, USA) and a holder needle (BD Vacutainer®) into 8.5 ml gel-separated vacuum tubes without anticoagulant (BD Vacutainer®). After collection, blood samples were centrifuged (NF 400R®, Nüve, Türkiye) at 3,000 rpm for 10 min at 4 °C. After centrifugation, serum samples were transferred into microcentrifuge tubes and stored at −20 °C until biochemical analyses were performed.

Biochemical analyses

Quantitative measurements of progesterone, estrogen, cortisol, glucose and nitric oxide were performed using a chemiluminescence immunoassay (CLIA) method. Analyses were carried out using a Siemens hormone analyzer and ADVIA Centaur® test kits (Siemens, Tarrytown, NY, USA). According to the manufacturer's specifications, the ADVIA Centaur assay has a sensitivity of 100% and a specificity of 95.5%. Serum Surfactant Associated Protein B (SP-B) concentrations were determined using a commercially available, sheep-specific sandwich ELISA kit (Assay Genie, Dublin, Ireland, Cat. No: AEKE11519) in accordance with the manufacturer's instructions. The assay is highly specific for sheep SP-B, with a dynamic detection range of 1.57 to 100 ng/ml and a minimum analytical sensitivity of 0.56 ng/ml. The optical density (OD) was measured spectrophotometrically at 450 nm using a microplate reader. Final SP-B concentrations were calculated by referencing the standard curve and expressed as ng/ml.

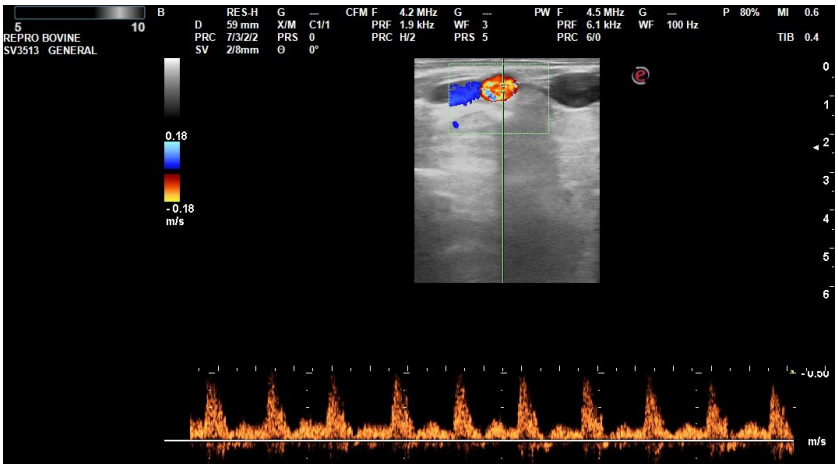

Fig. 2 Doppler mode image of the uterine artery.

Statistical analysis

All data obtained in the study are presented as mean ± standard error of the mean (SEM). Prior to inferential analyses, the distributional properties of the variables across sampling days were assessed using the Shapiro–Wilk test for normality. Maternal Doppler ultrasonographic and biochemical variables were measured repeatedly in the same ewes throughout the last two weeks of gestation; therefore, these longitudinal data were analyzed using a two-way repeated-measures analysis of variance (ANOVA). In this repeated-measures framework, treatment group (G1, G2, and G3) was defined as the between-subject factor, whereas time (days 136, 138, 140, 143, 145, and 147 of gestation) was defined as the within-subject repeated factor. Individual ewe was specified as the matched subject factor, thereby accounting for the dependency of repeated observations obtained from the same animal over time. Accordingly, the main effects of group and time, as well as the group × time interaction, were evaluated for each maternal parameter. For repeated-measures analyses, Geisser–Greenhouse correction was applied. When a significant main effect or interaction was detected, post hoc pairwise comparisons were performed to identify differences between groups at specific sampling days and differences between sampling days within each group. Multiple comparisons were adjusted using Tukey's multiple comparisons test. Variables measured only once in lambs at birth were analyzed separately using one-way ANOVA, followed by Tukey's post hoc multiple comparisons test when a significant overall group effect was observed. Pearson correlation coefficients were calculated to assess the relationships between selected Doppler ultrasonographic and biochemical variables. All statistical analyses were performed using GraphPad Prism (Version 9.5.1, GraphPad Software Inc., USA) and SPSS (Version 26.0, IBM Corp., USA). For all analyses, a p-value of $\leqslant 0.05$ was considered statistically significant.

RESULTS

Biochemical changes

Changes in glucose, estradiol, progesterone, cortisol, and nitric oxide concentrations in ewes

The time-dependent change in serum glucose concentration during the prepartum period was statistically significant ($p < 0.001$, Fig. 3). Glucose concentrations were significantly higher in the G1 and G2 that received isoxsuprine injection on days 136 and 143 of gestation ($p < 0.05$). Glucose concentrations remained unchanged throughout the sampling period in G3 throughout the sampling period ($p > 0.05$, Table S1).

Estradiol concentration did not differ significantly between the groups during the period close to lambing ($p > 0.05$, Fig. 3). According to the two-way analysis of variance, the time effect was statistically significant ($p < 0.001$). In G1 and G2, estradiol concentrations were significantly higher on day 147 compared with earlier sampling days ($p < 0.01$, Table S2).

The time effect on progesterone concentration was statistically significant (Fig. 3, $p < 0.001$). Progesterone concentrations in G1 and G2 on day 147 were significantly lower than on days 136 and 138 ($p < 0.01$). There were no differences in progesterone concentration between the groups ($p > 0.05$, Table S3).

During the prepartum period, cortisol concentration in the ewes significantly increased over time ($p < 0.01$, Fig. 3). In G3, cortisol concentrations were significantly higher on day 147 compared to day 143 ($p = 0.02$).

No significant group or group × time interaction was found ($p > 0.05$). However, the time effect was statistically significant ($p = 0.04$, Fig. 3). Nitric oxide concentrations were significantly higher on day 147 compared to day 136 in G2 ($p < 0.03$, Fig. 3).

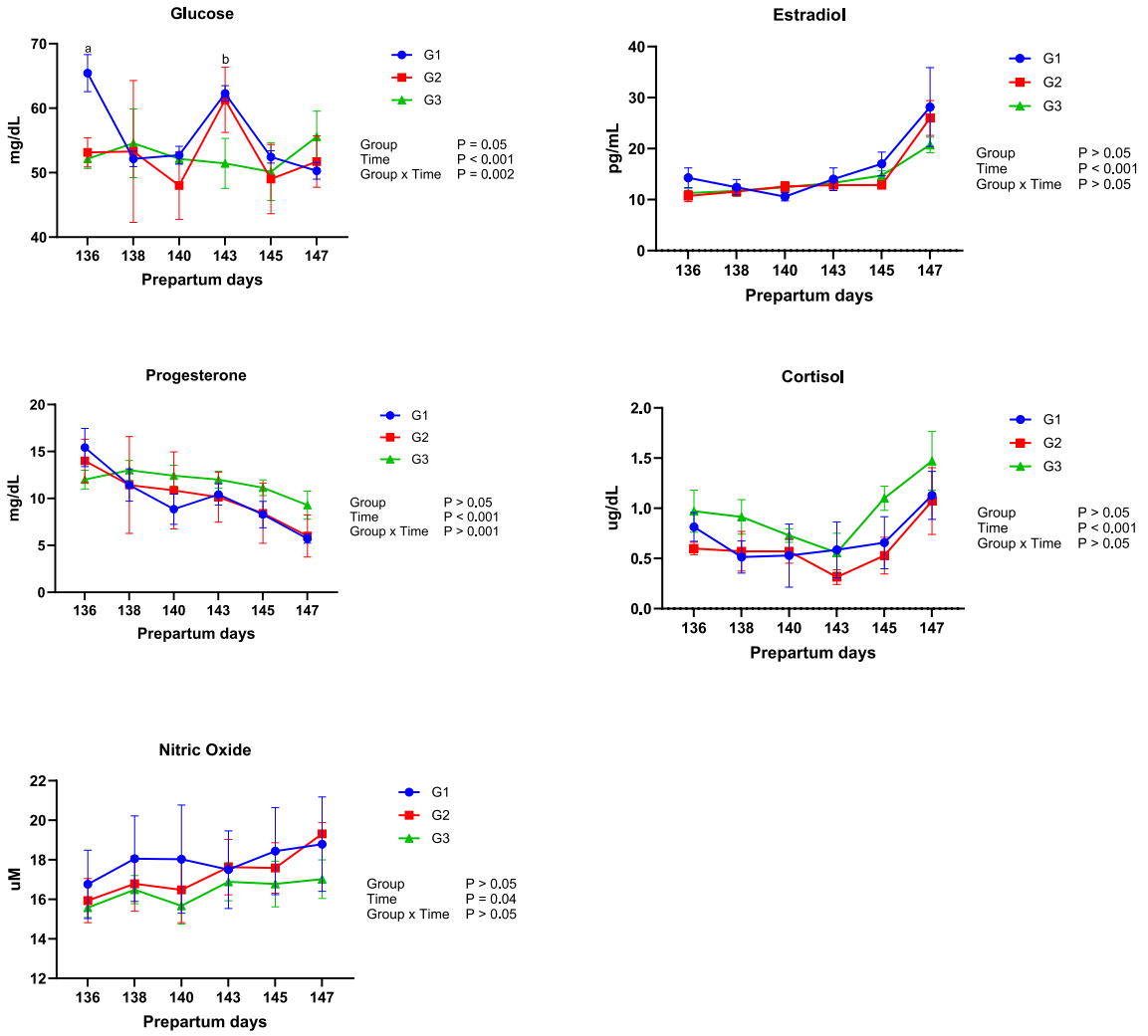

Fig. 3 Serum glucose, estradiol, progesterone, cortisol, and nitric oxide concentrations in the groups during the prepartum period. G1: Double-dose isoxsuprine group, G2: Single-dose isoxsuprine group, G3: Control group. a: Indicates a statistically significant difference in G1 compared to G2 and G3 on day 136. b: Indicates a statistically significant difference of G1 and G2 compared to G3 on day 143.

Changes in glucose, surfactant and birth weight changes in lambs

The changes in parameters of lambs are presented in Fig. 4. Serum SP-B levels were significantly lower in G3 compared to G2 ($p = 0.01$) and G1 ($p = 0.03$). Lamb birth weight was significantly higher in G1 than in G3 ($p = 0.02$).

Changes in ultrasonographic parameters

According to two-way repeated measures analysis of variance, UTA-BFVe was different in terms of time ($p < 0.001$). UTA-BFVe was significantly higher in G1 compared to G2 and G3 on day 136, and significantly lower in G3 compared to G1 and G2 on day 143 ($p < 0.001$). Additionally, the group × time interaction for UTA-BFVe was statistically significant ($p < 0.001$, Fig. 5, Table S4).

UTA-BFVo exhibited significant differences in terms of both groups ($p = 0.009$) and time ($p < 0.001$) according to two-way repeated measures analysis of variance. Furthermore, a significant group × time interaction was determined for UTA-BFVo ($p < 0.001$, Fig. 5, Table S5).

The effect of time on UTA-DM was statistically significant ($p < 0.001$). UTA-DM showed a significant decrease from day 136 to day 143 in G1 ($p < 0.001$, Fig. 5, Table S6).

The effects of group ($p = 0.001$) and time ($p < 0.001$) on UTA-RI were statistically significant. Additionally, the group × time interaction for UTA-RI was also statistically significant ($p < 0.001$). UTA-RI was lower on day 136 in G1 compared with G2 and G3, and significantly higher on day 143 in G3 compared to

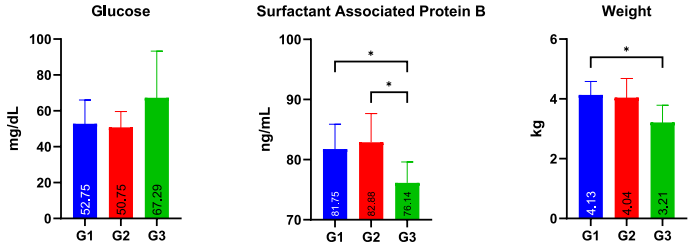

Fig. 4 Serum glucose, surfactant concentrations and birth weight changes of lambs. G1: Double-dose isoxsuprine group, G2: Single-dose isoxsuprine group, G3: Control group. *: Indicates a statistically significant difference between two groups ($p < 0.05$).

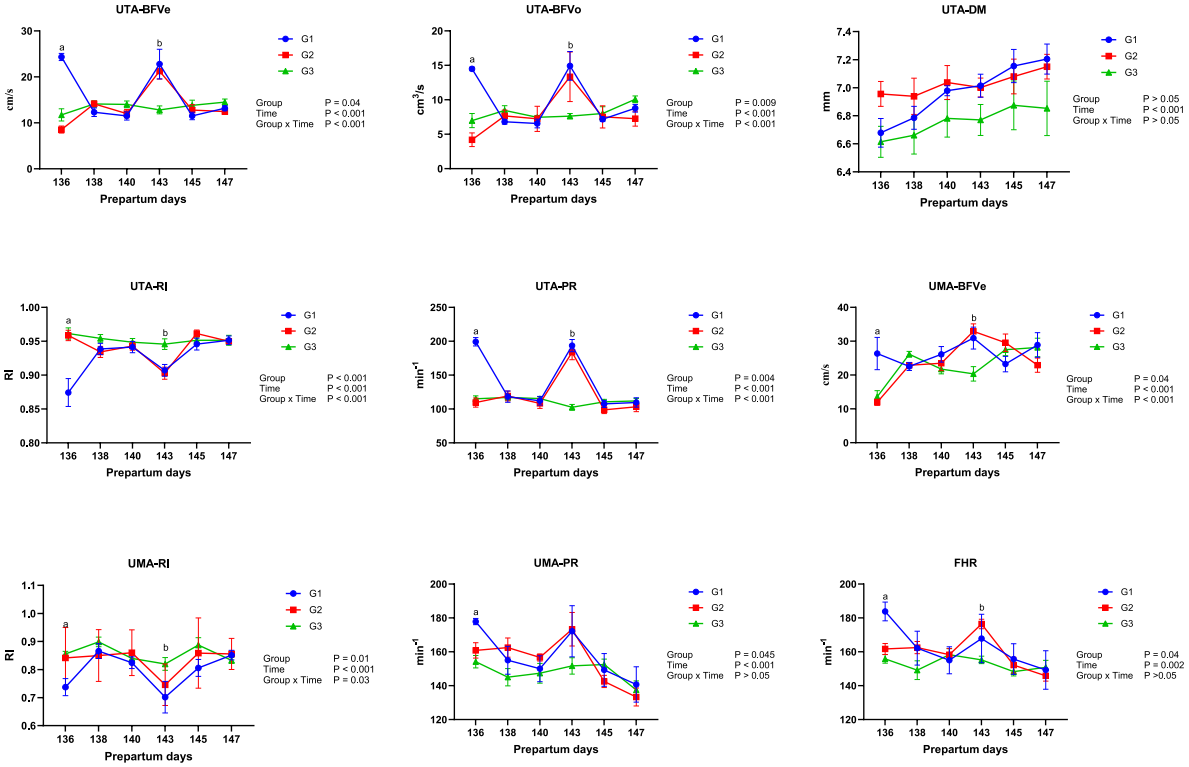

Fig. 5 Changes in uterine artery-blood flow velocity (UTA-BFVe), blood flow volume (UTA-BFVo), vessel diameter (UTA-DM), resistance index (UTA-RI), pulse rate (UTA-PR), and umbilical artery-blood flow velocity (UMA-BFVe), resistance index (UMA-RI), pulse rate (UMA-PR), fetal heart rate (FHR) during the prepartum period. G1: Double-dose isoxsuprine group, G2: Single-dose isoxsuprine group, G3: Control group. a: Indicates a statistically significant difference of G1 compared to G2 and G3 on day 136. b: Indicates statistically significant differences between G1 and G2 compared to G3 on day 143 (excluding UMA-PR: G1 was statistically different from G3 on day 136 for UMA-PR; excluding FHR: only G2 and G3 were statistically different on day 143 in FHR).

G1 and G2 ($p < 0.005$, Fig. 5, Table S7).

The effects of group ($p = 0.004$) and time ($p < 0.001$) on UTA-PR was statistically significant. UTA-PR was statistically higher in G1 compared to G2 and G3 on day 136, and significantly higher in G1 and G2 compared to G3 on day 143 ($p < 0.001$). In addition, group × time interaction for UTA-PR was statistically significant ($p < 0.001$, Fig. 5, Table S8).

For UMA-BFVe, the effect of time ($p < 0.001$) and group ($p = 0.04$) was statistically significant. UMA-BFVe was higher in G1 compared to G2 and G3 on day 136, and significantly higher in G1 and G2 compared to G3 on day 143 ($p < 0.01$). Group × time interaction was also statistically significant for UMA-BFVe ($p < 0.001$, Fig. 5, Table S9).

For UMA-RI, the effect of group ($p = 0.01$) and time ($p < 0.001$) was significant. UMA-RI was significantly higher in G2 and G3 compared to G1 ($p < 0.005$) on day 136, and significantly higher in G3 than in G1 and G2 on day 143 ($p < 0.05$). No significant differences were detected in G3 over time (Fig. 5, Table S10s).

In UMA-PR, group ($p = 0.045$) and time ($p < 0.001$) effects were significant. UMA-PR was significantly higher in G1 compared to G3 on day 136 ($p = 0.05$, Fig. 5, Table S11).

For fetal heart rate (FHR), the effect of group ($p = 0.04$) and time ($p = 0.002$) was statistically significant. FHR was higher in G1 compared to G2 and G3 on day 136 ($p < 0.05$), and significantly higher in G2 than in G3 on day 143 ($p = 0.04$, Fig. 5, Table S12).

Correlation between doppler ultrasonographic parameters and hormonal values

The correlation results between Doppler USG parameters and hormonal values measured during the study are presented in Table 1. A significant positive correlation was identified between UMA-BFVe and nitric oxide ($r = 0.207$, $p < 0.05$). Additionally, glucose showed significant negative correlations with UTA-RI ($r = -0.270$, $p < 0.01$) and UMA-RI ($r = -0.264$, $p < 0.01$).

DISCUSSION

Isoxsuprine is a drug commonly used in both veterinary and human medicine. In veterinary practice, it is generally used as a uterine smooth muscle relaxant during labor, while in humans it is used to delay premature labor. The study was designed to investigate how the vascular and metabolic changes following prenatal isoxsuprine injections affect both ewes and their lambs. In line with this hypothesis, the primary objectives were to measure the effects of isoxsuprine administration during the final stage of pregnancy on maternal and neonatal endocrine changes and to evaluate hemodynamic parameters using USG. Additionally, ewes were chosen as study animals for two main reasons: first, their gestation period is shorter than in cows, and second, both maternal and fetal ultrasonographic imaging is more feasible in these animals.

Biochemical changes

Several studies have indicated that isoxsuprine administration during pregnancy alters glucose concentrations [17]. Although the precise mechanism by which isoxsuprine stimulates glucose production remains unclear, it is hypothesized that its glycemic effect results from direct β-adrenergic stimulation of glucose production. It has been stated that sympathomimetic agents with tocolytic properties, such as isoxsuprine, stimulate glycogen phosphorylase and lipase, leading to elevated blood glucose and insulin concentrations [19]. In a study conducted on rats, isoxsuprine administration reduced hepatic glycogen concentrations to minimal levels. These findings suggest that isoxsuprine infusion nearly depletes hepatic glycogen stores in rats. Thus, it may cause hyperglycemia [17]. In the present study, glucose concentration on the day of injection was statistically elevated in the groups that received isoxsuprine injection during the final stage of pregnancy compared to the other groups. These results, consistent with previous studies, demonstrate that isoxsuprine increases serum glucose concentrations.

Various hemodynamic effects of maternal β-sympathomimetic administration were determined in our study. Significant changes were observed in blood flow parameters, such as UTA-BFVe and UMA-BFVe, following isoxsuprine injection. These hemodynamic changes exhibited a positive correlation with glucose concentrations; however, this effect may be attributed to the action of isoxsuprine, which may influence both vascular pathways (via vasodilation and increased blood flow) and metabolic pathways (via β-adrenergic stimulation affecting glucose metabolism).

β-adrenergic agonists not only inhibit uterine contractions but also increase progesterone concentrations. Isoxsuprine administered to humans with preterm labor symptoms significantly increased progesterone compared to the placebo group [20]. Ritodrine hydrochloride, a β-adrenergic agonist, did not cause any change in plasma progesterone concentration in ewes. Isoxsuprine hydrochloride also did not cause any change in blood progesterone concentration in ewes [21]. Although no significant differences were observed between the groups, progesterone concentrations decreased toward day 147, which is consistent with the physiological endocrine changes associated with the onset of parturition. Therefore, these findings do not indicate a direct effect of isoxsuprine on progesterone concentration.

Cortisol is a glucocorticoid hormone produced by the adrenal cortex in response to stress. Cortisol leads to an increase in estrogen concentration and a decrease in progesterone concentration. As a result, it stimulates the release of prostaglandin F2α, which leads to an increase in oxytocin, thereby enhancing myometrial contractions. Isoxsuprine hydrochloride administered to sheep 14 and 15 days after insemination, did not alter cortisol concentrations [21]. In cows with uterine torsion, isoxsuprine hydrochloride injection before surgery had no significant impact on cortisol concentrations [19]. In our study, no statistically significant difference was found in cortisol concentration between the groups, which suggests that isoxsuprine did not affect cortisol concentrations. Cortisol concentration varied over time during the sampling period, likely due to the hormonal mechanism of labor.

Nitric oxide regulates placental perfusion and increases uterine blood flow. Nitric oxide also has a very strong vasodilatory effect. It plays a central role in the uterus and cervix at the beginning of labor [22]. This suggests that nitric oxide is involved in the mechanisms preparing the uterus for labor. In ewes, nitric oxide concentrations increase progressively from day 135 of pregnancy until lambing [23]. In this study, nitric oxide concentrations were consistent with the findings obtained from other studies, and it can be inferred that its increase at the end of pregnancy may affect blood flow. However, the absence of any statistical difference

Table 1 Correlation coefficients between Doppler ultrasonographic parameters and selected biochemical variables in ewes during the prepartum period.

Variable	Glucose	Estradiol	Progesterone	Cortisol	Nitric oxide
UTA-BFVe	0.255**	0.007	0.181*	−0.039	0.021
UTA-BFVo	0.260**	0.085	0.099	0.029	0.057
UTA-DM	−0.146	0.226*	−0.295**	−0.064	0.111
UTA-RI	−0.270**	0.090	−0.245**	0.010	0.037
UTA-PR	0.404**	0.043	0.327**	0.019	0.174
UMA-BFVe	0.190*	0.188*	−0.076	−0.073	0.207*
UMA-RI	−0.264**	−0.063	−0.111	0.041	−0.089
UMA-PR	0.188*	−0.170	0.165	0.041	−0.131
FHR	0.225*	−0.080	−0.056	0.197*	−0.122

*: $p < 0.05$, **: $p < 0.01$. UTA = uterine artery; UMA = umbilical artery; BFVe = blood flow velocity; BFVo = blood flow volume; DM = vessel diameter; RI = resistance index; PR = pulse rate; FHR = fetal heart rate.

between the groups suggests that this increase is not related to isoxsuprine.

The response of the fetus or neonate to a drug given to pregnant ewes can be highly variable. Isoxsuprine injection can also cause biochemical or metabolic changes in the fetus either by altering maternal circulatory homeostasis or, since it easily crosses the placenta, by directly affecting the fetus [24]. Isoxsuprine injection into rats during parturition also significantly increased in fetal plasma levels [17]. This provides evidence that isoxsuprine administered to the dam passes directly to the fetus via the placenta. Isoxsuprine passes into the plasma of the newborn and triggers glycogenolysis in the fetus before birth, leading to depletion of hepatic glycogen stores. These findings strongly suggest that isoxsuprine treatment induces hypoglycemia after parturition [24]. In the present study, although no statistical difference was found between the lamb glucose concentrations, they were numerically higher in the control group. Other studies have also measured the glucose levels in the lamb immediately after isoxsuprine administration [17]. After the last isoxsuprine injection, blood samples were collected from the lambs at birth for glucose measurement. During this period, the lamb's metabolism may have adapted to tolerate hypoglycemia.

The survival of premature infants after birth depends on the presence of sufficient pulmonary surfactant on the alveolar surface to prevent alveolar collapse. Previous studies on the fetal lung conducted *in vivo* [10] and *in vitro* [9] have shown that β-adrenergic drugs stimulate surfactant secretion. In rabbits, injection of isoxsuprine led to increased surfactant secretion in newborn pups [25]. After isoxsuprine was given to pregnant rabbits on day 27 of gestation, there was a decrease in surface tension and an increase in the lecithin/sphingomyelin (L/S, used to assess fetal lung development) ratio in the lung fluid of the fetuses [26]. In another study, lung stability was increased in fetal rabbits after isoxsuprine injection [10]. β-adrenergic receptors are the final mediators of surfactant release before the initiation of air inspiration. A higher L/S ratio in tracheal lavage further supports these results. In another study, isoxsuprine injection into pregnant rabbits enhanced fetal lung maturation [9]. In the present study, surfactant levels were found to be higher in the isoxsuprine-administered groups. These findings suggest a possible association between isoxsuprine administration and increased surfactant levels; however, further studies are required to confirm a causal relationship.

It has been suggested that if β-sympathomimetic therapy induces fetal hyperglycemia and hyperinsulinism, long-term treatment may lead to fetal macrosomia (larger than normal) offspring [27]. However, from a hemodynamic point of view, β-adrenergic agonists are of limited value in the treatment of intrauterine fetal growth retardation [28]. For this purpose, women with normal carbohydrate metabolism were given oral ritodrine from the 25th week of pregnancy until delivery. None of the babies born to these women showed an increased birth weight. In a separate study, isoxsuprine administered to women between 32 and 35 weeks of gestation significantly increased birth weight [24]. In the present study, the live weights of the lambs born to the group that received double dose of isoxsuprine were higher than those of the control group. As pregnancy progresses, UTA-RI are expected to gradually decrease, allowing for greater fetal blood flow. With isoxsuprine administration, an even greater increase in blood flow occurs, thereby enhancing fetal perfusion. It is thought that these changes can help the fetus meet its increased nutritional needs more easily during the final days of pregnancy, thereby having a positive effect on fetal growth.

Ultrasonographic parameters

β-adrenergic agonists have significant cardiovascular effects [19]. In pregnant ewes, a study on isoxsuprine showed a significant decrease in mean maternal arterial pressure during infusion. In addition, maternal tachycardia, hyperglycemia, and metabolic acidosis were observed [16]. In the present study, UTA-PR was statistically higher on the days when isoxsuprine was injection compared to the control group. This may be due to the tachycardia caused by isoxsuprine

administration. Tachycardia can result from peripheral vasodilation, which decreases venous return, leading to increased peripheral blood flow [19]. β -adrenergic agonists can also directly increase heart rate by stimulating β-2 adrenergic receptors in the heart muscle, both in the left ventricle and the right atrium.

In a study conducted on sheep and goats, there was a decrease in the UTA-RI as a result of USG monitoring throughout pregnancy [29]. Sheep pregnancies were examined on days 60, 90, and 120 and it was determined that there was a decrease in the UTA-RI in parallel with the progression of pregnancy [30]. In the follow-up of pregnant ewes from day 21 to birth, the UTA-RI value was high in the first weeks but showed a significant decrease as pregnancy progressed [31]. In addition, in a study conducted on sheep in the second half of pregnancy, the RI decreased again [32]. The reason for the decrease in RI as pregnancy progresses is thought to be the increase in blood flow to the fetus [16]. In the control group, no statistically significant differences were observed over time during the last two weeks of pregnancy. This may be due to the limited 2-weeks sampling period of our study. However, in the groups administered isoxsuprine, UTA-RI was lower on the day of injection compared to the control, and this decrease was statistically significant. This may be because isoxsuprine transforms uterine vessels into a low-resistance system, increasing blood flow during placentation.

Isoxsuprine injections in sheep significantly increased FHR [16]. Isoxsuprine infusion in women also caused fetal tachycardia [8]. In line with other studies, an increase in FHR was determined after isoxsuprine injections in our study. The increase in FHR occurs after the increase in maternal heart rate. This means that the passage of isoxsuprine into the fetal circulation directly affects the fetal heart. Its ability to pass through the placenta is exemplified by adrenaline, a compound with a similar structure [33]. In another study, it was determined that isoxsuprine passes directly through the placenta and has a direct effect on the fetal heart [34]. In the present study, a statistical difference was found between the group administered isoxsuprine on day 136 and the other groups, and this finding is similar to other studies. However, no difference was found between the group administered isoxsuprine on day 143 (G1) and the control group (G3) in terms of FHR. The reason for this may be that the FHR increased individually in some ewes in the control group due to stress, or that the hemodynamic effect of isoxsuprine transferred to lambs was weaker than in ewes.

BFVe is considered one of the most important blood flow parameters in cardiac and blood vessel studies. UTA-BFVo increased significantly in the control group during the study period. In another study, UTA-BFVo also increased between 2 and 20 weeks of gestation in ewes [35]. The present results are also consistent with the findings during pregnancy in other species, including cows [36], mares [37] and pregnant women [38]. In the present study, the increased UTA-BFVo and decreased RI during the sampling period were due to increased blood vessel diameter and blood flow velocity [36]. The increase in the BFVo in the control group in our study may be due to the increase in intrauterine birth weight of the fetus, the rapid increase in fetal fluid, and the increase in the weight of the uterus, placenta, and fetal membranes in the period close to birth. The increase in blood flow may be attributed to the direct vasodilatory effect of isoxsuprine, as mentioned earlier. Therefore, UTA-BFVe/UTA-BFVo was found to be higher in the ewes injected with isoxsuprine in our study compared to the control groups. In addition, there is a high level of correlation between these two parameters.

The reason for choosing UMA for Doppler study compared to other fetal vessels is that it can be visualized more easily during ultrasonographic examination, its location is easy to determine, and in most cases, it is evaluated more accurately since angular correction is not required. In addition, UMA is a good and more accurate vessel for monitoring fetal growth. UMA waveform is recommended as the primary surveillance tool for fetuses detected to have growth retardation or small for gestational age in the prenatal period [39]. UMA-RI values decreased gradually throughout pregnancy in ewes [23], but in some studies this value did not show a statistical change in the late stages of pregnancy [40]. In our study, no statistical difference was determined in UMA-RI in the control group. This may be due to the short observation interval.

Isoxsuprine treatment significantly increased maternal heart rate. Possible changes in uteroplacental circulation during isoxsuprine administration are indirectly due to changes in cardiac output or directly due to dilation of uteroplacental vasculature [28]. Similarly, in Rhesus monkeys, vasodilation occurs during the infusion of β-adrenergic agonists, which results in increased placental blood flow [41]. In general, accelerated circulation causes changes in blood flow values, and there are important correlations between them. The presence of a strong negative correlation between UMA-RI and UMA-PR supports this situation. Although there was a difference between the group that received isoxsuprine and the control group on day 136 in our UMA-PR study, this difference did not occur on day 143 when isoxsuprine was administered. This situation may indicate that the placental hemodynamic effect of isoxsuprine on transfer from ewes to lambs is not as effective on the cardiovascular system as in the maternal circulation.

The sample size in this study was relatively small ($n = 7$ per group), which may limit statistical power, particularly for variables with high biological variability. Therefore, the findings should be interpreted as exploratory and require confirmation in larger stud-

ies. In addition, baseline (pre-treatment) Doppler and biochemical measurements were not obtained, which limits the ability to assess initial comparability between groups.

CONCLUSION

In conclusion, the effects of single and double-dose injections of isoxsuprine in pregnant ewes on maternal and fetal metabolism were evaluated in this study. Isoxsuprine significantly changed both maternal and fetal hemodynamics. Although isoxsuprine has risks such as tachycardia and hypoglycemia in ewes and lambs, it also exerts beneficial effects on surfactant production and weight gain in lambs. This study presents promising preliminary evidence that isoxsuprine administration during late gestation may improve uteroplacental blood flow and neonatal outcomes in ewes. However, before clinical application can be considered in sheep, further studies are required to evaluate the safety profile, pharmacokinetics, optimal dosing strategies, and potential long-term effects of isoxsuprine administration during the prepartum period. In addition, studies investigating its impact on parturition timing and neonatal outcomes under field conditions would enhance the translational relevance of these findings.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.057.

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Appendix A. Supplementary data

Table S1 Comparison of serum glucose concentrations between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−12.29	−21.30 to −3.271	**	0.005
G3 vs. G1	−13.29	−22.30 to −4.271	**	0.002
143				
G3 vs. G1	−10.86	−19.87 to −1.842	*	0.01
G3 vs. G2	−9.857	−18.87 to −0.842	*	0.03
G1				
138 vs. 136	−13.29	−23.15 to −3.419	**	0.002
140 vs. 136	−12.71	−22.58 to −2.847	**	0.004
145 vs. 136	−13	−22.87 to −3.133	**	0.003
147 vs. 136	−15.14	−25.01 to −5.276	***	<0.001
143 vs. 138	10.14	0.276 to 20.01	*	0.04
147 vs. 143	−12	−21.87 to −2.133	**	0.008
G2				
143 vs. 140	13.29	3.419 to 23.15	**	0.002
145 vs. 143	−12.29	−22.15 to −2.419	**	0.006

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine. G2: Single dose isoxsuprine. G3: Control group.

Table S2 Comparison of serum estradiol concentrations between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
G1				
147 vs. 136	13.86	4.221 to 23.49	***	<0.001
147 vs. 138	15.71	6.078 to 25.35	***	<0.001
147 vs. 140	17.57	7.935 to 27.21	***	<0.001
147 vs. 143	14.14	4.507 to 23.78	***	<0.001
147 vs. 145	11.14	1.507 to 20.78	*	0.01
G2				
147 vs. 136	15.29	5.650 to 24.92	***	<0.001
147 vs. 138	14.43	4.792 to 24.06	***	<0.001
147 vs. 140	13.43	3.792 to 23.06	**	0.001
147 vs. 143	13.14	3.507 to 22.78	**	0.002
147 vs. 145	13.14	3.507 to 22.78	**	0.002

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine. G2: Single dose isoxsuprine. G3: Control group.

Table S3 Comparison of serum progesterone concentrations between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
G1				
140 vs. 136	−6.571	−10.83 to −2.309	***	<0.001
143 vs. 136	−5	−9.263 to −0.738	*	0.01
145 vs. 136	−7.143	−11.41 to −2.880	***	<0.001
147 vs. 136	−9.714	−13.98 to −5.452	***	<0.001
147 vs. 138	−5.714	−9.977 to −1.452	**	0.003
147 vs. 143	−4.714	−8.977 to −0.452	*	0.02
G2				
145 vs. 136	−5.571	−9.834 to −1.309	**	0.004
147 vs. 136	−8	−12.26 to −3.737	***	<0.001
147 vs. 138	−5.429	−9.691 to −1.166	**	0.005
147 vs. 140	−4.857	−9.120 to −0.595	*	0.02

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine. G2: Single dose isoxsuprine. G3: Control group.

Table S4 Comparison of UTA-BFVe value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−15.79	−19.10 to −12.48	***	<0.001
G3 vs. G1	−12.59	−15.91 to −9.278	***	<0.001
143				
G3 vs. G1	−9.971	−13.29 to −6.658	***	<0.001
G3 vs. G2	−8.446	−11.76 to −5.132	***	<0.001
G1				
138 vs. 136	−12.03	−16.08 to −7.967	***	<0.001
140 vs. 136	−12.83	−16.89 to −8.775	***	<0.001
145 vs. 136	−12.84	−16.90 to −8.779	***	<0.001
147 vs. 136	−11.16	−15.22 to −7.102	***	<0.001
143 vs. 138	10.48	6.419 to 14.54	***	<0.001
143 vs. 140	11.29	7.228 to 15.35	***	<0.001
145 vs. 143	−11.29	−15.35 to −7.232	***	<0.001
147 vs. 143	−9.614	−13.67 to −5.555	***	<0.001
G2				
138 vs. 136	5.586	1.527 to 9.645	**	0.002
143 vs. 136	12.72	8.658 to 16.78	***	<0.001
145 vs. 136	4.229	0.169 to 8.288	*	0.04
143 vs. 138	7.131	3.072 to 11.19	***	<0.001
143 vs. 140	9.261	5.202 to 13.32	***	<0.001
145 vs. 143	−8.489	−12.55 to −4.429	***	<0.001
147 vs. 143	−8.809	−12.87 to −4.749	***	<0.001

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine. G2: Single dose isoxsuprine. G3: Control group.

Table S5 Comparison of UTA-BFVo value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−10.29	−12.61 to −7.968	***	<0.001
G3 vs. G1	−7.506	−9.825 to −5.187	***	<0.001
G3 vs. G2	2.781	0.4626 to 5.100	*	0.01
143				
G3 vs. G1	−7.293	−9.612 to −4.974	***	<0.001
G3 vs. G2	−5.713	−8.032 to −3.394	***	<0.001
147				
G3 vs. G2	2.803	0.4840 to 5.122	*	0.01
G1				
138 vs. 136	−7.686	−10.53 to −4.845	***	<0.001
140 vs. 136	−7.924	−10.76 to −5.084	***	<0.001
145 vs. 136	−7.307	−10.15 to −4.467	***	<0.001
147 vs. 136	−5.713	−8.553 to −2.872	***	<0.001
143 vs. 138	8.126	5.285 to 10.97	***	<0.001
143 vs. 140	8.364	5.524 to 11.20	***	<0.001
145 vs. 143	−7.747	−10.59 to −4.907	***	<0.001
147 vs. 143	−6.153	−8.993 to −3.312	***	<0.001
G2				
138 vs. 136	3.466	0.6252 to 6.306	**	0.008
140 vs. 136	3.036	0.1952 to 5.876	*	0.03
143 vs. 136	9.147	6.307 to 11.99	***	<0.001
145 vs. 136	3.319	0.4781 to 6.159	*	0.01
147 vs. 136	3.066	0.2252 to 5.906	*	0.03
143 vs. 138	5.681	2.841 to 8.522	***	<0.001
143 vs. 140	6.111	3.271 to 8.952	***	<0.001
145 vs. 143	−5.829	−8.669 to −2.988	***	<0.001
147 vs. 143	−6.081	−8.922 to −3.241	***	<0.001
G3				
147 vs. 136	3.087	0.2467 to 5.928	*	0.03

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S6 Comparison of UTA-DM value within groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
G1				
145 vs. 136	0.4757	0.1271 to 0.8243	**	0.002
147 vs. 136	0.5257	0.1771 to 0.8743	***	<0.001
145 vs. 138	0.3686	0.01995 to 0.7172	*	0.03
147 vs. 138	0.4186	0.06995 to 0.7672	**	0.01

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S7 Comparison of UTA-RI value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	0.08429	0.05582 to 0.1128	***	<0.001
G3 vs. G1	0.08714	0.05867 to 0.1156	***	<0.001
143				
G3 vs. G1	0.03857	0.01010 to 0.06704	**	0.005
G3 vs. G2	0.04286	0.01439 to 0.07133	**	0.002
G1				
138 vs. 136	0.06429	0.02941 to 0.09916	***	<0.001
140 vs. 136	0.06714	0.03227 to 0.1020	***	<0.001
145 vs. 136	0.07143	0.03655 to 0.1063	***	<0.001
147 vs. 136	0.07714	0.04227 to 0.1120	***	<0.001
145 vs. 143	0.03857	0.003698 to 0.07345	*	0.020
147 vs. 143	0.04429	0.009412 to 0.07916	**	0.005
G2				
143 vs. 136	−0.05571	−0.09059 to −0.02084	***	<0.001
143 vs. 140	−0.04000	−0.07487 to −0.00513	*	0.020
145 vs. 143	0.05857	0.02370 to 0.09345	***	<0.001
147 vs. 143	0.04714	0.01227 to 0.08202	**	0.002

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S8 Comparison of UTA-PR value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−89.78	−103.4 to −76.14	***	<0.001
G3 vs. G1	−84.29	−97.93 to −70.64	***	<0.001
143				
G3 vs. G1	−91.07	−104.7 to −77.43	***	<0.001
G3 vs. G2	−81.25	−94.90 to −67.60	***	<0.001
G1				
138 vs. 136	−81.07	−97.79 to −64.36	***	<0.001
140 vs. 136	−87.14	−103.9 to −70.43	***	<0.001
145 vs. 136	−91.79	−108.5 to −75.07	***	<0.001
147 vs. 136	−89.64	−106.4 to −72.93	***	<0.001
143 vs. 138	75.36	58.64 to 92.07	***	<0.001
143 vs. 140	81.43	64.71 to 98.14	***	<0.001
145 vs. 143	−86.07	−102.8 to −69.36	***	<0.001
147 vs. 143	−83.93	−100.6 to −67.21	***	<0.001
G2				
143 vs. 136	74.25	57.53 to 90.96	***	<0.001
143 vs. 138	64.58	47.87 to 81.30	***	<0.001
145 vs. 138	−20.48	−37.19 to −3.761	**	0.008
143 vs. 140	75.42	58.70 to 92.13	***	<0.001
145 vs. 143	−85.06	−101.8 to −68.34	***	<0.001
147 vs. 143	−80.42	−97.13 to −63.70	***	<0.001

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S9 Comparison of UMA-BFVe value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−14.39	−22.37 to −6.416	***	<0.001
G3 vs. G1	−12.74	−20.72 to −4.768	***	<0.001
143				
G3 vs. G1	−10.58	−18.56 to −2.606	**	0.006
G3 vs. G2	−12.59	−20.56 to −4.611	***	<0.001
G2				
138 vs. 136	10.95	1.212 to 20.69	*	0.02
140 vs. 136	11.52	1.786 to 21.26	*	0.01
143 vs. 136	20.98	11.24 to 30.72	***	<0.001
145 vs. 136	17.60	7.860 to 27.33	***	<0.001
147 vs. 136	10.97	1.236 to 20.71	*	0.02
143 vs. 138	10.03	0.2944 to 19.77	*	0.04
147 vs. 143	−10.01	−19.74 to −0.2701	*	0.04
G3				
138 vs. 136	12.55	2.816 to 22.29	**	0.004
145 vs. 136	13.89	4.154 to 23.63	***	<0.001
147 vs. 136	14.56	4.822 to 24.30	***	<0.001

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S10 Comparison of UMA-RI value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	0.1286	0.03467 to 0.2225	**	0.004
G3 vs. G1	0.1343	0.04038 to 0.2282	**	0.003
143				
G3 vs. G1	0.1471	0.05324 to 0.2410	***	<0.001
G3 vs. G2	0.1029	0.00896 to 0.1968	*	0.03
G1				
138 vs. 136	0.1286	0.01846 to 0.2387	*	0.01
147 vs. 136	0.1143	0.00418 to 0.2244	*	0.04
143 vs. 138	−0.1643	−0.2744 to −0.0542	***	<0.001
143 vs. 140	−0.1229	−0.2330 to −0.0128	*	0.02
147 vs. 143	0.15	0.03989 to 0.2601	**	0.002
G2				
143 vs. 136	−0.12	−0.2301 to −0.00989	*	0.02
143 vs. 140	−0.1143	−0.2244 to −0.00418	*	0.04
145 vs. 143	0.1129	0.00275 to 0.2230	*	0.04

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S11 Comparison of UMA-PR value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G3 vs. G1	−23.69	−47.35 to −0.0252	*	0.05
G1				
147 vs. 136	−37.14	−66.04 to −8.249	**	0.004
147 vs. 143	−31.43	−60.32 to −2.535	*	0.02
G2				
147 vs. 138	−29.17	−58.06 to −0.2737	*	0.05
145 vs. 143	−30.83	−59.73 to −1.939	*	0.03
147 vs. 143	−40	−68.89 to −11.11	**	0.001

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.

Table S12 Comparison of FHR value within and between groups.

Tukey's multiple comparisons test	Mean differences	95.00% Confidence interval for difference	Summary	Adjusted *p*-value
136				
G2 vs. G1	−22.19	−44.18 to −0.2033	*	0.05
G3 vs. G1	−28.02	−50.01 to −6.038	**	0.009
143				
G3 vs. G2	−22.62	−44.61 to −0.6319	*	0.04
G1				
140 vs. 136	−28.86	−55.70 to −2.013	*	0.03
145 vs. 136	−28.14	−54.99 to −1.298	*	0.03
147 vs. 136	−34.57	−61.42 to −7.727	**	0.004
G2				
147 vs. 143	−32.02	−58.87 to −5.180	**	0.01

The statistically significant days in comparisons are provided in the table. Asterisks (*) indicate the level of significance. G1: Double dose isoxsuprine, G2: Single dose isoxsuprine, G3: Control group.