

Acute stress response to winter pre-lambing shearing in ewes with and without maternal experience

Adriana Nusspaumer¹, Gonzalo Suárez², Martín Claramunt³, Victoria Pons⁴, Jorge Gil⁴, Damian González⁵, Elize van Lier⁶, Georgget Banchemo⁵, Juan Pablo Damián^{1,7*}

¹Departamento de Biociencias Veterinarias, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.

²Departamento de Clínicas y Hospital Veterinario, Facultad Veterinaria, Universidad de la República, Montevideo, Uruguay.

³Centro Universitario de la Región Este, Universidad de la República, Treinta y Tres, Uruguay.

⁴Laboratorio de Reproducción Animal, Facultad de Veterinaria, CENUR-LN, Universidad de la República, Paysandú, Uruguay.

⁵Unidad de Ovinos, INIA "La Estanzuela", Colonia, Uruguay.

⁶Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Estación Experimental Facultad de Agronomía, Salto, Uruguay.

⁷Núcleo de Bienestar Animal, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.

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Corresponding author

* Juan Pablo Damián

jpabldamian@gmail.com

ABSTRACT. This study aimed to compare the acute stress response to winter pre-lambing shearing between ewes with and without maternal experience. Twenty-four Finnish Landrace x Polwarth ewes in the last month of gestation were used. Twelve ewes had previous maternal experience (ME), and 12 ewes were undergoing their first pregnancy and were therefore considered without maternal experience (WME). Heart rate, rectal temperature, and eye temperature were recorded immediately before shearing (0 min) and immediately after shearing (5 min). Blood samples were collected, and hormonal (cortisol) and biochemical (total protein, albumin, globulin, and glucose) indicators were determined. Heart rate, rectal temperature, eye temperature, serum cortisol concentration, and plasma glucose level increased after shearing ($P < 0.05$). Eye temperature significantly increased after shearing only in WME ewes ($P = 0.004$). The rectal temperature tended to be higher in WME ewes than in ME ewes ($P = 0.07$). WME ewes had higher serum cortisol concentrations after shearing than ME ewes ($P = 0.049$). Biochemical indicators did not vary according to the ewes' maternal experience. In conclusion, ewes' maternal experience influenced the acute stress response to shearing during late gestation. WME had a greater stress response to shearing than ME, which was evidenced by a higher increase in serum cortisol concentration and eye temperature.

Keywords: Animal Welfare, Biochemistry, Cortisol, Sheep, Temperature

INTRODUCTION

In sheep, maternal experience is known to play a key role in the mother-lamb bond and in the future of the offspring. The behavioral response of mothers is fundamental to establishing the bond with their offspring; for example, a delay in the display of maternal behavior, the acceptance of only one of the twin lambs, or a complete absence of the maternal behavioral response can compromise the survival of the offspring (Nowak, 1996; Dwyer, 2014). Ewes that do not have maternal experience present worse maternal behavior and care for their offspring than do mothers with previous experience (Nowak, 1996; Dwyer, 2014). Among the factors that help explain why, unlike multiparous mothers, primiparous mothers have worse maternal behavior are the following: a) ewes without maternal experience (primiparous) give birth to smaller offspring; b) they delay the time they start licking the lamb; c) their lambs take longer to stand up and search for the udder and suckle; d) they tend to be more aggressive towards the lamb; e) they withdraw or avoid the lamb when it approaches them; f) they often do not allow the lamb access to suck on their teats; and g) they spend less

time searching for and being with them (Dwyer, 2003, 2014; Dwyer & Lawrence, 1998, 2000, 2005; Corner *et al.*, 2013; González-García *et al.*, 2015; Damián *et al.*, 2020; Karaca *et al.*, 2023). Furthermore, using the maternal behavior score (MBS), Damián *et al.* (2020) and Madani *et al.* (2013) reported that multiparous ewes are better mothers than primiparous ewes on the day of lambing. It has also been observed that ewes with better MBS have heavier lambs and lower lamb mortality rates (Madani *et al.*, 2013; Damián *et al.*, 2020). Beyond these studies carried out on sheep after the birth of their offspring, little is known about how mothers with and without maternal experience respond differentially to stressors during the gestation phase.

Stress during gestation not only affects the mother, but also her offspring, as stressors can generate intrauterine changes that influence the offspring and its development, a phenomenon known as fetal programming (Godfrey & Barker, 2001). In mammals, including humans, prenatal stress is known to affect the development and behavior of offspring throughout life (Charil *et al.*, 2010; Sartori *et al.*,

2022; Dieckmann & Czamara, 2024). Since physiological, hormonal and biochemical changes during gestation can affect the uterine environment and have effects on fetal programming (Meyer & Redifer, 2024), differences in stress response according to maternal experience during gestation could also affect the development of the offspring and contribute to maternal behavior.

Among the practices involved in sheep production, pre-lambing shearing is of great importance. Pre-lambing shearing is a management practice that has allowed the improvement of productive indices and the conditions of the ewes and their offspring at birth, via greater feed consumption, greater milk/colostrum production, increased live weight of the lamb at birth and its vigor, improving therefore the survival of the lambs (Rutter *et al.*, 1971; Banchemo *et al.*, 2010; López-Mazz *et al.*, 2017, 2020). However, shearing itself is an important stressor for sheep, and can affect their welfare (Hargreaves & Hutson, 1990; Sanger *et al.*, 2011; Contreras-Aguilar *et al.*, 2019). Several indicators have been used to evaluate the acute stress response to shearing in sheep, such as serum cortisol concentration (Hargreaves & Hutson, 1990; Arfuso *et al.*, 2022a, 2022b), heart rate (Contreras-Aguilar *et al.*, 2019), eye and rectal temperatures (Arfuso *et al.*, 2022a), and changes in blood protein concentrations, such as total protein, albumin, and globulins (Arfuso *et al.*, 2022b; Ungerfeld *et al.*, 2018). The use of these hormonal (cortisol), physiological (heart rate and rectal and eye temperature), and blood biochemical (total proteins, albumin, and globulins) indicators allows for a comprehensive view of the response of sheep to the acute stress of shearing.

To the best of our knowledge, no studies have evaluated and compared acute stress responses to shearing in ewes with or without maternal experience. Furthermore, Viérin and Bouissou (2022) reported that primiparous ewes showed a greater fear response to human presence than multiparous ewes three weeks after offspring weaning. In this regard, it is possible to hypothesize that ewes without previous maternal experience (during their first gestation) show a greater stress response than ewes with maternal experience during pre-lambing shearing at the end of gestation. Therefore, this study aimed to compare the acute stress response to winter pre-lambing shearing between ewes with and without maternal experience by evaluating hormonal, biochemical, and physiological indicators.

MATERIALS AND METHODS

Location, animals, and management

The study was conducted at the Experimental Sheep Unit, INIA La Estanzuela, Uruguay (34°19'S;57°40'E) in July 2024.

Twenty-four Finnish Landrace x Polwarth ewes were used during the last month of gestation. Based on sample size calculations, the sample size per group was $n = 12$, considering a 95% confidence level, a 5% margin of error, and 80% power. Twelve ewes had previous maternal experience (ME,

4 ± 1 years old, number of prior lambings: 2 ± 1 , 56.7 ± 2.1 Kg of live weight: LW) and 12 ewes were in their first pregnancy, without maternal experience (WME, 2 years old, 43.9 ± 1.4 Kg LW). All sheep were shorn in previous years.

The ewes grazed on improved pastures composed of *Bromus inermis* and *Trifolium pratense*, or *Dactylis glomerata* and alfalfa (*Medicago sativa*), *Avena sativa*, and ryegrass (*Lolium multiflorum*). All ewes grazed together in the same pasture. They received daily supplementation of 250 g of whole-grain barley per animal. The ewes were bred naturally on the same ram. Pregnancy was diagnosed using ultrasound (ALOKA Prosound 2 ultrasound) with a transrectal probe and a 7.5 MHz linear transducer (Regueiro *et al.*, 2022). For this study, only twinning ewes were used. All sheep used in this study ($n = 24$) had unassisted parturition with no clinical complications.

Pre-lambing shearing

To evaluate the stress response to shearing, the ewes from each group were shorn alternately from each group and in a random order within each group. There were two waiting pens, one before shearing and another where the animals went after they had been shorn, both of which had a slatted wooden floor. In both pens, there was never just one animal; they were always in groups to minimize separation stress. Each ewe was individually moved to the shearing pen, which was located inside the same stable, but with a concrete floor. The shearing time was 5 min and was carried out by an experienced operator who was familiar with the animals, which allowed it to be performed under optimal conditions. The ewes were shorn using the Tally Hi technique and a shearing comb (R13 comb), which leaves a wool stubble between 8 and 10 mm (Sphor *et al.*, 2011).

Physiological parameters

Heart rate was recorded by auscultation using a stethoscope (Littmann® Classic stethoscopes, USA) before (time zero) and immediately after shearing (at 5 min). Eye and rectal temperatures were measured before (time 0) and after (5 min) shearing. A FLIR E5PRO thermal camera (FLIR Systems, Oregon, USA) with a focal plane sensor array size of 160×120 pixels was used. The camera was positioned 30 cm from the head of the sheep. Recordings were made perpendicular to the sheep eye and an emissivity of $\epsilon = 0.98$ was set. Ambient temperature and relative humidity were recorded in the shearing shed every 30 min using a portable logger (HTC-1 Clock/Temperature/Hygrometer), which were then considered for eye temperature recordings in the software. Eye temperature was measured at the medial canthus, as described by Arfuso *et al.* (2022a), who reported that this area of the eye was the most sensitive to temperature changes in sheep induced by shearing. The mean temperature of the medial canthus area of the eye was used for analysis. Rectal temperature was measured using a digital thermometer, inserted 3 cm into the rectum (DMT-433, Joytech Healthcare Co Ltd).

Blood sample collection

Blood samples (for serum and plasma) were collected by jugular venipuncture immediately before (0 min) and immediately after shearing (5 min), and at 25, 45, and 70 min post-shearing. Blood samples were centrifuged at 2500 rpm for 10 min, and serum and plasma were separated and stored at -20°C until hormonal, serum protein, and glucose levels were determined. Serum cortisol concentrations were measured in all samples, and serum protein and plasma glucose concentrations were measured in samples collected at 0, 25, and 70 min.

Cortisol, serum protein and plasma glucose determination

Serum cortisol concentrations were measured at the Laboratorio de Endocrinología y Metabolismo Animal (Facultad de Veterinaria, Universidad de la República, Uruguay) using ELISA Kits (Neogen Corporation®, Lexington, KY, USA), as described by Damián *et al.* (2024). The limit of detection of the kit was 0.04 ng/mL. The intra- and inter-assay CV percentages were all below 10%.

Serum protein and plasma glucose concentrations were determined using commercial kits (Bio-System, Barcelona, Spain) at the Laboratorio de Bioquímica (Facultad de Veterinaria, Universidad de la República, Uruguay). The concentration of globulins was estimated based on the difference between the total proteins and albumin (Damián *et al.*, 2021).

Statistical analysis

Data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (v. 3.1.0, SAS Institute Inc., Cary, NC, USA). Repeated measurements analysis was performed

on the different variables and according to their distribution: cortisol with gamma and the rest of the variables with Gaussian distribution. The fixed effects were maternal experience (ME vs. WME), time, and the interaction between them. Animal was considered as a random effect. Body weight (four days before shearing) was included as a covariate in the model. Post-hoc comparisons were performed using Tukey-Kramer tests. Data are expressed as mean \pm standard error of the mean (SEM). Significance was considered at an alpha value ≤ 0.05 , and a tendency with values between 0.05 and 0.10.

RESULTS

Physiological parameters

There was no maternal experience effect ($P = 0.77$) or interaction between maternal experience and time ($P = 0.95$) on heart rate. The heart rate increased in all animals (both groups) ($P < 0.0001$) from 0 to 5 min after shearing (Figure 1A).

Rectal temperature tended to be higher in WME ewes than in ME ewes ($39.6 \pm 0.1^{\circ}\text{C}$ vs $39.2 \pm 0.1^{\circ}\text{C}$, $P = 0.07$). There was no interaction between maternal experience and time in rectal temperature ($P = 0.40$); however, it increased ($P < 0.0001$) between 0 and 5 min after shearing (Figure 1B).

There was no effect of maternal experience on eye temperature ($P = 0.66$), but there was an interaction between maternal experience and time ($P = 0.036$). Eye temperature increased ($P = 0.004$) between 0 and 5 min after shearing in WME ewes only (Figure 1C).

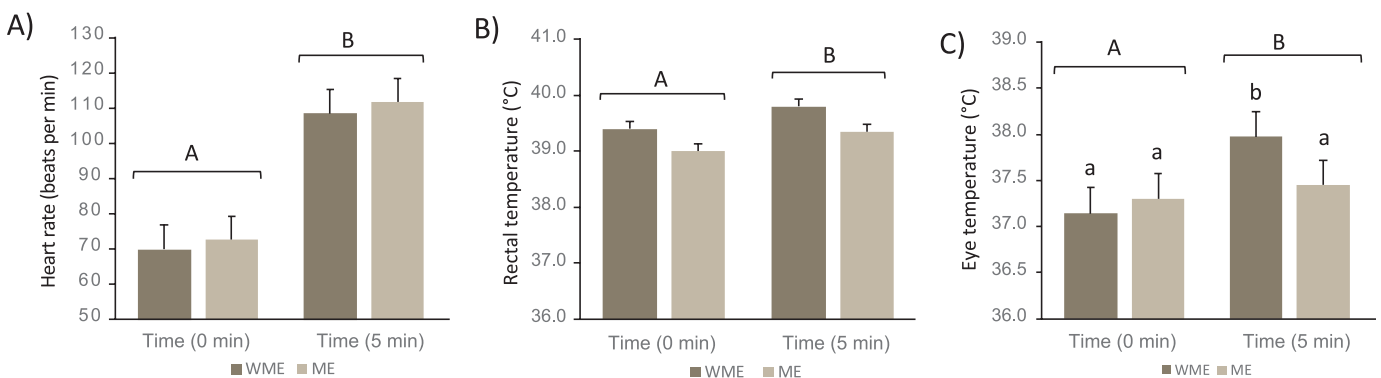


Figure 1.

Heart rate (A), rectal temperature (B), and eye temperature (C) (mean \pm SEM) before (0 min) and after (5 min) shearing of ewes with maternal experience (ME: white bar) and without maternal experience (WME: black bar). Different capital letters indicate differences between times ($P < 0.05$). Different lowercase letters indicate differences between times for the same group ($P < 0.05$).

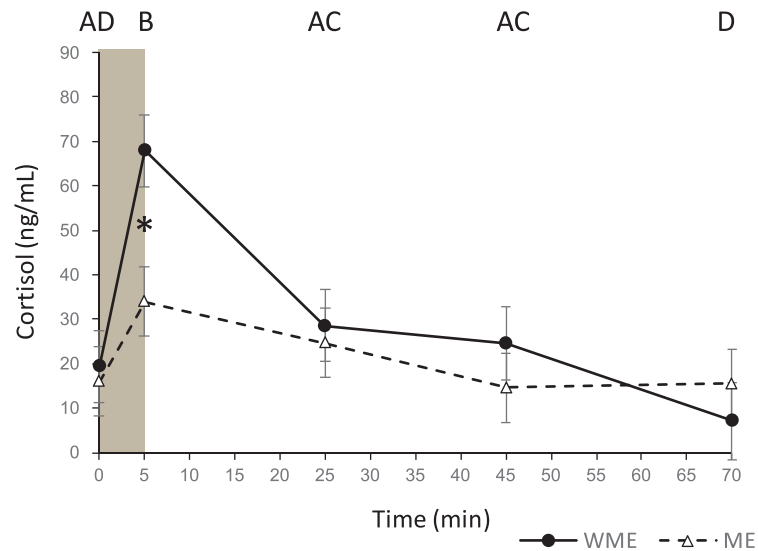


Figure 2.

Serum cortisol concentrations (mean \pm SEM) before and after shearing of ewes with maternal experience (ME: $-\Delta-$) and without maternal experience (WME: $-\bullet-$). The shaded area represents the shearing period (5 min). Different capital letters indicate differences between times ($P < 0.05$). Asterisks indicate significant differences between groups (ME vs. WME) at the same time ($P < 0.05$).

Serum cortisol concentrations

There was an interaction between maternal experience and time in serum cortisol concentration ($P = 0.004$). The WME ewes had higher serum cortisol concentrations immediately after shearing (5 min) than the ME ewes (WME: 68.50 ± 8.07 ng/mL vs ME: 33.40 ± 7.68 ng/mL, $P = 0.049$, Figure 2). Time affected the serum cortisol concentrations ($P < 0.0001$), which increased between 0 and 5 min after shearing, and then gradually decreased towards 25 and 45 min, reaching the lowest values at 70 min (Figure 2).

Serum protein and plasma glucose concentrations

Serum total protein concentrations were not affected by maternal experience or time; however, there was a trend ($P = 0.069$) in the interaction between maternal experience and time (Table 1). For serum albumin and globulin concentrations, there was no significant effect of maternal experience, time, or the interaction between maternal experience and time (Table 1). Plasma glucose concentrations were not affected by maternal experience or by the interaction between maternal experience and time (Table 1). However, glucose concentration varied over time ($P < 0.0001$), increasing between 0 and 25 min ($P < 0.0001$), and continuing to increase between 25 and 70 min ($P = 0.015$) (Table 1).

DISCUSSION

In this study, ewes showed a significant acute stress response to shearing, as evidenced by physiological, biochemical, and hormonal indicators. The response to shearing varied with maternal experience. Inexperienced

ewes had a greater stress response to shearing than those with maternal experience. Therefore, this study showed that during gestation, maternal experience plays a key role in the stress response to shearing.

According to this hypothesis, this study shows for the first time that maternal experience influences the response of ewes to shearing stress at the end of gestation. Ewes without maternal experience showed a greater stress response to shearing than did those with experience. The differential response according to the ewes' maternal experience to the stress of shearing at the end of gestation was evidenced by the rapid increase in cortisol after shearing, as well as the higher eye temperature and trend in rectal temperature, but not in relation to heart rate or biochemical indicators. In this study, we refer to mothers without previous maternal experience as a whole as those who are going through their first pregnancy, but since they are younger and still growing, they are also lighter in weight (González-García *et al.*, 2015; Damián *et al.*, 2020). It is known that during gestation, sheep without previous maternal experience have a greater mobilization of body reserves, given that in addition to meeting the requirements of the fetus, they are still growing (González-García *et al.*, 2015; Damián *et al.*, 2020). Therefore, ewes undergoing their first pregnancy have higher energy demands than those with previous maternal experience (Damián *et al.*, 2020). In addition, it is important to mention that, in our study, the ewes were pregnant with twins, which implies a higher nutrient demand than those with singletons (González-García *et al.*, 2015). Future studies are needed to understand how single or twin gestation can influence the response to shearing stress according to

Table 1.

Serum protein concentration (total protein, albumin and globulin) and glucose concentration (mean \pm SEM) of ewes with and without maternal experience before (time 0 min) and after (time 25 and 70 min) the shearing procedure, with the respective probability values (P) for the effects of maternal experience (ME), Time (Ti) and interaction between ME and Time (ME*Ti)

	Time (min)			P-value		
	0	25	70	ME	Ti	ME*Ti
Total protein (g/L)	73.7 \pm 2.4	73.5 \pm 2.3	73.1 \pm 2.3	0.933	0.978	0.069
Albumin (g/L)	31.7 \pm 1.8	35.3 \pm 1.9	32.5 \pm 2.0	0.645	0.338	0.474
Globulin (g/L)	42.5 \pm 2.5	37.9 \pm 2.5	38.5 \pm 2.6	0.685	0.308	0.897
Glucose (mg/dL)	65.1 \pm 1.8 ^a	79.0 \pm 2.1 ^b	86.5 \pm 1.9 ^c	0.436	<.0001	0.324

^{a, b, c}: indicate significant differences among time points within lines ($P \leq 0.05$)

maternal experience. It is also important to consider that the WME ewes had only been shorn once before, whereas the ME ewes had more frequent experience with shearing, as these had already been shorn several times. Although we do not know how the repetition of shearing with increasing age may affect the stress response, it is important to consider this element as an additional factor that could be involved in the differential response between the ewes categories. Therefore, all these elements together in relation to the lack of maternal experience may have determined that ewes without previous experience had a greater stress response to shearing. In this sense, the results of this study on the stress response to pre-lambing shearing highlight the vulnerability of WME ewes that are going through their first pregnancy, which is added to the set of metabolic needs that accompany pregnancy. Therefore, WME ewes would need greater care of handling in stressful situations during pregnancy. Our results agree with those of Viérin and Bouissou (2002) in the sense that inexperienced mothers respond with greater magnitude to stress than those with previous maternal experience. However, unlike our study, it is important to mention that the work done by Viérin and Bouissou (2002) evaluated the behavioral response to fear in relation to humans and in a different physiological state, since they evaluated it after weaning. Except for the study by Viérin and Bouissou (2002), to our knowledge, no other study has evaluated how ewes respond to different stressors according to previous maternal experience. Furthermore, the present study has the additional peculiarity that the shearing stressor occurs during gestation, and therefore not only affects the mothers, but could also have possible consequences for the offspring.

Stressful situations in mothers during pregnancy can affect the uterus and the fetus and its development (Godfrey & Barker, 2001; Dieckmann & Czamara, 2024). According to our results, the lack of maternal experience that leads to a greater stress response during shearing during gestation

may be another additional factor to the set off metabolic and physiological stressors that ewes experience during gestation and birth, which contributes to poor performance as mothers (Dwyer, 2003, 2014; Damián *et al.*, 2020) and possibly also influences the development of the offspring. Recently, Meyer and Redifer (2024) suggested that further research is needed to explore how primiparous and multiparous mothers respond differently to stressors, as well as their effects on their offspring. While our study contributed to clarifying how ewes with and without previous maternal experience responded differently to the stress of shearing during gestation, further work is needed to investigate how this different stress response to shearing during pregnancy, depending on maternal experience, can affect the development of their offspring.

In humans, mothers without maternal experience have higher cortisol concentrations and higher distress during gestation than those with previous experience (Vleugels *et al.*, 1986; Stirrat *et al.*, 2016; Bleker *et al.*, 2017; Gillespie *et al.*, 2018). Higher cortisol concentrations during gestation have also been reported in women with a lower body mass index (BMI) (Stirrat *et al.*, 2016; Bleker *et al.*, 2017). In addition, Bleker *et al.* (2017) reported higher cortisol concentrations in younger mothers during pregnancy. All of these results reported in humans are in line with our results in ewes during gestation. In addition, since mothers without previous experience are younger, have lower body weight (Dwyer, 2003, 2014; Damián *et al.*, 2020), and have a higher stress response during pregnancy as shown in this study, it is also possible to speculate that the sheep model during gestation and according to parity may be a good model for future studies associated with humans.

Although shearing was carried out by an experienced worker who was familiar with the animals, the shearing generated a clear stress response, which was evidenced by the increase in different parameters, such as physiological (eye and rectal temperature, heart rate), hormonal (corti-

sol), and biochemical (glucose) indicators. These results coincide with those of other studies on shearing stress in sheep (Sanger *et al.*, 2011; Ungerfeld *et al.*, 2018; Arfuso *et al.*, 2022a). Although physiological and hormonal indicators were affected by shearing, in the case of biochemical indicators only plasma glucose concentration was affected by shearing, not serum proteins. The increase in glucose concentration over time after shearing stress coincides with that reported in sheep subjected to other stressors, such as social isolation and movement restriction (Damián *et al.*, 2021), transport (Ali *et al.*, 2006), or thermal stress (Nazifi *et al.*, 2003). Furthermore, it is known that the increase in glucose concentration over time after stress is due to the hyperglycemic effect of cortisol, which stimulates gluconeogenesis (Sapolsky *et al.*, 2000; Damián *et al.*, 2015).

In conclusion, maternal experience in ewes influences the acute stress response to shearing during late gestation. Ewes without maternal experience had a greater stress response to shearing than ewes with previous experience, as evidenced by a greater increase in serum cortisol concentration and higher eye temperature.

Competing Interests Statement

The authors have no conflicts of interest or financial conflicts of interest to declare.

Ethics Statement

All the experimental procedures were approved by the Comisión de Ética en el Uso de Animales (CEUA) of the Instituto Nacional de Investigación Agropecuaria (INIA, No. 2023.17).

Author contributions

Conceptualization, JPD.; methodology, AN, GS, MC, VP, JG, DG, JPD.; software, JPD, AN.; formal analysis, JPD; investigation, AN, GS, MC, VP, JG, DG, JPD; resources, JPD, GB; data curation, JPD, AN; writing—original draft preparation, JPD, writing—review and editing, JPD, AN, GS, MC, VP, JG, EVL, GB; visualization, JPD; supervision, JPD; project administration, JPD, GB; funding acquisition, JPD, GB All authors have read and agreed to the published version of the manuscript.

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