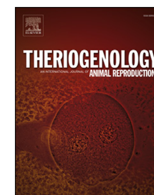
Theriogenology 173 (2021) 32–36

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com



Response of rams to electroejaculation following the administration of oxytocin and cloprostenol with or without GnRH

Daniel Andrews de Moura Fernandes [a], Clara Vieira de Souza [a], Mario Felipe Alvarez Balaro [a], Juliana Dantas Rodrigues Santos [a], Vanessa Moreira Barbosa dos Santos [a], Marta Maria Campos Pereira da Costa [a], Ana Beatriz da Silva Carvalho [a], André Luís Rios Rodrigues [a], Rodolfo Ungerfeld [b], Felipe Zandonadi Brandão [a, *]

[a] *Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, Rio de Janeiro, 24230-340, Brazil*
[b] *Departamento de Biociencias Veterinarias, Facultad de Veterinaria, Universidad de la República, Lasplaces 11 1550, Montevideo, 11600, Uruguay*

ARTICLE INFO

Article history:
Received 13 November 2020
Received in revised form
27 June 2021
Accepted 29 June 2021
Available online 30 June 2021

Keywords:
Glucocorticoids
Pain
Sheep
Semen
Welfare

ABSTRACT

The present study aims to evaluate the effect of administering prostaglandin (250 μg cloprostenol) and oxytocin (10 UI) or a GnRH agonist (4.2 μg buserelin acetate) on rams' physiological responses to electroejaculation and the ejaculate's characteristics. The study was performed with a 2 × 2 factorial arrangement, according to whether it used oxytocin and prostaglandin (OXPGF) or GnRH. Therefore, there were four treatments: GControl = saline; GOXPGF = administration of PGF2α and oxytocin; GGnRH = administration of GnRH; administration of GOXPGF + GnRH = GnRH and PGF2α + oxytocin. An interaction between the hormonal treatments in the heart rate occurred: while the heart rate decreased when using OXPGF alone (control: 113.7 bpm vs. GOXPGF: 103.5 bpm, pooled SEM; P = 0.02), it did not modify when applying both treatments simultaneously and administering GnRH (GGnRH: 109.1 bpm vs. GOXPGF + GnRH: 111.5 bpm respectively, pooled SEM = 4.5). The respiratory rate also decreased with the administration of OXPGF (38.7 vs. 46.3 with and without OXPGF, pooled SEM = 10.0, P = 0.003). Administering OXPGF also tended to decrease the temperature (38.77 °C vs. 38.94 °C, with and without OXPGF, respectively, pooled SEM = 0.06; P = 0.056). Blood glucose increased with the administration of OXPGF from 58.7 mg/dL to 62.4 mg/dL (pooled SEM = 1.3, P = 0.014) and varied with time. CK concentrations increased from 641.8 mg/dL to 881.7 mg/dL (pooled SEM = 50.6) with the administration of OXPGF. GnRH administration decreased cortisol concentration from 7.3 ng/mL to 2.1 ng/mL (pooled SEM = 1.4; P = 0.04). The treatments had no effects on the time required for EE, the pulse at which the animals began and ended the ejaculation, or the vocalizations emitted during EE. There were no effects in any evaluated sperm variable. The research concluded that the administration of oxytocin and analogs of PGF2alpha decreased the stress response to electroejaculation, as well as administering GnRH agonist was slightly effective as it only decreased cortisol concentration. Also, these treatments, either alone or combined, did not affect the characteristics of the ejaculate collected.

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1. Introduction

Electroejaculation (EE) is a common technique for collecting semen in various farm animals, but its use is under debate as it generates stress and pain to the animals (see review [1]). EE also induces several undesirable responses, including increased heart rates and creatine kinase, alkaline phosphatase, and aminotransferase blood concentrations (deer: [2]). Physiological, biochemical, hormonal, and hematological changes in sheep submitted to electrostimulation include increases in cortisol and decreases in testosterone concentrations [3]. These responses are also associated with vocalizations in sheep [3] and bulls [4], which indicate pain [3].

These responses raise negative concerns, which one can reduce by shortening the time or process needed for EE and then applying

* Corresponding author.
E-mail address: fzbrandao@id.uff.br (F.Z. Brandão).

https://doi.org/10.1016/j.theriogenology.2021.06.019
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fewer pulses. In that sense, there are important releases of oxytocin and prostaglandin-F2alpha (PGF2a) during the spontaneous ejaculation process, which stimulate the muscle fibers of the male genital tract [5]. Ungerfeld et al. [6] recently demonstrated that the administration of cloprostenol (a PGF2alpha analog) and oxytocin before EE reduce the time and number of electrical stimuli required to achieve ejaculation without affecting sperm quality in goat bucks. One should also consider that testosterone facilitated the ejaculatory reflex [7,8]. Therefore, a strategy for increasing testosterone concentation before EE may be to administer GnRH, as administering GnRH analogs trigger increases in testosterone concentration [9].

Since administering oxytocin and cloprostenol improves the process of semen collection in goat bucks, the present study aims to evaluate if administering GnRH per se or its association with the former treatment decreases welfare concerns about semen collection by EE or enhances semen quality.

2. Materials and methods

The Ethics Committee on the Use of Animals of Universidade Federal Fluminense approved this research (CEUA nº 5526080119). Additionally, this study followed the guidelines adopted by the Brazilian Society of Science in Laboratory Animals.

2.1. Animals and management

The study took place during December (the transition from the non-breeding to the breeding season; Souza et al., 2007) at the Experimental Research Unit on Goats and Sheep (UniPECO), located in Rio de Janeiro, Brazil (22° 27 'S). It used 15 healthy young Santa Inês rams [1.1 ± 0.2 years old; 47.7 ± 8.7 kg; 3.0 ± 0.1 of body condition; scale 1 to 5 according to Suiter [10] (mean ± SEM)]. All rams were healthy, and clinical and andrological evaluations approved their sperm quality (scrotal circumference ≥ 32 cm; swirling ≥ 3; sperm motility ≥ 70%; sperm vigor ≥ 3; sperm concentration $\geq 2 \times 10^9$; abnormal sperm <20%) before beginning the study [11]. These rams had been used before in other experimental studies that needed to collect semen with EE, so they were previously handled and habituated to the general procedures. One month before and throughout the experimental period, the animals remained confined and were fed chopped Napier grass (*Pennisetum purpureum*) and concentrate (400 g/animal/day; 18% crude protein) for their maintenance. Water and mineral salt (Salinas Ovinos, Salminas, Juiz de Fora, Minas Gerais, Brazil) were provided ad libitum.

2.2. Experimental design

All the rams were subjected to the four treatments in a crossover design, with blocks of 3–4 rams/treatment undergoing electroejaculation weekly. The treatments were organized in a 2 × 2 factorial arrangement according to the hormonal treatments the rams received before electroejaculation. These were: 1) 250 μg of cloprostenol (Ciosin-MSD Saúde Animal, São Paulo, São Paulo, Brazil) 5 min before semen collection and 10 IU oxytocin (Ocitocina Forte UCB, UCBVET Saúde Animal, Jaboticabal, São Paulo, Brazil) 30 s before collection (OXPGF), and 2) 4.2 μg of buserelin acetate (Sincroforte OuroFino Saúde Animal, Cravinhos, São Paulo, Brazil) 120 min before collection. Therefore, there were four treatments applied to the same rams: 1) Control (no treatment), 2) GOXPGF: received oxytocin and cloprostenol; 3) GGnRH: received buserelin acetate; 4) GOXPGF + GnRH: received both treatments. The researchers expected that testosterone and, thus, aggressiveness might increase after the buserelin treatment, so the GGnRH and GOXPGF + GnRH rams remained separated from the other rams after its administration.

2.3. Responses to the procedures

The researchers determined heart rates through auscultation, respiratory frequency by observing the respiratory movements, and rectal temperature using a digital thermometer immediately before beginning the EE, immediately after the EE ended, and 30, 90, 150, and 240 min later. They collected blood samples in tubes containing a vacuum, separated the serum by centrifugation immediately afterward, and maintained it at −20 °C until the analysis. Samples were collected 1 h and 5 min before EE, and 10, 30, 90, 120, 180, and 240 min after EE.

The cortisol concentration was measured in samples collected 1 h before EE and 10, 30, 90, and 180 min after EE using a solid phase radioimmunoassay with a commercial kit (ImmuChem, MP Biomedicals, Santa Ana, California, USA). The assay's sensitivity was 0.17 ng/mL, and the intra-assay variation coefficient was 12%. Additionally, all data were within the minimum and maximum points of the curve.

The researchers determined the creatinine kinase concentration with commercial colorimetric kits (Liquiform, Labtest, Lagoa Santa, Minas Gerais, Brazil) using an automatic system (Labmax Premium 240, Labtest Diagnostica, Lagoa Santa, Minas Gerais, Brazil) in samples collected 1 h and 5 min before EE and 10, 120 and 240 min after EE. Glycaemia was measured in samples collected 1 h and 5 min before EE and 10, 30, 90, and 180 min after EE with a commercial colorimetric kit (Glucose Liquiform, Labtest, Lagoa Santa, Minas Gerais, Brazil) in the same equipment.

2.4. Electroejaculation and semen evaluation

Each ram was individually immobilized in a sheep crush. The penis's prepuce and glans were cleaned, the rectum was emptied from feces, and the electroejaculator's probe was introduced. The probe measured 30 cm long and had a diameter of 1.5 cm, with longitudinal electrodes (TK Reprodução Animal, Uberaba, Minas Gerais, Brazil). It was coated with carboxymethyl cellulose, introduced into the rectum, and electrical pulses were applied, beginning with 2 V and increasing 1 V every ten pulses until a maximum of 5 V. Each pulse lasted three to 5 s, with resting periods of 2 s. During the semen collection procedure, the researchers recorded the time and the number of electrical pulses required to begin and end ejaculating, and the number of vocalizations.

After semen collection, the ejaculated volume was measured using graduated micropipettes. A diluted aliquot (1/100) was held in heated saline to perform the analysis in phase-contrast microscopy. Motility parameters were evaluated by computerized semen analysis (SCA - Sperm Class Analyzer – Microptic, Automatic Diagnostic Systems - Barcelona, Spain). Five fields from each sample were evaluated automatically for the sperm kinetics parameters: total motile sperm (TM, %); sperm with progressive motility (PM, %); average path velocity (VAP, μm/s); curvilinear velocity (VCL, μm/s); straight-line velocity (VSL, μm/s); amplitude of lateral head displacement (ALH, μm/s); beat/cross frequency (BCF, Hz); straightness (STR, %); linearity (LIN, %); and WOB (defined as the mean value of the ratio between VAP and VCL, %). Then, the sperm concentration was determined using a Neubauer chamber after diluting 10 μL of semen in 8.0 mL of distilled water, obtaining a dilution of 1: 800. The researchers calculated the number of sperm ejaculated (volume X concentration) and used this value to calculate the number of motile sperm ejaculated and sperm with progressive motility ejaculated.

2.5. Statistical analysis

The researchers determined the homoscedasticity and normality utilizing the Lilliefors test. They analyzed the data with a mixed model that included the treatments (OXPGF or GnRH) and time (in repeated data) as main factors and all their interactions. The model included the block and day of collection as random effects and considered time as a repeated factor. The analysis was performed with the SAS University Edition software. Results appear as LSmeans ± SEM and are considered as significant differences when $P < 0.05$ and as tendencies when $0.05 < P < 0.1$.

3. Results

3.1. Collection procedures

There were no treatment effects on the time required for EE (116.6 ± 16.4 s), the pulse at which the animals began ejaculating (4.1 ± 1.2) or the vocalizations emitted during EE (3.8 ± 1.8). The main effects of the treatment, the time, and their interactions in heart rate, respiratory frequency, rectal temperature, glycemia, CK, and cortisol concentrations appear in Table 1.

There was an interaction between the hormonal treatments in heart rates: While the heart rate decreased with the use of GOXPGF (control: 113.7 bpm vs. OXPGF: 103.5 bpm, pooled SEM; P = 0.02), it was not modified when also administering GnRH (109.1 bpm vs. 111.5 bpm respectively, pooled SEM = 4.5). Heart rates also varied with time. They increased during EE from 90.8 bpm to 176.8 bpm (P < 0.0001), decreasing at 30 min to 113.2 (P < 0.0001), and returning to basal values 90 min after EE 94.1 (P = 0.0003) (pooled SEM = 4.9). The respiratory frequency also decreased with the administration of OXPGF (38.7 vs. 46.3 with and without OXPGF, pooled SEM = 10.0, P = 0.003). Moreover, it tended to vary with time (P = 0.07). Administering OXPGF tended to decrease the temperature (38.77 °C vs. 38.94 °C with and without OXPGF, respectively, pooled SEM = 0.06; P = 0.056). It also varied with time, increasing from the end of EE to 30 min later (38.7 °C to 39.0 °C, P < 0.0001) and decreasing from this value 90 min after the end of EE (38.9 °C; P = 0.007) (pooled SEM = 0.07).

Glycemia increased with the administration of OXPGF from 58.7 mg/dL to 62.4 mg/dL (pooled SEM = 1.3, P = 0.014). It also varied with time: It increased during EE from 44.4 mg/dL to 79.8 mg/dL (P < 0.0001). It then decreased 30 min after EE to 69.7 (P < 0.0001), and 30 min later to 62.3 mg/dL (P = 0.005) without change by the study's completion (62.0 mg/dL) (pooled SEM = 2.0). The administration of GnRH decreased the cortisol concentration from 7.3 ng/mL to 2.1 ng/mL (pooled SEM = 1.4; P = 0.04). Cortisol concentration also varied with time. There was an interaction between the administration of OXPGF and time (Fig. 1A), a tendency toward interactions between the administration of GnRH and time, and a triple interaction between the treatments and time (Table 1). CK concentrations increased from 641.8 mg/dL to 881.7 mg/dL (pooled SEM = 50.6) upon administering OXPGF. It, too, varied with time. Furthermore, there was an interaction between OXPGF and time (Fig. 1B).

3.2. Sperm quality

There were no effects of treatments on the ejaculate volume (2.0 ± 0.26 mL), mass motility (3.2 ± 0.3 – 0-5), total sperm per ejaculated/mL (2649.3 × 10^6 ± 477.6), TM (90.7 ± 5.8%), PM (31.3 ± 2.8%), VAP (62.5 ± 5.9 μm/s), VCL (92.5 ± 7.3 μm/s), VSL (44.2 ± 4.4 μm/s), ALH (3.5 ± 0.3 μm/s), BCF (8.4 ± 0.6 Hz), STR (64.8 ± 5.0%), LIN (45.2 ± 3.5%), or WOB (63.4 ± 4.3%).

4. Discussion

Administering OXPGF did not modify the process for semen collection, including the time and the number of pulses required for ejaculation. Although the researchers hypothesized that using OXPGF would shorten and simplify the process due to its action on the male tract's motility, as in previous reports regarding Gabon bucks [6], this effect did not occur. Possibly, the treatment's positive effects relate to each species' or breed's sensitivity to EE and, thus, to the time and the number of pulses needed for ejaculation. In this sense, the goat bucks that Ungerfeld et al. [6] utilized required more than double the time to ejaculate than the rams that this study used. Thus, one may detect the positive effect if the species or breed requires more time spent immobilized and receiving electrical pulses than this study's rams. The rams' faster response, earlier ejaculation, and the shorter process might have masked possible treatment effects.

Although administering OXPGF did not shorten the process, it decreased significant stress responses because it lowered increases in heart and respiratory frequencies. It also tended to reduce the rectal temperature. These responses are triggered immediately after a stressor's action and relate to the sympathetic nervous system's activation and the release of catecholamines [12]. Since the treatment's local effects were non-evident in the collection process, oxytocin itself may also exert anti-stress effects [13]. At the same time, as oxytocin is rapidly metabolized with a half-life of not more than 2–3 min [14], the anti-stress effect might disappear quickly. This disappearance prevents its possible effects in cortisol secretion, which require more time than the sympathetic response. Thus, the greater cortisol increase, which in electroejaculated goat bucks and bulls also triggers an increase in glycemia [15,16], may result from a rebound effect in response to EE after oxytocin quickly disappears from the central nervous system. Similarly, the CK concentration, which also requires more time, increased with this treatment. In turn, possible sustained treatments with oxytocin may be more beneficial than administering a single dose, particularly for species or breeds in which the short time that EE needs might mask these hormones' possible positive direct effects in the male tract.

Administering GnRH decreased the cortisol increase, concording with the expected response in individuals with presumably greater testosterone concentrations. Reports claim that administering similar doses of GnRH stimulates notable increases of testosterone secretion in rams [17] and goat bucks [9]. In turn, high concentrations of testosterone decrease their response to stressors. In effect,

Table 1
Physiological and biochemical on rams submitted electroejaculated, previously treated or not with oxytocin and PGF (OXPGF) and/or GnRH.

Variable	OXPGF	GnRH	Time	OXPGF*GnRH	OXPGF*Time	GnRH* Time	OXPGF*GnRH*Time
Heart rate	ns	ns	<0.0001	0.04	ns	ns	ns
Respiratory frequency	0.0030	ns	0.07	ns	ns	ns	ns
Rectal temperature	ns	ns	0.09	ns	ns	0.08	ns
Glycaemy	0.0144	ns	<0.0001	ns	ns	ns	ns
CK	0.0009	ns	<0.0001	ns	0.014	ns	ns
Cortisol	ns	0.044	0.045	ns	0.03	0.09	0.027

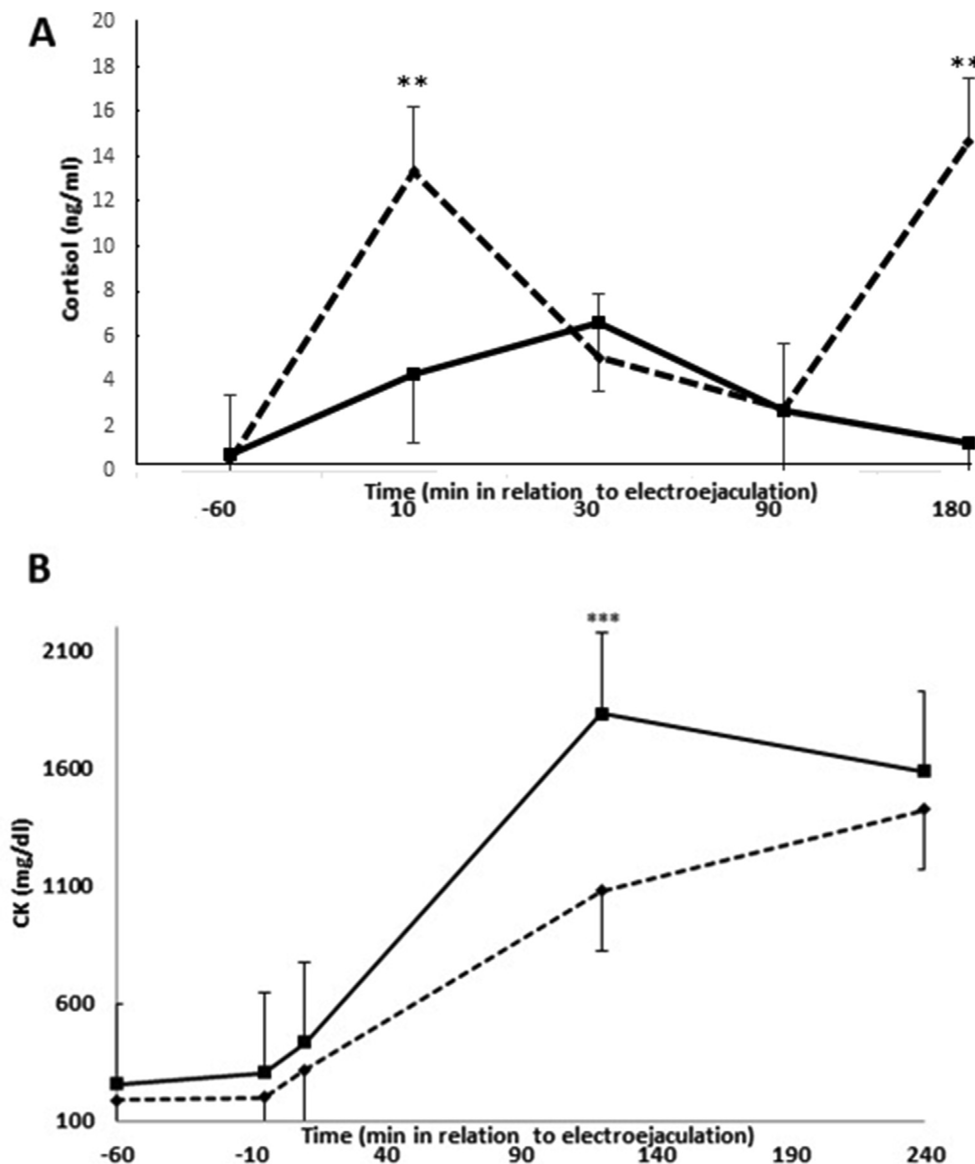

Fig. 1. A. Cortisol concentration (mean ± SEM) and B. CK concentration (LSmean ± SEM) on rams electroejaculated, previously treated or not with oxytocin and PGF2α(with or without GnRH), (−■−)or not (−◆--).

the increase of ACTH and cortisol in response to different stressors (the presence of barking dogs or hypoglycemic stress) reduces in wethers treated with testosterone [18]. Nevertheless, treatment with GnRH did not modify the process for semen collection itself, so it seems that the effect related more to the sensitivity to stressors or how they perceived them than to a direct effect in the ejaculation process. However, GnRH could not modify the increased heart rate that EE produced or even block OXPGF's positive effect in this variable, perhaps because testosterone did not affect the sympathetic nervous system response [18].

Treatments had no effect on semen quality. As all these hormones stimulate the ejaculatory process, the researchers expected the number of sperm ejaculated to enhance its characteristics. The explanation relates to the rams' easy and rapid response to electroejaculation, which may have thwarted improving the result. Although, since oxytocin and PGF2alpha stimulate the male genital tract's motility [5] and testosterone promotes the ejaculatory process [7], it seems that a single study cannot discard this possible effect. Moreover, it would be thought-provoking to determine these hormones' possible positive effects when two or three ejaculates are collected, as they may be evident if ejaculation requires more stimuli than this study utilized. One may expect the process to take longer [9] and, thus, require greater stimuli in such cases, standard processes in the practical application of sperm collection.

5. Conclusion

The research concluded that administering oxytocin and analogs of PGF2alpha decreased the stress response to electroejaculation, as well as administering GnRH agonist, under this study's conditions, was slightly effective as it only decreased cortisol concentration. Also, these treatments, either alone or combined, did not affect the characteristics of the ejaculate collected.

Declaration of interest

None of the authors has any conflict of interest to declare.

Data availability statement

No additional data are available.

CRediT authorship contribution statement

Clara Vieira de Souza: Data curation, collected data, revised, and worked on the manuscript, and approved the final version. **Mario Felipe Alvarez Balaro:** Data curation, discussed the general study design, collected data, revised and worked on the manuscript, and approved the final version. **Juliana Dantas Rodrigues Santos:** Data curation, collected data, revised the manuscript, and approved the final version. **Vanessa Moreira Barbosa dos Santos:** Data curation, collected data, revised the manuscript, and approved the final version. **Marta Maria Campos Pereira da Costa:** Data curation, collected data, revised the manuscript, and approved the final version. **Ana Beatriz da Silva Carvalho:** Data curation, collected data, revised the manuscript, and approved the final version. **André Luís Rios Rodrigues:** Data curation, discussed the general study design, collected data, revised and worked on the manuscript, and approved the final version. **Rodolfo Ungerfeld:** Formal analysis, Data curation, Writing – original draft, proposed the initial hypothesis, organized the study, analyzed the data, wrote the first draft, revised and worked on the manuscript, and approved the final version. **Felipe Zandonadi Brandão:** Data curation, discussed the general design, collected data, revised and worked on the manuscript, and approved the final version.

Acknowledgments

The present study was supported by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ (E-26/010.002238/2019). FZB is a fellow of the CNPq.

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