Administration of cloprostenol and oxytocin before electroejaculation in goat bucks reduces the needed amount of electrical stimulation without affecting seminal quality

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ARTICLE INFO

ABSTRACT

Electroejaculation (EE) is a widely used semen collection technique; but, it is stressful and painful for the animals. Considering these concerns, it may be important to develop practices to decrease the negative implications of EE on animals. Oxytocin and prostaglandin-F2alpha (PGF2α) stimulate the contractions of the muscles of the male genital tract. Therefore, the aim of this study was to analyze the effectiveness of the administration of oxytocin and/or a PGF2α analogue (cloprostenol) to bucks in relation to their stress response and sperm parameters before semen collection by EE was performed. Semen was collected with EE from 12 Gabon bucks in a 2 × 2 factorial arrangement (factors: with or without oxytocin, with or without cloprostenol). Each treatment was applied to different animals every 3 to 6 days, allowing all the animals to receive all the four treatments. The treatments applied to bucks before EE were as follows: 1) control (ConT), bucks received no hormonal treatment; 2) oxytocin (OxyT), bucks received 10 IU of oxytocin intramuscularly (IM) 30 s before beginning the EE; 3) PGF2α (PgT), bucks received 250 μg of cloprostenol IM 5 min before beginning the EE; and 4) oxytocin plus PGF2α (OxPgT), animals received treatment with both OxT and PgT. The number of electrical pulses, time length needed to achieve ejaculation, number of vocalizations, creatine kinase (CK) concentration and sperm parameters in goat bucks were recorded. The administration of cloprostenol and oxytocin before EE shortened the procedure and decreased the number of pulses and the pulse/voltage applied (P = 0.02 for all). This treatment also tended to decrease the number of vocalizations (P = 0.067). There were no treatment effects in the initial values; neither were there increases in heart rate and rectal temperature, or CK concentration. Seminal variables were not affected by the treatments. In conclusion, it would be important to consider the combined application of cloprostenol and oxytocin before EE, as it can shorten the process, reducing the electrical stimulus with positive effects on animal welfare and without affecting seminal quality in goat bucks.

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1. Introduction

In ruminants, semen is frequently collected via electro-ejaculation (EE). This practice is stressful and painful for the animals, which raises concerns about the use of this technique [1—4]. In bucks, rams, and bulls, EE provokes increases in cortisol concentration, rectal temperature (RT), and heart, pulse, and respiratory rates, as well as changes in hematological and biochemical parameters, all indicative of a stress response [1,4—7]. The main negative responses are mostly triggered by the application of electrical pulses rather than by the general procedure (rams: [8]; bulls [7]). The electrical pulses also induce strong muscular contractions, increasing creatine kinase (CK) serum concentrations [5,9], which indicate muscle damage [10]. When EE is applied in conscious rams and bulls [5,11,12], and even in anesthetized deer, animals vocalize, suggesting that this procedure is painful for them. Although similar responses than those observed during EE were

reported during spontaneous ejaculation, these responses are also observed in electrically stimulated ewes demonstrating a direct relation to EE [13]. These observations have led several authors to point out that EE may be pose animal welfare issues and that improvements to the technique are needed to reduce stress to animals [14,15].

The use of anesthetic methods and Transrectal Ultrasound-Guided Massage of the Accessory Sex Glands (TUMASG) have been studied as alternatives to decrease the negative effects of EE [16–18]. However, TUMASG requires an intensive training of the operators, and its effectiveness compared to EE seems to differ according to the species, and to the use or not of general anesthesia [19,20]. Therefore, it should be a priority to develop practices that help to reduce the number of electrical pulses, the voltage, and/or the length of time during which the animals are restrained during EE. During the normal ejaculation process, there is an increase in oxytocin and prostaglandin-F2alpha (PGF2α) concentrations [21,22], and both hormones stimulate the contractions of the muscles of the male genital tract. Therefore, the administration of these hormones may be effective to reduce the number of electrical pulses needed to achieve ejaculation.

Oxytocin is released from the neurohypophysis, but is also synthesized and secreted in the male genital tract, including the testes, epididymis, and prostate [23]. There are oxytocin receptors in the cauda epididymis and ductus deferens of rams [24], and it has been reported that oxytocin stimulates epididymal contractions acting directly on smooth muscle cells and indirectly inducing the secretion of endothelin-1 [25], promoting the transport of sperm into the vas deferens [26]. Oxytocin is also involved in corpus cavernosum contractility during the ejaculatory process, at least in rabbits, rats, and humans [27,28]. Thus, administration of oxytocin before rectal massage and EE shortened the procedure time and tended to decrease the amount of electrical stimuli needed for semen emission in bulls [14,29]. It also shortened the time needed for ejaculation with rectal massage in Mithun bulls [30] and TUMASG in goat bucks [31], without negative effects on seminal characteristics. In addition, PGF2α stimulates the fluid output from the testicle [32], and the contractions of the smooth muscles involved in the ejaculation process [33]. The administration of analogues of PGF2α induces an increase in the volume of semen collected, and also the sperm concentration in the semen collected from rams [34]. Both hormones stimulate different muscular fibres in the myometrium of the bitch [35]; therefore it may be interesting to test wheter both hormones have a cumulative effect, and thus, if the association of both can decrease the negative implications of EE on animal welfare without affecting or even improving the semen quality collected by EE.

Therefore, the aim of this study was to analyze the effectiveness of the administration of oxytocin and/or a PGF2α analogue (cloprostenol) to bucks in relation to their stress response and sperm parameters before semen collection by EE was performed.

2. Materials and methods

2.1. Animals and management

All the procedures were approved by the Comisión de Ética en el Uso de Animales of the Facultad de Veterinaria (CEUA, UdelaR, Uruguay). The study was performed in the Departamento de Fisiología, Facultad de Veterinaria (35°S, Montevideo, Uruguay) in March (late summer-early autumn; mid-breeding season [36]) with 12 Gabon bucks [8endash;9 years old, body weight: 31.4 ± 2.3 kg (mean ± SEM)]. The bucks were housed in a 17 × 10 m pen and received alfalfa hay and free access to water. All the animals in this study had undergone EEseveral times before the study.

2.2. Experimental design

Electroejaculation was used to collect semen four times in each animal; 3 to 6 days separated each procedure. Each animal received all four procedures (2 × 2 factorial arrangement: with or without oxytocin and with or without cloprostenol as main factors). All procedures were applied to all the bucks. Thus, three bucks received each treatment each day of semen collection (periods 1 to 4), and each triad of bucks received another treatment in each period.

The treatments were as follows: 1) control (ConT), bucks received no hormonal treatment; 2) oxytocin (OxyT), bucks received 10 IU of oxytocin (Hipofamina, Laboratorios Dispert, Montevideo, Uruguay) intramuscularly (IM) 30 s before beginning the EE; 3) PGF2α (PgT), bucks received 250 μg of cloprostenol (Ciclase DL, Syntex, Buenos Aires, Argentina) IM 5 min before beginning the EE; and 4) oxytocin plus PGF2α (OxPgT), animals received both OxT and PgT treatments.

2.3. Electroejaculation

Electroejaculation was performed with a probe of 30 cm length × 1.5 cm width, with 1 cm ring electrodes (Fuhijira Industry, Tokyo, Japan), covered with duct tape on the upper side to avoid stimulating the dorsal region of the animal. Briefly, the animal was manually restrained by the stock-keeper, and the penis was manually protruded. Then, the rectal probe coated with carboxymethyl cellulose gel was inserted into the rectum, with electrodes positioned ventrally. The equipment had manual control of pulses, which were applied for 4 to 5 s alternated with rest periods of approximately 2 s. The bucks were stimulated with 10 pulses of 2 V, increasing by 1 V in each series of 10 pulses, until ejaculation.

2.4. Responses to the procedures

During each procedure, the time required for ejaculation, the number of electric pulses applied until the animals began to ejaculate, the overall number of pulses, until the end of ejaculation, and the number of vocalizations were recorded. A pulse/voltage variable was calculated as the Σ (number of pulses × voltage) applied.

The heart rate (HR) was recorded by auscultation, and the rectal temperature (RT) was measured with a digital thermometer. The HR and the RT were determined immediately before and after EE, and the increase in the HR and the RT were calculated.

Blood samples were collected by jugular venipuncture immediately before and after EE, as well as 120 and 240 min later. Samples were allowed to clot and were centrifuged for 10 to 15 min at 1500 g. The serum was separated and maintained at −20° C until measurement of serum CK concentration was performed. The CK concentration was determined by colorimetry using a commercial kit (Wiener Lab, Rosario, Argentina) at the Laboratorio de Análisis Clínicos, Facultad de Veterinaria, Montevideo, Uruguay.

2.5. Semen evaluation

Semen ejaculated volume, sperm concentration (using a Neubauer chamber), sperm mass motility (scale 0-5), and the percentage of motile and progressive motile sperm were determined under phase contrast microscopy. The sperm membrane integrity was determined with the hypo-osmotic swelling test (HOST) as previously described [37]. The percentage of sperm with morphological abnormalities was determined in samples fixed in formol citrate solution at 37 °C, using phase-contrast microscopy. The total number of spermatozoa in the ejaculate was also calculated.

2.6. Statistical analysis

Normal distribution of the data was determined with the Shapiro-Wilk test. Time to ejaculation, pulse/voltage, and number of vocalizations were root square transformed to normalize them before analysis. The time of the procedure, number of electric pulses applied for EE (total number of pulses, pulses until ejaculation began, pulses while ejaculating), the number of vocalizations, the increase in the HR and the RT, and all the semen parameters (ejaculated volume, sperm concentration, sperm mass motility; the percentages of motile, progressive motile, sperm with membrane integrity, and with morphological abnormalities; and the total number of spermatozoa in the ejaculate) were compared between treatments with a mixed procedure or a Friedman test in a Latin-square design, including the procedures as main effect, and the period and animal as random effects. The CK concentration was compared with a mixed procedure, including in the model the treatments, the interaction between the treatments, the time, and the interaction between both treatments and the time as main effects, and period and animals as random effects. A first order autoregressive covariance structure was assumed to adjust the difference in data according to the differences over time. Statistical analyses were performed with the SAS University Edition software. Data are presented as mean $\pm$ SEM. Differences were considered statistically significant if $P \leq 0.05$.

3. Results

Time needed for EE, total number of pulses, and pulse/voltage were not affected by the administration of oxytocin or cloprostenol alone. However, in the three variables, the interactions between both hormones were significant, shortening the procedure and decreasing the number of pulses and the pulse/voltage applied ($P = 0.02$ in the three variables; Table 1). When both hormones were administered (group OxPgT), the number of vocalizations tended to decrease ($P = 0.067$; Table 1). There were no treatment effects or interactions in the number of pulses needed to begin the ejaculation, in the number of pulses needed while ejaculating, nor in the initial values and the increases in the HR and the RT (Table 1).

Concentrations of CK varied only with time ($P < 0.0001$), but there were no effects of the administration of cloprostenol, oxytocin, their interaction, or their interaction with time. Concentrations were similar immediately before and after EE (264 U/L vs 243 U/L; pooled SEM = 26), but increased 120 and 240 min after EE ended (319 U/L, $P = 0.007$ and 395 U/L, $P = 0.007$, respectively).

Seminal variables were not affected by the administration of oxytocin, cloprostenol, or their interactions (Table 2).

4. Discussion

The combination of cloprostenol and oxytocin decreased the negative effects of EE on the bucks in our study, as this treatment shortened the process of semen collection and required fewer electric pulses (reduced by approximately 18% and 15%, respectively); vocalisations emitted by the bucks decreased by 18%. It should be noted that the electrical pulses are the primary cause of the stress response of animals to EE (rams: [38]; bulls [7]), and with the protocol used, the reduction in the number of electrical pulses implied a reduction of the pulses of higher voltages. Furthermore, in agreement with previous observations in bulls, these hormonal treatments did not modify the seminal characteristics [29]. It is possible that EE itself provoked the maximum muscular contractions for sperm propulsion, and therefore, the administration of cloprostenol and oxytocin did not have any benefit in semen characteristics. Despite this, the combined application of cloprostenol and oxytocin before EE should be considered for routine application as it can shorten the process, reducing the electrical stimulus with positive effects on animal welfare.

The positive effects were observed only with the application of the combination of both hormones, but each hormone *per se* did not modify any parameter during the EE procedures. Although in other studies oxytocin alone had positive results on welfare indicators when it was associated to EE in bulls [29] or to TUMASG in bucks [31], different doses, administration routes, collection methods, and latency from administration to collection limit making direct comparisons. The positive effects observed in our study with the combination of both hormones may be consequence of a synergic action, as both hormones stimulate directly or indirectly the contractions of the male genital tract muscle cells promoting the transport of sperm in the ejaculation of rams [26]. Although we could not find any information in males, both hormones stimulate different muscular fibres in the myometrium of the bitch [35], and thus, may have an accumulative effect, thereby increasing the general contractility of the bucks' genital tract. Another non-opposed explanation is a possible priming effect of cloprostenol increasing the sensitivity of the muscles involved in ejaculation, and thus, improving the response to oxytocin or even stimulating endogenous oxytocin secretion [22], provoking an accumulated effect of both sources of oxytocin. However, both hormones have a short half-life [39,40], and the lapse from cloprostenol to oxytocin administration was too short to expect this type of effect. However, the application of PGF2α 1 h before semen collection did not produce any benefit in seminal characteristics [41], or produced slight transient benefits [42] in bulls. It may be interesting to study whether the use of long-acting presentations

Table 1
Variables recorded in electroejaculated goat bucks without treatment (control), or that received oxytocin 30 s before the beginning of electroejaculation (OxyT), prostaglandin-F2α 5 min before beginning the EE (PgT), or both treatments (OxPgT). (Mean and pooled SEM).

	Control	OxT	PgT	OxPgT	Pooled SEM	P Ox	P Pg	POx*Pg
Time length (s)	249	286	290	204	25	ns	ns	0.02
Pulses								
Total number	42.7	49.0	47.0	36.3	3.7	ns	ns	0.02
Pulses/Voltage	119	151	146	93	19	ns	ns	0.02
Number until ejaculation began	21.8	22.8	23.4	14.3	3.6	ns	ns	ns
Pulses ejaculating	20.8	26.3	23.6	22.0	2.7	ns	ns	ns
Number of vocalizations	29.1	34.8	33.1	23.8	6.1	ns	ns	0.067
HR (beats/min)								
Initial	96.0	93.3	93.3	92.3	2.1	ns	ns	ns
Increase	36.3	50.7	34.3	34.3	9.1	ns	ns	ns
RT (°C)								
Initial	38.1	38.0	38.2	38.2	0.1	ns	ns	ns
Increase	0.40	0.27	0.41	0.24	0.12	ns	ns	ns

Table 2
Sperm characteristics recorded in electroejaculated goat bucks without treatment (control), or that received oxytocin 30 s before the beginning of electroejaculation (OxyT), prostaglandin-F2α 5 min before beginning the EE (PgT), or both treatments (OxPgT). (Mean and pooled SEM).

	Control	OxT	PgT	OxPgT	Pooled SEM
Volume (μL)	1.42	1.21	1.33	1.04	0.19
Sperm concentration (million sperm/mL)	950	1295	1419	1441	336
Total sperm in the ejaculate (million)	1458	1506	1868	1487	493
Sperm mass motility (0-5)	2.1	2.3	2.7	2.6	0.5
Sperm motility (%)	68.3	65.0	65.8	65.7	3.8
Sperm with progressive motility (%)	60.0	56.7	58.3	58.3	4.2
Sperm with functional membrane (%)	70.3	64.3	72.3	69.5	4.9
Sperm with normal morphology (%)	57.3	58.0	51.9	58.1	5.7

There were no significant effects of treatments.

or the repeated application of a single hormone induces responses similar to those reported in our study. The scarce information available on the use of these hormones to stimulate ejaculation is an important limitation.

The positive results obtained with the application of these hormones open other possibilities to shorten the processes of sperm collection. For example, in wild ruminants, which are anesthetized for semen collection [9], the longer the process, the higher the mortality risk. Therefore, testing the inclusion of these hormones in the darts used for anesthetics, researchers may find that the EE process is shortened because the muscles of the genital tract have been sensitized.

Overall, the administration of cloprostenol and oxytocin before EE shortened the procedure and reduced the amount of electric stimulation needed; the vocalizations emitted by the male goats also tended to be reduced. Therefore, it would be important to consider the application of these hormones in semen collection with EE to decrease animal welfare concerns.

Author contributions

RU proposed the initial hypothesis, organized the study, analyzed the data, and wrote the first draft. DC discussed the general study design, collected data, revised and worked on the preparation of the manuscript, and approved the final version. JG organized the experimental procedures, analyzed the semen samples, revised and worked on the preparation of the manuscript, and approved the final version. AFM discussed the general design, collected data, revised and worked on the preparation of the manuscript, and approved the final version. PS organized the experimental procedures and the lab work, analyzed the semen samples, revised and worked on the preparation of the manuscript, and approved the final version. FZB collected data, revised and worked on the preparation of the manuscript, and approved the final version.

Competing interests

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

Acknowledgements

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