Theriogenology 98 (2017) 82–87

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Sperm collection by transrectal ultrasound-guided massage of the accessory sex glands is less stressful than electroejaculation without altering sperm characteristics in conscious goat bucks

Silvia Abril-Sánchez [a, 1], Aline Freitas-de-Melo [b], Florencia Beracochea [a], Juan Pablo Damián [b], Julia Giriboni [a], Julián Santiago-Moreno [c], Rodolfo Ungerfeld [a, *]

[a] Departamento de Fisiología, Facultad de Veterinaria, Universidad de la República, Lasplaces 1550, Montevideo, 11600, Uruguay
[b] Departamento de Biología Molecular y Celular, Facultad de Veterinaria, Universidad de la República, Lasplaces 1550, Montevideo, 11600, Uruguay
[c] Departamento de Reproducción Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain

ARTICLE INFO

Article history:
Received 4 February 2017
Received in revised form
20 April 2017
Accepted 3 May 2017
Available online 4 May 2017

Keywords:
Animal wellbeing
Distress
Ejaculation
Ruminants
Pain

ABSTRACT

In anesthetized non-domestic ruminants transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) is an alternative method to collect semen slightly less stressful than electroejaculation (EE). However, some sperm characteristics are better when semen is collected with EE than with TUMASG. As anesthesia reduces the response to stressors, the advantages of TUMASG may be reduced in anesthetized animals, and thus, TUMASG may be even more advantageous in conscious animals. Therefore, the aim of the present study was to compare the stress response and the characteristics of the sperm collected with TUMASG and EE in conscious goat bucks. Semen was collected in 10 bucks with both procedures. During each procedure, the time required for ejaculation, the number of electric pulses applied and the number of vocalizations were recorded. Rectal temperature, heart rate, serum cortisol concentration, biochemical and hematological parameters were measured before and after each procedure. Sperm characteristics [ejaculated volume, sperm concentration, sperm mass motility (scale 0–5), sperm vigor (scale 0–5), the percentages of motile and progressive motile sperm, of sperm vitality, of sperm with plasma membrane integrity, and with acrosome damage and morphological abnormalities] were also determined. Electroejaculation required more electric pulses than TUMASG ($P < 0.0001$), but TUMASG took more time than EE ($P < 0.0001$). The EE provoked more vocalizations ($P = 0.02$) and a greater increase of cortisol concentrations than TUMASG ($P = 0.04$). Heart rate also tended to be greater with EE than with TUMASG ($P = 0.07$). The sperm characteristics did not differ between TUMASG and EE. In conclusion, TUMASG was less stressful and probably less painful than EE without affecting the semen quality. Thus, although it required more time, TUMASG is an alternative procedure to decrease the welfare concerns raised by EE in conscious goat bucks.

1. Introduction

Electroejaculation (EE) is most common used technique in domestic ruminants for semen collection when artificial vagina cannot be used, i.e.: in untrained animals or outside the breeding season. However, EE is stressful, as it provokes increases in cortisol serum concentrations, rectal temperature (RT), pulse, respiratory and heart rates (HR), as well as hematological and biochemical changes indicative of stress [1–3]. Creatine kinase serum concentration (CK) also increases after EE indicating that there is muscle damage [4]. Some species, as pampas deer males, also vocalize during EE even under general anesthesia [5], suggesting that the procedure is also painful [6]. All those changes indicate that the EE affects negatively animal welfare [7,8].

A recently developed alternative method to collect semen in small ruminants is transrectal ultrasound-guided massage of the accessory sex glands (TUMASG). It has been originally used in anesthetized wild ruminants to substitute EE, and thus avoid its negative effects on animal welfare [9,10]. The use of TUMASG

* Corresponding author.
E-mail address: rungerfeld@gmail.com (R. Ungerfeld).
[1] Universidad de Murcia, Campus de Espinardo (*Campus Mare Nostrum*), 30100 Murcia, Spain.

http://dx.doi.org/10.1016/j.theriogenology.2017.05.006
0093-691X/

requires few or none electrical pulses to achieve ejaculation in anesthetized ibexes, provoking slight lower increases of RT, and cortisol and CK concentrations than EE [10]. However, similar advantages could not be confirmed in other species as mouflons [10]. Nevertheless, as anesthesia reduces the response to stressors, it is possible that differences in favor of the use of TUMASG may be reduced in those animals, and thus, be even greater in conscious animals.

The method of sperm collection may also affect semen characteristics [11–13]. For example, semen collected with transrectal massage of the ampullary region of bulls has less motile and live sperm than that collected with EE [11]. In the same direction, a smaller volume of semen with lower motility is collected with TUMASG than with EE in anesthetized mouflons, and semen with a lower percentage of sperm with progressive motility is ejaculated by anesthetized ibexes [10]. Furthermore, in ibexes subjected to EE high doses of ketamine may reduce erection and protrusion of penis, without affecting sperm quality and quantity [14]. However, we have recently observed that the combination of ketamine plus xylazine before EE improved some sperm characteristics in Gabón bucks (unpublished data).

Considering all these information, the aim of the present study was to compare the stress response and the characteristics of the sperm collected with TUMASG and EE in conscious goat bucks.

2. Material 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 on the Departamento de Fisiología, Facultad de Veterinaria (35°S, Montevideo, Uruguay) during February (summer; breeding season [15]) with 10 Gabon bucks [8–9 years old, 34.8 ± 5.7 kg (mean ± SEM)]. The bucks were housed in a 17 m × 10 m pen and received alfalfa hay and had free access to water. All the animals were electroejaculated several times before.

2.2. General procedures

Semen was collected with TUMASG in five animals and with EE in the other five. One week later, each procedure was applied to the five animals treated with the other procedure the week before (total = 10 collections/technique from the same 10 animals). During each procedure, the time required for ejaculation, the number of electric pulses applied and the number of vocalizations were recorded.

2.3. Transrectal ultrasound-guided massage of the accessory sex glands

Briefly, the bulbourethral glands, the seminal vesicles and the ampulla were observed using real-time transrectal ultrasonography (7.5 MHz linear array probe, Aloka, Tokyo, Japan) as previously described Ungerfeld et al. [16]. Before massage, the penis was protruded and then, a probe coated with carboxymethyl cellulose gel was placed into the rectum. The TUMASG was performed alternating massages between the ampulla of the vas deferens and the bulbourethral gland. Simultaneously, the manual massage was applied on the penile, perineal and pelvic area of the urethra to improve the transport of ejaculatory fluid through the urethra. During TUMASG, the ampulla of the vas deferens was observed by ultrasound scanning, ending when the glands were completely empty. On the cases in which the animal did not ejaculate, electrical stimuli (3 V lasting 5 s) were provided with an electroejaculator (30 cm length, 1.9 cm width, with 3 cm electrodes; model 303, PT electronics, Boring, Oregon, USA) with intermittent breaks for TUMASG.

2.3.1. Electroejaculation

Electroejaculation was performed with the same electroejaculator used in the TUMASG procedure. The penis was manually protruded and then, rectal probe coated with carboxymethyl cellulose was inserted into the rectum, with electrodes positioned ventrally. Electrical pulses were applied during 5 s alternating with rest periods of 2 s. The bucks were stimulated with 10 pulses of 3 V, increasing 1 V in each series of 10 pulses until ejaculation.

2.4. Heart rate and rectal temperature

The HR was recorded by auscultation and RT was measured with a digital thermometer. The HR and RT were determined before and after semen collection as well as 10, 20 and 30 min after TUMASG and EE ended.

2.5. Blood samples

Blood samples were collected from all animals by jugular venipuncture immediately before and after semen collection, as well as 10, 20, 30, 45, 60 and 120 min after TUMASG and EE ended. Samples from all times until 60 min after TUMASG and EE were collected in tubes without anticoagulant to measure serum cortisol concentration. Serum CK, total protein and albumin concentrations were measured in blood samples collected before and immediately after the procedures and 30 and 120 min later.

Blood samples for hematological analysis were collected in tubes with EDTA (Eurotubo, Deltalab, Spain) and samples for plasma glycaemia measurement were collected in tubes with iodoacetate and heparin (Eurotubo, Deltalab, Rubi, Spain) in the same time points than samples for CK, total protein and albumin measurements.

Samples for hematological analysis were maintained at 4 °C for 24 h, when measurements were performed. Samples collected for serum CK, total protein, albumin and cortisol measurements were allowed to clot for 1 h at room temperature. All blood samples collected for extract plasma and serum were centrifuged for 20 min at 1500*g* and they were separated and frozen at −20° C.

2.6. Serum cortisol concentration

Serum cortisol concentration was measured at the Laboratorio de Endocrinología y Metabolismo Animal, Facultad de Veterinaria, Montevideo, Uruguay, using a solid-phase radioimmunoanalysis kit (Cort-Ct2, Cisbio Bioassays, Parc Marcel Boiteux, France). The analytical sensitivity of the assay was 10.4 nmol/L; the intra-assay coefficient of variation was 8.6%.

2.7. Biochemical and hematological analysis

Total protein, albumin and glycaemia concentrations were determined by colorimetry using commercial kits (Bio-Systems, Barcelona, Spain). Serum globulin concentration was calculated by subtracting the serum albumin concentration from the serum total protein concentration [17]. The CK concentration was determined by colorimetry using commercial kits (Wiener Lab, Rosario, Argentina) at the Laboratorio de Análisis Clínicos, Facultad de Veterinaria, Montevideo, Uruguay. All biochemical analyses were assessed using a WienerLab. BT 3000 Plus/CB 350i (Rosario, Argentina) automated chemistry analyzer.