The 4th CZU Prague hybrid seminar
"Biotechnology in small ruminant reproduction: an international experience - 2023"

Semen collection techniques and welfare in small ruminants

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Aim:

- To present recent information on semen collection techniques, welfare problems generated by electroejaculation in ruminants, and treatments/alternative techniques that can reduce these problems and enhance semen collection results.

- Semen collection techniques
- Electroejaculation and wellness
- Alternatives:
  - ✓Anesthesia, analgesia and sedation
  - ✓Alternative techniques
  - ✓Improved effectiveness
- Synthesis

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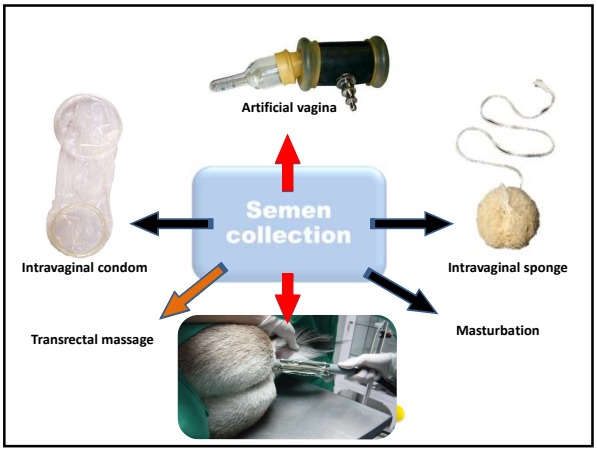

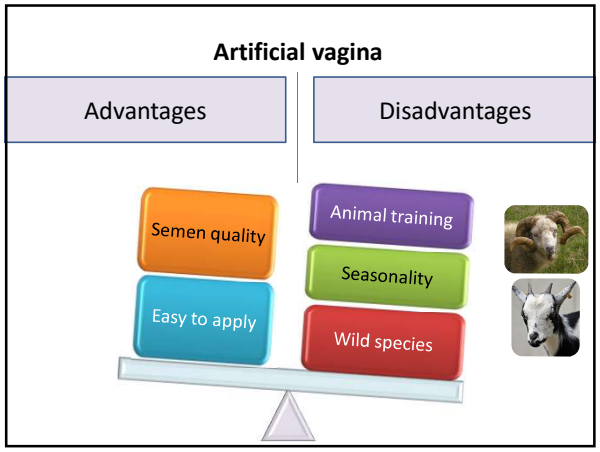

Electroejaculation

Advantages	Disadvantages
No training required	Electrical pulses
Seasonality	Pain
	Stress

- Electroejaculation:
  - ✓Application of electrical stimuli by means of a rectal probe placed over the accessory glands of the male
  - ✓Stimulate sympathetic nerve pathways that determine ejaculation
  - ✓Parasympathetic stimulation causes penile erection and contraction of the pelvic llimb muscles

- Semen collection techniques
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Reproduction in Domestic Animals

Reprod Dom Anim 46, 646–650 (2011); doi: 10.1111/j.1439-0531.2010.01722.x
ISSN 0936-6768

The Stress Response of Frequently Electroejaculated Rams to Electroejaculation: Hormonal, Physiological, Biochemical, Haematological and Behavioural Parameters

JP Damián[1] and R Ungerfeld[2]

EE in domestic ruminants

- Habituated rams

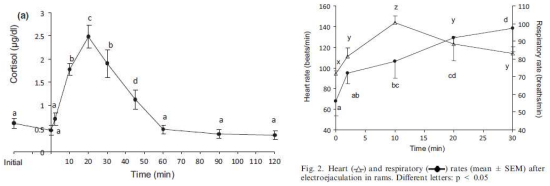

Fig. 2. Heart (○–○) and respiratory (□–□) rates (mean ± SEM) after electroejaculation in rams. Different letters: $p < 0.05$

Courting
Mating
Ejaculation

- Endocrine → ↑ Cortisol
- Bioquemistry → EE causes changes
- Fisiológica → ↑ Heart rate, ↑Rectal temperature
- Hematologic → Hematocrit, Hemoglobin

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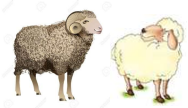
ORIGINAL ARTICLE

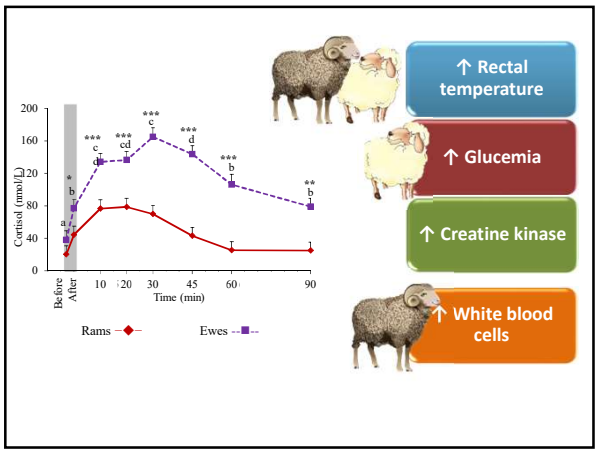
WILEY

Ejaculation does not contribute to the stress response to electroejaculation in sheep

S Abril-Sánchez[1,2] | A Freitas-de-Melo[3] | JP Damián[3] | J Giriboni[1] | A Villagrá-García[4] | R Ungerfeld[1]

- How much depends on EE?
- Objetive: to isolate the effect of ejaculation by comparing the response of rams and ewes





Physiological changes under general anesthesia

Reproduction in Domestic Animals

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ISSN 0936-6768

Physiological and Biochemical Parameters in Response to Electroejaculation in Adult and Yearling Anesthetized Pampas Deer (*Ozotoceros bezoarticus*) Males

F Fumagalli[1,2], M Villagrán[2], JP Damián[3] and R Ungerfeld[2]

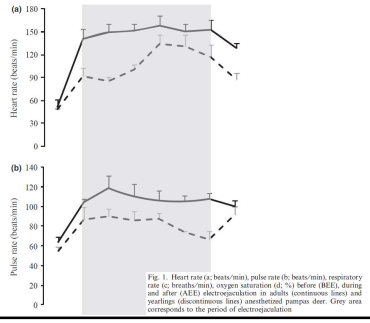

Fig. 1. Heart rate (a; beats/min), pulse rate (b; beats/min), respiratory rate (c; breaths/min), oxygen saturation (d; %) before (BEE), during and after (AEE) electroejaculation in adults (continuous lines) and yearlings (discontinuous lines) anesthetized pampas deer. Grey area corresponds to the period of electroejaculation

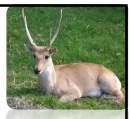

Table 2. Rectal temperature, blood enzymes and cortisol concentration obtained before and after electroejaculation (BEE and AEE) in adult (CA) and yearlings (CJ) anaesthetized pampas deer. The results are presented as mean ± SEM

	CA		CJ		p		
	BEE	AEE	BEE	AEE	Tr	T	Int
RT (°C)	37.5 ± 0.8	36.7 ± 0.7	37.6 ± 0.4	37.1 ± 0.4	NS	0.0001	NS
AP (UI/l)	251 ± 85	323 ± 118	404 ± 85	444 ± 96	<0.05	<0.001	NS
CK (UI/l)	157 ± 125	370 ± 40	303 ± 56	788 ± 251	<0.05	<0.01	NS
AST (UI/l)	71 ± 24	75 ± 12	76 ± 19	99 ± 18	NS	<0.01	NS
Cortisol (µg/dl)	0.21 ± 0.03	0.99 ± 1.01	0.45 ± 0.31	0.87 ± 0.90	NS	NS	NS

RT, rectal temperature; AP, alkaline phosphatase; CK, creatine kinase; AST, aspartate aminotransferase; Tr, effect of categories (adults vs yearlings); T, time effect (BEE vs AEE); Int, interaction between categories and time.

- There was a clear stress response and possibly pain despite the animals being anesthetized

Reproduction in Domestic Animals

Reprod Dom Anim doi: 10.1111/rda.12494
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Vocalizations During Electroejaculation in Anaesthetized Adult and Young Pampas Deer (*Ozotoceros bezoarticus*) Males

F Fumagalli[1], JP Damián[2] and R Ungerfeld[3]

Vocalizations

- Analysis of:
  - ✓Quantity
  - ✓Duration
  - ✓Sonogram characteristics
  - ✓Voltage effects

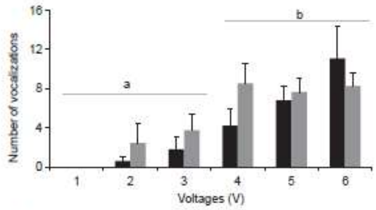
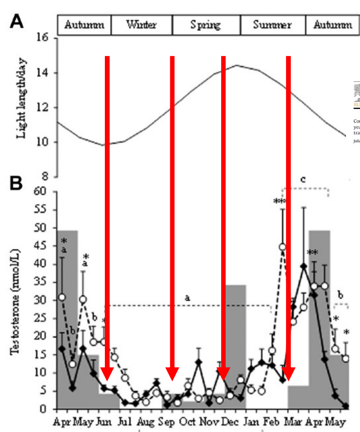


Fig. 1. Number of vocalizations emitted in each voltage used during electroejaculation in adults (black column n = 13) and young (grey column n = 13) anaesthetized pampas deer males. Values are expressed as mean ± SEM. a vs b: p < 0.05

- In most small ruminants there is a seasonal pattern of reproduction
- Differences in testosterone and melatonin secretion
- Both hormones affect the stress responses



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Theriogenology
journal homepage: www.theriojournal.com

Continuous contact with females in estrus throughout the year enhances testicular activity and improves seminal traits of male goats

Julia Giriboni*, Lorena Lacuesta, Rodolfo Ungerfeld

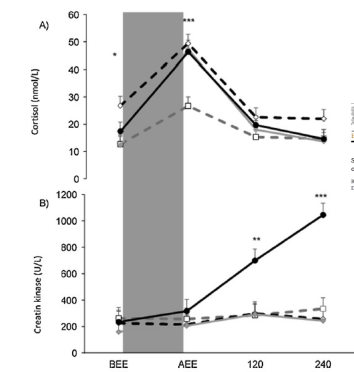

Fig. 1. Cortisol (A) and CK (B) concentrations in adult Gabon bucks after electroejaculations in different seasons (autumn: –□–; winter: –◇–; spring –◆–: summer: –●–) (LSmeans ± SEM); The grey area indicates the period in which electroejaculation procedures were impoased.
BEE: immediately before electroejaculation; AEE: immediately after electroejaculation;
Asterisks indicate that there are differences between groups in a specific moment: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.0001$ (for group to group differences, see the text).

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journal homepage: www.elsevier.com/locate/anireprosci

Seasonality of the stress response in goat bucks when there is use of electroejaculation for semen collection

Rodolfo Ungerfeld *, María Noel Viera, Aline Freitas-de-Melo, Julia Giriboni, Daniela Casuriaga, Patricia Silveira

Repeatability of the response

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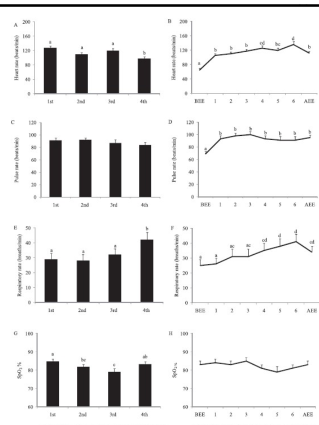
journal homepage: www.elsevier.com/locate/eas

The repetition of semen collection does not affect the physiological and biochemical response to electroejaculation of anesthetized adult and yearling pampas deer (*Ozotoceros bezoarticus*) males

Fernando Fumagalli[a,*,1], Matías Villagrán[b], Rodolfo Ungerfeld[b]



- Semen collection techniques
- Electroejaculation and wellness
- Alternatives:
  - ✓Anesthesia, analgesia and sedation
  - ✓Alternative techniques
  - ✓Improved effectiveness
- Synthesis

- EU requeriments
- Negative effects of anesthesia:
  - ✓Risk to the animals due to anesthesia
  - ✓fasting
  - ✓possible anesthesia-ejaculation and anesthesia-semen interactions
  - ✓post-anesthetic recovery
  - ✓ Aggressiveness of other animals
  - ✓Repetition in semen collection

Anesthetic protocol

- α-2 adrenergic agonists (xylazine, detomidine, medetomidine)
- Comparison of xylacine and medetomidine in rams: no differences
- Comparison of xylazine and detomidine
  - ⇨Xylazine:
    - ✓Lower increase in heart rate
    - ✓Collection of more spz

The administration of sedatives reduces the stress caused by EE

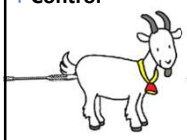
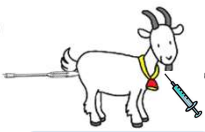
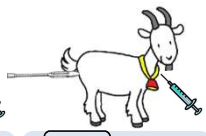
animal

Sedation or anaesthesia decrease the stress response to electroejaculation and improve the quality of the collected semen in goat bucks

S. Abril-Sánchez[†], N. Crosignani[†], A. Freitas-de-Melo[‡], A. Terrazas[§], J. P. Damián[‡], F. Beracochea[†], P. Silveira[†] and R. Ungerfeld[†]

Alternative: sedation

Experimental groups

Control	Sedation	Anesthesia
		
No treatment	0,5 mg/kg ketamine + 0,05 mg/kg xylacine	5 mg/kg ketamine + 0,05 mg/kg xylazine
EE	EE	EE
	0,01 mg/kg yohimbine	0,01 mg/kg yohimbine

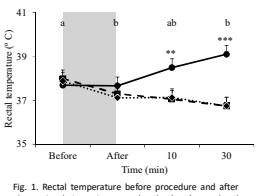
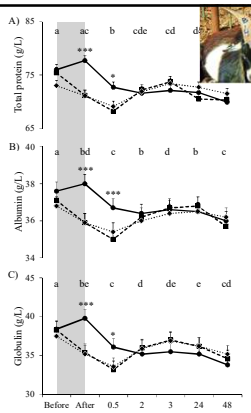


Fig. 1. Rectal temperature before procedure and after electroejaculation in unsedated (–●–), sedated (···◆···) and anesthetized bucks (–■–) (mean ± SEM). Shaded area shows the period in which electroejaculation was performed. Different letters mean significant differences over time (p = 0.006). Asterisks indicate differences between treatments in each time point: **: p < 0.001; ***: P < 0.0001. The RT was greater in unsedated than sedated and anesthetized bucks without differences between them (p = 0.002).



	Control	Anesthesia	Sedation	P
Volume (mL)	0.51 ± 0.08	0.56 ± 0.08	0.46 ± 0.08	ns
Concentration (10^6/mL)	989.2 ± 450.3	1733.2 ± 450.3	2649.3 ± 481.3	0.08
Mass motility (0-5)	1.4 ± 0.4	2.7 ± 0.4	3.0 ± 0.5	0.049
Vigor (0-5)	3.0 ± 0.3	3.4 ± 0.3	3.5 ± 0.3	ns
Total motility total (%)	64.1 ± 5.6	66.0 ± 5.6	67.0 ± 6.0	ns
Progressive motility (%)	59.7 ± 6.10	61.0 ± 6.1	61.7 ± 6.5	ns
Vitality (%)	71.8 ± 4.0	79.3 ± 4.0	73.4 ± 4.2	ns
HOST (%)	55.1 ± 8.1	54.7 ± 8.1	50.2 ± 8.5	ns
Acrosome damage (%)	18.2 ± 5.0	16.0 ± 5.0	16.3 ± 5.0	ns
Morphology (%)	72.1 ± 5.8	74.5 ± 5.9	66.7 ± 5.9	ns

Sedation was more effective than general anesthesia in improving wellbeing animal and semen quality

Vocalizations during EE:

- EE:

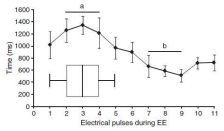
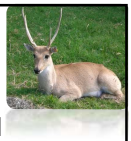


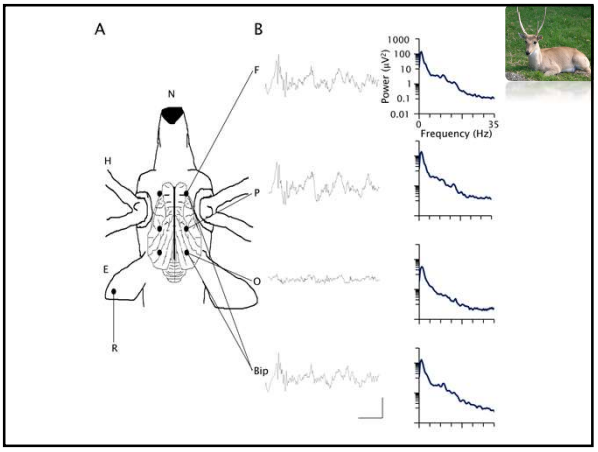
Fig. 3. Duration of vocalizations (mean ± SEM) and the moment in which ejaculation began (box plot inserted) in relation to number of electric pulses during electroejaculation in rams. Different letters: $p < 0.05$

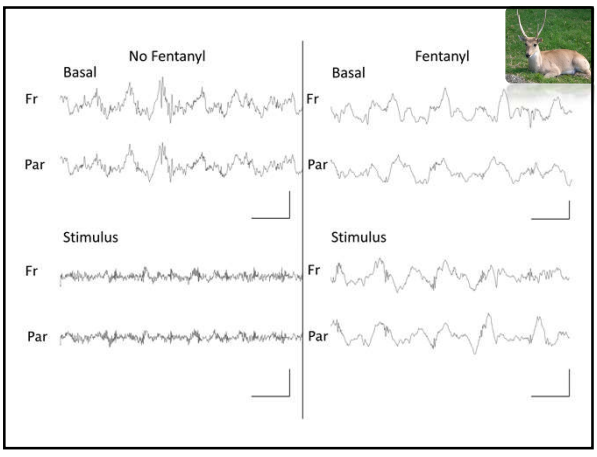
- With epidural anesthesia: no vocalization
- Hypothesis: vocalizations are can be used as pain markers

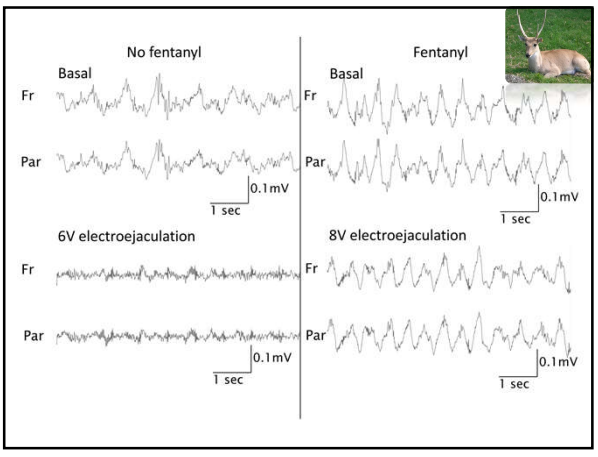
Anesthetic protocol



- Deer vocalized even under general anesthesia
- Alternative: epidural analegesia
- Fentanyl and bupivacaine
- Difficulty in assesing pain:
  - ✓subjetive perception
  - ✓lack of specific markers
  - ✓stress-like responses







Complementary studies

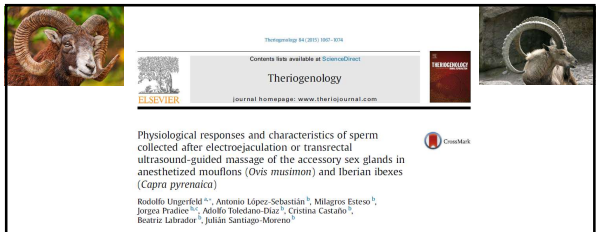
Butorphanol Rams Reduced stress/pain markers

Xylacine vs Detomidine Deer Xylacine better semen

- Semen collection techniques
- Electroejaculation and wellness
- Alternatives:
  - ✓Anesthesia, analgesia and sedation
  - ✓Alternative techniques
  - ✓Improved effectiveness
- Synthesis

Development of alternative techniques

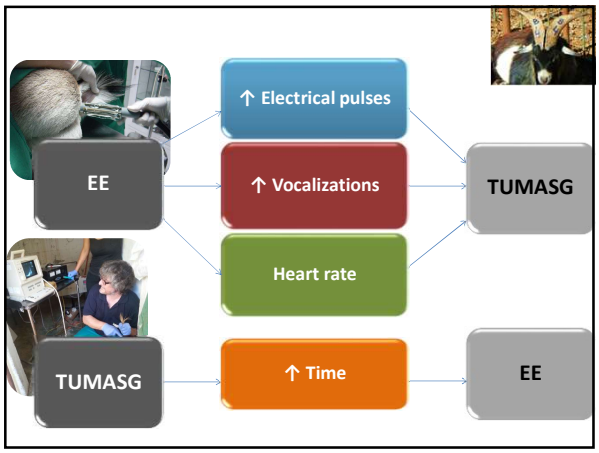
- In bulls, manual massage of accessory glands
- In small ruminants is not posible manual access to the glands
- TUMASG protocol: transrectal ultrasound-guided massage of the accessory glands

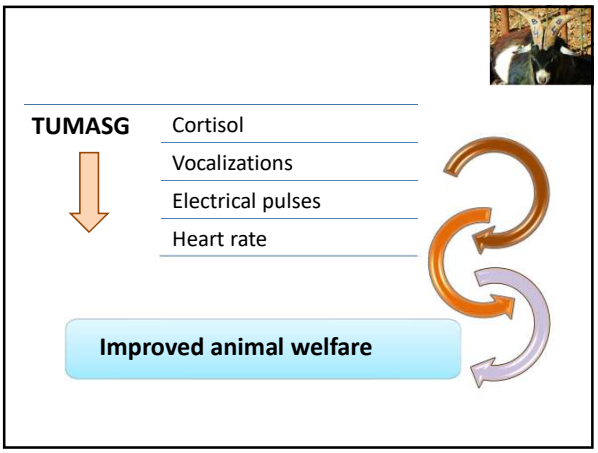


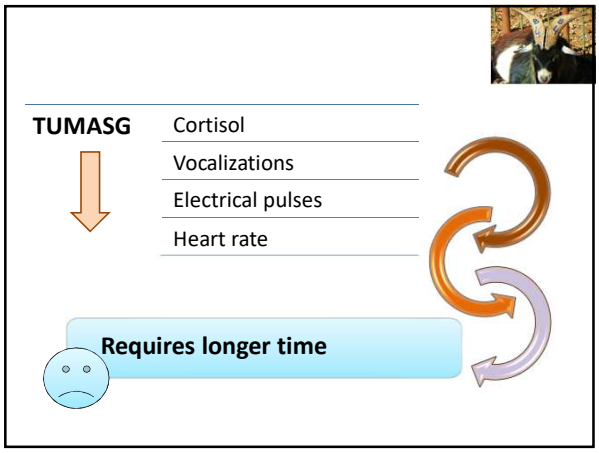
- Aim: to compare physiological responses and semen characteristicis to EE and TUMASG in anesthetized muflón and ibex

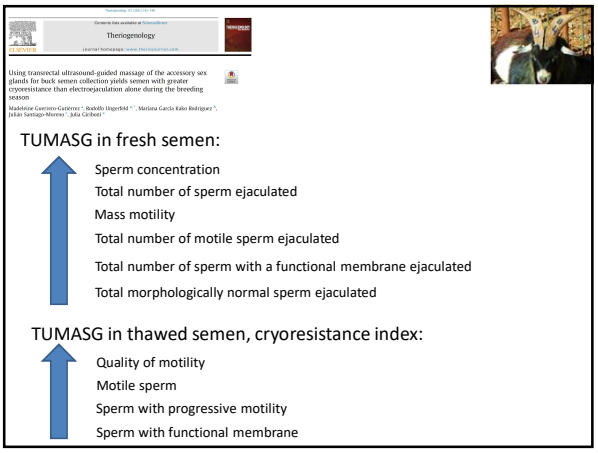
- TUMASG improved some indicators
- Differences between species
- EE: better semen
- TUMASG decreases risks of capture myopathy
- Differences in non-anesthetized animals?

- Aim: to compare physiological responses and semen characteristics to EE and TUMASG in unanesthetized goat bucks









- Semen collection techniques
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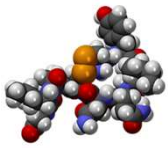
Aim: to determine whether administration of oxytocin before TUMASG improves the procedure and seman quality in anesthetized muflons and non-anesthetized bucks.

Oxytocin + TUMASG

Shorten the time required for the procedure

Decreases the number of electrical pulses

Improves semen quality



Oxytocin + TUMASG

- ↓Electrical pulses
- ↓Time
- No semen affected

Oxytocin + TUMASG

- ↓Electrical pulses
- ↓Time
- No semen affected

Oxytocin + TUMASG

- ↓Electrical pulses
- ↓Time
- No semen affected

Increased pulse variability

Inter-specific differences

(Pradiee et al., 2016)

No pulses required to ejaculate

Similar effect with carbetocin:

Other hormonal treatments:

Oxytocin + PGF2alpha:

- EE time
- Number of pulses
- Total sum of voltages applied
- Number of pulses until ejaculation began
- Vocalizations

Oxytocin + PGF2Alpha with or without GnRH:

GnRH not needed for reduction of:

- Cortisol
- CK

Other hormonal treatments:

Carbetocin (long-acting oxytocin) with TUMASG:

- Tended EE time

Carbetocin (long-acting oxytocin) with EE:

- Time
- Electrical pulses
- Voltages applied
- Tended vocalizations

Table 3
Characteristics of fresh semen collected by electroejaculation in rams treated with carbetocin 20 min (CB-20) or 10 min (CB-10) before electroejaculation or without treatment (CON). (LSmean ± pooled SEM). The P value indicates the mean effect of the treatments.

	CB-20	CB-10	CON	SEM	P
Volume of the ejaculate (mL)*	4.8 ^{ab}	5.6 ^{a}	3.4 ^{b}	0.9	0.02
Semen concentration (x10^6)	4412 ^{a}	4210 ^{a}	2461 ^{b}	553	0.02
Sperm mass motility (0–5)	3.2	3.7	2.5	0.4	0.07
Total number of ejaculated sperm x10^6	20176 ^{a}	21324 ^{a}	10113 ^{b}	4387	0.01
Number of insemination doses	135 ^{a}	142 ^{a}	67 ^{b}	29.2	0.01
Number of insemination doses per electrical pulses administered	1.9 ^{a}	2.0 ^{a}	1.1 ^{b}	0.4	0.05
Percentage					
Motile sperm	80.3	69.1	81.2	4.9	ns
Sperm with progressive motility	56.3	52.8	59.1	4.5	ns
Sperm with functional membrane	71.1 ^{a}	61.2 ^{ab}	54.3 ^{b}	4.9	0.02
Sperm without bent tails	90.0	88.2	87.4	1.3	ns
Total number sperm x10^6					
Motile sperm	15603 ^{a}	15141 ^{a}	7293 ^{b}	3003	0.01
Sperm with progressive motility	10884 ^{a}	10964 ^{a}	5130 ^{b}	2013	0.01
Sperm with functional membrane	14976 ^{a}	12810 ^{a}	5959 ^{b}	2922	0.003
Sperm without bent tails	16163 ^{a}	13879 ^{a}	9063 ^{b}	448	0.01

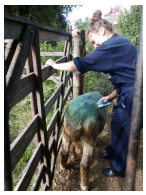
^{ab} Different literals in the same row indicate statistically significant differences ($P \leq 0.05$).
* Indicates tendencies for differences ($P \leq 0.1$); CB-20 vs CON: $P = 0.08$.
ns: not significant.

Positive reinforcements

- Brushing produces positive effects in rams, facilitating animal-human interactions
- It triggers releases of oxytocin and dopamin
- Dopamin binds to sperm receptors and promote motility


Applied Animal Behaviour Science

Rams recognize and prefer the human who regularly brushed them




Animal Reproduction Science

Brushing rams before and during electroejaculation improves sperm motility and kinetics with slight changes in stress biomarkers

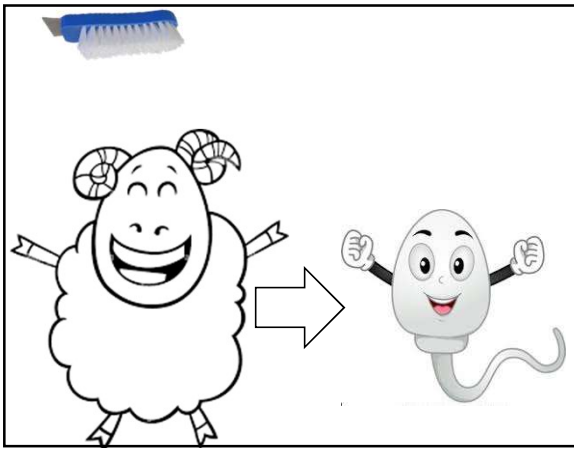
- Aim: to determine whether brushing rams before and during EE reduces their stress response and improves the characteristics of the ejaculate.

Brushing tended to:

- ↑ Time
- ↑ Electrical pulses
- ↑ Voltages applied
- ↓ Head movements during collection

Brushing increased:

- ↑ Sperm concentration
- ↑ Mass motility
- ↑ Curvilinear velocity
- ↑ Linear velocity
- ↑ Average path velocity



- Semen collection techniques
- Electroejaculation and wellness
- Alternatives:
  - ✓Anesthesia, analgesia and sedation
  - ✓Alternative techniques
  - ✓Improved effectiveness
- Synthesis

Synthesis

- EE is a very simple technique to apply, but provoques stress and pain.
- There are tools to minimize it, and/or alternatives:
  - ⇨Use of analgesia
  - ⇨Alternative techniques
  - ⇨Treatments to improve its´effectiveness
- A reference technique is not yet available
- There are many alternatives that reduce pain and stress, and others that improve seminal quality, which can be incorporated into the routing without apparent dissadvantages



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- ¿?