Animal Reproduction Science 193 (2018) 90–97

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Modeling of behavioral responses for successful selection of easy-to-train rams for semen collection with an artificial vagina

Claudia Pamela Ambrosi[a], Natalia Rubio[b], Gustavo Giménez[b], Andrés Venturino[a], Eduardo Gabriel Aisen[a], María Fernanda López Armengol[a,*]

[a] *Centro de Investigaciones en Toxicología Ambiental y Agrobiotecnología del Comahue (CITAAC), Instituto de Biotecnología Agropecuaria del Comahue (IBAC), Facultad de Ciencias Agrarias, Universidad Nacional del Comahue y CONICET. Ruta 151, km 12, (8303) Cinco Saltos, Río Negro, Argentina*

[b] *Departamento de Estadística, Facultad de Economía y Administración, Universidad Nacional del Comahue, Buenos Aires 1400, (8300) Neuquén, Argentina*

A R T I C L E I N F O

Keywords:
Rams
Behavioral events
Training
Semen collection

A B S T R A C T

The aim of this study was to analyze the reproductive behavioral responses in Australian Merino rams, to identify those related to a faster training for semen collection with an artificial vagina. Eight Australian Merino rams, aged 1.5 years and with no prior sexual experience, were randomly selected from an extensively grazed flock. One immobilized ewe with no hormone stimulation was used for rams to sexually interact and mount. The frequencies of approaching, sniffing, flehmen, pushing, pawing with chin resting, and tongue flicking were recorded during eight training and three post-training assessments periods. In addition, the duration of sniffing and flehmen responses, as well as the time from when the ram started to approach the ewe until the mount with ejaculation (completed mount) were recorded. Descriptive, correlation, and modeling analyses were performed. Amongst the rams, four mounted the ewe and ejaculated for the first time during the training phase, and three mounted and ejaculated for the first time after the training phase. The remaining ram mounted the ewe and ejaculated for the first time during the post-training evaluation in the following year. A great variability in the behavior repertoire was observed among rams. The correlation analysis indicated that the completed mount was associated with the behaviors during the approaching response. The expression of the sniffing response decreased between the training phase and post-training evaluation, while the responses of pushing the ewe and tongue flicking ceased to occur. Pawing the side of the ewe with the chin resting on the back of the ewe and flehmen responses, however, continued between the training and post-training phases. This led to a decrease in the time from when the ram started to approach the ewe until the completed mount. It is concluded that the responses of approaching the ewe, pawing the side of the ewe with chin resting on the ewe, and sniffing of the ewe (the latter occurring only during the training phase) are behavioral indicators that could be used for selection of easy-to-train rams for purposes of semen collection with an artificial vagina.

1. Introduction

The use of artificial vagina (AV) for semen collection results in greater quality semen samples, compared with semen obtained by

* Corresponding author at: Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, Ruta 151, CC N° 85, (8303) Cinco Saltos, Río Negro, Argentina.
E-mail address: m.lopezarmengol@conicet.gov.ar (M.F. López Armengol).

https://doi.org/10.1016/j.anireprosci.2018.04.003
Received 31 August 2017; Received in revised form 30 March 2018; Accepted 5 April 2018
Available online 07 April 2018
0378-4320/

electroejaculation. Semen characteristics such as volume, concentration, live sperm and sperm motility are generally affected (Matthews et al., 2003; Nikolovski et al., 2012; Malejane et al., 2014). The use of the AV for semen collection also has advantages with respect to animal welfare (Ortiz-de-Montellano et al., 2007; Alomar et al., 2016). Semen collection with the AV, however, requires previous training of the males, which includes habituation to the presence of operators and the use of the AV.

For training and semen collection, different approaches for ram interactions and mounting can be used: ewes in estrus, hormone-treated ewes, ewes not in estrus, and manual constructed devices (*i.e.*, "dummies"). Because of the sexual seasonality of the sheep and the negative effects of continuous hormonal treatment in the ewes, the recommended option for achieving continuous semen collection is training with ewes that are not in estrus (Aguirre Flores et al., 2005).

Rams express a series of sexual behavioral responses during natural mating as well as in semen collection with an AV. These responses may be categorized into two groups of responses: one, where the male identifies the physiological status of the ewe by sniffing the genital area and sensing its urine; and another, where the male assesses the receptivity of the female through tactile stimulation (Orihuela Trujillo, 2014). For the identification of physiological status, rams smell the genitals and urine of the ewe, after which the flehmen response indicates the passage of pheromone into the vomeronasal organ for sensing (Lindsay, 1965; Ungerfeld et al., 2006). The receptivity of the female is ascertained by ram assessment of the immobilization state of the ewe by licking of the genital area, pushing of the ewe, pawing the side of the ewe, and chin resting on the ewe (Cárdenas-Gallegos et al., 2015).

Sexual behavioral responses have been studied for the identification of mating competence such as amount of libido, sexual performance (Pepelko and Clegg, 1965; Katz, 2008; Haulenbeek, 2009; Gouletsou and Fthenakis, 2010), serving capacity (Price et al., 1992; Cárdenas-Gallegos et al., 2015), and sexual partner preference (Roselli et al., 2002). There, however, are no detailed studies about the sexual behavioral responses indicating the relative ease of rams training for semen collection with an AV. Abramson and Kieson (2016) described different conditioning techniques that could help understand the learning responses in livestock. There are two categories of learning: associative and non-associative, the latter including habituation as a response to repeated stimuli. Hence, the animal modifies an already existing behavior because of the stimuli, instead of learning a new response. From these previous descriptions, ram training for use of an AV for semen collection would encompass habituation with non-associative learning.

The aims of this study were: (1) to analyze the reproductive behavioral responses of Australian Merino rams during and after the training for semen collection with an AV, (2) to assess by statistical analysis the association of the various responses with the mounting of a ewe; and (3) to ascertain those responses that would lead to selection of easy-to-train rams for semen collection with an AV (short-term training of rams).

2. Materials and methods

2.1. Animal handling

Eight Australian Merino rams, aged 1.5 years, were randomly selected from an extensively grazed flock in Paraje La Angostura (40°25′S, 69°34′W, Río Negro, Argentina). These rams had been separated from ewes as lambs, after weaning from their dams and had no sexual experience prior to the experiment. The animals were transported to the facilities of the Facultad de Ciencias Agrarias (FCA), Universidad Nacional del Comahue (38°51′S, 68°04′W, Río Negro, Argentina). The experiment was conducted complying with the animal welfare conditions according to the institutional policy. The rams were housed in two pens with access to the natural environment and had free access lucerne pellets (*Medicago sativa*) and water.

The experiment started 1 week after the rams were transported to the FCA, and consisted of three phases: taming, training phase (T), and post-training phase (PT). The taming consisted of the habituation of rams to the presence of people, handling with a rope, and approaching the ewe that was used to conduct the study. The taming phase lasted for 41 days from 30 January to 12 March 2014 and was immediately followed by the T phase. The aim of the T phase was the training of the rams for semen collection with an AV by two operators, and started before the beginning of the mating period performed by farmers from Northern Patagonia. It lasted from 13 March to 16 April 2014, and consisted of eight assessment periods of 10 min each (two per week), in which one operator moved the ram using a 3.5 m rope to let it approach the immobilized ewe. During the first four assessments periods, a second operator approached with the AV only when a mounting attempt was observed, to avoid distracting the rams. At this time, the ram was briefly restrained by using the rope to avoid natural mating. The PT phase was conducted to evaluate the changes in the sexual behavioral responses after the rams had been trained for collection of semen with an AV, and consisted of three assessments periods between 5 February and 17 March 2015, also before the beginning of the mating period in Northern Patagonia.

Between the T and PT phases, the routine with the rams continued once per week to avoid the loss of habituation to use of the AV for semen collection. Every time the rams completed mounting (CM), they received a small portion of oat seeds (*Avena sativa*) as a stimulatory experience to facilitate conditioning and to reinforce the habituation to the semen collection method. During the entire experimental period, only one ewe was used for the semen collection regimen, and the ewe was not treated with hormones.

2.2. Sexual behavioral data

During the T and PT phases, the same researcher assessed the ram activities and reported them on a voice recorder. The behavioral responses analyzed in this experiment were: approaching to the ewe (A), sniffing the ewe (S), flehmen expression (F), pushing the ewe (Pu), pawing the side of the ewe with chin resting on it (PaCR), tongue flicking (TF), mount attempts (MA = mount without intromission), and completed mount (CM = mount with ejaculation: Bernon and Shrestha, 1984; Odagiri et al., 1995; Darwish and Mahboub, 2011; Morales-Piñeyrúa and Ungerfeld, 2012; Orihuela Trujillo, 2014). Consistent with the findings of Odagiri et al.

(1995), PaCR was considered a single behavioral response. Duration of sniffing (tS), duration of flehmen (tF), and the total time from the approaching to the completed mount, termed reaction time (RT) were also recorded (Chenoweth, 1981; Bernon and Shrestha, 1984; Fahey et al., 2012; Orihuela Trujillo, 2014). The RT was analyzed only in rams that mounted the ewe during the T phase.

2.3. Statistical analyses

2.3.1. Analysis of sexual behavioral responses

Correlation analyses between the behavioral responses and CM during the T and PT phases were performed using the Pearson's coefficient *r*. The probability value that was considered to be statistically significant was $P = 0.05$.

The RT was analyzed by means of one-way ANOVA followed by use of the Fisher least-squares difference *post-hoc* test, with a probability value of $P = 0.05$ indicating significance. For each ram that responded with a CM in both phases of the study (Tag numbers 1, 5, 6, and 8) the average values of the two first assessment periods in which CM (Ti) occurred and the average of the two last assessment periods of the T phase (Tf) were analyzed. For the PT phase, the observational findings from the first and the last periods of assessment were analyzed (PTi and PTf, respectively).

2.3.2. Completed mount modeling

2.3.2.1. Generalized linear model (GLM). The response variable CM was modeled using logistic regression because this variable had a binomial distribution (Agresti, 2010). The modeling was initiated using a saturated model that included the following explanatory variables: S, F, PaCR, and date of assessment periods (D), and the 2nd, 3rd, and 4th-degree interactions among variables to achieve a parsimonious model.

For the binary response variable *Y* describing the CM, and an explanatory variable X (*e.g.*, S, F, PaCR), the probability model was

$$\pi(x) = P(Y = 1|X = x) = 1 - P(Y = 0|X = x)$$

with Y = presence or absence of CM.

The logistic regression model was:

$$\pi(x) = \frac{\exp(\alpha + \beta x)}{1 + \exp(\alpha + \beta x)}$$

The model for the log odds (logit) followed a linear relationship:

$$logit\,[\pi(x)] = \log\frac{\pi(\mathrm{x})}{1-\pi(x)} = x_i'\beta$$

This equates to the logit link function of the linear predictor.

x_i = characterizes the systematic part of the design, where *i* is the number of fixed effects of the model.

β = is the parameter vector of the fixed effects of the model that completes the systematic part of the model. Because the observations were made on the same rams, it was necessary to consider the individual ram effect, for which the representation of the confidence intervals for each ram was analyzed. If this effect was present, a generalized linear mixed model was applied as indicated below.

2.3.2.2. Generalized linear mixed model (GLMM). For the GLMM, y_{it} denoted any observation *t* in cluster *i* (ram), $t = 1, \ldots, T_i$; x_{it} denoted the column vector of values of explanatory (S, F, PaCR, D) variables for the fixed effect model parameters β; u_i denoted the vector of random effect values for cluster *i*; and *z* denoted a column vector of explanatory variables.

The linear predictor for a GLMM is described by:

$$g(\mu_{it}) = x_{it}'\beta + z_{it}'u_i$$

For link function g (μ_{it}), the random effect vector u_i was assumed to have a multivariate normal distribution N (0, Σ). The covariance matrix Σ depended on unknown variance components, and possibly also on correlation parameters (Agresti, 2010). The statistical analyses were performed with statistical language R version 3.3.0. The libraries used were: *anova*, *stats*, *effects*, and *lme4* (R CoreTeam, 2016).

3. Results

Training of rams for semen collection with AV could be successfully achieved, although the CM occurred at varying times during this experiment. There was CM in four rams during the T phase, in the following order: Tag No. 5, 8, 1, and 6 (Fig. 1a–d). The other three rams expressed the CM after the T phase concluded and prior to the PT phase, in November 2014 (Tag No. 2 and 3; Fig. 1e, g) and January 2015 (Tag No. 4; Fig. 1f). The remaining ram (Tag No. 7; Fig. 1h) had a CM after the experiment concluded.

Fig. 1 depicts the absolute frequency of S, F, and PaCR for the eight rams during the T and PT phases. Note that, independent of date of first CM, some rams had great variability in the repertoire of sexual behaviors (Tag No. 5, 1, 6, 2, and 4; Fig. 1a, c–f), and others did not (Tag No. 8, 3, and 7; Fig. 1b, g, and h).

Rams that had a CM during the T phase had less S during the PT phase compared with the T phase. Throughout the experiment, there was continual expression of PaCR and the incidence of this response did not vary among the periods of assessments. Not all the

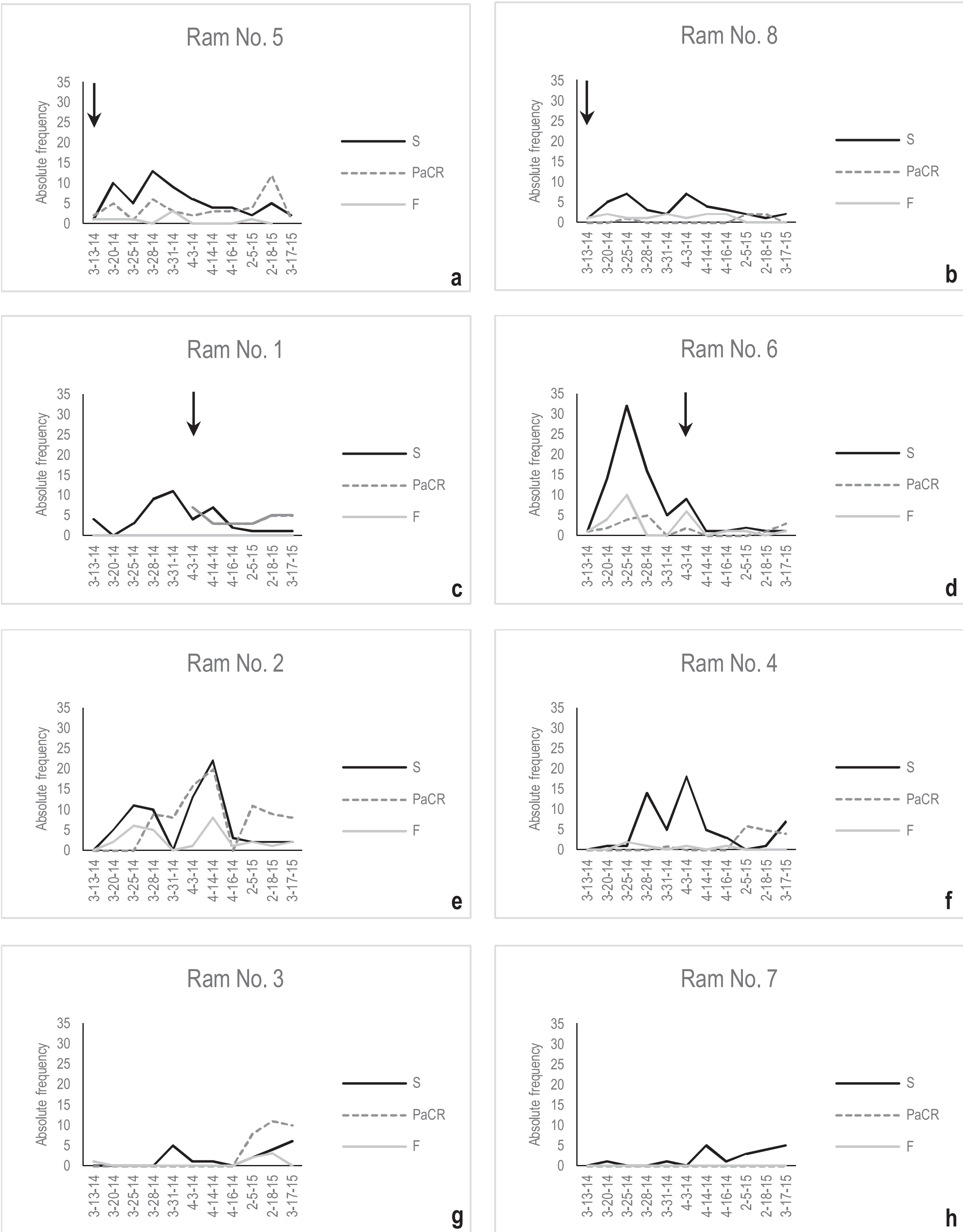

Fig. 1. Frequency of behavioral responses of rams during the training and post-training phases. Fig. a–h are ordered chronologically, according to the date of first completed mount. Rams that completed mounting for the first time during the training phase (Tag No. 5, 8, 1, and 6; a–d). Rams that completed mounting for the first time between the training and post-training phases (Tag No. 2, 4, and 3; e–g). Ram that completed mounting for the first time after the conclusion of the experiment (Tag No. 7, h). S: sniffing; F: flehmen; PaCR: pawing the side of the ewe with chin resting on the ewe. Arrows indicate the exact date of first completed mounting.

Table 1
Relationship among approaching, mount attempt, and completed mount.

Approaching (A)	Mount attempt (MA)	Completed mount (CM)	T (%)	PT (%)
–	–	–	50	12
–	+	–	6	0
+	–	–	2	0
+	+	–	8	0
+	+	+	23	25
+	–	+	11	63

– = absence; + = presence; T = training phase; PT = post training phase; % = percentage of assessments periods.

rams expressed a F response, and when this response occurred, it was at a low frequency compared with S and PaCR, and there was little variation in the incidence of F between the T and PT phases (Fig. 1).

During the T phase, TF was observed only in two rams (Tag No. 3 and 5), and Pu in one ram (Tag No. 5), with there being neither of these responses during the PT phase. Thus, neither of these variables was included in the statistical analyses.

There were different combinations of the patterns (presence/absence) of the behavioral responses A, MA, and CM during the T and PT phases, and in the percentage of assessment periods in which such combinations occurred. During the T phase, the combinations of the behavioral responses, A/MA, A/MA/CM, or A/CM occurred in 42% of the assessments periods. In 50% of the assessments periods during the T phase, A, MA, and CM did not occur. There were exceptions to the direct relationship between A and MA or CM, where in 6% of the assessments periods rams had a MA response when there was not an A response. Also, there was an A response during 2% of the assessment periods when there was neither a MA nor a CM response. It was noted that CM was always preceded by A.

Comparing responses during the T and PT phases (Table 1), responses of A and CM increased markedly, either with or without a MA response occurring (from 23% to 25% and from 11% to 63%, respectively). The assessment periods in which A, MA, and CM were absent, decreased markedly (from 50% to 12%), and furthermore, during the PT phase the exceptions mentioned above did not occur.

The pattern was analyzed for changes of the reaction time (RT) in rams that achieved CM in both phases (Tag No. 1, 5, 6 and 8; Fig. 2). At the beginning of the T phase (Ti), the average RT was 209 s, whereas at the end (Tf), it was significantly reduced (*i.e.*, 79.5 s; $P = 0.00017$). The average RT during the PT phase was 38.5 s at the beginning (PTi) and 44.75 s at the end (PTf; $P = 0.69$). When comparing Tf with PTi, there was a significant reduction in the RT ($P = 0.01$).

There was great variability in tS and tF without a discernible trend in the changes of these responses (data not shown). The correlations among behavioral responses during the T phase (Table 2a) and the PT phase (Table 2b) were statistically assessed. During the T phase, the behavioral responses that were correlated with CM were: A (0.82) and tF (0.34) (Table 2a). During the PT phase, A (1.00) and PaCR (0.47) were correlated with CM (Table 2b). Furthermore, S was positively correlated with F, PaCR, tS, and tF during the T phase, whereas there were no significant correlations between these variables during the PT phase.

3.1. CM analysis with GLM

With the first modeling (saturated), 2nd, 3rd, and 4th-degree interactions were not significant. When analyzing the explanatory variables, only S and PaCR were significant for CM. The estimate of the S coefficient was negative (−0.128), whereas the estimated coefficient for PaCR was positive (0.213) (Table 3a).

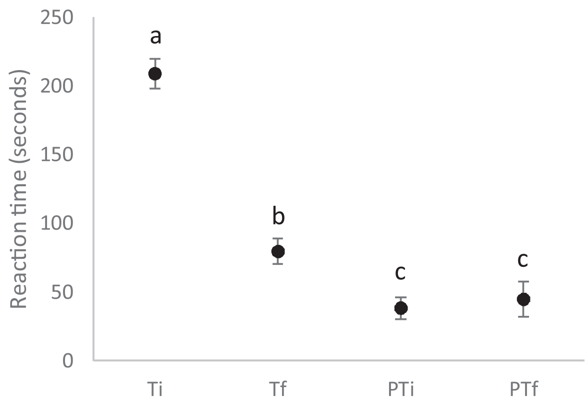

Fig. 2. Reaction time (*i.e.*, time from when rams started to approach the ewe until CM) for four rams (Tag No. 1, 5, 6, and 8) during the training and post-training phases. Mean and SEM values are shown for the data. Different letters indicate differences ($P \leq 0.05$) as analyzed using the one-way ANOVA test and Fisher least-squares difference *post hoc* test. Ti = mean initial reaction time of the training phase; Tf = mean final reaction time of the training phase; PTi = initial reaction time for the post-training phase; PTf = final reaction time for the post-training phase.

Table 2
Correlations among behavioral responses, duration of sniffing and flehmen, and completed mount during training and post-training phases.

A. Training phase

	Approaching	Sniffing	Flehmen	Pawing-Chin resting	Duration of sniffing	Duration of flehmen
Sniffing	0.22					
Flehmen	0.16	0.68*				
Pawing-Chin resting	0.05	0.51*	0.40*			
Duration of sniffing	0.08	0.45*	0.37*	0.18		
Duration of flehmen	0.34*	0.33*	0.53*	0.09	0.31*	
Completed mount	0.82*	-0.03	0.02	0.04	0.10	0.34*

B. Post-training phase

	Approaching	Sniffing	Flehmen	Pawing-Chin resting	Duration of sniffing	Duration of flehmen
Sniffing	−0.33					
Flehmen	0.25	−0.03				
Pawing-Chin resting	0.47*	0.19	0.53*			
Duration of sniffing	0.13	−0.15	0.52*	0.18		
Duration of flehmen	0.25	−0.09	0.87*	0.42*	0.40	
Completed mount	1.00*	−0.33	0.25	0.47*	0.13	0.25

* $P < 0.05$.

The GLM formula explaining the CM variable for the rams was:
Glm (formula = CM ~ −1 + S + PaCR, family = binominal, data = base)

$$logit\,[\pi(x)] = -0.1280 \quad * \text{S} + 0.2130 * \text{PaCR}$$

3.2. CM analysis with GLMM

There was a large amount of variability among rams with the GLMM calculated being:
Specified model in R: CM ~ −1 + S + PaCR + (1 | ram)
Thus, the estimation of the model was performed (Table 3c) as:

$$logit\,[\pi(x)] = -0.5192 \; * \text{S} + 0.8715 \; * \text{PaCR}$$

The comparison of the Akaike Information Criterion (AIC, Sakamoto et al., 1986) for both models conclusively indicated the best fit for the GLMM, validating that the random effects by rams were highly significant (Table 3b). When the explanatory variables were analyzed, S and PaCR were again the only significant variables for the model. As for the previous case, there was a negative coefficient for S, whereas for PaCR there was a positive coefficient (Table 3c). Moreover, according to the model, CM was always associated with PaCR expression.

Table 3
Modelling completed mount as response variable.

A. Generalized linear model

	Estimate	Std. Error	z value	Pr(> |z|)
S	−0.1280	0.0507	−2.523	0.0116
PaCR	0.2130	0.0734	2.903	0.0037

B. Likelihood test

	DF	AIC	BIC	logLik deviance	Chisq Chi	Pr(> Chisq)
CM ~ −1 + S + PaCR	2	113.441	118.40	−54.721	109.441	
CM ~ −1 + S + PaCR + (1 | ram)	3	63.028	70.46	−28.514	52.413	$4.5E^{-13}$

C. Generalized Linear Mixed Model

	Estimate	Std. Error	z value	Pr(> |z|)
S	−0.5192	0.1592	−3.262	0.00111
PaCR	0.8715	0.2657	3.280	0.00104

Abbreviations: CM = Completed mount, S = Sniffing, PaCR = Pawing-Chin resting.

4. Discussion

Amongst all rams, 50% had a CM during the training phase (50% success), whereas the rest of the rams had a CM later in the experimental period. A plausible explanation for this would be the adapted social interactions among rams (Synnott and Fulkerson, 1984; Lindsay et al., 1976; Vázquez et al., 2012), and the fear of the novel environment and presence of humans (Romeyer and Bouissou, 1992; Lankin, 1997).

According to some previous reports (Orihuela Trujillo, 2014; Bernon and Shrestha, 1984), there is great overall variability in behavior among rams, regarding the response to the training process and the presence and frequency of sexual behavioral responses. Some of these behavioral responses, such as pushing and tongue flicking occurred at very low frequencies and there were very few rams expressing these behaviors, whereas other responses were much more common among rams (Fig. 1).

In the present study, the approaching response was a sexual behavior closely related to CM from the beginning of the T phase for the rest of the experiment. Rams that did not have a CM during the T phase also failed to approach the ewe spontaneously. Among these, 6% of the times, the rams attempted to mount after being manually manipulated towards the ewe.

The lack of approaching of ewes by rams could be explained by the fact that these rams were still habituating to the novel environment. In addition, the reluctance to approach the ewe could have been affected by social interactions among males (Synnott and Fulkerson, 1984; Lindsay et al., 1976) or by the fear of the presence of humans (Lankin, 1997; Romeyer and Bouissou, 1992).

The amount of training of the rams did not alter the presence of F, the duration of F, or the duration of S. These findings are consistent with those of Odagiri et al. (1995), where F and S were thought to have a secondary and independent role in mating behavior, and not be exclusively limited to the detection of estrus. Although there was a great amount of S at the beginning of the T phase, it decreased gradually among the assessment periods of the experiment in all rams. Thus, there was a positive correlation of the S response with F and PaCR during the T phase (Table 2a), and there was no correlation with these behavioral responses during the latter part of the study (Table 2b). This resulted in a negative correlation being determined with the model for S as related to the CM during the study (Table 3).

Considering the correlations and model used to assess results in the present experiment, pawing the side of the ewe with chin resting was a reliable indicator for the CM. These results are consistent with those of Price et al. (1992) when studying serving capacity in rams.

After the training phase, rams no longer expressed many of the sexual behaviors that they had earlier in the study for detection of estrus in the ewe. Instead, they responded by assessment of the immobilization response of the ewe. This would imply there was a decrease in the RT from when rams started to approach the ewe until the time of CM. Although the present study was conducted under different conditions (training), Orihuela Trujillo (2014) described similar responses during intensive breeding. Although the rams had different manifestations of the behavioral repertoire during the present experiment, results allow for the conclusion that there is a reduction in the variety and frequency of the behavioral responses between the phases of the study. As a consequence the RT decreased.

From the present results, training of rams for semen collection with an AV is thought to be a habituation which is a type of non-associative learning (Abramson and Kieson, 2016), and thus, if the stimulus (ejaculation plus reward) is repeated frequently, young rams could be trained for semen collection with an AV.

In conclusion, all young rams could be successfully trained for semen collection with an AV. The duration of the training is dependent on the individual response to the training process. Furthermore, the training does not necessarily require hormone-treated ewes for the rams to be habituated for the use of an AV for semen collection. The training process will occur in a shorter time period if rams approach the ewe voluntarily in human presence, and even more so in those that additionally paw the side of the ewe, rest their chin on her back and sniff the ewe. During the training period, rams easily adapt to use of the AV for semen collection, and as they habituate to the procedure, the sexual behavior repertoire preceding the CM decreases and ejaculation occurs in a shorter time period from when the ram is placed in the presence of the ewe.

Conflict of interest

The authors have not declared any conflict of interest.

Acknowledgements

This research was supported by the Universidad Nacional del Comahue [grant 04/A 126 from].

References

Abramson, C.I., Kieson, E., 2016. Conditioning methods for animals in agriculture: a review. Cienc. Anim. Bras. 17, 359–375.

Aguirre Flores, V., Rosales, R., Orihuela Trujillo, A., 2005. Entrenamiento de carneros para recolección de semen mediante vagina artificial, utilizando como estímulo objetos inanimados. Vet. Mex. 36, 105–111.

Agresti, A., 2010. Analysis of Ordinal Categorical Data, vol. 656 John Wiley & Sons Inc., New York, USA.

Alomar, M., Soukouti, A., Alden Alzoabi, M., Zarkawi, M., 2016. Testosterone and cortisol patterns and the effects of electro-ejaculation and copulation in Awassi rams. Arch. Anim. Breed. 59, 139–144.

Bernon, D.E., Shrestha, J.N., 1984. Sexual activity patterns in rams. Can. J. Comp. Med. 48, 42.

Cárdenas-Gallegos, M.A., Aké-López, J.R., Magaña-Monforte, J.G., Centurión-Castro, F.G., 2015. Libido and serving capacity of mature hair rams under tropical

environmental conditions. Arch. Med. Vet. 47, 39–44.
Chenoweth, P.J., 1981. Libido and mating behavior in bulls, boars and rams. A review. Theriogenology 16, 155–177.
Darwish, R.A., Mahboub, H.D.H., 2011. Breed and experience effect on the sexual behaviors of Damascus and Egyptian-Nubian goat bucks. Theriogenology 76, 1386–1392.
Fahey, A.G., Duffy, P., Fair, S., 2012. Effect of exposing rams to a female stimulus before semen collection on ram libido and semen quality. J. Anim. Sci. 90, 3451–3456.
Gouletsou, P.G., Fthenakis, G.C., 2010. Clinical evaluation of reproductive ability of rams. Small Ruminant Res. 92, 45–51.
Haulenbeek, A.M., 2009. Partner preference and sexual performance in male goats. Capra Hircus. Doctoral dissertation, Rutgers University-Graduate School, New Brunswick.
Katz, L.S., 2008. Variation in male sexual behavior. Anim. Reprod. Sci. 105, 64–71.
Lankin, V., 1997. Factors of diversity of domestic behaviour in sheep. Genet. Sel. Evol. 29, 73.
Lindsay, D.R., 1965. The importance of olfactory stimuli in the mating behaviour of the ram. Anim. Behav. 13, 75–78.
Lindsay, D.R., Dunsmore, D.G., Williams, J.D., Syme, G.J., 1976. Audience effects on the mating behaviour of rams. Anim. Behav. 24, 818–821.
Malejane, C.M., Greyling, J.P.C., Raito, M.B., 2014. Seasonal variation in semen quality of dorper rams using different collection techniques. S. Afr. J. Anim. Sci. 44, 26–32.
Matthews, N., Bester, N., Schwalbach, L.M.J., 2003. A comparison of ram semen collected by artificial vagina and electro-ejaculation. SA-Anim. Sci. 4, 28–30.
Morales-Piñeyrúa, J.T., Ungerfeld, R., 2012. Pampas deer (*Ozotoceros bezoarticus*) courtship and mating behavior. Acta Vet. Scand. 54, 60.
Nikolovski, M., Atanasov, B., Dovenska, M., Petkov, V., Dovenski, T., 2012. Variations in semen quality parameters of Ovchepolian Pramenka rams according to the method of collection and the meteorological season. Mac. Vet. Rev. 35, 79–86.
Odagiri, K., Matsuzawa, Y., Yoshikawa, Y., 1995. Analysis of sexual behavior in rams (*Ovis aries*). Exp. Anim. Tokyo 44, 187–192.
Orihuela Trujillo, A., 2014. La conducta sexual del carnero: Revisión. Rev. Mex. Cienc. Pecu. 5, 49–89.
Ortiz-de-Montellano, M., Galindo-Maldonado, F., Cavazos-Arizpe, E.O., Aguayo-Arceo, A.M., Torres-Acosta, J.F.J., Orihuela, A., 2007. Effect of electro-ejaculation on the serum cortisol response of Criollo goats (*Capra hircus*). Small Ruminant Res. 69, 228–231.
Pepelko, W.E., Clegg, M.T., 1965. Studies of mating behaviour and some factors influencing the sexual response in the male sheep Ovis aries. Anim. Behav. 13, 249–258.
Price, E.O., Erhard, H., Borgwardt, R., Dally, M.R., 1992. Measures of libido and their relation to serving capacity in the ram. J. Anim. Sci. 70, 3376–3380.
R CoreTeam, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Romeyer, A., Bouissou, M.F., 1992. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. Appl. Anim. Behav. Sci. 34, 93–119.
Roselli, C.E., Resko, J.A., Stormshak, F., 2002. Hormonal influences on sexual partner preference in rams. Arch. Sex. Behav. 31, 43–49.
Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike Information Criterion Statistics. pp. 1515–1524.
Synnott, A.L., Fulkerson, W.J., 1984. Influence of social interaction between rams on their serving capacity. Appl. Anim. Ethol. 11, 283–289.
Ungerfeld, R., Ramos, M.A., Möller, R., 2006. Role of the vomeronasal organ on ram's courtship and mating behaviour, and on mate choice among oestrous ewes. Appl. Anim. Behav. Sci. 99, 248–252.
Vázquez, R., Orihuela, A., Aguirre, V., 2012. Effect of dominance-subordinate relationship and familiarity of an audience male on young rams' libido and semen characteristics. J. Vet. Behav. 7, 80–83.