RESEARCH REGARDING EVALUATION OF RAM'S SEMEN, COLLECTED BY ELECTROEJACULATION, OUT OF THE BREEDING SEASON

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Abstract

Our study was conducted out of the breeding season (March, 2012). An experimental study was conducted to assess the external genitalia in rams, along with artificial collection of semen, using the electroejaculation method by electrostimulation with an electroejaculator standardized for small ruminants, produced by MINITUBE-Germany. The semen obtained was macroscopically (number of stimulus necessary for obtaining the semen, volume, color, smell) and microscopically (sperm waves, mobility, viability, concentration) evaluated.

***Key words**: electroejaculation, microscopical and macroscopical evaluation, reproduction, ram, semen.*

INTRODUCTION

Adult males of many ruminant species present importand changes of involution and growth of testicular volume in season and out of the breeding season, including a tranzition between the intense activity and the arrest of spermatogenesis. (Asher et all., 1999).

In season, the morphologic changes of the entire reproductive sistem, production of spermatozoa and testosteron ensure the success of breeding in the short period of the breeding season. (Goeritz et all., 2003).

Collection of semen is made by ejaculation, without sexual contact between male and female (Noakes D.E. et all, 2009) Being an very important operation, many scientists tried to improve the collection methods for obtaining the semen, but without affecting the health state of the male (Ptaszynska M., 2009). The methods for semen collection have to assure the collection of semen without losses, without affecting the spermatozoa, and also a high quality sanitation for the collected semen is required (Grazyna P.et all., 2002). If electroejaculation is practiced for too long time, the reproduction potential of the animal can be compromised **(**Lasley B.L. 1994, Groza I.Ș. 2006).

The existing methods for collectin the semen from reproductive males are divided into: urethral methods, which allow obtaining the semen directly from the male urethra, and vaginal methods, in which semen is collected in the female vagina, after the matting act.

MATERIAL AND METHOD

An experimental study was conducted to evaluate the reproductive potential of rams, outside of the breeding season. For this purpose, testicol size and semen were evaluated. The semen was obtained by electrical stimulation and then a microscopical and macroscopical evaluation was conducted.

The study was carry out in March 2012, out of the breeding season, on a batch of 11rams.

The including criteria of the rams in the study were: males, sexual mature, clinically healthy.

The excluding criteria from the study were: sexual imature rams or at andropause.

Clinical examination. The general examination was made on the main systems and then the male genitalia was examined. The examination was conducted on natural light and the general evolution, attitudes and conformation were evaluated.

The skin from different areas, the joints, the hooves were palpated, being very important in the examination of the reproduction rams.

Local and general changes that could affect the reproductive abillity and behaviour of the rams were evaluated.

The examination was done standing and in movement by inspection, palpation and ascultation.

Examination of the male genitalia. The examination was made by inspection and palpation of the genitaila, or the scrotum examination, testes, epididyms, the testicular cord, prepuce and penis.

In testes it was noted: mobility, size, consistency, tenderness to palpation, the position of testes and testicular measurements were made to lenght and circumference of the testes. Note that all this findings were made out of the breeding season.

The examination of head, body and tail of epididym was conducted.

The deferend ducts were easy to feel on palpation, the thickness and sensibility was assessed in the testicular and funnicular segment.

Also , the testicular cord was palpated and thickness, consistency and mobility of the cord was evaluated, by comparison with the other.

The penis was examined, then it was exteriorised and volume and integrity was assesed. Also an examination of the prepuce was made. (Table 1)

Table 1. Clinical examination of genitals and testicular measurement in the studied rams

Ram	Breed	Testicular length		Testicular circumfe r-ence (cm)	Testis	Penis	Prepuce
		Left testis (cm)	Right testis (cm)				
RO112932623	Schwarzkopf	9	9	36	Normal	Normal	Normal
RO110293577	Schwarzkopf	10	10	38	Normal	Normal	Normal
RO1552592912	Schwarzkopf	9,5	9,5	37	Normal	Normal	Normal
RO1102932644	Schwarzkopf	9	10	39,5	High consistence on the right testis	Normal	Normal
RO1551948912	Schwarzkopf	11	10	39	High consistence on the left testis	Normal	Normal
RO1102932601	Schwarzkopf	10,5	10	38	Normal	Normal	Normal
RO1075345130	IDF (Ille de France)	9,5	9,5	40	Normal	Normal	Normal
RO1075345140	IDF (Ille de France)	10,5	10,5	39,5	Normal	Normal	Normal
RO1075345069	IDF (Ille de France)	10	10	39	Normal	Normal	Normal
RO1075345070	IDF (Ille de France)	9	10,5	41	High consistence on the right testis; hard epididimis	Balano-posthitis	Balano-posthitis
Ram	**Breed**	**Testicular length**		**Testicular circumfe r-ence (cm)**	**Testis**	**Penis**	**Prepuce**
		Left testis (cm)	**Right testis (cm)**				
RO11210002323	Schwarzkopf	10	10	40	Normal	Normal	Normal
Average		9.71	9.86	38.67			
STDEV		0.76	0.38	1.58			

Collection of semen

The first step for evaluating of the semen is the collection. The collection of semen was made using the electrostimulation method.

Semen was collected with an manual electroejaculator standardized for small ruminants. In this type of electroejaculator, the electrical stimulation is made by the user and not by a program, like in electronic electroejaculators which have an diferent program that changes automaticlly the stimuli voltage depending on the species.

For collection, after restraining, the ram is laid on the left side. A local toiled was performed by shaving and washing the prepuce with sodium bicarbonate 3% followed by the drying of the area. An enema was performed to eliminate the feces and for a better conductibility. The penis was exteriorised from the prepuce, acting on the sigmoid flexure, and the vermiform appendix was introduced in the collection recipient.

The electrode of the electroejaculator (30cm long and 2 cm in diameter) was inserted into rectum after the lubrication, for about 15 cm.

The collector recipient was placed in front of the urethra to collect the semen.

Collection was made in special sterile glass (sperm friendly), dry and heated at body temperature, which were changed at every semen ejaculate, to avoid the risk of urine contamination in the whole ejaculate.

The electrical stimulus was applied for 5 seconds, and breaks of 10 seconds between stimuli. The device is connected to a source of continuous electric charge (accumulator) with tension variations of 2.5-9V.

The semen was obtained after 3-5 stimuli, and the process occurs without erection.

The semen was obtained with the same protocol at all the collections and to all studied individuals. Then a macroscopical and microscopical examination was performed.

The macroscopical examination included the measurement of the ejaculate volume with an graduated cylinder, also the color and smell of semen were assessed.(Table 2)

Table 2. Macroscopic examination of semen

No crt	**Ram**	**Breed**	**Number of stimulations**	**Semen volume (ml)**	**Color**	**Consistency**	**Density**	**Smell**
1	RO112932623	Schwarzkopf	6	2,5	White	creamy	dense	normal
2	RO110293577	Schwarzkopf	5	2	White	creamy	dense	normal
3	RO1552592912	Schwarzkopf	8	2	White	creamy	dense	normal
4	RO11029326 44	Schwarzkopf	7	2	White	creamy	dense	normal
5	RO1551948912	Schwarzkopf	8	2	White	creamy	dense	normal
6	RO11029326 01	Schwarzkopf	9	0,75	White	creamy	dense	normal
7	RO1075345130	IDF (Ille de France)	8	2	White	creamy	dense	normal
8	RO1075345140	IDF (Ille de France)	8	2	White	creamy	dense	normal
9	RO1075345069	IDF (Ille de France)	8	1,5	White	creamy	dense	normal
10	RO1075345070	IDF (Ille de France)	8	-	-	-	-	-
11	RO11210002 323	Schwarzkopf	9	5	White	creamy	dense	normal

The microscopical examiantion of semen evaluated the presence of sperm waves, the mobility, viability and concentration of spermatozoa.
The mobility was evaluated by the number of spermatozoa with forward movements and their movement energy, on a slide at 37^0C.
Viability of spermatozoa was assessed by Hancook-Dott method- the intravital staining with eosin and nigrozin.
Over 500 spermatozoa were evaluated and then expressed as a percentage their share (Table 3).

Table 3. Microscopic examination of semen

No. crt	**Ram**	**Breed**	**Sperm waves**	**Mobility (%)**	**Viability (%)**	**Concentration**	**Observation**
1	RO112932623	Schwarzkopf	+ + +	90	92	over 1 x 10^9/ml	-
2	RO110293577	Schwarzkopf	+ + +	90	93	over 1 x 10^9/ml	-
3	RO1552592912	Schwarzkopf	+ + +	95	95	over 1 x 10^9/ml	Exfoliate cells
4	RO1102932644	Schwarzkopf	+ + +	50	60	over 1 x 10^9/ml	Exfoliate cells and cellular detritus
5	RO1551948912	Schwarzkopf	+ + +	85	80	over 1 x 10^9/ml	-
6	RO1102932601	Schwarzkopf	+ + +	95	90	over 1 x 10^9/ml	-
7	RO1075345130	IDF (Ille de France)	+ + +	95	93	over 1 x 10^9/ml	-

No. crt	Ram	Breed	Sperm waves	Mobility (%)	Viability (%)	Concentration	Observation
8	RO1075345140	IDF (Ille de France)	+ + +	90	90	over 1 x 10^9/ml	-
9	RO1075345069	IDF (Ille de France)	+ + +	100	100	over 1 x 10^9/ml	-
10	RO1075345070	IDF (Ille de France)	-			-	-
11	RO11210002323	Schwarzkopf	+ + +	80	80	over 1 x 10^9/ml	-

CONCLUSIONS

At clinical evaluation 3 rams presented testicular modifications, and one ram was diagnosticated with balanoposthitis.
Collection of semen failed on one ram (aspermia) and 5-9 electrical stimulations were needed to obtain semen.
Volume of semen ranged between 0,75 and 5 ml and had white-creamy color.
At microscopical examination sperm waves were present, mobility ranged between 50 and 100%, viability ranged between 60 to 100% and concentration was over 109sperm/ml semen.
Total number varied between 1 and 7 x 109 sperm.
After andrological evaluation, 2 out of 11 rams were excluded from reproduction process.

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