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Preliminary report on observed differences in goat sperm characteristics based on scrotal morphology

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INTRODUCTION

ROBERTSHAW (1982), in discussing goat adaptation to thermoregulation, mentions an anatomical scrotum adaptation observed in goats raised in the arid and semi-arid areas of Eastern Africa, which consists of having the testicles encapsulated by separate scrotum pouches, thus increasing surface area and enhancing heat loss in a region which demands preferential cooling.

Observation of this trait, which we call the "divided scrotum trait'", in some bucks and their male progeny as well as comments from the goat producers that the animals presenting this trait are more fertile, led to the design of the present study. Results presented correspond to data recorded during the dry season period between the months of august through october.

MATERIAL AND METHODS

The Centro Nacional de Pesquisa de Caprinos (CNPC), is located in the hot semi-arid region of Northeast Brasil. Climatological data was reported elsewhere (FIGUEIREDO & PANT, 1982 ; MELO LIMA et al., 1982).

A score system from zero to five was developed to indicate the degree of scrotal division observed in the animals, zero, meaning no division and five a total division of the scrotum in such a manner that each testicle is encapsulated in a separate scrotum pouch (Figure 1).

Semen from ten males of the native Moxoto breed, ranging from 18 to 24 months of age was collected twice a week, with an artificial vagina. Six of these animals presented some degree of scrotum division, having at least the region of the tail of the epididymis fully separated (Score 2). The following observations on semen quality were recorded:

1) Ejaculate volume in 0,1 ml units.
2) Concentration, determined by the use of a spectrophotometer.
3) Total number of sperm cells per ejaculate.
4) Mass motility, determined by a scoring system from zero to five (CORTEEL, 1974).

5) Individual progressive motility and percentage of motile sperm: to varying quantitaties of a solution, which contained 20g of skimmedmilk powder, 194mg of glucose and 100ml of double distilled water, raw semen was added, to reach an average final dilution of 4 x 10^5 sperm cells/ml, which was maintained in a 37°C water bath. The individual progressive motility and percentage of motile sperm were evaluated at 5, 30, 60, 90 and 120 minutes, by means of a phase contrast microscope at 37°C. The former was estimated using a scoring system from zero to five, as described by SMYTH & GORDON (1967), and the latter by a method proposed by CORTEEL (1975).

6) Sperm morphological abnormalities: using the same sperm dilution described above for the individual progressive motility and percentage of motile sperm determinations, slides of sperm smears were prepared and stained with a solution containing 1 % eosin, 3 % nigrosin and 3 % sodium citrate.

A total of 150 sperm cells per slide were examined under a phase contrast microscope equiped with a warming stage at 37°C and at a magnification of 200X. The number of abnormal sperm cells was expressed as a percentage of the total number of cells examined.

A nested analysis of variance was used for analysis of data. If a animal had two successful semen collections within a week the average value of the two observations was used as the statistical unit for analysis. Otherwise, the values obtaines from one collection within a week were used. The only exeption to this was volume per ejaculate which was evaluated as total volume of semen produced per week, to take into account the inability of certain animals to produce two ejaculations per week. Data for the percentage of motile sperm at 90 and 120 minutes as well as for the percentage of abnormal sperm cells were transformed to Arcsin values.

RESULTS

Results of the analysis of variance are presented in Table 1 and means in Table 2.

In all parameters studied means reported for the group of animals which presented some degree of division of the scrotum were superior, although not significantly different, to means obtained for the group which did not present the divided scrotum trait. The only exeption to this trend was the concentration of sperm cells. At the analysis of variance the group effect showed a tendency to become significant for the percent sperm motility ($P = 0.0559$) and individual sperm progressive motility ($P = 0.0615$) at 120 minutes after ejaculation. The only sperm characteristics which showed a significant group effect ($P<0.01$) was the percentage of abnormal sperm cells. The incidence of the most common sperm abnormalities observed are presented in Table 3.

DISCUSSION

In general animals which presented a divided scrotum showed superior semen quality, according to our criteria.

It is known that storage and maturation of sperm cells occurs in the epididymis (ORTAVANT, 1953; COUROT, 1981). Probably, animals with a divided scrotum exposed epididymal regions to better ventilation and or

transpiration, hence, achieving better cooling of those regions, and, as a consequence insuring much better control over temperatures stress. Results presented here indicate that this trait could be advantageous, since sperm from these animals proved to be more motile up to 120 minutes after ejaculation, and presented a lower percentage of abnormal cells. This is important to fertility, because, matings occur indiscriminately during the heat period of the doe, although ovulation only occurs during the last half of it. Thus maintenance of sperm motility for long periods after ejaculation becomes an important trait to insure fertilization of the ovum. The mass motion of raw semen may not be as important, as the individual sperm cell progressive motility for goat fertility (CORTEEL, 1975). CORTEEL (1981) also demonstrated a high correlation between the degradative process of individual sperm progressive motility and doe fertility. In this regard, sperm from animals with divided scrota showed that the degradative individual progressive motility process was much slower per unit of time than in animals which did not present the trait.

RIMBERT (1980) calculated the percent degradation of individual sperm progressive motility as a ratio of the difference between individual progressive motility at 5 and 120 minutes over the individual progressive motility at 5 minutes, expressed as a percentage. The same author scored 48 Alpine and 18 Saanen bucks according to the parameter described above to test the effect of the percent degradation of individual progressive motility on doe fertility, measured by kidding percentages after intracervical artificial insemination. It was found that does inseminated with semen which showed percent degradation values ranging from 30 % to 60 %, kidded in the range of 59 to 61 %. Semen with percent degradation higher than 60 % showed a significant drop in the kidding percentage, to levels of 49 % when the percent degradation was equal to or above 70 %. Applying the same calculation to our observed values of individual sperm progressive motility within groups under comparison, our figures would be 55.7 % and 73.4 % degradation for animals which presented divided and non-divided scrotum respectively, thus indicating the formers as more fertile on the average.

Another important aspect is that of percentage of abnormal sperm cells. COLAS (1980) working with the Ile-de-France breed found that exposure of rams to 29°C for 3 days or 30° C for 2 consecutive days, is enough to cause an increase in the proportion of abnormal cells and demonstrated that this breed is thermosensible. Increases in percentage of abnormal sperm cells have been reported to be detrimental for sheep (COLAS, 1981), and goat (HIROE & TOMIZUKA, 1968) fertility. It appears that temperature stress is an important environmental effect causing an increase in sperm abnormalities, specially those of the flagela which amounted to about 27 % of all the abnormalities observed in the present study.

Native goats in Brazil are fairly well adapted on the average, to the environment. However bucks are showing temperature stress as reflected in the sperm characteristics studied during the dry period. It has been reported that native does in Northeast Brazil are polyestric (SIMPLICIO et al., 1980). Based on that conclusion, initiation of the breeding season was proposed for the months of october and november for the last third of the gestation period and kidding to occur during the wet season, when forage availability peaks. This proposed breeding season will occur during the driest period of the year when mean maximum temperatures are about 37°C. If temperature stress is reducing fertility, and the trait under discussion counteracts this to a certain extent, it is logical to consider the possibility of selecting animals which present this favorable trait, thus

increasing the buck performance during the breeding season recommended for this region.

Results of a survey of all male goats born during the 1982 kidding season at the Centro Nacional de Pesquisa de Caprinos (CNPC) are presented in Table 4. From a total of 121 male kids born, 59 of them scored from 2 to 5, corresponding to 48.8 % of animals presenting some degree of the trait under discussion (RIERA et al., 1982). Another survey conducted in 1981 on private farms, indicated that 21 out of 61 male kids born of bucks with divided scrotum, presented varying degrees of the same trait (PONDE DE LEON & MELO LIMA, 1982). The relatively high incidence of the trait suggests it is a heritable character which could be taken into consideration in future selection programs.

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RESUME

CARACTERISTIQUES DE LA SEMENCE DES CAPRINS LOCAUX MOXOTO EN RELATION AVEC LA MORPHOLOGIE DU SCROTUM

Dix boucs, âgés de 24 mois de la race native Moxoto du Nordeste du Brésil, ont été récoltés deux fois par semaine au vagin artificiel, pendant les mois d'août à novembre période sèche. Six animaux avaient les testicules séparés par une division du scrotum et les quatre autres ne possédaient pas cette caractéristique. Le volume, la motilité massale, la concentration et le nombre de spermatozoïdes sont appréciés dans chaque éjaculat. Après dilution dans le lait écrémé, le pourcentage de spermatozoïdes et leur motilité individuelle ont été mesurés au microscope à contraste de phase après 5, 30, 60, 90 et 120 minutes d'incubation à +37°C. Un frottis de la semence colorée avec une solution à base d'éosine - nigrosine a été employé pour voir les anomalies des spermatozoïdes des deux groupes d'animaux. Le volume moyen de l'éjaculat est de 0,37 et 0,30 ml pour les animaux avec le scrotum divisé et non divisé respectivement. La concentration et la motilité massale ne sont pas significativement différentes. Le pourcentage de spermatozoïdes mobiles et la motilité individuelle au cours du test de thermo-résistance sont très significativement différents pendant toute la période d'observation ($P<0,01$). Après 60 minutes, la différence est nette, puisque le pourcentage de cellules mobiles est respectivement de 42,9 et 31,9 chez les animaux avec le scrotum divisé et non divisé. Le pourcentage total de spermatozoïdes anormaux est de 5,42 et 32,94 respectivement pour les animaux avec et sans division du scrotum. Les anomalies les plus fréquentes sont celles du flagelle, avec 2,40 et 16,80 %, ce qui pourrait s'expliquer par une meilleure adaptation des animaux avec le scrotum divisé, aux températures très élevées pendant la saison sèche du Nordeste du Brésil.

SUMMARY

PRELIMINARY REPORT ON OBSERVED DIFFERENCES IN GOAT SPERM CHARACTERISTICS BASED ON SCROTAL MORPHOLOGY.

Observations were recorded on a peculiar variation of scrotal morphology in native goats of Northeast Brasil. A scoring (0 to 5) of the degree of division of the scrotum was developed; zero, meaning no division, and five a total division of the scrotum in such a manner as having each testicle in a separate scrotum pouch. Semen from 10 males of the Moxoto native breed, varying from 18 to 24 months of age, was collected by means of an artificial vagina twice a week during the months of august through october. This corresponded to about half of the dry season period, during which daily maximum and minimum temperatures ranged from 39°C to 35°C and from 25°C to 19°C respectively. Six of these males presented some degree of division of the scrotum, having at least the region of the tail of the epididymus totally separated (Score 2). Means and pooled stardard errors are reported for several semen characteristics such as, total ejaculate volume per week, sperm concentration, total number of sperm cells in the ejaculate, mass motility, percentage of motile sperm and individual progressive motility at 5, 30, 60, 90 and 120 minutes after ejaculation, and percent sperm abnormalities. A trend of superior semen quality for animals showing the "divided scrotum trait" was observed. The most striking significant difference ($P<0.01$) was that of sperm morphological abnormalities, which showed a mean of 5.4 % and 32.9 % for animals presenting divided and non divided scrotum respectively. Hence, this trait could be related to enhanced fertility. If so, it should be taken into consideration in future selection programs.

REFERENCES

COLAS G., 1980. Variations saisonnières de la qualité du sperme chez le bélier Ile-de-France. I. Etude de la morphologie cellulaire et de la motilité massale. Reprod. Nutr. Develop., 20 (6), 1789-99.

COLAS G., 1981. Variations saisonnières de la qualité du sperme chez le bélier Ile-de-France. II. Fécondance : relations avec les critères qualitatifs observés "in vitro". Reprod. Nutr. Develop., 21 (3), 399-407.

CORTEEL J.M., 1975. Production du sperme chez le bouc : variation saisonnière de la quantité et de la qualité du sperme récolté selon l'âge des animaux. In : Journée de la Recherche Ovine et Caprine, 3. Paris, p 4-17.

CORTEEL J.M., 1981. Collection, processing and artificial insemination of goat semen. In : GALL, C. Goat Production. London, Academic Press, p 171-91.

CORTEEL J.M., 1974. Viabilité des spermatozoides de bouc conservés et congelés avec/ou sans leur plasma seminal : effet du glucose. Ann. Biol. anim. Biophys., 14 (4B), 741-5.

COUROT M., 1981. Transport and maturation of spermatozoa in the epididymis of mammales. Prog. Reprod. Biol., 8, 67-9.

FIGUEIREDO E.A.P., PANT K.P., 1982. Evaluation of goat breeds in the tropical Northeast Brazil. II. An analysis of age at death of kids. Pesq. Agrop. Bras., 17, 803-8.

HIROE K., TOMIZUKA T., 1966. Effect of high environmental temperature on the semen production in domsetic animals. Bull. Nat. Inst. Anim. Ind. (Chiba), 9, 27-35.

MELO LIMA F.A., FIGUEIREDO E.A.P., SIMPLICIO A.A., PONCE DE LEON F.A. Traditional system of goat manamente. II. Pre-weaning growth performance of the SRD (non-descript) goat. Pesq. Agrop. Bras. (Submited for publication).

ORTAVANT R., 1953. Le cycle spermatogénétique chez le bélier. Thèse, Paris.

PONCE DE LEON F.A., MELO LIMA F.A., 1982. Dados nao publicados.

RIERA G.S., NUNES F.F., SILVA A.E.F.D.,1982. Dados nao publicados.

RIMBERT M., 1980. Recherche des voies possibles pour l'amélioation des résultats de fertilité après insémination artificielle chez les caprins. Rennes, Thèse 48 p.

ROBERTSHAW D., 1982. Concepts animal adaptation thermoregulation of the goat. In: International conference on goat production and disease,3. Tucson, Arizona. Proceedings p 395-7.

SHELTON M., FIGUEIREDO E.A.P., 1981. Types of sheep and goats in Northeast Brazil. Int. Goat and Sheep Res., 1, 258-68.

SIMPLICIO A.A., RIERA G.S., FOOTE W.C., 1980. Caracteristicas de reproducao de tres tipos de cabras influenciadas pelo nivel nutricional e epocas do ano no Nordes te do Brasil. Sobral, CE. EMBRAPA/CNPC. Mimeografado.

SMYTH P., GORDON I., 1967. Seasonal and breed variations in the semen characteristics of rams in Ireland. Irish Vet. J., 21, 222-33.

Table 1

Analysis of variance of the sperm characteristics evaluated in animals with divided and non-divided scrotum

Source of Variation	d.f.	Total semen volume	Concen-tration	Total sperm cell/ ejaculate	Mass Motility	Percent sperm motility at 5 min	30 min	60 min	90 min[a]	120 min[a]
Groups	1	0.72 NS	0.07 NS	1.75 NS	0.12 NS	260.68 NS	596.28 NS	1586.73 NS	661.72 NS	910.48[e]
Animals.Groups	8	0.55 **	0.25 NS	1.97 **	1.29 *	256.31 NS	282.84 NS	442.45 **	218.19 **	178.61 *
Collection/Animals	+	0.08	0.14	0.39	0.53	130.63	145.51	156.07	66.11	67.45
Groups		(64)	(64)	(64)	(63)	(65)	(65)	(65)	(65)	(65)

Note: a = Original data was transformed to arcsin.

e = (P = 0.0559)

f = (P = 0.0615)

NS = Non-significant

* (P<0.05)

** (P<0.01)

\+ degrees of freedom within parenthesis are presented at the bottom of each column.

Table 1 (suite)

Individual sperm progressive motility					Percent[a] abnormal sperm cells
5 min	30 min	60 min	90 min	120 min	
0.63 NS	1.71 NS	4.01 NS	5.56 NS	7.09[f]	7674.65 **
0.61 NS	0.74 NS	1.57 **	2.07 **	1.45 **	79.38 NS
0.42	0.49	0.46	0.53	0.51	62.88
(65)	(65)	(65)	(65)	(65)	(58)

Table 2

Means for the sperm characteristics evaluated in animals

with divided and non-divided scrotum

Sperm characteristics	Pooled Standard Error	Divided scrotum			Non-divided scrotum		
		n	⋇	⋇ Arcsin	n	⋇	⋇ Arcsin
Pooled ejaculate volume per week (ml)	0.176	(44)	0.711[a]		(30)	0.510[a]	
Concentration/Ejaculate (x 10^9) ml	0.118	(44)	3.897[a]		(30)	3.962[a]	
Total of sperm cells/Ejaculate (x 10^9)	0.332	(44)	1.564[a]		(30)	1.250[a]	
Mass motility Score (0 - 5)	0.272	(44)	3.489[a]		(29)	3.405[a]	
Percent sperm motility at (%)							
5 minutes	3.774	(45)	59.09[a]		(39)	55.28[a]	
30 minutes	3.964	(45)	52.19[a]		(30)	46.43[a]	
60 minutes	4.958	(45)	41.72[a]		(30)	32.33[a]	
90 minutes	3.482	(45)	29.02	38.66[a]	(30)	19.01	32.59[a]
120 minutes	3.150	(45)	23.34	35.28[a]	(30)	12.29	28.17[b]
Individual sperm progressive motility score (0 - 5) at							
5 minutes	0.184	(45)	3.261[a]		(30)	3.075[a]	
30 minutes	0.203	(45)	2.933[a]		(30)	2.625[a]	
60 minutes	0.295	(45)	2.389[a]		(30)	1.917[a]	
90 minutes	0.339	(45)	1.822[a]		(30)	1.267[a]	
120 minutes	0.284	(45)	1.444[a]		(30)	0.817[b]	
Percent of abnormal sperm cells	0.333	(42)	5.33	13.35[a]	(26)	33.24	35.21[b]

Note: Means within rows showing the same supercript letter are not significantly different (P>0.05)

Table 3

Incidence of sperm abnormalities observed in animals with divided and non-divided scrotum

Groups	Anomalias abnormalities (%)					
	AH	AF	PCD	DGD	FA	TOTAL
Divided scrotum	1.20	1.10	0.32	0.40	2.40	5.42
Non-divided scrotum	5.00	6.74	2.00	2.40	16.80	32.94

AH = Abnormal heads

AF = Absence of flagela

PCD = Proximal citoplasmic drop

DGD = Distal citoplasmic drop

FA = Flagela abnormalities

Table 4

Incidence of the divided scrotum trait

Observed on male kids born during the 1982 kidding season at the CNPC[1,2]

Breeds[3]	Scoring system						Total
	0	1	2	3	4	5	
Anglo-Nubian	9	5	2	-	-	-	16
Bhuj	2	-	5	-	-	1	8
Caninde	-	4	13	-	-	-	17
Marota	2	8	9	1	1	-	21
Moxoto	8	9	11	1	-	-	29
Repartida	-	6	5	1	-	-	12
SRD	-	9	9	-	-	-	18
Total	21	41	54	3	1	1	121
%	17.4	33.9	44.6	2.5	0.8	0.8	100.0

[1] RIERA et al., 1982

[2] Centro Nacional de Pesquisa de Caprinos

[3] Breeds description, is available in SHELTON & FIGUEIREDO (1981).

COMMUNICATION DU DOCTEUR : J.F. NUNES

Question du Dr. HARRICHARAN :

Based on your present knowledge of the scrotal division and sperm production, could you say whether we could use scrotal division as a criteria to select flock sires ? Can we eliminate the animal with undivided scrotum ?

Réponse :

Il faudrait introduire dans les fermes des animaux avec le scrotum divisé et mesurer les fréquences des petits mâles nés avec le scrotum divisé. Il y a une tendance pour cette caractéristique d'être de très haute héritabilité, donc nous pourrions après une sélection rigoureuse des mâles avec le scrotum divisé, améliorer l'efficacité reproductive des troupeaux, et progressivement éliminer les animaux qui n'ont pas cette caractéristique.

Question du Dr. MONTY :

Do the temperatures of the testicles of animals with divided scrotum differ from those with undivided scrotum ? Are testicular temperatures higher in animals with divided scrotum ?

Réponse :

Nous avons mesuré avec un thermomètre digital la température du scrotum et nous avons trouvé une température plus élevée chez les animaux avec le scrotum non divisé. La température la plus basse a été enregistrée au niveau de la queue de l'épididyme chez les animaux avec le scrotum divisé.

Question du Dr. THIMONIER :

Avez-vous essayé de réaliser chirurgicalement une division du scrotum pour voir l'effet sur la qualité de la semence ?

Réponse :

Cette expérience n'a pas été faite, mais nous pensons qu'il sera très difficile de retrouver les conditions naturelles de division du scrotum.

Question du Dr. SERGENT :

Avez-vous étudié l'anatomie du scrotum divisé, y-a-t-il des différences d'irrigation sanguine par rapport au scrotum non divisé ?

Réponse :

Nous n'avons pas encore étudié le système d'irrigation sanguine du scrotum, mais nous avons commencé actuellement un travail sur ce sujet.