

# THE MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE CONSECUTIVE EJACULATES FROM IL-DE-FRANCE RAMS AT VARIOUS AGES\*\*

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**Abstract :** In the agricultural practice one ram is used for the insemination a lot of ewes according to the breeding plan. For the insurance of the successful insemination of ewes reached the heat simultaneously, it is necessary to obtain some consistently ejaculates from one ram.

The aim of the present work was the estimation of the sperm quality in consecutive ejaculates from Il-de-France rams at various ages. It was investigated over 40 ejaculates from 5 rams of the IAS-Kostinbrod herd between 1, 5 and 4 years old during the breeding season. The sperm was collected by artificial vagina. It was estimated the following morphological and biochemical parameters: volume, count of the spermatozoids in 1ml, motility, survivability at 39°C and activity of the alkaline phosphatase in the semen plasma.

In the consecutive ejaculates of both groups no significant morphological changes of spermatozoids were established. The obtained results are shown that the concentration of the spermatozoids and the activity of the alkaline phosphatase in the semen plasma reduce in the third consistently ejaculate, but the keeping of Aph activity in second ejaculates despite decreasing of the sperm volume and concentration of spermatozoid .On the base of these results we could give reference for use of the first and second ejaculates in the practical insemination of ewes.

**Key words:** consistently ejaculates, sperm quality, Il-de-France rams, alkaline phosphatase

## Introduction and literature review

It is known that reproductive potential of different males give possibility to obtain some ejaculates from one male per day. The ability to collect two or more consecutive ejaculates in a short period could be valuable for gamete resource banking and preserving genetic diversity of the native breeds in different animal species. In this way, there are a former and present investigations aimed to estimate the quality of the consecutive ejaculates (*Parker and Thwaites*, 1962; *Mattner and Voglmayr*, 1962; *Ritar et al.*, 1992; *Shamsuddin, et al.*, 2000; *Nel-Themaat et al.*, 2006). It was noticed the differences in sperm volume and concentration of spermatozooids between first and consistently received ejaculates in different species. In rabbits there are the cyclic repeated changes in quality of consecutive ejaculates (*Ambriz D et al.* 2002).

In the agricultural practice one ram is used for the insemination a lot of ewes according to the breeding plan. For the insurance of the successful insemination of ewes reached the heat simultaneously the obtaining of consecutive ejaculates from one ram is necessarily. How many ejaculates can be collected and if all of them have the good quality? It is impossibility to give exactly answer, because the literature data are very scanty.

*Parker and Thwaites* (1962) noticed the progressively decreasing of semen quality (volume, density, and motility of ejaculate, and methylene blue reduction time) from first to eight consecutive ejaculates of Merino rams.

*Nel-Themaat et al.* (2006) reported that the mean semen volume, sperm concentration and number of spermatozoa per ejaculate obtained from the first ejaculate of Gulf Coast Native rams were significantly greater ( $P < \text{or} = 0.01$ ) than those of the second ejaculate. On the other hand, the mean motility of pre-cooled (22 degrees Celsius), cooled (5 degrees Celsius) and frozen (-196 degrees Celsius) post-thawed spermatozoa was less ( $P < \text{or} = 0.01$ ) in the first ejaculate compared with that of the second ejaculate.

The main objective of our study was the estimation of the sperm quality in consecutive ejaculates from Il-de-France rams at various ages obtained during the breeding season.

## Material and methods

It was investigated the 5 rams of the Il-de-France breed from the IAS-Kostinbrod herd during the breeding season. They were between 1, 5 and 4 years old. The three consecutive ejaculates per ram were obtained within a 30 minutes period twice in week. Total more than 40 ejaculates were examined in the experiment.

The sperm was collected by artificial vagina. The assessment of the sperm on the percent of alive and mobile spermatozoids was performed visually on a light microscope immediately after the obtaining. After this primary estimation the ejaculates were diluted with extender 6A (1:4). The following spermal parameters: volume, count of the spermatozoids in 1ml, survivability at 39°C were estimated.

For production and dye of histological samples were used routine methods. During morphological analysis were taken into account: 1 – normal spermatozoa; 2 – with injured acrosoma; 3 – with other injures (without heads, with cytoplasm droplets, with curled tails).

After centrifuge of semen samples the activity of the alkaline phosphatase (Aph) in the seminal plasma was evaluated. p-Nitrophenolphosphate is used as the substrate for the determination of it activity. The Aph activity is directly proportional to the amount of p-nitorhenol liberated per unit time ( by *Bessey et al.*, 1965).

The statistical processing of the data was done in the STATISTIC computer programme (Stat Soft Company).

## Results and discussion

Table 1 reflects sperm characteristics of the consecutive ejaculates from rams of Ill-de-France breed at different ages. It was observed that the sperm volume and concentration of spermatozoids in mature rams was greater than in young in each consecutive ejaculate. In both groups the concentration of spermatozoids significantly decreased from first to third ejaculate ( $P < 0, 01$  for mature,  $P < 0, 05$  for young rams). The motility of sperm in first and second ejaculates was higher in mature rams than in young ( $P < 0,05$ ). It wasn't defined the significantly differences in survivability of sperm at 39° C depending of age or number of ejaculate.

**Table 1. Spermograms of the consecutive ejaculates from rams of Ill-de-France breed at different ages, year 2006.**

Parameters	Concentration, bil/ml			Volume, ml			Motility, %			Survivability, min		
	1	2	3	1	2	3	1	2	3	1	2	3
Number of ejaculates												
Mature rams 2,5-4 years old	1,9 ± 0,04	1,6 ± 0,1	1,4** ± 0,2 (1-3)	1,35 ± 0,2	1,01 ± 0,3	1,08 ± 0,2	66,6 ± 3,3	69,2 ± 4,5	60,0 ± 7,0	260,0 ± 29,1	286,0 ± 21,5	258,0 ± 66,6
Young rams 1,5-2 years old	1,2*** ± 0,04 (1-1)	1,1* ± 0,4 (2-2)	1,0* ± 0,02 (1-3)	0,84* ± 0,3 (1-1)	0,8 ± 0,1	0,9 ± 0,1	56,0* ± 2,4 (1-1)	53,0* ± 4,8 (2-2)	53,0 ± 3,3	308,0 ± 115,3	339,0 ± 48,5	136,0 ± 86,8

\*\*\* -  $P < 0,001$ ; \*\* -  $P < 0,01$ ; \* -  $P < 0,05$ ; (1-1) - numbers of comparative ejaculates

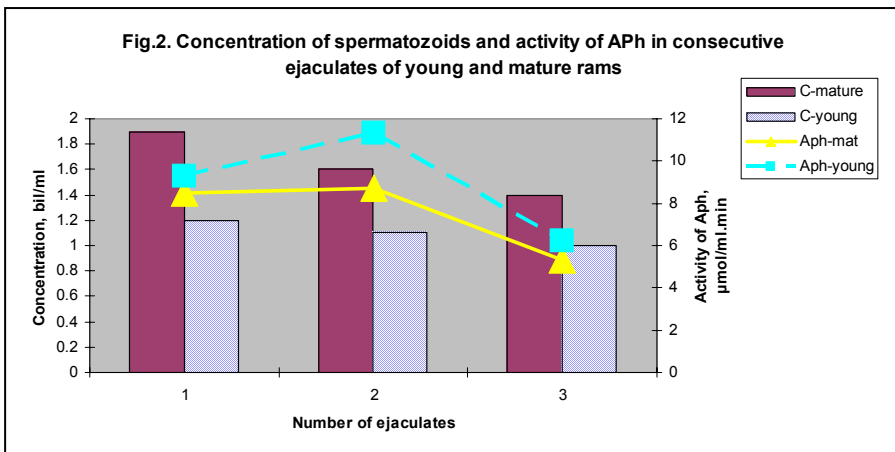
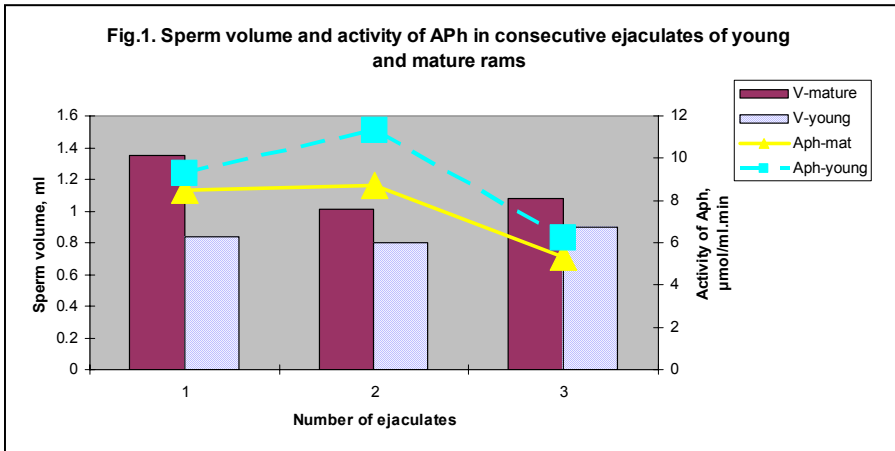
The morphological characteristics of consecutive ejaculates from rams of Ill-de-France breed at different ages are shown in the table 2. All consecutive ejaculates had morphologically normal spermatozooids about 80% and more. The percent of injuries (acrosomal and other) is within normal parameters. No age depending differences in morphology of spermatozooids was established.

**Table 2. The morphological characteristic of consecutive ejaculates from rams of Ill-de-France breed at different ages, year 2006.**

Parameters	Normal			Acrosomal injuries			Other injuries		
	1	2	3	1	2	3	1	2	3
Number of ejaculate									
Mature rams 2,5-4 years	84,2 ± 2,6	81,5 ± 2,7	84,4 ± 2,1	1,2 ± 0,2	1,5 ± 0,5	1,5 ± 0,5	14,5 ± 2,3	17,1 ± 2,3	13,9 ± 1,7
Young rams 1,5-2 years	77,1 ± 1,7	83,6 ± 1	86,7 ± 0,9	1,95 ± 0,9	2,1 ± 0,5	1,5 ± 0,1	15,1 ± 3,2	14,4 ± 3,2	12,6 ± 1,1

The dynamic of alkaline phosphatase activity corresponding with changes of sperm volume and concentration of spermatozooids are present at figures 1 and 2. Despite decreasing of the sperm volume and concentration of spermatozoa in second ejaculate, the activity APH in mature rams doesn't change. Moreover, in young rams is observed the tendency for increasing of the APH activity in second ejaculate (from 9,3 to 11,3  $\mu\text{mol}/\text{min}.\text{ml}$ ,  $P > 0,05$ ). It is not significant, because the wider individual variability of the APH

activity in ram ejaculates was defined. In third ejaculates of both groups the activity of APh is significantly less then in first and second ejaculates (  $P < 0,05$  for mature rams,  $P < 0,01$  for young rams).



The correlatives analyses show that in mature rams the high positive correlation presents between activity of APh and sperm volume in first ejaculate (Table 3). In young rams the close correlations between sperm volume, concentration of spermatozoids and activity of APh in all consecutive ejaculates was observed (Fig 3 and 4). It needs to remark the presence of negative relative relationships between APh and sperm

parameters in second ejaculates of both groups (table 3)

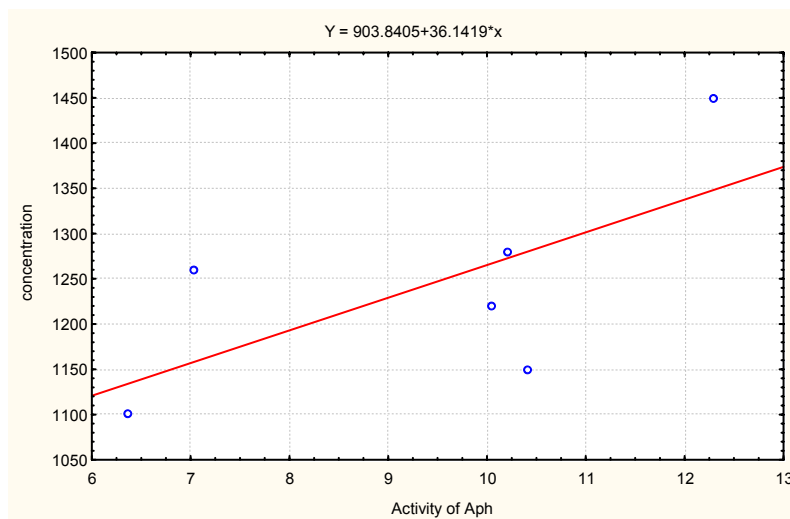
**Table 3. Correlation between activity of Aph ,sperm volume and concentration of spermatozoids in consecutive ejaculates of rams**

Ejaculate number	Correlative coefficients, $r$ , $P < 0,05$			
	Young		Mature	
	Aph - concentration	Aph- sperm volume	Aph - concentration	Aph- sperm volume
1	0,67	0,57	0,14	0,67
2	-0,41	-0,70	-0,37	-0,31
3	0,61	0	0,1	0,14

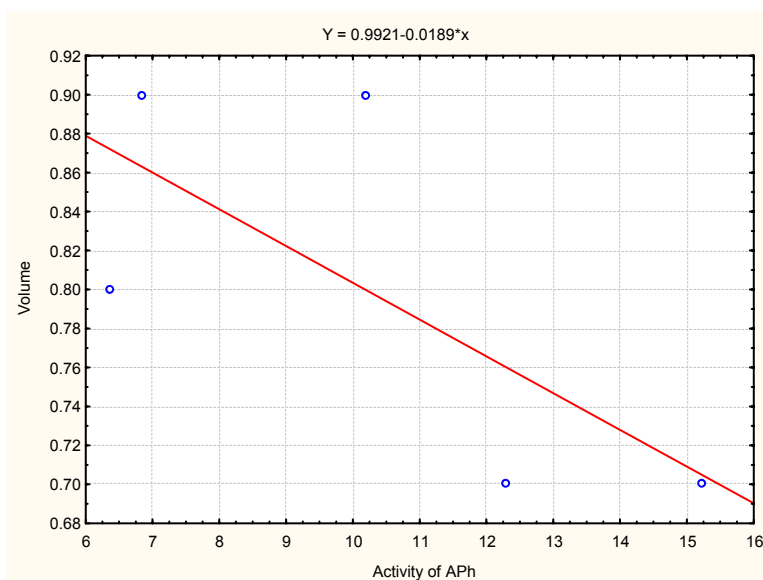
The obtained results related sperm parameters are in agreement with data received of *Parker and Thwaites* (1962) and *Nel-Themaat et al* (2006). These authors reported about decreasing of sperm volume and concentration of spermatozoids from first to second ejaculates in rams. The data related to the motility of spermatozoids in consecutive ejaculates is opposite. *Parker and Thwaites* (1962) observed the decreasing of the motility of sperm in consistently received ejaculates, but *Nel-Themaat et al* (2006) pointed out greater motility of spermatozoids from second ejaculates not only in fresh, but also in cooled and in frozen - post-thawed sperm of rams. We didn't receive significantly differences in sperm motility between consecutive ejaculates, but the tendency of enhancement of motility and survivability of spermatozoids from second ejaculates in compare with first was observed. Probably, it depends of changes in biochemical parameters of sperm. Our study had shown the keeping of Aph activity in second ejaculates despite decreasing of the sperm volume and concentration of spermatozoids. It is known that Aph ensures the substrate for cytoplasmic glycolyse by the dephosphorylation of the monosacharidephosphates in seminal plasma (*Uzunov et al.*, 1998) and participates in transport processes of the nutritional substances through cell membranes, too. *Singer et al.* (1980) in their study point out the correlation between the level of acid phosphatase and the total number of spermatozoids, while the activity of alkaline phosphatase correlates only with the number of vital and active spermatozoids. *Turner and McDonnell* (2003) reproted that majority of semen plasma Aph activity originates from the epididymis and testicle. In this way Aph activity can be used clinically as a marker to differentiate testicular origin of spermatozoids disorders from ejaculatory failure. The tendency of decreasing of sperm motility and survivability in third ejaculates corresponding with significantly going down Aph activity. Thus allow us to consider the third ejaculates as a

non full values and non suitable for successfully artificial insemination.

**Fig.3. Correlative relationship between activity of ApH and concentration of the spermatozoa in first ejaculates of young rams ( $r = 0,67$  ;  $Y = 903.8405+36.1419*x$ ;  $P<0,05$ ).**



**Fig.4. Correlative relationship between activity of APH and sperm volume in second ejaculates of young rams ( $r = - 0,70$  ;  $Y = 0.9921-0.0189*x$ ;  $P<0,05$ ).**



## Conclusion

The obtained results allow following conclusions:

1. It was established the age depending significant differences in concentrations of spermatozoids between young and mature rams of Il-de-France breed in each consecutive ejaculate (Table 1).
2. In both groups the concentration of spermatozoids significantly decreased from first to third ejaculate ( $P < 0,01$  for mature,  $P < 0,05$  for young rams).
3. In the consecutive ejaculates of both groups no significant morphological changes of spermatozoids were defined (Table 2)
4. The activity of the alkaline phosphatase in the semen plasma significantly reduce in the third consecutive ejaculates of both ram groups in compare with first and second ejaculates ( $P < 0,05$  for mature rams,  $P < 0,01$  for young rams).
5. In first ejaculates the activity of ApH correlates positively in young rams with sperm volume and concentration of spermatozoids, in mature rams - with sperm volume only (Table 3).
6. In second ejaculates of both groups the negative correlation between sperm volume, concentration of spermatozoids and activity of alkaline phosphatase was observed (Table 3).
7. On the base of these results we could give reference for use of the first and second ejaculates in the practical insemination of ewes.

## MORFOLOŠKE I BIOHEMIJSKE KARAKTERISTIKE KONSEKUTIVNIH EJAKULATA OVNOVA RASE IL DE FRANS RAZLIČITIH UZRASTA

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### Rezime

U poljoprivrednoj praksi, ovan se koristi za osemenjavanje većeg broja ovaca prema odgajivačkom planu. Na primer, za obezbeđivanje uspešne inseminacije ovaca koje su dostigle estrus istovremeno, neophodno je dobiti nekoliko konsekutivnih ejakulata od jednog ovna.



Cilj ovog rada je ocena kvaliteta asperme konsektivnih ejakulata ovnova rase il de frans različitih uzrasta. Više od 40 ejakulata od 5 ovnova iz stada koje se nalazi u Institutu za stočarstvo u Kostinbrodu, u uzrastu od 1, 5 i 4 godine je ispitano tokom sezone parenja. Životinje su podeljene u dve grupe: mladi i zreli ovnovi. Sperma je sakupljana pomoću veštačke vagine. Ocenjivani su sledeći morfološki i biohemijski parametri: zapremina, broj spermatozoida u 1ml, pokretljivost, procenat opstanka na 39°C i aktivnost alkalne fosfataze u plazmi semena.

Utvrđeno je da je uzrast imao signifikantan uticaj na koncentraciju spermatozoida između mladih i zrelih ovnova rase il de frans u svakom konsektivnom ejakulatu.

U obe grupe koncentracija spermatozoida se signifikantno smanjivala od prvog do trećeg ejakulata ( $P < 0,01$  kod zrelih,  $P < 0,05$  kod mladih ovnova). U konsektivnim ejakulatima obe grupe nisu utvrđene morfološke promene spermatozoida. Aktivnost alkalne fosfataze u plazmi semena je signifikantno smanjena u trećim konsektivnim ejakulatima ovnova obe grupe u poređenju sa prvim i drugim ejakulatima ( $P < 0,05$  za zrele ovnove,  $P < 0,01$  za mlade ovnove). U prvim ejakulatima aktivnost ApH je bila u pozitivnoj korelaciji kod mladih ovnova sa zapreminom sperme i koncentracijom spermatozoida, kod zrelih ovnova – samo sa zapreminom sperme. U drugim ejakulatima ovnova obe grupe utvrđena je negativna korelacija između zapremine sperme, koncentracije spermatozoida i aktivnosti alkalne fosfataze. Na osnovu dobijenih rezultata možemo preporučiti korišćenje prvih i drugih ejakulata za osemenjivanje ovaca.

## References

- AMBRIZ D., ROSALES A.M., SOTELO R., MORA J.A., ROSADO A., GARCIA A.R. (2002): Changes in the quality of rabbit semen in 14 consecutive ejaculates obtained every 15 minutes. *Arch Androl.* 48(5):389-95.
- BERGMEYER, H., E. BERNT (1965): *Methods of Enzymatic Analysis*, Acad. Press, New-York-London
- MATTNER P. AND VOGLMAYR J. (1962): A comparison of ram semen collected by the artificial vagina and by electro-ejaculation. *Australian Journal of Experimental Agriculture and Animal Husbandry* 2(4) 78 – 81.
- NEL-THEMAAT L, HARDING G.D, CHANDLER J.E, CHENEVERT J.F, DAMIANI P, FERNANDEZ JM, HUMES PE, POPE CE, GODKE R.A. (2006): Quality and freezing qualities of first and second ejaculates

collected from endangered Gulf Coast Native rams. *Anim Reprod Sci.* ,95(3-4):251-61.

PARKER G. AND THWAITES C. (1962): The effects of undernutrition on libido and semen quality in adult Merino rams. *Australian Journal of Agricultural Research* 23(1) 109 – 115.

RITAR A., MENDOZA, G., SALAMON, S. AND WHITE I.G. (1992): Frequent semen collection and sperm reserves of the male Angora goat (*Capra hircus*) *Journal of Reproduction and Fertility* ( 95): 97-102.

SHAMSUDDIN, M., AMIRI Y. AND BHUIYAN M.M.U. (2000): Characteristics of Buck Semen with Regard to Ejaculate Numbers, Collection Intervals, Diluents and Preservation Periods. *Reproduction in Domestic Animals* 35 (2), 53–57.

SINGER R., BARNET, M., ALLALOUF, D., SCHWARTZMAN, S., SAGIV, M., LANDAU, B., SEGENREICH, E., SERVADIO C. (1980): Some properties of acid and alkaline phosphatase in seminal fluid and isolated sperm *Arch. Androl.*, 5(2), p.195-199.

UZUNOV G., PETKOVA, O., POPOVA M. (1998): *Biochemistry of animals*. Sofia, 345 pp