

MODIFICATIONS OF SOME PARAMETERS OF THE RABBIT EJACULATE AFTER ABLATION OF THE VESICULAR GLAND

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ABSTRACT : An evaluation is made of the influence which the vesiculectomy may have on the ejaculate in the rabbit. To do this we have studied three different factors in the semen, before and after the operation: quality control parameters (volume, vitality and concentration), presence or not of the mucous plug and levels of different ions (sodium, potassium, calcium and magnesium) in the rabbit's seminal plasma. At the same time we performed periodical determinations of these ions in the blood plasma, in order to rule out

the fact that the variations of the cation levels in the seminal plasma could be due to alterations of these levels in the blood reduction in the seminal quality parameters analysed (46,4% for concentration, 29,5% for vitality and 45,6% for volume). On the other hand, as far as the concentration of the different ions in seminal plasma is concerned, a significant fall in the case of sodium (21,1%) is observed and a parallel increase of the calcium, magnesium and potassium ions, although the difference is only significant in the first of the three (19%).

RESUME : Modifications de quelques paramètres de l'éjaculat du lapin après ablation de la vésicule séminale.

Cette étude évalue l'influence que peut avoir la vésiculectomie sur l'éjaculat du lapin. Pour cela nous avons étudié 3 paramètres de la semence avant et après l'opération : contrôle de la qualité (volume, vitalité et concentration), présence ou non de mucus, taux des différents ions (Sodium, Potassium, Calcium et Magnésium) dans le plasma séminal du lapin. Parallèlement nous avons contrôlé périodiquement la concentration de ces ions dans le plasma sanguin afin de vérifier si la

variation du taux de ces cations dans le liquide séminal n'est pas dû à leur variation dans le plasma sanguin. Nos résultats montrent qu'une vésiculectomie partielle conduit à la disparition du mucus dans l'éjaculat, ainsi qu'à une réduction significative des paramètres de qualité analysés (46,4 % pour la concentration, 29,5 % pour la vitalité et 45,6 % pour le volume). La concentration des différents ions dans le plasma séminal est peu affectée, bien que l'on observe une diminution significative du taux de Sodium (21,1 %) et une augmentation parallèle du Calcium, du Magnésium et du Potassium, avec un différence significative (19%) seulement pour le premier des trois.

INTRODUCTION

Over the last few decades rabbit breeding has increased considerably within modern stockbreeding. Traditional family farms have given way to an industry which employs all the technological and production advances that exist on the market. The rapid evolution of the consumption models by an industrialised society has meant that the demand for food of animal origin has undergone a great increase (AUXILIA, 1985). Therefore, in the future, the majority of the meat will come from animals with a short life cycle, the rabbit being one of the most outstanding; no other domestic mammal can produce as much protein in such a short time as the rabbit (VERVUEREN, 1981).

Rabbit breeders will include all those techniques in their production systems which lead to increasing the production, artificial insemination being one of the most important reproduction techniques. Perfecting these techniques requires quality semen, so this point must be studied in depth. Different organs contribute to the formation of the ejaculate throughout the genital tract, especially the accessory glands. The contribution made by these secretions will not only help towards the maturing and discapacitating of the sperm, but they will also nourish them and help them progress along the genital tract. Thus, the mobility and metabolic activity of these cells are stimulated as the different secretions are incorporated from the accessory glands during ejaculation (Mc.DONALD, 1989).

Within the accessory glands, the role played by the seminal vesicle is very important. This gland contributes to a great extent to the volume of the fluid which bathes the sperm; it affords substances such as citric acid, of great importance for the nutrition and viability of the sperm, and cations, the most important being: Na⁺, K⁺, Ca₂⁺ and Mg₂⁺. Sodium and potassium carry out essential functions, supporting the basic-acid equilibrium, working on the hydric balance and its distribution and the osmotic equilibrium remaining normal.

Calcium and magnesium are necessary to maintain the mobility of that specialised cell which is the sperm (ALVAREZ and STOREY, 1983).

The vesicular gland, *glandula seminalis* or seminal vesicle (HOLTZ and FOOTE, 1978), is an azygous, medium organ, the front end being bilobate. From the histological viewpoint, it is a simple alveolar gland with an extremely puckered surface and with a uniform pseudo-stratified secretory epithelium (SCHULT, 1977), the greatest mucosa thickness being found in the cranial area. It presents several lateral evaginations, which start from an irregularly ramified lumen (FAWCETT, 1987) and which are often anastomosed forming cavities. Metabolically it presents an inconstant alkaline phosphatase activity which makes the production of vesiculase possible. This substance, on joining the prostatic vesiculase will give rise to the vaginal plug (RACHAIL-BOURCIER, 1973). When those two secretions mix together the viscosity of the ejaculate increases and the vaginal mucous plug is formed (ARVEUX, 1985), preventing the sperm from flowing back after the mount.

The aim of this work has been to determine the influence of the partial vesiculectomy on the seminal quality and on the formation of the vaginal plug. An analysis is also made of the role of this gland in the intake of certain ions in the seminal plasma: sodium, potassium, calcium and magnesium.

MATERIAL AND METHODS

Animals

Fifteen male rabbits crossed industrially between the California and Spanish Giant have been used. The average weight was 4 kg and the age varied between 11 months and two years. During the experiment the animals were housed in individual cages with an upper opening, and they had water and food *ad libitum*. The animals were kept in buildings at a controlled temperature (16 °C) and exposed to a light cycle of 10L:14D (From 8.00 am to 6.00 pm)

Surgical operation. Vesiculectomy

To carry out the operation, and after 24 h fasting, the animals were tranquillised with a Droperidol and Phentanyl compound. Then, they were submitted to an anaesthetic induction with a gaseous mixture of oxygen and 5% halothane, also performing an epidural anaesthesia with 2% Xylocain. The anaesthetic was maintained throughout the operation with the same gaseous mixture at 1.5%, using an Ayre T-system and mask. In all the cases a partial vesiculectomy was performed with acusector, which covered approximately 80% of the gland surface, always extirpating the cranial area which is where the mucosa is the thickest

Obtaining ejaculates

The collection technique used was that of an artificial vagina, using a live female as a support. The bucks were submitted to a collection rhythm of two per week. After carrying out the vesiculectomy, and after a variable recovery period, the collections were continued, using the same methodology.

Seminal analysis

The ejaculates were studied in a similar way before and after the surgical operation.

1 - At the time of the collection the presence or not of the mucous plug in the ejaculate was recorded. Later the volume of the sample was checked and two seminal contrast tests were performed: spermatic concentration by microscopic visual recount in a Bürber cell-count chamber and vital staining of the sperm, by means of the nigrosin-eosin technique.

2 - Then, the ejaculates were centrifuged at 2,500 r.p.m. for 10 minutes. The supernatant seminal plasma was deposited in Ependorf tubes which were suitably identified and frozen at -20°C until the time of their analysis. The different ions were determined with a variable technology in each case. For the sodium, potassium and calcium ions, a Jenway Ltd. flame spectrophotometer, model PFP7, was used, with air-butane flame and special filters for each one of them. To determine the concentration of the magnesium ion a Thermo Jarrel Ash atomic absorption spectrophotometer, model Smith-Hieftje, was used, with air-acetylene flame.

3 - During the whole experiment a periodical analysis of the levels of these same cations in the blood was carried out in order to verify if the changes observed in their concentrations in seminal plasma was exclusively due to the vesiculectomy or if on the contrary they could be due to variations on a general organic level

Statistical analysis

In order to homogenise the great viability which this species presents in its ejaculates, both among the different bucks and among the ejaculates of the same individual, 45 pre-operative ejaculates and 45 post-operative ejaculates were taken at random (after a period of not less than two months and when all of them showed a normal jumping rhythm) and were analysed as if they were from a pool. The statistical study was carried out by variance analysis (ANOVA) using a computer program, Sigma Horus.

RESULTS.

The animals did not show any pathological alterations or variations in their sexual behaviour as a result of the surgical operation. However, the bucks needed a recovery period which

varied from individual to individual from a month and a half to three months, before being able to obtain the first ejaculate after the operation and progressively reach the same collection rhythm as described in the previous section.

Presence of the mucous plug

Of the 45 ejaculates collected before the operation, 10 presented a mucous plug (22.2%), all of them corresponding to only 6 animals. After the operation no mucous plug was recorded from the ejaculates obtained ($P < 0.01$) from the same 6 males. The ejaculates of these rabbits did not show any significant differences in any of the parameters studied compared with those of the rabbits which did not have this mucous plug. After the operation none of the forty-five ejaculates presented a mucous plug ($P < 0.01$)

Seminal feature (Table 1)

The analysis of the three factors studied for the seminal feature did not show any variations among the ejaculates which presented a vaginal plug and those which did not. In all the cases, the parameters analysed have shown statistically significant differences before and after the operation ($P < 0.01$). In this sense, a reduction of 45.6% is observed in the mean volume values, 46.4% in the case of the concentration and a lower vitality index in 29.5% after the vesiculectomy.

Study of the cation concentration (Table 2)

The analysis of the different ions in the blood plasma has not varied throughout the whole experiment, so we can only report on the values obtained in the seminal plasma.

On comparing the concentration of the different ions before and after the surgical operation, a significant reduction in the sodium ion is observed ($P < 0.01$). Parallel to this, an increase in the mean values of the rest of the ions is observed, although this variation is only statistically significant in the case of calcium.

DISCUSSION

The mean pre-operative values in the seminal parameters obtained in our study are similar to those shown by other authors, such as ESPINOSA *et al.* (19819), HAFEZ (1989), M.A.P.A. (1989) or BATTAGLINI *et al.* (1990).

In the bibliographic review carried out, we have hardly found any works which analyse the values of the main ions in the rabbit's seminal plasma. In our results we find higher concentrations than those described by QUINN *et al.* (1965) and those observed by USON (1975) in almost all the ions analysed. From our viewpoint, the reason lies in that for his study QUINN only used five rabbits, so the variability between individuals will be greater. USON, on his part, used a different methodology to ours, as he analysed the ion concentrations in the seminal plasma based on ejaculates obtained in the first or/and second collection.

We have not found any reference in literature to the parameters used in our study after performing a partial vesiculectomy. According to RACHAIL-BOURCIER (1973) the vesicular gland contributes to a great extent to the volume of ejaculate, as well as to the intake of sodium ion in the seminal plasma. Therefore, we believe that the fall observed in these two parameters is due only to the nature of the gland itself.

Although a decrease in volume would involve a relative increase in the concentration of sperm, an operation such as that carried out could result in a rise in temperature which would cause a transitory reduction in spermatic production

Table 1: Variations observed in the parameters studied in the seminal feature before and after the surgical operation. All differences are significant (P<0.01)

	Before vesiculectomy		After vesiculectomy	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Volume(ml.)	0.92 ± 0.05	(0.4 - 1.7)	0.50 ± 0.03	(0.2 - 1.4)
Vitality(%)	72.4 ± 3.8	(7 - 100)	51.0 ± 4.1	(0 - 99)
Concentration (spz.x10 ³ /mm ³)	649 ± 68	(55 - 1557)	348 ± 7	(5 - 1812)

Table 2: Variations observed in the concentration of the ions analysed in plasma before and after the surgical operation.

	Before vesiculectomy		After vesiculectomy	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Sodium (p.p.m.)	1516 ± 66 ^a	(842-3064)	1196 ± 53 ^b	(563-1928)
Potassium (p.p.m.)	997 ± 45	(400-1941)	1069 ± 52	(421-2417)
Calcium (p.p.m.)	206 ± 8 ^a	(115-329)	245 ± 8 ^b	(133-2417)
Magnesium (p.p.m.)	438 ± 31	(123-946)	459 ± 34	(113-1378)

a, b = P<0.01

(PLÖEN, 1972). On the other hand, the post-operative ejaculates analysed corresponded to bucks whose jumping frequency was similar to that before the operation, at least two months having elapsed in the buck which first recovered the ejaculate rhythm after the operation. We believe that the descent observed in the spermatid concentration is not due to the gland acting as a reservoir, but to the drawing effect which occurs at the time of ejaculation, which entails a carrying of sperms - we must remember that the reduction in volume is 45.6%. The decrease in the vitality index would be due exclusively to the alteration in the medium where the sperm are located (MANN, 1964).

On the other hand, a post-surgical increase in the concentration of the potassium, calcium and magnesium ions has been recorded, although when carrying out the statistical study it is observed that this is only significant in the case of the calcium ion. This ion, according to MORTON *et al.*, (1978) is essential in the gelification process of the mucous plug, a fact, which we must remember, does not occur in any case after the vesiculectomy.

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