

SPERM ABNORMALITIES AS POSSIBLE INDICATORS OF RABBIT CHRONIC HEAT STRESS

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ABSTRACT : Rabbit sperm abnormalities have been studied in conditions of chronic heat stress. Very significant increases ($P < 0.001$) have been observed. The maximum was in the fourth week of stress when total abnormalities were $40.5 \pm 13.2\%$ (+122.5 % in comparison with the pre-stress period) ; tail abnormalities were $26.5 \pm 10.5\%$ (+94.9 %) ; broken spermatozoa $11.1 \pm 7.1\%$ (+552.9 %) ; bent tails $2.3 \pm 1.7\%$ (+76.9 %) ; coiled tails $24.1 \pm 10.3\%$ (+164.8 %). Head abnormalities and cytoplasmic droplets showed a decrease in

the first weeks followed by a late increase. Combined rates have been studied to be used as chronic heat stress indexes. Ratio between percentages of broken spermatozoa and tail abnormalities showed a quick and constant increase, while ratio between percentages of cytoplasmic droplets and coiled tails decreased to zero from the second to the fourth week to increase regularly after this period. Simultaneous observation of the two ratio trends should permit to judge about the time lag rabbits have been exposed to heat stress.

RÉSUMÉ : Anomalies des spermatozoïdes comme possibles indicateurs de stress de chaleur chronique chez le lapin.

On a étudié les anomalies des spermatozoïdes de lapins soumis au stress de chaleur chronique. De très significatives augmentations ($P < 0.001$) de ces anomalies ont été observées. Le maximum se situe à la 4ème semaine de stress quand le total des anomalies a atteint la valeur de $40.5 \pm 13.2\%$ (+122.5 % comparé à la période de préstress) ; les queues anormales représentent $26.5 \pm 10.5\%$ (+94.9 %) ; les spermatozoïdes cassés $11.1 \pm 7.1\%$ (+552.9 %) ; les queues incurvées $2.3 \pm 1.7\%$ (+76.9 %) ; les queues frisées $24.1 \pm 10.3\%$ (+164.8 %). Le nombre de têtes anormales et

de gouttes cytoplasmiques a diminué dans les premières semaines, puis augmenté les semaines suivantes. On a étudié les rapports éventuellement utilisables comme index du stress thermique chronique. Le rapport entre les pourcentages de spermatozoïdes cassés et les pourcentages de queues anormales a montré des valeurs rapidement et régulièrement croissantes, tandis que le rapport entre les pourcentages de gouttes cytoplasmiques et les pourcentages des queues frisées a montré une diminution jusqu'à zéro de la 2ème à la 4ème semaine, suivi par un accroissement régulier. L'observation contemporaine des deux tendances permettrait d'évaluer pendant combien de temps les lapins ont été exposés au stress de chaleur.

INTRODUCTION

Rabbit buck sperm output is strongly affected by high ambient temperatures. A number of characters have been taken into consideration by different Authors (KUZMINSKY *et al.*, 1990 ; PANELLA and CASTELLINI, 1990) but information about morphological abnormalities of rabbit spermatozoa is still limited to peculiar topics (BAGLIACCA *et al.*, 1987 ; KASA and THWAITES, 1992 ; VIRAG *et al.*, 1992).

Heat stress, depending on high temperatures, is a seasonal condition, lasting some months in Mediterranean area, and semen evaluation is getting a growing importance in the practice of artificial insemination.

A research has been planned to investigate morphological abnormalities of spermatozoa in rabbit bucks exposed to high temperature for a long period.

MATERIALS AND METHODS

An experimental group of ten rabbit bucks, homogeneous according to age (9 months) and body weight (4.3 ± 0.2 Kg), was set from a larger group controlled for a two months period to choose animals homogeneous also for sperm output (0.6 ± 0.1 ml in the first of two consecutive ejaculations).

The bucks were trained to artificial vagina and were located into a climatic chamber for an adaptation period of three weeks. In this period mean environment temperature was $20.3 \pm 1.1^\circ\text{C}$ (programmed 20°C), relative humidity was $69.1 \pm 3.5\%$ (programmed 70%), light/dark rate was 12/12 hours as indicated for bucks (LEBAS *et al.*, 1984).

The animals were then exposed for two months to a mean environment temperature of $29.8 \pm 0.6^\circ\text{C}$ (programmed 30°C) for 22 hours a day, with a relative remission to $24.7 \pm 0.8^\circ\text{C}$ (programmed 25°C) for 2 hours.

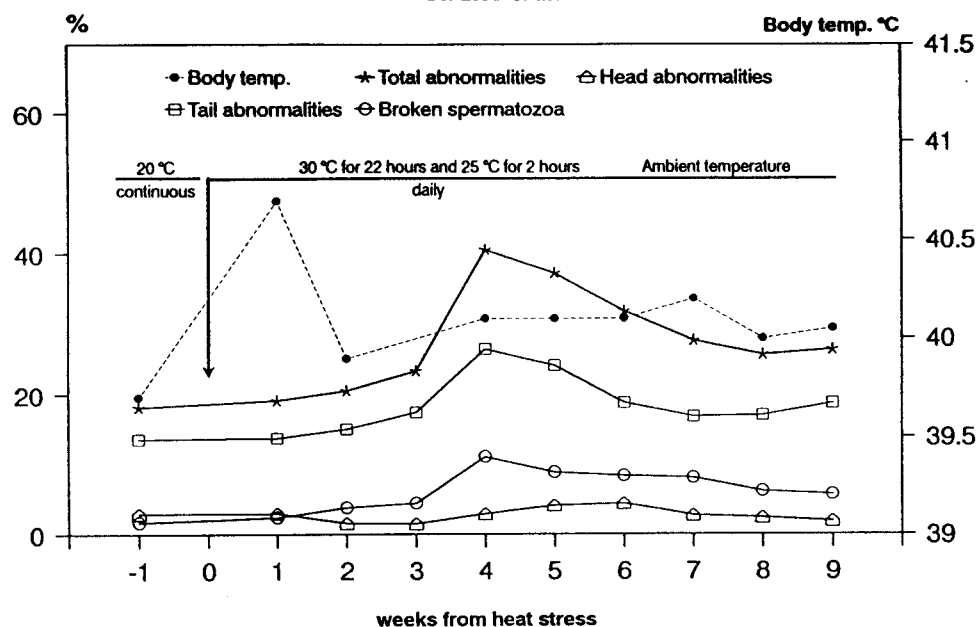


Figure 1 : Body temperature and spermatic abnormalities percentages (on total observed spermatozoa ; percentages based on 6000 cells observed for week "-1", and on 4000 cells for the others) in rabbits exposed to chronic heat stress.

These periods have been set considering that 30°C is a temperature very near to the limit of 32°C at which, if continuous, together with an high relative humidity, mortality of young rabbits is observed (FINZI *et al.*, 1986) and 25°C is a temperature slightly higher than thermoneutrality (CAMPS *et al.*, 1985 ; COLIN, 1985 ; LEYUN, 1985). Relative humidity and photoperiod were maintained unchanged as in the adaptation phase.

To reproduce standard conditions semen was collected three times a week as in most commercial rabbit breeding farms specialized in production of sperm doses for artificial insemination. As in field practice each buck made two consecutive ejaculations in the lag of about 10-20 minutes. Observations were done twice a week on the first ejaculation. To remain into the limits of field practice which must be quick, simple and performed with the means at disposal, spermatozoa were neither fixed nor stained. The analyses have been performed in a maximum of 5 hours from the semen collection. This time is much shorter than the 55 hours of storage when still no morphological alterations of spermatozoa can be observed (BERGONZONI *et al.*, 1994). To perform the quantitative analysis, fresh sperm has been diluted 1:100 by a blood diluting pipette in a 3% NaCl solution to kill the cells and count them. A drop of this solution has been put in a Bürker chamber and, after two minutes (the time required to have a stabilized sample), the analysis begun. The samples tested were 210 (30 in the pre-stress period and 180 in the next 9 weeks). For each sample 200 spermatozoa have been observed. The total was 42000 analysed spermatozoa.

Sperm abnormalities were investigated by a light microscope at x400 as it is done frequently in farm

condition in Italy. The observed abnormalities were numbered and classified (KUZMINSKY *et al.*, 1995) as:

- ◆ normal ;
- ◆ head abnormalities (acrosome abnormalities and abnormalities in shape and dimensions) ;
- ◆ tail abnormalities (bent tails, coiled tails, swollen tails, cytoplasmic droplets) ;
- ◆ broken spermatozoa (1/2 ♂ headless + tailless).

The observed percentages were compared by a chi-square test.

Rectal temperature was weekly controlled six hours after the beginning of the hot period. It was utilised a digital thermometer with a probe introduced in the rectum for about 5 cm.

RESULTS AND DISCUSSION

In comparison with the pre-stress period ($\bar{X} = 18.2 \pm 10.9$ %), total morphological abnormalities (figure 1) showed a significant increase yet in the second week of heat stress (+13.2 % ; $P < 0.05$). The peak occurred at the fourth week of thermal stress ($\bar{X} = 40.5 \pm 13.2$ % ; increase 122.5 % ; $P < 0.001$) and it was followed by decreasing values that, at the ninth week, still remained significantly higher ($P < 0.001$) in comparison with the control period. This result indicates that chronic heat stress seems to induce a defence mechanism leading, after some weeks, to a lowering of morphological abnormalities in comparison with the maximum. This decrease can be interpreted in terms of acclimatization to hot environment (FINZI *et al.*, 1988 ; WELCH, 1993). The result is possibly mediated by body temperature which, after a quick increase (40.7°C, figure 1), succeeded in

RABBIT SPERM ABNORMALITIES

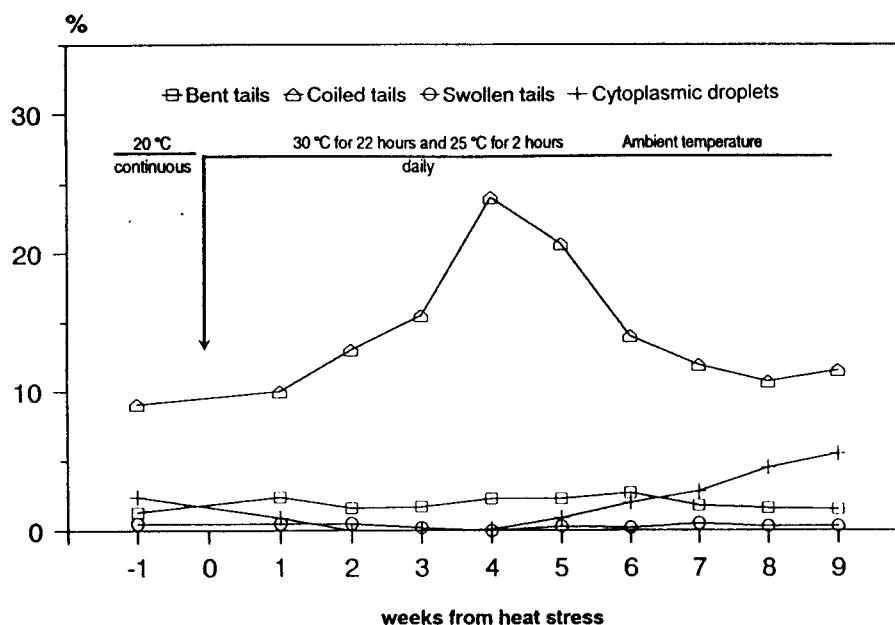


Figure 2 : Percentage trend of spermatozoa with tail abnormalities in rabbits exposed to chronic heat stress.

stabilizing itself (about 40.1°C), so that a situation of moderate hyperthermia persisted in comparison with physiological conditions.

The observed data are coherent with literature on rabbit spermatogenesis which was observed to last 43-44 days plus 8-10 days for epididymal transport (SWIESTRA and FOOTE, 1965). Maximum sensitivity towards the formation of abnormal traits can be calculated about the fifth week of spermatogenesis. This period, as analytically described by the over mentioned Authors, would correspond to the stage of spermatid formation.

Components of total abnormalities are also analysed in figure 1. Trend of total abnormalities was mainly determined by tail abnormalities and broken spermatozoa. Significant differences in relation to the pre-stress period (tail abnormalities $\bar{X} = 13.6 \pm 6.5\%$; broken spermatozoa $\bar{X} = 1.7 \pm 2.0\%$) appeared from the third week for tail abnormalities (+28.7%; $P < 0.001$) and from the second week for broken spermatozoa (+123.5%; $P < 0.001$). The peak was at the fourth week for both parameters (increase 94.9% and 552.9%, respectively; $P < 0.001$). As it can be observed variability is very high, sometimes even higher than the mean. This condition is very commonly observed in literature (SAACKE, 1970; GALLI and BOSISIO, 1986).

Tail abnormalities are analysed in detail in figure 2. In the pre-stress period the mean value of coiled tails was $9.1 \pm 4.2\%$. Heat stress caused a significant increase of this abnormality already in the second week of stress (+42.9%; $P < 0.001$). Also in this case the peak occurred in the fourth week (+164.8%; $P < 0.001$) and it was followed by decreasing values. Nevertheless

they remained significantly higher than in the control period till the end of the experiment ($P < 0.01$). Cytoplasmic droplets showed a trend opposite to other abnormalities. In comparison with the pre-stress value ($\bar{X} = 2.4 \pm 1.9\%$) a significant decrease ($P < 0.01$) was observed from first to fifth week of stress. In the second and third week no cytoplasmic droplets were observed. The parameter began to increase again from the fifth week and in the ninth week the values were significantly higher than in the pre stress period (+129.2%; $P < 0.001$). No particular trend was showed by bent and swollen tails.

These results suggest that, as spermatozoa are very sensitive cells in regard to high temperature, morphological abnormalities could be assumed as indicators of heat stressing conditions suffered by the animals.

The limit could be established by the value of 23.4% total abnormalities observed in the third week of stress and significantly higher in comparison with the control ($P < 0.001$). Until effects of stressing conditions and other factors on sperm abnormalities will be better studied it could be stated that, if total sperm abnormalities are, as a mean, more than 23.4% of total observed spermatozoa, it is possible that bucks (as to say all the animals in the breeding) have undergone a chronic heat stress (longer than three weeks).

To simplify the analysis only some abnormalities could be tested. For instance it could be utilised as a limit only the number of tail abnormalities (more than 17.5%) or the number of coiled tails (more than 13.0%). The latter are very easy to be observed with a light microscope in field conditions and it could be

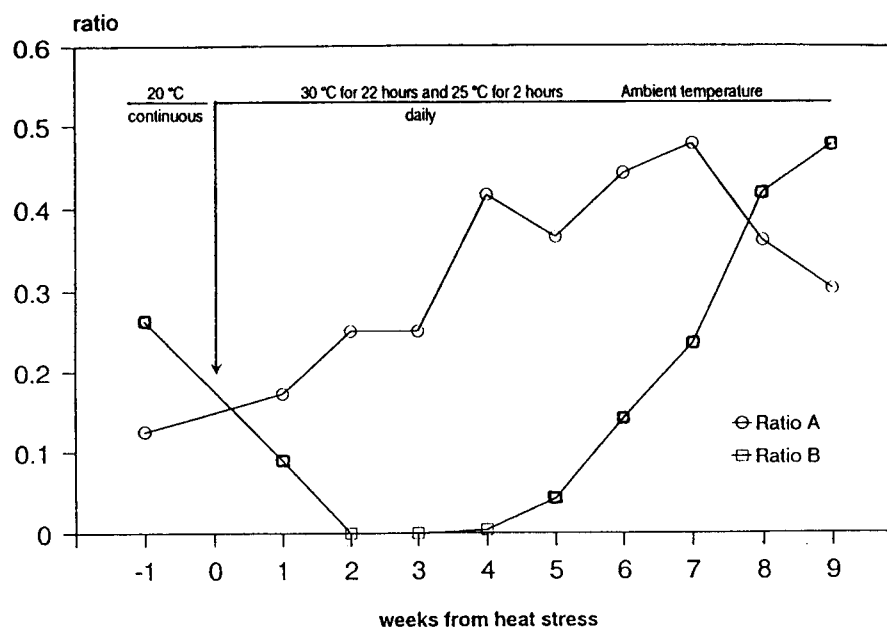


Figure 3 : Trend of ratio between broken spermatozoa and spermatozoa with tail abnormalities (Ratio A) and between spermatozoa with cytoplasmic droplets and spermatozoa with coiled tails (Ratio B).

utilised in the practical breed management. Total tail abnormalities and coiled tails are obviously correlated with total sperm abnormalities ($r = 0.92$ and 0.80 respectively ; $P < 0.001$).

In field conditions it could be useful to verify if rabbit breedings have suffered chronic heat stresses. To get more sensible indicators combined rates able to amplify the observed responses have been studied. The best ones are shown in figure 3. The ratio between percentages of broken spermatozoa to percentages of spermatozoa with tail abnormalities (ratio A) was quickly increasing. In the pre-stress period it was 0.13. From the very beginning of the heat stress the increase of the ratio was significant (first week: +30.8 % ; $P < 0.05$). It reached the maximum at the seventh week (+269.2 % ; $P < 0.001$) and it was still sensible after 9 weeks of heat stress ($P < 0.001$).

The trend is obtained though the two parameters are positively correlated ($r = 0.883$; $P < 0.001$), since variations are not proportional.

On the contrary the ratio between percentages of spermatozoa with cytoplasmic droplets and percentages of spermatozoa with coiled tails (ratio B) showed a quick decrease. In the pre-stress period it was 0.26. In the first week it was 0.09 (-65.4 % , $P < 0.001$). The trend showed an increase after the fourth week of heat stress.

If the two trends are read contemporary it can be said that an increase of ratio A with a decrease of ratio B indicate a recent heat stress from one to five weeks. When both values are high it means that the animals

have been exposed to high temperatures for a period of at least eight weeks, mainly if ratio B has become again higher than ratio A.

In conclusion the analysis of morphological abnormalities of spermatozoa indicates that the observed parameters are sensible to heat stress and they change according to the exposure time. Morphological abnormalities could thus be used as a routine to test both sperm quality and breeding conditions with reference to high ambient temperature, mainly in tropical and subtropical countries where lowering of fertility can be due to this factor.

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REFERENCES

- BAGLIACCA M., CAMILLO F., PACI G., 1987. Temperatura e performance di conigli maschi riproduttori. *Riv. di Coniglicoltura*, 24 (10), 61-65.
- BERGONZONI M.L., ZAMBELLI D., SAMOGGIA G., 1994. Influenza del diluitore, della temperatura e del periodo di conservazione del seme nella F.A. cunicola. *Riv. di Coniglicoltura* 31 (4), 37-40.
- CAMPS J., CEREZA J.M., ROSELL V., 1985. Efectos del calor y frio excepcionales en la producción de los conejares. in: *Act. X Symp. de Cunicultura ASESCU, Barcelona, nov. 1985*, 237-248.

- COLIN M., 1985. Les problèmes liés a l'été dans l'élevage du lapin. *Cuniculture*, 12 (3), 177-180.
- FINZI A., KUZMINSKY G., MORERA P., AMICI A., 1986. Alcuni aspetti della termotolleranza nel coniglio. *Riv. di Coniglicoltura*, 23 (12), 51-55.
- FINZI A., MORERA P., KUZMINSKY G., 1988. Acclimation and repeatability of thermotolerance parameters in rabbit. in: *Proc. 4th World Rabbit Congr., Budapest, oct. 1988, vol. 2, 419-426.*
- GALLI A., BOSISIO M., 1986. Quality semen stored at +15°/16°C and fertility in swine artificial insemination. In : *Proc. Int. Congr. on Fertility Laboratory Techniques II, Bergamo, 155-159.*
- KASA I.W., THWAITES C.J., 1992. Semen quality in bucks exposed to 34°C for 8 hours or either 1 or 5 days. *J. Appl. Rabbit Res.* 15, 560-568.
- KUZMINSKY G., MORERA P., FINZI A., 1990. Tempi di raccolta dell'ejaculato come misura della libido nel coniglio. In: *Atti III Meet. Naz. Studio della efficienza riproduttiva degli animali di interesse zootecnico, Bergamo, nov. 1990, 139-143.*
- KUZMINSKY G., FAUSTO A.M., MORERA P., 1995. Morphological abnormalities of rabbit spermatozoa studied by scanning electron microscope and quantified by light microscope. (to be published).
- LEBAS F., COUDERT P., ROUVIER R., ROCHAMBEAU H. de, 1984. Le lapin. Elevage et pathologie. F.A.O., Rome, Italy.
- LEYUN M., 1985. Control ambiental. *Boletín de Cunicultura*, 30 (8), 30-33.
- PANELLA F., CASTELLINI C., 1990. Fattori ambientali e genetici che influiscono sulle caratteristiche del seme di coniglio. *Riv. di Coniglicoltura* 27 (8), 39-41.
- SAACKE R. G., 1970. Morphology of the sperm and its relationships to fertility. In : *Proc. III Techn. Cong. Anim. Reprod. Artif. Ins. Nat. Assoc. Animal Breeding, Chicago, 17-30.*
- SWIESTRA E.E., FOOTE R.H., 1965. Duration of spermatogenesis and spermatozoon transport in the rabbit based on cytological changes, DNA synthesis and labelling with tritiated thymidine. *Am. J. Anat.*, 116 (2), 401-412.
- VIRAG G., MEZES M., BERSENYI A., 1992. Effect of independent factors on semen characteristics in rabbits. *J. Appl. Rabbit Res.* 15, 499-504.
- WELCH W. J., 1993. Come le cellule reagiscono allo stress. *Le Scienze*, 51 (299), 50-57.