

with fresh or deep-frozen semen 24 or 36 hours after injection of 500 U.I. hCG at proestrus. After slaughtering, oocytes were collected, treated according to the classical histological techniques (10  $\mu$ -sections) and observed by photon microscopy.

Results showed that the percentage of oocytes fertilized per sow and the number of spermatozoa per egg were significantly lower in sows inseminated with frozen semen than in those inseminated with fresh semen (37.2 vs 81 % fertilized oocytes and 0.9 vs 36.9 spermatozoa/egg, respectively).

In contrast, histological analysis of the eggs did not show any difference between frozen and fresh semen relative to fertilization quality. Each oocyte penetrated by a frozen spermatozoon emitted the second polar globule and developed. No specific abnormality related to insemination with frozen semen was observed in the activation process.

Results showed a predominance of the pronucleus stage in all groups and the presence of segmented eggs only after insemination with fresh semen.

Whatever the previously studied parameters there was no difference between animals inseminated at different times after hCG.

### **Effect of freezing on the nucleus of boar spermatozoa**

S. HAMAMAH <sup>(1)</sup>, M. PAQUIGNON <sup>(2)</sup>, J.C. NICOLLE <sup>(1)</sup>

- (1) *INRA, Station de Physiologie de la Reproduction, Nouzilly, 37380 Monnaie.*  
(2) *Institut Technique du Porc, M.N.E., 149, rue de Bercy, 75595 Paris Cedex XII.*

A study was made with the aim of testing the effect of deep-freezing on the nucleus quality of boar spermatozoa. Two techniques of analysis were used : staining sperm with acridine orange to study DNA denaturation and cytophotometric measurement of chromatin after staining DNA by Feulgen method.

Results showed that after staining with acridine orange, the percentage of spermatozoa whose nucleus possessed denatured DNA did not change before and after freezing (7.1 vs 10.7 %, respectively). In contrast, freezing caused a significant reduction in DNA-Feulgen content of the nucleus (4.83 vs 4.27, respectively before and after freezing) and in its surface (31.3  $\mu\text{m}^2$  vs 30.5  $\mu\text{m}^2$ , before and after freezing, respectively). Quantitative analysis of DNA-Feulgen showed an effect of freezing on the structure of chromatin.

### **Bacteriological characteristics of boar semen used in artificial insemination**

F. MADEC

*Ministère de l'Agriculture, Direction de la Qualité,  
Station de Pathologie Porcine, B.P. 9, 22440 Ploufragan*

Two trials were conducted in order to assess the bacterial contamination of extended semen under practical farm conditions. A preliminary trial involving 20 semen samples prepared for artificial insemination showed that semen microflora remained quite steady during the first 24 hours of storage at 18-20 °C. However, after 48 hours of storage at 20 °C a significant increase in contamination was observed. A second trial involving 60 ejaculates from 22 boars (4 herds) confirmed the previous findings : extended semen routinely prepared on-farm (without addition of antibiotics) may be severely contaminated and figures as high as 10<sup>5</sup> bacteria per ml of extended semen were found in 8 % of the samples. After 48 hours of storage the number of contaminated