implantation) were obvious and commonly encountered. Apart from a few instances of acrosome lipping, no acrosome defects were observed in the material studied.

The principal tail defects were double tails, stump tails, kinked tails and bent tails. SEM of double-tailed sperm generally revealed a normal head and two perfectly formed tails consisting of a midpiece, chief-piece and end-piece. Separation of the two tails was variable along the length of the double flagellum. The morphology and alignment of the axoneme, mitochondrial sheath and connecting piece of the neck region all appeared normal when viewed by TEM. Stump tails displayed a variety of forms and were best visualized by SEM. In some instances the tail was rudimentary, consisting only of a small bead of cytoplasm attached to the base of the head. In other cells the tail was of variable but shortened length and generally displayed no obvious regional characteristics. Kinked tails generally appeared normal except that the head and tail were oriented at right angles to each. Tail bending most often occurred in the region of the midpiece where it was sharply reflected and often associated with a cytoplasmic droplet. Tail coiling, typical of the "Dag defect" was only occasionally seen in the material examined.

Multiple defects were common in cane rat sperm. Nuclear vacuolation (the diadem defect), for example, was often associated with double-tailed sperm as well as with stump-tailed sperm.

The sperm defects observed in cane rat sperm have also been described in various other mammalian species. Nuclear invaginations (vacuolation) have specifically been identified in elongating spermatids and epididymal sperm in two other hystricomorph rodents and this defect would appear to be particularly common to this group of animals. Although no causative factors could be determined for the presence of the various defects in the animals studied, the fact that a number of the defects have been associated with infertility/subfertility makes it important to consider these defects when evaluating cane rat semen.

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Application of variable pressure scanning electron microscopy (VP-SEM) to non-coated biological samples: Improvement of the image quality by using helium gas in a low-voltage, low-vacuum environment

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The variable pressure scanning electron microscope (VP-SEM) has been applied to the observation of biological samples without metal coating. It is also helpful for the observation of wet and/or oily samples. The VP-SEM further has the potential to observe a variety of specimens without the need of conventional specimen preparation. However, the range of the proper observation condition (*i.e.*, the relation between the accelerating voltage and pressure) is generally rather narrow in each sample.

We recently showed that the quality of the VP-SEM image signals (by backscattered electrons) is dramatically improved by introducing helium gas into the specimen chamber (Oho et al. 2000). This method is especially useful in low-voltage as well as in a variety of SEM operating conditions, because helium gas can relatively keep the amount of unscattered primary electrons. In the present study, we mainly describe the quality improvement of the image signals obtained from the newly developed environmental secondary electron detector (ESED), which was originally introduced for low-vacuum SEM (Danilatos 1990; Farley and Shah 1991).

Figures 1a and 1b are ESED images operated under air and helium gas, respectively. The accelerating voltage is 5kV, the working distance 14.6 mm, and the pressure 80 Pa for air and 330 Pa for helium. SEM imaging using the ESED under helium gas was helpful in a whole range of pressure. In contrast, imaging in air was practical only under a relatively low pressure. Under the pressure of 330 Pa, no clear image was obtained under air. Surprisingly, it was very difficult to observe the surface structure of samples even under the pressure of 80 Pa (b), which is a common pressure frequently employed in the VP-SEM routine work.

One of the problems for the practical use of helium gas in the SEM is that the charge neutralization effect is reduced to a certain extent in exchange for the increase of unscattered electrons. However, a high pressure condition increases the charge neutralization effect, which may cancel the charging problem. As described above, the VP-SEM is useful for the observation of wet and/or oily samples without the need of conventional specimen preparation. In general, as the pressure of the specimen chamber increases, a wet sample keeps its natural shape. However, the water content of the specimen under the helium gas is unknown. It is necessary to investigate the effective condition of helium gas for achieving practical charge neutralization effect and for retaining a certain amount of water in specimen as well as for obtaining high quality SEM images.

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