透明帯除去ハムスター卵内におけるヘアピン型尾部を有する豚精子の雄性前核形成

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Male Pronuclear Formation by a Boar Spermatozoon with Hairpin-Curved Tail in Zona-Free Hamster Egg

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Semen contains much or less the morphologically abnormal spermatozoa, but they had been considered not to achieve the fertilization because of the selection in the female reproductive tracts [4] and their differential motility [8].

However, there are some reports describing the structually abnormal human spermatozoa had fused with zona-free hamster eggs and undergone the nuclear decondensation *in vitro* [1, 9]. Since zona-free hamster egg penetration test have also been utilized to evaluate the fertilizing ability of spermatozoa of boar [5, 6], bull [2, 5] and stallion [2], there have been no reports on the penetration of abnormal spermatozoa into zona-free hamster eggs in farm animals.

This paper describes a case of male pronuclear formation derived from a boar spermatozoon with "hairpin-curved" tail in zona-free hamster egg *in vitro*.

The sperm rich fractions were obtained from a mature Large White boar of known fertility using the gloved hand method. The evalution of sperm morphology was made on the smears stained with eosin-nigrosin. Washed sperm suspensions were incubated for 6 hr before insemination. Mature female golden hamsters were superovulated by intraperitoneal injection of 50 IU PMSG followed by 50 IU hCG 56 hr later. The eggs were recovered from the oviducts at 16–17 hr after hCG injection. The cumulus mass and the zona pellucida were removed by the treatment with 0.1% hyaluronidase (Sigma, USA) and 0.1% trypsin (Sigma, USA), respectively.

The procedures for *in vitro* capacitation and *in vitro* fertilization, culture media and culture conditions were the same as described in the previous report [11].

After the 6 to 7 hr incubation, the assessment of penetration was carried out twice under the phase-contrast and differential interference-contrast microscope; firstly, of the freshly mounted egg, and once again after being fixed with methanol: acetic acid (3: 1) followed by staining with 1% aceto-orceine. The eggs having the enlarged sperm head or the male pronucleus with sperm tails were regarded as being penetrated.

The mean value (n=3) for sperm concentration, the percentage of sperm motility and the percentage of spermatozoa with normal morphology were 6.4×10^8/m*l*, 76.7% and 30.8%, respectively. And the proportion of spermatozoa with the hairpin-curved abnormality was 30.8% (34.3–42.3%).

The percentage of penetrated eggs was 83.1% (59/71). Fig. 1 showed two enlarged sperm heads with accompanying normal tails in an egg fixed and stained. The total number of penetrating spermatozoa was 159 and 31 of these (19.4%) had developed into the male pronucleus. Among these penetrating spermatozoa, one had a hairpin-curved tail (Fig. 2) attached to the male

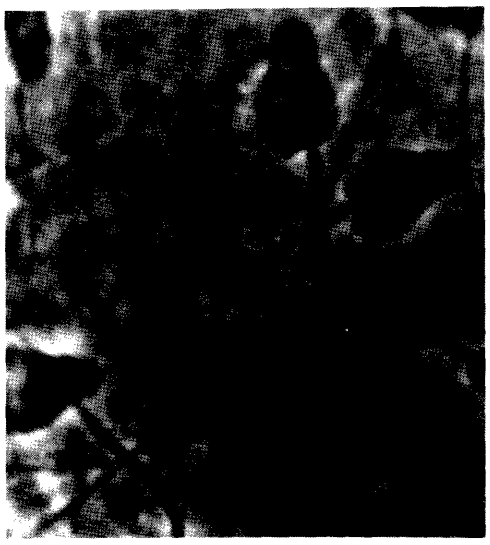

Fig. 1. A fixed and stained egg showing two enlarged sperm heads with accompanying normal tails. Phase-contrast. ×1023.

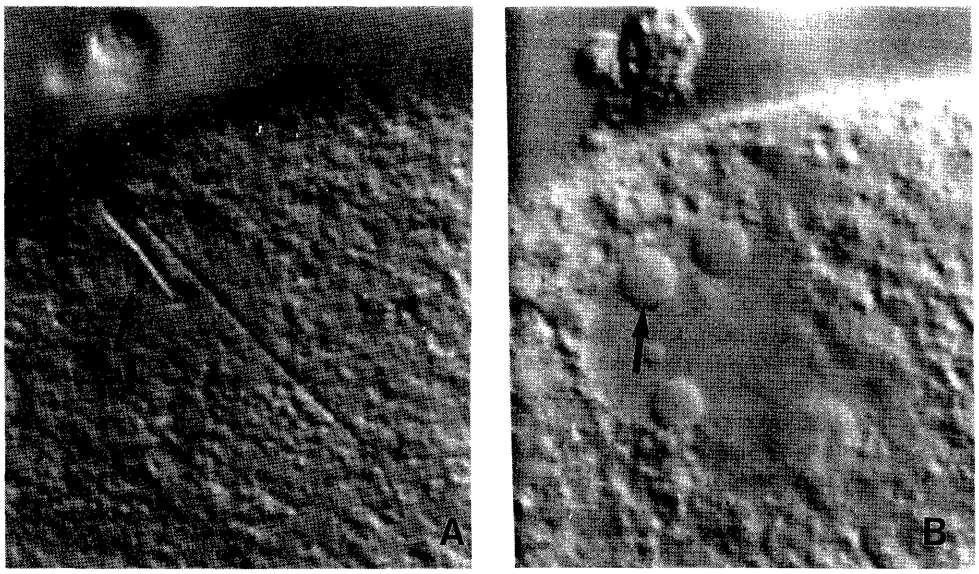

Fig. 2. A freshly mounted egg showing a hairpin-curved tail (A) attached to the pronucleus (B). A and B are different foci. Sperm tail is on the surface of egg, but sperm neck is in the cytoplasma of egg. Arrows indicate the same nucleolus in the pronucleus. Interference-contrast. ×1425.

pronucleus (Fig. 3) in a living egg. The egg penetrated by this spermatozoon was monospermic and had the female pronucleus and the second polar body.

Zona-free hamster eggs permit entry of only alien spermatozoa that have completed both capacitation and the acrosome reaction [10]. Therefore, the result of present study shows clearly that the boar spermatozoon with hairpin-curved tail had capacity to undergo the process of fertilization involving capacitation, the acrosome reaction, fusion with the vitelline membrane and nuclear decondensation. The hairpin-curved spermatozoa have a sharp folding of the tail at the caudal area of the middle piece and show the characteristic backward swimming [7]. This abnormality is commonly observed in boar semen; it is strongly supported that the etiology of this deformity resulted from the disfunction of epididymis [3]. However, it appears that they could not penetrate the zona-intact homogeneous oocytes *in vivo* or *in vitro* because of their differential motility, even though they would have the capacity to undergo capacitation and the acrosome reaction.

On the other hand, immotile human spermatozoa, which characterized by the typical absence of dynein arms or the microtubular defects in their tail, demonstrated the penetration of zona-free hamster eggs [1, 9]. However, this deformity results from a hereditary condition [1] and differs from the present case in morphology and etiology.

Although zona-free hamster egg penetration test may afford an effective information in predicating the fertilizing ability of spermatozoa [9], it should be noted that the morphologically abnormal spermatozoa have the ability to penetrate into those eggs *in vitro*.

REFERENCES

1. Aitken, R. J., Ross, A., and Lees, M. M. 1983. Analysis of sperm function in Kartagener's syndrome. *Fertil. Steril.* 40: 696–698.
2. Brackett, B. G., Cofone, M. A., Boice, M. L., and Bousquet, D. 1982. Use of zona-free hamster ova to assess sperm fertilizing ability of bull and stallion. *Gamete Res.* 5: 217–227.
3. Gusstafsson, B., Einarsson, S., Nicander, L., Holtman, M., and Soosalu, O. 1974. Morphological, physical and chemical examination of epididymal contents and semen in a bull with epididymal dysfunction. *Andrologia,* 6: 321–331.
4. Hafez, E. S. E. 1980. Transport and survival of gametes. pp. 203–225. *In:* Reproduction in farm animals, 4th ed. (Hafez, E. S. E. ed.), Lea & Febiger, Philadelphia.

5. Hanada, A. and Nagase, H. 1981. Effects of sperm preincubation in rabbit uterus and imidazol on the penetration of zona-free hamster eggs by bull and boar spermatozoa *in vitro*. *Jpn. J. Anim. Reprod.* 27: 113–118.
6. Imai, H., Niwa, K., and Iritani, A. 1977. Penetration *in vitro* of zona-free hamster eggs by ejaculated boar spermatozoa. *J. Reprod. Fertil.* 51: 495–497.
7. Kojima, Y. 1977. Light and electron microscopic studies of "hairpin curved deformity" on boar spermatozoa. *Jpn. J. Vet. Sci.* 39: 265–272.
8. Mortimer, D., Leslie, E. E., Kelly, R. W., and Templeton, A. A. 1982. Morphological selection of human spermatozoa *in vivo* and *in vitro*. *J. Reprod. Fertil.* 64: 391–399.
9. Moryan, A. I., Guay, A. T., and Tulchinsky, D. 1986. Normal penetration of hamster ova by human spermatozoa with dyskinetic motility. *Fertil. Steril.* 45: 735–738.
10. Yanagimachi, R. 1984. Zona-free hamster eggs: Their use in assessing fertilizing capacity and examining chromosomes of human spermatozoa. *Gamete Res.* 10: 187–232.
11. Yoshida, M. 1987. *In vitro* fertilization of pig oocytes matured *in vivo*. *Jpn. J. Vet. Sci.* 49: 711–718.

要　　約

透明帯除去ハムスター卵内におけるヘアピン型尾部を有する豚精子の雄性前核形成（短報）：吉田光敏・小島義夫（静岡大学農学部家畜繁殖学教室）——体外培養した豚射出精子を透明帯除去ハムスター卵へ媒精したところ，ヘアピン型尾部を有する精子に雄性前核の形成が観察された．このことから尾部異常精子の中にも，受精能獲得および先体反応を完了する精子の存在が示唆された．