

Effective activation method of *in vitro* maturation of pig ovum

THIS STUDY sought to establish maturation culture and activation methods for immature pig ovum, where *in vitro* maturation of ovum occurs at a high rate during the blastocyst stage of the embryo. In the activation method, butyrolactone-1 (BL-1), which shows inhibitory effect, was used in addition to electric stimulation of kinase in the cell cycle.

Maturation rates of 79.6, 81.6, and 83.6% were obtained for immature pig ovum in the maturation culture solution, when cultivated for 36, 42, and 48 hours, respectively, but the difference was not significant.

The activation rate in the 36-hour culture was significantly lower ($P < 0.05$) compared to those in other culture time. Also, the proportion of normal activation ovum was low in any of the culture time, as a result of carrying out the activation by combined use of BL-1 processing and single electric stimulation.

For the mature ovum cultivated for 48 hours, after the activation was carried out by the combined use of electric stimulation and BL-1 processing, generation culture was carried out using Whitten and mNCSU37 culture solution. The division rate and incidence of blastocyst stage embryo were then examined in the second and sixth culture. In addition, the blastocyst stage embryo was also examined for cell population.

Results of the study show that giving electric and activation stimulation after 48 hours of maturation culture using butyrolactone-1, as well as the use of mNCSU37 for the extracorporeal generation of immature pig ovum, are effective.

For further information, see Bing, Y.Z., Hirao, Y., Iga, K., Che, L.M., Takenouchi, N., Kuwayama, M., Fuchimoto, D., Rodriguez-Martinez, H., and Nagai, T. 2002. In Vitro Maturation and Glutathione Synthesis of Porcine Oocytes in the Presence or Absence of Cysteamine under Different Oxygen Tensions: Role of Cumulus Cells. *Reproduction, Fertility and Development* 14: 125-131.

Imai, K., Kobayashi, S., Kaneyama, K., Kojima, T., and Nagai, T. 2002. Effects of Butyrolactone-I on GVBD in Bovine Oocytes and Subsequent Maturation, Fertilization and Development In Vitro. *Journal of Reproduction and Development* 48:249-255.

Nguyen, V.T., Kure-bayashi, S., Harayama, H., Nagai, T., and Miyake, M. 2003. Stage-specific effects of the osmolarity of a culture medium on the development of parthenogenetic diploids in the pig. *Theriogenology* 59:719-734.

Long-term storage procedure for silkworm fertilized egg

AMETHOD for preserving silkworm fertilized eggs for 2 years was developed. After subjecting 60-day-old eggs to high temperature protection for 20 days, they were stored at -2.5°C for 370 days. Then, the eggs went through a method of protection at 5°C for 90 days, with middle care at $10-15^{\circ}\text{C}$ for 9 days and re-refrigeration for another 120 days at 0°C .

In order to achieve system preservation of one voltinism silkworm and two voltinism silkworm, it is necessary to breed once a year. For this reason, a great amount of time and labor is required. In order to attain labor-saving system preservation, the system-saving method which allows breeding in a 2-year cycle is the most effective. Therefore, the development of a long-term saving method for fertilized ovum had become necessary.

After performing high temperature (25°C) protection on the eggs for 2 months, they passed

through four stages (20°C , 15°C , 10°C , and 5°C) for 10 days, and at 0°C for 5 days each, then refrigerated for 368 days at -2.5°C . In order to achieve activation of the embryo, after protecting the eggs for 90 days at 5°C and performing middle care (10°C for 2 days; 15°C for 4 days), these were re-refrigerated for 120 days at 0°C .

The biggest difference of this newly-developed method from the existing double refrigerating method is the shortened period from high temperature protection to refrigeration, with minimal energy consumption. Temperature change and middle repair frequencies under storage were also reduced in comparison with the prolonged cold storage test of previous reports. The storage process has also been simplified, and the work made easier.

Under patent application 2003-057940

High-precision and high-efficient QTL analysis for iron resistance of rice and fusarium disease resistance of barley

THIS STUDY developed a time- and cost-saving QTL precision analysis for iron depletion resistance of rice plant with complicated genotypic composition, as well as for rust resistance of barley, through the use of High Efficiency Genome Scanning for adequate recombination inbred lines.

The QTL analysis is a powerful technique for analyzing complicated characteristics involving multiple genes. However, the precision of the analysis may not be sufficient when a large number of genetic markers, such as 100-200 individuals, are involved; also, considerable time and costs are needed. Hence, it is necessary to develop a high-precision QTL analysis which is both time and cost effective, and involves high-efficient genome scanning to enable the manufacture of precise genome map.

In the tropical zone, red soil which mainly contains iron oxide called the laterite is widely distributed, and when such land is made into paddy field and begins to dissolve in water, non-resistant rice plants suffer from leaf discoloration, becoming reddish brown, causing growth inhibition. The rice type Indica, which is mainly

grown in the tropical zone, has weak resistance to iron, but the Japonica rice type grown in the temperate zone has comparatively higher resistance to iron.

This study was able to produce high-density map that consists of 1300 markers within half a year, using recombination inbred lines between high-resistant Ginbouzu (Japonica) and low-resistant Kasalath (Indica).

The high-density map was produced by using HEGS/AFLP which was a recombination of inbred lines H.E.S.4 which is sensitive and Russia No. 6 which is rust resistant, in cooperation with the Okayama University resources biology laboratory. Simultaneously, the sitting position of three almost equivalent titers as a result of the QTL analysis of resistance by using the limits spike method was clearly confirmed. The strong interaction existing between this gene and the other genes was also clarified.

For further information, see Hori, K.; Shimizu, T.; Satou, K.; Takeda, K.; Kawasaki, S. *Theor. Appl. Genet.* (Accepted).

Reduction technique for herbicide run-off from paddy fields using charcoal made from rice hull

BY SPRAYING chaff briquette powder over the paddy field 1 day after spraying 50 g/m² of herbicide, the amount of runoff was reduced by about 50%. Moreover, by using the model PCPF-1 which estimates agricultural chemical contents in the paddy field, the optimal diameter of a particle of chaff briquette powder, carbonization temperature, and rate of application can be determined, and an outflow system outside technology can be developed efficiently.

Using the PCPF-1 model, the aging variation of agricultural chemical concentration of paddy field water in the soil surface, the desorption kinetics from the soil surface, and the optimization of soil adsorption ability (coefficient) were estimated and analyzed in order to reduce herbicide runoff from the paddy field by 50%. The chaff briquette (agricultural chemical adsorbent) was produced experimentally to achieve optimized conditions, and an outside system runoff reduction technique of agricultural chemical was developed in the demonstration field.

In the calculation according to the PCPF-1 model, the desorption kinetics of the herbicide was reduced by about one-third, and it was possible that the outside system outflow of agricultural chemicals from the paddy rice was reduced by about 50%, when the soil adsorption coefficient was raised to about 1.5.

Herbicide adsorption ability of the chaff briquette was dependent on the specific surface of the briquette (0.25-0.5 mm), and on the particle size of the 650 μ carbide (the 145 m²/g BET PLC specific surface), which was smaller than that of the 500 μ carbide (45 m²/g). The carbonization temperature was also higher at 0.5-1.0 mm. The adsorption ability was 20-300 times higher in paddy soil with 650 μ carbide, and 80-500 times higher in 500 μ carbide.

For further information, see Watanabe and Takagi, *Environmental Technology*, 21, 1379-1391 (2000).