

**Improvement of Reproductive Efficiency of Artificial Insemination
Following Estrus Induction in Dog
II. Semen Freezing and Artificial Insemination in Dog**

Y. R. Lee¹ and S. Y. Choe

*¹Pusan Animal Hospital & College of Veterinary Medicine, College of Veterinary
Medicine, Gyeongsang National University*

Considerable attention has been focused on the cryopreservation of semen and estrus induction in dog, as consequence of poor productivity caused by long anestrus period, in order to enhance the productivity of youngs and to preserve the breeds. The objectives of this study were to evaluate semen quality after cryopreservation and to evaluate the Pregnancy rate after insemination (AI).

Fifty infertile dogs (age 2~3 years) were selected for the study and divided into three different estrus induction treatment groups. Group 1: dogs (n=15) were given clomifene (0.1 mg/kg) orally for five days at 12 hr intervals. Group 2: dogs (n=15) were given bromocriptine (50 µg/kg) orally for five days at 12 hr intervals, followed by single injection intravenously of 500 IU GnRH (Group 3, n=20) when pro-estrus occurred. The rates of pregnancy in estrus induced dogs mated naturally compared to those inseminated artificially with ejaculated fresh semen and frozen-thawed semen. Estrus detection was performed using the method of vaginal smear and confirmed by the plasma progesterone assay.

The ejaculated semen to freeze was exposed to a mixture of Tris extender with cryoprotectant (Trisma, 81 mM; TES, 209 mM; citric acid, 6 mM; glucose, 5 mM; glycerol, 8%) and cryopreserved gradually by slow-cooling at 17 cm above the surface of liquid nitrogen (LN₂) for 23 min. The motility of frozen-thawed spermatozoa was assessed by phase-contrast microscopy. To assess their viability and acrosome content, spermatozoa were stained with a vital stain and Fluorescence conjugated lectin *Pisum Sativum Agglutinin* (FITC/PAS), respectively. Pregnancy was confirmed by ultrasonography on day 25, 35 and 55 post insemination.

The use of fresh semen, the pregnancy rates were observed 66.6, 66.6, 75.0 and 83.3% in natural estrus, clomifene induced, bromocriptine induced and a combination of GnRH and bromocriptine, respectively. The use of frozen-thawed semen, the pregnancy rates were observed 66.6, 33.3, 50.0 and 60.0% in natural estrus, clomifene induced, bromocriptine induced and a combination of GnRH and bromocriptine, respectively. No difference was observed in the number of offspring produced among natural estrus and treated groups inseminated with fresh or frozen-thawed semen.

In conclusion, the pregnancy rate of dogs treated with a combination of GnRH and bromocriptine was more effective than use of clomifene or bromocriptine only. In addition, frozen-thawed semen can be used successfully for artificial insemination in dog.

Key words) *Semen Freezing, Artificial insemination, Dog, Estrus induction*