SUMMARY
A comprehensive study of the various aspects of freezing of buffalo semen is presented.

The importance of the study in relation to the distribution of buffaloes over the world as well as the potentiality of buffaloes as a milk producer is discussed.

An exhaustive review of the literature on the various aspects of cattle semen freezing along with the few reported work on freezing of buffalo semen is presented.

A procedure was evolved for the first time for the successful freezing of buffalo semen and was named "Dairysearch method". This method involves the use of a diluter containing skim milk, egg yolk, lactose and citrate in addition to glycerol. The diluted semen was cooled from room temperature to 5°C in one hour, followed by glycerolation and equilibration for five hours. The final dilution rate of semen was about 1:10. Freezing could be successfully done by three different methods: in alcohol bath with dry ice, over liquid nitrogen vapour and in powdered dry ice.

It was observed that after freezing in alcohol bath by this method nearly 50% of initial motility could be preserved. Out of this a loss of about 5% was recorded during a storage period of 30-90 days.
Freezing in liquid nitrogen vapour gave better but not significant average survival than in alcohol bath freezing. Freezing in powdered dry ice gave slightly lower but not significant average survival than in alcohol bath freezing. Freezing as pellets over dry ice gave only poor survival rates.

Out of 81 inseminations conducted with semen collected from two donor bulls and frozen by the new method, a 60-90 day nonreturn rate of 79% was recorded. The nonreturn rate for the same bulls during the same period for chilled semen inseminations was 30.5% and for all bulls put together it was 79.6%. It was concluded that the fertility rate for frozen semen was as good as that of chilled semen.

It was observed that milk-yolk diluent with added lactose and citrate was better than that containing either lactose or citrate alone or without any addition. A five hour equilibration period was found to be better than that of 18 hours for alcohol bath freezing. This was better than pellet freezing. A final glycerol concentration of 7% was found to be optimum with milk diluters.

In using a sodium citrate-yolk diluter, a level of 2% citrate was found better than 1.6 and 2.5%. A glycerol level of 9% was found to give optimum results with citrate diluters. For freezing in alcohol bath, an equilibration period of 5 hours was superior to 18 hours.
It was found that there was much variation in the freezability of semen from different bulls, and only the semen of two out of five bulls gave satisfactory results after freezing.

Reconstituted skim milk was inferior to fresh skim milk for the preparation of the diluent for the freezing of buffaloe semen. The three levels of glycerol combined with three levels of milk solids in reconstituted skim milk did not show significant difference in survival rates of sperm after freezing.

There was no significant difference among survival rates after freezing with 40% and 50% milk containing two levels of 20 and 30% egg yolk.

The pH of milk at 6.6, 7.0 and 7.4 were found to have no effect on survival rate of spermatozoa after freezing.

Skim milk of cows as well as of buffaloes gave similar results regarding survival rates after freezing.

It was observed that citrate diluter reduced the motility during equilibration period. In all the cases the diluter for pellet freezing reduced the motility more than that of alcohol bath freezing during equilibration. The motility in lactose diluter was almost similar to that in milk diluter before freezing but after freezing motility in lactose diluter was much lower than that in milk diluter.

It was observed that the abnormalities produced during the processing were highest in citrate diluter especially
that for pelleting. Most of the abnormalities were caused before freezing itself.

It was observed that there were significant differences in survival rates after freezing with different concentrations of spermatozoa. The optimum level was found to be 100 million spermatozoa per ml. of diluter.

Addition of EDTA to the milk diluent had no effect on the motility of spermatozoa in the frozen thawed semen.

Incorporation of Dimethyl sulphoxide instead of glycerol as protectant did not prove to be beneficial in the levels tried.

With the Dairysearch method, an equilibration period of five hours was found to give best results.

It was observed that a faster rate of freezing of about 5 or 10°C per minute in liquid nitrogen vapour gave slightly better survival rates than slow freezing in alcohol bath with dry ice.

The equilibrated semen was frozen by placing them in alcohol baths having initial temperatures of +5, -10, -20, -30 and -70°C. It was found that a starting temperature of -20°C gave better result than other temperatures.

Storage over liquid nitrogen vapour gave better results than storage in alcohol bath with dry ice.
Motility of frozen-thawed semen kept at 38°C was maintained up to 15 minutes but reduced much after 30 minutes whereas the frozen-thawed semen stored at 5°C maintained motility for four hours.

The Dairysearch diluter was also found to be suitable for storing buffalo semen at refrigeration temperature.