EFFECT OF INCUBATION ON SPERM MOTILITY AND LIVABILITY OF LABRADOR RETRIEVER DOG SPERMATOZOA PRESERVED AT 5ºC IN DIFFERENT EXTENDERS

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ABSTRACT

Four ejaculates from each of two adult Labrador Retriever dogs collected by digital manipulation method were extended @ 1:4 in Tris-Egg Yolk-Citric Acid-Glucose (TEYCAG), Tris-Egg Yolk-Citric Acid-Fructose (TEYCAF) and Egg Yolk-Citrate-Glycine-Glucose (EYCGG) extender by split sample technique. The extended semen samples were preserved at 5^0C for 24, 48, 72 and 96h. Thereafter, the aliquots of preserved semen samples were incubated at 37°C for 0, 2, 4, 6 and 8h. Irrespective of preservation period and incubation period, the mean sperm motility and live sperm percentage differed ($p<0.05$) between TEYCAG and EYCGG extenders, and between TEYCAF and EYCGG extenders, but was similar ($p>0.05$) between TEYCAG and TEYCAF extenders. Mean sperm motility and live sperm percentage were higher ($p<0.05$) in TEYCAG and TEYCAF extenders at all hours of preservation at 5^0C for different incubation periods than that in EYCGG extender. The sperm motility and live sperm dropped ($p<0.05$) as the incubation period increased from 0 to 8h irrespective of the extender and preservation period. In brief, canine semen extended in TEYCAG extender and preserved for 48h at 5^0C maintained the semen quality suitable for artificial insemination up to 4h of incubation.

Keywords: Incubation, Labrador -Retriever dog, live sperm, semen preservation, sperm motility

The intensity of dog rearing has increased in recent times which has increased the demand of superior quality dogs for breeding. The non-availability of male of desired breed in a locality and the possibility of obtaining superior germplasm of selected dogs elsewhere have aroused interest among the dog breeders for adopting artificial insemination (AI) in bitches. Short-term storage of semen is highly desirable when AI cannot be resorted to immediately after its harvest. The evaluation of sperm parameters during preservation of semen of a few breeds of dog and their admixture were carried out earlier (Varela Junior *et al.*, 2009). Like other farm animals, sometimes AI in dogs cannot be done immediately after preparation of AI dose or thawing. Some dogs are very uncooperative during AI process and often take longer time to become familiar with the situation. This causes gradual deterioration of semen quality as long as the animal is not inseminated. Hence, the present work was carried out to find out the extent of time during which the quality of preserved dog semen could be maintained after bringing it to normal room temperature for a fertile AI.

Four ejaculates from each of two adult Labrador Retriever dogs were collected by digital manipulation method and the first and second fractions of the ejaculates were collected in a single collection cup. The semen samples were then extended @ 1:4 in Tris-Egg Yolk-Citric Acid-Glucose (TEYCAG; Verstegen *et al.*, 2005), Tris-Egg Yolk-Citric Acid-Fructose (TEYCAF; Foote, 1970) and Egg Yolk-Citrate-Glycine-Glucose (EYCGG; Foote and Leonard, 1964) extenders by split sample technique. The extended semen samples preserved at refrigeration temperature for 24, 48, 72 and 96h were taken in aliquots, incubated at 37°C for 0, 2, 4, 6 and 8h. The semen samples were evaluated for sperm motility and live sperm percentage and

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results were subjected to statistical analysis.

The mean percent motile and live sperm of preserved semen decreased with increase in incubation time for all the three extenders. In a previous study, the mean sperm motility and live sperm of fresh dog semen diluted with physiological phosphate declined on incubation at 22^{0}C when assessed at 2h interval up to 6h of incubation (Veznik *et al*., 2003). Others reported that when dog semen frozen in Tris-egg yolk-citric acid-glucose extender was thawed and incubated at 38^{o}C for 8h, the percent progressive sperm motility and live sperm decreased with increase in hour of incubation (Pena and Linde-Forsberg, 2000). In present study, the mean percentage of sperm motility and live sperm irrespective of preservation period and incubation period was lower ($p<0.05$) in EYCGG extender (25.76±5.76 and 39.21±5.86, respectively) in comparison with TEYCAG (40.81±9.13 and 53.85±5.45, respectively) and TEYCAF (35.88±8.03 and 48.81±5.75, respectively) extenders, but was similar ($p>0.05$) between TEYCAG and TEYCAF extenders. The mean percentage of sperm motility and live sperm irrespective of extender and preservation period decreased ($p<0.05$) between successive hours of incubation.

Higher ($p<0.05$) percent mean sperm motility and live sperm obtained in TEYCAG and TEYCAF extenders than in EYCGG extender irrespective of preservation period and incubation period might reveal superiority of the former two extenders over the latter in withstanding the stress of incubation for a prolonged period. Although similar, the percent mean sperm motility and live sperm were rather higher in TEYCAG than in TEYCAF extender indicating better ability of TEYCAG extender in maintaining the sperm motility and survivability of canine semen preserved at 5^{o}C on elevation to a higher temperature. It was found in the present study that more than 50% mean sperm motility was maintained in semen preserved for 72h on incubation for 2h (55.00±4.56%) and in that preserved for 48h on incubation for 4h (51.25±7.18%) using TEYCAG extender. It was also observed that higher mean percent live sperm was maintained in semen preserved for 72h on incubation for 2h (66.77±4.23%) and in that preserved for 48h on incubation for 4h (59.44±6.57%) utilizing TEYCAG extender than TEYCAF extender.

Laboratory stress tests were designed to indicate the efficacy of preserved semen in maintaining fertilization potentiality of spermatozoa in bovine (Roussel *et al*., 1963). The present findings might imply that canine semen extended in TEYCAG and TEYCAF extenders and preserved at 5^{o}C up to 96h could possess higher potentiality in sustaining sperm motility and liveability during passage through the female genital tract at body temperature that was comparable with the incubation temperature in the present study before involving in the process of fertilization as compared to that preserved in EYCGG extender.

It could be concluded that Labrador-Retrieveer dog semen extended in TEYCAG and preserved for 48h maintained the quality suitable for AI up to incubation of 4h.

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