A DNA FINGERPRINTING SCHEME TO IDENTIFY THE MIX UPS IN SEMEN SAMPLES OF CATTLE BREEDS IN ARTIFICIAL INSEMINATION PROGRAMS OF SRI LANKA

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Dairy cattle industry plays a prominent role in Sri Lankan economy because of the very high consumption of dairy milk and dairy products. Artificial Insemination is frequently used to inculcate improved performances such as high milk yield and better adaptability to the cows. However, the efficiency of Artificial Insemination is often hampered due to the mix-ups of semen samples belonged to different breeds. This could happen when semen samples are imported and distributed among regional artificial insemination centers. Hence, the availability of a robust DNA based method to identify cattle breeds at the semen stage is very important. The present study was conducted to develop a method to discriminate the five commonly used cattle breeds (Sahiwal, Jersey, Friesian, Ayrshire and Australian Friesian Sahiwal) used in artificial insemination programs by using DNA fingerprinting with microsatellite markers and to assess the accuracy of Artificial Insemination using recently born calves and their parents.

The semen samples were collected from Ambewela Farm, Nuwara-Eliya, Sri Lanka (Ayrshire) and Central Artificial Insemination Station-Kundasale, Sri Lanka (Jersey, Friesian, Sahiwal, AFS). DNA was extracted from semen samples and genotypic analysis was done using 14 cattle specific microsatellite markers (*ETH152, ETH225, HEL1, CSSM66, RM180, RM011, RM192, BM6425, BMS1678, BMS1941, BM3517, TGLA304, BMS1747* and *ILSTS011*). Blood samples were collected from a total of 25 animals including stud bulls, calves born from AI and their mothers. Stud bulls were chosen from Central Artificial Insemination Center, Kundasale, Sri Lanka and calves and cows were selected from Kundasale Veterinary Range. The DNA samples extracted from blood were subjected to PCR using two SSR markers, *ETH225* and *ET125* to verify the parentage (i.e. paternity). The PCR products of blood DNA samples were size separated using 6% denaturing polyacrylamide gel electrophoresis.

Only four out of fourteen markers generated polymorphic bands with semen DNA (*ETH225, RM011, BM3517* and *BM6425*). The markers *BM3517* and *BM6425* can be suggested for the precise discrimination of five breeds. *BM6425* generated three different bands with size ranging from 123-200 bp. Marker *BM3517* displayed four different bands; in size range from 100-150 bp. In combination these two markers can be successfully used to authenticate the exact breed from which semen sample is coming and avoid any ambiguities. In addition marker *BM3517* and *RM011* in combination can be used to independently verify the results.

Out of calves tested eight produced bands; four calves had the wrong parentage and four calves had correct parentage with respect to the expected father showing the use of mixed up semen samples in Artificial Insemination or fertilization by a contaminator bull. Hence DNA authentication of the semen samples can be used to increase the accuracy of Artificial Insemination.