OPTIMAL HORMONAL REGIME AND FEASIBILITY OF USING PHOSPHATE BUFFERED SALINE AND UNDISSOCIATED CUMULUS OOCYTE COMPLEX FOR EMBRYO TRANSFER IN MICE

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Embryo transfer (ET) is one of the modern Assisted Reproductive Technologies (ART) that allows the utilization of advantageous qualities of superior males and superior females. This technique can be used for improving the efficiency of reproduction not only in farm animals but also in humans. However, this valuable technique is yet to be developed and established in Sri Lanka due to obstacles such as lack of facilities and financial constraints. In an attempt to develop and establish ET in developing countries such as Sri Lanka, it is advisable to examine the feasibility of achieving success using less expensive locally available reagents and animal models such as mice. This research aimed in determining the optimal hormonal concentration and anesthesia regime to be applied, and examining the feasibility of performing ET using phosphate buffered saline (PBS) and undissociated cumulus oocyte complex (COC) to establish a low cost protocol for ET in Sri Lanka.

Mice (BALP/cJ) were used for the experiment. During the preparatory stage all the required micro tools were improvised using locally available glassware and instruments. To establish a suitable anesthesia regime, groups of mice were given different doses (16 mg/Kg to 150 mg/Kg) and combinations of Ketamine and Xylasine with or without chloroform. Several males (12-16 weeks old) were surgically vasectomized to be used for making pseudopregnant recipients. Three groups of mice (4-6 weeks old, 25-30 g body weight) were given a single intraperitoneal (IP) injection of 4, 5 or 6 IU of PMSG followed by 6 IU of HCG to determine the superovulatory response for PMSG. After placing with male studs, the females having vaginal plugs were used for embryo retrieval. Collected one-cell embryos were counted under a stereo zoom microscope. To determine the superovulatory response for HCG, three groups of female mice (4-6 weeks old, 25-30 g body weight) were treated with 5 IU of PMSG followed by 3, 4.5 or 6 IU of HCG. Above described procedure was adapted to determine the numbers of one-cell embryos. For the feasibility study of ET, one cell embryos with COC obtained from donor mice treated with best PMSG and HCG regime, were surgically transferred through the infundibulum of the pseudopregnent synchronized recipients. Data were analyzed by using SAS computer package.

The improvised glassware and tools were effective in performing ET. Intraperitoneal administration of 100 mg/Kg Ketamine resulted in 20–30 minutes duration anesthesia with better survival rate (male 33%, female 66%). Higher number of embryos could be collected from the mice treated with PMSG and HCG compared to control ($P<0.05$). Treatments with 5 IU of PMSG and 4.5 IU of HCG produced superior ($P<0.05$) superovulatory response. Embryo transfer using COC in PBS medium was possible. Based on the above results it can be concluded that Ketamine (100 mg/Kg) is suitable for anesthetize mice for about 20 minutes. The optimum hormonal regime for superovulation was 5 IU of PMSG and 4.5 IU of HCG for about 25-30 g body weight. ET can be performed locally using PBS medium and COC following the above protocol.