EFFECT OF DIFFERENT CULTURE MEDIA AND INCUBATION METHODS ON
CULTURING MURINE EMBRYOS IN VITRO USING A SEMEN STRAW AS AN
ALTERNATIVE RECEPTACLE

BY

LUFUNO ROSHEEN MADZHIE

Student no: 11575368

A dissertation submitted in fulfilment of requirements for the degree Master of
Science in Agriculture degree (Animal Science)

Department of Animal Science

School of Agriculture

University of Venda

South Africa

Supervisor: Prof D.M. Barry (UNIVEN)

Co-supervisor: Prof T.L. Nedambale (TUT and UNIVEN)

UNIVEN LIBRARY
Library Item: 20162018

Date: May 2016
The aim of the study was to fertilize mouse oocytes and culture the resulting zygotes in bi-gas incubator and in a goat vagina and compare the in vitro embryo development in TCM-199 and Ham's F10 culture media until the blastocyst formation. The spermatozoa and oocytes of an F1 generation (Balb C x C57) were fertilized in Ham's F10 and TCM-199 in semen straws and in micro-drops in petri dishes. The embryos were cultured in TCM-199 and Ham's F10 in semen straws and micro-drops in petri dishes. The semen straws were incubated in a bi-gas incubator and goat vagina. The blastocyst-stage embryos were stained using Hoechst 33528 solution and blastomeres count. The results were analyzed by 2 X 3 factorial design and the student t-test was used to separate the mean and they showed that there was no statistical difference (p > 0.05) between the media, receptacles and incubators. The overall fertilization rate was 94 % to 99 %. The semen straw with Ham's F10 incubated in the bi-gas incubator had the highest rate (80.5 %), reaching the blastocyst-stage of the embryos. The lowest rate of murine embryos reaching the blastocyst-stage were those cultured in semen straws incubated in the goat vagina, with its highest rate of 80 %. The embryos blocking at the 8-cell and morula stage recorded the lowest rate on both culture media and incubation methods, compared to the other embryo developmental stages. The overall mean number of blastomeres in the blastocyst-stage of the embryos ranged from 85±9 - 90±9 cells in all receptacles and incubators. It was concluded that the fertilization and culturing of murine embryos are possible in French semen straws incubated in a bi-gas incubator and in the goat vagina as an alternative method of fertilizing oocytes and culturing murine embryos in addition TCM-199 and Ham's F10 can both be used to fertilize oocytes and culture murine embryos until blastocyst formation embryo in vitro, incubated in a bi-gas incubator or in the vagina.

Keywords: in vitro fertilization, semen straw, TCM-199, Ham's F10, bi-gas incubator, goat vagina.