

CHAPTER 1

GENERAL INTRODUCTION

Malaysia currently imports 80% of its lamb and mutton consumption worth RM35 million a year (Ministry of Agriculture Statistics, 1990). This meat contributed only 4.6% of the country's meat supply compared to 95% from beef (Devandra, 1986). Despite the high demand for goat meat, supply remained low (Devandra, 1986), and with a current number of 220,000 goats for 18 million population; this insufficiency is said to be very serious. Realising this, the Malaysian government has committed to embark a project which includes encouraging the estimated 566,000 small holders throughout the country to rear goat and sheep not only to increase their incomes, but also to make Malaysia self sufficient in mutton by the year 2000 (Ministry of Agriculture Statistics, 1990).

In order to make the government policy a reality, it is felt that basic scientific research and the application of the data obtained for the commercial production of good quality goats should be given a priority. It should be emphasised that conventional or traditional methods of animal production will lead to no where because of global competition. The present study, therefore, was designed with the intention to establish basic research involving embryo

technology in local set-up so that the data, could be used to facilitate more applied research in the future.

Embryo technology such as *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes and subsequent *in vitro* culture (IVC) of embryos have allowed the study of mammalian embryos under controlled environmental conditions. This has resulted not only in the understanding of mammalian fertilization and preimplantation development of embryos, but also in the enhancement of new related biotechniques including micromanipulation of embryos, e.g. embryo splitting to produce monozygotic twins (Moore *et al.*, 1969; Willadsen, 1979; Gatica *et al.*, 1984; Szell *et al.*, 1994), embryo sexing (Handyside *et al.*, 1989; Herr and Reed, 1991), gene transfer to produce transgenic animals (Thomas *et al.*, 1993), chimaera (Mwenya *et al.*, 1988; Picard *et al.*, 1990), nuclear transfer (Kanka *et al.*, 1991) and microinsemination (Keefer *et al.*, 1990). Besides, this technique also provides a means of conserving embryos, especially from elite animals and rare breeds which may become economically important in the future (Kanagawa, 1980).

In vitro maturation and *in vitro* fertilization-embryo transfer (IVF-ET) studies in some laboratory animals such as mice have been well established. Laboratory animals have been extensively used as experimental model animals because they are cheap, can be maintained in colonies, respond well

to superovulatory treatment with high yields of oocytes and have short generation intervals (Hahn, 1984). Research in laboratory animals has become a model for research in farm animals which directly benefits the livestock industry. For example, embryo sexing has been used to obtain female animals in dairy industry and to obtain male animals in meat industry. Therefore, these and their related techniques including IVM, IVF, ET and cryopreservation, together, have improved animal reproduction and breeding performance with a consequent improved yield in livestock products such as milk and meat. The development of these techniques has also enabled a rapid multiplication of superior genotype, better disease-resistant animals and animals with better adaptation to environment. Genetic improvement can be accelerated by increasing the number of offspring that could be generated from genomic combination of elite females and progeny tested sires.

At present, despite extensive studies on IVM and IVF of oocytes involving two domestic ruminant species, namely cattle and sheep, the literature concerning these subjects in goats is scarce. Although embryo studies in cattle have been established, rigorous advancement of IVF-ET technology in goat can be advantageous economically, especially in developing countries due to its shorter generation intervals, lesser use of land to rear and breed, easier to handle, which can contribute towards cheaper labour costs.

Although attempts to study in vitro maturation-fertilization-culture (IVMFC) in goat have been carried out, the results were variable and of limited success. Studies in goat by Kim (1981), Hanada (1985), Song and Iritani (1985) and Jufen *et al.* (1991), while achieving varying degrees of success, all were using oocytes retrieved from superovulated goats. By using superovulated goats, we not only limit the reproductive capability of the animal concerned after one or two treatments, but also wasted the rich oocytes source of ovaries from slaughtered goat which otherwise would have been capitalised. Recently Younis *et al.* (1991, 1992) reported successful IVF and pregnancy using non-superovulated and superovulated goats, however, in both studies the pregnancies did not continue to term. In their studies on the relationship between the methods of oocytes recovery, namely, aspiration, puncturing and slicing, and the rate of fertilization of the oocytes in the CR 1 aa medium, Pawshe *et al.* (1994) found that the rate of fertilization was not different between the three methods (76.1, 80.7 and 86.9% respectively).

Characterization of the matured goat oocytes in vitro also has not been systematically elucidated, particularly from the ultrastructural and chromosomal point of view. This knowledge is important to understand the changes in the developing oocytes before mechanisms of subsequent in vitro fertilization can really be understood. Besides, there are

various factors affecting the viability of embryos during IVMFC. Some of the factors are source of ovaries, medium, temperature (Eng et al., 1986; Lavy et al., 1988); osmotic pressure and pH (Brinster, 1965; Naglee et al., 1969; Bondioli, 1981; Walker et al., 1989) and epithelial cell co-culture system (Goto et al., 1988; Eyestone and First, 1989; Iwasaki and Nakahara, 1990; Goto et al., 1994).

The importance of co-culture system for the success rate of preimplantation embryonic development in bovine has been established. In goat, attempt to undertake this procedure has been carried out by Sakkas et al. (1989) but in those studies the embryos were obtained through in vivo fertilization. Younis et al. (1992) have introduced the embryos with co-culture, however this co-culture system consisted of cumulus cells. Thus, reports on the use of oviductal epithelial cells for co-culture of embryos obtained through IVF is scarce.

Finally, the primary goal of the present study was to obtain embryos through IVF procedure using oocytes matured in vitro. In order to achieve this, the following investigations were carried out:

1. To characterize the changes of the morphology and the cytoplasmic organelles of the developing goat

cumulus-oocytes complexes (COCs) and cumulus-free oocytes (CFOs) cultured in vitro. This study was based on:

- (a) light microscopy
- (b) electron microscopy

2. To study the changes of chromosome configuration of goat COCs and CFOs during development in vitro.

3. To fertilize the developing goat oocytes in vitro using frozen-thawed sperm and subsequently to culture the embryos in vitro with oviductal epithelial cells as co-culture.