PREFACE

In vitro production (IVP) of embryos provides an excellent source of low cost embryos for basic research on developmental biology and physiology, and for the application of emerging biotechnologies such as nuclear transfer and production of transgenic animals. The successful development and application of IVP of embryos and related technologies depend on a whole range of basic protocols including *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes and *in vitro* culture (IVC) of fertilized oocytes.

Gametes are sensitive to many toxic compounds, and the damage in gametes has severe consequences for fertility and subsequent normal development (Hartshorne, 1997). The oxygen (O_2) tension in the gas atmosphere represents such a potential deleterious impact on the IVP of mammalian embryos through the generation of toxic reactive oxygen species (*e.g.*, hydrogen peroxide, superoxide anion and hydroxyl radical). These free oxygen radicals have been shown to cause severe cellular injury (Umaoka *et al.*, 1992).

The culture medium used for oocyte maturation affects the subsequent embryonic development in mice (van de Sandt *et al.*, 1990) and cattle (Rose and Bavister, 1992). Waymouth medium, which contains a relatively high concentration of glucose, was found to be superior to other media in supporting the maturation of mouse oocytes and subsequent embryonic development (van de Sandt *et al.*, 1990). However, a previous study (Hashimoto *et al.*, 2000ab) suggested that high concentrations of glucose support the nuclear maturation and subsequent developmental competence of bovine oocytes only under low (5%) O_2 tension. At present, the optimal O_2 tension during the various steps of IVP is still unclear and in many cases contradictory especially in mice. In chapter 1, the effects of O_2 tension during IVM, IVF and IVC on the efficiency of IVP of mouse embryos using Waymouth medium as a maturation medium were investigated.

Mouse oocytes have been subjected to IVM for periods ranging between 15 and 18 h (Schroeder and Eppig 1994; Merriman *et al.*, 1998; Smitz *et al.*, 1998; de la Fuente *et al.*, 1999). However, prolongation of the culture period for IVM may cause aging of oocytes (Schroeder *et al.*, 1988). It was, therefore, necessary to optimize the maturation

period to harvest oocytes with high developmental capacity. The aim in chapter 2 was to confirm the superiority of 5% O_2 for IVM and to optimize the culture period for IVM.

Current methods for IVP of embryos are dependent upon the supply of oocytes from antral or preovulatory follicles, which are present in the ovary in a relatively small number. The ovaries contain a large number of preantral follicles, which could be a potential source of fertile oocytes. Therefore, the culture of preantral follicles for *in vitro* growth (IVG) has the potential to produce large quantities of oocytes for IVP. To date, the culture of preantral follicles has been used most successfully with mouse follicles (Eppig and Schroeder, 1989; Spears *et al.*, 1994; Eppig and O'Brien, 1996; Cortvrindt *et al.*, 1998). However, the growth characteristics of follicles differ among the culture systems. The individual culture of intact preantral follicles maintains the spherical structure of follicles, and has advantages for investigating the mechanism of folliculogenesis, oocyte growth and ovarian function including ovulation over other types of cultures (Nayudu *et al.*, 2001). So far, only a few studies have examined the developmental competence of oocytes ovulated from individually cultured preantral follicles using membrane inserts (Rose *et al.*, 1999) and droplets (Boland *et al.*, 1993). Refining the culture conditions to maximize the yields of developmentally competent oocytes from preantral follicles remains a major challenge. In chapter 3, to develop a reliable IVG system, the efficiency of the insert and droplet culture systems in follicular growth and preimplantation development of the ovulated oocytes was determined.