CHAPTER 1

INTRODUCTION

1.1 Principle, Rationale and Hypotheses.

Ovaries of slaughtered donors have been used as the main source of oocytes for in vitro embryo production in cattle. Very large numbers of embryos have been produced from slaughterhouse materials in some commercial programs. This system may have the potential for commercial application, if individual identification of the donors is possible or when a unique population of cattle is slaughtered. It may, under certain circumstances, be possible to think in terms of recovering oocytes from cattle that have undergone superstimulation treatment prior to the time slaughter. The cattle given follicle stimulating hormone (FSH) preparations some 48-72 hours before slaughter may possess ovaries containing a number of follicles that have commenced their final growth phase and which may be capable of yielding oocytes of higher than average quality (Pavlok et al., 1989). A study reported by Lu et al. (1992) compared oocytes recovered from slaughterhouse ovaries 2 days after commencement of superstimulation treatment with those from untreated animals and it was found that a significantly higher yield of embryos was produced by gonadotrophin-treated animals. Lonergan et al. (1994) showed that FSH treatment prior to slaughter in cattle led to a greater proportion of larger follicles and a higher number of good-quality oocytes for in vitro maturation (IVM). If follicle size is to be regarded as an important

determinant of oocyte quality, then techniques for increasing the number of larger-sized follicles may be one means of increasing embryo yield per ovary.

Presently, oocytes from valuable donors are more frequently recovered from live animals by transvaginal ultrasound-guide oocyte collection (ovum pick-up, OPU). The OPU provides several advantages over standard embryo transfer procedure. This technique, in conjunction with in vitro fertilization (IVF) technology, greatly reduce generation interval by producing more progeny per year than conventional superovulation and embryo transfer (Galli et al., 2001). The practical application of in vitro embryo production depends primarily on the number of immature oocytes being obtained, especially from genetically superior cows. This number could increase if immature oocytes could be collected repeatedly from the same animals over a period of several months (Kruip et al., 1994). In vitro embryo production from live donors is an extremely useful technique because it can be used for dry and lactating donors and even during pregnancy up to the third and fourth month of gestation. It does not interfere with the physiological status of the donors, since no hormonal stimulation is required and the cost of hormones is eliminated (Galli et al., 1996).

Although hormonal stimulation is not required, it has been suggested that low doses of hormonal supplement may increase the number of antral follicles, which in turn, increase the number of retrieved oocytes. However, it is still debatable whether, in long-term, this procedure would yield the highest embryo production. Pretreatment of the oocyte donors with FSH allows the oocyte retrieval to be performed at best every two weeks. However, twice-weekly collection can be done in donors that have not received FSH treatment. Thus, over a period of several months, it may be that a

higher number of embryo are produced from donors that are subjected to twice-weekly collection without pretreatment with FSH than those treated with FSH.

The OPU technique has continuously been improved in order to create a proper system leading to the optimal condition for oocyte recovery. Increasing the frequency of aspiration and pretreatment with gonadotrophins hormone have also increased the number of retrieved oocytes per aspiration session.

Several researches have developed new OPU devices that are practical and economical for routine use in oocyte retrieval. To optimize the OPU technique and to increase the recovery rate of oocytes, it is important to reconsider those procedures. The use of disposable needles is inexpensive and because of their small dead volume, they are very well suited for collection of small amounts of follicular fluid from individual follicles. However, the disadvantages of disposable needles are that it require a more complex needle guidance system to permit changing the needle, and its implications concerning the type of ultrasonographic scanner used. Some parts of the system are difficult to be rinsed so that the cumulus oocyte complexes (COC) can be trapped (Bols et ., 1995). Therefore, new systems should be of simple construction to permit easy changing of the needles and to prevent loss of oocytes. From our preliminary study (unpublish data) the use of Hill Aspirator eliminates this problem. A simple method of OPU technique has also been developed for collection oocytes from live donors under field situations that does not require ultrasound visualization of the ovary (Hill, 1995).

The hypothesis of this study is that treatment with FSH can be used to increase the number of retrievable oocytes and their developmental competency from non-ultrasound guided transvaginal ovum pick up (n-OPU) technique in the cow.

1.2 Purposes of the Study

1. To apply n-OPU technique use to collect oocytes from live cows.
2. To apply n-OPU technique and in vitro embryo production to produce bovine embryos.
3. To determine whether the procedure can be performed with or without the use of FSH treatment before n-OPU technique.

1.3 Educational Advantages

1. The commercial value of n-OPU technique for in vitro embryo production lies in that it would allow breeders to produce a high number and low cost of embryos.
2. In vitro embryo production using this technique may also be a valuable source for basic research on studying of oocyte biology.
3. This technique is simple, non-invasive, repeatable and efficient for application onto other species.