Oocyte Collection

Development and application of assisted-reproduction technologies like *in vitro* embryo production through *in vitro* maturation, fertilization and culture of oocytes, production of cloned and/or transgenic cattle, establishment of oocyte banks, etc. can be expected to bring about a significant increase in the population of superior genetic merit cattle. The availability of enough number of oocytes is the pre-requisite to any investigation for the development and optimization of reproduction techniques.

Methods of oocyte collection:

1- Originally, *in vitro* procedures in cattle primarily were conducted for research purposes and used oocytes collected from superovulated females. When collected either from preovulatory follicles or oviducts 20 to 24 hours after onset of estrus, these oocytes had already undergone maturation and thus were ready for *in vitro* fertilization and *in vitro* culture.

a- surgical procedures, was expensive, inefficient, and risked the formation of adhesions with subsequent loss of fertility.

b- The introduction of real-time transrectal ultrasonic imaging led to the development of techniques for the repeated collection of oocytes from bovine females and has become the predominant technique for oocyte collection from living cattle. This technique has become widely known as ovum pick-up (OPU).

An ultrasound transducer and attached needle guide of the type used in cattle and horses is shown in Figure 8.16. A diagram of how the transducer is inserted into the vaginal fornix so that a needle can be guided through the vaginal wall and into the ovary is also shown.

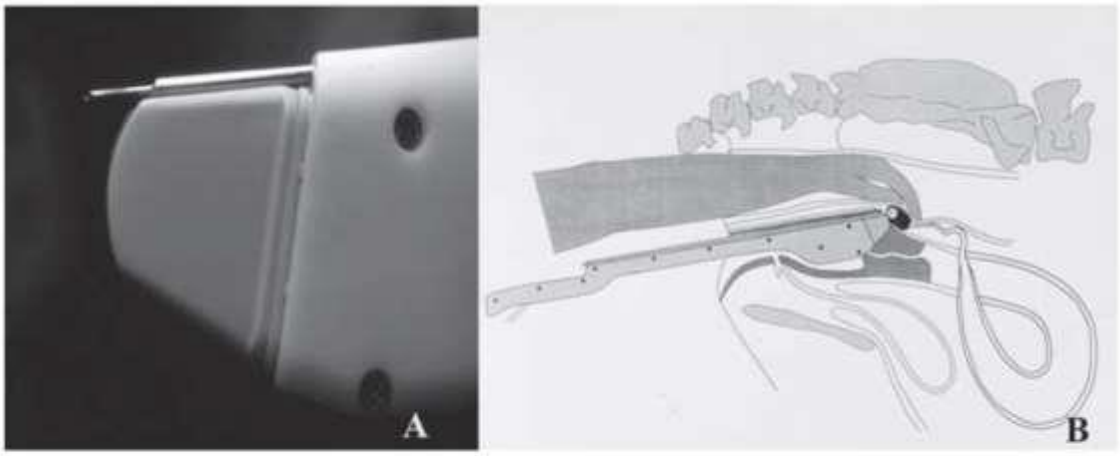

Figure 8.16. (A) Ultrasound transducer with oocyte aspiration needle protruding from needle guide. (B) Diagram showing position of the ultrasound transducer pressed against vaginal fornix, with ovary manually manipulated per rectum and held up against the vaginal wall.

2- Oocytes collected from slaughterhouse-procured ovaries were not useful for studying fertilization and culture until *in vitro* maturation techniques were improved. Ovaries of the slaughtered animals are the cheapest and the most abundant source of primary oocytes for large scale production of embryos through *in vitro* maturation (IVM) and *in vitro* fertilization (IVF)

Although superovulation remains the chosen method of producing high-quality bovine embryos for most commercial ET purposes, cost may well make this prohibitive for many research programmes. Abattoir materials, on the other hand, form an inexpensive .and readily available source of oocytes for research in embryo production and for use in cloning and the production of transgenic animals. In the production of cattle embryos from slaughterhouse ovaries, it was necessary to develop methods that permit the recovery of several good-quality oocytes per ovary; the number actually recovered will vary with different collection procedures.

Oocytes were harvested by many following techniques.

(1) **Slicing**:

ovaries were placed in a graded plastic Petri dish containing a saline solution and were chopped into small pieces with a surgical blade. The cumulus-oocyte complexes (COCs) were selected from the saline solution.

(2) **Puncture(slashing)**:

follicles visible on the surface ranging from 2.0 to 6.0 mm in diameter were punctured with an 18-g needle. The COCs were selected from the follicular fluid.

(3) **Aspiration**:

the follicular fluid from surface follicles (2.0 to 6.0 mm) was aspirated through a sterile 18-g needle attached to a 5 ml syringe containing a sterile saline solution. Aspirated contents were expelled into a fresh Petri dish containing the saline solution and COCs were selected from it.

Aspiration

Slicing

slashing

The COCs recovered were then classified under an **inverted microscope**. The COCs were graded as good, fair and poor according to the character of cumulus cells as follow:

1- **good** (A nad B): oocytes with many complete layers of cumulus cells and uniform cytoplasm.
2- **fair (C)**: oocytes with thin or incomplete layers of cumulus cells and uniform cytoplasm.
3- **poor (D)**: oocytes with few or no cumulus cells.