in vitro fertilization (IVF)

From its Latin origin, *in vitro* fertilization (IVF) means "fertilization in glass," although today it could more accurately be described as "fertilization in polystyrene."

IVF has been adopted as a generic phrase that often includes the procedures of *in vitro* maturation (IVM) and *in vitro* culture (IVC). All three procedures usually are conducted in sequence to produce embryos exclusively *in vitro* (IVP). In 1959, the rabbit was the first mammalian species in which live offspring were known to have been produced by IVF (Chang, 1968). The next reported success was with laboratory mice in 1968 (Whittingham, 1968). Subsequent progress with *in vitro* technology in mice, however, often did not extend to livestock species, because the mouse did not prove to be a good procedural model.

In 1977, *in vitro* fertilization in cattle was first accomplished with semen capacitated in the oviduct or uterus of cows in estrus or the uterus of a rabbit (Iritani and Niwa, 1977). The first live calf resulting from IVF was born in 1981 as the result of the transfer of a 4-cell embryo into the oviduct of a recipient cow. The first calves produced entirely from IVM, IVF, and IVC were born in 1987 (Fukuda et al., 1990).

Advantages of *In Vitro*-produced Embryos

There are many reasons for interest in embryos that can be produced in the laboratory, rather than recovered from the living animal. Progress in cellular and molecular embryology in farm animals has been difficult in the past due to the limited availability of suitable experimental material at an acceptable cost. Although oocytes and embryos can come from superovulated donor animals, this is likely to be expensive and not always free from animal welfare concerns. For such reasons, *in vitro* production (IVP) techniques, particularly those based on ovaries recovered after the donor's death, have received much attention in the past 10–15 years (Galli and Lazzari, 2003).

Oocyte Collection

Transvaginal aspiration of ovaries manipulated per rectum and guided by a vaginally-inserted ultrasound probe is used in cattle and horses.

Smaller species require a laparoscopic approach in which follicles are visibly identified and aspirated.

Evaluation and maturation of the oocyte

Selection criteria

The methods of selection of the cumulus– oocyte complexes (COCs) are usually based on parameters such as the morphology of the cumulus, the combined morphology of the cumulus and of the ooplasm, the size of the follicle and the oocyte and the level of follicular atresia.

COCs with a compact and complete cumulus mass and a uniform appearance seem to present a higher developmental ability (Fig. 4.6).

Many reports have proposed classification schemes based on the compactness and number of layers of cumulus cells surrounding the oocyte and on the appearance of the oocyte itself. Oocytes with the highest developmental competence are expected to possess an even, smooth, finely granulated cytoplasm, surrounded by fewer than three compact layers of cumulus cells.

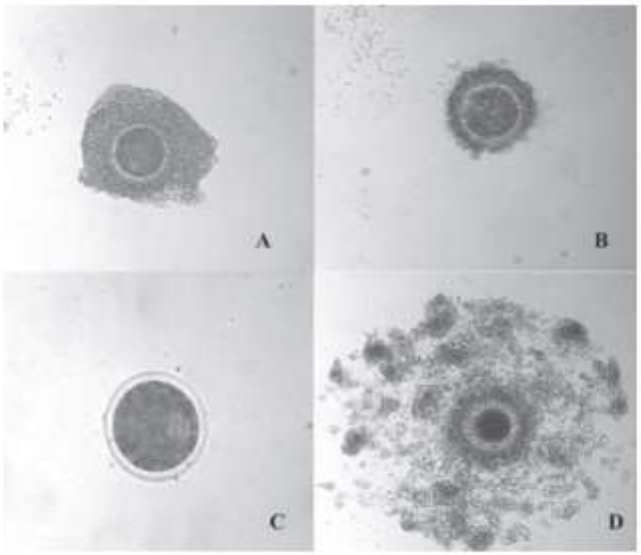

Figure 8.17. Variety of immature bovine oocytes aspirated by OPU. (A) Type I oocyte with more than four layers of compact cumulus; (B) type II oocyte with one to three layers of cumulus; (C) type 3 nude oocyte; (D) type 4 oocyte with expanded cumulus.

Maturation system

Production of zygotes, however, depended on the development of semen capacitation procedures and suitable fertilization media as well as the availability of mature oocytes. Except for the small numbers removed from super ovulated females, mature oocytes were not readily available until IVM techniques were perfected. Thus, IVF could not be efficiently studied without IVM, but the only definitive proof of complete IVM is fertilization followed by normal cleavage and embryonic development. Such development, however, depends on a suitable IVC system. Currently, the efficiency of IVM is high enough to make feasible extensive research using immature oocytes obtained from slaughterhouse ovaries.

Two aspects of IVM in the bovine are rather remarkable. Oocytes aspirated from a variety of the follicles in any given wave can be induced to mature in approximately 24 hours. Second, maturation can be induced *in vitro* with a wide variety of media. Although it is known that oocytes from follicles less than 2 mm in diameter exhibit lower rates of maturation than oocytes from larger follicles. a high percentage of oocytes from larger follicles are competent to undergo IVM.

The maturation system employed in many laboratories involves the use of **TCM-199, supplemented with 10% fetal calf serum (FCS) and gonadotrophins (FSH, LH), in 5% carbon dioxide in air at 38.5°C. After incubation for 24 h, the bovine oocyte is mature, extrudes the first polar body and is ready for fertilization**. Under conditions in which donor cattle dealt with by OPU are located far from the processing laboratory maturation may be initiated during transport from the collection site to the laboratory, using portable battery-powered incubators. Under optimal IVM conditions, more than 90% of oocytes can be expected to reach metaphase II; just prior to fertilization, cumulus cells are partially removed to leave fewer cell layers surrounding the oocyte.

Sperm preparation and *in vitro* fertilization (IVF)

Certain preliminaries must occur before sperm are in a position to effect fertilization. In embryo production, one of the first steps is the selection of sperm for use in IVF. A common practice is to select frozen–thawed sperm on the basis of a Percoll separation method.

IVF is a complex procedure involving oocyte maturation, sperm separation and sperm capacitation. Sperm capacitation is the biochemical modification sperm must undergo within the female tract before the cell can bind to the zonapellucida and undergo the acrosome reaction (AR).

Capacitation is possible *in vitro* in the absence of reproductive-tract fluids and several compounds are known to induce *in vitro* capacitation. the most common of these is the glycosaminoglycan (GAG) heparin.

IVF is usually achieved by co-incubating sperm and oocytes for 18–20 h; the oocytes are denuded of cumulus cells before transfer to the IVC system used for development to the blastocyst stage.

In vitro embryo culture

Traditionally, the commercial development of bovine embryos derived from IVM, IVF and IVC has employed co-culture of somatic cell lines, such as buffalo-rat liver (BRL) cells or Vero cells in complex TCM supplemented with serum for embryo culture.

The oviduct for embryo culture

Several culture systems have been developed to culture the fertilized oocyte (the zygote) to the blastocyst stage; these include an *in vivo* culture

procedure using the surrogate sheep oviduct, as well as various co-culture and cell-free systems. The sheep oviduct method is one that yields embryos of a quality almost comparable to those recovered from the cow itself.

Sequential media

Encouraging results are found using media in which composition changes according to the embryo's stage of development. Data presented by Lane *et al*. (2003), for example, using a physiologically based, sequential, serum-free culture system (G1.2/G2.2), showed equivalent pregnancy and cryosurvival rates to those achieved with the traditional BRL co-culture system.

Transfer of IVF-Derived Embryos

In most cases, IVF-derived embryos of a given species are transferred in the same manner as *in vivo*-derived embryos. Once removed from the incubator, IVF-derived

embryos are usually transferred immediately; whereas, at least with some species, *in vivo*-derived embryos can be held at room temperature for up to 24 hours prior to transfer. Also, as described below for cattle, IVF-derived embryos require that synchrony—the matching of the age of the embryo to the estrous stage of the recipient—be calculated more tightly than is necessary for *in vivo*-derived embryos.