In vitro Production of Bovine Embryos - A Review

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ABSTRACT : Over the years, the embryo transfer industry has grown from the simple collection & transfer of embryos into an advanced field of embryo biotechnology. Currently a large demand exists for bovine oocytes and early embryos in both research and commercial settings. Bovine embryos can now be produced in-vitro. Primary oocytes collected from antral follicles of abattoir - obtained ovaries can be induced to undergo the maturation process. In-vitor maturation system, however must ensure that the resulting oocyte is capable of undergoing normal fertilization and yields a zygote competent of developing to term after embryo transfer. Sperm preparation for IVF has improved with the use of heparine. The use of co-culture system has proved beneficial in circumventing the developmental block in IVM/IVF bovine embryos. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 9 : 1342-1351)*

Key Words : *In-vitro*, Maturation, Fertilization, Embryo, Bovine

INTRODUCTION

In vitro production of viable oocytes,fertilized ocytes and embryos has become an essential part of contemporary embryo technology in cattle.The technical aspects of *in vitro* production have been subject to several reviews (see e.g. Leibfried-Rutledge et al., 1989;Gordon and Lu, 1990; Greve and Madison, 1991), and its practical implications in animal breeding schemes has recently been addressed (Betteridge et al.,1989; Gordon 1991). From a basic science standpoint, the area of *in vitro* production is very exciting. It has given us a better understanding of the basic events of reproduction, as *in vivo* processes are relatively inaccessible for study. A program for *in vitro* production of transferable stage bovine embryos from immature ovarian oocytes involves three main stages, (1) *in vitro* maturation (IVM) of oocytes, (2) sperm capacitation and *in vitro* fertilization (IVF), and (3) *in vitro* culture of embryos.

1) *IN VITRO* MATURATION OF OOCYTES

Oocyte maturation involves nuclear and cytoplasmic events, including changes in the plasma membrane, and nuclear and cytoplasmic maturation. Pincus and Enzmann (1935) were the first to recognize that oocytes once liberated from follicles and maintained in serum containing culture media without added hormones would spontaneously resume meiosis (i.e. nuclear maturation). In cattle, the first attempts to mature and fertilize primary oocytes recovered from slaughterhouse ovaries were those of Sreenan (1968; 1970). Other investigators have demonstrated that oocytes will readily undergo nuclear maturation when removed from follicles (Edwards, 1965; Toyoda and Chang, 1974; Mahi and Yanagimachi, 1976; Ball et al., 1983a) but their potential to be fertilized was limited (Sreenan, 1970). Oocytes matured under such conditions failed to form normal pronuclei after sperm penetration (Thibault et al., 1975a, b) or did not continue development into blastocysts following transfer to the oviducts of inseminated recipients (Moor and Trounson, 1977).

In addition to nuclear maturation, cytoplasmic and membrane maturation are critical components of the maturation process for continued viability of oocytes following fertilization (Thibault and Gerard, 1973). Cytoplasmic maturation remains ill-defined, but involves both reorganization of cytoplasmic components (Hyttel et al., 1986; Ducibella et al., 1990) and synthesis of ooplasmic proteins (Moor et al., 1980, 1983; Kastrop et al., 1990) in preparation for fertilization and embryonic development. Though the identity and function of these newly synthesized proteins remains unclear, decreased fertilization and development of oocytes matured in the presence of translational inhibitors (e.g. cycloheximide) suggest a role in the developmental potential of the embryo (Hunter and Moor, 1987).

OOCYTE IVM METHODS IN DOMESTIC ANIMALS

Ovary storage conditions

Previous studies utilized ovaries obtained at a local slaughterhouse and transported to the laboratory as quickly as possible. Transportation conditions reported included: temperature maintained between 20°C and 37°C; interval from slaughter to oocytes recovery 2 to 6 h, and normal saline or Dulbecco's phosphate buffered saline as a transportation medium (Staigmiller, 1988; Kim et al., 1990; Voelkel and Hu, 1992). Work by Yang et al. (1990) suggests that cattle ovaries can be stored for up to 11 h at a temperature of 25°C without compromising the outcome of

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IVM/IVF and subsequent embryo development. Such information is likely to be valuable in planning the collection of ovaries from abattoirs located some distance from the embryo processing laboratory.

Oocyte recovery

Aspiration of follicular oocytes, using either a pipette or a syringe and needle (18 g) has been the common method of recovering bovine follicular oocytes (Sreenan, 1968; Iritani and Niwa, 1977; Leibfried-Rutledge and First, 1979; Hanada, 1985; Xu et al., 1988; Sirard et al., 1988). Lu et al. (1987; 1988) used dissection with subsequent rupture of the intact follicles and obtained a higher yield of oocytes per ovary acceptable for maturation. Scoring the ovarian surface with a sterile surgical blade yielded greater number of morphologically good oocytes than the aspiration method (Khan et al., 1977; Samad et al., 1998).

Stage of the estrous cycle and size of the follicle

Bovine *in vitro* fertilization studies have involved recovery of oocytes from ovaries without regard to the stage of the estrous cycle. There is little evidence to show that the estrous cycle is an important factor influencing oocyte maturation (Leibfried-Rutledge et al., 1985; Tan and Lu, 1990). However, the appearence of an antral cavity in the follicle and the size of oocytes have been correlated with acquisition of meiotic competence (Motlik and Fulka, 1986). Primary oocytes from all species studied show an acquired ability of meiotic competence after they have reached full-size. The work of Tan and Lu (1990) indicated that bovine oocytes recovered from follicles smaller than 1.5 mm in diameter can contain growing oocytes that have not yet become meiotically competent.

Maturation culture system

Both intrafollicular and extrafollicular culture systems are used for oocyte maturation. Intrafollicular oocytes (ovine: Moor and Trounson, 1977; bovine: Fukui et al., 1987) matured in medium containing hormones have been shown to be developmentally competent. Oocytes for extrafollicular maturation are selected based on their general appearence and the density of granulosa cells surrounding them. Most granulosa cells in mammalian antral follicles constitute the wall of the follicle (mural granulosa) while a few closely surround the oocytes (cumulus cells) to form the germ cell somatic complex i.e., oocyte-cumulus cell complex (OCC). Cumulus cells form processes that penetrate the cortex of the oocytes, where they form gap juctions with the oolema. This intimate association has been shown to be important for in vivo and *in vitro* oocyte growth (Eppig, 1979; Crosby et al., 1981; Eppig and Schroeder, 1986; Critser et al., 1986) and regulation of meiotic maturation (Schultz, 1986; Kastrop et al., 1991). The importance of cumulus cells for *in vitro* fertilization and development is shown by the lower proportion of oocytes that mature if the oocytes are denuded before maturation (Fukui and Sakuma, 1980; Staigmiller and Moor, 1984; Eppig and Schroeder, 1986). Also premature breakdown of the cell contacts reduces the developmental potency of the oocytes (Mattioli et al., 1988). The number of layers of cumulus cells surrounding the oocytes during IVM also influence their subsequent developmental capacity. Yang and Lu (1990) presented evidence showing best results were obtained with bovine oocytes that possesed more than 5 layers of cumulus cells. Bovine OCCs graded on the basis of their morphological appearence, i.e. homogeneity of the ooplasm, color, size and thickness and compactness of the cumulus investment are different in their ability to mature, fertilize and develop (Younis et al., 1989; De Loos et al., 1991; Hazeleger and Stubbings, 1992). A positive identification of oocytes with very high developmental potency is not yet possible, but using stringent selection, the variation which occurs in immature bovine OCC can be eliminated, thereby enhancing developmental results.

Culture media

A wide variety of media have been used for IVM in domestic animals, ranging from simple physiological solutions to complex culture media containing amino acids, vitamins, purines, and other compounds regarded as essential for general cell culture. Although acceptable rates of nuclear maturation have been obtained with most media, TCM-199 has emerged as the most commonly used medium for bovine oocytes (Staigmiller, 1988). The medium used for oocyte maturation can strongly affect the developmental capacity of *in vitro* produced bovine embryos (Bavister et al., 1992).

Hormone and serum supplements

It is well known that the follicle destined for ovulation in the bovine, is exposed to a changing environment of gonadotrophins, steroids, growth factors and other molecules. These factors acting separately or in concert can influence cytoplasmic maturation of the oocyte. To achieve full maturation of oocytes *in vitro*, several important factors, such as addition of hormones, growth factors and serum to the culture medium have been studied. The importance of the hormonal environment during *in vitro* maturation of mammalian oocytes on subsequent fertilization and development have been demonstrated in sheep (Moor and Trounson, 1977; Moor et al., 1980) and cattle (Fukushima and Fukui, 1985; Zuelke et al., 1989). For maturation of bovine oocytes, supplementation of the medium with gonadotrophins and steroids has been found beneficial (First and Parrish, 1987; Fayrer-Hosken et al., 1988; Sirard

et al., 1989). However, Lu and Gordon (1987) found that, far from improving the outcome of IVM/IVF, the use of hormone supplements had an unfavourable effect. Fukui and Ono (1989) and Fukushima et al. (1991) and Jenner et al. (1995) found no effect of added hormones on bovine oocyte maturation and development to the blastocyst stage.

In most IVM studies, the basic medium is supplemented with serum or crystallized albumin. Fetal calf serum (FCS), Estrus cow serum (ECS) and bovine serum albumin (BSA) have found the most use. FCS supported nuclear maturation of cow oocytes at a greater rate than did BSA (Leibfried-Rutledge et al., 1986). Sanbuissho and Threlfall (1985) reported that the presence of ECS during maturation significantly improved fertilization and development of bovine oocytes over serum collected at ovulation or 24 h post-ovulation. Younis et al. (1989) obtained higher cleavage rates following IVF of bovine oocytes matured in the presence of day 0 (obtained at estrus) or day 20 (obtained at proestrus) cow serum. ECS proved to be superior to FCS in bovine IVM/IVF studies (Lu and Gordon, 1987; Schellander et al., 1990). Addition of serum (FCS or ECS) enhanced the maturation rates of bovine oocytes compared to serum free medium (Lorenzo et al., 1996). Using serum free medium, Zuelke and Brackett (1990) and Saeki et al. (1991) reported that the addition of serum during oocyte maturation was not required but the addition of gonadotrophins (LH and FSH) and estadiol 17B enhanced the rate of fertilization and developmental ability of bovine oocytes matured *in vitro*. Conflicting reports in the literature regarding optimum conditions for *in vitro* maturation, especially serum and hormones, indicate that continued research is needed.

2) SPERM CAPACITATION AND *IN VITRO* FERTILIZATION

Mammalian spermatozoa, matured in the epididymis and ejaculated, are not immediately capable of fertilizing oocytes. These sperm must undergo a period of capacitation, which normally occurs in the female reproductive tract (Austin, 1951; Chang, 1951).

There are several factors controlling or affecting capacitation in vivo or *in vitro*. *In vitro* heparine was shown to be the most potent glycosaminoglycan in its ability to induce the acrosome reaction in bovine epididymal spermatozoa (Handrow et al., 1982) and to capacitate ejaculated bovine spermatozoa (Parrish et al., 1985b). Several investigators have observed that uncapacitated spermatozoa fail to penetrate the cumulus cells, while capacitated spermatozoa move freely within the cumulus mass (Cummins and Yanagimachi, 1986; Corselli and Talbot, 1987).

In vitro fertilization has been accomplished in more than 20 mammalian species (for refrences and reviews see Wright and Bondioli, 1981; Brackett, 1983; 1985; Ball et al., 1984; First and Parrish, 1987; Staigmiller, 1988; Leibfried-Rutledge et al., 1989; Gordon and Lu, 1990). The first report of successful IVF was documented by Chang in 1959 by obtaining live rabbit offspring. Chang (1959), cultured freshly ovulated rabbit oocytes with in vivo capacitated spermatozoa which were obtained by flushing the uterine horns of females mated 12 h beforehand.Yanagimachi and Chang (1963) were the first to demonstrate that hamster spermatozoa could be prepared for fertilization outside of the female genital tract. Since then many investigators have succeeded in fertilizing eggs *in vitro* with epididymal or ejaculated spermatozoa or both.

The first bovine offspring from IVF was a bull calf born in 1981 (Brackett et al., 1982). The initial success with bovine IVF followed use of in vivo matured ova inseminated with *in vitro* capacitated ejaculated bull spermatozoa. Later, initiation of pregnancy with embryos resulting from IVM and IVF has been well documented and serves to validate the physiological relevance of data obtained from these procedures (Critser et al., 1986; Lu et al., 1987; Xu et al., 1987; Goto et al., 1988; Sirard et al., 1988; Stubbings et al., 1988; Younis et al., 1989; Brackett et al., 1989).

The intended outcome of IVF is to produce a viable embryo, except when using IVF as a means of detecting sperm capacitation or perhaps as a predictor of in vivo fertility.The success of fertilization *in vitro* relies upon completion of both oocyte maturation and sperm capacitation. Failure of either of these steps will result in fertilization failure. In addition, the sperm concentration, time of sperm-egg interaction, medium utilized and culture conditions play a role in successful fertilization which will result in developmentally competent zygotes.

3) *IN VITRO* CULTURE OF EARLY EMBRYOS

Mammalian embryos have been cultured in a variety of chemically defined and undefined media. A chemically defined medium has been described as a liquid prepared from bench chemicals and containing four or less basic components: inorganic salts, amino acids, vitamins and an energy source at known concentrations (for lists of ingredients of several common defined media see, Wright and Bondioli, 1981; Freshney, 1987; Biggers, 1987; Wright and O'Fallon, 1987). In contrast, undefined medium is a liquid containing any biological fluid where the composition and the components can vary considerably. Early embryo studies utilized undefined media such as blood plasma, blood sera, follicular fluid and chick egg extracts for culture (see Wright and Bondioli, 1981 for references).

In vitro culture of mammalian embryos was first accomplished in undefined media such as serum or plasma (Pincus and Enzmann, 1935). Progress in embryos culture has originated from studies involving laboratory species (mouse: Brinster, 1965a-d; rabbit: Maurer et al., 1968; Kane and Foote, 1971; Ogawa et al., 1971; Kane, 1972; hamster: Bavister et al., 1983; ferret: Whittingham, 1975) and provided models for the culture of domestic animal embryos from early stages through to the hatched blastocyst stage. Basic culture conditions, such as, media (types and supplements), pH, osmolarity, gaseous atmosphere and temperature, that can influence *in vitro* development of the preimplantation stage farm animal embryos have been discussed in detail by Wright and Bondioli (1981) and Wright and O'Fallon (1987).

For *in vitro* production of non-surgically transferable stage bovine embryos, a suitable *in vitro* culture system, capable of efficiently supporting development to the morula or blastocyst stage is an essential prerequisite. In initial studies a wide variety of culture media were used but only limited *in vitro* development of preimplantation cow embryos could be obtained (Brock and Rowson, 1952; Brinster, 1968; Sreenan, 1968; Sreenan et al., 1968; Onuma and Foote, 1969; Seidel et al., 1971; McKenzie and Kenney,1973). Although, Wright et al. (1976a, b) reported the first *in vitro* expansion and hatching of blastocysts from bovine embryos cultured at 8-cell and 1- to 2-cell stages. However, several invesigators reported that early bovine embryos when cultured *in vitro* failed to develop past the 8- to 16-cell stage, whereas embryos cultured from the 16-cell stage or later frequently developed into compact morulae and blastocysts (Thibault, 1966; Wright and Bondoli, 1981; Camous et al., 1984; Heyman et al., 1987). This observation led Thibault (1966) to suggest the existance of a "block" to *in vitro* development at the 8- to 16-cell stage.

The cattle is not the only species which exhibits a block to embryonic development *in vitro*. Blocks to *in vitro* development have been described at the 2-cell stage in outbred mouse (Cole and Paul, 1965; Goddard and Pratt, 1983) and hamster (Yanagimachi and Chang, 1964; Whittingham and Bavister, 1974; Farrel and Bavister, 1984) embryos, the 4-cell stage in porcine embryos (Davis and Day, 1978; Herrmann and Holtz, 1981; Davis, 1985) and the 8 to 16-cell stage in ovine embryos (Bondioli and Wright, 1980; Gandolfi and Moor, 1987). Interestingly, embryos of these species stay within the oviduct during that critical period when block to development occurs *in vitro*. According to First and Parrish (1987), the stage at which block to *in vitro* development occurs indicate that (1) the oviduct and not the culture medium contains factors or conditions condusive to early embryonic development and (2) that certain developmental events occuring between the 1 and 16-cell stage require specific environmental factors or conditions normally provided by the oviduct.

The stage of blocked development occurs at a time of prolonged cell cycle, DNA synthesis and a transition from maternal to zygotic control of development for the mouse (Goddard and Pratt, 1983), cow (King et al., 1985), sheep (Calaraco and McLaren, 1976) and pig (Norberg, 1973). It is difficult to rescue the embryo once the block has initiated (Eyestone and First, 1986). The mechanism(s) of the blocks to *in vitro* development is unclear. Whether the culture medium fails to provide critical substrates for synthesis of essential protein, cell cycle controlling signals, or a proper environment for transcription or translation is unknown (First and Parrish, 1987).

Various methods have been employed to circumvent the obvious limitations imposed by these blocks. For example Whittingham and Biggers (1967) used organ culture (explanted oviducts) and were successful in overcoming 2-cell block in mice. *In vitro* fertilized cattle zygotes or early embryos have been cultured through 8-to 16-cell block to a transferable morula or blastocyst stage by utilizing the oviducts of sheep (Eyestone et al., 1985) and rabbits (sirard et al., 1985) in situ. This procedure, however, adds another level of complexity.

One suggested approach in an attempt to define optimum conditions for culture of farm animal embryos was selection of media and additives, gas atmosphere, and stage of development of embryos (Wright and Bondioli, 1981). Following this approach, there have been reports of the development *in vitro* of 1-cell zygotes to the blastocyst stage by utilizing modifications of prexisting media for certain blocking strains of mice (Pomp et al., 1988; Chatot, et al., 1989, 1990; Spindle, 1990). Similarly recent reports indicate that a simple medium, CR1aa, supports bovine embryo development very well without cell co-culture (Rosenkrans and First, 1991; Keefer, 1992; Dominko and First, 1992).

Another approach to overcome the developmental arrest *in vitro* is to culture the embryos in the presence of other cell types (feeder cells) that provide stimulus for the development of the embryos. Feeder cells used for embryo co-culture falls into 3 groups: (1) feeder cells from reproductive tissue, (2) Feeder cells from nonreproductive tissues, and (3) Trophoblastic vesicles (see reviews by Heyman et al., 1987; Rexroad, 1989; Bongso et al., 1991).

Prior to 1980's, the use of feeder monolayers for the *in vitro* culture of farm animal embryos had not been reported. Kuzan and Wright (1981) reported the first study on porcine blastocyst development, testing the fibroblast cells from bovine uterine or testicular tissues as feeder monolayers. They found that uterine fibroblast monolayers provided a superior substratum for blastocyst attachment and trophoblatic outgrowth of porcine embryos *in vitro*. In another study, Kuzan and Wright (1982) observed that

bovine morulae hatched equally well on both bovine uterine and testicular fibroblast monolayers. The use of fetal bovine uterine fibroblast cells (FBUFC) for the culture of bovine (Wiemer et al., 1987; Pool et al., 1988), equine (Wiemer et al., 1988) embryos has proved superior over that of medium alone.

Currently, the oviductal epithelial cells are commonly used for co-culture of cow (Ellington et al., 1990; Nagao et al., 1991; Xu et al., 1992), sheep (Holm et al., 1991; Powell and Rexroad, 1992), goat (Prichardet al., 1991), pig (Reed et al., 1992) and equine (Ball and Miller, 1991; Ball et al., 1992) embryos. However, Rexroad and Powell (1986) were the first to report the use of ovine oviductal epithelial cell monolayer for embryo co-culture and found improved development of co-cultured 1- to 8-cell ovine embryos over embryos cultured in the medium alone. Gandolfi et al. (1986) reported that one-cell sheep embryos cultured in TCM-199 with 10% fetal calf serum (M199FCS) on an oviductal cell monolayer developed better compared to medium alone. Eyestone et al. (1987) introduced a similar oviductal epithelial cell culture system for early-stage bovine embryos. They reported that 5- to 8-cell bovine embryos co-cultured on the bovine oviductal epithelial cell (BOEC) monolayer for 4 to 5 days resulted in 46% of the embryos developing to late morula or blastocyst stage compared to zero development in Hams's F-10 alone. The results of this study were confirmed by a later report utilizing a BOEC co-culture system (Eyestone and First, 1988).

More recently, investigators have reported the use of BOEC (Eyestone and First, 1989; Kim et al., 1990; Boccart et al., 1991; Nagao et al., 1991; Rorie et al., 1992; Xu et al., 1992) and bovine cumulus/granulosa cell (Goto et al., 1988; Fukuda et al., 1990; Kajihara et al., 1990; Younis and Brackett, 1991; Wang et al., 1992) co-culture systems for *in vitro* production of transferable stage bovine embryos from IVM\IVF oocytes. In most of these studies the basic culture medium used for co-culture was TCM 199, which was supplemented with different additives and varying concentrations of serum of bovine origin.

Jiang et al. (1991) compared the *in vitro* development of early bovine embryos (2 to 8-cell stage) to the blastocyst stage on seven different monolayers of bovine origin (granulosa cell, oviducal cell, uterine cell and the combination of these cells) and found no differences except for granulosa and uterine cells (37.2% versus 26.1%). Goto et al. (1992) obtained 23% blastocyst development *in vitro* from 2- to 8-cell IVM/IVF bovine embryos in medium alone which were morphologically of poor quality compared with embryos produced by co-culture.

The positive effect exerted by the reproductive tract cells may be non-specific as other types of somatic cells (skin, testis, liver) from different species could support the development of bovine embryos *in vitro* (Goto et al., 1992). This result is not surprising because others have also reported the positive effects of co-culture system on the development of bovine (Kuzan and Wright, 1982; Camous et al., 1984; Voelkel et al., 1985), murine (Ouhibi et al., 1990) and human (Menezo et al., 1990) embryos using various types of feeder cells. Recently, Buffalo rat liver cells (BRLC) monolayer prepared from commercially available permanent cell line have been reported to support the *in vitro* development of IVM/IVF bovine embryos (van Inzen et al., 1993). Voelkel and Hu (1992) reported that post-freezing viability of IVM/IVF bovine embryos co-cultured with BRLC monolayers was significantly higher compared to those cultured with BOEC monolayers. BRLC have been shown to synthesize and secrete multiplication stimulating activity, a family of polypeptides, which can replace the multiplication stimulating activity present in serum and support proliferation of serum-dependent cells in serum-free medium (Dulak and Temin, 1973; Temin et al., 1974). The use of commercially available cell lines for co-culture are very useful because they are quality-control tested for microbes and endotoxins.

While these co-culture systems can be criticized for being undefined, they do achieve the desired effect of circumventing the "*in vitro* block" and producing viable embryos. The rationale behind these co-culture systems is that the feeder cells are providing some factor(s) to the embryos that improve their *in vitro* development This "positive conditioning" (Kuzan and wright, 1982; Bavister et al., 1992) may involve a growth factor or some other factor normally produced by such cells. An alternative explanation may be that the cells remove an inhibitory component from the culture environment i.e. "the negative conditioning" (Kuzan and wright, 1982; Bavister et al., 1992). Moreover, both positive and negative conditioning may be occuring simultaneously.

The use of co-culture systems have proved beneficial in circumventing the development block in IVM/IVF bovine embryos according to the reported studies. However, the proportion of these embryos reaching the morula / blastocyst stage was small (25-40% of inseminated oocytes) and few hatched (Bavister et al., 1992). This may be due to some fundamental underlying problem, such as inadequacy of the culture conditions, or possibly abnormal oocyte maturation. Further improvement including *in vitro* culture systems comparable with the in vivo environment, using defined medium for both oocyte maturation and embryo development, is needed.

CONCLUSIONS

Successful establishment of procedures for *in-vitro* production of bovine embryos have laid down the

foundations for advanced reproductive technologies like transgenesis, cloning and totipotent cell production. However, IVM, IVF and *in-vitro* embryo culture systems vary greatly among different labs, which makes comparing the results between the groups much more difficult. There is no consensus to date, for example, on to which medium is the best for bovine embryo culture and one is left with the one that works best in his own laboratory. A concentrated effort from research labs and commercial settings is required to understand and overcome some of the existing problems. For mass scale production of developmentally competent bovine embryos further improvement in embryo culture system is still needed.

REFERENCES

Austin, C. R. 1951. Observations on the penetration of the sperm into the mammalian egg. Aust. J. Sci. Res. (B), 4:581-596.

Ball, B. A. and P. G. Miller. 1991. Viability of equine embryos co-cultured with equine oviductal epithelium from the 4-8-cell to blastocyst stage. Theriogenology 35:183(Abstr.).

Ball, B. A., P. G. A. Thomas, S. P. Brinsko, P. G. Miller and J. E. Ellington. 1992. Development of 1- to 2-cell equine embryos cocultured with oviductal epithelial cells. Theriogenology 37:189(Abstr.).

Ball, G. D., M. L. Leibfried, R. L. Ax and N. L. First. 1983a. Maturation and fertilization of bovine oocytes *in vitro*. J. Dairy Sci. 67:2775-2785.

Bavister, B. D., M. L. Leibfried and G. Lieberman. 1983. Development of preimplantation embryos of the golden hamster in a defined culture medium. Biol. Reprod. 28:235-237.

Bavister, B. D., T. A. Rose-Hellekant and T. Pinyopummitr. 1992. T. Development of *in vitro* matured/*in vitro* fertilized bovine embryos into morulae and blastocysts in defined culture media. Theriogenology 37:127-146.

Betteridge, K. J., C. Smith, R. B. Stubbings, K. P. Xu and W. A. King. 1989. Potential genetic improvement of cattle by fertilization of fetal oocytes *in vitro*. J. Reprod. Fert.(Suppl.) 38:87-98.

Biggers, J. D. 1987. Pioneering mammalian embryo culture. In: The Mammalian Preimplantation Embryo: Regulation of Growth and Differentiation *in vitro* (Ed. B. D. Bavister). Plenum Press, N.Y., 1987. pp. 1-22.

Boccart, C. P. Mermillod, C. Defecoeuillerie and F. Dessy. 1991. Bovine oviduct cell monolayers for supporting the blastocyst formation of bovine embryos. Theriogenology 35:187(Abstr.).

Bondioli, K. R. and R. W. Jr. Wright. 1980. Influence of culture media on *in vitro* fertilization of ovine tubal oocytes. J. Anim. Sci. 51:660-667.

Bongso, A., S. C. Ng, C. T. Fong and S. S. Ratnam. 1991. Cocultures: a new lead in embryo quality improvement for assisted reproduction. Fertil. Steril. 56:179-191.

Brackett, B. G., D. Bousquet, M. L. Boice, W. J. Donawick, J. F. Evans and M. A. Dressel. 1982. Normal development following *in vitro* fertilization in the cow. Biol. Reprod. 27:147-158.

Brackett, B. G. 1983. A review of bovine fertilization *in vitro*. Theriogenology 19:1-15.

Brackett, B. G. 1985. *In vitro* oocyte maturation and fertilization J. Anim. Sci. 61:14-24.

Brackett, B. G., A. I. Younis and R. A. Fayre-Hosken. 1989. Enhanced viability after *in vitro* fertilization of bovine oocytes matured in vivo with high concentrations of luteinizing hormone. Fertil. Steril. 72:2826-2833.

Brinster, R. L. 1965a. Studies on the development of mouse embryos *in vitro*. I. The effect of osmolarity and hydrogen ion concentration. J. Exp. Zool. 158:48-58.

Brinster, R. L. 1965b. Studies on the development of mouse embryos *in vitro*. II. The effect of energy source. J. Exp. Zool. 158:58-68.

Brinster, R. L. 1965c. Studies on the development of the mouse embryos *in vitro*. III. The effect of fixed nitrogen source. J. Exp. Zool. 158:69-78.

Brinster, R. L. 1965d. Studies on the development of mouse embryos *in vitro*. IV. Interaction of energy sources. J. Reprod. Fert. 10:2270240.

Brinster, R. L. 1968. *In vitro* culture of mammalian embryos. J. Anim. Sci. 27:1-15.

Brock, H. and L. E. A. Rowson. 1952. The production of viable bovine ova. J. Agic. Sci., Camb. 42:479-482.

Calarco, P. G. and A. McLaren. 1976. Ultrastructural observations of preimplantation stages of sheep. J. Embryol. Exp. Morph. 36:609-622.

Camous, S., Y. Heyman, W. Meziou and Y. Menezo. 1984. Cleavage beyond the block stage and survival after transfer of early bovine embryos cultured with trophoblastic vesicles. J. Reprod. Fertil. 72:479-485.

Chang, M. C. 1951. Fertilizing capacity of spermatozoa deposited in fallopian tube. Nature 8:997-998.

Chang, M. C. 1959. Fertilization of rabbit ova *in vitro*. Nature 184:466-467.

Chatot, C. L., C. A. Ziomek, B. D. Bavister, J. L. Lewis and I. Torres. 1989. An improved culture medium supports development of random bred 1-cell mouse embryos *in vitro*. J. Reprod. Fertil. 86:679-688.

Chatot, C. L., J. L. Lewis, I. Torres and C. A. Ziomek. 1990. Development of 1-cell embryos from different strains of mice in CZB medium. Biol. Reprod. 42:432-440.

Cole, R. J. and J. Paul. 1965. Properties of preimplantation mouse and rabbit embryos, and cell strains derived from them. In: Preimplantation Stages of Prgnancy (Ed. G. E. W. Wolstenholme and M. O'Connor). Churchill, London, pp. 82-122.

Critser, E. S., M. L. Leibfried, W. H. Eyestone, D. L. Northey and N. L. First. 1986. Acqisition of developmental competence during maturation *in vitro*. Theriogenology 25:151(Abstr.).

Crosby, I. M., J. C. Osborn and R. M. Moor. 1981. Follicle cell regulation of protein synthesis and development competence in sheep oocytes J. Reprod. Fertil. 62:575-582.

Crozet, N. and M. Domont. 1984. The site of the acrosome reaction during *in vivo* penetration of the sheep oocyte Gamete Res. 10:97-105.

Davis, D. L. and B. N. Day. 1978. Cleavage and blastocyst formation by pig eggs *in vitro*. J. Anim. Sci. 46:1043-1053.

Davis, D. L. 1985. Culture and storage of pig embryos. J. Reprod.

Fertil. 33(Suppl):115-124.

De Loos, F., P. Kastrap, P. Van Maurik, T. H. Van Benedon and Th. A. M. Kruip. 1991. Heterologous cell contacts and metabolic coupling in bovine cumulus oocyte complexes. Mol. Reprod. Dev. 28:255-259.

Dominko, T. and N. L. First. 1992. Kinetics of bovine oocyte maturation allows selection for developmental competence and is affected by gonadotropins. Theriogenology 37:203(Abstr.).

Ducibella, T., P. Duffy, R. Reindollar and B. Su. 1990. Biol Reprod. 43:870-876.

Dulak, N. C. and H. M. Temin. 1973. A partially purified polypeptide fraction from rat liver cell conditioned medium with multiplication-stimulating activity for embryo fibroblast. J. Cell Physiol. 81:153-160.

Edwards, R. D. 1965. Maturation *in vitro* of mouse, sheep, cow, pig, Rhesus monkey and human ovarian oocytes. Nature (London) 208:349-351.

Ellington, J. E., E. W. Carney, P. B. Farrel, M. E. Simkin and R. H. Foote. 1990. Bovine 1-2-cell embryo development using a simple medium in three oviductal epithelial cell coculture systems. Biol. Reprod. 43:97-104.

Eppig, J. J. 1979. A comparison between oocyte growth in coculture with granulosa cells and oocytes with granulosa cell-oocyte contact maintained *in vitro*. J. Exp. Zoo. 209:345-353.

Eppig, J. J. and A. C. Schroeder. 1986. Culture systems for mammalian oocyte development: progress and prospects. Theriogenology 25:97-106.

Eyestone, W. H. and N. L. First. 1986. A study of the 8- to 16-cell developmental block in bovine embryos cultured *in vitro*. Theriogenology 25:152(Abstr.).

Eyestone, W. H., D. L. Northey and M. L. Leibfried. 1985. Culture of 1-cell bovine embryos in the sheep oviduct. Biol. Reprod. 32 (Suppl. 1):100(Abstr.).

Eyestone, W. H., J. Vignieri and N. L. First. 1987. Co- culture of early embryos with oviductal epitheium. Theriogenology 27:228(Abstr.).

Eyestone, W. H. and N. L. First, 1988. Co-culture of bovine embryos with oviductal tissue. Proc. 11th Int. Congr. Anim. Reprod. & A.I., Dublin, Ireland. 4:471(Abstr.).

Eyestone, W. H. and N. L. First. 1989. Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or conditioned medium. J. Reprod. Fertil. 85:715-720.

Farrell, P. S. and B. D. Bavister. 1984. Short term exposure of two-cell hamster embryos to collection media is detrimental to viability. Biol. Reprod. 31:109.

First, N. L. and J. J. Parrish. 1987. *In-vitro* fertilization of ruminants. J. Reprod. Fertil. 34:151-165.

Freshney, R. I. 1987. Culture of animal cells: A manual of basic techniques. John Wiley & Sons, Inc., N.Y. pp. 227-229.

Fukuda, Y., M. Ichikawa, K. Naito and Y. Toyoda. 1990. Birth of normal calves resulting from bovine oocytes matured, fertilized and cultured with cumulus cells *in vitro* up to the blastocyst stage. Biol. Reprod. 42:114-119.

Fukui, Y. and Y. Sakuma. 1980. Maturation of bovine oocytes cultured *in vitro*: Relation to ovarian activity, follicular size and the presence or absence of cumulus cells. Biol. Reprod. 22:669-673.

Fukui, Y., K. Imai, N. F. Alfonso and H. Ono. 1987. Follicle culture enhances fertilizability and cleavage of bovine oocytes matured *in vitro*. J. Anim. Sci. 64:935-941.

Fukui, Y. and H. Ono. 1989. Effects of sera, hormones and granulosa cells added to culture medium for *in vitro* maturation, fertilization, cleavage and development of bovine oocytes. J. Reprod. Fertil. 86:501-506.

Fukushima, M. and Y. Fukui. 1985. Effects of gonadotropins and steroids on the subsequent fertilizability of extrafollicular bovine oocyte cultured *in vitro*. Anim. Reprod. Sci. 9:323-332.

Fukushima, M., Y. Shioya, M. Kuwayama, S. Iwasaki and A. Hanada. 1991. A. Effects of gonadotropins and estradiol added into maturation medium of bovine oocytes *in vitro* on the subsequent capacity for fertilization and embryonic development. Jpn. J. Anim. Reprod. 37:127-132.

Gandolfi, F. and R. M. Moor. 1987. Stimulation of early embryonic development in the sheep by co-culture with oviductal epithelial cells. J. Reprod. Fert. 81:23-29.

Go, K. J. and D. P. Wolf. 1985. Albumin mediated changes in the sperm sterol contents during capacitation. Biol. Reprod. 32:145-153.

Goddard, M. J. and H. D. M. Pratt. 1983. Control of events during early cleavage of the mouse embryo: An analysis of the "2-cell block".J. Embryol. Exp. Morphol. 73:111-133.

Gordon, I. and K. H. Lu. 1990. Production of embryos *in vitro* and its impact on livestock production. Theriogenology 33:77-87.

Gordon, I. 1991. Potential Application of *in vitro* fertilization in commercial practice and research. Embryo Transfer Newsletter 9:4-9.

Goto, K., Y. Kajihara, S. Kosaka, M. Koba, Y. Nakanishi and K. Ogawa. 1988. Pregnancies after coculture of cumulus cells with bovine embryos derived from *in vitro* fertlization of *in vitro* matured follicle oocytes. J. Reprod. Fertil. 83:753-758.

Goto, K., N. Iwai, Y. Takuma and Y. Nakanishi. 1992. Co-culture of *in vitro* fertilized bovine embryos with different cell monolayers. J. Anim. Sci. 70:1449-1453.

Greve, T. and V. Madison. 1991. *In vitro* fertilization in cattle: A review. Reprod. Nutr. Dev. 31:147-157.

Hanada, A. 1985. *In vitro* fertilization in cattle, with particular reference to sperm capacitation by Ionophore A23187 Jpn. J. Anim. Prod. 31:56-61.

Hazeleger, N. L. and R. B. Stubbings. 1992. Developmental potential of selected bovine oocyte cumulus complexes. Theriogenology 37:219(Abstr.).

Herrmann, H. H. and W. Holtz. 1981. Culture of pig embryos collected in situ or after slaughter. Anim. Reprod. Sci. 4:43-47.

Heyman, Y., Y. Menezo, P. Chesne, S. Camous and V. Gardinier. 1987. *In vitro* cleavage of bovine and ovine early embryos: Improved development using coculture with trophoblastic vesicles. Theriogenology 27:59-68.

Holm, P., B. Irvine, D. T. Armstrong and R. F. Seamark. 1991. *In vitro* production of sheep blastocyst from IVM-oocytes using frozen semen and oviductal epithelial cell co-culture for IVF. Theriogenology 35:214(Abstr.).

Hu, Y. X. and S. A. Voelkel. 1992. Effect of gas atmosphere on the development of one-cell bovine embryo in two culture systems. Theriogenology 37:1117-1131.

Hyttel, P., K. P. Xu, S. Smith and T. Greve. 1986. Ultrastucture of *in vitro* maturation in cattle. J. Reprod. Fertil. 78:615-625.

Iritani, A. and K. Niwa. 1977. Capacitation of bull spermatozoa

and fertilization *in vitro* of cattle follicular oocytes matured in culture. J. Reprod. Fertil. 50:119-121.

Jenner, L. J., M. D. Fray, P. J. Ross, H. P. Prentice and J. Brownlie. 1995. Development of an IVM-IVF-IVC system for bovine embryo production. J. Reprod. Fertil. Abstract Series 15. Abs.171.

Jiang, H. S., W. L. Wang, K. H. Lu, I. Gordon and C. Polge. 1991. Roles of different cell monolayers in co-culture of IVF bovine embryos. Theriogenology 35:216(Abstr.).

Khan, I. Q., H. A. Samad and N. U. Rehman. 1997. Quantity and quality of buffalo follicular oocytes recovered by aspiration and scoring methods for *in vitro* studies. Pakistan Vet. J., 17(4):187-189.

Kajihara, Y., N. Kometani, S. Kobayashi, Y. Shitanaka, Y. Koshiba, K. Hishiyama, K. Shiraiwa and K. Goto. 1990. Pregnancy rates and births after co-culture of cumulus cells with bovine embryos derived from *in vitro* fertilization of matured follicular oocytes. Theriogenology 33:264(Abstr.).

Kane, M. T. and R. H. Foote. 1971. Factors affecting blastocyst expansion of rabbit zygotes and young embryos in defined media. Biol. Reprod. 4:41-47.

Kane, M. T. 1972. Energy substrates and culture of single cell rabbit ova to blastocysts. Nature 238:468-469.

Kastrop, P. M. M., M. M. Bevers, O. H. J. Destree and A. M. Kruip, Th. 1990. Changes in protein synthesis and phosphorylation pattern during bovine oocyte maturation *in vitro*. J. Reprod. Fertil. 90:305-310.

Kastrop, P. M. M., S. C. J. Hulshaf, M. M. Bevers, O. H. J. Destree and A. M. Kruip, Th. 1991. Effects of a-amanitin and cyclohexamide on nuclear progression, protein synthesis and phosphorylation during bovine oocyte maturation *in vitro*. Mol. Reprod. Dev. 28:249-254.

Keefer, C. L. 1992. Development of *in vitro* produced bovine embryos cultured individually in a simple medium: Effects of EGF and TGFB1. Theriogenology 37:236(Abstr.).

Kim, C. I., J. E. Ellington and R. H. Foote. 1990. Maturation, fertilization and development of bovine oocytes *in vitro* using TCM199 and a simple defined medium with co-culture. Theriogenology 33:433-440.

King, W. A., A. Niar and K. J. Betteridge. 1985. The nucleolus organizer regions of early bovine embryos. J. Dairy Sci. 68(Suppl.2):249(Abstr.).

Kuzan, F. B. and R. W. Wright, JR. 1981. Attachment of porcine blastocysts to fibroblast monolayers *in vitro*. Theriogenology 16:651-658.

Kuzan, F. B. and R. W. Wright, Jr. 1982. Observations on development of bovine morulae on various cellular and non-cellular substrata. J. Anim. Sci. 54:811-816.

Leibfried-Rutledge, M. L. and N. L. First. 1979. Characterization of bovine follicular oocytes and their ability to mature *in vitro*. J. Anim. Sci. 48:76-86.

Leibfried-Rutledge, M. L., E. S. Critser and N. L. First. 1985. Fertilization potential of follicular oocytes classified by stage of cycle and size of follicle. Theriogenology 23:753(Abstr.).

Leibfried-Rutledge, M. L., E. S. Critser and N. L. First. 1986. Effects of calf serum and bovine serum albumin on *in vitro* maturation and fertilization of bovine and hamster cumulus-oocyte complexes. Biol. Reprod. 35:850.

Leibfried-Rutledge, M. L., E. S. Critser, J. J. Parrish and N. L. First. 1989. *In vitro* maturation and fertilization of bovine oocytes. Theriogenology 31:61-74.

Lorenzo, P., M. J. Illera, J. C. Illera and M. Illera. 1995. Chronological aspects of the nuclear maturation of bovine oocytes cultivated *in vitro*. Anat. Histol. Embryol., 24(2):139-144.

Lu, K. H. and I. Gordon. 1987. Efect of serum, hormones and cumulus cells on the *in vitro* maturation of bovine oocytes. Annu. Conf. Soc. Study Fert. abstr. No. 81.

Lu, K. H., M. Gallagher and M. McGovern. 1987. Pregnancy established in cattle by transfer of embryos derived from *in vitro* fertilization of oocytes matured *in vitro*. Vet. Rec. 121:259-260.

Lu, K. H., I. Gordon, H. B. Chen, H. Gallagher and H. McGovern. 1988. Births of twins after transfer of cattle embryos produced by *in vitro* techniques. Vet. Rec. 122:539-540.

Mahi, C. A. and R. Yanagimachi. 1976. Maturation and sperm penetration of canine ovarian oocytes *in vitro*. J. Exp. Zool. 196:189-196.

Mattioli, M., G. Galeati, M. L. Bacci and E. Seren. 1988. Follicular factors influence oocyte fertilizability by modulating intercellular cooperation between cumulus cells and oocytes. Gamete Res. 21:223-232.

Maurer, R. B., W. L. Hunt, L. D. VanVleck and R. H. Foote. 1968. Developmental potential of superovulated rabbit ova. J. Reprod. Fertil. 15:171-175.

McKenzie, B. E. and R. M. Kenney. 1973. *In vitro* culture of bovine embryos. Am. J. Vet. Res. 34:1271-1276.

Menezo, Y. J. R., J. F. Guerin and J. C. Czyba. 1990. Improvement of human early embryo development *in vitro* by co-culture on monolayers of vero cells. Biol. Reprod. 42:301-306.

Moor, R. M. and A. D. Trounson. 1977. Hormone and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity. J. Reprod. Fertil. 49:101-109.

Moor, R. M., C. Polge and S. M. Willadsen. 1980. Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. J. Embryol. Exp. Morphol. 56:319-334.

Moor, R. M., I. M. Crosby and J. C. Osborn. 1983. Growth and maturation of mammalian oocytes. In: *In vitro* Fertilization and Embryo Transfer. (Ed. P. G. Crosignani and B. L. Rubin). Academic Press, London.

Motlik, J. and J. Fulka. 1986. Factors affecting meiotc competence in pig oocytes. Theriogenology 25:87-96.

Nagao, Y., K. Saeki and H. Kainuma. 1991. Development of bovine *in vitro* matured and fertilized oocytes co-cultured with oviductal tissue in serum free medium. Theriogenology 35:249(Abstr.).

Norberg, H. 1973. Ultrastructural aspects of the preattached pig embryo: Cleavage and early blastocyst stages. Z. Anat. EntwGesch. 143:95-114.

Ogawa, S., K. Satoh and H. Hashimoto. 1971. *In vitro* culture of rabbit ova from the single cell to the blastocyst stage. Nature 233:422-424.

Onuma, H. and R. H. Foote. 1969. *In vitro* development of ova from pre-puberal cattle. J. Dairy Sci. 52:1985-1987.

Ouhibi, N., J. Hamidi, J. Guillaud and Y. Menezo. 1990. Co-culture of 1-cell mouse embryos on different cell supports. Human Reprod. 5:737-743.

Pincus, G. and E. V. Enzmann. 1935. The comparative behavior of mammalian eggs in vivo and *in vitro*. The activation of mammalian eggs. J. Exp. Med. 63:665-675.

Pool, S. H., K. E. Weimer, R. W. Rorie and R. A. Godke. 1988. The use of trophoblastic vesicles and fetal uterine monolayer cells for the culture of pre-compaction stage bovine embryos. Proc. 11th Int. Congr. Anim. Reprod. & A. I. Dublin, Ireland. 4:479.

Pomp, D., E. S. Critser and J. J. Rutledge. 1988. Lower Sodium lactate in Whitten's medium improves *in vitro* developmental capacity of one-cell mouse embryo. Theriogenology 29:1019-1025.

Powell, A. M. and C. E. Rexroad, Jr. 1992. Co-culture of sheep zygotes on long-term cultures of estrus or luteal-phase oviductal cells on millicell inserts, Theriogenology 37:276(Abstr.).

Prichard, J. F., S. H. Pool, E. G. Blakewood, Y. Menezo and R. A. Godke. 1991. Culture of early-stage caprine embryos using goat oviductal cell monolayer. Theriogenology 35:259(Abstr.).

Reed, M. L., M. J. Illera and R. M. Petters. 1992. *In vitro* culture of pig embryos. Theriogenology 37:95-109.

Rexroad, C. E., Jr. and A. M. Powell. 1986. Co-culture of sheep ova and cells from sheep oviduct vesicles. Theriogenology 25:187(Abstr.).

Rexroad, C. E. Jr. 1989. Co-culture of domestic animal embryos. Theriogenology 31:105-114.

Rorie, R. W., T. D. Lester, G. F. Miller and D. L. Gliedt. 1992. *In vitro* development of bovine embryos co-cultured with bovine oviductal epithelial cells (BOEC) in either synthetic oviductal fluid (SOF) or M- 199 medium. Theriogenology 37:287(Absr.).

Rosenkrans, C. F. Jr. and N. L. First. 1991. Culture of bovine zygotes to the blastocysr stage: Effects of amino acids and vitamins. Theriogenology 35:266(Abstr.).

Saeki, K., M. Hoshi, M. L. Leibfried-Rutledge and N. L. First. 1991. *In vitro* fertilization and development of bovine oocytes matured in serum free medium. Biol. Reprod. 44:256-260.

Sanbuissho, A. and W. R. Threfall. 1985. The effect of estrous cow serum on the maturation and fertilization of bovine follicular oocyte *in vitro*. Theriogenology 23:226(Abstr.).

Schellander, K., F. Fuhrer, B. G. Brackett, B. Korb and W. Schleger. 1990. *In vitro* fertilization and cleavage of bovine oocytes matured in medium supplemented with estrous cow serum. Theriogenology 33:477-485.

Schultz R. M, 1986. Molecular aspects of mammalian oocyte growth and maturation. In: Experimental approaches to mammalian embryonic development. Ed. Rossant and Pederson, Cambridge Univ. Press, pp. 195-237.

Seidel, G. E. Jr., L. L. Larson, C. H. Spilman, J. Hahn and R. H. Foote. 1971. Culture and transfer of cow ova. J. Dairy Sci. 54:923-926.

Sirard, M. A., R. D. Lambert, D. P. Menard and M. Bedoya. 1985. Pregnancies after *in vitro* fertilization of cow follicular oocytes, their incubation in rabbit oviduct and their transfer to the cow uterus. J. Reprod. Fert. 75:551-556.

Sirard, M. A., J. J. Parrish, C. B. Ware, M. L. Leibfried and N. L. First. 1988. Culture of bovine oocytes to obtain developmentally competent embryos. Biol. Reprod. 39:546-552.

Spindle, A. 1990. *In vitro* development of one-cell embryos from outbred mice: Influence of culture medium composition. *In vitro* Cell Dev. Biol. 25:151-156.

Sreenan, J. 1968. *In vivo* and *in vitro* culture of cattle eggs. Proc. 6th Int. Congr. Anim. Reprod. & A.I. Paris, pp.577-580.

Sreenan, J., P. Scanlon and I. Gordon. 1968. Culture of fertilized cattle eggs. J. Agric. Sci. Camb. 70:183-185.

Sreenan, J. M. 1970. *In vitro* maturation and attempted fertilization of cattle follicular oocytes. J. Agric. Sci. Camb. 74:593-594.

Staigmiller, R. B. and R. M. Moor. 1984. Effect of follicle cells on the maturation and developmental competence of ovine oocytes matured outside the follicle. Gamete Res. 9:221-229 (1984).

Staigmiller, R. B. 1988. *In vitro* methods for production of viable oocytes. J. Anim. Sci. 66(Suppl. 2):54-64.

Stubbings, R. B., K. J. Betteridge and P. K. Basrur. 1988. Investigations of culture requirements for bovine oocyte maturation *in vitro*. Theriogenology 29:313(Abstr.).

Tan, S. J. and K. H. Lu. 1990. Effects of different estrous cycle stages of ovaries and sizes of follicles on generation of IVF early bovine embryos. Theriogenology 33:335(Abstr.).

Temin, H. M., G. L. Smith and N. C. Dulak. 1974. Control of multiplication of normal and rous sarcoma virus-transformed chicken embryo fibroblasts by purified multiplication-stimulating activity with nonsuppresible insulin-like and sulfation factor activities. In: Clarkson, B. and Baserga, R. (Ed) Control of proliferation in animal cells. Cold Spring Harbor, Vol 1, pp. 19-26.

Thibault, C. La. 1966. culture *in vitro* de l' oeuf de vache. Ann. Biol. Anim. Biochem. Biophys. 6:159-164.

Thibault, C., M. Gerard and Y. Menezo. 1975a. Acquisition par l ovocyte de lapine et de veau factuer decondensation due spermoatozoide fecondant (PMGF). Ann. Biol. Anim. Biochem. Biophy. 15:705.

Thibault, C., M. Gerard and Y. Menezo. 1975b. Preovulatory and ovulatory mechanisms in oocyte maturation. J. Reprod. Fertil. 45:605-610.

Thibault, C. and M. Gerard. 1973. Cytoplasmic and nuclear maturation of rabbit oocytes *in vitro*. Ann. Biol. Anim. Biochem. Biophy. (suppl.)13:145-156.

Toyoda, Y. and M. C. Chang. 1974. Fertilization of rat eggs *in vitro* by epididymal spermatozoa and the development of eggs following transfer. J. Reprod. Fert. 31:9-22.

Van Inzen, W. G., A. M. Kruip, Th. and S. M. Weima. 1993. Use of conditioned medium for IVM-IVF bovine embryos *in vitro* culture system. Theriogenology 39:236(abstr.).

Voelkel, S. A., G. F. Amborski, K. G. Hill and R. A. Godke. 1985. Use of a uterine-cell monolayer culture system for micromanipulated bovine embryos. Theriogenology 24:271-281.

Voelkel, S. A. and Y. X. Hu. 1992. Effect of gas atmosphere on the development of one-cell bovine embryos in two culture systems. Theriogenology 37:1117-1131.

Wang, W. L., H. S. Jiang, K. H. Lu, I. Gordon and C. Polge. 1992. The effect of gas phase on the *in vitro* development of bovine embryos derived from *in vitro* maturation and fertilization of ovarian oocytes. Theriogenology 37:320(Abstr.).

Whittingham, D. G. and J. D. Biggers. 1967. Fallopian tube and early cleavage in the mouse. Nature 213:942-943.

Whittingham, D. G. 1975. Fertilization, early development and storage of mammalian ova. In: The Early Development to

Mammals (Ed. M. Ballis and A. E. Wild). Cambridge Univ. Press, pp. 1-24.

Whittingham, D. G. and B. D. Bavister. 1974. Development of hamster eggs fertilized *in vitro* or *in vivo*. J. Reprod. Fertil. 38:489-492.

.Wiemer K. E., G. F. Amborski, R. S. Denniston and R. A. Godke. 1987. Use of a hormone treated fetal uterine fibroblast monolayer for *in vitro* development of bovine embryos. Theriogenology 27:294(Abstr.).

Wiemer, K. E., P. L. Casey and R. A. Godke. 1988. Short term storage of equine embryos on a fetal bovine uterine fibroblast monolayer followed by transfer to recipients. Proc. 11th Int. Congr. Reprod. & A.I., Dublin, Ireland. 2:198.

Wright, W. R., Jr., G. B. Anderson, P. T. Cupps and M. Drost. 1976a. Blastocyst expansion and hatching of bovine ova cultured *in vitro*. J. Anim. Sci. 43:170-174.

Wright, W. R., Jr., G. B. Anderson, P. T. Cupps and M. Drost. 1976b. Successful culture *in vitro* of bovine embryos to the blastocyst stage. Biol. Reprod. 14:157-162 (1976b).

Wright, R. W., Jr. and K. R. Bondioli. 1981. Aspects of *in vitro* fertilization and embryo culture in domestic animals. J. Anim. Sci. 53:702-729.

Wright, R. W., Jr. and J. V. O'Fallon. 1987. Growth of domesticated animal embryos *in vitro*. In: The mammalian preimplantation embryo: Regulation of growth and differentiation *in vitro* (Ed. B. D. Bavister). Plenum Press, N.Y., pp. 251-271.

Xu, K. P., T. Greve, H. Callesen and P. Hytell. 1987. Pregnancy resulting from cattle oocytes matured and fertilized *in vitro*. J. Reprod. Fertil. 81:501-504.

Xu, K. P., T. Greve, H. Callesen and P. Hytell. 1988. Birth of a calf following *in vitro* fertilization of *in vitro* matured oocytes. J. Reprod. Fertil. Abstr. Series No. 1:18.

Xu, K. P., B. R. Yadav, R. W. Rorie, L. Plante, K. J. Betteridge and W. A. King. 1992. Development and viability of bovine embryos derived from oocytes matured and fertilized *in vitro* and co-cultured with bovine oviductal epithelial cells. J. Reprod. Fertil. 94:33-43.

Yanagimachi, R. and M. C. Chang. 1963. Fertilization of hamster eggs *in vitro*. Nature 200:281-282.

Yanagimachi, R. and M. C. Chang. 1964. *In vitro* fertilization of golden hamster ova. J. Exp. Zool. 156:361-376.

Yang, Y. B. and K. H. Lu. 1990. the influence of bovine oocyte type on *in vitro* fertilization and subsequent *in vitro* development. Theriogenology 33:355(Abstr.).

Younis, A. I., B. G. Brackett and R. A. Fayrer-Hosken. 1989. Influence of serum and hormones on maturation and fertilization of bovine oocytes *in vitro*. Gamete Res. 23:189-201.

Zuelke, K. A., A. I. Younis and B. G. Brackett. 1989. Enhanced oocyte maturation of bovine oocytes with and without protein supplementation. In: Fertilization in Mammals (Ed. B. D. Bavister, J. Cummins and E. R. S. Roldan). Serono Symposium, Aug. Newton, p. 423.

Zuelke, K. A. and B. G. Brackett. 1990. Luteinizing hormone-enhanced *in vitro* maturation of bovine oocyte with and without protein supplementation. Biol. Reprod. 43:784-787.