

A single medium supports development of bovine embryos throughout maturation, fertilization and culture

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Oocytes and embryos are typically exposed sequentially to varying culture media in standard in-vitro protocols. Expenditures of energy may be required following each medium change to adjust to the changing environment. Therefore, a single base medium was evaluated for its ability to support in-vitro maturation, fertilization and pre-implantation development (IVM/F/C) of bovine oocytes and embryos. Four treatments were examined: a standard maturation [tissue culture medium (TCM) 199 with bovine calf serum (BCS)], fertilization (modified Tyrode's medium with albumin, lactate and pyruvate) and culture (hamster embryo culture medium/TCM with BCS) system (control) and three synthetic oviductal fluid (SOF) treatments; maturation in SOF with bovine serum albumin (SOFBSA), SOF with bovine calf serum (SOFBCS) or the control maturation medium (TCM199 with BCS; SOF199), followed by fertilization and culture in SOF medium. The percentage of total inseminated oocytes successfully developing to the morula and blastocyst stage did not differ ($P > 0.05$) between treatments (control, 30.5 ± 3.5 ; SOFBSA, 24.6 ± 3.2 ; SOFBCS, 22.4 ± 4.7 ; SOF199, 27.3 ± 3.2). Embryos cultured in SOFBCS (92.1 ± 6.4) had significantly higher cell numbers ($P < 0.05$) than those cultured in control (74.8 ± 4.8) and SOFBSA (71.6 ± 6.6) but not SOF199 (81.2 ± 6.8). In conclusion, a single medium can be used successfully throughout maturation, fertilization and pre-implantation embryo development. Moreover, inclusion of serum during maturation in the single medium system resulted in significantly greater cell numbers, possibly reflecting increased quality of the embryos produced.

Key words: bovine/culture medium/embryo culture/in-vitro fertilization/oocyte maturation

Introduction

Numerous studies have examined the ability of bovine oocytes and embryos to develop *in vitro* using a wide variety of culture media (Rosenkrans and First, 1994; Avery *et al.*, 1995; Liu

and Foote, 1995; Keskinetepe and Brackett, 1996). Typically, a different medium is used for each phase of in-vitro embryo production; maturation, fertilization and culture. In many cases, the culture period is further divided into two phases, each requiring a separate medium (Pinyopummintr and Bavister, 1996). Thus, an embryo may be exposed to as many as four discrete media during in-vitro development. The effect of transferring the developing embryo between different culture environments is unknown. Because different media may have varying concentrations of ions and energy substrates, cellular adjustments and expenditure of energy may be necessary due to changing osmolarity and/or pH. Metabolic pathways of the embryo may also be forced to adjust to changing substrate availability. Continual adjustment of the embryo to the changing culture environment may result in reduced developmental potential.

Synthetic oviductal fluid (SOF) is one medium commonly used for bovine embryo culture *in vitro*. This medium was originally based upon the biochemical analysis of ovine oviductal fluid (Tervit *et al.*, 1972). Synthetic oviductal fluid has subsequently been modified by the addition of amino acids (Gardner *et al.*, 1994). Other modifications have included the addition of citrate (Keskinetepe *et al.*, 1995), the removal of glucose (Takahashi and First, 1992), and the addition of EDTA for the initial 72 h of the culture period (Gardner *et al.*, 1997).

A standard medium for in-vitro maturation of bovine oocytes is tissue culture medium 199 (TCM199), either with serum supplementation (Loneragan *et al.*, 1994; Rosenkrans and First, 1994; Avery *et al.*, 1995; Liu and Foote, 1995; Pinyopummintr and Bavister, 1996; Thompson *et al.*, 1998) or without serum supplementation (Keskinetepe *et al.*, 1995; Keskinetepe and Brackett, 1996). Synthetic oviductal fluid has been examined for maturation of bovine oocytes, although its success was reduced compared to TCM199 supplemented with serum (Loneragan *et al.*, 1994).

The objective of this study was to determine if SOF medium alone could support in-vitro bovine oocyte maturation, fertilization and pre-implantation embryo development at a level comparable to standard multiple media protocols.

Materials and methods

Oocyte collection and in-vitro maturation

Bovine ovaries were collected from an abattoir and transported in 0.9% saline at 27–31°C. Follicles were aspirated with an 18 gauge needle using vacuum suction (100 mmHg; 28 ml/min). Aspirated oocytes with at least three layers of compact cumulus cells and homogeneous cytoplasm were selected and washed three times in SOF HEPES medium. Oocytes were placed in groups of 10 into

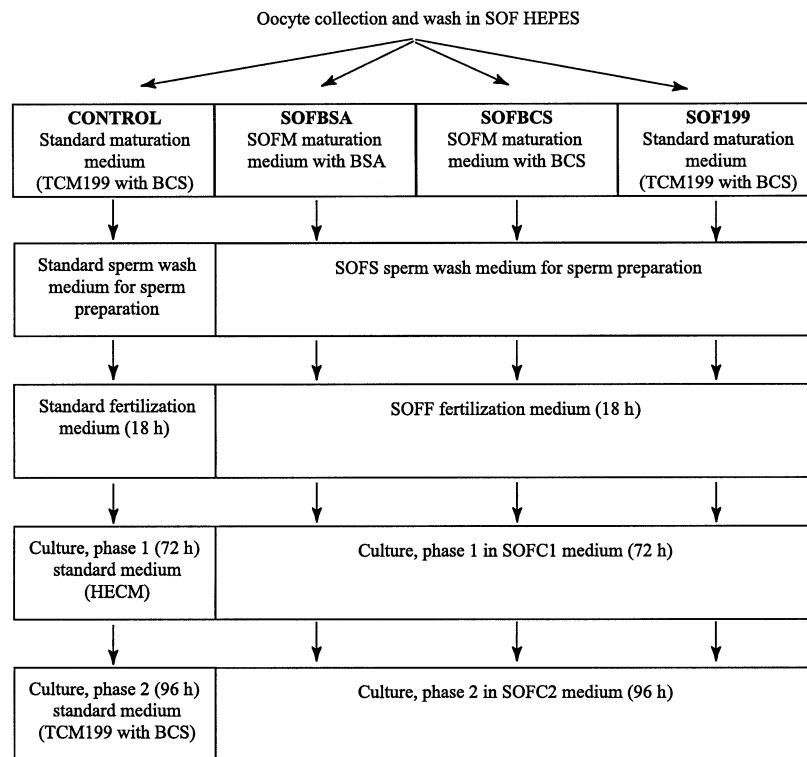


Figure 1. In-vitro maturation, fertilization and culture of bovine oocytes in control and synthetic oviductal fluid (SOF)-based medium systems. After aspiration, selected oocytes were placed into one of three maturation media; TCM199 with bovine calf serum (BCS) (control and SOF199), SOF maturation medium with bovine serum albumin (BSA) (SOFBSA) or bovine calf serum (BCS) (SOFBCS). Control oocytes were then fertilized and cultured according to a standard protocol. All other oocytes were fertilized and cultured in the SOF system.

Table I. Composition of synthetic oviductal fluid (SOF) medium used to mature, fertilize and culture bovine oocytes and embryos *in vitro*

| Reagent (mmol/l) | SOFM (maturation) | SOFF (fertilization) | SOFS (sperm wash) | SOFC1 (culture 1) | SOFC2 (culture 2) |
|----------------------|-------------------|-------------------------|--------------------|----------------------|----------------------|
| Sodium pyruvate | 0.33 0.33 | 1.0 | 0.33 | 0.33 | |
| Glutamine | 1.0 | – | – | 1.0 | 1.0 |
| Glucose | 1.5 | – | – | 1.5 | 3.0 |
| Sodium lactate | – | – | 18.3 – | – | – |
| HEPES | – | – | 12.5 | – | – |
| Bovine serum albumin | See text | 6 mg/ml fatty acid free | 3 mg/ml fraction V | 8 mg/ml crystallized | 8 mg/ml crystallized |
| EDTA | – | – | – | 0.1 | – |
| Taurine | – | – | – | 0.1 | – |
| MEM, NEAA | 1× | 2× | – | 1× | 1× |
| MEM, EAA | 1× | – | – | 1× | 1× |
| MEM, vitamins | – | – | – | – | 1× |

MEM = Eagle’s minimum essential medium (Eagle, 1959); NEAA = non-essential amino acids; EAA = essential amino acids. Supplements added to base SOF medium for specific developmental stages.

50 µl droplets of each maturation medium (Figure 1) under 10 ml mineral oil (Sigma, St Louis, MO, USA). Maturation proceeded for 22–24 h at 38.7°C in an environment of 5% CO₂ in air.

Oocytes were matured in one of three maturation media: (i) standard [TCM 199 (Gibco, Grand Island, NY, USA), 10% bovine calf serum (BCS; HyClone Laboratories, Logan, UT, USA), 0.23 mmol/l sodium pyruvate (Sigma)], (ii) SOFM medium (Table I) containing 8 mg/ml bovine serum albumin (BSA; Bayer, Kankakee, IL, USA), or (iii) SOFM medium containing 10% BCS. In addition, 50 ng/ml epidermal growth factor (EGF; Sigma), 0.01 U/ml each bovine luteinizing hormone (LH) and bovine follicle stimulating hormone (FSH) (from bovine pituitary; Sioux Biochemical, Sioux Center, IA, USA)

and penicillin/streptomycin/amphotericin (PSA; 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 ng/ml amphotericin; Gibco) were added to all maturation media. Base SOF medium consisted of 99.70 mmol/l NaCl, 7.16 mmol/l KCl, 1.19 mmol/l KH₂PO₄, 0.49 mmol/l MgCl₂·6H₂O, 3.30 mmol/l DL-lactic acid, sodium salt (60% syrup), i.e. sodium lactate, 25.07 mmol/l NaHCO₃, and 1.71 mmol/l CaCl₂·2H₂O.

In-vitro fertilization

Frozen mixed bull semen (Select Sires; Plain City, OH, USA) was thawed and divided into two treatments; SOFS (Table I) or standard (Sperm TALP; Parrish *et al.*, 1985) sperm wash medium for a 1 h

swim-up procedure. After swim-up, spermatozoa were further washed by centrifuging at 700 g twice for 5 min to remove any remaining semen extender.

Before transfer to fertilization drops, oocytes were washed three times in SOF HEPES without glucose. Oocytes were then transferred (Figure 1) into 44 μ l drops of standard fertilization medium [(Parrish *et al.*, 1988); fertilization in modified Tyrode's medium with albumin, lactate and pyruvate (Bavister and Yanagimachi, 1977), 6 mg/ml fatty acid-free BSA (Sigma), 0.24 mmol/l pyruvate, 1% penicillin/streptomycin (Gibco; 10 000 U and 10 000 μ g respectively per ml) and 2 \times Eagle's minimal essential medium (MEM) non-essential amino acids [NEAA; 100 μ l NEAA/5 ml total volume; ICN (Eagle, 1959)] or SOFF medium (Table I) under 10 ml mineral oil. Insemination was carried out by adding 1×10^6 spermatozoa/ml, 2 μ g/ml heparin, and PHE (Ball *et al.*, 1983; Parrish *et al.*, 1988: penicillamine, 20 μ mol/l; hypotaurine, 10 μ mol/l; epinephrine, 1 μ mol/l). Oocytes were coincubated with spermatozoa for 18 h at 38.7°C in 5% CO₂ in air.

Embryo culture

Embryos were denuded by vortexing for 4.5 min in 0.5 ml SOF HEPES with 0.11 mg/ml hyaluronidase (Sigma). After washing, 10 embryos were placed in 50 μ l droplets of either standard medium [basic medium 3 (McKiernan *et al.*, 1991) with 1 \times NEAA, 1 \times MEM essential amino acids (EAA), 0.10 mmol/l glutamine (Sigma)] or SOFC1 medium (Table I). There were a total of four treatment groups (Figure 1). Oocytes matured in SOFM with BSA (SOFBSA) and BCS (SOFBCS) were cultured in SOFC1/2 medium, while oocytes matured in standard maturation medium were cultured in both standard (control) and SOFC1/2 (SOF199) culture medium. Embryos were cultured at 38.7°C in an atmosphere of 5% CO₂ and 10% O₂, 85% N₂. After 72 h of culture, embryos were again washed three times in SOF HEPES and transferred into a second step medium [standard (TCM 199 with 10% BCS) or SOFC2 medium (Table I)] and incubated under the same conditions for an additional 96 h. At that time embryos were examined for development and stained for determination of cell number.

Determination of cell number

Staining of morulae and blastocysts was accomplished using Hoechst 33342 (Pursel *et al.*, 1985). Briefly, embryos were placed in a drop of 0.01% Trypan Blue solution for 1 min. The stain was then removed and replaced with a drop of 0.01 mg/ml solution of Hoechst 33342. The slide was incubated for 3 min at 37°C. The stain was then removed and the embryos covered with Permount (Fisher Scientific, Pittsburgh, PA, USA) and a cover slip and stored in the dark. The total number of cells in each embryo was counted using fluorescence microscopy.

Statistical analysis

Data were collected in eight replicates over 8 days, one replicate per day. For evaluation of differences between cleavage, development to the morula and/or blastocyst stage and hatching blastocysts as a percentage of both cleaved embryos and total inseminated oocytes, data were arc sine transformed. The data were analysed using a randomized block analysis of variance (ANOVA), blocked for replicates (i.e. days, since days and replicates were the same). Treatment differences were determined using a Bonferroni (all-pairwise) multiple comparison test for normally distributed data or a Kruskal-Wallis multiple comparison Z-value test for non-normally distributed data. The *P*-value used to determine significance in all tests was 0.05.

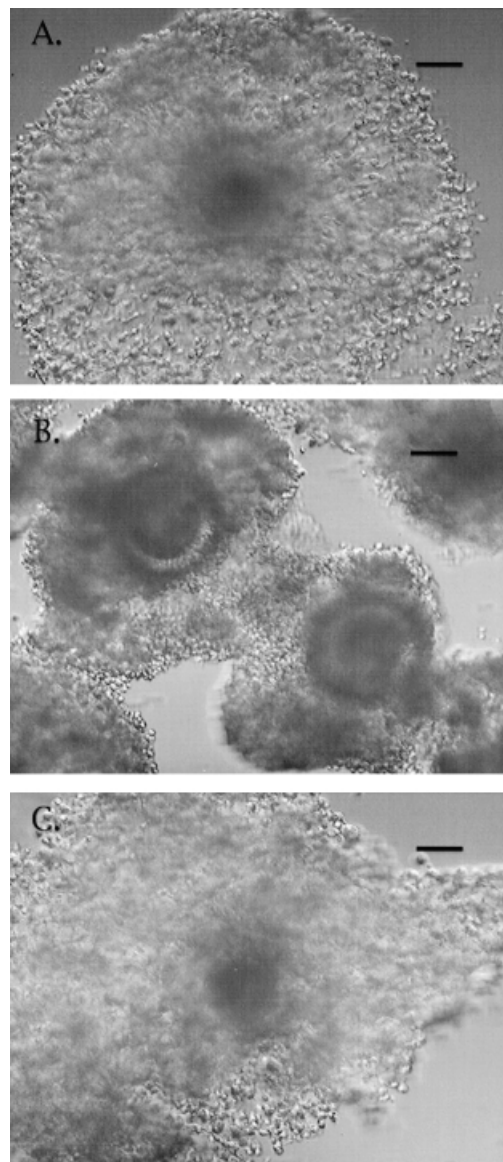


Figure 2. Cumulus expansion of bovine oocytes matured for 24 h *in vitro* in (A) TCM199 with BCS; (B) SOFM with BSA; (C) SOFM with BCS. Scale bars = 100 μ m. For abbreviations see Figure 1.

Results

A single base medium was evaluated for its ability to support in-vitro maturation, fertilization and preimplantation embryo development (IVM/F/C). Four treatments were examined: control (a standard IVM/F/C production system) and three SOF treatments; maturation in SOF with BSA (SOFBSA), SOF with bovine calf serum (SOFBCS) or the control maturation medium (TCM199 with BCS; SOF199), followed by fertilization and culture in SOF medium (Figure 1). Cumulus-oocyte complexes matured in standard maturation medium (control and SOF199 treatments) and SOFBCS were fully expanded at 24 h post maturation, while oocytes matured in SOFBSA displayed reduced cumulus expansion (Figure 2). However, there were no differences in the completion of nuclear maturation (metaphase II) between treatments (data not shown).

There was no difference in cleavage to at least the 2-cell

Table II. Development of bovine embryos resulting from different conditions of oocyte maturation, fertilization and embryo culture

| Culture treatment | Cleavage (n) | Percentage of total oocytes | | | Percentage of cleaved zygotes | | | Mean blastocyst cell no. \pm SEM (n) |
|-------------------|-----------------------------|-----------------------------|----------------------------|---|-------------------------------|----------------------------|---|--|
| | | Morulae and blastocysts (n) | Blastocysts (n) | Hatching blastocysts (n) | Morulae and blastocysts (n) | Blastocysts (n) | Hatching blastocysts (n) | |
| Control | 64.2 \pm 4.9 (96/148) | 30.5 \pm 3.5 (47/148) | 30.5 \pm 3.5 (47/148) | 9.4 ^{ab} \pm 2.5 (14/148) | 47.4 \pm 4.7 (47/96) | 47.4 \pm 4.7 (47/96) | 13.2 ^{ab} \pm 3.7 (14/96) | 74.8 ^a \pm 4.8 (40) |
| SOFBSA | 63.5 \pm 3.8 (117/191) | 24.6 \pm 3.2 (46/191) | 24.1 \pm 3.1 (45/191) | 3.5 ^a \pm 1.3 (7/191) | 40.1 \pm 5.0 (46/117) | 39.3 \pm 4.9 (45/117) | 6.2 ^a \pm 2.3 (7/117) | 71.6 ^a \pm 6.6 (35) |
| SOFBCS | 63.0 \pm 4.7 (106/171) | 22.4 \pm 4.7 (40/171) | 20.9 \pm 5.0 (38/171) | 6.1 ^a \pm 2.2 (12/171) | 33.6 \pm 6.6 (40/106) | 31.8 \pm 7.1 (38/106) | 9.5 ^a \pm 3.6 (12/106) | 92.1 ^b \pm 6.4 (30) |
| SOF199 | 55.8 \pm 4.4 (89/156) | 27.3 \pm 3.2 (43/156) | 27.3 \pm 3.2 (43/156) | 12.8 ^b \pm 2.5 (20/156) | 49.7 \pm 5.8 (43/89) | 49.7 \pm 5.8 (43/89) | 24.6 ^b \pm 5.2 (20/89) | 81.2 ^{ab} \pm 6.8 (32) |

^{ab}Mean values within columns with different superscripts are significantly different ($P < 0.05$).
SOF = synthetic oviductal fluid; BSA = bovine serum albumin; BCS = bovine calf serum.

stage between embryos in control, SOFBSA, SOFBCS or SOF199 in-vitro maturation, fertilization and culture treatments (64.2, 63.5, 63.0, 55.8% respectively; Table II). There were no differences between treatments in the percentage of embryos successfully developing to the blastocyst stage, whether expressed as a percentage of either total inseminated oocytes (control 30.5%, SOFBSA 24.1%, SOFBCS 20.9%, SOF199 27.3%) or cleaved embryos (control 47.4%, SOFBSA 39.3%, SOFBCS 31.8%, SOF199 49.7%; Table II). For blastocysts cultured in the SOF199 treatment, hatching from the zona pellucida was significantly more likely than for blastocysts cultured in SOFBSA or SOFBCS when expressed as a percentage of total inseminated oocytes (12.8, 3.5, 6.1% respectively) or cleaved embryos (24.6, 6.2, 9.5% respectively; Table II). Hatching was not different between controls and any SOF treatment. Morulae and blastocysts developing in the SOFBCS culture treatment had significantly higher mean cell numbers than morulae and blastocysts resulting from culture in control and SOFBSA (92.1, 74.8, 71.6 respectively; Table II). The mean cell numbers of morulae and blastocysts cultured in the control system were not different from those cultured in SOFBSA or SOF199 treatments.

Discussion

Results from the present study clearly demonstrate that bovine oocytes can be matured, fertilized and cultured in a single base medium appropriately supplemented for specific stages of development. In the present study, modified SOF was used successfully to mature and culture bovine oocytes and embryos, resulting in development equal to that in a standard multiple media system used in our laboratory. Embryos developed in the single medium system which included serum during maturation had higher cell numbers, suggesting that these embryos were of a higher quality.

Synthetic oviductal fluid (SOF) is a common medium used for the culture of cleaving embryos from sheep and cattle. This medium was originally developed based on the biochemical analysis of ovine oviductal fluid (Tervit *et al.*, 1972). The SOF medium used in this study was based upon this original formulation and subsequent modifications (Gardner *et al.*,

1994, 1997). In addition, several other modifications have been used in the present formulation. The SOF medium used for embryo culture has been separated into two phases, one designed to support early cleavage stages during the initial 72 h of culture and the second stage for development of morulae and blastocysts in the remaining 96 h of culture. In the first stage of culture, 0.1 mmol/l taurine (Barnes *et al.*, 1995) was included. The concentration of glucose in the culture medium for the second stage was increased from 1.5 to 3.0 mmol/l; both EDTA and taurine were excluded and MEM vitamins were added. Vitamins have been reported to increase blastocyst hatching in rabbit and hamster embryos (Kane *et al.*, 1986; Kane, 1988; Kane and Bavister, 1988). Studies in cattle and sheep, however, have provided conflicting results including negative effects or no effect on development to an increase in glucose uptake by blastocysts (Takahashi and First, 1992; Gardner *et al.*, 1994; Rosenkrans and First, 1994).

Taurine has been shown to be beneficial to the development of porcine, murine, hamster and rabbit embryos, particularly in defined, protein-free medium and/or high oxygen conditions (Reed *et al.*, 1992; Li *et al.*, 1993; Petters and Wells, 1993; Liu and Foote, 1995; McKiernan *et al.*, 1995; Spindle, 1995). Taurine is naturally found in fluids of the female reproductive tract, is a major component of the free amino acid pool and it is present *in vivo* in mammalian oocytes and embryos (Meizel *et al.*, 1980; Schultz *et al.*, 1981; Miller and Schultz, 1987; Dumoulin *et al.*, 1992; Guerin and Ménézo, 1995). Taurine has been shown to support development of cleavage stage human embryos through blastulation (Devreker *et al.*, 1999). The beneficial effect of taurine on embryo development may be due to the antioxidant properties of taurine, or its ability to serve as an osmolyte or chelating agent (Li *et al.*, 1993).

A great deal has been published regarding the inclusion of glucose as an energy substrate in culture medium for mammalian preimplantation embryos. Initially, glucose was found to be inhibitory to hamster (Schini and Bavister, 1988; Seshagiri and Bavister, 1989) and human (Conaghan *et al.*, 1993; Quinn *et al.*, 1995) embryo development. Research in mice showed glucose to be inhibitory to cleavage stage embryos but necessary for blastocyst development (Chatot *et al.*, 1989; Chatot *et al.*, 1994). However, glucose is present in oviductal fluid

(Nichol *et al.*, 1992; Gardner *et al.*, 1996). It has been suggested that the inhibitory effect of glucose can be attributed to sub-optimal in-vitro culture environments, and is thus an artefact (Gardner, 1998). Indeed, a recent study reports increased fetal development of hamster embryos cultured with 0.5 mmol/l glucose when phosphate is removed from the medium (Ludwig *et al.*, 1998). In cattle, high concentrations of glucose (>3 mmol/l) in the early cleavage stages are inhibitory, whereas glucose at levels up to 5 mmol/l stimulate blastocyst development when added after day 4 of culture (Kim *et al.*, 1993; Matsuyama *et al.*, 1993). It appears that, as in most mammalian species, glucose utilization increases with development as the bovine embryo shifts toward glycolysis (Thompson, 1996). These results suggest that a two-step culture system with varying levels of glucose may be optimal for bovine embryo development.

A factor that must be taken into consideration is the preparation of medium to be used in an in-vitro bovine embryo production system. Preparation of media solutions requires exacting, time-consuming measurements. Traditional culture medium must be prepared weekly or biweekly to protect against degradation of some components (Stewart-Savage and Bavister, 1988). The modified synthetic oviductal fluid medium used in this study was prepared from a series of stock solutions. This method provides several advantages. Many of the stock solutions containing stable salts can be prepared and kept for several months. Components that have short shelf-lives in solution, such as pyruvate, glutamine and bicarbonate, can be prepared separately and thus are always fresh when used. All media throughout the maturation, fertilization and culture period are prepared from the same basic stock solutions, so less time is spent on medium preparation. In addition, the use of stock solutions lends itself very well to experimental work, as several variants of a medium can be compared simultaneously with minimal variation.

Many investigators have used complex medium containing serum and/or co-culture with somatic cells to culture bovine embryos successfully *in vitro* (Bavister, 1995). While these systems often do produce successful development to the blastocyst stage, they introduce many unknown components into the culture environment and result in a great deal of variability between and within laboratories and experiments. In addition, the unknown environment makes it difficult if not impossible to examine effects of specific components of culture medium on oocyte and embryo development. Serum also has the effect of masking problems in the culture system, and can buffer the embryo from less than optimal environmental conditions. Replacement of serum with BSA or other macromolecules has the effect of making the culture system exquisitely sensitive to perturbations in the culture system. Consequently, the culture system must be well suited to the needs of the embryo before serum can be successfully removed. We have developed a semi-defined system containing BSA throughout maturation, fertilization and culture that results in development and hatching percentages equal to systems with serum present during maturation and portions of the culture period. This system will allow us to examine further experi-

mental maturation and culture treatments with increased repeatability and confidence.

In this study, although cumulus expansion in maturation medium containing BSA appeared to be reduced compared to that in maturation medium with serum, there were no differences in the percentages of oocytes successfully completing nuclear maturation (metaphase II) when preliminary observations were made. This reduced cumulus expansion did not adversely affect development of the resulting embryos to the blastocyst stage, although cell numbers were reduced when compared to an identical IVMFC system including serum during maturation. It is unclear if the presence of serum during the maturation period actually increases viability of the resulting blastocysts or simply speeds up the rate of cell division. Examination of inner cell mass:trophoblast ratios and embryo transfers in future studies may help to answer this question.

The culture of human embryos to the blastocyst stage offers many advantages. The overall efficiency of the in-vitro fertilization procedure is improved by producing higher quality embryos for transfer. Thus, fewer embryos can be transferred which reduces the occurrence of multiple gestations. The ability to culture human embryos successfully past the early cleavage stages has been dependent upon the development of new embryo culture medium to meet the embryo's metabolic needs without relying upon serum or somatic cell co-culture which may introduce pathogens. Recent studies have shown that semi-defined sequential media systems can work as effectively as co-culture or serum-supplemented media (Behr *et al.*, 1999; Fong and Bongso, 1999). Like the SOF system used in this study, this medium can be further studied and modified by the addition of amino acids (Devreker *et al.*, 1999) or growth factors (Lighten *et al.*, 1998) to support embryonic development better. Significantly, development of sequential medium for culture of embryos to the blastocyst stage in human IVF procedures has resulted in increased implantation rates with fewer embryos transferred when compared to traditional embryo transfer of cleavage stage embryos (Gardner *et al.*, 1998). The potential now exists to develop an entire culture protocol from oocyte maturation through to blastocyst development in the human based upon a single medium, as we have shown in these studies in cattle.

The main experimental endpoint of most embryo culture experiments, this study included, is the percentage of embryos developing to the morula and/or blastocyst stage. However, it is recognized that the morphology of these developed embryos may not be a good predictor of embryo viability (Bavister, 1995; Overstrom, 1996). Embryos representing a wide range of viability may appear morphologically normal. In this study, we have evaluated two additional parameters to address the viability of embryos developed in our culture systems; total cell numbers and blastocyst hatching. Reduced cell numbers and the inability of a blastocyst to hatch from the zona pellucida may be signs of developmental incompetence. However, because the hatching mechanism remains to be completely elucidated, the functional significance of hatching *in vitro* is uncertain (Bavister, 1995). In this study, we have demonstrated that the inclusion of serum during maturation in the SOF

system increases total cell numbers over those oocytes matured in the presence of BSA, even though the fertilization and culture media are identical. This emphasizes the importance of the in-vitro maturation environment on developmental parameters measured days later. Embryos cultured in the SOF system with serum during maturation also had greater total cell numbers than embryos cultured in the standard protocol with serum during maturation and the final stage of culture, suggesting that the SOF system is better able to support development of higher quality embryos. Hatching was not different between embryos cultured in the SOF system with serum or BSA during maturation and the standard protocol, again reflecting the ability of the SOF system to support a high level of embryo viability even in the absence of serum. Hatching was significantly improved when TCM199 with serum was used for maturation in the SOF system although the total cell number was not different, suggesting that a component of TCM199 has an effect during maturation that results in increased hatching of subsequently developing blastocysts. The relationship between total cell numbers, hatching and embryo viability remains unclear. Only embryo transfer data will conclusively determine their association.

In conclusion, embryos can be successfully developed in a single medium from maturation through fertilization and culture. This system is easy to use and maintain compared to media employed in standard production systems. The single medium system can be modified for use in both applied production and basic research. Embryos can be developed successfully to the blastocyst stage in a completely serum-free environment, or with serum in the maturation medium. A serum-free maturation and culture system allows researchers to examine more accurately the requirements of embryos *in vitro*. Hatching is not significantly reduced, even though serum is completely absent in the second stage of culture. This system provides a simplified method to develop high quality bovine embryos in a semi-defined environment.

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