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In Vitro-Matured/In Vitro-Fertilized Bovine Oocytes Can Develop into Morulae/Blastocysts in Chemically Defined, Protein-Free Culture Media[1]

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ABSTRACT

Development of bovine embryos derived from in vitro-matured/in vitro-fertilized (IVM/IVF) oocytes was examined in two culture media: hamster embryo culture medium (HECM), a relatively simple, chemically defined, protein-free medium containing 20 amino acids; and tissue culture medium (TCM)-199, a more complex medium designed for culture of somatic cells. The first experiment studied (1) effects of glucose and/or phosphate (P_i) using HECM and (2) the development of bovine IVM/IVF embryos in four different conditions: HECM, TCM-199, TCM-199 + 10% unheated bovine calf serum (BCS), and oviduct cell-conditioned TCM-199 + 10% BCS. After IVF, 45% of the inseminated oocytes developed to the morula/blastocyst stages (M&B) when cultured in HECM; blastocyst development was depressed in the presence of glucose and P_i when compared to P_i alone. When the four culture conditions were compared, there was no significant difference in M&B development (42–51% of inseminated oocytes). However, blastocyst development in TCM-199 supplemented with 10% BCS (29.7%) or with BCS + oviduct cell-conditioned medium (21.6%) was significantly greater than in nonsupplemented HECM (9.7%) or TCM-199 (13.8%). In the second experiment, the effects of serum supplementation and/or oviduct cell conditioning on HECM and TCM-199 were examined in a 2 × 2 × 2 factorial experiment. Oviduct cell conditioning of either HECM or TCM-199 without serum supplementation did not enhance bovine embryo development. Serum supplementation exhibited a biphasic effect, with inhibition at the first cleavage and stimulation of morula compaction and blastocyst formation. These data show that bovine embryos derived from IVM/IVF oocytes can develop in vitro to the morula stage in chemically defined, protein-free media, with no advantage of using oviduct cell contributions or serum; however, serum factors are needed for blastocyst development.

INTRODUCTION

In many laboratories, bovine morulae and blastocysts are routinely obtained from in vitro-matured/in vitro-fertilized (IVM/IVF) oocytes. When media supplemented only with serum are used, most embryos cease development before or at the 8–16-cell stage [1–3]. This block to development at the 8–16-cell stage can be overcome by culturing embryos in a medium containing cumulus cells or oviduct cell monolayers [4]. Alternatively, cell-free medium, typically tissue culture medium (TCM)-199 plus serum, in which oviductal tissue has been cultured for 24–48 h (conditioned medium), is capable of supporting early bovine embryo development equally as well as oviduct cell monolayers [1, 5]. However, with both procedures, the proportion of embryos that cleave to the blastocyst stage is quite variable (25–40% [2, 6–9]) and needs to be increased to maximize the usefulness of IVM/IVF embryos for research and commercial purposes. Moreover, although a few IVM/IVF bovine embryos have developed to term after transfer to recipient cattle [e.g., 1, 10–12], these have been highly selected, and the viability of the great majority of embryos is not known.

At present, the actual role of oviduct cells in the coculture or conditioned medium has not been elucidated. The oviduct cell monolayers could provide embryonic growth stimulatory components, or remove embryotoxic substances from the culture medium, or both. However, determination of the actual role of oviduct cells in supporting bovine embryo development through use of a conventional protocol (oviduct cells + TCM-199 + bovine serum) will be very difficult. The presence of serum, somatic cells, and their metabolites in the complex medium may obscure isolation and identification of any embryotrophic components, and/or analysis of conditioned medium for oviduct cell-induced modifications. An alternative hypothesis is that oviduct cells detoxify inhibitory substances present in the serum-supplemented complex medium. This hypothesis would be supported if it were possible to use a chemically defined, serum-free medium to culture bovine embryos. Therefore, we have conducted a study of development of bovine IVM/IVF embryos under both "conventional" culture conditions and with use of two chemically defined media: (1) hamster embryo culture medium (HECM), a relatively simple medium that supports development of hamster 2-cell embryos to the blastocyst stage [13–16], and (2) TCM-199, a more complex medium designed for culture of somatic cells. Glucose and/or phosphate (P_i), common components of embryo culture media, were responsible for blocks to development of hamster 2- and 8-cell embryos [13, 17, 18]. These components were examined in the present study. Finally, the effects of serum supplementation and/or oviduct tissue conditioning on HECM and TCM-199 were examined in a 2 × 2 × 2 factorial experiment.

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MATERIALS AND METHODS

Culture Media

Media used in this study were as follows: TALP and TL-HEPES, used for IVF and oocyte washing, respectively, were prepared as described by Parrish et al. [19]. Medium TCM-199, used for IVM and embryo culture, was prepared weekly from a powdered medium (cat. no. 400-1100EB; Gibco, Grand Island, NY). Bicarbonate was added, and the medium was made to volume with water purified by reverse osmosis and filtration through a 4-bowl Milli-Q system (Millipore Corporation, New Bedford, MA). Water was prepared weekly and tested with the hamster sperm motility assay [20]. HECM was prepared as described by Schini and Bavister [13]; this medium is chemically defined (protein-free), contains no glucose or phosphate (P_i), and is supplemented with 20 amino acids. To reduce the possibility of intrinsic inhibitory effects of the culture medium, pyruvate was deleted from HECM because of its inhibitory effect on hamster 1-cell embryos [21]. TCM-199 and HECM were supplemented in some treatment groups with 10% unheated bovine calf serum (BCS; Hyclone Labs, Logan, UT). BSA was fatty acid-free (cat. no. 5711, lot. no. 29F-9315) from Sigma Chemical Co. (St. Louis, MO).

The oocyte maturation medium was TCM-199 containing 10% BCS, 0.25 mM sodium pyruvate, antibiotics/antimycotic (100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B; PSA), 50 ng/ml epidermal growth factor, and hormones (5 μg/ml LH, 0.5 μg/ml FSH, and 1 μg/ml 17β-estradiol). The IVF medium was TALP containing 0.25 mM sodium pyruvate, 6 mg/ml BSA, and 50 μg/ml gentamicin.

Preparation of Conditioned Media

Bovine oviducts were obtained at a local abattoir, regardless of the donors' endocrine status, and transported on ice to the laboratory. The oviducts were trimmed free from surrounding connective tissue and washed three times in saline containing PSA. Then the oviducts were scraped gently from the isthmus toward the infundibulum with a glass microscope slide [1]. The extruded tissue was transferred to a 12-ml conical test tube containing 10 ml TALP-HEPES medium and allowed to settle. The supernatant was discarded, and the sediment was redispersed in 10 ml TALP-HEPES and allowed to settle. This washing step was repeated five times. After the final wash, 0.25 ml of tissue sediment was introduced into a 50-ml flask containing 10 ml of medium TCM-199 supplemented with 10% BCS and PSA. The tissue suspension was cultured under 5% CO_2 in air with high humidity at 39°C for 48 h; during this time, viable oviduct cell cultures grew as free-floating spheres of epithelial cells, with outwardly directed, actively beating cilia.

For experiment 1, after the 48-h incubation for cell growth, the tissue suspension was centrifuged at 250 × *g* for 10 min; the supernatant was collected and used as the conditioned medium. For experiment 2, after centrifugation, the oviduct tissue pellet was washed separately in five changes of either HECM or TCM-199 (without serum) corresponding to subsequent treatments. Each tissue pellet was then resuspended and allowed to condition one assigned medium (HECM, HECM + 10% BCS, TCM-199, or TCM-199 + 10% BCS) during culture for another 48 h. At the end of this second 48-h incubation period, oviduct cell cultures were re-examined for signs of viability, as described above. Only conditioned medium from viable cultures was used; the conditioned media were sterilized by passage through a 0.2-μm millipore filter and stored at −20°C.

In Vitro Maturation (IVM)

Cow ovaries from random breeds were collected immediately post mortem at a local abattoir and transported to the laboratory in saline (25–28°C) containing PSA. The ovaries were pooled regardless of stages of the estrous cycle of donors. Fluid was aspirated from small antral follicles (2–6 mm) using an 18-g needle connected to a 10-ml syringe, then pooled and allowed to settle in 50-ml conical test tubes wrapped with aluminum foil to protect the aspirated fluid from exposure to light. The aspiration was completed within 4–6 h after ovary collection. The supernatant was discarded and the sediment was divided into 1–2-ml amounts in sterile petri dishes. By use of a low-power (20–30×) stereo-microscope, only cumulus-intact oocytes with evenly granulated cytoplasm were selected from follicular fluid with a fine-tip "Unopette" (Becton, Dickinson and Co., Rutherford, NJ). The oocyte-cumulus complexes (OCCs) were washed two times in 10 ml TL-HEPES supplemented with 10% BCS and PSA; then a group of 10–14 randomly selected oocytes was allocated to each drop of maturation medium. For IVM, oocytes were cultured for 24 h in 50-μl drops of medium overlaid with 10 ml of paraffin oil in sterile 60 × 15-mm petri dishes (Falcon 1007, Becton, Dickinson and Co.). The culture conditions were 5% CO_2 in air with saturated humidity at 39°C.

In Vitro Fertilization (IVF)

The OCCs contained in each drop of maturation medium were removed, washed three times in TL-HEPES containing 10% BCS and PSA, then introduced into a 50-μl drop of IVF medium under 10 ml paraffin oil. For each experiment, 2 straws of frozen semen containing 5×10^7 sperm/straw (American Breeders Service, DeForest, WI) were thawed in a 35°C water bath for 1 min, then subjected to swim-up separation in Sperm-TL medium [19] in a 39°C water bath for 1 h to increase the proportion of motile sperm. The final sperm concentration used in IVF was 0.6–2.0 × 10^6/ml. Sperm capacitation was enhanced by including 2

TABLE 1. Effect of glucose and/or P_i on the development of bovine IVM/IVF embryos.*

Treatment	No. of inseminated oocytes	No. of replicates	Stage of embryo development (%)**					
			1-Cell	2–7-Cell	8–16-Cell	Morula	Compact morula	Blastocyst
HECM	136	6***	4.1[a]	29.6[b]	21.2[cd]	14.3[e]	21.2[f]	9.7[hi]
HECM + glucose	163	7	5.6[a]	25.3[b]	18.9[cd]	9.9[e]	29.2[f]	11.1[hi]
HECM + P_i	166	7	3.0[a]	34.0[b]	12.5[c]	9.3[e]	26.9[f]	14.3[h]
HECM + glucose + P_i	166	7	3.2[a]	27.4[b]	22.6[d]	14.7[e]	26.5[f]	5.7[i]

*Results shown in Tables 1 and 2 were obtained from the same experiment.
**Stage of embryo development was evaluated on Day 8 after the IVF (percentage calculated from total inseminated oocytes).
***One replicate lost due to contamination.
[a–i]Numbers in same column with different superscripts differ ($p < 0.05$).

μg/ml heparin sulfate in the IVF medium [19]. Incubation conditions for IVF were 5% CO_2 in air with high humidity at 39°C for 18 h.

In Vitro Embryo Development (ED)

The oocytes in each IVF drop were stripped of cumulus cells by passing them through a fine-tip Unopette and then were washed four times in medium corresponding to designated treatments. The majority (9–10) of each group of inseminated oocytes was then allocated to randomly preassigned embryo culture media; the remaining oocytes were fixed and examined to verify fertilization (pronuclear formation).

Experiment 1. This preliminary study was to determine whether bovine IVM/IVF embryos could develop in a relatively simple, chemically defined medium, HECM, without supplementation by serum. Effects of glucose and/or P_i on bovine embryo development were also studied. For supporting bovine embryo development, HECM was compared to more complex media: TCM-199, TCM-199 + 10% BCS, and conditioned TCM-199 + 10% BCS. The inseminated oocytes were randomly allocated to (1) simple medium, nonsupplemented: HECM, HECM + glucose, HECM + P_i, HECM + glucose + P_i; (2) TCM-199 + 10% BCS; or (3) oviduct cell-conditioned TCM-199 + 10% BCS.

Experiment 2. Effects of media (HECM vs. TCM-199) ± serum supplementation ± oviduct cell conditioning on supporting bovine embryo development were examined using a complete 2 × 2 × 2 factorial experiment. This design permitted study of oviduct cell conditioning in the absence of serum; serum used for the oviduct cell growth cultures (first 48-h incubation) was removed for conditioning in protein-free media during the second 48-h incubation. The inseminated oocytes were randomly placed in (1) unconditioned HECM, HECM + 10% BCS, TCM-199, or TCM-199 + 10% BCS; or (2) the same media as above, conditioned by oviduct cells.

Embryo culture in both experiments 1 and 2 was done in 50-μl drops of medium overlaid by 10 ml paraffin oil under 5% CO_2 in air with saturated humidity at 39°C. Embryos were incubated undisturbed for 8 days (range 188–192 h).

Data Analysis

At the end of each experiment (=24 h of IVM + 18 h for IVF + 8 days of embryo culture), all embryos were evaluated with Nomarski optics for the morphological stages of development reached. In some experiments, the number of nuclei in each embryo (all embryos counted) were counted by staining with the fluorescent, DNA-specific dye Hoechst 33342 [22].

The data were analyzed by use of a randomized block design ANOVA followed by protected least-significant difference comparison of means.

TABLE 2. Development of bovine IVM/IVF embryos in four different media*

Treatment	No. of inseminated oocytes	No. of replicates	Stage of embryo development (%)**					
			1-Cell	2–7-Cell	8–16-Cell	Morula	Compact morula	Blastocyst
HECM	136	6***	4.1[a]	29.6[c]	21.2[d]	14.3[g]	21.2[i]	9.7[l]
TCM-199	153	7	6.4[a]	28.9[c]	18.4[de]	19.2[g]	13.4[ij]	13.8[l]
TCM-199 + 10% BCS	129	6***	32.5[b]	18.6[c]	7.0[f]	0.8[h]	11.5[j]	29.7[m]
Cond. TCM-199 + 10% BCS	154	7	7.6[a]	28.9[c]	12.8[ef]	11.0[gh]	18.1[ij]	21.6[n]

*Results shown in Tables 1 and 2 were obtained from the same experiment.
**Stage of embryo development was evaluated on Day 8 after the IVF (percentage calculated from total inseminated oocytes).
***One replicate lost due to contamination.
[a–n]Numbers in same column with different superscripts differ ($p < 0.05$).

RESULTS

Experiment 1

The possibility of growing bovine embryos in a relatively simple, chemically defined medium (HECM) and effects of glucose and/or P_i were examined first. No differences in development were noted across the treatments up to the morula stage (Table 1, $p > 0.05$). In addition, there were no significant differences in mean number of nuclei per embryo (range 27–32) or per blastocyst (99–130) across the treatments (all embryos counted, data not shown). Although the presence of glucose or P_i alone had no effect on bovine IVM/IVF embryo development to the blastocyst stage ($p > 0.05$), development to blastocyst in medium containing both glucose and P_i was significantly lower than with P_i alone (Table 1).

Tables 2 and 3 show results from comparing four different media: HECM, TCM-199, TCM-199 + 10% BCS, and conditioned TCM-199 + 10% BCS, on the development of bovine IVM/IVF embryos. Some bovine IVM/IVF-derived embryos were able to cleave beyond the 8–16-cell stage in all media. When development was expressed as total (morulae + blastocysts), there was no significant difference between the treatments (range 42–51% of inseminated oocytes, Table 2). Thus, neither serum supplementation nor oviduct cell conditioning was necessary. However, about half the embryos failed to develop past the critical 8–16-cell stage in all treatments (Table 2).

There was no significant difference between HECM and TCM-199 in supporting embryos across all stages of development reached (Table 2, $p > 0.05$). No difference was detected in the mean number of nuclei per embryo or per blastocyst cultured in HECM or TCM-199 (Table 3, $p > 0.05$).

Either serum supplementation or oviductal cell conditioning increased the proportion of embryos reaching the blastocyst stage (Table 2, $p < 0.05$). There was no increase in development when oviduct cell conditioning was used rather than serum supplementation alone. The highest number of embryos that failed to cleave to the 2-cell stage was noted in TCM-199 + serum without oviduct cell conditioning. However, serum supplementation did significantly enhance the compaction of morulae and formation of blastocysts. Both serum supplementation and serum + conditioning of TCM-199 significantly enhanced blastocyst hatching (14/37 [39%] and 15/33 [40%] of blastocysts hatched, respectively) compared with TCM-199 alone (3/20 [14%]). Furthermore, these treatments also had main effects on increasing the mean number of nuclei per embryo and per blastocyst (Table 3, $p < 0.05$).

Experiment 2

Table 4 summarizes results from the factorial experiment comparing HECM and TCM-199 supplemented with serum or without. This experiment reaffirmed that there was no significant difference between these two media for culture of bovine IVM/IVF embryos to the blastocyst stage ($p > 0.05$). Serum supplementation had no significant effect on enhancing bovine embryo development in comparison to nonsupplemented media, although there was a numerical increase. Supplementing either medium with serum produced an approximately 5-fold increase in the proportion of oocytes that failed to cleave to the 2-cell stage ($p < 0.05$). Paradoxically, serum supplementation enhanced the compaction of morulae (2- to 8-fold). This biphasic effect of serum supplementation was notable in both HECM and TCM-199. Moreover, the combination of complex medium and serum supplementation, TCM-199 + 10% BCS, was superior to the relatively simple medium, HECM, in terms of embryos reaching the blastocyst stage ($p < 0.05$).

Conditioning media without serum supplementation slightly improved embryo development, but these effects were not statistically different. However, for HECM, serum supplementation plus conditioning with oviductal tissue significantly increased the proportion of blastocysts ($p < 0.05$). In contrast, conditioned TCM-199 + 10% BCS suppressed embryo development to the morula and blastocyst stages ($p < 0.05$).

DISCUSSION

The principal finding from this study is that bovine IVM/IVF embryos can develop to the morula/blastocyst stages in chemically defined media, without any serum supplementation or contribution from oviduct cells. This demonstration supports the hypothesis that oviduct cells and, by inference, other types of somatic cells assist in overcoming the usual blocks to development at the 8- or 16-cell stages by reducing levels of inhibitory components typically present in conventional culture media such as TCM-199

TABLE 3. Mean cell number of embryos cultured in four different media.

Treatment	No. of embryos	*Mean no. of nuclei/embryo	No. of blastocysts	*Mean no. of nuclei/blastocyst
HECM	136	27.7[a]	13	118.7[cd]
TCM-199	153	30.3[a]	20	111.1[c]
TCM-199 + 10% BCS	129	48.8[b]	37	148.6[de]
Cond. TCM-199 + 10% BCS	154	45.9[b]	33	152.8[e]

*Means were obtained from two-way analysis of variance.
[a–e]Numbers in same column with different superscripts differ ($p < 0.05$).

TABLE 4. Effect of serum supplementation and/or oviduct cell conditioning on the development of bovine IVM/IVF embryos.

Treatment	No. of inseminated oocytes	No. of replicates	Stage of embryo development (%)*					
			1-Cell	2–7-Cell	8–16-Cell	Morula	Compact morula	Blastocyst
HECM	166	6	5.3[a]	35.1[d]	20.7[ghij]	15.7[m]	10.6[op]	12.6[st]
HECM + 10% BCS	181	6	24.7[b]	22.1[ef]	15.5[ijk]	1.4[n]	20.4[q]	15.9[tuv]
Cond. HECM	176	6	6.3[a]	37.2[d]	21.6[ghi]	15.2[m]	5.6[or]	14.1[stu]
Cond. HECM + 10% BCS	177	6	23.2[b]	20.0[f]	13.0[jk]	2.2[n]	15.2[pq]	26.4[w]
TCM-199	175	6	3.4[a]	33.5[d]	25.9[g]	15.4[m]	2.2[r]	19.6[tuvw]
TCM-199 + 10% BCS	177	6	18.7[b]	27.6[def]	10.8[k]	2.3[n]	17.5[q]	23.1[uvw]
Cond. TCM-199	176	6	4.4[a]	27.0[def]	25.2[gh]	13.8[m]	5.2[or]	24.4[vw]
Cond. TCM-199 + 10% BCS	176	6	37.8[c]	31.6[de]	17.6[hijk]	1.7[h]	6.3[or]	5.1[a]

*Stage of embryo development was evaluated on Day 8 after the IVF (percentage calculated from total inseminated oocytes).
[a–w]Numbers in same column with different superscripts differ ($p < 0.05$).

supplemented with serum. Since the percentages of IVM/IVF embryos developing to the morula and blastocyst stages in this study were similar to those reported by other investigators using somatic cell cocultures or conditioned media, our data also argue against the hypothesis that embryotrophic factors secreted by these cells are responsible for overcoming the blocks to development. Nevertheless, our results also clearly show that serum stimulated differentiation of morulae into blastocysts.

In a preliminary experiment, we examined effects of P_i and glucose on bovine embryo development (Table 1). Glucose or P_i alone had no effect on the development of bovine embryos, but glucose together with P_i inhibited embryo development to the blastocyst stage in comparison to the treatment with P_i alone ($p < 0.05$). This result resembles that obtained with cultured hamster 8-cell embryos, in which development was inhibited by glucose and P_i together, but not separately [17, 18]. However, unlike cultured bovine embryos that were not blocked prior to the blastocyst stage by P_i + glucose (Table 1), hamster embryos were totally blocked by P_i at the 2-cell stage in vitro [13]. Petters et al. [23] also found an interaction of glucose and P_i, with a negative effect on pig embryo development in modified Krebs-Ringer-bicarbonate medium. Deletion of glucose during the first 36–48-h period of coculture was beneficial to bovine embryo development [24, 25]; however, it is difficult to determine from these studies whether glucose affected embryos directly or indirectly via the cocultured somatic cells. Further research is needed to clarify the role of glucose and/or P_i in mammalian embryo development.

Development up to the morula stage occurred equally well in HECM or TCM-199, with or without supplementation by serum or oviduct cell conditioning (Tables 2 and 4). This result was surprising because most laboratories have consistently found that, in TCM-199 ± serum, a developmental block occurs at the 8–16-cell stages [e.g., 1, 3]. There are several possible explanations for this difference. Our water quality control program is very rigorous, using a sensitive sperm motility bioassay [20]; variations in water used for culture medium preparation underlie many of the problems and inconsistencies encountered both in embryo culture research and clinical applications [26–29]. We did not heat-inactivate serum used for supplementing culture media, in contrast to most other studies; the way in which serum is processed markedly alters its properties for cell culture [30]. However, we have not observed any difference in bovine embryo development using heated versus unheated serum (unpublished data). Additionally, the source of serum may be critical since the quality of serum from different suppliers and even between batches can vary widely. Another difference between the present study and procedures followed in some other laboratories is that we allowed embryo development to continue undisturbed for 8 days; frequent (e.g., daily) examination of embryos can be detrimental to development, probably because of pH and temperature changes in the culture environment.

Although not essential for embryo development prior to the compact morula stage, serum supplementation clearly influenced development in a strikingly biphasic manner: (1) serum caused inhibition of development at or before the first cleavage division (Tables 2 and 4); this was not due to inhibition of fertilization because all oocytes were inseminated in the absence of serum and then (after 18 h) randomly allocated to ± serum treatments. (2) Serum stimulated morula compaction and blastocyst formation (Tables 2 and 4); in addition, the mean numbers of nuclei per embryo (total embryos) and per blastocyst were significantly greater with serum supplementation (Table 3). Serum supplementation enhanced embryo development more in experiment 1 than in the factorial experiment (Tables 2 and 4, respectively). This disparity may derive from the increased number of treatments in the latter experiment, which required a longer period of time for embryo handling outside the incubator.

These responses with bovine embryos may be analogous to those obtained with rabbit and hamster preimplantation embryos, in which early stages are very sensitive to artificial culture conditions and require a relatively simple medium to undergo cleavage divisions [21, 31]. On the other hand,

components such as vitamins, fatty acids, growth factors, etc., which are present in serum, may become essential to development at later stages [31–34].

Oviduct cell conditioning seemed able to revoke the inhibitory effect of serum on completing the first cleavage division (Table 2), but the ability of the rescued embryos to sustain further development is questionable since there was no significant increase in the proportion of embryos reaching the morula/blastocyst stages (Table 2). Oviduct cell conditioning without serum supplementation did not enhance embryo development (Table 4). However, for HECM, oviduct cell conditioning together with serum supplementation did significantly augment the proportion of blastocysts. Possibly HECM alone is not sufficient for oviduct cells to maintain their normal activities; hence, adding serum to HECM would compensate the nutritional requirements of oviduct cells. Alternatively, oviduct cells may simply deplete components in serum that are unfavorable for embryo development. In contrast, for TCM-199, serum supplementation plus oviduct cell conditioning actually suppressed morula and blastocyst development (Table 4), an effect not found in experiment 1 (Table 2). We cannot explain this effect; strangely, no such depression of embryo development was seen when HECM was used as the conditioned medium (Table 4).

In conclusion, we have shown that bovine IVM/IVF embryos can be cultured to the morula/blastocyst stages in chemically defined, protein-free media, HECM, and TCM-199. Without serum supplementation, the relatively simple medium designed for hamster embryo culture, HECM, was as effective as the widely used, more complex TCM-199 in supporting bovine embryo development to the blastocyst stage. Serum supplementation of either medium exhibited a biphasic effect on bovine embryo development; however, the mean cell number was higher in embryos cultured in the presence of BCS. Using oviduct cells to condition media without serum supplementation did not significantly enhance the development of bovine embryos in vitro.

This is the first report that a high proportion (37–50%) of IVM/IVF embryos cultured in chemically defined, protein-free media did not exhibit the usual block at the 8–16-cell stages. However, the media studied here, HECM and TCM-199, are clearly not sufficient to meet the requirements of bovine IVM/IVF embryo development to the blastocyst stage in vitro. Use of chemically defined, protein-free culture media to support bovine early embryo development will facilitate investigations into actions of particular compounds, e.g., growth factors, different molecular-weight fractions of serum, energy substrates, hormones, etc. This approach will accelerate investigations into the regulatory mechanisms and the nutritional requirements of bovine early embryonic development.

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