1-1-1992

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Recommended Citation

Thibodeaux, J., Menezo, Y., Roussel, J., Hansel, W., Goodeaux, L., Thompson, D., & Godke, R. (1992). Coculture of In Vitro Fertilized Bovine Embryos with Oviductal Epithelial Cells Originating from Different Stages of the Estrous Cycle. *Journal of Dairy Science, 75* (6), 1448-1455. https://doi.org/10.3168/jds.S0022-0302(92)77900-4

Coculture of In Vitro Fertilized Bovine Embryos with Oviductal Epithelial Cells Originating from Different Stages of the Estrous Cycle[1]

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ABSTRACT

Bovine embryos derived from in vitro fertilization procedures were cocultured in vitro with oviductal cells obtained from heifers between d 4 and 6 or d 14 and 16 of the estrous cycle. In addition, proteins secreted by oviductal cells isolated between d 4 and 6 or d 14 and 16 of the cycle were monitored. Embryos (2- to 4-cell) were incubated in Tissue Culture Medium-199 with 10% fetal bovine serum with or without oviductal cells at 39°C for 10 d following in vitro insemination. There were more morulae, blastocysts, and hatched blastocysts following coculture with oviductal cells than with culture in medium alone. However, no differences were noted in embryo development following coculture with oviductal cells obtained between d 4 and 6 or d 14 and 16 of the estrous cycle. Also, no differences were detected in the amount of [^{35}S]methionine-labeled proteins secreted by oviductal cells isolated from different days of the estrous cycle. These results indicate that oviductal epithelial cells isolated from early and late luteal phases of the estrous cycle will effectively support early embryonic development following prolonged in vitro culture.

(Key words: oocytes, fertilization in vitro, embryo coculture, estrous cycle)

Abbreviation key: B-O = Brackett-Oliphant medium, BSA = bovine serum albumin, DMEM = Dulbecco's modified Eagle medium, DMEM-MET = methionine-free Dulbecco's modified Eagle medium, FBS = fetal bovine serum, MTCM-199 = modified TCM-199, TCM-199 = Tissue Culture Medium-199.

INTRODUCTION

Bovine embryos of early cleavage stages do not readily develop beyond the 8- to 16-cell developmental block under in vitro conditions [see review by Wright and Bondioli (32)]. During recent years, researchers have been able to enhance in vitro development with the use of "helper" cells in an embryo coculture system [see review by Rexroad (20)]. However, for almost a decade, a controversy has been growing as to which is the most effective helper cell to use for optimal embryo development beyond the in vitro block stage during coculture. Recently, success has been reported using trophoblastic vesicles (2, 13, 17) and oviductal epithelial cells (3, 6, 9) of sheep and cattle for embryo coculture, rather than traditional uterine cell (e.g. fibroblast) coculture systems. Oviductal cell coculture has been reported to enhance embryo development in sheep (11, 21, 22, 23, 24), goat (18, 19, 25), pig (30), and cattle (4, 5, 14) embryos. In addition, medium harvested from oviduct cells following 48 h of incubation will also support development of bovine embryos beyond the 8- to 16-cell stage to the blastocyst and hatched blastocyst stages (6).

The use of oviductal tissue in embryo coculture systems has received increased attention in an effort to understand the mechanisms by which helper cells interact with embryos to

Received September 23, 1991.
Accepted February 10, 1992.
[1]This manuscript was approved for publication by the director of the Louisiana Agricultural Experiment Station as Manuscript Number 91-15-5333.
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enhance in vitro development. Studies evaluating the use of fresh or subpassaged monolayers indicate that previously subpassaged cell monolayers may provide a more uniform and consistent coculture system for mammalian embryos (16). Other researchers have evaluated in vitro bovine embryo development after using either fresh or frozen-thawed oviductal cells for coculture (3, 22). In addition, oviductal cell coculture enhanced embryonic development in serum-free medium (3, 5, 24). These studies have provided the basis for proposing that embryotropic effects during coculture are directed by oviductal cells themselves and are not due directly to effects of added serum in the medium.

Evaluation of specific factors that influence oviductal cells prior to and during embryo coculture may provide information on the mechanism or mechanisms by which somatic cell embryotropic factors enhance embryonic development in vitro. Rexroad and Powell (24) suggested that the embryotropic effect of oviductal and uterine cells in vitro may be influenced by the hormonal environment to which the cells were exposed in vivo. This suggestion has been further strengthened by a recent report (29) in which significant decreases in percentage of viability and attachment of both uterine and oviductal epithelial cells in vitro were noted followed daily exposure of the cells to elevated circulating progesterone concentrations while in vivo. The results of in vitro cell culture data reported by Thibodeaux et al. (29) suggest that the stage of estrous cycle from which the cells are harvested in the cow influences the secretory capacity of both uterine and oviductal epithelial cells during in vitro incubation.

The objectives of the present study were twofold. The first was to evaluate the efficiency of bovine oviductal epithelial cells isolated between d 4 and 6 or d 14 and 16 of the estrous cycle to support early in vitro development of in vitro fertilized bovine embryos. The second was to measure the amount of [^{35}S]methionine-labeled proteins secreted by oviductal cells used for embryo coculture as an indicator of cell secretory capacity. The measurement of labeled proteins secreted by oviductal cells may provide an indication of the potential embryotropic capability of helper cells during embryo coculture.

MATERIALS AND METHODS

Experimental Embryos

Embryos used in the present study were produced from in vitro matured and in vitro fertilized bovine oocytes. Ovaries from random breeds were collected from a local abattoir and transported to the laboratory (25 to 28°C) in PBS (Gibco Laboratories, Grand Island, NY) immediately following collection. Cumulus-intact oocytes were aspirated from small antral follicles (3 to 8 mm) using a 10-ml syringe and a 20-g needle and washed two times in PBS supplemented with 5% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT). Cumulus-intact oocytes were washed two times in Tissue Culture Medium-199 (TCM-199) with 10% FBS and matured for 22 to 24 h in 4-well culture plates (Nunclon, Naperville, IL) with 500 μl of TCM-199 supplemented with 10% FBS under light mineral oil at 39°C and 5% CO_2 in humidified air. Following the maturation interval, oocytes were transferred to 50-μl drops of Brackett-Oliphant (B-O) medium (1) supplemented with 20 mg/ml of bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO). Two straws of frozen-thawed semen from one Holstein bull were washed two times in B-O medium with 10 m*M* caffeine sodium benzoate (Sigma) and exposed to .1 μ*M* Ca^{2+} ionophore A23187 (Sigma) for 1 min. A 50-μl portion of sperm cells containing 1.5×10^6 motile sperm cells/ml was added to oocytes (15 to 20) and incubated for an addition 5 to 6 h at 39°C and 5% CO_2 in humidified air. Following the insemination interval, oocytes were washed two times and then incubated in TCM-199 with 10% FBS for an additional 42 h at 39°C and 5% CO_2 in humidified air.

Isolation and Culture of Oviductal Cells

Bovine oviductal epithelial cells were isolated using procedures recently reported (29). Oviducts were obtained from heifers immediately following slaughter and transported to the laboratory on ice in PBS that was free of Ca^{2+} and Mg^{2+} with 200 units of penicillin, 200 μg of streptomycin, and .25 μg of amphotericin-B/ml. Oviducts were trimmed free of mesentery and washed three times with fresh PBS and antibiotics. Epithelial cells from both oviducts were then stripped with a pair of fine forceps

from the isthmus end distal to the infundibulum. Small clusters of ciliated epithelial cells were recovered and resuspended in a modified TCM-199 with 10% FBS, 100 units of penicillin and 100 μg streptomycin/ml of medium (MTCM-199). Oviductal cells were washed three times with fresh MTCM-199 by centrifugation (200 × *g*), and the cellular pellet was resuspended in 48 ml of MTCM-199. The cell suspension was then incubated in 24-well plates (1 ml of suspension per well) at 39°C and 5% CO_2 in humidified air. Oviductal cells were used for coculture experiments within 48 h of initial cell seeding.

Embryo Coculture

Oviductal epithelial cells were isolated from cyclic Holstein heifers between d 4 and 6 (n = 3) or between d 14 and 16 (n = 3) of the estrous cycle (estrus = d 0). Embryos derived from in vitro fertilization procedures (2- to 4-cell) were randomly allotted (approximately 20 embryos per treatment per replicate) in suspension coculture; oviductal cells originated from either cycle d 4 to 6 (treatment A) or cycle d 14 to 16 (treatment B) 48 h after cell isolation. Immediately prior to embryo coculture, fresh MTCM-199 was replaced on oviductal cell suspensions. The free-floating clusters of epithelial cells, in this case, were allowed to remain in the culture wells. A portion of oviductal cells often attached and would form monolayers (approximately 20%) during the 48-h preincubation period. The control culture (treatment C) consisted of similar stage embryos incubated in MTCM-199 alone. All cultures were maintained at 39°C and 5% CO_2 in 1 ml of MTCM-199 for 10 d; portions (750 μl) of the culture medium were replaced at 48-h intervals. Embryos were evaluated for their ability to develop to the morula (≥32 cells), blastocyst, and hatched blastocyst stages on d 6, 8, and 10 following in vitro insemination, respectively.

[^{35}S]Methionine-Labeled Proteins

The remaining portion of oviductal cells harvested from each experimental female that served as the cell source during embryo coculture were used for [^{35}S]methionine labeling. Initially, cells isolated between d 4 and 6 or d 14 and 16 were seeded in 8 wells of a 24-well tissue culture plate and allowed to form confluent monolayers (≈5 d following initial seeding). However, the initial washing prior to radiolabeling removed the remaining free-floating oviductal cells.

Oviductal cells were incubated with radioactive methionine according to procedures previously reported (16). Initially, cells were washed twice with methionine-free Dulbecco's Modified Eagle Medium (DMEM-MET) (Gibco) and subsequently incubated for 2 h in DMEM-MET, followed by a 90-min incubation period with DMEM-MET containing 50 μCi of [^{35}S]methionine/ml of DMEM-MET (specific activity of >1000 Ci/mmol; Amersham Corp., Arlington Heights, IL). Cells were incubated with radioactive methionine (750-μl volume) at 39°C in 24-well culture plates. Following a 90-min incubation period with radioactive methionine, individual wells were washed three times in Dulbecco's Modified Eagle Medium (DMEM) and further incubated in TCM-199 supplemented with 10% FBS for an additional 48 h.

At 24- and 48-h intervals, aliquots of the culture medium (500 μl) were removed and precipitated in .5*N* perchloric acid. Free-labeled methionine was separated from the bound methionine by centrifugation, and the protein pellet containing radioactive methionine was counted by liquid scintillation to determine [^{35}S]methionine incorporation into the proteins secreted by cells. In addition, cells were manually removed from the individual culture wells, precipitated in .5*N* perchloric acid, and centrifuged; the cellular pellet was then counted by liquid scintillation for [^{35}S]methionine incorporation into the cellular proteins. Control samples consisted of TCM-199 with 10% FBS alone and TCM-199 with 10% FBS and cells incubated without radioactive methionine. In addition, nonspecific activity was determined by incubating wells without cells in radioactive methionine. Cells in several wells (3 wells per treatment per replicate) were removed and counted to determine the number of cells at each sampling period to adjust the [^{35}S]methionine-labeled secreted and cellular proteins based on the number of cells sampled. The amount of [^{35}S]methionine-labeled secreted and cellular protein content of oviductal cells was determined from 4 wells for each experimental heifer across treatment

TABLE 1. Development of 2- to 4-cell bovine embryos with oviduct epithelial cells isolated between d 4 and 6 or d 14 and 16 of the bovine estrous cycle (3 replicate d).[1]

Treatment		Morula		BLST[2]		HBLST[3]	
	(n)	(n)	(%)	(n)	(%)	(n)	(%)
A (d 4 to 6)	65	35	54[a]	25	39[c]	9	14[c]
B (d 14 to 16)	65	32	49[a]	23	35[c]	10	15[c]
C (control)	65	21	32[b]	6	9[d]	0	0[d]

[a,b]Means within columns with different superscripts differ ($P < .05$).

[c,d]Means within columns with different superscripts differ ($P < .01$).

[1]Evaluations on embryo development were conducted on d 6 (morula), d 8 (blastocyst), and d 10 (hatched blastocyst) following in vitro insemination.

[2]BLST = Blastocyst.

[3]HBLST = Hatched blastocyst.

days of the estrous cycle at 24 and 48 h of incubation. The number of [^{35}S]methionine-labeled secreted protein samples was 24 duplicates for cells isolated between d 4 and 6 or d 14 and 16 of the estrous cycle.

Statistical Analysis

For embryo coculture results, the proportions of embryos developing to morula, blastocyst, and hatched blastocyst stages were compared by chi-square analysis. For data on radioactive methionine incorporated into cellular proteins and proteins secreted by cells, activity was expressed as counts per minute per 1×10^6 cells. Secreted and cellular proteins were compared across days of the estrous cycle using a split-plot design with repeated measures in time (12) and a mixed model with animal as a random effect in the model. Statistical comparisons of least squares means were conducted using predicted differences (27).

RESULTS

Embryo Culture

A higher percentage ($P < .05$) of 2- to 4-cell embryos developed to the morula stage when cocultured with oviductal cells than when cultured in medium alone (52 vs. 32%). The percentages of embryos developing to the morula stage were similar for oviductal cells isolated between d 4 and 6 and between d 14 and 16 (54 vs. 49%) (Table 1). Furthermore, blastocyst developmental rate was higher ($P < .01$) with oviductal cell coculture than with culture in medium alone (37 vs. 9%). Results at the hatched blastocyst stage were similar for embryos cultured with oviductal cells originating between d 4 and 6 and between d 14 and 16 of the estrous cycle (14 and 15%, respectively) and were higher ($P < .01$) than for those cultured in medium alone, where no embryos developed to the hatched blastocyst stage. Overall, approximately 15% of the embryos hatched from their zona pellucida during coculture, but no embryos hatched when incubated in medium alone.

[^{35}S]Methionine-Labeled Proteins

The percentage of [^{35}S]methionine uptake by cells, based on total counts per minute, averaged 16% and was similar for epithelial cells isolated between d 4 and 6 or d 14 and 16 of the estrous cycle. Control samples with and without epithelial cells not incubated with radioactive methionine exhibited low counts, indicating no nonspecific activity. Aliquots of culture medium containing radioactive methionine were harvested following a 90-min incubation period and counted to determine incorporation by oviductal cells. Because no differences were noted among individual wells within treatments for radioactive methionine incorporation, all data were subsequently combined for final analysis. The stage of the estrous cycle from which epithelial cells were isolated did not influence the amount of [^{35}S]methionine incorporation into proteins secreted by oviductal cells at either 24 or 48 h of

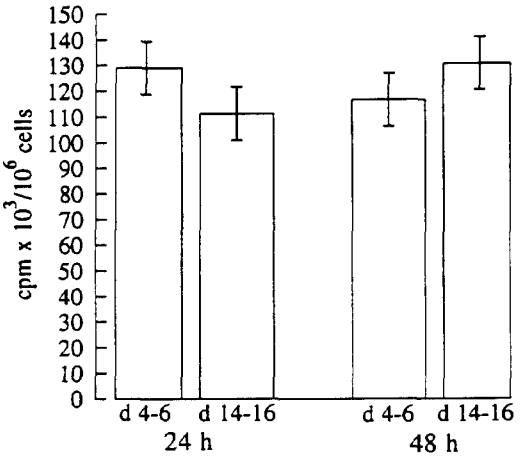

Figure 1. [^{35}S]Methionine-labeled proteins secreted by oviductal epithelial cells isolated between d 4 and 6 or d 14 and 16 of the estrous cycle. Evaluations were conducted at 24 and 48 h following labeling procedures. Data are least squares means and standard error of the mean expressed as counts per minute from 3 replicate d.

in vitro incubation (Figure 1). The mean counts and standard error of the mean of radioactive methionine incorporated into proteins secreted by cells following 24 h of incubation from cells isolated between d 4 and 6 and d 14 and 16 were 128.9 ± 10.3 and 111.2 ± 10.3 × $10^3/10^6$ cells, respectively. In addition, secretion rates were similar following 48 h of incubation for cells isolated between d 4 and 6 and d 14 and 16 of the estrous cycle (116.6 ± 10.3 and 130.8 ± 10.3 × $10^3/10^6$ cells, respectively). Oviductal epithelial cells isolated from both stages of the estrous cycle maintained a consistent level of secretion of labeled proteins following 48 h of in vitro culture.

The stage of the estrous cycle at which oviductal cells were harvested did not influence ($P > .05$) the amount of [^{35}S]methionine-labeled cellular proteins following 24 or 48 h of in vitro culture (Figure 2). Lower ($P < .05$) radioactive methionine incorporation into cellular proteins was noted for epithelial cells isolated between d 14 and 16 than for cells isolated between d 4 and 6 following 24 h of culture. However, cells isolated between d 14 and 16 had activity of labeled cellular protein at 48 h of incubation, which was similar ($P > .05$) to that of cells isolated between d 4 and 6 of the estrous cycle.

DISCUSSION

[^{35}S]Methionine incorporated into proteins and subsequently secreted by oviductal cells was evaluated to assess secretory capabilities of cells isolated between d 4 and 6 and d 14 and 16 of the estrous cycle. In this study, incorporation rates were similar for the two stages of the estrous cycle evaluated. Gandolfi et al. (9, 10) previously noted two major classes of proteins secreted by sheep oviductal cells during in vitro culture. The authors detected a small group of proteins exhibiting uniform secretion patterns throughout the estrous cycle and a larger group of proteins exhibiting cyclic variations in secretion patterns. It was further noted that the overall secretion of these proteins were reduced following 3 d of in vitro culture. The level of proteins secreted by bovine oviductal cells in the present study may have been reduced, which may have prevented detection of differences between stages of the estrous cycle. However, protein secretion by oviductal cells in the present study was similar to that reported for bovine oviductal cells following the fifth subpassage (16). The lower amount of

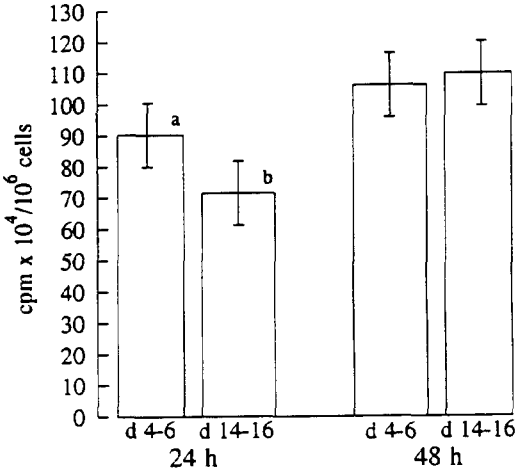

Figure 2. [^{35}S]Methionine-labeled cellular proteins in oviductal epithelial cells isolated between d 4 and 6 or d 14 and 16 of the estrous cycle. Evaluations were conducted 24 and 48 h following labeling procedures. Data are least squares means and standard error of the mean expressed as counts per minute from 3 replicate d. Means with different superscripts within sampling periods differ ($P < .05$).

cellular protein from cells isolated between d 14 and 16 following 24 h of in vitro incubation may be attributed to a higher turnover rate of protein synthesis and not necessarily to a decrease in cell quality. In an earlier study conducted in sheep, Salamonsen et al. (26) evaluated the effects of changing hormonal patterns associated with the estrous cycle on uterine epithelial cell protein production. These authors suggested that estrogen primarily influenced incorporation of radiolabeled methionine into proteins, whereas progesterone increased the secretion of newly synthesized proteins.

The results reported in this study indicate that the stage of the estrous cycle at which the oviductal cells are harvested has no significant effect on embryo development in vitro. In addition, cocultures with oviductal epithelial cells harvested from either early or late luteal stages were superior for culture of bovine embryos over that for medium alone. These results are in agreement with those recently reported by Eyestone et al. (7) using conditioned medium harvested from oviductal cells for culturing in vitro fertilized bovine embryos. In the latter report, no difference in development of embryos was noted using conditioned medium harvested from oviductal cells of cows either at estrus or in the luteal phase of the estrous cycle. Furthermore, embryo development results were similar following in vivo culture within oviducts of sheep in estrus or during the luteal phase or in anestrous and ovariectomized females (8, 15, 31).

Bovine embryo development in vivo or in vitro apparently is not affected following culture with oviductal cells or conditioned medium harvested from different stages of the estrous cycle. During primary cell culture, differences in cell viability and percentage of cell attachment of bovine uterine and oviductal epithelial cells harvested on different days of the estrous cycle were previously noted (29). Following the first cell passage, percentages of cell attachment were similar for uterine and oviduct cells collected among different stages of the cycle, indicating that a selection of certain cell populations occurred during primary culture (29).

The use of subpassaged cells during embryo coculture was suggested to provide a more uniform population of cells that may reduce individual animal variation contributing to embryo development (29). The present study used primary cell suspension cultures to prevent a cell selection process reported to occur during primary culture (29) and allowed an equal contribution of all cells to embryo development. However, a previous study using conditioned medium (7) and the present study using direct cell contact with oviductal cells harvested from different stages of the estrous cycle enhanced embryo development equally, indicating that a cell selection process during in vitro culture does not alter development capabilities of early stage bovine embryos. In addition, McCaffery et al. (14) reported enhanced early stage development of in vivo fertilized bovine embryos cocultured with primary cultures of oviductal cells in vitro (14). Also, the present study indicates that suspension cultures of oviductal cells are capable of stimulating embryo development and may create a coculture system with more in vivo properties because of cell to cell interactions within oviduct cell clusters. Recently, it has been demonstrated that oviductal cell monolayers enhanced in vitro development of early stage sheep (24) and cattle (3, 5) embryos. In each study, a simple serum-free medium combined with oviductal cell coculture stimulated embryonic development. These reports further suggest that oviductal cells are secreting embryotropic factors, and the use of serum-free medium during coculture does not alter the secretory capacity of the oviductal cells during coculture (3, 5, 24). It has been proposed that the embryotropic effects of oviductal cell coculture may reflect the ability of oviductal cells to maintain a required minimal amount of protein secretion (10). However, once bovine epithelial cells are placed in in vitro conditions, minimal protein secretion is maintained.

CONCLUSIONS

No differences were noted in development rates between bovine oviductal cells isolated from early and late luteal stages. However, oviductal cells isolated from the early luteal stages of the estrous cycle may be more suitable for embryo coculture experiments because of their increased ciliary activity, viability, and efficiency of attachment and growth, as reported for in vitro cell culture studies. Confluent monolayers of oviductal cells may not

be necessary to obtain good embryo development in vitro, because free-floating clusters of oviductal epithelial cells in primary culture provide an adequate in vitro system for bovine embryos. Finally, the simultaneous evaluation of embryo development and radioactive methionine incorporation into proteins secreted by cells may provide a useful way to evaluate an embryo coculture system. These criteria may be used in future studies to explain possible variation in monolayers used for embryo coculture studies.

REFERENCES

1 Brackett, B. G., and G. Oliphant. 1975. Capacitation of rabbit spermatozoa *in vitro*. Biol. Reprod. 12:260.

2 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.

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

4 Ellington, J. E., P. B. Farrell, and R. H. Foote. 1990. Comparison of six-day bovine embryo development in uterine tube (oviduct) epithelial cell co-culture versus *in vivo* development in the cow. Theriogenology 34: 837.

5 Ellington, J. E., P. B. Farrell, M. E. Simkin, R. H. Foote, E. E. Goldman, and A. B. McGrath. 1990. Development and survival after transfer of cow embryos cultured from 1-2 cells to morulae or blastocysts in rabbit oviducts or in a simple medium with bovine oviduct epithelial cells. J. Reprod. Fertil. 89:293.

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

7 Eyestone, W. H., J. M. Jones, and N. L. First. 1991. Some factors affecting the efficacy of oviduct tissue-conditioned medium for the culture of early bovine embryos. J. Reprod. Fertil. 92:59.

8 Eyestone, W. H., M. L. Leibfried-Rutledge, D. L. Northey, B. G. Gilligan, and N. L. First. 1987. Culture of one- and two-cell bovine embryos to the blastocyst stage in the ovine oviduct. Theriogenology 28:1.

9 Gandolfi, F., T.A.L. Brevini, and R. M. Moor. 1989. Effect of oviduct environment on embryonic development. J. Reprod. Fertil. 38(Suppl.):107.

10 Gandolfi, F., T.A.L. Brevini, L. Richardson, C. R. Brown, and R. M. Moor. 1989. Characterization of proteins secreted by sheep oviduct epithelial cells and their function in embryonic development. Development (Camb.) 106:303.

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

12 Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

13 Heyman, Y., and Y. Menezo. 1987. Interaction of trophoblastic vesicles with bovine embryos developing in vitro. Page 175 *in* The mammalian preimplantation embryo. B. D. Bavister, ed. Plenum Press, New York, NY.

14 McCaffery, C., T. G. McEvoy, M. G. Diskin, F. C. Gwazdauskas, M. T. Kane, and J. M. Sreenan. 1991. Successful co-culture of 1–4-cell cattle ova to the morula or blastocyst stage. J. Reprod. Fertil. 92:119.

15 Moore, N. W., B. G. Miller, and M. N. Trappl. 1983. Transport and development of embryos transferred to the oviducts and uteri of entire and ovariectomized ewes. J. Reprod. Fertil. 68:1129.

16 Ouhibi, N., J. Hamidi, J. Guillavd, and Y. Menezo. 1990. Co-culture of 1-cell mouse embryos on different cell supports. Hum. Reprod. (Ox.) 5:737.

17 Pool, S. H., R. W. Rorie, R. J. Pendleton, A. R. Menino, and R. A. Godke. 1988. Culture of early-stage bovine embryos inside day-13 and day-14 precultured trophoblastic vesicles. Ann. New York Acad. Sci. 541:407.

18 Prichard, J. F., S. H. Pool, E. G. Blakewood, and R. A. Godke. 1990. Culture of early-stage caprine embryos using goat oviductal and uterine cell monolayers. Theriogenology 33:300.(Abstr.)

19 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 monolayers. Theriogenology 35:259.(Abstr.)

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

21 Rexroad, C. E., and A. M. Powell. 1986. Co-culture of sheep ova and cells from sheep oviduct. Theriogenology 37:859.

22 Rexroad, C. E., and A. M. Powell. 1988. Co-culture of ovine eggs with oviductal cells and trophoblastic vesicles. Theriogenology 29:387.

23 Rexroad, C. E., and A. M. Powell. 1988. Coculture of ovine ova with oviductal cells in medium 199. J. Anim. Sci. 66:947.

24 Rexroad, C. E., and A. M. Powell. 1991. Effect of serum-free co-culture and synchrony of recipients on development of cultured sheep embryos to fetuses. J. Anim. Sci. 69:2066.

25 Sakkas, D., P. A. Batt, and A.W.N. Cameron. 1989. Development of preimplantation goat (*Capra hircus*) embryos *in vivo* and *in vitro*. J. Reprod. Fertil. 87:359.

26 Salamonsen, L. A., W. Sum, B. Doughton, and J. F. Findlay. 1985. The effects of estrogen and progesterone *in vivo* on protein synthesis and secretion by cultured epithelial cells from sheep endometrium. Endocrinology 117:2148.

27 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.

28 Sutton, R., C. D. Nancarrow, A.L.C. Wallace, and N. W. Rigby. 1984. Identification of an oestrus-associated glycoprotein in oviductal fluid of the sheep. J. Reprod. Fertil. 72:415.

29 Thibodeaux, J. K., L. L. Goodeaux, J. D. Roussel, Y. Menezo, G. F. Amborski, J. D. Moreau, and R. A. Godke. 1991. Effects of stage of the bovine estrous cycle on *in vitro* characteristics of uterine and oviductal epithelial cells. Hum. Reprod. (Ox.) 6:571.

30 White, K. L., K. Hehnke, L. F. Rickords, L. L. Southern, D. L. Thompson, and T. C. Woods. 1989. Early embryonic development *in vitro* by coculture with oviductal epithelial cells in pigs. Biol. Reprod. 41:425.

31 Willadsen, S. M. 1982. Micromanipulation of embryos of the large domestic species. Page 185 *in* Mammalian egg transfer. C. E. Adams, ed. CRC Press, Boca Raton, FL.

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