Identification of Retinol-binding Protein Produced by Caprine Endometrium during Periattachment Period of Early Pregnancy[a]

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ABSTRACT : Endometrial explants obtained from does between days 13 and 21 of pregnancy were cultured in a modified minimum essential medium in the presence of [^{35}S]methionine and [^{3}H]-leucine. Proteins synthesized and secreted into medium were analyzed by fluorography of two-dimensional polyacrylamide gel electrophoresis and fluorography. No marked qualitative changes in patterns of protein production by caprine endometrium between days 13-21 of pregnancy. At least 11 proteins showed consistently a clear spot or a grouping of spots with characteristic location on two-dimensional gels. A major low molecular weight protein consisted of two major isoforms (pI 5.3-6.0) of similar molecular mass (21 kDa). Limited N-terminal sequence analysis of these two isoforms showed that the protein had complete homology with bovine placental and plasma retinol-binding protein (RBP) over the first 20 amino acids. Through use of the antiserum raised against bovine placental RBP, immunoreactive RBP was detected in cultures conditioned by uterine explants prepared at days 13, 15 and 21 of pregnancy. In the present study, proteins synthesized and secreted by caprine endometrium during periattachment period of early pregnancy were characterized. The pregnant endometrium secreted a number of neutral-to-acidic proteins which constituted, in part, the histotroph. A vitamin A-transport protein, RBP, was identified in cultures conditioned by endometrium of days 13-21 of pregnancy. The uterine endometrium is the only source of retinol for embryonic tissues. The uterine RBP appears to transport retinol locally toward embryonic tissues. Secretion of RBP by caprine endometrium of days 13, 15 and 21 of pregnancy suggested that retinol played an important role in conceptus development during periattachment period of early pregnancy. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 12 : 1708-1713)***

Key Words : Goat, Uterus, Vitamin A

INTRODUCTION

In caprine, the period of placentation of conceptus to the uterine endometrium is relatively late. Adhesion of trophoblast and uterine epithelium occurs between day 19 and 23 after mating (Wongo et al., 1990). During this time period, uterine secretions, called histotroph, are particularly important to provide nourishment for the embryo. Besides, uterine secretions may be associated with maternal-fetal interactions. Presence of the conceptus must signal its presence to maternal system to prevent the regression of the corpora lutea (Homeida and Cook, 1982). The phenomenon, known as maternal recognition of pregnancy occurs between days 15-17 of pregnancy in goats (Gnatek et al., 1989). Several low molecular weight proteins secreted by caprine endometrium have been suggested to be involved in the process of maternal recognition of pregnancy (Weise et al., 1993).

Vitamin A is an essential nutrient and is needed for normal functions in mammalian biology. Because of its high degree of hydrophobisity, vitamin A needs the specific carrier protein to be solubilized and transported in the body fluids and in the cells (Vieira et al., 1995). In circulation, the specific protein that transports retinol (alcohol form vitamin A) from the liver parechymal cells to target tissues is plasma retinol-binding protein (RBP) (Hendriks et al., 1987). Liver is the major site of plasma RBP synthesis (Goodman et al., 1984). The interaction of retinol with plasma RBP serves to solubilize the retinol molecule against chemical degradation and to protect tissues from the toxic action of free retinol (Berni et al., 1990). In caprine, we have demonstrated that during post-attachment periods, extraembryonic membranes are extrahepatic tissue sites of RBP synthesis (Liu et al., 1995). These observations illustrate that besides systemic actions of retinol, it may play an important role in embryonic and placental development (Wolf, 1984; Brockers, 1989).

The long period of periattachment in goats suggests that local transportation of retinol between uterine and embryonic tissues probably exists to meet the rapid cellular growth and development of conceptuses. The present study was undertaken to characterize more thoroughly the proteins produced by caprine uterine endometrium during the periattachment period of early pregnancy. A group of low molecular weight proteins was identified as caprine uterine RBP. The uterine endometrium secreted continuously the RBP from the time periods of blastocyst expansion through maternal recognition of pregnancy and initiation of differentiation of extraembryonic membranes.

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MATERIALS AND METHODS

Materials

Eagle's minimal essential medium (MEM) and other supplies for tissue culture were purchased from GIBCO. [^{35}S]-methionine and [^{3}H]-leucine were obtained from New England Nuclear. X-Omat AR film was a product of Eastman Kodak. Polyvinylidene difluoride (PVDF) membrane was from Millipore Corporation.. Supplies for polyacrylamide gel electrophoresis (PAGE) and immune complex precipitation were used as described by Lifsey et al. (1989). Nitrocellulose and immunoblot assay kit were obtained from Bio-Rad Laboratories. All other chemicals were of reagent grade or better and were products of Sigma Chemical Co.

Animals

Adult crossbred female goats, primarily of Taiwan black (*Capra aegagrus hircus*), Alpine and Nubian breeding, were checked twice daily for estrus with vasectomized bucks. Estrous does (estrus=day 0) were mated by intact bucks.

Uteri of pregnant does were collected surgically via midventral laparotomy on days days 13 (n=4), 15 (n=4) and 21 (n=4) of pregnancy. Collected uteri were placed in sterile MEM and transported to a laminar flow tissue culture hood.

In vitro culture endometrial explants

Uteri of days 13-21 of pregnancy were dissected away from underlying myometrial and connective tissues. The endometrium was cut into 2 to 4 mm^3 explants, rinsed three times in phosphate buffered saline, and blotted on sterile gauze under sterile conditions in a laminar flow tissue culture hood. Approximately 400 mg of wet tissues were cultured in polystyrene culture dishes containing 12 ml of modified MEM with 30 μCi [^{35}S]-methionine and 100 μCi [^{3}H]-leucine. Incubations were carried out at 37°C in a gaseous atmosphere of 50%N_2, 47.5%O_2, 2.5%CO_2 (by volume) on a rocking platform. After 24 h, incubations were terminated by centrifuging to separate medium from tissue. Conditioned medium samples were dialyzed against 10 mM Tris-HCl buffer, pH 7.6.

Two-dimensional PAGE

Two-dimensional (2D) PAGE was performed according to the method of Roberts et al. (1984). Aliquots of dialyzed medium (200,000 cpm) from uterine explants of days 13-21 of pregnancy were lyophilized. Dried samples were dissolved in 75 μl of 5 mM K_2CO_3 containing 9.4 M urea, 2% (v/v) Nonidet P-40 and 0.5% (w/v) dithiothreitol for 2D-PAGE. The proteins were separated by isoelectric focusing in the first dimension and in 12% polyacrylamide gels in the second dimension. Following electrophoresis, Coomassie blue R-250-stained gels were dried after impregnation with 1 M sodium salicylate (Chamberlain, 1979). Fluorographs were prepared and radiolabeled proteins were detected using Kodak film.

N-terminal protein microsequencing

A modified method of Matsudaira (1987) was used to obtain sequence from protein after electrophoresis through polyacrylamide gel. Aliquots of dialyzed medium from culture of uterine explants of day 15 of pregnancy, containing 350 μg protein, were subjected to 2D-PAGE. After 2D electrophoresis, proteins were transferred to a PVDF membrane and visualized by staining with 0.5% Coomassie blue. Protein spots of interest were cut out of the membrane and rinsed extensively with water. A duplicate of the excised protein spot was counted by liquid scintillation spectrometry to verify incorporation of [^{35}S]-methionine and [^{3}H]-leucine. N-terminal amino acid analysis was carried out by gas-phase Edman degradation on an Applied Biosystems 477A sequencer. Cysteines were not reduced and alkylated, and thus could not be identified.

Immunological analyses

Immunoprecipitation were performed using rabbit antiserum against RBP purified from bovine allantoic culture medium (Liu et al., 1990). The anti-RBP antiserum (50 μl) was added to 1 ml of ^{35}S- and ^{3}H-labeled uterine explants culture medium from days 13-21 of pregnancy and incubated over night at 4°C. Immune complex were collected onto protein-A Sepharose CL-4B in 40 mM Tris-HCl (pH 7.5). Immune complexes were solubilized by boiling at 100°C for 5 min. Proteins contained in the supernatant were analyzed by one-dimensional (1D) PAGE. One-dimensional PAGE was performed according to the method of Laemmli (1970) in 12.5% (w/v) polyacrylamide gels and 5% of stacking gel. Following electrophoresis, Coomassie blue R-250-stained gels were dried after impregnation with 1 M sodium salicylate (Chamberlain, 1979). Fluorographs were prepared and radiolabeled proteins were detected using Kodak X-ray film.

RESULTS

Analysis of endometrial proteins by 2D-PAGE

The array of polypeptides synthesized and secreted by caprine endometrium between days 13-21 of pregnancy were characterized by 2D-PAGE. No marked qualitative changes in patterns of protein production by pregnant endometrium were observed over the time-periods examined. Because of this result, we have shown just one representative fluorogram of proteins produced by endometrium of day 15 of pregnancy (Figure 1). Table 1 lists the proteins (numbered 1-11) which showed consistently a clear spot or a grouping of spots with

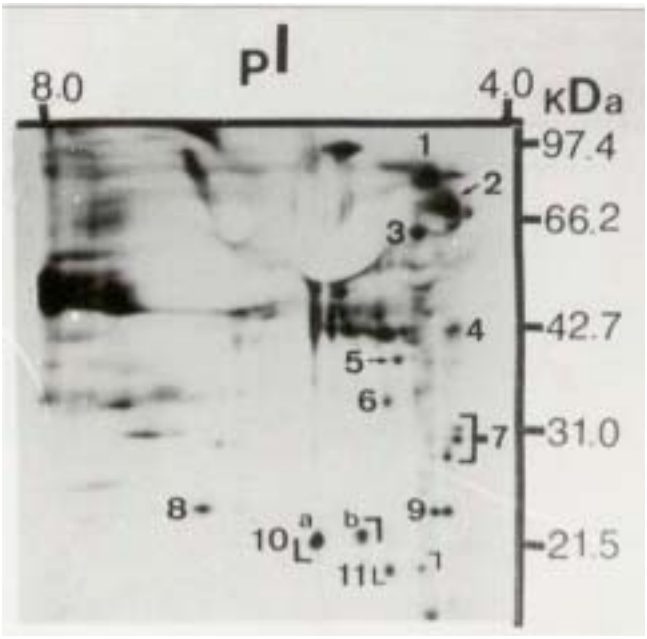

Figure 1. Fluorogram of 2D-PAGE gel for analysis of [^{35}S]methionine- and [^{3}H]leucine-labeled synthesized and secreted by bovine uterine explants at day 15 of pregnancy. Proteins (200,000 cpm) were loaded onto gel and fluorograph was exposed to dried gel for four weeks.

Table 1. Proteins identified in cultures of pregnant uterine endometrium

Protein	kDa[a]	pI[b]
1	85-90	4.8-5.1
2	70-75	4.8-4.5
3	60	5.1
4	43	4.5
5	40	5.2
6	35	5.3
7	31-27	4.5
8	26	7.0
9	26	4.7-4.5
10	21	6.0-5.3
11	18	5.3-4.8

[a] Apparent molecular mass (kDa) was estimated for each protein based on its relative mobility compared with protein standards in the second dimension electrophoresis.
[b] Apparent pI was estimated for each protein based on its relative mobility compared with isoelectric focusing in the first dimension electrophoresis.

characteristic location on 2D gels. The protein 10 consisting of two major isoforms (numbered 10a and 10b) with similar molecular masses of 21 kDa were observed to be the prominent products in cultures.

N-terminal amino acid microsequence

Proteins 10a and 10b were electroblotted onto a PVDF membrane and subjected together to N-terminal amino acid microsequencing. Only one major signal was determined over the first 20 cycles on analysis. Table 2 shows the comparison of the N-terminal amino sequence of the proteins with bovine placental and plasma RBP. The proteins exhibited complete homology with bovine placental (Liu et al., 1990) and plasma RBP (Berni et al., 1990) with the exception of the two unidentified residue (cycles 4 and 8).

Immunological identification

The anti-bovine placental RBP serum (Liu et al., 1990) was utilized to identify immunologically RBP in cultures conditioned by caprine uterine explants prepared from days 13, 15 and 21 of pregnancy. Immune-complex precipitation with anti-bovine placental RBP serum, followed by 1D-PAGE and fluorography clearly showed that anti-bovine placental RBP serum cross-reacted with the 21 kDa protein (Figure 2) which corresponded to the protein 10 on 2D-PAGE (Figure 1). In Figure 2, Immuno-precipitated bands (21 kDa) were detected in uterine cultures of days 13 (Figure 2A), 15 (Figure 2C) and 21 (Figure 2E) of pregnancy, Lanes B, D and F are the controls for lanes A, C and E, respectively. Non-immune serum did not react with the 21 kDa protein in above cultures. Occasionally, there are faint bands present in both positive and control lanes indicating nonspecific precipitates.

Table 2. N-terminal amino acid sequences of caprine uterine RBP, bovine placental RBP and bovine plasma RBP

Protein	Amino acid				
	1	5	10	15	20
caprine uterine RBP	ERD-[a]R	VS-FR	VKENF	DKARF	
bovine placental RBP[b]	ERD-R	VS-FR	VKENF	DKARF	
bovine plasma RBP[c]	ERDCR	VSSFR	VKENF	DKARF	

[a] The dashes indicate unidentified amino acid residues.
[b] Liu et al. (1990).
[c] Berni et al. (1990).

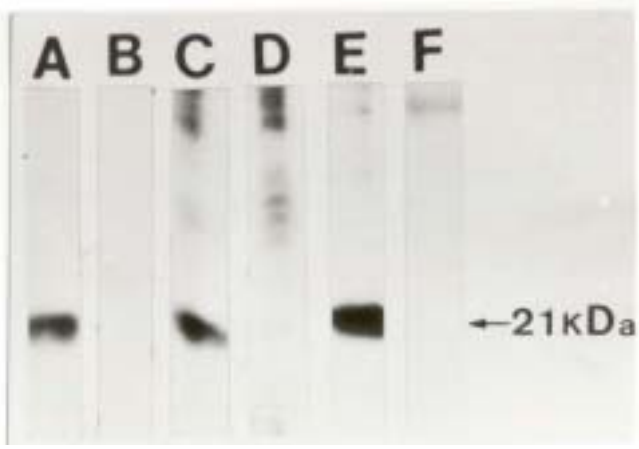

Figure 2. Immunoprecipitation of RBP from cultures of uterine explants at days 13 (A), 15 (C), and 21 (E) of pregnancy followed by 1D-PAGE and fluorography. Exposure time was four weeks. Lanes B, D and F are controls for lanes A, C and E, respectively.

Early caprine embryonic development

Events of early caprine embryonic development are shown in Figures 3 and 4. The day 12 blastocyst was a small spherical structure (0.4-.0.5 mm in diameter). (Figure 3A). On day 13 of pregnancy, the blastocyst expanded to form tubular shape (1.1-1.2×0.7-0.8 mm) (Figure 3B). Location of the inner cell mass (ICM) at the center of the day 13 blastocyst was observed (Figure 3B, arrow). On day 15 of pregnancy, the time of maternal recognition of pregnancy in the goats (Gnatek et al., 1989), the conceptus developed to elongated form. Prior to initiation of differentiation of extraembryonic membrane (chorion, allantois and amnion), the conceptus had undergone a tremendous growth resulting in a thin thread-like filamentous structure (150-180×2 mm) on day 21 of pregnancy (Figure 4).

DISCUSSION

Results from the present studies demonstrated that during the period of periattachment of pregnancy, the caprine endometrium was active in protein synthesis and secretion. Early pregnant uterus was the source of a number of neutral-to-acidic proteins, which constituted, in part, the histotroph. These uterine-specific proteins may play important roles in embryonic growth and development, and may also mediate maternal-fetal interactions such as implantation and transport of nutrients. A group of protein (numbered 10) was composed of at least two major isoforms of similar molecular weight (21 kDa) with pIs in the range of 5.3-6.0. The two isoforms were further characterized as RBP by N-terminal amino acid analysis. These two isoforms appeared to possess a common N-terminus over the first 20 amino acids residues. The first 20 amino acids of sequence were shown to be identical to those bovine placental (Liu et al., 1990) and plasma RBP (Berni et al., 1990). Molecular mass of caprine uterine RBP determined by protein electrophoresis was similar to that of bovine plasma RBP (Berni et al., 1990). Molecular microheterogeneity on certain polyacrylamide gel systems is a general characteristic of plasma RBP (Peterson et al., 1973). Recently, Minic et al. (1997) reported that RBP prepared from human urine was composed four isoforms: two retinol-containing (holo-) and two retinol-free (apo-) species. RBP under pH 7-10 condition favoring deamidation resulted in formation of the more acidic apo-isoform. Since plasma RBP is not glycosylated (Berni et al., 1990), the microheterogeneity of caprine uterine RBP is likely due to different amounts of retinol bound to the protein. The present study demonstrated by radiolabeled amino acid incorporation into protein and N-terminal amino acid analysis that RBP was a product of caprine endometrium during the periattachment period of pregnancy.

The uterine endometrium is the only source of retinol for embryonic tissues. The RBP of uterine origin appears to transport retinol locally toward embryonic tissues. Secretion

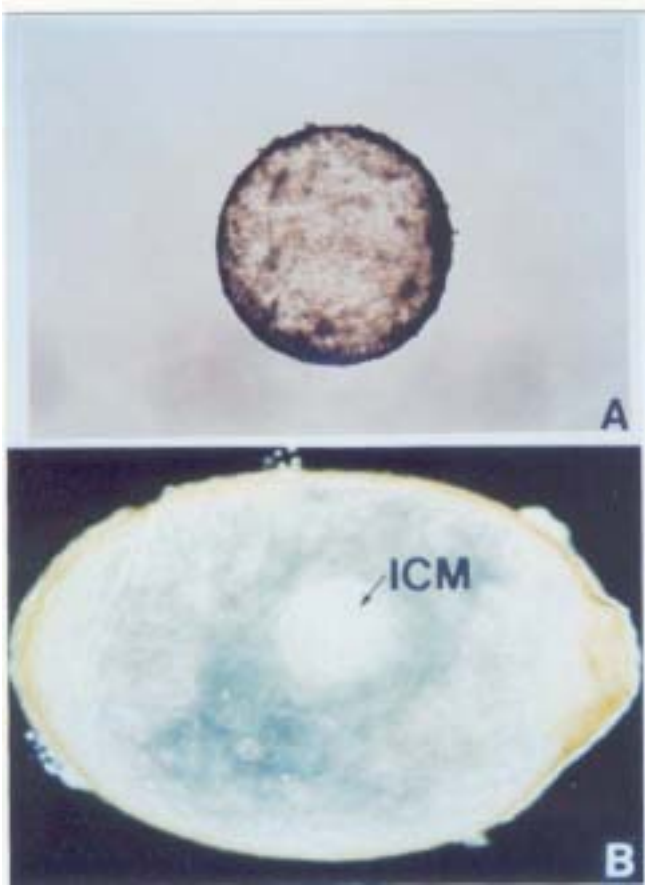

Figure 3. Early embryonic development of caprine blastocysts: day 12 spherical (A) and day 13 expanding (B) blastocysts. Location of inner cell mass (ICM) is indicated by an arrow in panel B. Panel A, 80x; B, 80x.

Figure 4. Early embryonic development of day 21 caprine conceptus. ×10.

of RBP by caprine endometrium of days 13, 15 and 21 of pregnancy determined by immunoprecipitation in this study suggested that retinol played an important role in conceptus development during periattachment period of early pregnancy. On day 13 of pregnancy, the conceptus was transformating from a spherical to a tubular morphology. On day 15 of pregnancy, the elongated conceptus is giving the signal for maternal recognition of pregnancy (Gnatek et al., 1989). The day 21 conceptus has undergone a tremendous growth resulting in a filamentous structure. On day 21 of pregnancy, differentiation of extraembryonic membranes (chorion, allantois and amnion) was initiating. These coincidences indicate that uterine RBP and its bound retinol may regulate the trophoblast hyperplasia and implantation.

The mechanism by which retinol exert their physiological role is not well understood. Embryonic development is believed to be mediated through gene activation within conceptus (Geisert and Malayer, 2000). Since changes of caprine conceptuses in morphology involve changes in the extracellular matrix, which is made of collagen and laminin, and cell adhesion, possible target genes include genes encoding proteins of the extracelluar matrix. Retinoic acid is an activated metabolite of retinol (Vieira et al., 1995). Retinoic acid has been reported to affect expression of transforming growth factor-β (TGF-β), a major modifier of extracelluar matrix and cell surface adhesive molecules (Schmid et al., 1991). In the mouse, TGF-β genes are expressed during embryonic development and that the total levels of their specific mRNA increase with the age of the embryo (Heine et al., 1987; Miller et al., 1989a,b). TGF-β and platelet-derived growth factor are synergistic mitogens for bovine trophoblastic and endometrial epithelial cells (Munson, 1992). These results implicated that impact of retinol on embryogenesis may be closely related to function and expression of peptide growth factors.

In summary, protein production by caprine uterine endometrium during the periattachment period of pregnancy was characterized. Early pregnant uterus was the major source of a number of neutral-to-acidic proteins in uterine secretions. A group of acidic proteins was identified as uterine-specific RBP determined by its N-terminal amino acid sequence and cross-reaction with anti-bovine placental RBP antibody. Secretion of uterine RBP was coincident with the rapid growth and differentiation of conceptus. Interestingly, RBP is also a product of extraembryonic membranes in caprine (Liu et al., 1985), in ovine (Liu et al., 1992a) and in bovine (Liu et al., 1990), and bovine uterine endometrium (Thomas et al., 1992; Liu et al., 1992b). However, the goat oocytes appear to acquire no abilities of protein secretion of RBP (Malakar and Majumdar, 2002). These findings suggested that an important function of the endometrial-placental unit is to regulate local supply of free retinol in domestic ruminants.

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REFERENCES

Berni, R. M., M. Stoppini, M. C. Zapponi, M. L. Meloni, H. L. Monaco and G. Zanotti. 1990. The bovine plasma retinol-binding protien:amino acid sequence, interaction with transthyretin, crystallization and preliminary X-ray data. Euro. J. Biochem. 192:507-513.

Brockes, J. P. 1989. Retinoids, homeobox genes, and limb morphogenesis. Neuron 2:1285-1294.

Chamberlain, J. P. 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water soluble fluor sodium salicylate. Anal Biochem. 98:132-135.

Geisert, R. D. and J. R. Malayer. 2000. Implantation. In: Reproduction in Farm Animals (Ed. E. S. E. Hafez and B. Hafez). Lippincott Williams and Wilkins, New York. pp. 126-139.

Gnatek, G. G, L. D. Smith, R. T. Duby and J. D. Godkin. 1989. Maternal recognition of pregnancy in thc goat: Effects of conceptus removal on interestrus intervals and characterization of conceptus protein production during ear1y pregnancy. Biol. Reprod. 42:655-663.

Goodman, D. S. 1984. Plasma retinol-binding protein. In: The Retinoids (Ed. M. B. Sporn, B. Roberts and D. S. Goodman). Academic Press, Orlando, Florida. pp. 41-88.

Heine, U. I., E. F. Munoz, K. C. Flanders, L. R. Ellingsworth, H. Y. Lam, N. L. Thompson, A. B. Roberts and M. B. Sporn. 1987. Role of transforming growth factor-β in the development of the mouse embryo. J. Cell Biol. 105:2861-2876.

Hendriks, H. F. J., J. Brouwer and D. L. Knook. 1987. The role of hepatic fat-strong (stellate) cells in retinoid metabolism. Hapatology 7:1368-1371.

Homeida, A. M. and R. G. Cook. 1982. Peripheral plasma concentrations of 13, 14-dihydro-15-keto-prostaglandin F_2 and progesterone around luteolysis and early pregnancy in the goat. Prostaglandins 24:313-321.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Lifsey, B. J., G. A. Baumbach and J. D. Godkin. 1989. Isolation, characterization and immunocytochemical localization of bovine trophoblast protein-1. Biol. Reprod. 40:343-352.

Liu, K. H., G. A. Baumbach, P. M. Gillevet and J. D. Godkin. 1990. Purification and characterization of bovine placental retinol-binding protein. Endocrinology 127:2696-2704.

Liu, K. H., K. X. Goa, G. A. Baumbach and J. D. Godkin. 1992a. Purification and immunolcalization of ovein placental retinol-binding protein. Biol. Reprod. 46:23-29.

Liu, K. H. and J. D. Godkin. 1992b. Characterization and Immunolocalization of bovine uterine retinol-binding protein. Biol. Reprod. 47:1099-1104.

Liu, K. H., J. C. Huang and J. D. Godkin. 1995. Characterization of protein production by caprine placental membranes: identification and immunolocalization of retinol-binding protein. J. Endocrinol. 146:527-534.

Malakar, D. and A. C. Majumdar. 2002. Secretory Proteins from goat oocytes matured in culture. Asian-Aust. J. Amin. Sci. 3:340-345.

Matsudaira, P. l987. Sequence of picomo1e quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. 262:10035-10040.

Miller, D. A., A. Lee, R. W. Pelton, E. Y. Chen, H. L. Moses and R. Derynck. 1989a. Murine transforming growth factor-β2 cDNA sequence and expression in adult tissues and embryos. Mol. Endocrinol. 3:1108-1114.

Miller, D. A., A. Lee, Y. Matsui, E. Y. Chen, H. L. Moses and R. Derynck. 1989b. Complementary DNA cloning of the murine transforming growth factor-β3 (TGF-β3) precursor and the comparative expression of TGF-β3 and TGF-β1 messenger RNA in murine embryos and adult tissues. Mol. Endocrinol. 3:1926-1934.

Minic, Z., J. Hranisavljevic and D. Vucelic. 1997. Isolation and characterization of isoforms of retinol binding protein by isoelectrofocusing. Biochem. Mol. Biol. Int. 41:1057-1066.

Munson, L. L., J. E. Wilkinson and M. K. Bechtel. 1992. Transforming growth factor-β and platelet-derived growth factor are synergistic mitogens for bovine trophoblastic and endometrial epithelial cells. Biol. Reprod. 46(suppl. 1):67 (Abstr.) .

Peterson, P. A., L. Rask, L. Ostberq, L. Anderson, F. Kamwendo and H. Pertoft. 1973. Studies on the transport and cellular distribution of vitamin A in normal and vitamin-A-deficient rats with special reference to the vitamin A binding plasma protein. J. Biol. chem. 248:4009-4022.

Roberts, R. M., G. A. Baumbach, W. C. Buhi, F. B. Denny, L. A. Fitzgerald, S. Babelyn and M. N. Horst. 1984. Analysis of membrane polypeptides by two–dimensional polyacrylamide gel electrophoresis. In: Molecular and Chemical Characterization of Membrane Receptors (Ed. J. C. Ventor and L. C. Harison). Allen R. Liss, Inc., New York. pp. 61-113.

Schmid, P., D. Cox, G. Bilbe, R. Maier and G. K. McMaster. 1991. Differential expression of TGF β1, β2 and β3 genes during mouse embryogenesis. Development 111:117-130.

Thomas, P. G. A., M. V. Leslie and P. J. Hansen. 1992. Retinol binding protein is produced by the bovine endometrium and accumulates in uterine secretions in a progesterone-dependent manner. Anim. Reprod. Sci. 27:55-66.

Vieira A. V., W. J. Schneider and P. M. Vieira. 1995. retinoids: transport, metabolism, and mechanisms of action. J. Endocrinology 146:201-217.

Weise, D. W., G. R. Newton and G. C. Emesih. 1993. Effect of day of the estrus cycle or pregnancy on protein secretion by caprine endometrial tissues. Biol. Reprod. 49:522-527.

Wolf, G. 1984. Multiple functions of vitamin A. Physiol. Rev. 64:873-937.

Wongo, E. O., F. B. P. Wooding and R. B. Heap. 1990. The role of trophoblast binucleate cells in implantation in the goat: a quantitative study. Placenta 11:381-394.

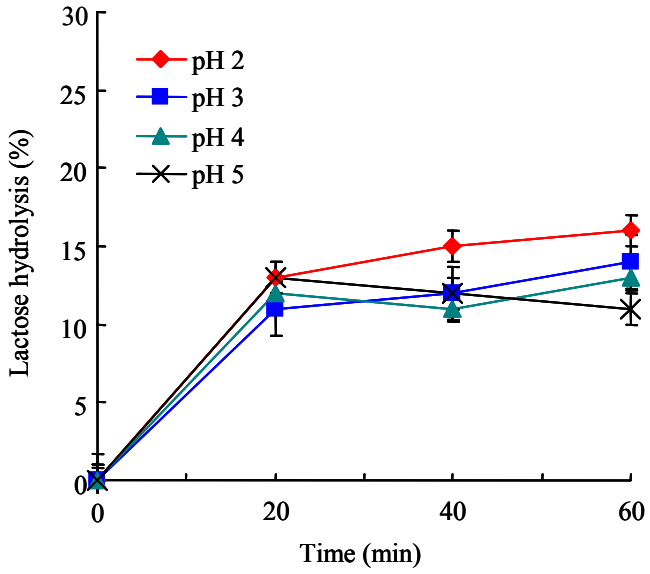

Figure 2. Hydrolysis pattern of lactose form PGMS microcapsules as a function of time during incubation in a simulated gastric fluid.