To the Graduate Council:

I am submitting herewith a thesis written by Linda Diane Smith entitled "Conceptus Proteins' Effects on Luteal Maintenance in the Ewe." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

James D. Godkin
J. D. Godkin, Major Professor

We have read this thesis
and recommend its acceptance:

H. G. Kattesh

S Polumbo

Accepted for the Council:

Lawrence Dewminkel
Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Linda D. Smith

Date 8/28/86

CONCEPTUS PROTEINS' EFFECTS ON LUTEAL MAINTENANCE IN THE EWE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Linda Diane Smith

December 1986

AG-VET-MED.
Thesis
86
.S652

ACKNOWLEDGMENTS

The author would like to express her appreciation and thanks to her major professor, Dr. James D. Godkin, for the opportunity to work with him, his encouragement, and assistance. Thanks are also extended to the other members of her committee, Dr. Steven P. Oliver and Dr. Henry G. Kattesh, for their assistance with her studies and this manuscript and for serving on her committee. She is grateful too for Dr. George A. Baumbach's advice and assistance.

She would especially like to thank Barbara B. Gillespie for her patience, friendship, and assistance with the progesterone radioimmunoassays and pen doors. Thanks also to her fellow graduate students for their help with her project and statistics, and to Tye Ardrey, Mark Short, and Judy Prescott for their endless help with the sheep and surgeries.

Appreciation is also extended to John Bacon for his help with the sheep, to Kathy Moore for her assistance with the clerical aspects of finishing this manuscript, and to Ben Lifsey and Brian Rice for their help with the photographs and graphs within this manuscript. The author also thanks Sandy Fletcher for the typing of this manuscript.

The author is grateful for her irreplacable roommate and friend, Joanne Maki, for putting up with her odd hours and for the endless supply of coffee, ice cream, and laughs. She would finally like to thank her parents, Kay and Carl Smith, for all their love, support, and encouragement throughout her academic career.

ABSTRACT

The functional lifespan of the ovine corpus luteum (CL) is prolonged when a viable conceptus is present. Infusion of unfractionated day 16 ovine conceptus protein (CP) prolongs CL function up to 52 days or more, while infusion of isolated ovine trophoblast protein-1 (oTP-1) prolongs CL function to a lesser extent (3-5 days).

Studies were performed to determine whether oTP-1 is a necessary component of total CP for antiluteolysis. Ovine TP-1 was removed from total CP via repeated diethylaminoethyl-cellulose (DEAE) ion exchange column chromatography. Removal of measurable ovine trophoblast protein-1 (oTP-1) was confirmed by two dimensional polyacrylamide gel electrophoresis (2D PAGE) and fluorography of dried gels.

Beginning on day 12 (estrus = day 0), either 3.4 ml (620 µg/ml) oTP-1 free CP or diluted sheep serum were infused daily into the uterine lumen of each of three animals for 7 days (days 12-18). Peripheral blood samples were collected daily for 13 days (days 12-24), and progesterone (P_4) concentration was determined by radioimmunoassay. On day 24, sheep infused with oTP-1 free CP were examined for maintenance of the marked CL. All sheep had ovulated and formed new CL. Results of P_4 analysis demonstrated luteal regression (P_4 < 1 ng/ml) by day 16 in all animals. Analysis by Wilcoxon's paired sample test indicated no significant difference in duration of P_4 production by the CL of treated or untreated ewes. Failure of the infused fractionated protein to prolong luteal function indicates that oTP-1 may be a necessary component of the CP antiluteolysin.

A separate but related experiment determined the feasibility of increasing availability of conceptus proteins of interest by changing the culture media at 24 hour intervals. Qualitative analysis of conceptus culture media by 2D PAGE and fluorography and determination of percent incorporation of radiolabelled tracer and concentration of total proteins synthesized *in vitro* showed no change in the profile of proteins synthesized after prolonged incubation. Therefore, an efficient way to increase the availability of conceptus proteins of interest is to prolong the amount of time a conceptus remains in culture by changing the culture media at various intervals.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION.	1
II.	LITERATURE REVIEW	3
	Estrous Cycle of the Ewe.	3
	Luteal Regression: Uterine $PGF_{2\alpha}$ and Ovarian Oxytocin.	6
	Early Pregnancy	10
	Uterine Environment	11
	Uterine/Conceptus Synchrony and Maternal Recognition of Pregnancy.	12
	Blastocyst Development and Conceptus Products	12
	Maintenance of Luteal Function by Conceptus Proteins.	13
	Mechanisms of Actions of oTP-1.	14
	Effects of PG on Luteal Maintenance	15
III.	MATERIALS AND METHODS	18
	Protein Isolation	18
	Materials	18
	Animals	18
	Culture Media Preparation	19
	In Vitro Conceptus Culture.	19
	Protein Purification	20
	Isolation of Peak 2 Proteins from oTP-1	21
	Infusion of oTP-1 Free Conceptus Media.	24
	Preparation of oTP-1 Free CP for Infusion	24
	Infusion of Proteins into the Uterine Lumen	24
	Progesterone Assay.	25
	Statistical Analysis.	26
	Comparison of Conceptus Culture Media	26
	Incorporation of 3H Leu	26
	Protein Content Determination	26
	Qualitative Analysis of Conceptus Proteins Synthesized	27
	Statistical Analysis.	27
IV.	RESULTS	28
	oTP-1 Free CP Infusion and Progesterone Response.	28
	Demonstration of the Absence of oTP-1	28
	oTP-1 Free CP Infusion.	28
	Progesterone Concentration.	32
	Comparison of Conceptus Culture Media Percent Incorporation and Total Protein	32

CHAPTER PAGE

IV. (Continued)
Percent 32
Total Protein Content 32
Qualitative Analysis of Culture Media 35

V. DISCUSSION. 43
Infusion of oTP-1 Free Conceptus Proteins 43
Possible Mechanisms of Action of oTP-1. 44
Analysis of Conceptus Culture Media 47

VI. SUMMARY AND CONCLUSIONS 49

LITERATURE CITED. 52

VITA. 61

LIST OF FIGURES

FIGURE		PAGE
1.	Pattern of elution of total conceptus secretory proteins from preliminary DEAE column chromatography procedure . . .	22
2.	Pattern of elution of fractionated conceptus proteins from the second DEAE column chromatography procedure.	23
3.	Comparison of Coomasie blue stained 2D gel of unfractionated CP with oTP-1 free CP	29
4.	Fluorograph of oTP-1 free CP.	31
5.	Progesterone concentrations of ewes infused with sheep serum on days 12-18 following estrus (day 0; n = 3).	33
6.	Progesterone concentrations of ewes infused with oTP-1 free conceptus proteins on days 12-18 following estrus (day 0; n = 3)	34
7.	Coomasie blue stained 2D gel of 24 hour culture media and its corresponding fluorograph	36
8.	Coomasie blue stained 2D gel of 48 hour culture media and its corresponding fluorograph	38
9.	Fluorograph of 72 hour culture media.	40
10.	Coomasie blue stained 2D gel of 96 hour culture media and its corresponding fluorograph	41

CHAPTER I

INTRODUCTION

Early embryonic mortality is a primary factor in decreased reproductive efficiency of livestock (Ayalon, 1978). Embryonic mortality is defined as fertility losses during the embryonic period; the period extending from conception to completion of differentiation (Committee on Reproductive Nomenclature, 1972). In the ewe, the largest percentage of embryonic mortality (20.4%) occurs during the first 18 days of pregnancy. Between day 18 and lambing, embryonic mortality increases to 9.3% (Hulet et al., 1956). Two factors believed to be involved in embryonic mortality are maternal hormone imbalances and a nonreceptive uterine environment (Hanly, 1961; Ayalon, 1978). Investigations of conceptus-maternal interactions during early pregnancy may lead to improved understanding of the causes of early embryonic mortality and possible means for reducing its occurrence.

As in all placental mammals, maintenance of pregnancy in the ewe requires continued progesterone (P_4) production by a functional corpus luteum (CL) (Amoroso, 1952). Elevated P_4 facilitates continued development of the uterine lining for implantation and the production of uterine secretions for the nourishment of the early embryo (Bazer and First, 1983).

Mechanisms which prolong the functional lifespan of the CL involve interactions between the female and the developing

conceptus. The conceptus must produce a biochemical signal(s) which elicits the maternal response of CL maintenance and continued P_4 production. This phenomenon is known as maternal recognition of pregnancy (Short, 1969). A protein named ovine trophoblast protein-1 (oTP-1), produced by the ovine conceptus between days 13-21, is believed to be the ovine signal for maternal recognition of pregnancy (Godkin et al., 1982; Godkin et al., 1984b). Whether oTP-1 acts alone to elicit the maternal response and the exact mechanism by which it does act is unclear.

Objectives of this study were to remove oTP-1 from other conceptus proteins (CP) produced by day 15-17 ovine conceptuses, confirm the absence of oTP-1, and determine the effects of these proteins on the functional lifespan of the CL when infused into the uteri of cyclic sheep.

In a separate but related study, attempts were made to increase the yield of proteins produced during conceptus culture by prolonging the incubation period from 24-48 hours without media change to 48, 72, and 96 hours with media changes every 24 hours. Total protein released by the conceptus into the culture media and percent incorporation of radiolabelled amino acid into radiolabelled protein (<u>in vitro</u> synthesized proteins) were determined and compared at each time interval. Qualitative differences in protein production at each time period were determined by two dimensional polyacrylamide gel electrophoresis (2D PAGE) and fluorography.

CHAPTER II

LITERATURE REVIEW

I. Estrous Cycle of the Ewe

The estrous cycle provides an animal with multiple opportunities to achieve gamete fertilization culminating with parturition and continuation of the species. If fertilization is not achieved, estrous cycles continue to allow for another chance of conception in a subsequent cycle. Continuing estrous cycles are dependent on the interactions between the pituitary gonadotrophin hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH); the ovarian steroids, P_4 and E_2; and uterine prostaglandins (PG). Ovarian oxytocin is also believed to play a role in the control of the estrous cycle. Reviews by Hansel et al. (1973) and Bazer et al. (1981) thoroughly cover the patterns of hormone secretion and the various interactions between pituitary-hypothalamus, ovary, and uterus. When fertilization results, conceptus-maternal interactions occur to halt the estrous cycle and allow for the maintenance of the pregnancy. A review by Bazer and First (1983) summarizes the endocrinological and physiological events associated with early pregnancy in the sow, ewe, cow, and mare.

Sheep are generally seasonally polyestrous with recurring estrous cycles during the short day seasons (fall and winter). Seasonal breeding is associated with the frequency and amplitude

of episodic LH releases which are increased at the onset of the breeding season (Yuthasastrakosal et al., 1977). The mean estrous cycle length is 16.7 days with a normal range of 14-19 days (Hulet and Shelton, 1980). Reports of numerous investigators (Scaramuzzi et al., 1970; Bjersing et al., 1972; Pant et al., 1972; Yuthasastrakosol et al., 1975; Baird and McNeilly, 1981) have established the patterns of FSH, LH, E_2, and P_4 release and relationships between these hormones which occur during the ovine estrous cycle. The estrous cycle can be divided into four distinct phases: estrus, metestrus, diestrus, and proestrus with which there is a certain profile of hormones and endocrinological events associated.

The length of estrus ranges from 24-36 hours with ovulation occurring spontaneously near the end of estrus (Hulet and Shelton, 1980). Estrus is characterized by the female's receptiveness to the male and is associated with increasing concentrations of E_2 and decreasing P_4. Peak values of E_2 are not observed until P_4 production by the CL declines. This occurs preceding ovulation (Obst et al., 1971). Progesterone values are lowest during and immediately after estrus (Stabenfeldt et al., 1969; Pant et al., 1972). Estrus is also characterized by an LH surge. Maximum concentrations of LH are found 6-15 hours after estrus, with a return to baseline values immediately following ovulation (Bjersing et al., 1972).

Metestrus is characterized by decreasing E_2 levels, ovulation, and luteinization of the theca interna and granulosa layers

of the follicle to form a functional CL (for review of CL formation, function, and regulation, see Niswender et al., 1985). Progesterone secreting cells of the CL are classified as either large luteal cells (diameters of 22-35 μm) or small luteal cells (diameters of 12-22 μm) (Fitz et al., 1982; Rodgers and O'Shea, 1982). The small cells possess numerous receptors for LH, but none for prostaglandins, and their P_4 production is stimulated through adenylate cyclase and cAMP (Glass et al., 1984; Niswender et al, 1985). Large luteal cells have receptors for prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$), but very few LH receptors (Glass et al., 1984). Progesterone production by large luteal cells is not stimulated by LH nor associated with increased activity of adenylate cyclase and cAMP production. Prostaglandin E_2 has been shown to stimulate P_4 production by large luteal cells, but this response does not appear to be mediated by activation of adenylate cyclase (Niswender et al., 1985). Although the mechanism for stimulation of P_4 production by large cells is unclear, it has been suggested that it is stimulated through a signal transduction system other than adenylate cyclase and cAMP such as the calcium, calmodulin, lipid-dependent protein kinase C system (Niswender et al., 1985). Large luteal cells also possess intracellular organelles necessary for synthesis and secretion of peptides and proteins (Fawcett et al., 1969) and seem to be responsible for production of the oxytocin and vasopressin identified in ovine luteal tissue (Wathes and Swann, 1982; Niswender et al., 1985).

High P_4 concentrations, a fully functional CL (Hansel et al., 1973), and peaks in luteal weight (Bartol et al., 1981) are associated with diestrus. Progesterone ranges between 2-7 ng/ml during diestrus (Bjersing et al., 1972). During proestrus (day 15 of the estrous cycle, or about 12 hours before the onset of estrus), P_4 concentrations are low ($\leq$ 0.2 ng/ml) (Bjersing et al., 1972; Pant et al., 1972) due to the regression of the CL. Estrogen values increase during proestrus due to the continued development of the graffian follicle in preparation for ovulation (Hansel et al., 1973). Luteal regression, which occurs during proestrus, is essential for recurring estrous cycles. Prevention of luteal regression, by a signal for the conceptus, results in continued P_4 production by the CL and the maintenance of early pregnancy (review by Bazer et al., 1986).

Luteal Regression: Uterine $PGF_{2\alpha}$ and Ovarian Oxytocin

Luteal regression and decreased P_4 production must occur in the ewe so that estrus occurs and the ewe remains cyclic. Wiltbank and Casida (1956) demonstrated that hysterectomy prolongs the functioning of the CL, indicating uterine involvement in the control of luteal regression. Unilateral hysterectomy resulted in CL maintenance ipsilateral, but not contralateral to the removed uterine horn (Inskeep and Butcher, 1966). Ovarian transplantation to the neck with the uterus remaining *in situ* resulted in CL maintenance (Goding et al., 1967), but transplantation of the ovary and uterus together resulted in luteal regression

and return to estrus (Harrison et al., 1968). Thus, the uterus and ovary must be in close proximity for regression of the CL to occur, indicating that the uterine luteolysin acts in a localized manner. Cross circulation studies between donor ewes of various estrous cycle stages and recipient ewes with ovarian transplants to the neck indicated the luteolysin secreted by the uterus was blood borne (McCraken et al., 1972). The first suggestion that prostaglandin might be the uterine luteolysin was by Babcock (1966). Prostaglandin $F_{2\alpha}$ has now been demonstrated to be the uterine luteolytic substance responsible for the demise of the CL in many species (McCraken et al., 1972; Goding, 1974; Horton and Poyser, 1976; McCraken et al., 1981). Prostaglandin $F_{2\alpha}$ is produced in the uterus and acts in a localized manner by traveling to the ovary via a "counter current" mechanism (Barrett et al., 1971; Horton and Poyser, 1976). The counter current mechanism states that uterine $PGF_{2\alpha}$ is drained by the uterine vein which in the ewe is closely associated with the ovarian artery. $PGF_{2\alpha}$ crosses from the uterine vein into the ovarian artery and is transported to the CL, resulting in luteal regression (McCraken et al., 1972; McCraken et al., 1981). Because $PGF_{2\alpha}$ is a vasoconstrictor, it is believed that one way of exerting its antiluteolytic effects on the CL may be to deplete the blood supply of the CL, resulting in its regression (Ford, 1984). It has been shown that decreased blood flow to the ovary and decreased production of P_4 occur simultaneously during regression of the CL in the ewe. The use of $PGF_{2\alpha}$ to induce luteal regression

is associated with decreased blood flow to the ovary (Bruce and Moor, 1976; Nett et al., 1976; Niswender et al., 1976; Ford et al., 1979b). Another possible mechanism by which $PGF_{2\alpha}$ causes CL regression is through the uncoupling of the luteal LH receptor (LH-R) from the plasma membrane adenylate cyclase (AC) (Henderson and McNatty, 1975). Buhr et al. (1979) demonstrated physical changes in the lipid structure of the luteal cell plasma membrane in the rat following treatment with $PGF_{2\alpha}$ which may be a physical representation of Henderson and McNatty's (1975) proposed LH-R and AC uncoupling. The model of the adenylate cyclase system is comprised of three protein components: receptors, guanine nucleotide-binding regulatory proteins (N_s and N_i), and the enzyme catalytic unit (C) (Sibley and Lefkowitz, 1985). It is also possible that homologous or heterologous densensitization of the adenylate cyclase system's three protein components is stimulated by $PGF_{2\alpha}$, resulting in the decreased P_4 production associated with luteal regression (for review of adenylate cyclase system desensitization see Sibley and Lefkowitz, 1985). Silvia et al. (1984) found that the secretion of P_4 by large luteal cells was inhibited when PGF_2 was administered <u>in vitro</u>. There was found to be no direct effect on the small luteal cells' secretion of P_4 by the administration of $PGF_{2\alpha}$. This was explained by the observation that the large luteal cells have receptors for $PGF_{2\alpha}$ and PGE_2 whereas small luteal cells do not (Niswender et al., 1985). It is hypothesized that $PGF_{2\alpha}$ induces secretion of a factor(s) by large luteal cells which may act directly

on large luteal cells in a cytotoxic manner, and acts to disrupt LH's ability to stimulate adenylate cyclase of small luteal cells (Silvia et al., 1984).

The initiation of luteolysis by $PGF_{2\alpha}$ requires very specific pulses and concentrations of $PGF_{2\alpha}$. Five 1 hour pulses of $PGF_{2\alpha}$ at 6 hour intervals for a 25 hour period is the minimum requirement for luteolysis (Schramm et al., 1983). Minimal elevations of 5-10 pg/ml of $PGF_{2\alpha}$ in ovarian arterial plasma initiates a large release of luteal oxytocin (5-10 ng/ml) into the ovarian vein which elicits a large luteolytic burst of $PGF_{2\alpha}$ into the uterine vein. After counter current transfer of the $PGF_{2\alpha}$ released, ovarian arterial plasma concentrations of $PGF_{2\alpha}$ increased to 100-500 pg/ml which appears to be sufficient to initiate luteolysis (Lamsa and McCraken, 1986). Subsequent pulses of $PGF_{2\alpha}$ are believed to be associated with the decline in P_4 concentrations which would allow maximal expression of the actions of E_2 and therefore facilitate resynthesis of the oxytocin receptor (Lamsa and McCraken, 1986).

Ovarian oxytocin is reported to have a role in the control of luteal regression. An increase in uterine oxytocin receptors has been observed at the end of the estrous cycle in the ewe (Roberts et al., 1976). Associated with pulses of uterine $PGF_{2\alpha}$ at the end of the cycle are episodes of release of oxytocin-neurophysin (Fairclough et al., 1980) or oxytocin alone (Flint and Sheldrick, 1983). When oxytocin receptor concentrations

rise at the end of the estrous cycle, the basal pulse interval of 20 minutes (20 ng released) (Lamsa and McCraken, 1985) caused small releases of $PGF_{2\alpha}$ into the uterine vein. This resulted in the minimal elevation of $PGF_{2\alpha}$ in the ovarian arterial plasma which elicited a large release of ovarian oxytocin (Lamsa and McCraken, 1986). Secretion of oxytocin by the ovary has been confirmed by measuring its concentrations in ovarious venous and utero-ovarian venous blood (Flint and Sheldrick, 1983). On days 10-14 of the estrous cycle, ovarian venous plasma contained concentrations of oxytocin 5-8 times that of utero-ovarian venous plasma and 10-100 times that of femoral or carotid arterial plasma (Flint and Sheldrick, 1983). Additional evidence indicating a role for oxytocin in luteal regression was illustrated in animals immunized against oxytocin which exhibited delayed luteal regression (Sheldrick et al., 1980).

II. Early Pregnancy

Maintenance of the functional lifespan of the CL and its continued production of P_4 is necessary for the maintenance of early pregnancy in all placental animals (Amoroso, 1952). However, the role of E_2 in early pregnancy maintenance is less established (Sauer, 1979). Length of dependency on P_4 secreted by the CL for the continuation of pregnancy varies among species (see Bazer and First, 1983). Maintenance of early pregnancy by P_4 is dependent upon a receptive uterine endometrium and an appropriate profile of uterine secretions which allow for the continued development of the conceptus, eventual implantation, and nutrient partitioning (Bazer and First,

1983). Other roles of P_4 in maintaining early pregnancy may include regulation of blood flow at implantation sites (Ford et al., 1979a) and acting as a precursor for various steroid metabolites (Thatcher et al., 1984). For a review of hormonal involvement in the establishment of pregnancy see Sauer (1979).

Uterine Environment

The endometrium synthesizes numerous proteins and enzymes for intermediary and steroid hormone metabolism which may contribute to continued development of the preimplantation blastocyst (for review of uterine protein synthesis and ovarian steroid control see Findlay, 1981). Protein content of the uterine lumen increased between day 13 and 17 of pregnancy (Ellinwood et al., 1979a), and Findley et al. (1981, 1982) demonstrated an increased rate of cellular protein leucine incorporation by the endometrium of pregnant ewes for implantation. Epithelial cells of endometrium obtained from day 13 pregnant ewes exhibited a higher incorporation of ^{35}S-methionine than the cells of ewes at day 13 of the estrous cycle. The increased synthesis and secretion of specific proteins by endometrium of day 13 pregnant ewes can be mimicked by addition of blastocyst secretions to endometrial cells of day 13 nonpregnant ewes (Salamonsen et al., 1986). The mechanism by which blastocyst secretions enhance endometrial protein production and the exact function of increased endometrial proteins requires further investigation.

Uterine/Conceptus Synchrony and Maternal Recognition of Pregnancy

Synchrony between the uterus and embryo is necessary for successful continuation of pregnancy (Wilmut et al., 1985). In cattle, a close synchrony (± 1 day) of embryo donor and recipient cows is necessary for successful maintenance of pregnancy following embryo transfer (Rowson et al., 1969; Betteridge et al., 1980). Presence of the conceptus within the uterus must occur by a specific time period in order to prevent luteal regression. Its presence in the uterus prolongs elevated P_4 levels by lengthening the functional lifespan of the CL. This phenomenon has been termed "maternal recognition of pregnancy" (Short, 1969). Embryo transfer studies indicated that the embryo's presence in utero on or before day 13 of the estrous cycle is necessary for continued luteal function. Embryos transferred after day 13 had no effect on CL lifespan (Moor and Rowson, 1966). Based on embryo transfer data, it has been inferred that maternal recognition of pregnancy in the ewe occurs by day 13. For complete reviews of maternal recognition of pregnancy in large domestic animals see Bazer et al. (1986) and Thatcher et al. (1986).

Blastocyst Development and Conceptus Products

Bindon (1971) described sheep blastocysts as basically spherical between days 4-10, and elongated into a filamentous form by days 12-14. Elongation was the result of a dramatic outgrowth of the trophoblast. On day 15, the blastocyst was greatly elongated, but remained located in the uterine horn ipsilateral to the CL, while

the trophoblast extended through the uterine body and into the contralateral horn by day 16-17. For a review of elongation and the various fetal membranes see Perry (1981). It is during the period of rapid elongation that secretory activity of the blastocyst increases. Increased protein secretion by the ovine, pig, and cow conceptus (Bazer et al., 1986; Thatcher et al., 1986), and increased E_2 synthesis by the pig conceptus (Gadsby et al., 1980; Fischer et al., 1985) has been demonstrated. Estrogen synthesis by the bovine conceptus has been suggested (Thatcher et al., 1984) but not fully documented. Evidence for (Ellinwood, 1978) and against (Reynolds et al., 1982) E_2 secretion by the ovine conceptus has been presented. Estrogen appears to be the conceptus agent necessary for maternal recognition of pregnancy in the pig (for review see Bazer et al., 1986), but because E_2 production by the ovine/bovine conceptuses is limited or nonexistent, investigators have focused their attention on proteins secreted by these conceptuses.

Maintenance of Luteal Function by Conceptus Proteins

Intrauterine infusion of embryo homogenates demonstrated that synthesis of the conceptus signal occurs up until day 21. Day 14-16 trophoblast homogenates prolonged CL function for over a month while homogenates of embryos beyond day 21 failed to extend CL function (Rowson and Moor, 1967; Ellinwood et al., 1979a; Martal et al., 1979). Heat treated day 12-14 sheep embryos and heat or pronase treated day 14 trophoblast homogenates failed to extend the CL lifespan, indicating that the conceptus signal is proteinaceous in nature (Rowson

and Moor, 1967; Martal et al., 1979). Failure of human chorionic gonadotrophin and ovine chorionic somatomammotrophin to maintain the CL suggested that the conceptus signal was not a gonadotrophin-like protein (Martal et al., 1979). Godkin et al. (1982) analyzed proteins produced and released by conceptuses in culture by 2D PAGE and observed one major protein, oTP-1 (ph 5.5; MW 17,000) at day 13 which predominated until day 21. Production of oTP-1 occurred at the exact times when conceptus homogenates and/or extracts were capable of prolonging CL function, suggesting that oTP-1 may be the conceptus signal for maternal recognition of pregnancy (Godkin et al., 1982).

Attempts were made to characterize the major conceptus protein produced between days 13-21, and determine its mechanism of action. Daily infusion of isolated oTP-1 into the uterine lumen of cyclic ewes resulted in a prolongation of CL function for 3-5 days compared to prolongation of up to 50 days by the infusion of total conceptus protein. It was suggested that either infused oTP-1 was denatured during purification or within the uterine lumen, not present in a high enough concentration to exert a biological response, or was only part of a complex of conceptus proteins which act to prevent CL regression (Godkin et al., 1984b).

Mechanisms of Actions of oTP-1

Numerous experiments have been conducted to determine the location and mechanisms of action of oTP-1 in eliciting maternal recognition of pregnancy. Immunocytochemical studies demonstrated that oTP-1 is associated with the blastocyst trophectoderm cells

and accumulated in the uterine surface and upper glandular epithelium. Competitive binding studies showed that oTP-1 binds specifically to membrane preparations from the uterine endometrium (Godkin et al, 1984a). Ovine TP-1 stimulated synthesis of secretory proteins by day 12 nonpregnant ewe uterine endometrium explants in vitro. When iodinated oTP-1 was infused into the uterine lumen the majority of of radioactivity remained in the uterus. Very small amounts of the protein entered the maternal bloodstream, and none was found associated with the CL or ovaries (Godkin et al., 1984a). These data indicated that rather than directly influencing CL maintenance, oTP-1 may act on the endometrium to elicit the maternal response which results in CL maintenance.

Effects of PG on Luteal Maintenance

It is possible that the effect exerted by oTP-1 on the uterine endometrium may result in changes in concentration of $PGF_{2\alpha}$ and PGE_2 which prevents CL regression leading to maternal recognition of pregnancy. Prostaglandin $F_{2\alpha}$ has been shown to be the substance responsible for CL regression if fertilization does not occur (Goding, 1974; Horton et al., 1976). Corpus luteum regression may be initiated by changes in frequency of pulsatile release of $PGF_{2\alpha}$. When the CL regresses between days 15 and 17, $PGF_{2\alpha}$ is released at a frequency of 5-6 episodes per day, but it is released at only 1 or 2 episodes per day in pregnant sheep (McCraken et al., 1984; Zarco et al., 1984). Fincher et al. (1986) demonstrated that sheep conceptus proteins suppress $PGF_{2\alpha}$ release by measuring its metabolite, 15-keto-13,14-

dihydro-$PGF_{2\alpha}$ (PGFM) after uterine infusion of conceptus proteins. Whether the conceptus proteins affected the frequency or the amount of $PGF_{2\alpha}$ released, or whether they functioned to sequester $PGF_{2\alpha}$ within the uterine lumen as Bazer and Thatcher (1977) hypothesized in the pig is still unclear.

The possibility of a role for PGE_2 in luteal maintenance has been investigated. Increased $PGF_{2\alpha}$ and PGE_2 in pregnant ewes has been reported (Ellinwood et al., 1979b; Marcus, 1981; Lacroix and Kann, 1982), and preimplantation embryos have been shown to have PGE_2 synthesizing activity (Marcus, 1981). Prostaglandin E_2 is a vasodilator, and infusion of it into the ovarian artery or uterine lumen delayed luteal regression (Henderson et al., 1977; Pratt et al., 1977; Magness et al., 1978). Although PGE_2 was antiluteolytic when infused into the ewe, no in vivo stimulation of steroidogenesis by the CL was observed (Pratt et al., 1977). It may be that delayed luteal regression by PGE_2 is mediated through its vasodilatory effects which counteract the vasoconstrictive effects of $PGF_{2\alpha}$ (Henderson et al., 1977). It is also possible that secretion of PGE_2 prevents $PGF_{2\alpha}$ from inducing the synthesis of a factor(s) by large luteal cells which are cytotoxic. PGE_2 may also act as a luteotropin, substituting for LH as a stimulator of adenylate cyclase in small luteal cells (Silvia et al., 1984).

Over the past 5 years a great deal of information has been obtained concerning conceptus products and their effects on luteal maintenance during early pregnancy. Various actions and interactions of maternal uterine products (proteins and PG), conceptus products

(oTP-1, other proteins, PG, and possibly steroids), and luteal products (oxytocin and steroids) in the maternal response to the presence of a conceptus within the uterus remain unclear. The effect of conceptus proteins on PG metabolism and secretion by the uterine endometrium remains to be determined, and the mechanisms of action and target(s) of conceptus proteins needs further examination.

CHAPTER III

MATERIALS AND METHODS

I. Protein Isolation

Materials

Tissue culture supplies were purchased from Gibco Life Technologies, Inc., Chagrin Falls, OH. Silastic tubing for catheters was from Dow Corning Co., Midland, MI. ICN Radiochemicals, Irvine, CA, was the source of L-[4,5- H] leucine. Diethylaminoethyl cellulose was obtained from Whatman, Clifton, NJ. Fluorothane was from Fort Dodge Laboratories, Fort Dodge, IA, and Scinti Verse II scintillation cocktail was bought from Fisher Scientific Co., Fair Lawn, NJ. Protein standards and Tris-HCl were purchased from Sigma Chemical Co., St. Louis, MO. All inorganic chemicals used were reagent grade or better.

Animals

Adult crossbred ewes (Finn, Suffolk, Dorset, or Hampshire breeding) were checked twice daily for estrus with vasectomized rams. At estrus, ewes were bred to two intact rams. Between day 15-17 (estrus = day 0) conceptuses were collected by the following surgical procedure. Ewes were anesthetized (Fluorothane), and the reproductive tract was exposed by a midventral abdominal laparotomy. Ovaries were observed for a well vascularized CL indicating a pregnancy. The cervix was gently clamped closed, a 25 cm length of sterile silastic

tubing (id = .157 cm; od = .318 cm) was inserted into the gravid horn, and conceptuses were flushed from the uterus by injecting 20 ml sterile Hank's balanced salt solution (HBSS) into the nongravid horn. This procedure was conducted twice. The HBSS was massaged through the tract, and conceptuses were collected in a sterile serum bottle. Ewes were bred again on the second heat after surgery so that a maximum number of embryos could be collected.

Culture Media Preparation

A modified Eagle's minimum essential media (MEM; 500 ml) was sterilely prepared by the addition of 10 ml (2% v/v) antibiotic-antimycotic mixture: penicillin 10,000 IU/ml, fungizone 25 mcg/ml, and streptomycin 10,000 mcg/ml (ABAM), 5 ml (1% v/v) vitamins, 5 ml (1% v/v) nonessential amino acids, 1 ml (.1% v/v) insulin (Eli Lilly and Co., Indianapolis, IN; 100 units/cc), and 5 ml (4% v/v) 1M glucose, and was used for conceptus culture. Ten ml (2% v/v) ABAM was aseptically added to HBSS (500 ml) and transferred to sterile serum bottles for conceptus flushing during surgery. The procedure used for preparation of culture media is described by Godkin et al. (1982).

In Vitro Conceptus Culture

Conceptuses were aseptically transferred to the lab in the sterile serum bottle in which they were collected. They were placed in petri dishes containing 15 ml of the prepared culture media. One hundred microcuries (μCi) 3H-L-leucine (3H Leu; 1 μCi/1 μl) was added to each culture. Conceptus proteins synthesized *in vitro*

incorporated the radiolabelled amino acid, allowing proteins to be traced by scintillation counting during the purification and isolation procedures. Conceptuses were incubated at 37°C in a controlled atmosphere chamber (Bellco Biological Glassware, Vineland, NJ) containing 5% CO_2, 45% N_2, and 50% O_2 and were placed on a rocker platform at 3 cycles per minute. The majority of conceptus cultures were terminated after 24 hours (24 hour media). This technique for culture of sheep blastocysts was adapted by Godkin et al. (1982). It was observed that some blastocysts accumulated fluid within the trophoglast membrane and tissues remained intact. Others did not accumulate fluid, fragmented, and cellular breakdown was apparent. After a subjective evaluation of viability based on fluid accumulation and degree of tissue fragmentation, the intact conceptuses were placed in fresh media with another 100 µCI 3H Leu and incubated for an additional 24 hours (48 hour media). One conceptus was cultured for 48 additional hours beyond the first 48 hours of culture; media was changed and additional 3H Leu (100 µCI) added at 72 hours beyond initiation of the culture (72 and 96 hour media). After termination of a culture, media was centrifuged at 12,100 x g, 4°C, for 15 minutes, and frozen and stored at -20°C.

Protein Purification

Conceptus culture media was dialysed, molecular weight cut off (MWCO) 6-8,000 against 4 L 10 mM Tris-HCl buffer (pH 8.2) changed 4 times to remove low molecular weight compounds. The conceptus media protein solution was then submitted to diethylaminoethyl

(DEAE)-cellulose ion exchange column chromatography (2.0 x 10 cm column). Proteins were eluted with a 300 ml 0.0-0.5 M NaCl linear gradient and collected in 3 ml fractions. Twenty-five µl of each fraction were subjected to scintillation counting, and the resulting counts per minute (cpm) were plotted against fraction number. This procedure separated the conceptus proteins into three major peaks. The first peak primarily contained a high molecular weight glycoprotein (HMWGP) which eluted between 0.02-0.07 M NaCl. The third peak eluted between 0.20-0.33 M NaCl and contained oTP-1 (Godkin et al., 1982). The second peak eluted between 0.07-0.20 M NaCl and contained most other conceptus proteins (Figure 1). This procedure for purification of ovine CP is described by Godkin et al. (1982).

Isolation of Peak 2 Proteins from oTP-1

Proteins in the second peak were thoroughly dialysed against 4 L 10 mM Tris-HCl changed 4 times and then submitted to a second DEAE column chromatography procedure (2.0 x 8 cm column). Elution of these proteins was by a 300 ml 0.0-0.3 M NaCl gradient into 3 ml fractions. Preliminary DEAE chromatography separations demonstrated that oTP-1 began eluting at approximately .20 M NaCl. Therefore, all fractions eluting before .175 M NaCl were pooled and found to be oTP-1 free by 2D PAGE (O'Farrell, 1975; Horst and Roberts, 1979) and fluorography (Figure 2) (Laskey and Mills, 1975).

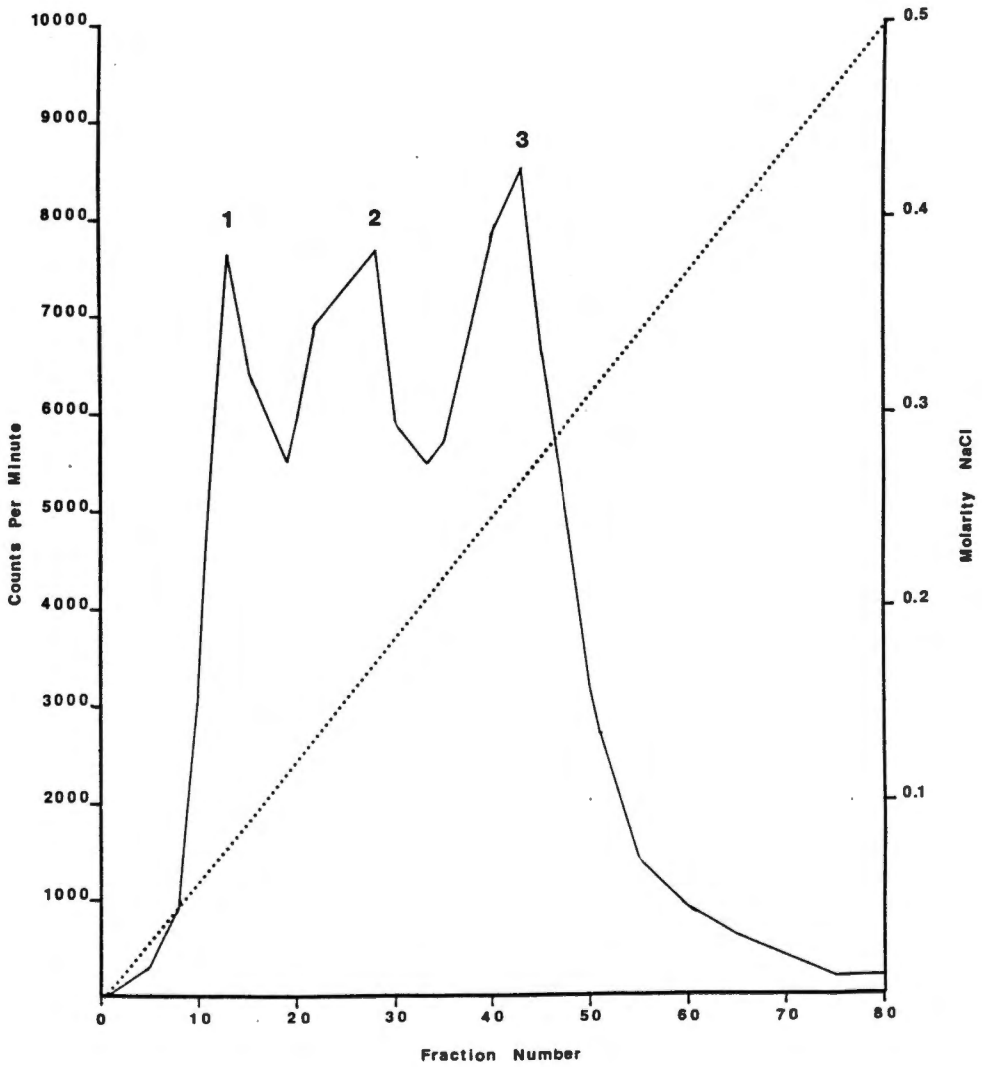


Figure 1. Pattern of elution of total conceptus secretory proteins from preliminary DEAE column chromatography procedure.

Peak 1 = HMWGP
Peak 2 = various conceptus proteins
Peak 3 = oTP-1
..... = linear NaCl gradient

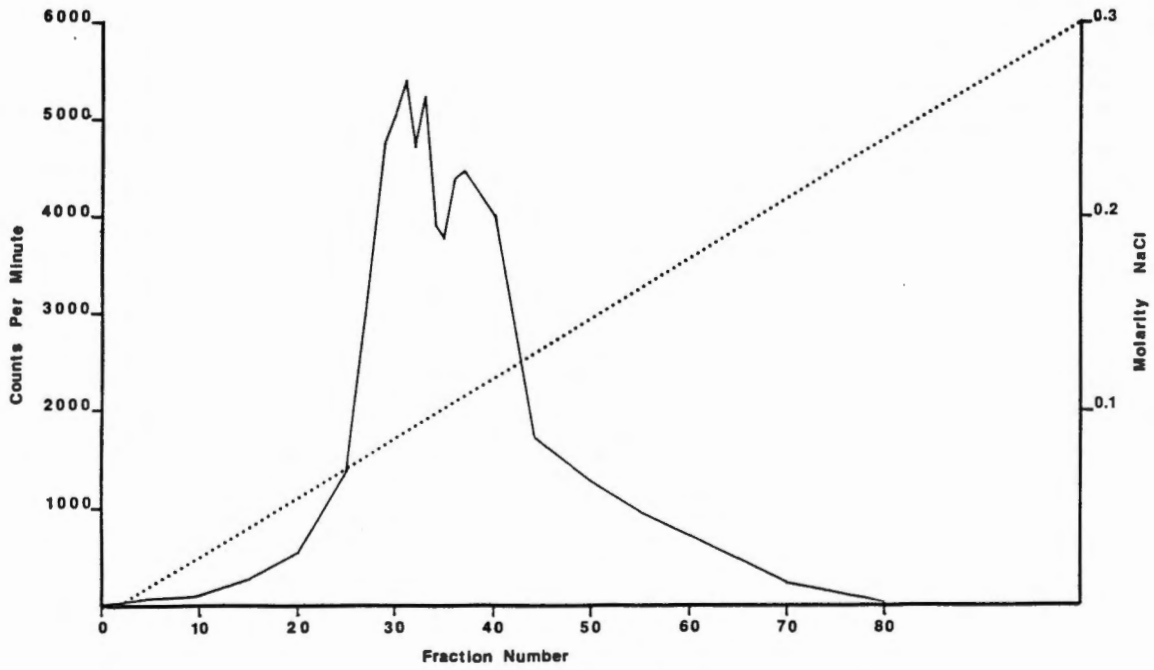


Figure 2. Pattern of elution of fractionated conceptus proteins from the second DEAE column chromatography procedure.

••••••• = linear NaCl gradient

II. Infusion of oTP-1 Free Conceptus Media

Preparation of oTP-1 Free CP for Infusion

Ovine TP-1 free CP were concentrated by ultrafiltration and dialysed (4 L, 4 changes, MWCO 6-8,000) against Dulbecco's phosphate-buffered saline (PBS), pH 7.6 (Dulbecco and Vogt, 1954). Sheep serum, the control infusate, was dialysed against PBS. Protein concentration of oTP-1 free CP and control serum was determined (Lowry et al., 1951), and the infusates were prepared at equal concentration and volume. Ovine TP-1 free CP and serum were filter sterilized (0.2 μ), and transferred to sterile capped culture tubes. Tubes were frozen and stored at -20°C until infused.

Infusion of Proteins into the Uterine Lumen

Records of ewes' estrous cycles were kept for three consecutive cycles, and six ewes with cycles of 16-17 days were chosen for infusion. Ewes (3 for protein infusion, 3 for control) were scheduled to undergo surgery on day 11 of the estrous cycle. The reproductive tract was exteriorized through a midventral laparotomy, and a silastic catheter (id = .157 cm, od = .318 cm) with three silastic cuffs (0.5 cm wide) spaced at 4, 5, and 15 cm from the tip was inserted into the uterine lumen ipsilateral to the CL just below the tubo-uterine junction. Approximately 2 cm tubing was fed into the lumen, and silk ligatures placed on either side of each cuff secured to the uterine horn and body were used to hold the catheter in place. Catheter volume was determined by infusion of sterile saline before placement. Once

secured, the catheter was exteriorized through the ewes' left flank with a trocar, and the end was closed off with a sterile blunt needle and screw-on top to maintain sterility. Catheters were stored in pouches sewn to the skin over the left flank. The CL was marked by injection of 0.1 ml india ink for future identification. The surgical and infusion procedures used are described by Godkin et al. (1984b). In the event of a CL on each ovary in the oTP-1 free CP infused sheep, one ovary was removed.

Beginning on day 12, 3.4 ml (620 µg/ml) oTP-1 free CP or sheep serum was thawed and infused into the uterus at 8:00 A.M. After protein infusion, catheters were flushed with a volume of sterile 0.9% saline equal to the catheter's volume. Protein infusion was continued for 7 days (day 12-18). Blood samples were taken from the jugular vein in the A.M. after protein infusion and again in the P.M., 9 hours later, from days 12-24. On day 24, oTP-1 free CP infused ewes were laparotomized, and the location and patency of the catheters were checked. Ovaries were examined for the maintenance of the india ink marked CL or new ovulations.

Progesterone Assay

Peripheral blood plasma was analyzed for P_4 by a variation of the radioimmunoassay procedure described by Niswender (1973). Mean P_4 concentration of plasma used to determine the variation in the assay was 4.94 ng/ml. The intra and interassay coefficients of variation were 11.68 and 11.74 respectively. Cross reactivities of the antibody used were: $< 0.1\%$ with cortisol, β-estradiol, and

estrone; 0.1% with cortisone, β-estradiol 3-benzoate, 5α-androstan-17β-ol-3-one, and cholesterol; 0.2% with hydrocortisone 21-acetate; 2.0% with corticosterone; 3.0% with pregnenolone; and 5.0% with 17α-hydroxy-progesterone. The sensitivity of the assay was 0.1 ng/ml.

Statistical Analysis

Differences in the duration of P_4 production by the CL in treated and control ewes were analyzed for significance by Wilcoxon's two sample test (Steele and Torrie, 1960).

III. Comparison of Conceptus Culture Media

Incorporation of 3H Leu

Percent incorporation of the 3H Leu into radiolabelled proteins by conceptuses during culture was determined by scintillation counting of each sample before and after dialysis. Three 25 µl samples were taken from each culture media (n = 24 for 24 and 48 hour media), and means were used to determine percent incorporation. Percent incorporation was also determined for 72 and 96 hour media (n = 1 for each).

Protein Content Determination

Total protein content of each culture media (n = 6 for 24 and 48 hour media, n = 1 for 72 and 96 hour media) was determined after dialysis. Two 100 µl samples were taken from each culture media and analyzed for protein content (Lowry et al., 1951).

Qualitative Analysis of Conceptus Proteins Synthesized

Determination of a qualitative difference in proteins synthesized during the various times of conceptus incubation (24, 48, 72, and 96 hours of culture) was conducted by submitting samples to 2D PAGE (O'Farrell, 1975; Horst and Roberts, 1979). A sample containing a total of 400,000 cpm was taken from each culture media after dialysis. Each sample was frozen and lyophilized and then subjected to 2D PAGE. In the first dimension (isoelectric focusing), a sample size of 100 µl containing 200,000 cpm was used. Molecular weight standards used in the second dimension were: transferrin molecular weight (MW) = 78,000; ovalbumin MW = 43,000; chymotrypsinogen MW = 25,000; and ribonuclease A MW = 13,899. After electrophoresis, gels were soaked in 1M sodium salicylate for 30 minutes (Chamberlain, 1979) and dried. Fluorographs were made (Laskey and Mills, 1975) with Kodak XRP-1 x-ray film.

Statistical Analysis

Differences in percent incorporation of 3H Leu into conceptus proteins synthesized, and protein content of different culture media were tested for significance by Wilcoxon's two sample test (Steele and Torrie, 1960).

CHAPTER IV

RESULTS

I. oTP-1 Free CP Infusion and Progesterone Response

Demonstration of the Absence of oTP-1

Two dimensional gel electrophoresis and fluorography demonstrated that the conceptus proteins to be infused were indeed free of oTP-1. Gels of unfractionated CP and oTP-1 free CP are compared in Figure 3. Ovine TP-1 is easily identifiable as a slightly acidic, low molecular weight compound: MW = 17,000 and isoelectic point = 5.5 (Godkin et al., 1982) (Figure 3, Protein A). A low molecular weight protein was also apparent on the gel produced from oTP-1 free CP, but this polypeptide was distinct from oTP-1. It is of lower molecular weight (less than 17,000) and more basic than oTP-1 (Figure 3, Protein B). In addition, fluorography of the gel failed to show any incorporation of radioactivity by the protein B. It was concluded that the fractionated CP for infusion is oTP-1 free (Figure 4).

oTP-1 Free CP Infusion

Placement and patency of catheters of the oTP-1 free CP infused sheep were checked on day 24 and all were found to be undisturbed. Infusion of sterile saline through each catheter showed each to have remained functional. The india ink marked CL of each ewe regressed, becoming a corpus albicans. A corpus hemorrhagicum, indicating a new ovulation, was seen in each ewe.

Figure 3. Comparison of Coomasie blue stained 2D gel of unfractionated CP (top) with oTP-1 free CP (bottom). Absence of protein A (oTP-1) from bottom gel indicates removal of oTP-1 from total CP.

A = oTP-1
B = lower MW, slightly more basic protein than oTP-1
+ = acidic end; - = basic end
cpm loaded = 200,000

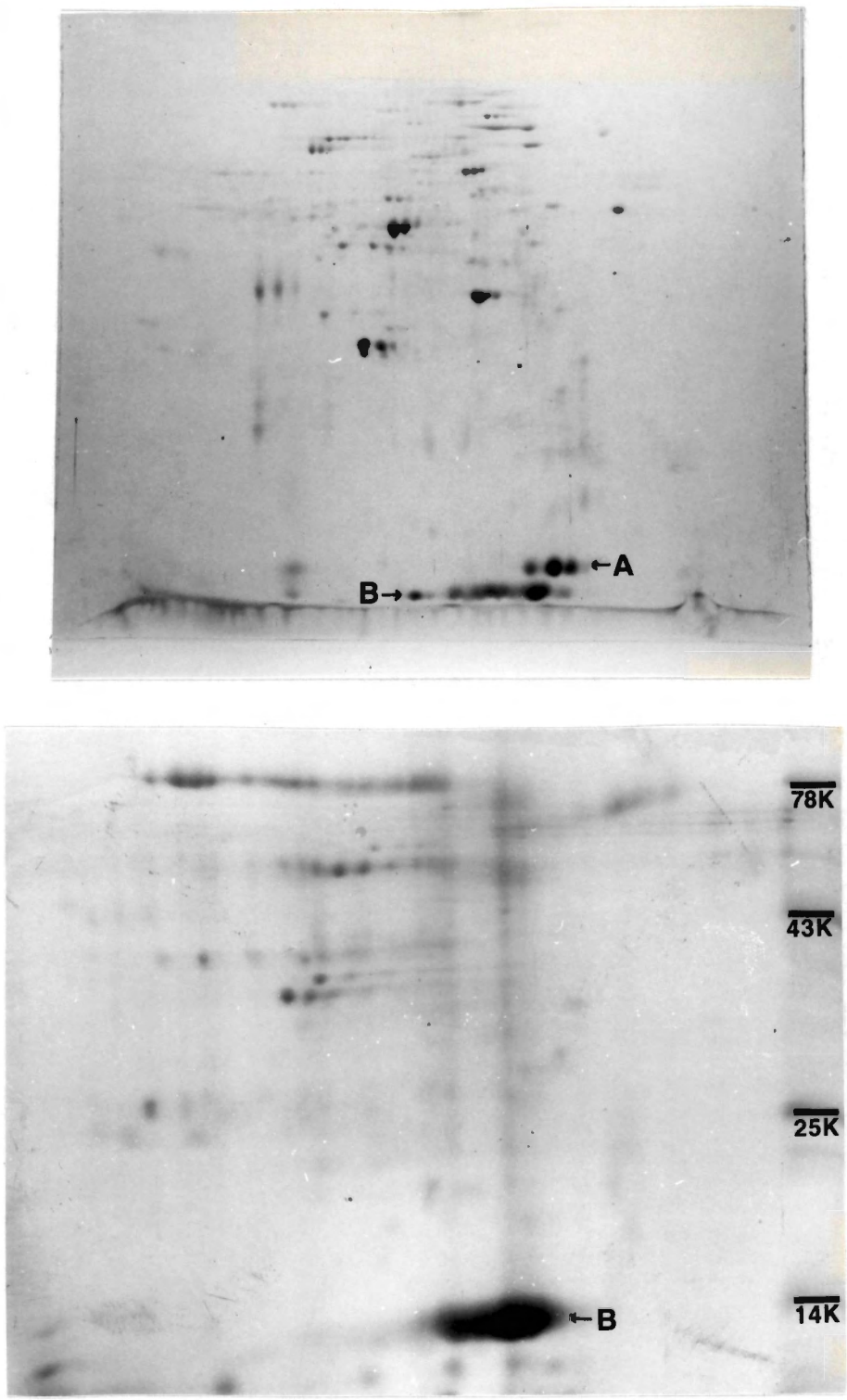


Figure 3.

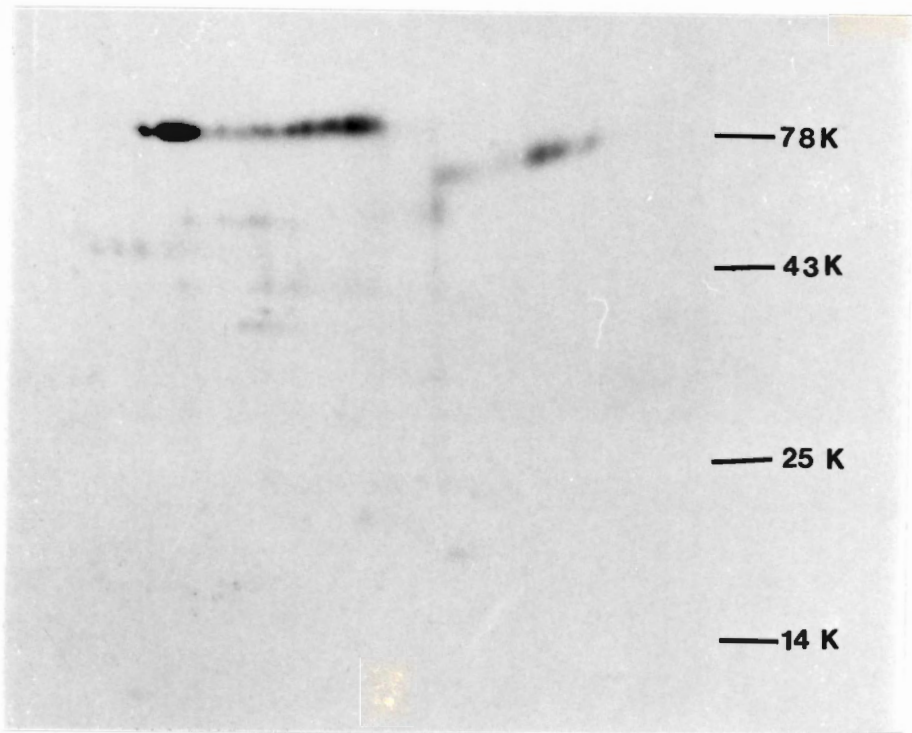


Figure 4. Fluorograph of oTP-1 free CP. Absence of radioactivity at MW = 17,000; the acidic end indicates oTP-1 is not present.

+ = acidic end; - = basic end
cpm loaded = 200,000
exposure time = 14 days

Progesterone Concentration

Analysis of peripheral plasma by radioimmunoassay demonstrated that all sheep, oTP-1 free CP treated and controls, had not undergone prolonged luteal maintenance. Five of six ewes exhibited peripheral P_4 concentrations < 1.0 ng/ml by day 15, indicating luteal regression. The sixth's peripheral P_4 fell below 1.0 ng/ml by day 16 (Figure 5 and Figure 6). There were no significant differences ($P > .10$) in length of P_4 production by the CL between days 15-18 in oTP-1 free CP treated and control ewes.

II. Comparison of Conceptus Culture Media Percent Incorporation and Total Protein

Percent Incorporation

Analysis of percent incorporation of 3H Leu into conceptus proteins in the first 24 hours of culture and the second 24 hours of culture indicated no significant ($P > .10$) difference between the two. Mean and standard error percent incorporation in the first 24 hours was 9.85% ± 4.01 and mean and standard error percent incorporation during the second 24 hours was 6.92% ± 2.36. The percent incorporation of the 72 hour medium was 13.18% and that of the 96 hour medium was 11.87%. Because only one sample existed for 72 and 96 hour media, statistical analysis of the percent incorporation by these samples could not be performed.

Total Protein Content

Mean and standard error total protein content of unfractionated CP after dialysis for the first 24 hours of culture was 256 ± 4.60 µg/ml

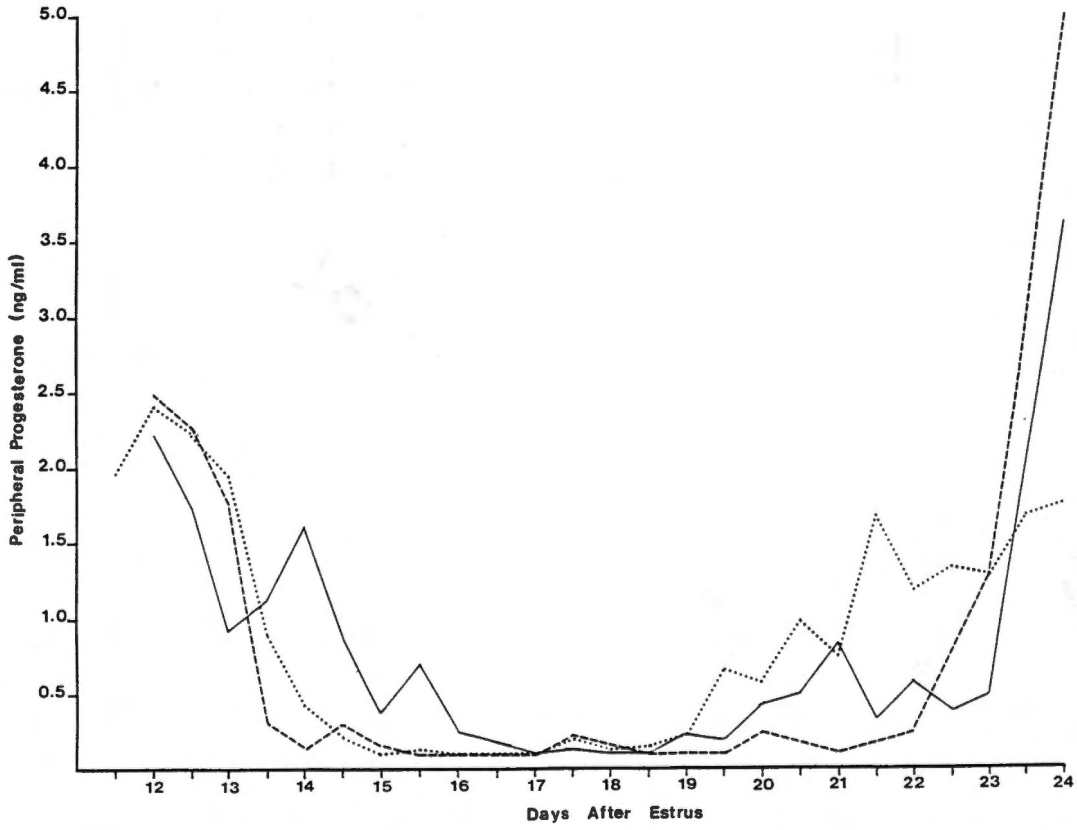


Figure 5. Progesterone concentrations of ewes infused with sheep serum on days 12-18 following estrus (day 0; n = 3).

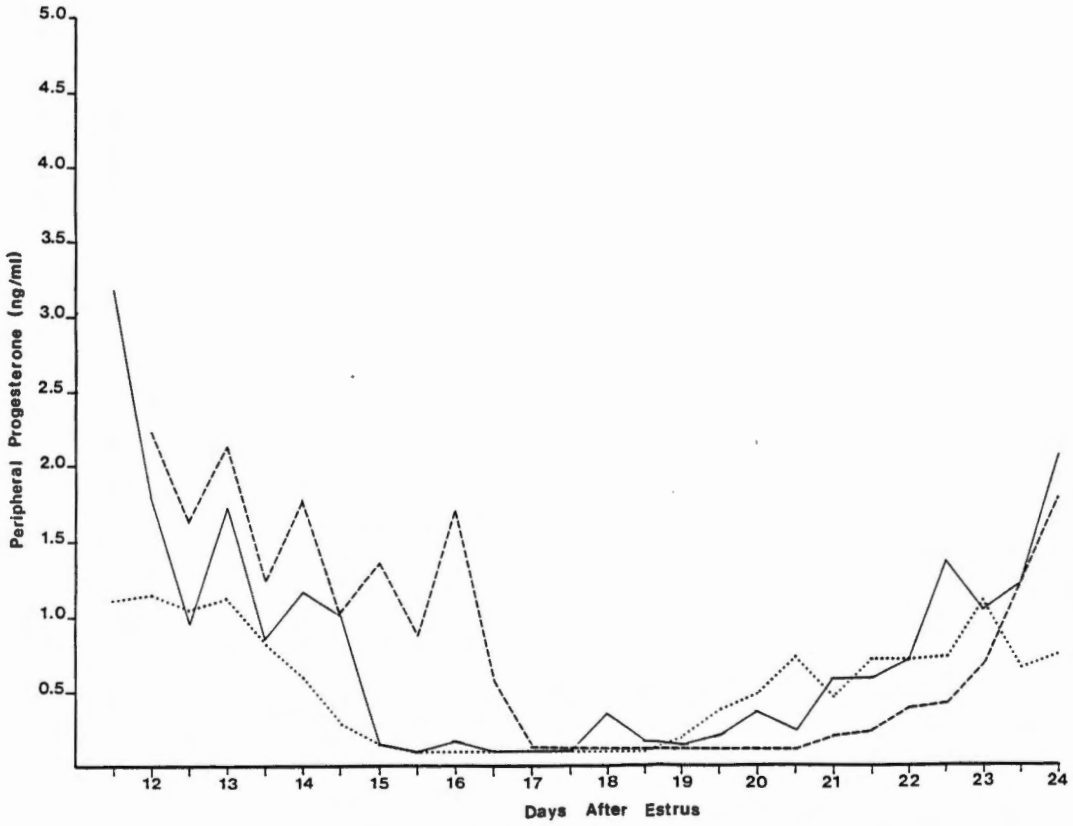


Figure 6. Progesterone concentrations of ewes infused with oTP-1 free conceptus proteins on days 12-18 following estrus (day 0; n = 3).

and 200 ± 3.51 µg/ml for the second 24 hours of culture. There was no significant ($P > .10$) difference in total protein content between the two time periods. Total protein concentrations of 72 and 96 hour media were 205 µg/ml and 195 µg/ml, respectively. Because only one sample existed for each, no statistical analysis of the total proteins found in 72 and 96 hour media were done.

Qualitative Analysis of Culture Media

Two dimensional gel electrophoresis and fluorography of the 48, 72, and 96 hour conceptus media demonstrated that the conceptus continues to produce oTP-1 and other conceptus proteins beyond 24 hours of culture. Comparison of gels and fluorographs indicated that changing the media and the prolonged time in culture did not alter or prevent secretion of major conceptus proteins (Figures 7-10). After 72 to 96 hours, the conceptus continued to secrete oTP-1 into the culture media.

Figure 7. Coomasie blue stained 2D gel of 24 hour culture media (top) and its corresponding fluorograph (bottom).

cpm loaded = 200,000
exposure time = 14 days
+ = acidic end; - = basic end

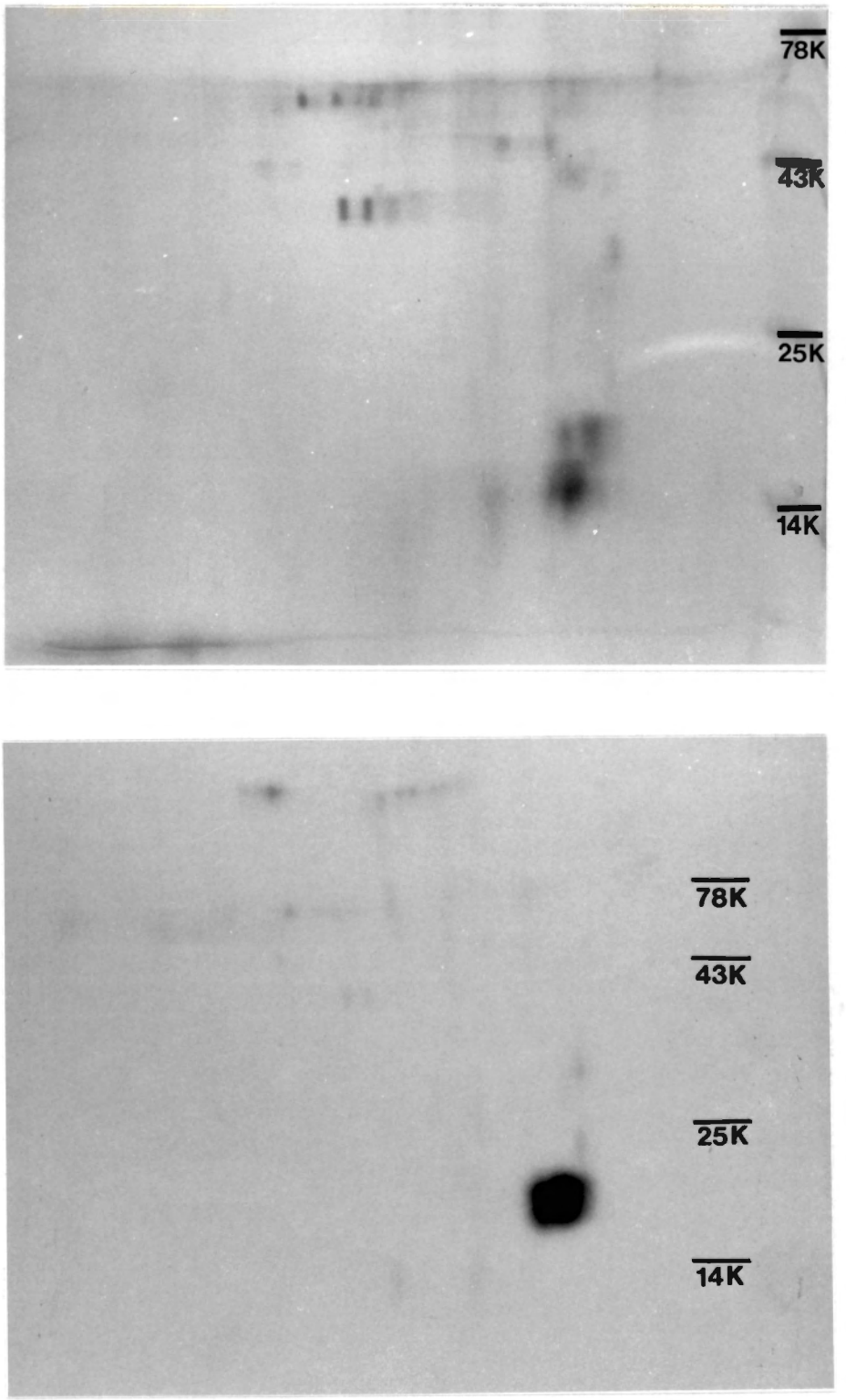


Figure 7.

Figure 8. Coomasie blue stained 2D gel of 48 hour culture media (top) and its corresponding fluorograph (bottom).

cpms loaded = 200,000
exposure time = 14 days
+ = acidic end; - = basic end

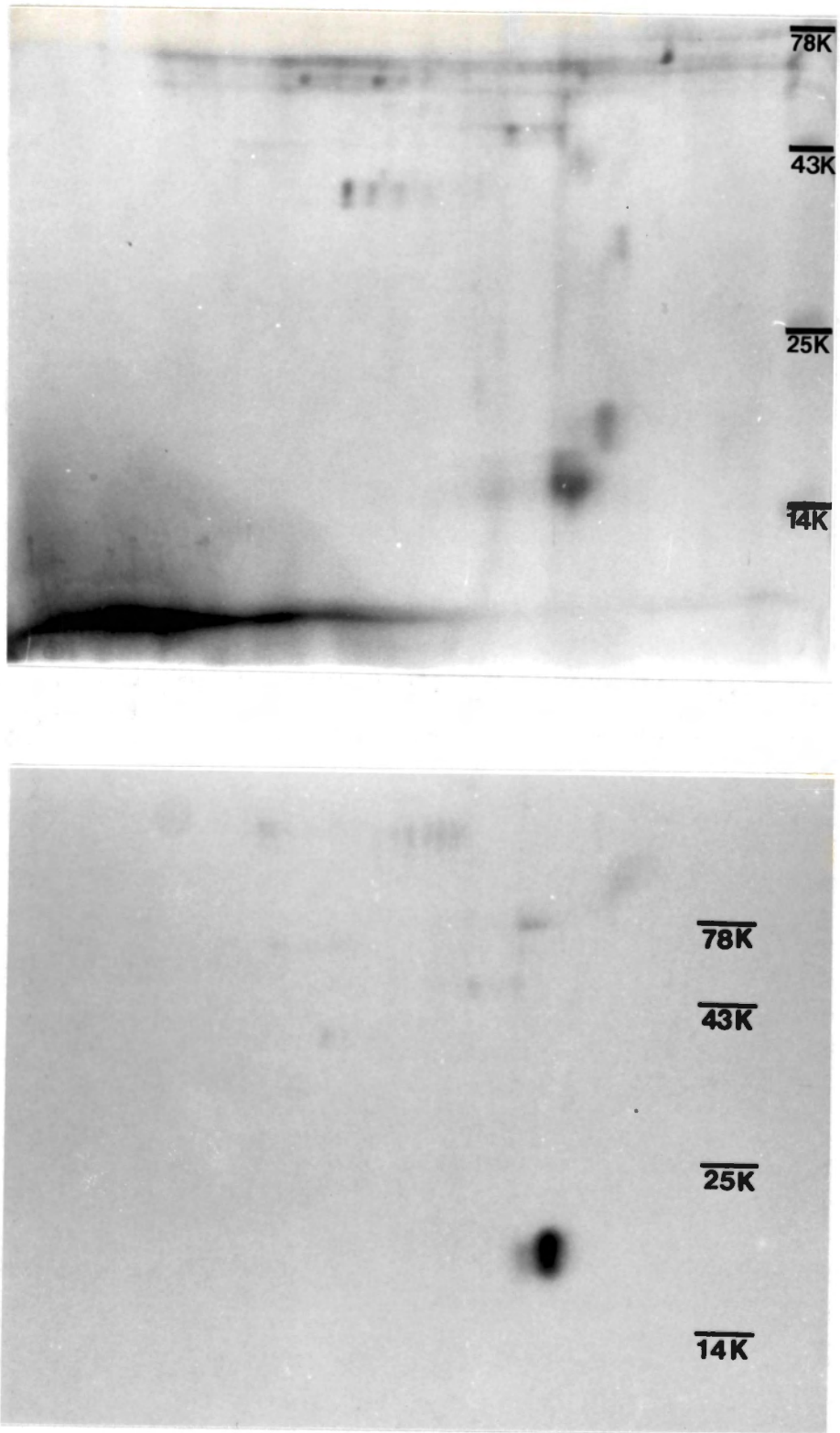


Figure 8.

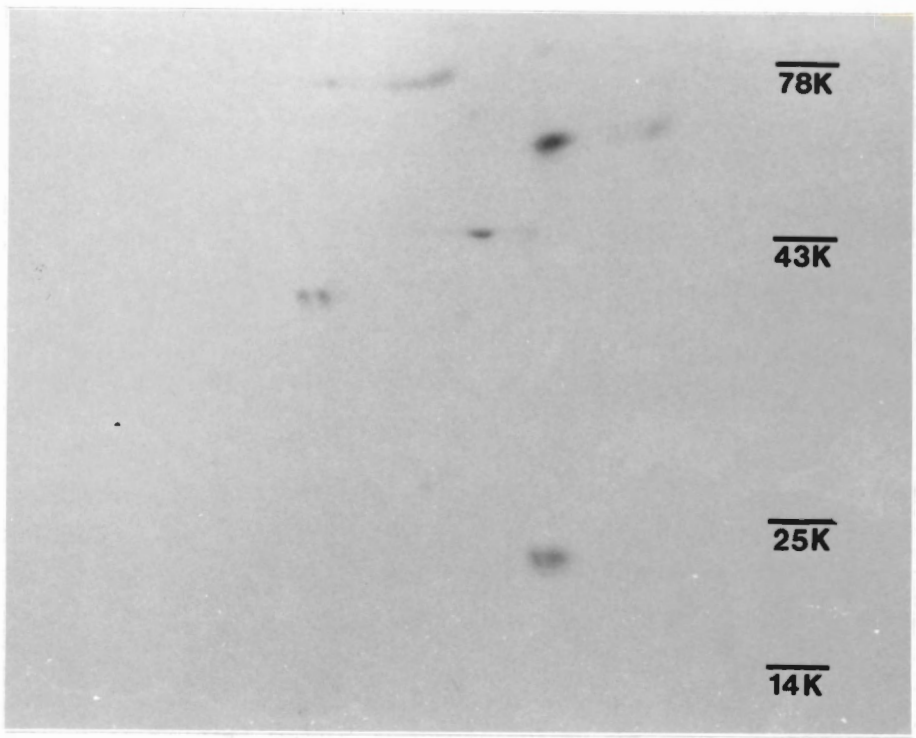


Figure 9. Fluorograph of 72 hour culture media (photo of 2D gel unavailable).

cpm loaded = 400,000
exposure time = 10 days
+ = acidic end; - = basic end

Figure 10. Coomasie blue stained 2D gel of 96 hour culture media (top) and its corresponding fluorograph (bottom).

cpm loaded = 400,000
exposure time = 10 days
+ = acidic end; - = basic end

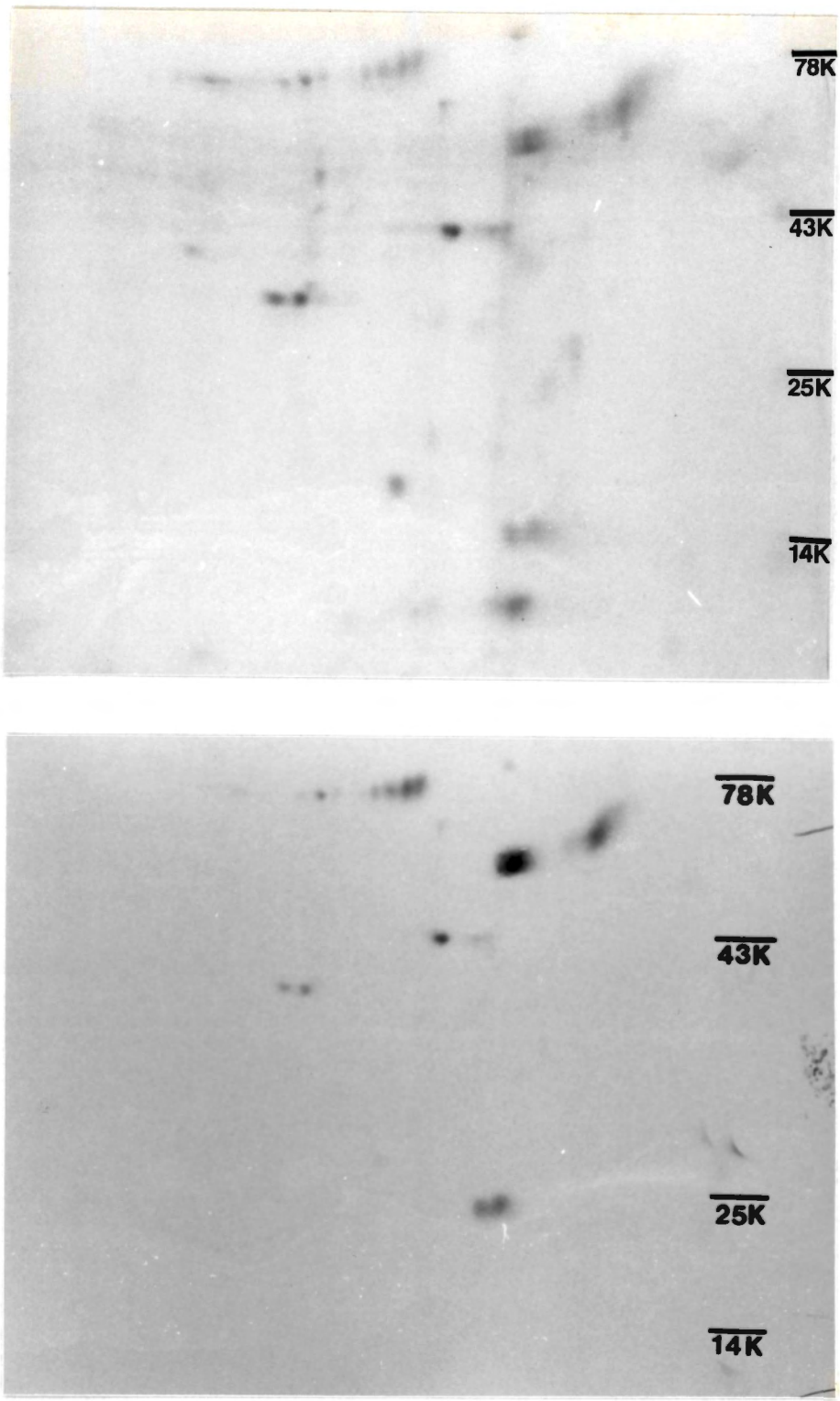


Figure 10.

CHAPTER V

DISCUSSION

Infusion of oTP-1 Free Conceptus Proteins

Infusion of oTP-1 free CP into the uterine lumen of cyclic ewes did not prolong luteal maintenance. Peripheral P_4 values indicated that all ewes, oTP-1 free CP treated and controls, underwent luteal regression and returned to estrus. Progesterone on days 12 and 13 was within the range of 2-7 ng/ml as reported for peak production of P_4 between days 7-13 (Bjersing et al., 1972; Pant et al., 1972). On days 15 and 16, P_4 values for all ewes was < 1.0 ng/ml which agrees with reported values on day 15 (Bjersing et al., 1972; Pant et al., 1972). At about day 22, the majority of ewes began to demonstrate a rapid increase in P_4 which would have corresponded with the P_4 synthesis by the newly formed CL. Fluctuations in P_4 values between A.M. and P.M. bleedings are consistent with the report that plasma P_4 concentrations in all ewes varies considerably throughout each 24 hours period (McNatty et al., 1973). Plasma P_4 concentrations are significantly higher on days 8-9 and 12-13 during daylight hours than the hours of darkness. Progesterone concentration during the daylight hours and darkness hours was not significantly different on days 4-5 and 15-16 (McNatty et al., 1973). The P_4 values obtained in this study agree with McNatty et al. (1973).

Failure of oTP-1 free CP to extend luteal function and P_4 production indicates that removal of oTP-1 from total CP results in the loss of its antiluteolytic properties. Godkin et al. (1984b)

hypothesized that decreased effectiveness of oTP-1 in maintaining CL function (3-5 days) when compared with maintenance stimulated by total CP (> 50 days) may have been due to several factors: oTP-1 may have been degraded by uterine enzymes within the uterine lumen before being able to exert any effects on uterine endocrinology, the concentration of oTP-1 infused may not have been high enough to exert a biological response, or oTP-1 may be only part of an anti-luteolytic complex of conceptus proteins which initiate maternal recognition of pregnancy.

The later hypothesis may not be correct since results of this study indicate that oTP-1 may be the necessary antiluteolytic protein secreted by the conceptus. One or both of the other theories may be a more likely explanation for the failure of oTP-1 to extend luteal function and P_4 production for as long a time as total CP. Studies by others indicate that the ability of oTP-1 to maintain luteal function for only 3-5 days (Godkin et al., 1984b) may have been due to its degradation in the uterus. A protein carrier, ovine serum, was added to the oTP-1 infusate to protect oTP-1 from uterine degradation. Luteal maintenance and P_4 production was comparable to that of total CP with the protection of the protein carrier (Bazer and Vallet, personal communication).

Possible Mechanisms of Action of oTP-1

The exact mechanism by which oTP-1 acts to maintain luteal function remains unclear. Most of available evidence suggests that the site of action is the luminal epithelium of the endometrium. Immunocytochemical studies have shown that oTP-1 accumulates at the

uterine surface and upper glandular epithelium, and binds specifically to membrane preparations of uterine epithelium (Godkin et al., 1984a). Only very small amounts of iodinated oTP-1 enter the maternal bloodstream after infusion into the uterine lumen, and no iodinated oTP-1 was found associated with the ovaries or CL (Godkin et al., 1984a). Ovine TP-1 stimulates synthesis of secretory proteins by day 12 nonpregnant ewe uterine endometrial explants in vitro (Godkin et al., 1984a); however, these endometrial proteins were shown to have no effect on secretion of P_4 by the CL (Vallet et al., 1985). Conversely, oTP-1 has been shown to bind specifically to membranes prepared from the CL (Godkin et al., 1984a). The protein does not stimulate P_4 synthesis by luteal slices or dissociated luteal cells. Binding of oTP-1 to luteal membranes cannot be explained at this time. It is paradoxical that there is no evidence to indicate that oTP-1 reaches the general circulation, but the protein appears to bind to luteal receptors.

Because oTP-1 appears to have no direct effect on steroidogenesis by the CL, and neither do endometrial proteins stimulated by oTP-1, the mechanism by which oTP-1 maintains luteal function may be through endometrial PG ($PGF_{2\alpha}$ and PGE_2). Fincher et al. (1986) demonstrated that uterine infusion of sheep conceptus proteins suppressed release of $PGF_{2\alpha}$ as determined by measuring its metabolite, 15-keto-13,14-dihydro-PGF (PGFM). Whether it is an actual suppression of the release of $PGF_{2\alpha}$, a change in the frequency of PGF pulses, or whether $PGF_{2\alpha}$ is still released at the same levels but sequestered within the uterus has not been fully explained.

It is also possible that oTP-1 exerts its effects by increasing uterine PGE_2 . Increased PGE_2 in the uterine lumen has been reported to be associated with early pregnancy (Ellinwood et al., 1979b; Marcus, 1981; Lacroix and Kann, 1982). The infusion of PGE_2 into the uterine lumen delays luteal regression (Henderson et al., 1977; Magness et al, 1978), but no in vivo stimulation of steroidogenesis by the CL was observed with treatment of luteal tissue by PGE_2 (Pratt et al., 1977). Actions of PGE_2 may be to antagonize those of $PGF_{2\alpha}$ either by the vasodilatory effects of PGE_2 or the stimulation of P_4 synthesis via increased levels of adeylate cyclase.

The role of oxytocin, if any, in maternal recognition of pregnancy remains unclear. Oxytocin causes the release of $PGF_{2\alpha}$ from the uterus at the end of the estrous cycle, resulting in luteal regression (Lamsa and McCraken, 1986). Oxytocin receptors within the uterus increase towards the end of the estrous cycle (Roberts et al., 1976). This occurs at approximately the same time as the initiation of oTP-1 secretion. It is possible that oTP-1 or endometrial protein production stimulated by oTP-1 may exert some effect on the oxytocin receptor, resulting in a disruption of the $PGF_{2\alpha}$ pulses necessary for the occurrence of luteal regression. Release of oxytocin from the ovary and its binding to receptors in the uterus appears to be necessary for the surge of $PGF_{2\alpha}$ released which ultimately results in CL regression. Perhaps oTP-1 inhibits synthesis of the oxytocin receptor within the uterus or oTP-1 may bind to the oxytocin receptor, thereby preventing oxytocin from binding it. Effects of conceptus proteins on oxytocin release or its receptors located in the uterus must be considered.

This could be accomplished via infusion of total CP and oTP-1 into cyclic ewes and measuring oxytocin and oxytocin receptors. Competitive binding assays using oTP-1 and oxytocin may be helpful in determining whether oTP-1 exerts its effects at the level of the oxytocin receptor. Direct measurement of $PGF_{2\alpha}$ and PGE_2 after infusion of total CP or oTP-1 via catheterization of the uterine vein and ovarian artery may also indicate what influence if any oTP-1 has on levels and/or release of the PG.

Analysis of Conceptus Culture Media

The supply of conceptus proteins available is limited due to many factors: ewes are seasonal breeders, therefore conceptuses can only be collected from September through March; the number of conceptuses which can be collected is limited by the number of surgeries a ewe can undergo and the number of eggs she ovulates; and length of time a conceptus can be kept in culture. The average length of time a conceptus has been left in culture ranged from 24-48 hours without media change or additional radiolabelled tracer (Godkin et al, 1982; Godkin et al., 1984a,b; Fincher et al., 1986). No qualitative difference in conceptus protein profile and reasonable incorporation of the radiolabelled tracer and protein concentration implies availability of conceptus proteins may be increased by changing the conceptus media at 24 hour intervals.

Qualitative analysis of conceptus proteins synthesized throughout incubation indicates that the general pattern of protein synthesis does not change after 24, 48, 72, or 96 hours of culture. Similar

incorporation of the radiolabelled tracer and total protein concentration were found for all incubation times. These results indicate that the incubation of conceptuses can be extended by changing the culture media at 24 hour intervals which results in increased production of the conceptus proteins by as much as four fold. Culture media change and extended incubation can be used to increase amount of conceptus proteins collected, thereby increasing experimentation which can be performed with these proteins.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Infusion of oTP-1 free CP into the uterine lumen of cyclic ewes from days 12-18, which is known to be the time of maternal recognition of pregnancy in the ewe, did not result in luteal maintenance and the continued production of P_4. All ewes had P_4 concentration < 1.0 ng/ml by day 16, indicating luteal regression. Visual evidence of luteal regression was seen on day 24 when india ink marked CL were observed and corpora albicans were found marked with the ink. The corpora hemorrhagica of new ovulations were also observed.

It is concluded that removal of oTP-1 from total CP results in the loss of the antiluteolytic properties of total CP which had been shown earlier to maintain luteal function in cyclic ewes for longer than 50 days. Failure of oTP-1 free CP to maintain luteal function strongly indicates that oTP-1 may be the necessary conceptus protein which elicits the maternal recognition of pregnancy in the ewe. Godkin et al. (1984b) hypothesized that the limited ability of oTP-1 to maintain luteal function when infused into the uterine lumen of cyclic ewes (3-5 days maintenance versus > 50 days with total conceptus secretory protein) may have been due to: the degradation of oTP-1 by uterine enzymes after infusion, a level of oTP-1 which was not concentrated enough to exert a biological response, or that oTP-1 may be only part of an antiluteolytic complex of conceptus proteins. Preliminary results indicate that infusion of oTP-1 with a carrier

protein to protect it from uterine degradation prolongs luteal function for an extended period of time (Bazer and Vallet, personal communication). Results of this study and those of Bazer and Vallet (personal communication) indicate that oTP-1 may be the conceptus protein signal for maternal recognition of pregnancy and not just a component of an antiluteolytic protein complex.

Qualitative analysis of the profile of conceptus proteins synthesized in vitro at various hours of incubation was conducted to determine if there were any major changes in proteins produced. Fluorography and 2D PAGE of samples of the various conceptus media indicate that there is no major difference in the profile of proteins produced. Incorporation of the radiolabelled tracer into conceptus proteins synthesized in vitro was determined and found to be 9.85% and 6.92% for 24 and 48 hours of culture, respectively. One culture each of 72 and 96 hour incubation medium exhibited incorporation of 13.18% and 11.87% of tracer. Total protein synthesized during the first 24 hours of culture was 256 µg/ml and 200 µg/ml for the second 24 hours. Total proteins produced by one culture each of 72 and 96 hour incubation medium were 205 µg/ml and 195 µg/ml, respectively. Incorporation of the radiolabelled tracer into the proteins synthesized was comparable at the later hours of culture to that of the first 24 hours of culture. Total protein concentration of later hour media was similar to that of the initial incubation, indicating the ongoing synthesis of conceptus proteins even after prolonged incubation. Based on results of qualitative analysis (2D-PAGE and fluorography) and quantitative analysis (percent

incorporation and concentration of total proteins synthesized) it is concluded that an efficient method for obtaining increased amounts of conceptus proteins of interest is to change the culture media at 24 hour intervals. Further analysis of the 72 and 96 hour incubation media is required, but preliminary results indicate that conceptuses can be cultured for such an extended length of time without losing their protein synthesizing abilities.

LITERATURE CITED

LITERATURE CITED

Amoroso, E. C. 1952. In: Marshall's Physiology of Reproduction, 3rd ed. Vol. 2 (Parker, A. S. ed.), Little Brown & Co., Boston, p. 127.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fert. 54:483.

Babcock, J. C. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. J. Reprod. Fert. (Suppl. 1):47.

Baird, D. T., and A. S. McNeilly. 1981. Gonadotrophic control of follicular development and function during the oestrous cycle of the ewe. J. Reprod. Fert. (Suppl. 30):119.

Barrett, S., M. A. de B. Blockey, J. M. Brown, I. A. Cumming, J. R. Goding, B. L. Mole, and J. M. Obst. 1971. Initiation of the oestrous cycle in the ewe by infusion of $PGF_{2\alpha}$ to the autotransplanted ovary. J. Reprod. Fert. 24:136.

Bartol, F. F., W. W. Thatcher, F. W. Bazer, F. A. Kimball, J. R. Chenault, C. J. Wilcox, and R. M. Roberts. 1981. Effects of the estrous cycle and early pregnancy on bovine uterine, luteal, and follicular responses. Biol. Reprod. 25:759.

Bazer, F. W., D. C. Sharp, W. W. Thatcher, and R. M. Roberts. 1981. Comparative approach to mechanisms in the maintenance of early pregnancy. In: Reproductive Processes and Contraception (McKerns, K. W., ed.), Plenum Press, New York, p. 581.

Bazer, F. W., J. L. Vallet, R. M. Roberts, D. C. Sharp, and W. W. Thatcher. 1986. Role of conceptus secretory products in establishment of pregnancy. J. Reprod. Fert. 76:841.

Bazer, F. W., and N. L. First. 1983. Pregnancy and parturition. J. Anim. Sci. 57(Suppl.2):425.

Bazer, F. W., and W. W. Thatcher. 1977. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin $F_{2\alpha}$ by the uterine endometrium. Prostaglandins 14:397.

Betteridge, K. J., M. D. Eaglesome, G.C.B. Randall, and D. Mitchell. 1980. Collection, description, and transfer of embryos from cattle 10-16 days after oestrus. J. Reprod. Fert. 59:205.

Bindon, B. M. 1971. Systematic study of preimplantation stages of pregnancy in the sheep. Aust. J. Biol. Sci. 24:131.

Bjersing, L., M. F. Hay, G. Kann, R. M. Short, F. Naftolin, R. J. Scaramuzzi, R. V. Short, and E. V. Younglai. 1972. Changes in gonadotrophins, ovarian steroids, and follicular morphology in sheep at oestrus. J. Endocr. 52:465.

Bruce, N. W., and R. M. Moor. 1976. Capillary blood flow to ovarian follicles, stroma, and corpora lutea of anesthetized sheep. J. Reprod. Fert. 46:299.

Buhr, M. M., J. C. Carlson, and J. E. Thompson. 1979. A new perspective on the mechanism of corpus luteum regression. Endocrinology 105:1330.

Butler, W. R., P. V. Malven, L. B. Willett, and D. J. Bolt. 1972. Patterns of pituitary release and cranial output of LH and prolactin in ovariectomized ewes. Endocrinology 91:793.

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

Committee on Reproductive Nomenclature. 1972. Recommendations for standardizing bovine reproductive terms. Cornell Vet. 62:216.

Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. 99: 167.

Ellinwood, W. E. 1978. Maternal recognition of pregnancy in the ewe and the rabbit. PhD dissertation. Colorado State University, Fort Collins.

Ellinwood, W. E., T. M. Nett, and G. D. Niswender. 1979a. Maintenance of the corpus luteum of early pregnancy in the ewe. I. Luteotrophic properties of embryonic homogenates. Biol. Reprod. 21:281.

Ellinwood, W. E., T. M. Nett, and G. D. Niswender. 1979b. Maintenance of the corpus luteum of early pregnancy in the ewe. II. Prostaglandin secretion by the endometrium *in vitro* and *in vivo*. Biol. Reprod. 21:845.

Fairclough, R. J., L. G. Moore, L. T. McGowan, A. J. Peterson, J. F. Smith, H. R. Tervit, and W. B. Watkins. 1980. Temporal relationships between plasma concentration of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ and neurophysin I/II around luteolysis in the sheep. Prostaglandins 20:199.

Fawcett, D. W., J. A. Long, and A. L. Jones. 1969. The ultrastructure of endocrine glands. Recent Prog. Horm. Res. 25:315.

Fincher, K. B., F. W. Bazer, P. J. Hansen, W. W. Thatcher, and R. M. Roberts. 1986. Proteins secreted by the sheep conceptus suppress induction of uterine prostaglandin $F_{2\alpha}$ release by oestradiol and oxytocin. J. Reprod. Fert. 76:425.

Findlay, J. K. 1981. Blastocyst-endometrial interactions in early pregnancy in the sheep. J. Reprod. Fert. (Suppl. 30):171.

Findlay, J. K., I. J. Clarke, J. Swaney, N. Colvin, and B. Doughton. 1982. Oestrogen receptors and protein synthesis in caruncular and intercaruncular endometrium of sheep before implantation. J. Reprod. Fert. 64:329.

Findlay, J. K., N. Ackland, R. D. Burton, A. J. Davis, F. M. Maule Walker, and R. B. Heap. 1981. Protein, prostaglandin, and steroid synthesis in caruncular and intercaruncular endometrium of sheep before implantation. J. Reprod. Fert. 62:361.

Fischer, H. E., F. W. Bazer, and M. J. Fields. 1985. Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in the swine. J. Reprod. Fert. 75:69.

Fitz, T. A., M. H. Mayan, H. R. Sawyer, and G. D. Niswender. 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod. 27:703.

Flint, A.P.F., and E. L. Sheldrick. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. J. Reprod. Fert. 67:215.

Ford, S. P. 1984. Maternal recognition of pregnancy in the ewe, cow, and sow: Vascular and immunological aspects. Theriogenology 23:145.

Ford, S. P., J. R. Chenault, and S. E. Echternkamp. 1979a. Uterine blood flow of cows during the oestrous cycle and early pregnancy: Effect on conceptus on the uterine blood supply. J. Reprod. Fert. 56:53.

Ford, S. P., R. K. Christenson, and J. R. Chenault. 1979b. Patterns of blood flow to the uterus and ovaries of ewes during the period of luteal regression. J. Anim. Sci. 49:1510.

Gadsby, J. E., R. B. Heap, and R. D. Burton. 1980. Oestrogen production by blastocyst and early embryonic tissue of various species. J. Reprod. Fert. 60:409.

Glass, J. D., T. A. Fitz, and G. D. Niswender. 1984. Cytosolic receptors for estradiol in the corpus luteum of the ewe: Variation throughout the estrous cycle and distribution between large and small steroidogenic cell types. Biol. Reprod. 31:367.

Godkin, J. D., F. W. Bazer, J. Moffatt, F. Sessions, and R. M. Roberts. 1982. Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. J. Reprod. Fert. 65:141.

Godkin, J. D., F. W. Bazer, and R. M. Roberts. 1984a. Ovine trophoblast protein 1, an early secreted blastocyst protein bind specifically to uterine endometrium and affects protein synthesis. Endocrinology 114:120.

Godkin, J. D., F. W. Bazer, W. W. Thatcher, and R. M. Roberts. 1984b. Proteins released by cultured day 15-16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. J. Reprod. Fert. 71:57.

Goding, J. R., F. A. Harrison, R. B. Heap, and J. L. Linzell. 1967. Ovarian activity in the ewe after autotransplantation of the ovary or uterus to the neck. J. Physiol. Lond. 191:129.

Goding, J. R. 1974. The demonstration that $PGF_{2\alpha}$ is the uterine luteolysin in the ewe. J. Reprod. Fert. 38:261.

Hanly, C. V., H. P. Voigtlander, A. L. Pope, L. E. Casida. 1961. Prenatal mortality in farm animals. J. Reprod. Fert. 2:182.

Hansel, W., P. W. Concannon, and J. H. Lukaszewska. 1973. Corpora lutea of large domestic animals. Biol. Reprod. 8:222.

Harrison, F. A., R. B. Heap, and J. L. Linzell. 1968. Ovarian function in the sheep after autotransplantation of the ovary and uterus to the neck. J. Endocr. 40:xiii.

Henderson, K. M., and K. P. McNatty. 1975. A biochemical hypothesis to explain the mechanism of luteal regression. Prostaglandins 9: 779.

Henderson, K. M., R. J. Scaramuzzi, and D. T. Baird. 1977. Simultaneous infusion of prostaglandin E_2 antagonizes the luteolytic action of prostaglandin $F_{2\alpha}$ *in vivo*. J. Endocr. 72:379.

Horst, M. N., and R. M. Roberts. 1979. Analysis of peptide turnover rates in Chinese Hamster ovary cells plasma membranes using two dimensional electrophoresis. J. Biol. Chem. 254:5000.

Horton, E. W., and N. L. Poyser. 1976. Uterine luteolytic hormone: A. physiological role for prostaglandin $F_{2\alpha}$. Physiol. Rev. 56: 595.

Hulet, C. V., H. P. Voigtlander, A. L. Pope, and L. E. Casida. 1956. The nature of early season infertility in sheep. J. Anim. Sci. 15:607.

Hulet, C. V., and M. Shelton. 1980. Sheep and goats. In: Reproduction in Farm Animals (Hafez, E.S.E., ed.), Lea & Febiger, Philadelphia, p. 346.

Inskeep, E. K., and R. L. Butcher. 1966. Local component of utero-ovarian relationships in the ewe. J. Anim. Sci. 25:1164.

King, G. J., B. A. Atkinson, and H. A. Robertson. 1982. Implantation and early placentation in domestic ungulates. J. Reprod. Fert. (Suppl.31):17.

Lacroix, M. C., and G. Kann. 1982. Comparative studies of prostaglandins $F_{2\alpha}$ and E_2 in late cyclic and early pregnant sheep: In vitro synthesis by endometrium and conceptus. Effect of in vivo indomethacin treatment on establishment of pregnancy. Prostaglandins 23:507.

Lamsa, J. C., and J. A. McCraken. 1985. The effect of estrogen on the pulse frequency of oxytocin release from the brain of ovariectomized sheep. Biol. Reprod. 32(Suppl.1):38 (Abstr).

Lamsa, J. C., and J. A. McCraken. 1986. Luteolytic pulses of $PGF_{2\alpha}$ secretion from the ovine uterus: Mechanisms regulating their frequency and triggering their release. Biol. Reprod. 34(Suppl.1): 208 (Abstr).

Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. 193:265.

Lowry, O. H., N. J. Rosenbrough, A. L. Fair, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.

Magness, R. R., J. M. Huie, and C. W. Weems. 1978. Effect of contralateral and ipsilateral intrauterine infusion of prostaglandin E_2 (PGE_2) on luteal function in the nonpregnant ewe. J. Anim. Sci. 47(Suppl.1):376.

Marcus, George J. 1981. Prostaglandin formation by the sheep embryo and endometrium as an indication of maternal recognition of pregnancy. Biol. Reprod. 25:26.

Martal, J., M. C. Lacroix, C. Loudes, M. M. Saunier, and S. Wintenberger-Torres. 1979. Trophoblastin, and antiluteolytic protein present in early pregnancy in sheep. J. Reprod. Fert. 56:63.

McCraken, J. A., J. C. Carlson, M. E. Glew, J. R. Goding, and D. T. Baird. 1972. Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. Nat. New Biol. 238:129.

McCraken, J. A., W. Schramm, B. Barcihowski, and L. Wilson, Jr. 1981. The identification of prostaglandin $F_{2\alpha}$ as a uterine luteolytic hormone and the hormonal control of its synthesis. Acta. Vet. Scand. 77:71.

McCraken, J. A., W. Schramm, W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31.

McNatty, K. P., K.J.A. Revfeim, and A. Young. 1973. Peripheral plasma progesterone concentration in sheep during the oestrous cycle. J. Endocr. 58:219.

Moor, R. M., and L.E.A. Rowson. 1966. The corpus luteum of the sheep: Functional relationship between the embryo and the corpus luteum. J. Endrocr. 34:233.

Nett, T. M., M. C. McClellan, and G. D. Niswender. 1976. Effects of prostaglandins on the ovine corpus luteum: Blood flow, secretion of progesterone and morphology. Biol. Reprod. 15: 66.

Niswender, G. D. 1973. Influence of the site of conjunction on specificity of antibodies to progesterone. Steroids 22:413.

Niswender, G. D., T. J. Reimers, M. A. Diekman, and T. M. Nett. Blood flow: A mediator of ovarian function. Biol. Reprod. 14: 64.

Niswender, G. D., R. H. Schwall, T. A. Fitz, C. E. Farin, and H. R. Sawyer. 1985. Regulation of luteal function in domestic ruminants: New concepts. Recent Prog. Horm. Res. 41:101.

Obst, J. M., R. F. Seamark, and J. M. Brown. 1971. Application of a competitive protein-binding assay for oestrogens to the study of ovarian function in sheep. J. Reprod. Fert. 24:140.

O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007.

Pant, H. C., C. Hopkinson, and R. J. Fitzpatrick. 1972. Plasma oestradiol, progesterone, and luteinizing hormone concentrations during the ovine estrus cycle. J. Reprod. Fert. 71:501.

Perry, J. S. 1981. The mammalian fetal membranes. J. Reprod. Fert. 62: 321.

Pratt, B. K., R. L. Butcher, and E. K. Inskeep. 1977. Antiluteolytic effect of the conceptus and of PGE_2 in ewes. J. Anim. Sci. 46: 784.

Reynolds, L. P., R. R. Magness, and S. P. Ford. 1982. Uterine blood flow and uterine arteriovenous steroid concentration on days 11, 13, and 15 of the estrous cycle and pregnancy in ewes. J. Anim. Sci. 55(Suppl.1):382 (Abstr).

Roberts, J. S., J. A. McCraken, J. E. Gavagan, and M. S. Soloff. 1976. Oxytocin stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: Correlation with estrus cycle and oxytocin receptor binding. Endocrinology 99:1107.

Rodgers, R. J., and J. D. O'Shea. 1982. Purification, morphology, and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. Aust. J. Biol. Sci. 35:441.

Rowson, L.E.A., and R. M. Moor. 1967. The influence of embryonic tissue homogenate infused into the uterus, on the life-span of the corpus luteum in the sheep. J. Reprod. Fert. 13:511.

Rowson, L.E.A., R. M. Moor, and R.A.S. Lawson. 1969. Fertility following egg transfer in the cow: Effect of method, medium and synchronization of oestrus. J. Reprod. Fert. 18:517.

Salamonsen, L. A., B. W. Doughton, and J. K. Findlay. 1986. The effects of the preimplantation blastocyst in vivo and in vitro on protein synthesis and secretion by cultured epithelial cells from sheep endometrium. Endocrinology 119:1192.

Sauer, M. J. 1979. Hormone involvement in the establishment of pregnancy. J. Reprod. Fert. 56:725.

Scaramuzzi, R. J., B. V. Caldwell, and R. M. Moor. 1970. Radioimmunoassay of LH and estrogen during the estrous cycle of the ewe. Biol. Reprod. 3:110.

Schramm, W., L. Bovaird, M. E. Glew, G. Schramm, and J. A. McCraken. 1983. Corpus luteum regression induced by ultra-low pulses of prostaglandin $F_{2\alpha}$. Prostaglandins 26:347.

Sheldrick, E. L., M. D. Mitchell, and A.P.F. Flint. 1980. Delayed luteal regression in ewes immunized against ocytocin. J. Reprod. Fert. 59:37.

Short, R. V. 1969. Implantation and the maternal recognition of pregnancy. In: Fetal Autonomy (Wolstenholme, G.W.W., and M. O'Connor, eds.), J & A Churchill Ltd., London, p. 2.

Sibley, D. R., and R. J. Lefkowitz. 1985. Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317:124.

Silvia, W. J., T. A. Fitz, M. H. Mayan, and G. D. Niswender. 1984. Cellular and molecular mechanisms involved in luteolysis and maternal recognition of pregnancy in the ewe. Anim. Reprod. Sci. 7:57.

Stabenfeldt, G. H., J. A. Holt, and L. L. Ewing. 1969. Peripheral plasma progesterone levels during the ovine estrous cycle. Endocrinology 85:11.

Steele R.G.D., and J. H. Torrie. 1960. In: Principles and Procedures of Statistics. McGraw-Hill, New York.

Thatcher, W. W., F. F. Bartol, J. J. Knickerbocker, J. S. Curl, D. Wolfenson, F. W. Bazer, and R. M. Roberts. 1984. Maternal recognition of pregnancy in cattle. J. Dairy Sci. 67:2797.

Thatcher, W. W., F. W. Bazer, D. C. Sharp, and R. M. Roberts. 1986. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: Sheep, cattle, pigs and horses. J. Anim. Sci. 62(Suppl.2):25.

Vallet, J. L., F. W. Bazer, and R. M. Roberts. 1985. Effect of ovine trophoblast protein-1 induced endometrial proteins on progesterone production by corpus luteum and cAMP response of endometrium to oxytocin. J. Anim. Sci. 61(Suppl.1):377 (Abstr).

Wathes, D. C., and R. W. Swann. 1982. Is oxytocin an ovarian hormone? Nature 297:225.

Wilmut, I., D. I. Sales, and C. J. Ashworth. 1985. The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. Theriogenology 23:107.

Wiltbank, J. N., and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134.

Yuthasastrakosal, P., W. M. Palmer, and B. E. Howland. 1975. Luteinizing hormone, oestrogen, and progesterone levels in peripheral serum of anoestrous and cyclic ewes as determined by radioimmunoassay. J. Reprod. Fert. 43:57.

Yuthasastrakosal, P., W. M. Palmer, and B. E. Howland. 1977. Release of LH in anoestrus and cyclic ewes. J. Reprod. Fert. 50:319.

Zarco, L., G. H. Stabenfeldt, H. Kindahl, G. E. Bradford, and S. Basu. 1984. A detailed study of prostaglandin $F_{2\alpha}$ release during luteolysis and establishment of pregnancy in the ewe. Biol. Reprod. 30(Suppl.1):153 (Abstr).

VITA

Linda Diane Smith, daughter of Carl and Kathryn Smith, was born and raised in Pittsfield, Massachusetts. She graduated from Taconic High School in Pittsfield in June 1980.

In September 1980, she entered the University of New Hampshire and received her Bachelor of Science degree in Animal Science in May 1984. Linda began studying for a Master of Science degree in Animal Science in September 1984. The degree was awarded in December 1986.