ARACHIDONIC ACID METABOLISM BY EARLY OVINE EMBRYOS AND THE
ROLE OF PROSTAGLANDINS IN ONE ASPECT OF
EMBRYONIC DEVELOPMENT

By

Brian L. Sayre

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

in

Animal Science
(Physiology of Reproduction)

APPROVED:

G.S. Lewis, Chairman

J.W. Knight

R.G. Saacke

December, 1991

Blacksburg, Virginia
ARACHIDONIC ACID METABOLISM BY EARLY OVINE EMBRYOS AND THE
ROLE OF PROSTAGLANDINS IN ONE ASPECT OF
EMBRYONIC DEVELOPMENT

Brian L. Sayre

(ABSTRACT)

Most embryonal mortality occurs during early embryonic
development. Two experiments were designed to study
aspects of early embryonic development. Experiment 1
was to determine if early ovine embryos were capable of
metabolizing arachidonic acid. Cyclic ewes were
estrous synchronized with 6α-methyl-17β-hydroxy
progesterone acetate (MPA) pessaries, superovulated
with follicle stimulating hormone (FSH) and bred
artificially. Embryos were collected on d 4, 8, 10, 12
or 14 of pregnancy and incubated with 1 μCi of [14C]
arachidonic acid in an atmosphere of 5% CO2, 45% O2 and
50% N at 37°C for 24 h. Embryos from all days of
pregnancy metabolized arachidonic acid to a number of
compounds. Embryos produced primarily an unidentified
polar compound, 6-keto-prostaglandin F1α (6-keto-
PGF1α), prostaglandin F2α (PGF2α), prostaglandin E2
(PGE2), 13,14-dihydro-15-keto prostaglandin F2α (PGFM),
prostaglandin B2 (PGB2) and 12L-hydroxy-5,8,10-
heptadecatrienoic acid (HHT). Experiment 2 was to
determine whether prostaglandins have a role in embryo hatching from the zona pellucida. Ewes were superovulated and bred artificially, and embryos were collected on d 7 of pregnancy. Embryos were incubated with ethanol (control), indomethacin, PGE\(_2\) or indomethacin and PGE\(_2\) in an atmosphere of 5% CO\(_2\) and 95% air at 37°C for 24 h. Indomethacin appeared to decrease embryo hatching rate (indomethacin, 34.5% vs control, 46.4%). Prostaglandin E\(_2\) appeared to increase embryo hatching rate (PGE\(_2\), 60.0% vs. control, 46.4%). However, hatching rates for indomethacin and PGE\(_2\) treatment groups were not different from control (P > .05). When compared to any group with indomethacin treatment, PGE\(_2\) increased (P < .05) embryo hatching rate. The results of this study indicated that early ovine embryos can convert arachidonic acid to various compounds in vitro. Although not conclusive, indomethacin may decrease and PGE\(_2\) may increase embryo hatching rate. Therefore, embryo-produced prostaglandins may be involved in hatching of sheep embryos from the zona pellucida.
ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks and appreciation to all the following:

Dr. G. S. Lewis, committee chairman and advisor, for all his patience, advice and encouragement as I worked through all my setbacks.

Drs. J. W. Knight and R. G. Saacke for their insights and willingness to serve on the graduate committee.

Lee Johnson for his patience and all of the help that he supplied on short notice.

Suyapa Fortin for all of her assistance in preparing the surgeries and the moral support and stress relief contributions.

Stacey Wilson for all of her moral support and stress relief contributions.

Wimpy Williams and Phil Keefer for all of their help in maintaining the ewes and for putting up with me.

Kathy Smith for her help in surgery and in laboratory procedures.

Sean Bowman, Michelle Daigneau and all of the people that assisted with preparing my surgeries.
Drs. D. L. Thomas, T. R. Carr and D. F. Parrett their for encouragement and advice to convince me to enter graduate studies. Most of all, Mom and Dad for all the patience and encouragement they have given through it all.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statement of the problem</td>
<td>1</td>
</tr>
<tr>
<td>Review of literature</td>
<td>3</td>
</tr>
<tr>
<td>Embryonal mortality</td>
<td>3</td>
</tr>
<tr>
<td>Early embryonic development</td>
<td>15</td>
</tr>
<tr>
<td>Prostaglandin production</td>
<td>22</td>
</tr>
<tr>
<td>Embryonal prostaglandin production</td>
<td>27</td>
</tr>
<tr>
<td>Shedding the zona pellucida</td>
<td>30</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>40</td>
</tr>
<tr>
<td>General</td>
<td>40</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>45</td>
</tr>
<tr>
<td>Day-4, -8, and -10 embryos</td>
<td>46</td>
</tr>
<tr>
<td>Day-12 and -14 embryos</td>
<td>46</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>49</td>
</tr>
<tr>
<td>Statistical analyses</td>
<td>52</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>52</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>52</td>
</tr>
<tr>
<td>Results</td>
<td>54</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>54</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>55</td>
</tr>
<tr>
<td>Discussion</td>
<td>69</td>
</tr>
<tr>
<td>Implications</td>
<td>74</td>
</tr>
<tr>
<td>Literature cited</td>
<td>75</td>
</tr>
<tr>
<td>Appendix I</td>
<td>93</td>
</tr>
<tr>
<td>Vita</td>
<td>94</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Arithmetic means of amounts of various compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>57</td>
</tr>
<tr>
<td>2.</td>
<td>Means of log transformed data for amounts of various compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>58</td>
</tr>
<tr>
<td>3.</td>
<td>Arithmetic means of amounts of major unknown compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>59</td>
</tr>
<tr>
<td>4.</td>
<td>Means of log transformed data for amounts of major unknown compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>60</td>
</tr>
<tr>
<td>5.</td>
<td>Arithmetic means of amounts of minor unknown compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>61</td>
</tr>
<tr>
<td>6.</td>
<td>Means of log transformed data for amounts of minor unknown compounds in media after embryos were incubated with $\text{\textsuperscript{14}C}$ arachidonic acid for 24 h.</td>
<td>62</td>
</tr>
<tr>
<td>7.</td>
<td>Embryo hatching percentages after incubation with indomethacin and PGE$_2$ for 6 days at 37°C in an atmosphere of 5% CO$_2$ and 95% air in modified Ham's F-10 media.</td>
<td>62</td>
</tr>
<tr>
<td>8.</td>
<td>Contrasts of maximum-likelihood estimates in CATMOD in SAS.</td>
<td>63</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

1. Pathways of arachidonic acid metabolism..............26

2. Chromatographic profile of arachidonic acid and its metabolites. Peaks are identified, from left to right, as 6-keto-PGF$_1$α, PGF$_2$α, PGE$_2$, PGFM, PGB$_2$, and arachidonic acid (----). Gradient profile of solvent B for separation of arachidonic acid metabolites (---)..............................50

3. Reversed-phase HPLC chromatogram representative of d-4 embryonal metabolism of [14C] arachidonic acid.......................................................64

4. Reversed-phase HPLC chromatogram representative of d-8 embryonal metabolism of [14C] arachidonic acid..........................................................65

5. Reversed-phase HPLC chromatogram representative of d-10 embryonal metabolism of [14C] arachidonic acid.......................................................66

6. Reversed-phase HPLC chromatogram representative of d-12 embryonal metabolism of [14C] arachidonic acid.......................................................67

7. Reversed-phase HPLC chromatogram representative of d-14 embryonal metabolism of [14C] arachidonic acid.......................................................68
STATEMENT OF THE PROBLEM

Because of embryonal mortality, livestock producers are hindered financially by losses of potential calves, lambs and pigs. Embryonal mortality may be as high as 40% in cattle, sheep and pigs (Edey, 1969; Ayalon, 1978; Pope and First, 1985). Therefore, 40% of the potential calf and lamb crops may be lost each breeding season, dramatically reducing the profit opportunities of producers. Calving rate, in studies with about 25,000 cows, was only 47% after one artificial insemination (Spalding et al., 1975; Pelissier, 1976). Fertilization rates are expected to be around 90%; therefore, the decrease in calving rate is due primarily to embryonal deaths (Hawk, 1979).

Embryonal mortality can be caused by many environmental and genetic factors. Environmental factors do not appear to be responsible for a large percentage of embryonal deaths, and many can be controlled by producers. Genetic factors and the lack of a proper uterine environment may be the main causes of embryonal deaths.

Early embryonic development is often the critical period for embryonal mortality. The factors involved directly in losses of early embryos are unclear. Although genetic factors are certainly an important cause of embryonal deaths, but they are difficult to control and may
effectively remove undesirable genetics from a population. Therefore, relationships between the uterine environment and the developing embryo are thought to be the most important, and perhaps controllable, factors affecting embryonal deaths.

Edey (1969) explained that, "until we can define the conditions in the normal uterus, we are in no position to detect the abnormal". Similarly, until one can understand normal embryonic development, one cannot accurately define embryonal mortality. The purpose of the studies described in this thesis was to focus on one embryonic developmental stage, shedding of the zona pellucida, and the role of prostaglandins in that process. First, one must determine if early ovine embryos have the capacity to produce prostaglandins. After determining embryonal arachidonic acid metabolism and the specific prostaglandins produced, then those specific prostaglandins can be used in embryo hatching experiments.
REVIEW OF LITERATURE

Embryonal Mortality

Ayalon (1978) defined embryonal mortality as "fertility losses during the embryonic period". The embryonic period is often referred to as the period between fertilization and attachment of the conceptus to the endometrium (Robinson, 1951; Averill, 1955; Moore et al., 1960; Quinlivan et al., 1966). One can further break down the embryonic period into the pre-hatching, hatching, maintenance of the corpus luteum and attachment periods.

In cattle, fertilization rates of approximately 90% are generally expected, but calving rates may be only 50% after a single insemination (Laing, 1949; Kidder et al., 1954; Bearden et al., 1956; Hendricks et al., 1971; Spalding et al., 1975; Pelissier, 1976; Hawk, 1979). Hendricks et al. (1971) reported a fertilization rate of 89%, but only 60% of embryos survived to d 42. High percentages of oocytes fertilized do not continue development to parturition.

Early embryonic development may be the most critical time for embryonal mortality. In sheep, the highest percentage of embryonal mortality is within the first month of pregnancy (Quinlivan et al., 1966; Edey, 1969).Diskin and Sreenan (1980) reported that most embryos die between d
8 and 12 in cattle. Ayalon (1978) suggested that d 7 is the most critical period for embryonal mortality. Embryonal losses are due to a variety of factors, such as environmental factors, asynchrony between embryo and uterine environment, genetic abnormalities and abnormal embryonic development (Edey, 1969; Ayalon, 1978).

Hanley (1961) suggested that even if all environmental factors could be controlled, embryonal mortality would continue; it would be caused by an "unknown factor". Bishop (1964) proposed that Hanley's unknown factor could be categorized as genetic abnormalities, such as lethal mutations and chromosomal aberrations. He considered these losses inevitable and a necessary means of removing unwanted genetics. Inherited defects, errors at fertilization or a direct effect of the environment on the embryo may cause embryos to be abnormal. Studies during early pregnancy in sheep (Long & Williams, 1980), cattle (McFeely & Rajakoski, 1968; Gayerie de Abreu et al., 1984) and pigs (McFeely, 1967) indicated that the incidence of abnormalities was 14.6%, 7.5% and 10%, respectively. However, other studies indicate a lower incidence of abnormalities for sheep (0%; Long, 1977) and cattle (1.9%; Hare et al., 1980) embryos. Mutations affecting prenatal development have been found in mice. For example, mouse embryos homozygous for the yellow allele at the agouti locus die during the late blastocyst
stage (Pedersen, 1974). Similar mutations may be present in livestock species, but they are undefined. Although, Long and Williams (1980) reported that 6% of sheep embryos contained chromosomal abnormalities. Genetic abnormalities cause embryonal mortality, but they are difficult to define and control, and they may effectively remove undesirable genetics from a population.

Environmental conditions have been the most widely studied causes of embryonal mortality. Following a review of the literature, Edey (1969) and Ayalon (1978) determined that breed, age and parity have no demonstrable effect on embryonal mortality in sheep and cattle. However, ewes bred at less than 2 yr of age tended to have lower embryonal survival (Edgar, 1962). Edgar (1962) found 10% embryonal mortality in 1.5 yr-old ewes compared to 0% in mature ewes. In contrast, Mullaney (1966) reported no difference in rates of embryonal mortality between 2 yr-old maiden ewes and mature ewes, 23.7 vs. 22.8%, respectively. A possible effect of age on embryonal mortality is implied, but the evidence is unclear.

Average environmental temperature changes do not appear to affect embryonal mortality. However, extremely high temperatures during the first 35 days of pregnancy may increase embryonal deaths in cattle (Ayalon, 1978). Beef heifers exposed to 32°C for 72 h immediately after mating
had reduced embryonal survival rates; none of the treated heifers became pregnant compared to 48% of heifers maintained at 21°C (Dunlap and Vincent, 1971). In sheep, constant elevated temperatures during early pregnancy can increase embryonal mortality (Yeates, 1953; Dutt et al., 1956). Continuous exposure to thermal stress that raises body temperature 1.5° to 3.0°C above normal will dramatically increase embryonal deaths (Thwaites, 1967). The embryo is most vulnerable to thermal stress during the pre-hatching period, especially the early cleavage stages of development (Dutt, 1963; Thwaites, 1967). In addition, embryonal survival in ewes is reduced by cold temperatures at mating and during the first three weeks of pregnancy (Griffiths et al., 1970; Doney et al., 1976). A response similar to heat stress was found during a general stress, reflected by a period of increased ACTH concentrations (Doney et al., 1976). Embryonal mortality was also increased from 17.2 to 37.7% when ACTH was injected daily for the first 20 days of pregnancy in sheep (Doney et al., 1976). Extreme temperature change affects embryonal mortality rate by changing body temperature or causing a generalized stress response.

Time of mating during the breeding season may affect embryonal mortality (Edey, 1969). Hulet et al. (1956) reported embryonal mortality rates of 28.6% in ewes bred
early in the breeding season and only 9.9% in those bred late in the season; however, time of the breeding season and ambient temperature may have been confounded. Wilmut et al. (1986) reported a higher embryonal mortality rate in ewes bred in March than in ewes bred in September or November. In addition, fertilization of aged ova in cattle is associated with high embryonal mortality (Hawk, 1979). In heifers bred 6 to 20 h after ovulation, 50% had cleaved embryos at 2 to 4 d after estrus, but only 22% had embryos at d 21 to 35 (Casida, 1950). Apparently the optimum breeding period for reducing embryonal mortality is during the middle of the breeding season and near ovulation.

The effects of level of nutrition on embryonal mortality has been studied extensively. In cattle and pigs, no clear effect of feeding level has been found. Some reports indicate a possible adverse effect of a high plane of nutrition on fertility in heifers (Joubert, 1954). However, Reid et al. (1964) stressed that it was not known whether fertilization or embryonal survival was affected by feeding level. In a more controlled experiment that accounted for fertilization rate, Hill et al. (1970) reported that decreased energy and protein in the diet reduced fertilization rate, but the diet had no clear effect on embryonal mortality. After mating gilts, an increase in feed levels increased embryonal mortality, while fasting
improved embryonal survival (Rattray, 1977). These responses were not found in sows (Rattray, 1977). In sheep, underfed ewes have increased embryonal deaths. Young ewes underfed during the first 90 days of pregnancy lost 24% of their initial body weight while controls gained 15%. Underfed ewes had fewer lambs born per ewe mated than did controls, 6/32 vs. 21/32, respectively (Bennett et al., 1964). In two studies, mature ewes were underfed for 7 days during the first 20 days of pregnancy. Results indicated an overall increase in prenatal mortality of 16% compared to controls (Edey, 1965, 1966). The time of embryonal deaths is unclear, but these data indicate that animals need an intermediate level of nutrition for optimal embryonal survival.

In addition to feeding levels, embryonal mortality may be affected by changes in feed constituents. Sheep grazing plants containing compounds with estrogenic activities have increased embryonal mortality (Jainudeen and Hafez, 1987). Also, selenium and vitamin E deficiency may affect embryonal mortality. Hartley (1963) reported that the high incidence of barren ewes in selenium deficient areas of New Zealand could be lowered with an injection of selenium. Vitamin E administration before d 89 of pregnancy had a favorable effect on lambing percentage (Boyazoglu et al., 1967).
Nutrition can affect embryonal mortality, but nutrition can be controlled in most livestock operations.

The previous paragraphs contain an overview of classical studies of losses due to embryonal mortality. Those studies were important for indicating to producers the need for controlling the environment of their livestock to maximize embryonal survival, but these studies often did not determine the stage of development at which embryonal losses occurred.

Although the possibility exists that embryonal mortality may be due to maternal inadequacies for supporting pregnancy (Wilmut et al., 1986), most embryonal deaths occur in cattle between d 8 and 16, which is during the hatching and maintenance of pregnancy periods (Diskin and Sreenan, 1980). This may indicate that embryonal inadequacies are a greater contributor to embryonal mortality than are maternal inadequacies. Cole (1967) found that if mouse embryos did not hatch from the zona pellucida, implantation could not occur. At d 12, some cattle embryos were found hatched, but they had failed to expand (Diskin and Sreenan, 1980). A critical period of embryonal survival appears to be the late blastocyst stage (Jainudeen and Hafez, 1987).

In sheep and pigs, there may be a relationship between ovulation rate and embryonal losses (Edey, 1969; Xie et al., 1990). Embryonal mortality was 34.2% in 174 twin-ovulating
ewes and 23.6% in 267 single-ovulating ewes (Casida et al., 1966). In sheep with increased ovulation rates, there are increases in embryonal losses (Edey, 1969). Later ovulating ova resulted in smaller blastocysts by d 11 (Pope et al., 1988) and d 12 (Xie et al., 1990) in pigs. The time of ovulation of each ovum that develops into an embryo may result in differences in development of individual embryos, which may result in the loss of lesser developed embryos.

Asynchrony between embryo and uterine environment may be the main cause embryonal mortality (Dickmann and Noyes, 1960; Adams 1971; Wilmut and Sales, 1981). Embryonal mortality may be caused by embryos that have not developed in synchrony with changes in uterine environment during the early stages of the luteal phase (Wilmut et al., 1986). Initially, embryonic development is relatively independent of the uterine environment, but by the second week of development, an appropriate uterine environment is required for proper embryonic development (Wilmut et al., 1985). An association between progesterone profiles and embryonal survival has been reported (Ashworth et al., 1984). In pigs, it appears that a threshold number of embryos, about 4, must be present in the uterus to maintain progesterone concentrations necessary for pregnancy to continue (Webel et al., 1975). Parr et al. (1982) found a dose-dependant effect of progesterone on embryonal survival following
embryo transfer to ovariectomized ewes. Fox et al. (1989) reported that exogenous progesterone treatment can improve embryonal survival rates after asynchronous embryo transfer in cattle. However, Diskin and Niswender (1989) reported no effect of exogenous progesterone administration to intact ewes on embryonal survival rates. Ovarian steroids appear to control a sequence of uterine secretions that is required for normal embryonic development (Miller and Moore, 1976; Wilmut and Sales, 1981). In addition, asynchrony between embryo and uterine hormonal environment can cause abnormalities (Dickmann and Noyes, 1960; Adams, 1971; Wilmut and Sales, 1981; Lawson et al., 1983).

Estrogen-progesterone imbalances can lead to accelerated or delayed transport of the egg and increase preimplantation deaths (Jainudeen and Hafez, 1987). Gates (1965) reported that cleavage rate of mouse embryos can affect embryonal mortality. He compared slow-cleaving and fast-cleaving embryos and found no difference in the proportion that implanted, but a higher percentage of the slow-cleaving embryos died after implantation (Gates, 1965).

In pigs, early embryonic development varies within a litter, and the more advanced embryos are more likely to survive (Ford et al., 1982a; Geisert et al., 1982a; Xie et al., 1989; Pusateri et al., 1990). Pope and First (1985) reported that d-7 porcine embryos were more likely to
survive when transferred to a d-6 recipient than were d-6 embryos transferred to a d-7 recipient. Early embryonal mortality in pigs may be related to estrogen production by the elongating embryo (Pope, 1988; Pusateri et al., 1990). Porcine embryos produce estrogens during the development from a spherical form through elongation (Pope, 1988; Pusateri et al., 1990). Filamentous embryos do not appear to produce as much estrogen as do earlier stage embryos; thus the estrogen profile decreases after elongation of the embryo. The more advanced embryos begin producing estrogens and elongating earlier, which may reduce the survivability of the less advanced embryos.

Porcine embryonal estrogens appear to be important in embryonal spacing (Pope et al., 1982) within the uterus and maintenance of the corpus luteum (Ford et al., 1982b; Geisert et al., 1982b). Morgan et al. (1987) and Gries et al. (1989) have shown that d 9 and 10 embryos will elongate but will not attach to an estrogen-altered environment. Advanced embryos may affect their littermates by secreting estrogens earlier and not allowing proper spacing within the uterus or attachment to the uterus by less advanced embryos. Dzuik (1985) suggested that embryos can create a space for development by influencing uterine contractions and repelling other embryos from that area. Polge and Dzuik (1970) reported that filamentous embryos were unable to
migrate in the pig uterus. In sheep, embryonal migration also appears to occur during the elongation stage of development (Nephew et al., 1989) and when estrogen production by the ovine embryo begins (Nephew et al., 1989a). In addition, progesterone does not appear to be involved in embryo migration (Nephew et al., 1988b). Recognition of pregnancy appears to occur before ovine embryonal migration (Nephew et al., 1989). After multiple ovulations, more advanced embryos may create an environment that is unsuitable for less developed embryos. The changing environment may restrict migration and spacing by less developed embryos within the uterus, which may increase embryonal deaths.

An underdeveloped embryo may not be able to overcome the luteolytic effect of the uterus (Jainudeen and Hafez, 1987). If the embryo has not developed fast enough, then the corpus luteum may regress at the normal time of the cycle, resulting in expulsion of the embryo. However, if embryonic development is too much ahead of the uterine environment, then attachment may not be possible (Wilmut et al., 1986). Thus, the question is: Does the embryo die and allow subsequent regression of corpora lutea (CL) or does CL regression cause embryos to be lost (Hawk, 1979)? Embryonal death appears to precede CL regression (Jainudeen and Hafez, 1987), but the time sequences are still controversial. The
maintenance of the corpus luteum during pregnancy is controlled by the embryo (Bazer et al., 1986; Bazer et al., 1989; Stewart et al., 1989; Thatcher et al., 1989). The early embryos of livestock secrete various compounds between d 12 and 24, which may be involved in maintenance of the corpus luteum by the dam (Bazer et al., 1986; Bazer et al., 1989; Hansel et al., 1989). The possible mechanism of corpus luteum maintenance will be discussed in detail later, but failure of secretion of the embryonal signal or recognition by the dam could lead to increased embryonal mortality.

Some recent evidence indicates a possible effect of the male on subsequent embryonic development. Fukui et al. (1988) reported that different rams can affect fertilization rate, percentage of polyspermy and cleavage rate of the developing embryo. This could cause some embryos to be less advanced during the critical periods of development and increase embryonal deaths.

In summary, the exact causes of embryonal mortality are unknown. However, embryonal mortality is affected by the external environment, the uterine environment and genetic factors. In sheep and cattle, embryonal deaths occur during the first month of pregnancy (Quinlivan et al., 1966; Edey, 1969; Ayalon, 1978) In pigs, most embryonal losses are between d 12 and 17, but some fetal losses occur after d 60
(Knight et al., 1977). An important relationship exists between the embryo and the uterine environment for development and survival. External environmental factors can often be controlled and generally are not a major factor in embryonal mortality. Genetic factors are difficult to control and not well understood. These genetic factors may effectively remove undesirable genetics from a population. For a better understanding of embryonal mortality, one must first develop a better understanding of normal embryonic development.

Early Embryonic Development

Embryonic development begins at fertilization, but some causes of embryonal mortality may occur during development of the ovum. The first meiotic division begins during prenatal development and stops during prophase near the time of birth. The first meiotic division continues when the follicle is recruited for ovulation. The second meiotic division is not completed until fertilization occurs (Davies and Hesseldahl, 1971; Anderson, 1977). The meiotic divisions are when most genetic abnormalities can occur. The delay from the initiation of the second meiotic division to completion at fertilization can increase developmental complications (Hawk, 1979).
Fertilization occurs in the oviducts at the ampullary-isthmic junction. The embryo develops through the first stages in the oviduct and enters the uterus around d 4 in cattle and sheep. In sheep, the 2-celled stage was found at 39 h after fertilization (Green and Winters, 1945). Green and Winters (1945) flushed both 4- and 8-celled embryos from the uterus at 42 h; however, their flushing procedures were not well defined, and they probably flushed the oviducts and uterus. Blastomeres of the 2- to 8-celled stages have been found to each have the ability to develop into a new embryo (Bazer et al., 1987). These cells are said to be totipotent (Bazer et al., 1987). Tight junctions begin to form between the blastomeres of the 8-celled embryo (Ducibella, 1977). These tight junctions are thought to seal the blastocoele and allow the active transport of ions into the blastocoele (Bazer et al., 1987). The 16-celled stage appeared at around 77 h (Green and Winters, 1945). After the 16-celled stage, the embryo appeared as a solid ball of cells, called a morula (Anderson, 1977). Development to the 16-celled stage is called cleavage. During cleavage, the embryo does not increase in mass, only made divisions, which increases cell number within the mass (Anderson, 1977; Bazer et al., 1987). Embryo mass decreases during cleavage, but the size of nuclei increases (McLaren, 1974; Bazer et al., 1987). There is little increase in metabolic rate during cleavage,
but it increases rapidly between morula and blastocyst stages (Bazer et al., 1987).

Following the morula stage, embryos develop a cavity called a blastocoele, resulting in formation of the blastocyst (Anderson, 1977). Green and Winters (1945) first visualized the blastocoele on d 6 post coitum in sheep. Two cell types are found in the blastocyst: the outer layer of cells, called the trophoblast, and an inner-cell mass, which becomes the embryo proper (Anderson, 1977; Bazer et al., 1987). The inner cell mass develops into three primary germ layers, ectoderm, mesoderm and endoderm (Bazer et al., 1987). Differentiation of cells and the development of the blastocoele indicates a change in embryonic development.

The blastocyst increases fluid accumulation within the blastocoele and undergoes a series of contractions and expansions (Anderson, 1977). The contractions and expansions are thought to be involved in the mechanism of shedding of the zona pellucida, called hatching (Biggers et al., 1978). In sheep, hatching occurs on about d 8 (Green and Winters, 1945). After escaping from the zona pellucida, embryos continue rapid fluid accumulation and growth (Anderson, 1977). In cattle, blastocysts change from a 3 mm spherical shape on d 13 to a 25 mm filamentous shape on d 17, and by d 18, they have expanded into the contralateral uterine horn (Bazer et al., 1987).
During the pre-hatching and hatching periods porcine embryos migrate from the tip of the uterine horns to all parts of the uterus. This process continues until around d 12 (Dhindsa et al., 1967) and appears to require a progesterone-dominant uterine environment (Day and Polge, 1968). The number of embryos migrating does not appear to influence rate of movement (Dziuk et al., 1964). In addition, embryos do not appear to influence each other during migration (Dziuk et al., 1964).

Maintenance of the corpus luteum appears to be controlled by the conceptus. The critical time of the conceptus signal is around d 12 in sheep (Bazer et al., 1987) and pigs (Dzuik, 1985) and d 15 in cattle (Betteridge et al., 1980). Porcine embryos are actively secreting estrogens at d 12 (Pope, 1988; Pusateri et al., 1990), and estrogens can extend the life of the corpus luteum for several weeks (Ford et al., 1982b; Geisert et al., 1982b). This is evidence that embryonal estrogen production may be the factor involved in maintenance of the corpus luteum in pigs. Prostaglandins appear to be the luteolysin in pigs, and it has been postulated that conceptus estrogens change the ratio of uterine venous to uterine luminal prostaglandin secretion (for review see, Bazer et al., 1986).

In addition to steroids, embryos may secrete proteins that may be a signal for maintenance of the corpus lutea by
the mother. The developing bovine and ovine conceptus secretes many proteins, one of which, [sheep: ovine trophoblastin protein-1 (oTP-1); cattle: bovine trophoblastin protein-1 (bTP-1); Rowson and Moor, 1967; Martal et al., 1979; Godkin et al., 1982], has been implicated in maintenance of the corpus luteum. The protein has been shown to delay luteal regression in cyclic ewes after intrauterine administration (Godkin et al., 1984a). The conceptus protein has been reported to bind to endometrial receptors (Godkin et al., 1984b). As reviewed by Bazer et al. (1989), oTP-1 does not stimulate progesterone production, and they implied that oTP-1 inhibited the oxytocin-induced secretion of PGF₂α. Bazer et al. (1989) suggested that oTP-1 is the compound responsible for maintenance of the corpus luteum in sheep. In contrast, Stewart et al. (1989) reported only marginal success in extending luteal life with oTP-1, and oTP-1 did not inhibit the cyclic increase in oxytocin receptor levels, as is seen during pregnancy. Therefore, Stewart et al. (1989) suggest that oTP-1 may not be the only conceptus signal required for maintenance of the corpus luteum. The literature indicates a similar role for bTP-1 for maintenance of pregnancy in cattle (Thatcher et al., 1989).

In addition to proteins, conceptuses may secrete platelet-activating factor (PAF), which may have a role in
maintenance of pregnancy (Hansel et al., 1989). Platelets activated by PAF can release serotonin (Battista and Condon, 1986) and prostaglandins (Habenicht et al., 1985). Serotonin has been reported to increase progesterone secretion by bovine luteal cells (Battista and Condon, 1986), and Hansel and Dowd (1986) have shown that PGE\textsubscript{2} and PGI\textsubscript{2} have luteotrophic effects on bovine luteal cells. Although not conclusive, this evidence indicates that the conceptus is probably secreting a compound or series of compounds that are necessary for the maintenance of the corpus luteum and continuation of pregnancy.

In cattle and sheep, the embryo begins attachment to the uterus on about d 15 (Eckstein and Kelly, 1977). Implantation in livestock is a superficial and noninvasive process (King et al., 1982). Therefore, most authors avoid using the word, implantation, to describe the process in ruminants. In ruminants, attachment involves uterine caruncular and intracaruncular epithelium (Bazer et al., 1987). Attachment begins with a loose apposition of trophoblastic microvilli and uterine glands. The trophoblast temporarily loses microvilli in specific areas. This is followed by a close apposition of uterine epithelium to the trophectoderm and redevelopment of trophoblastic microvilli, which redevelop into the uterine epithelium (Bazer et al., 1987). Around d 15, the embryo and the
uterus develop a close appositional relationship, which is essential for continued embryonic development.

To summarize, the embryonic period is usually considered as the period between fertilization and attachment. Embryonic development begins at fertilization with the union of sperm and ovum and the conclusion of meiosis. The newly formed embryo consists of one diploid cell. The early stages of development, termed cleavage, are mitotic divisions within the embryo. During cleavage, the number of cells increases within the embryo without increasing the mass of the embryo. After the morula stage, a cavity develops within the mass of cells, and the cavity is called the blastocoele. Blastocoele formation begins the blastocyst stage. After a period of growth, the blastocyst becomes restricted by the zona pellucida. The embryo must escape from the zona pellucida for growth to continue. After escaping from the zona pellucida, the blastocyst expands and elongates rapidly. Before the end of the period of a normal cyclic corpus luteum, the embryo begins to attach to the uterine epithelium and communicates with the maternal system for maintenance of pregnancy.
Prostaglandin Production

The classical definition of a hormone is "a chemical substance produced in one part of the body that diffuses or is transported to another area, where it influences activity and tends to integrate component parts of the organism" (Baylis and Starling, 1902). Hormones, in the classical sense, are generally thought to be localized in production and action. Prostaglandins are compounds that fit only part of that definition. Prostaglandins are produced by a cell, but they may affect the same cell or another target cell. The main deviation from the classical definition is that prostaglandins are not localized in production, but in fact, are produced by most cells (Tepperman and Tepperman, 1987). Prostaglandins are also metabolized quite rapidly and are involved in many physiological systems; thus, they are often difficult to study. Prostaglandins are involved in circulatory functions (McGiff, 1974), platelet aggregation (Hamberg et al., 1974; Malmsten et al., 1975), kidney functions (McGiff, 1981; Gerber et al., 1982) and a variety of other nonreproductive functions. In addition, prostaglandins are involved in numerous reproductive functions, such as uterine contractions (Pope et al., 1982), corpus luteum regression (McCcracken et al., 1970; Inskeep
and Murdoch, 1980), embryo hatching (Biggers et al., 1978) and maternal-embryo relationships (Hoos and Hoffman, 1983; Pakrasi et al., 1985).

Prostaglandins are a family of lipid substances derived from arachidonic acid, and they have potent and diverse physiological and pharmacological actions (van Dorp et al., 1964; Kelly, 1981). Kurzrok and Lieb (1930) reported that fresh semen could elicit a strong contraction or relaxation of the uterus. During the early 1930's, von Euler and Goldblatt discovered independently that compounds in the seminal fluid and vesicular gland caused the uterine responses. The compounds were called prostaglandins. Thirty years later, Bergström, Samuelsson and co-workers determined the unique chemical structures of some of the prostaglandins (Bergström and Sjövall, 1960; Bergström et al., 1962; Samuelsson, 1963).

Arachidonic acid, a 20 carbon chain with 4 double bonds (20:4), is formed from the essential fatty acid, linoleate, a 18 carbon chain with 2 double bonds (18:2), by elongation and desaturation (Lands, 1979). Arachidonic acid is found mainly in phospholipids and triglycerides, and is released by phospholipase A₂, a process that requires Ca²⁺, and lipase, respectively (Figure 1; Lands, 1979; Tepperman and Tepperman, 1987). Free arachidonic acid concentration in unstimulated cells is low, and the phospholipase A₂ reaction
is often considered the rate limiting step for prostaglandin synthesis (Oliw et al., 1983).

Free arachidonic acid can be metabolized by two enzymes, cyclooxygenase and lipoxygenase. Prostaglandins are derived via the cyclooxygenase pathway (Tepperman and Tepperman, 1987). Arachidonic acid is metabolized to prostaglandin G2 (PGG2) by cyclooxygenase in the presence of molecular O2 (Figure 1; Oliw et al., 1983). Prostaglandin G2 is rapidly converted to prostaglandin H2 (PGH2) by peroxidase activity (Figure 1; Oliw et al., 1983). This series of reactions is common for all prostaglandins, and various enzyme systems within a tissue convert PGH2 to different prostaglandins.

Prostaglandin F2α (PGF2α) synthesis is a good example of prostaglandin synthesis (Figure 1). Prostaglandin F2α is synthesized by PGF synthase (Oliw et al., 1983). Prostaglandin F synthase causes a reductive cleavage of PGH2, but the exact nature of the enzyme is still unclear (Oliw et al., 1983). Prostaglandin F2α is reduced by 15-hydroxy-PG-dehydrogenase to 15-keto-PGF2α, which is quickly metabolized to 13,14-dihydro-15-keto-PGF2α (PGFM) by Δ13-PG-reductase (Oliw et al., 1983). In addition, PGF2α can be converted to PGE2 and visa versa by 9-hydroxy-PG-dehydrogenase and 9-keto-PG-reductase, respectively (Oliw et
al., 1983). Most prostaglandins are synthesized in a similar manner.

Two other arachidonic acid metabolites, prostacyclin and thromboxane, appear to be produced via pathways that are independant of the other prostaglandins (Figure 1). Prostacyclin (PGI₂) is synthesized from PGH₂ by prostacyclin synthase, 6(9)oxy-cyclase (Pace-Asciak and Gryglewski, 1983). Prostacyclin is unstable, especially at acidic pH, and is quickly hydrolyzed to 6-keto-PGF₁α (Pace-Asciak and Gryglewski, 1983). During the production of thromboxane (TXA₂), 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) is often produced. Thromboxane and HHT have been found to be produced by the same enzyme, thromboxane synthase (Yamamoto, 1983). The hypothetical mechanism involves the protonation of PGH₂, which can be isomerized to TXA₂ or decomposed to HHT (Granström et al., 1983). The biological effects of HHT are not yet clear.

In summary, prostaglandins are lipid compounds derived from arachidonic acid. Cyclooxygenase converts arachidonic acid to two intermediate compounds, PGG₂ and PGH₂. Prostaglandin H₂ is metabolized to the various prostaglandins by specific enzymes. These compounds have a variety of important biological roles, including reproductive functions.
Figure 1. Pathways of arachidonic acid metabolism.
Embryonal Prostaglandin Production

Dickmann and Spilman (1975) first reported that d 6 rabbit embryos contained PGE$_2$ and PGF$_2\alpha$. Since that report, many groups have studied embryonal prostaglandins at various stages of development. Prostaglandins have been suggested to be involved in embryo migration in pigs (Pope et al., 1982), sodium transport and fluid movement (Barth and Barth, 1972; Lewis, 1986b), embryo hatching (Biggers et al., 1978, Basker et al., 1981; Hurst and MacFarlane, 1981; Chida et al., 1986), increased endometrial capillary permeability preceding implantation (Hoos and Hoffman, 1983; Pakrasi et al., 1985) and energy metabolism in blastocysts (McCarther et al., 1985; Khurana and Wales, 1987).

Prostaglandin synthesis by d 12 to 24 ovine embryos has been reported. Marcus (1981) reported that d 12 and 20 ovine conceptuses converted $[^{14}\text{C}]$ arachidonic acid to a nonpolar substance, PGE$_2$ and 6-keto-PGF$_1\alpha$, while d 15 conceptuses produced a larger quantity of PGF$_2\alpha$. This is substantiated by findings that d 14 and 16 conceptuses produced high quantities of PGF$_2\alpha$ (Hyland et al., 1982; Lacroix and Kann, 1982; Lewis and Waterman, 1985). In addition, Lewis (1987) reported that d 14 and 16 conceptuses metabolized PGF$_2\alpha$ to a substance more polar than PGF$_2\alpha$, while endometrium converted PGF$_2\alpha$ to PGFM, which is less
polar than PGF$_2\alpha$. The largest percentage of [³H] arachidonic acid was found to be incorporated into the free fatty acid pool, followed by incorporation into triglycerides and phospholipids in d 24 ovine conceptuses (Waterman and Lewis, 1983). Moreover, indomethacin decreased prostaglandin production by d 20 conceptuses, but it did not reduce arachidonic acid utilization (Lewis and Waterman, 1985).

Bovine embryos from d 6 to 20 of pregnancy released prostaglandins into incubation media (Shemesh et al., 1979; Lewis et al., 1982; Lewis and Waterman, 1983a; Lewis, 1986a; Hwang et al., 1988). Day-6 to -10 bovine embryos metabolized [³H] arachidonic acid to PGE$_2$ (Hwang et al., 1988). Day-13 bovine conceptuses metabolized arachidonic acid mainly to PGE$_2$ and PGF$_2\alpha$ (Hwang et al., 1988). Embryonic discs and trophoblastic tissues from d-13 bovine conceptuses, incubated separately, metabolized arachidonic acid to both PGE$_2$ and PGF$_2\alpha$ (Hwang et al., 1988). High-performance liquid chromatography (HPLC) indicated that bovine conceptuses collected after d 15 metabolized arachidonic acid to PGI$_2$ (as assessed by 6-keto-PGF$_{1\alpha}$), PGE$_2$ and PGF$_2\alpha$ (Hwang et al., 1988).

Lewis and Waterman (1983a) suggested a possible role of endometrium in controlling conceptus production of prostaglandin. Lewis (1986a) found that trophoblastic
tissue produced more prostaglandins than did endometrium. Day-20 conceptuses were found to produce a larger amount of PGFM, and d-16 conceptuses converted PGF$_2$α to a compound other than PGFM (Lewis et al., 1982; Lewis, 1986a). When d-16 conceptuses were co-incubated with endometrium, the media contained a higher concentration of PGFM than did media that contained either conceptuses or endometrium incubated alone (Lewis, 1986a).

Prostaglandin production increased with age of porcine embryo from d 7 to 16. Prostaglandin E$_2$ was the primary prostaglandin synthesized, but PGF$_2$α and 6-keto-PGF$_1$α were also produced (Davis et al., 1983; Lewis and Waterman, 1983b; Stone et al., 1986). The embryo has the ability to secrete estrogen (Perry et al., 1973; Stone et al., 1986), and estrogen stimulates phospholipase A$_2$ activity (Dey et al., 1982). Therefore, Davis et al. (1983) suggested that estrogen and prostaglandin synthesis may be interrelated.

Cultured rabbit embryos increased prostaglandin production from d 4 to 7 (Dey et al., 1980; Harper et al., 1983; Pakrasi and Dey, 1983). Indomethacin decreased prostaglandin production, but it reduced PGF$_2$α production significantly more than PGE$_2$ production (Harper et al., 1983). Harper et al. (1983) reported that concentrations of prostaglandins were higher in embryonal tissue than in culture media and that most of the prostaglandins measured
in intact rabbit blastocysts were found within the blastocoelc fluid, not with embryonic cells. Jones and Harper (1984) hypothesized that rabbit embryos produced prostaglandins and stored them in the blastocoele. In addition, Racowsky and Biggers (1983) found that radiolabelled arachidonic acid was incorporated mainly into mouse embryonal phospholipid pools.

As an overview, arachidonic acid was incorporated into the lipid pools of early embryos (Waterman and Lewis, 1983; Racowsky and Biggers, 1983). Prostaglandin production by embryos has been reported from early morula stage in rabbits (Harper et al., 1983) through d 24 in sheep (Lewis and Waterman, 1985). Patterns of prostaglandin production by embryos change with stage of development, but it is not yet known whether these changes affect development or are a result of development. Prostaglandin inhibitors can delay or stop development in some cases (Biggers et al., 1978). So, it appears that changes in prostaglandin production are involved in controlling embryonic development.

Shedding the Zona Pellucida

One of the most critical stages in embryonal development is the loss of the zona pellucida. The zona pellucida surrounds mammalian embryos, and it must be shed
before implantation and continued development can occur (Cole, 1967). The zona pellucida varies by species between 3 to 15 μm in thickness (Austin, 1961; Gwatkin, 1977; Bleil and Wassarman, 1980). The intact zona pellucida of bovine blastocysts is a slightly rough, continuous envelop that, at high magnification, appears porous (Fléchon and Renard, 1978). The zona pellucida consists of proteins and carbohydrates, possibly in the form of acidic glycoproteins (Gwatkin, 1964; Yanagimachi et al., 1973; McRorie and Williams, 1974; Gwatkin, 1977; Bleil and Wassarman, 1980). The mouse zona pellucida is approximately 80% protein (Bleil and Wassarman, 1980). Following fertilization, the zona pellucida becomes less susceptible to reducing agents (Inoue and Wolf, 1974), this appears to due to the release of cortical granular material from the oocyte into the perivitelline space (Barros and Yanagimachi, 1972; Gwatkin, 1977).

Two theories have been proposed for the loss of the zona pellucida. The first theory, called hatching, is physical tearing of the zona, which opens a channel that allows blastocystic expulsion (Mintz, 1962; Cole, 1967; Bergström, 1972; Fléchon and Renard, 1978; Biggers et al., 1978). The second theory is lysis of the zona pellucida (Pinsker et al., 1974; Maurer and Beier, 1976; Kane, 1983; Kane, 1986). Literature indicates that, although lysis is
possible, blastocysts appear to escape from the zona pellucida by hatching. For the hatching theory, embryos undergo a series of contractions and expansions before and during the hatching process. Embryos accumulate fluid and increase in diameter with each re-expansion. At some time in the process, the zona pellucida tears, which allows extrusion of trophoblastic cells. Continued contractions and re-expansions push the blastocyst out of the zona pellucida.

Using cinemicroscopy, Cole (1967) determined that contractions and expansions are an essential and normal part of blastocyst escape from the zona pellucida. Blastocyst contractions are rapid, occurring approximately every 4 to 5 min while re-expansion is slow, requiring 2 to 3 h (Cole, 1967). The mechanisms involved in embryonal contractions are unknown. Cole (1967) suggested that the cell membrane depolarizes, as a critical volume within the blastocoel is reached, this would open channels for fluid release. Bergström (1972) reported that the changes in diameter of blastocysts gradually thins the zona. The generally accepted theory is that increases in diameter are due to increasing fluid accumulation within the blastocoel. Hurst and MacFarlane (1981) reported that mouse embryos, which eventually hatched, obtained a diameter in excess of 140 µm, but the maximum diameter of unhatched embryos was 132 µm.
However, Cole (1967) suggested that fluid accumulation is not within the blastocoele, but within a specific set of cells. This may indicate that hatching is not just the mechanical opening of the zona, but that it may involve specific cells and cellular functions.

In mouse embryos at 2100 h on d 4, protruding trophoblastic buds were found on some embryos examined with electron microscopy, and the buds were described as an 'expulsion from an otherwise intact zona' (Bergström, 1972). After hatching, a slit in the zona pellucida about half the perimeter long was visible, although the zona pellucida basically retained its shape (Mintz, 1962; Cole, 1967; Bergström, 1972; Fléchon and Renard, 1978). The absence of zonal lysis and the presence of small cracks in the zona pellucida supports the hatching theory (Bergström, 1972).

The uterine environment may affect embryonic development and embryo hatching. In mice and rats, the uterus plays a minimal role in hatching, while in hamsters, the process is progesterone dependant (Orsini, 1963). Mouse blastocysts can free themselves from the zona pellucida when implanted in the anterior chamber of the eye (Runner, 1947) and when contained in diffusion chambers implanted into the peritoneal cavity of male mice (Bryson, 1964). Also in mice, loss of the zona pellucida was delayed for 24 h in females that were ovarietomized on d 2 or 3 of pregnancy or
when blastocysts were retained in the oviduct by ligation of the utero-tubal junction (Orsini and McLaren, 1967). The uterus appears to have little effect on embryo hatching, but studies in sheep have not been reported.

Proteases can remove the zona pellucida (Maurer and Beier, 1976; Kane, 1983; Kane, 1986), and proteases are present within uterine fluids (Pinsker et al., 1974). However, only large doses of proteases were effective in removing the zona pellucida, and afterwards embryo development was hindered (Maurer and Beier, 1976; Kane, 1983; Kane, 1986).

McLaren (1970) suggested that zona pellucida lysis occurred after hatching. Unfertilized ova undergo lysis late on d 5 in intact pseudopregnant female mice (McLaren, 1970). McLaren (1970) also reported that the zona pellucida surrounding an unfertilized ovum in pseudopregnant female mice persisted longer than the zona pellucida of a blastocyst, probably due to the activity of blastocysts. Kane (1983) suggested that this was evidence for a mechanical hatching process through a weak point in the zona pellucida and not an enzymatic process.

What causes the blastocystic expansion that appears necessary for the hatching theory? Expansion of blastocysts is due to accumulation of fluid within the blastocoele (Smith, 1970; Cross, 1973; Borland et al., 1976; Borland et
al., 1977). Sodium and chloride ions appear to be actively transported across the trophectoderm into the blastocoele (Cross, 1973; Biggers et al., 1977), followed by passive transport of water (Smith, 1970; Borland et al., 1976). Sodium appears to require Na/K pumps on the blastocoelic cell membrane to be transported into the blastocoele (Borland et al., 1977). Membrane junctional complexes found at the apex of trophoblastic cells connect to the blastocoelic cavity through intracellular spaces (Gamow and Daniel, 1970; Enders, 1971; Hastings and Enders, 1975; Ducibella et al., 1975). Diamond and Bossert (1967) suggest that these junctions may be the site of active sodium transport. After formation of membrane junctional complexes, embryos appear to obtain the ability to transport solutes and water (Borland et al., 1976). Fluid accumulation within the blastocoele appears to cause increased blastocystic diameter.

Prostaglandins may be involved in the control of fluid accumulation within the blastocyst. Barry and Hall (1968) reported that FGE₁ increased short-circuit current in and increased Na movement across frog skin. They concluded that increases in short-circuit current were a result of increased inward sodium movement. Indomethacin, mefenamic acid and acetylsalicylic acid decreased sodium transport across frog skin (Hall et al., 1976). Prostaglandin E₁
increased and indomethacin decreased cAMP activity in frog skin (Hall et al., 1976). Therefore, prostaglandins appear to be involved in sodium transport across frog skin, and prostaglandin activity may be mediated by cAMP (Hall et al., 1976). Prostaglandin E₁ was found to increase Na uptake by frog embryos (Barth and Barth, 1972). Prostaglandin E₁ allowed for Na uptake by displacing membrane Ca. Therefore in frog embryos, a decrease in Ca was accompanied by an increase in Na uptake (Barth and Barth, 1972). In addition, indomethacin significantly decreased uptake of $^{22}$Na by ovine trophoblastic tissue (Lewis, 1986b). Prostaglandins appear to increase Na influx into the blastocoele, which increases fluid accumulation within the blastocoele.

Hatching appears to result from accumulation of fluids in the blastocyst. Fluid transport may be controlled by prostaglandins. Therefore, Biggers et al. (1978) hypothesized that embryo hatching is controlled by prostaglandins. To test their hypothesis, mouse embryos were cultured with prostaglandin inhibitors. Prostaglandin inhibitors prevented embryo hatching (Biggers et al., 1978). Further studies with mouse embryos have produced similar results (Basker et al., 1981; Hurst and MacFarlane, 1981; Chida et al., 1986). Biggers et al. (1978) observed, in treated embryos, that a space appeared between the embryo and the zona pellucida. This indicates that the inhibitors
prevented fluid accumulation by the embryo (Biggers et al., 1978). Cinemicrographic observations confirmed that inhibitors prevented hatching by limiting blastocyst expansion and the attainment of a diameter greater than 140 \( \mu m \) (Hurst and MacFarlane, 1981). Chida et al. (1986) added PGE\(_2\) and PGF\(_2\alpha\) to embryos cultured with indomethacin. They reported that PGF\(_2\alpha\) reversed the inhibition of hatching, while PGE\(_2\) seemed to possess an increased, irreversible inhibitory effect on hatching embryos. However, Baskar et al. (1981) reported that 18,18,20-trimethyl PGE\(_2\), a purported specific inhibitor of PGE\(_2\) action, was effective in inhibiting hatching, yet other PGE\(_2\)- and PGF\(_2\alpha\)-specific inhibitors were not effective. Chida et al. (1986) suggested that prostaglandins may have a role in fluid transport into the blastocyst and local protease production, which together facilitate hatching. Therefore, prostaglandins may control embryo zonal loss by increasing fluid accumulation within the blastocoele.

In summary, the literature indicates that two possible mechanisms for zonal loss exist: hatching and lysis. The hatching theory appears the most likely process of zonal loss, because embryo zonal loss can occur in the absence of uterine fluids. Uterine proteases probably remove the zona pellucida from the uterus after the blastocyst escapes. The hatching process is caused by the contractions and
expansions of the blastocyst. The processes involved in contraction are not clear, but blastocyst expansion is due to fluid accumulation within the blastocoele. Prostaglandins are involved in the transport of sodium across trophoblastic tissue, which is followed passively by water. Therefore, the hatching process may be controlled in part by prostaglandins, but information on early embryonal prostaglandin production is not complete. If prostaglandins are involved in blastocyst formation and expansion, then the early embryo must be capable of metabolizing arachidonic acid to prostaglandins.

Cole (1967) concluded that if embryos did not escape from the zona pellucida, continued embryonic development would not occur. Lack of embryonic development will result in embryonal mortality. Embryonal mortality will decrease potential lamb crops. For financial reasons, producers should maximize reproductive efficiency of the flock. Therefore, an understanding of embryonal mortality is essential for improving reproductive performance of livestock and increasing a producer's opportunity for profit.

As indicated previously, to understand abnormal conditions, one must first understand normal conditions (Edey, 1969). Thus, one may hypothesize that prostaglandins are involved in the control of embryo hatching in sheep. To
test this hypothesis, one must first determine if early ovine embryos are capable of metabolizing arachidonic acid to prostaglandins. Then, using prostaglandin inhibitors and specific prostaglandins, one can focus on the mechanism of embryo hatching. With this information, one can begin to understand the control of embryo hatching in sheep.
MATERIALS AND METHODS

General

Mature ewes of various breeds and ages from the Virginia Tech flock were checked for signs of estrus twice daily using vasectomized rams. The ewe breeds were primarily Suffolk, Hampshire, Dorset and Finnish Landrace, and ages ranged from 8 mo to 13 yr. Ewes were maintained on pasture and supplemented with grain and hay during the winter. Ewes were assigned to experiments following detection of estrus. Estrus was synchronized with 6α-methyl-17β-hydroxy progesterone acetate (MPA; Sigma Chemical Co., St. Louis, MO)—containing pessaries. Pessaries were made using a large needle to sew monofilament fishing line (6.8 kg test) through an expanded polyether foam sponge (Jaece Industries Inc., North Tonawanda, NY). Maintaining a loose loop through the sponge, the line was cut to approximately 25 cm in length and was secured to the sponge with a square knot near the sponge surface. A plastic button was threaded in at the bottom of some sponges to reduce the incidence of the line being pulled through the sponge during removal.

After making the sponges, MPA was dissolved in ethanol (EtOH) at a concentration of 10 mg MPA/ml EtOH. The sponges
were hung by the monofilament lines from a rod in a fume hood. Each sponge was inverted, injected with 2 ml of MPA-EtOH solution (MPA-EtOH solution was evenly distributed by making four injections of .5 ml each), returned to the hanging position and allowed to dry. This procedure was repeated 3 times for a total of 6 ml of MPA-EtOH solution and a content of 60 mg MPA in each pessary. Pessaries were dried and placed in groups of ten in plastic bags, and the bags were labelled and sealed.

Pessaries were inserted into the vagina of ewes between d 8 and 12 of the estrous cycle using a disinfected, modified 12 cc disposable syringe. The syringe was modified by removing the closed end, which made a tube with a plunger. With the plunger removed, the pessary was rolled into a cylindrical shape and pushed into the top of the syringe. The plunger was inserted into the syringe with the pessary lines passing by it. Each ewe was restrained, and a pessary was pushed into the vagina with the syringe device. Pessaries were removed after 12 d. Ewes that lost pessaries prior to removal were removed from the studies.

Ewes were superovulated with 20 mg of porcine-derived follicle-stimulating hormone (FSH; Schering Corp., Kenilworth, NJ) administered as a series of five injections: 5, 5, 5, 2.5 and 2.5 mg at -40, -32, -8, 0 and 16 h,
respectively, relative to pessary removal (Rexroad and Powell, 1991).

Two rams, known to produce offspring, were trained for semen collection with an artificial vagina (AV). The rams were introduced to an estrous ewe restrained in a stanchion. After allowing the rams to become accustomed to the presence of a collector, they were allowed to mount the ewe, and their penises were directed into the AV. This process was repeated until the rams would consistently ejaculate into the AV. The semen was collected into a glass, graduated tube, and was stored in a water bath at 37°C for experiment 1 or allowed to cool slowly to 25°C and stored in the dark for experiment 2. Semen storage was modified for the second experiment to improve the quality of stored semen, and all semen collections were used within one hour. Motility was estimated in each ejaculate, and only collections with greater than 50% motility were used.

Ewes were artificially inseminated surgically 40 h after sponge removal. Ewes were restricted from feed and water for 24 h before surgery. They were anesthetized with i.v. injections of sodium pentobarbital (Sigma Chemical Co.) in sterile .9% saline (65 mg sodium pentobarbital/ml saline). Anesthetized ewes were lifted onto a mobile, stainless-steel surgical table. The ewes were placed in the dorsal recumbency position and sheared from the hocks to the
sternum. The surgical field, from the udder anterior approximately 20 cm and approximately 20 cm wide, was clipped closer with small animal clippers and then scrubbed at least twice with disinfectant soap. Following the final scrub, a 10% iodine solution was sponged over the surgical area.

Aseptic surgical techniques were used. A sterile drape was placed over approximately 2/3 of the ewe's body, and a longitudinal opening was cut directly anterior to the udder. A 10-cm skin incision was made with a scalpel to one side of the midline, to avoid cutting the major mammary vein. The subcutaneous layer of tissue was blunt dissected to the body wall. At midline, the body wall was scored with a scalpel to create a weak point. A closed, curved hemostat was pushed through the body wall at the weak point and opened to increase the size of the opening. After removing the hemostat, the blunt tip of bandage scissors was placed into the opening and lifted slightly. The scissors were pushed forward allowing the body wall to cut against the blade of the scissors; the body wall opening was slightly shorter than the skin incision. The uterus was brought to the surface using fingers, attempting to avoid disturbing the ovaries. With only the uterine tips exposed, .2 ml of neat semen was injected into each uterine horn using a 1 ml syringe and a blunt 20-gauge needle. After the semen was
injected, the uterus was allowed to return to its usual position. The body wall and skin incisions were closed with size 1 polyamide suture.

For collection of embryos, each ewe was sacrificed on a specified day of pregnancy, and the uterus and a portion of the cervix were removed and placed into a plastic bag, which was labelled with the ewe number, and transferred to the laboratory within 30 min. For Experiment 1, embryos were collected on d 4, 8, 10, 12 and 14 of pregnancy; for Experiment 2, embryos were collected on d 7 of pregnancy. Uteri were trimmed of the ligaments, ovaries and oviducts, and placed onto a paper towel in an aseptic laminar-flow hood.

A large, sterile renal-vascular clamp was used to clamp the cervix. The tip of one uterine horn was picked up and the utero-isthmus junction was excised with sterile scissors. The opening in the uterine tip was enlarged by inserting the tip of sterile scissors and making a longitudinal cut approximately 5 mm in length. The uterine tip was grasped with a sterile hemostat and placed over an open, sterile 100 X 15 mm Petri dish (Fisher Scientific Co., Pittsburgh, PA). The uterine horn was clamped near the cervix with the thumb and forefinger. Using a 20 cc disposable syringe with a 20-gauge needle, 20 ml of either sterile saline (Experiment 1) or medium (Experiment 2) were
injected into the uterine lumen at a slow, steady rate to allow for complete flushing of the uterine contents into the Petri dish. The remaining fluid in the uterine horn was removed with a gentle massaging action with the thumb and forefinger in the direction of the uterine tip. The procedure was repeated for the opposite uterine horn. The Petri dishes containing uterine flushings were stored in an incubator at 37°C until embryos were processed further, approximately 30 min.

Experiment 1

This experiment was designed to determine if early embryos produce prostaglandins. Embryos were recovered from 60 ewes. The recovery rate averaged 3.5 embryos/ewe. The average ovulation rate of ewes, assessed by number of corpora lutea (CL), was 9.5 CL/ewe. The embryo recovery rate was 36.8%. Embryos were incubated in a modified minimum-essential medium (MEM; GIBCO, Grand Island, NY). The medium was modified by adding 500 mg glucose, 20 IU insulin, 1 ml antibiotic and antimiycotic (GIBCO; 10,000 IU sodium penicillin G, 10,000 μg streptomycin sulfate and 25 μg amphotericin B) and 1 ml nonessential amino acids to 100 ml MEM with Earle's salts, L-glutamine and 92 mg/L D-valine substituted for L-valine.
Day-4, -8, and -10 Embryos.

Day-4, -8, and -10 embryos stored in Petri dishes in an incubator at 37°C were moved to a laminar-flow hood. A dissecting microscope was used to locate embryos. The embryos, along with 10 μl of saline, were recovered with an Eppendorf pipet and placed into a well of a microtiter plate (48 wells; Nunc; Roskilde, Denmark) containing 500 μl of modified MEM. The embryos, approximately 5/incubation, were then transferred to a second well containing 500 μl of modified MEM. This procedure was repeated for each uterus flushed. One μCi of [14C] arachidonic acid (52.8 mCi/mmol; New England Nuclear, Boston, MA) was added to the wells with embryos. The plates were incubated in an atmosphere of 5% CO₂, 45% O₂, 50% N at 37°C for 24 h. After incubation, the medium and embryos were transferred to polypropylene test tubes, capped and stored frozen until analyzed for prostaglandin production by HPLC (VanRollins et al., 1980).

Day-12 and -14 Embryos.

Day-12 embryos were recovered in 10 μl of saline with an Eppendorf pipet and placed into a sterile 60 X 15 mm Petri dish (Becton-Dickinson, Lincoln Park, NJ) with 4 ml of
modified MEM. The d-12 embryos were transferred, approximately 5/incubation, to another sterile 60 X 15 mm Petri dish containing 4 ml of modified MEM and 1 μCi of [14C] arachidonic acid. The d-14 embryos were recovered with stainless-steel forceps, transferred to a weigh boat, weighed and transferred, 1 to 2/incubation, to a Petri dish containing 4 ml of modified MEM and 1 μCi [14C] arachidonic acid. The Petri dishes were incubated on a rocking platform in an atmosphere of 5% CO2, 45% O2, 50% N at 37°C for 24 h. After incubation, the medium and embryos were transferred to polypropylene test tubes, capped and stored frozen until analyzed for prostaglandin production by HPLC (VanRollins et al., 1980).

Samples were analyzed using a Waters HPLC 810 system with a reversed-phase Nova-Pak C18 column. Samples were allowed to thaw at room temperature. A 250 μl volume of sample was transferred to a 16 X 125 mm test tube containing 250 μl of incubation medium. The sample was acidified with 62.6 μl of 1N HCl, and 4 ml of ethyl acetate were added. Samples were vortexed for 1 min and then centrifuged for 15 min at about 2000 x g. The ethyl acetate (top layer) was transferred with a disposable pipet to a clean test tube and evaporated under nitrogen in a water bath at 37°C. The ethyl acetate extraction procedure was then repeated. After ethyl acetate from the double extraction procedure had
evaporated, 1 ml of methanol was added around the upper edge of the storage tube to rinse the extract off of the sides of the tube and concentrate it in the bottom of the storage tube. The methanol was evaporated under nitrogen at 37°C in a water bath.

The Baseline® HPLC controller program was accessed (Waters Dynamic Solutions; Milford, MA) and used to control the HPLC system. The test tube with the extract was removed from the water bath, and the extract was reconstituted with 100 µl of the baseline HPLC solvent [97% solvent A (25% acetylnitrile (CNCH₃)/74% ultrapure water/1% acetic acid):3% solvent B (75% CNCH₃/24% ultrapure H₂O/1% acetic acid)]. This solution was injected into the HPLC column for fractionation. Separation of compounds was accomplished using a stepwise gradient from 97% solvent A:3% solvent B to 0% solvent A:100% solvent B (Figure 2).

The HPLC system separated the carrier solvents into .7 ml fractions every .7 min. Scintillation cocktail (5 ml) was added to each fraction, and radioactivity was estimated with a scintillation counter. A [¹⁴C] quench curve was developed based upon sample channels ratios (SCR). Disintegrations per minute (dpm) were calculated from counts per minute (cpm) using the sample SCR and the quench curve. Disintegrations per minute were imported into the Baseline® HPLC controller program and used to define HPLC peaks. The
Baseline® HPLC controller program detects peaks based upon the deviation of points from baseline. Peak area (total dpm/peak) was calculated using the trapezoidal calculation of areas between discrete data points. After peak area was calculated, the program subtracted the baseline region from the total area. Total dpm in peaks were converted to picograms using the specific activity of \([^{14}C]\) arachidonic acid in the equations:

\[
\frac{\text{mmol AA}}{52.8 \text{ mCi}} \times \frac{nCi}{2.22 \times 10^9 \text{ dpm}} \times \frac{305.5 \times 10^9 \text{ pg}}{\text{mmol AA}} = 2.606 \text{ pg/dpm}
\]

Integrated peak area (dpm) \(\times\) \(\frac{2.606 \text{ pg}}{\text{dpm}}\) = Calculated peak area (pg)

Peaks were identified from chromatograms of known arachidonic acid metabolites (Figure 2).

Experiment 2

The purpose of this experiment was to determine if prostaglandins have a role in embryonal zonal loss. Embryos were collected from 47 ewes that averaged 3.3 embryos/ewe. The ovulation rate, as assessed by the number of CL, was 9.8, and embryo recovery rate was 33.7%. Embryo hatching experiments were conducted with a modified Ham's F-10 medium (GIBCO). Added to 100 ml Ham's F-10 medium with L-glutamine
Figure 2. Chromatographic profile of arachidonic acid and its metabolites. Peaks are identified, from left to right, as 6-keto-PGF$_{1\alpha}$, PGF$_{2\alpha}$, PGE$_2$, PGFM, PGB, and arachidonic acid (---). Gradient profile of solvent B for separation of arachidonic acid metabolites (-----). Roman numerals were assigned to chromatographic regions, which were described in the text.
and 25 mg/L HEPES buffer was 1.5% defatted bovine serum albumin (BSA; Sigma Chemical Co.) and 1 ml antibiotic and antmyotic (GIBCO; 10,000 IU penicillin G sodium, 10,000 μg streptomycin sulfate and 25 μg amphotericin B).

Petri dishes containing uterine flushes were moved from the incubator to a sterile laminar-flow hood. The embryos were located and transferred in 2 μl of medium to a Petri dish containing 1 ml of modified Ham's F-10 medium. The embryos from all ewes flushed on each day were combined. Embryos judged to be of good quality or better were transferred to one of four wells, approximately 5 embryos/well, in a microtiter plate containing 500 μl of modified Ham's F-10 medium per well. The wells received one of four treatments: control, indomethacin (10^-4 M; Sigma Chemical Co.), PGE2 (2 ng/ml; Sigma Chemical Co.) or indomethacin and PGE2. The stage of embryo development and condition were noted, and the plate was incubated in an atmosphere of 5% CO2, 95% air at 37°C for 24 h.

After 24 h, the plate was moved to a laminar flow hood, and the embryos were examined under a dissecting microscope. The embryos were observed for changes in development, the number hatched was noted, and embryos were transferred to another well containing 500 μl modified Ham's F-10 medium with the same treatment. This procedure was repeated for 6 d.
Indomethacin was diluted in EtOH to deliver 17.89 μg/10 μl of EtOH (10^-4 M solution). Prostaglandin E2 was diluted in EtOH to deliver 2 ng/10 μl of EtOH. The dose of indomethacin was reported previously to inhibit hatching in mouse embryos (Chida et al., 1986). The dose of PGE2 was determined from synthesis of prostaglandins in Experiment 1.

Statistical Analyses

Experiment 1.

The Statistical Analysis System (SAS) was used to determine baseline and compare chromatographic curves between days of pregnancy (SAS, 1985). Baseline was determined by testing all dpm data from each sample for skewness and kurtosis and then truncating the tails until a normal distribution was derived (Brinkley et al., 1973). A change in the chromatographic profile of greater than three standard deviations from calculated baseline was considered a peak. Peak area was analyzed with GLM procedures to determine differences between days, and means were separated with Duncan's procedure (SAS, 1985).

Experiment 2.

The main effects of treatment on embryo hatching and interactions between treatments were evaluated with the
CATMOD procedure of SAS (SAS, 1985). Contrasts among treatments were performed to determine treatment differences.
RESULTS

Experiment 1.

Figures 3 to 7 show typical chromatographic profiles of arachidonic acid metabolism by embryos from each day of pregnancy studied. Regions II, VI, VII, VIII, X, XIV and XV correspond to 6-keto-PGF$_1$α, PGF$_2$α, PGE$_2$, PGFM, PGB$_2$, HHT and arachidonic acid, respectively. Regions I, III, IV, V, IX, XI, XII and XIII correspond to unknown peaks 1 to 8, respectively. For statistical analyses, chromatographic data were transformed to logarithms to reduce variability. Tables 1 to 6 contain geometric and arithmetic means of the amounts of various compounds measured after incubation. Tables of arithmetic means are for reference, all statistics reported are from analyses performed with logarithmic data. Average amounts of arachidonic acid and HHT did not differ (P>.05) with day of pregnancy (Table 2). Production of PGF$_2$α and PGB$_2$ was greater (P<.001; Table 2) on d 12 and 14 than on d 4, 8 and 10. The amount of PGE$_2$ increased in magnitude from d 4 to d 14 (P<.001; Table 2). Production of PGFM was greater on d 12 than it was on d 4, 8, and 10; PGFM production was greatest on d 14 (P<.001; Table 2). Production of 6-keto-PGF$_1$α was greatest (P<.03) on d 14 (Table 2). Total picograms of product in regions other than
the one that contained arachidonic acid were greater (P<.001) on d 12 and 14 than on d 4, 8 and 10 (Table 2).

Eight unknown peaks were detected after incubation with $[^{14}\text{C}]$ arachidonic acid. They were numbered from most polar to least polar. Production of unknown 1 was greater on d 8 and 12 than d 4 and 10, and it was greatest on d 14 (P<.001; Table 4). Unknown 2 was found in significant amounts only on d 10 (P<.001; Table 6). Unknown 3 was detected in significant amounts on d 10 (P<.001; Table 4). Unknowns 4, 5, and 6 were only detected in significant amounts in d 14 incubations (P<.001; Table 6), while unknown 7 was prominent in all incubations, except d 10 (P<.001; Table 4). Unknown 8 was detected in significant amounts on d 4, 8 and 10 (P<.001; Table 4).

**Experiment 2.**

Incubations containing indomethacin appeared to decrease the rate of embryo hatching (32.2 vs. 46.4%; Table 7). However, hatching rates of embryos incubated with indomethacin were not different from control (EtOH; P>.05; Table 8). Used alone, PGE$_2$ appeared to increase embryo hatching rate (60 vs. 46.4%; Table 7), but data for PGE$_2$ treatment were not different from control (P>.05; Table 8). Incubation of embryos with indomethacin and PGE$_2$ appeared to
decrease the rate of embryo hatching (30 vs. 46.4%; Table 7), but the difference from control was not significant (P>.05; Table 8). Hatching rate of embryos incubated with indomethacin and PGE₂ was not different from that with indomethacin alone (P>.05; Table 8). Hatching rate for all incubations with indomethacin was less than that for incubations with PGE₂ (P<.05; Table 8).
TABLE 1. ARITHMETIC MEANS OF AMOUNTS OF VARIOUS COMPOUNDS IN MEDIA AFTER EMBRYOS WERE INCUBATED WITH [14C] ARACHIDONIC ACID FOR 24 H

<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>MSE (x10^-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=4</td>
<td></td>
</tr>
</tbody>
</table>

| 6-keto-PGF1αb  | .74 | 3.39| 3.44| 7.79| 62.17| 2.54         |
| PGF2α          | NA  | NA  | .05 | 6.25| 68.30| 4.67         |
| PGE2           | NA  | .07 | .15 | 5.85| 61.03| 4.78         |
| AA             | 116.31| 51.11| 44.76| 76.23| 43.07| 44.75        |
| PGFM           | NA  | NA  | NA  | .71 | 58.06| 7.13         |
| PGB2           | .48 | 1.58| 1.17| 6.78| 66.89| 7.29         |
| HHT            | .69 | 1.75| 1.71| 1.49| 4.96 | .08          |
| TPGW0AA        | 10.20| 34.93| 22.38| 74.28| 718.99| 3.89        |

a. n=number of incubations (d-4, -8 and -10 had 4 to 8 embryos/incubation; d-12 and -14 had 1 to 5 embryos/incubation).
b. Abbreviations are explained in Appendix I.
c. The value was less than .1 pg, .1 was assigned as the value for the observation.
<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=4</td>
<td></td>
</tr>
<tr>
<td>6-keto-PGF$_2$$^a$$^{b}$</td>
<td>1.91$^c$</td>
<td>2.90$^c$</td>
<td>3.42$^{cd}$</td>
<td>3.26$^{cd}$</td>
<td>4.71$^d$</td>
<td>1.85</td>
</tr>
<tr>
<td>PGF$_2$$^a$</td>
<td>- .70$^c$</td>
<td>- .70$^c$</td>
<td>- .24$^c$</td>
<td>2.45$^d$</td>
<td>4.66$^e$</td>
<td>1.11</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>- .70$^c$</td>
<td>- .21$^c$</td>
<td>.69$^{cd}$</td>
<td>2.50$^{de}$</td>
<td>3.79$^e$</td>
<td>2.52</td>
</tr>
<tr>
<td>AA</td>
<td>4.94$^c$</td>
<td>4.55$^c$</td>
<td>4.55$^c$</td>
<td>4.84$^c$</td>
<td>4.65$^c$</td>
<td>0.12</td>
</tr>
<tr>
<td>PGFM</td>
<td>- .70$^c$</td>
<td>- .70$^c$</td>
<td>- .70$^c$</td>
<td>.52$^d$</td>
<td>4.54$^e$</td>
<td>.46</td>
</tr>
<tr>
<td>PGB$_2$</td>
<td>2.56$^c$</td>
<td>3.14$^d$</td>
<td>2.98$^d$</td>
<td>3.66$^e$</td>
<td>4.59$^f$</td>
<td>.13</td>
</tr>
<tr>
<td>HHT</td>
<td>2.75$^c$</td>
<td>3.20$^c$</td>
<td>3.06$^c$</td>
<td>1.69$^c$</td>
<td>1.58$^c$</td>
<td>1.55</td>
</tr>
<tr>
<td>TPGWOAA</td>
<td>3.92$^c$</td>
<td>4.49$^{de}$</td>
<td>4.23$^{cd}$</td>
<td>4.79$^e$</td>
<td>5.75$^f$</td>
<td>.09</td>
</tr>
</tbody>
</table>

$^a$n=number of incubations (d-4, -8 and -10 had 4 to 8 embryos/incubation; d-12 and -14 had 1 to 5 embryos/incubation).

$^b$Abbreviations are explained in Appendix I.

$^{cde}$Within a row, values with different superscripts differed (P < .05).
TABLE 3. ARITHMETIC MEANS OF AMOUNTS OF MAJOR UNKNOWN COMPOUNDS IN MEDIA AFTER EMBRYOS WERE INCUBATED WITH [14C] ARACHIDONIC ACID FOR 24 H.

<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7a</td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=4</td>
<td>(x10^-8)</td>
</tr>
<tr>
<td>UNK 1b (2)c</td>
<td>6.43</td>
<td>22.07</td>
<td>12.67</td>
<td>20.55</td>
<td>119.89</td>
<td>4.582</td>
</tr>
<tr>
<td>UNK 3 (14)</td>
<td>NA</td>
<td>.90</td>
<td>1.18</td>
<td>NA</td>
<td>NA</td>
<td>.005</td>
</tr>
<tr>
<td>UNK 7 (78)</td>
<td>.31</td>
<td>1.60</td>
<td>NA</td>
<td>3.15</td>
<td>40.75</td>
<td>3.932</td>
</tr>
<tr>
<td>UNK 8 (107)</td>
<td>.29</td>
<td>1.07</td>
<td>.87</td>
<td>NA</td>
<td>NA</td>
<td>.003</td>
</tr>
</tbody>
</table>

a n=number of incubations (d-4, -8 and -10 had 4 to 8 embryos/incubation; d-12 and -14 had 1 to 5 embryos/incubation).
b Abbreviations are explained in Appendix I.
c Parentheses indicate the average fraction in which the peak was found.
d The value was less than .1 pg, .1 was assigned as the value for the observation.
<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNK 1 (2)</td>
<td>3.75^d</td>
<td>4.31^e</td>
<td>3.98^d</td>
<td>4.31^e</td>
<td>5.05^f</td>
<td>0.06</td>
</tr>
<tr>
<td>UNK 3 (14)</td>
<td>0.70^d</td>
<td>1.02^e</td>
<td>2.52^f</td>
<td>0.0^de</td>
<td>0.0^de</td>
<td>1.48</td>
</tr>
<tr>
<td>UNK 7 (78)</td>
<td>2.03^d</td>
<td>2.61^d</td>
<td>0.70^e</td>
<td>2.95^d</td>
<td>2.79^d</td>
<td>2.30</td>
</tr>
<tr>
<td>UNK 8 (107)</td>
<td>2.37^d</td>
<td>2.96^d</td>
<td>2.35^d</td>
<td>0.0^e</td>
<td>0.0^e</td>
<td>0.47</td>
</tr>
</tbody>
</table>

^a^ n=number of incubations (d=4, -8 and -10 had 4 to 8 embryos/incubation; d=12 and -14 had 1 to 5 embryos/incubation).
^b^ Abbreviations are explained in Appendix I.
^c^ Parentheses indicate the average fraction in which the peak was found.
^d^ within a row, values with different superscripts differed (P < .05).
TABLE 5. ARITHMETIC MEANS OF AMOUNTS OF MINOR UNKNOWN COMPOUNDS IN MEDIA AFTER EMBRYOS WERE INCUBATED WITH [14C] ARACHIDONIC ACID FOR 24 H.

<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=7</td>
<td>n=4</td>
<td>(x10^-7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>ng/embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNK 2b (11)c</td>
<td>NA NA .57 NA NA .009</td>
</tr>
<tr>
<td>UNK 4 (21)</td>
<td>NA NA NA NA 14.15 5.135</td>
</tr>
<tr>
<td>UNK 5 (67)</td>
<td>NA NA NA NA 54.19 93.019</td>
</tr>
<tr>
<td>UNK 6 (76)</td>
<td>NA NA NA NA 30.26 20.791</td>
</tr>
</tbody>
</table>

a = number of incubations (d-4, -8 and -10 had 4 to 8 embryos/incubation; d-12 and -14 had 1 to 5 embryos/incubation).

b Abbreviations are explained in Appendix I.

c Parentheses indicate the average fraction in which the peak was found.

d The value was less than .1 pg, .1 was assigned as the value for the observation.
TABLE 6. MEANS OF LOG TRANSFORMED DATA FOR AMOUNTS OF MINOR UNKNOWN COMPOUNDS IN MEDIA AFTER EMBRYOS WERE INCUBATED WITH $^{14}C$ ARACHIDONIC ACID FOR 24 H.

<table>
<thead>
<tr>
<th>Region</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNK 2&lt;sup&gt;b&lt;/sup&gt; (11)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.39&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.85</td>
</tr>
<tr>
<td>UNK 4  (21)</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.53&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.86</td>
</tr>
<tr>
<td>UNK 5  (67)</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.84&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.08</td>
</tr>
<tr>
<td>UNK 6  (76)</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.73&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.99</td>
</tr>
</tbody>
</table>

<sup>a</sup>n=number of incubations (d-4, -8 and -10 had 4 to 8 embryos/incubation; d-12 and -14 had 1 to 5 embryos/incubation).

<sup>b</sup>Abbreviations are explained in Appendix I.

<sup>c</sup>Parentheses indicate the average fraction in which the peak was found.

<sup>d</sup>Within a row, values with different superscripts differed (P < .05).

---

TABLE 7. EMBRYO HATCHING PERCENTAGES AFTER INCUBATION WITH INDOMETHACIN AND PGE$_2$ FOR 6 DAYS AT 37°C IN AN ATMOSPHERE OF 5% CO$_2$ AND 95% AIR IN MODIFIED HAMS F-10 MEDIA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th># of Embryos</th>
<th># Hatched</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28</td>
<td>13</td>
<td>46.4</td>
</tr>
<tr>
<td>Indo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29</td>
<td>10</td>
<td>34.5</td>
</tr>
<tr>
<td>PGE$_2$&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>Indo + PGE$_2$</td>
<td>30</td>
<td>9</td>
<td>30.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>EtOH = Ethanol.

<sup>b</sup>Indo = Indomethacin.

<sup>c</sup>Prostaglandin E$_2$. 

<table>
<thead>
<tr>
<th>Contrast</th>
<th>df</th>
<th>Chi-square</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH\textsuperscript{a} vs. Indo\textsuperscript{b}</td>
<td>1</td>
<td>.84</td>
<td>.3595</td>
</tr>
<tr>
<td>EtOH vs. PGE\textsubscript{2}\textsuperscript{c}</td>
<td>1</td>
<td>1.07</td>
<td>.3020</td>
</tr>
<tr>
<td>EtOH vs. Indo + PGE\textsubscript{2}</td>
<td>1</td>
<td>1.64</td>
<td>.2003</td>
</tr>
<tr>
<td>Indo vs. PGE\textsubscript{2}</td>
<td>1</td>
<td>3.76</td>
<td>.0524</td>
</tr>
<tr>
<td>Indo vs. Indo + PGE\textsubscript{2}</td>
<td>1</td>
<td>.14</td>
<td>.7127</td>
</tr>
<tr>
<td>PGE\textsubscript{2} vs. Indo + PGE\textsubscript{2}</td>
<td>1</td>
<td>5.27</td>
<td>.0216</td>
</tr>
<tr>
<td>PGE\textsubscript{2} vs. Indo &amp; Indo+PGE\textsubscript{2}</td>
<td>1</td>
<td>6.10</td>
<td>.0135</td>
</tr>
<tr>
<td>Main effect</td>
<td>3</td>
<td>6.40</td>
<td>.0937</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Ethanol.
\textsuperscript{b}Indomethacin.
\textsuperscript{c}Prostaglandin E\textsubscript{2}.
Figure 3. Reversed-phase HPLC chromatogram representative of d-4 embryonal metabolism of $^{14}$C arachidonic acid. Roman numerals were assigned to chromatographic regions. The identity of compounds in various regions was indicated in the text.
Figure 4. Reversed-phase HPLC chromatogram representative of δ-8 embryonal metabolism of $[^{14}C]$ arachidonic acid. Roman numerals were assigned to chromatographic regions. The identity of compounds in various regions was indicated in the text.
Figure 5. Reversed-phase HPLC chromatogram representative of d-10 embryonal metabolism of $[^{14}\text{C}]$ arachidonic acid. Roman numerals were assigned to chromatographic regions. The identity of compounds in various regions was indicated in the text.
Figure 6. Reversed-phase HPLC chromatogram representative of d-12 embryonal metabolism of [14C] arachidonic acid. Roman numerals were assigned to chromatographic regions. The identity of compounds in various regions was indicated in the text.
Figure 7. Reversed-phase HPLC chromatogram representative of d-14 embryonal metabolism of [14C] arachidonic acid. Roman numerals were assigned to chromatographic regions. The identity of compounds in various regions was indicated in the text.
DISCUSSION

Data in this thesis indicate that, as early as d 4 of development, ovine embryos can metabolize arachidonic acid to prostaglandins. Day-4 and -8 embryos converted arachidonic acid to 6-keto-PGF1α, PGE2 and various other compounds. Prostaglandin E2 appeared to increase embryo hatching rate in vitro, while indomethacin appeared to decrease hatching rate. Hatching rate for neither treatment was statistically different from controls, but PGE2 treatment increased hatching rate when compared to all indomethacin treatments.

Previous literature indicates that ovine embryos actively metabolize arachidonic acid to prostaglandins (Marcus, 1981; Hyland et al., 1982; Lacroix and Kann, 1982; Waterman and Lewis, 1983; Lewis and Waterman, 1985; Lewis, 1987). Marcus (1981) reported that d-12 ovine embryos could metabolize arachidonic acid to prostaglandins, but results of the present study seem to be the first to indicate that d-4 ovine embryos can actively metabolize arachidonic acid to a variety of compounds. In media that contained d-4 ovine embryos, chromatographic peaks were found in the regions of an unidentified compound, 6-keto-PGF1α, PGB2 and HHT. Day-8 embryos metabolized arachidonic acid to similar compounds and amounts as did d-4 embryos. Regions
containing an unidentified polar compound, 6-keto-PGF$_1\alpha$, PGB$_2$ and HHT were the most prominent in the d-8 chromatograms.

All embryos harvested on d-10 of pregnancy were hatched, expanding blastocysts. Chromatograms of d-10 embryo incubations indicated a high degree of variability among incubations, possibly reflecting differing degrees of development and prostaglandin-synthesizing capability. Some d-10 embryo incubations had arachidonic acid metabolic profiles similar to those detected for d-4 and -8 embryo incubations, while chromatograms for some d-10 incubations indicated that embryos metabolized arachidonic acid more extensively. Day-10 embryos metabolized arachidonic acid to a polar unidentified peak, 6-keto-PGF$_1\alpha$, PGB$_2$ and HHT as did d-4 and -8 embryos, but d-10 embryos also produced PGE$_2$, PGF$_2\alpha$, PGFM and an unknown peak found in region III. Because all d-10 embryos were hatched and expanding embryos, changes in arachidonic acid metabolism by d-10 embryos may indicate a period of rapid growth, which may reflect the transition from spherical to elongated embryos. Day-12 and -14 embryos converted arachidonic acid to a variety of metabolites, primarily 6-keto-PGF$_1\alpha$, PGE$_2$, PGF$_2\alpha$, PGFM and PGB$_2$, which is consistent with previous reports (Marcus, 1981; Hyland et al., 1982; Lacroix and Kann, 1982; Lewis and Waterman, 1985; Lewis, 1987).
Because prostaglandin production is greater after embryo hatching, one may speculate that the increase in arachidonic acid metabolism by d-10, -12 and -14 embryos implicates the involvement of prostaglandins in embryonic development. Ovine embryos hatch from the zona pellucida around d 8 of pregnancy, and hatching is followed by rapid expansion of the blastocyst. Hatched, rapidly expanding embryos may require prostaglandins for fluid transport (Biggers et al., 1978; Lewis, 1986b), attachment to the endometrium (Hoos and Hoffman, 1983; Pakrasi et al., 1985) and maternal-embryo relationships to maintain pregnancy (Rawlings and Hyland, 1985).

Biggers et al. (1978) hypothesized that prostaglandins were involved in embryonal zonal loss. They proposed that prostaglandins controlled fluid movement into the blastocoele and that fluid movement was essential for embryo hatching. Hatching of mouse embryos was inhibited by prostaglandin inhibitors (Biggers et al., 1978; Baskar et al., 1981; Hurst and MacFarlane, 1982; Chida et al., 1986). Day-4 and -5 ovine embryos produced PGE$_2$ and PGI$_2$ (as assessed by 6-keto-PGF$_1\alpha$), which may be involved in controlling fluid movement into the blastocyst (Barth and Barth, 1972; Hall et al., 1976; Biggers et al., 1978; Lewis, 1986b). Thus, PGE$_2$ was evaluated for its effects on hatching. Because of its short half-life and chemical
instability, PGI\textsubscript{2} was not evaluated. Those characteristics of PGI\textsubscript{2} would make it difficult to determine whether PGI\textsubscript{2} could affect hatching rate, i.e., the lack of a PGI\textsubscript{2} effect could indicate that PGI\textsubscript{2} was not involved in hatching or that PGI\textsubscript{2} was degraded before it was able to exert a physiological effect on the blastocyst.

Even though embryos incubated with PGE\textsubscript{2} alone appeared to have increased hatching rates, embryos incubated with indomethacin and PGE\textsubscript{2} appeared to have decreased hatching rates. This indicates that PGE\textsubscript{2} was unable to reverse the effects of indomethacin on embryo hatching rate. These results are similar to those reported by Chida et al. (1986) with mouse embryos, which indicated that PGE\textsubscript{2} could not reverse the effects of indomethacin. In addition to inhibiting prostaglandin synthesis, indomethacin can also inhibit protein synthesis by the trophoblast (G.S. Lewis, personal communication) and it may affect ion movement by the cell membrane. Thus, because indomethacin has a variety of effects, PGE\textsubscript{2} may be unable to reverse the toxic effects of indomethacin. Indeed, PGE\textsubscript{2} may have a cooperative role in embryo expansion and hatching, i.e., it may require other prostaglandins, such as PGI\textsubscript{2}, to affect embryo hatching rate. Indomethacin inhibits the cyclooxygenase enzyme pathway, so arachidonic acid may have been metabolized by the lipoxygenase pathway, and the lipoxygenase products have
been demonstrated to decrease PGI$_2$ production by luteal cells (Milvae et al., 1986). The effect of lipoxygenase products on PGE$_2$ synthesis is unknown. Therefore, the lipoxygenase products may have blocked a PGE$_2$ response when coincubated with indomethacin. The use of specific prostaglandin inhibitors may help accurately determine the involvement of PGE$_2$ in embryonal hatching, but such inhibitors are not yet available for general use.

In summary, early ovine embryos can metabolize arachidonic acid to various prostaglandins. Prostaglandins may be involved in embryo hatching and other developmental events of the embryo. The biochemical relationships between the embryo and the uterine environment may control embryonal prostaglandin production and ultimately embryonic development. A better understanding of the relationships between the uterus, developing embryos and factors involved in embryonic development are essential for an understanding of embryonal mortality. Embryonal deaths reduce the reproductive performance and profitability of livestock production. Therefore, it is essential to understand the factors controlling embryonic development so that the proper uterine environment for embryonic development can be maintained and reduce the percentage of embryonal deaths.
IMPLICATIONS

Early embryonic development is a critical period for embryonal mortality. Embryonal mortality reduces the reproductive efficiency of livestock. Reduced reproductive efficiency burdens producers financially and restricts the use of embryo transfer procedures. Until one understands the development of a normal embryo, one cannot determine what is abnormal. This thesis focused on one developmental stage, zonal loss, and indicates a possible role of prostaglandins in that developmental stage. If one can understand normal embryonic development one may be able to determine the causes of and possibly reduce, embryonal mortality.
LITERATURE CITED


Austin, C.R. 1961. The Mammalian Egg. Thomas; Springfield, IL.


Appendix I

CL.................Corpus luteum
PGG2................Prostaglandin G2
PGH2................Prostaglandin H2
PGF2α...............Prostaglandin F2α
PGE2...............Prostaglandin E2
PGI2...............Prostacyclin
6-keto-PGF1α.........6-keto-prostaglandin F1α
HHT................12L-hydroxy-5,8,10-heptadecatrienoic acid
TXA2.................Thromboxane A2
PGFM.................13,14-dihydro-15-keto-prostaglandin F2α
PGA2...............Prostaglandin A2
PGB2...............Prostaglandin B2
TXB2...............Thromboxane B2
HPLC...............High performance liquid chromatography
CNCH3..............Acetylnitrile
dpm...............Disintergrations per minute
cpm...............Counts per minute
SCR................Sample channels ratio
PLA2...............Phospholipase A2
TPGWOAA..........Total picograms without the arachidonic acid region
NA....................Not available
VITA

Brian Lynn Sayre, son of Darryl and Ruth Sayre, was born August 9, 1967 in Jacksonville, Illinois. He graduated from Franklin High School in May, 1985. He received his Bachelor of Science degree in Animal Science from the University of Illinois in May, 1989. He began work on his Master of Science degree in Animal Science (Physiology of Reproduction) under the direction of Dr. G. S. Lewis at Virginia Polytechnic Institute and State University in August, 1989. He is a member of the American Society of Animal Science. His immediate plans are to pursue a Doctor of Philosophy degree in Animal Science (Physiology of Reproduction) at Virginia Polytechnic Institute and State University.

Brian L. Sayre