# THE EFFECT OF HEAT STRESS ON EARLY EMBRYONIC DEVELOPMENT IN THE BEEF COW

By

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# THE EFFECT OF HEAT STRESS ON EARLY EMBRYONIC DEVELOPMENT IN THE BEEF COW

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

# CHAPTER I

## INTRODUCTION

The ideal results of a perfect breeding season for the cow-calf operator is to achieve a 100 percent conception rate within the breeding herd. However, early embryonic mortality accounts for a large portion of pregnancy failure in all domestic animals. In cattle, prenatal death losses of up to 40 to 50% of all ova fertilized may occur, which is especially inefficiect to the dairyman as well as average calf weaning weight for the beef producer (Ayalon, 1978.) Approximately 20% of the pregnancy failure in cattle results from the death of the early embryo (Beardon et al., 1956). Environment can be a possible factor contributing to early embryonic mortality through its influences on the reproductive endocrine system as well as the local uterine environment (Ayalon, 1978).

With the use of artificial insemination, the effect of high environmental temperatures on cattle fertility point to the dam as a contributor to seasonal (late summer) infertility. If cows are exposed to a thermal environment of $37^0$C for 3 days postbreeding, conception rates of zero are not uncommon (Dunlap and Vincent, 1971). Besides the effect of heat stress during fertilization, it also appears that a

hyperthermic environment may be responsible for a significant portion of embryonic loss before 35 days of pregnancy (Stott and Williams, 1962).

Since the reproductive process is controlled by the hypothalamic-pituitary-ovarian endocrine system, any shift in the hormone balance has the potential to be detrimental to the establishment and maintenance of pregnancy. The effects of high ambient temperatures on the reproductive endocrine system is well documented, as heat stress can alter plasma concentrations of progesterone (Gwazdawskas et al., 1973; Collier et al., 1982), estradiol (Gwazdawskas et al., 1974; Folman et al., 1983), and luteinizing hormone (Madan and Johnson, 1973). Heat stress can also lead to irregular estrous cycle lengths and depress overt estrus expression, resulting in reproductive inefficiency (Fuquay et al., 1970).

The state of the uterine environment is of critical importance for the viability of the embryo, as this is the location of blastocyst hatching, growth, development and attachment. If any of these processes are hindered, the chain of normal events leading up to the maternal recognition of pregnancy and placental development could possibly be jeopardized (Thatcher, 1974; Alliston et al., 1961; Dutt, 1963).

Although it has been previously shown that early pregnacy in the cow (Day 1-4) can be affected by a

hyperthermic environment (Gwazdauskas et al., 1973), the effect of thermal stress during the time of blastocyst development from Day 10 to 17 of pregnancy is unknown. It is important to look at the hormonal and uterine changes that might take place during the time near establishment of pregnancy,(maternal recognition) by the conceptus during exposure to elevated ambient temperatures. Changes in hormonal and uterine secretion may cause embryonic mortality at the time of rapid blastocyst development and maternal recognition of pregnancy.

The review of literature which follows, attempts to present the current state of knowledge regarding the effects of thermal stress on the maternal endocrine system and early embryonic development and survival. Placentation, blastocyst development and uterine function will be discussed as to the effects that alteration of normal synchrony between the embryo and the uterus may cause pregnancy failure. Emphasis is placed on the bovine female as the model for this review.

# CHAPTER II

# LITERATURE REVIEW

# ENDOCRINE FUNCTION

## Estrous Cycle

The estrous cycle of the cow is approximately 21 days in duration with a range of 18 to 24 days. The estrous cycle is normally divided into four periods: proestrus, estrus, metestrus, and diestrus.

Proestrus is characterized by follicular growth and increased plasma estradiol-17B (E2) concentrations stimulated by rising levels of follicle stimulating hormone (FSH) from the anterior pituitary (Hansel and Snook, 1970). Estradiol-17B is produced by both the granulosa and thecal cells of the follicle (Lacroix et al., 1974; Hendricks and Mayer, 1977). The thecal cells also have the ability to produce substantial amounts of androstenedione (an estrogen precursor) while granulosa cells have a greater capacity to transform androstenedione to E2 when compared with the thecal cells. This supports the view of a positive interaction between granulosa and thecal cells and demonstrates that E2 production occurs chiefly within the granulosa cells of the follicle (Lacroix et al., 1974). Thus, increased follicular development results in

elevated plasma E2, which rises from a concentration of 4 pg/ml to a level of 6 pg/ml before estrus (Mellin and Erb, 1965; Erb et al., 1971; Garverick et al., 1971; Schams et al., 1977). Plasma progesterone (P4) declines to baseline levels of less than 1 ng/ml 2 days before estrus due to the regression of the corpus luteum (CL) (Gomes and Erb, 1965; Melampy and Anderson, 1968; Hansel and Snook, 1970; Erb et al., 1971; Garverick et al., 1971). Proestrus occurs about 1-2 days before the expression of standing heat (estrus).

Estrus in the bovine averages approximately 18 hours with a range of 6 to 30 hours (Schams et al., 1977). This is the time period in which the cow becomes receptive to the bull, with E2 levels rapidly increasing in the circulating plasma (10-12 pg/ml) due to continued follicular growth and development (Wettemann et al., 1972; Glencross et al., 1973; Schams et al., 1977). Rapid development of the preovulatory follicle is stimulated by FSH, which is initially elevated during estrus (Schams et al., 1977). However, towards the end of estrus, FSH begins to decline while Luteinizing Hormone (LH), also from the anterior pituitary, begins to increase in concentration (Hackett and Hafs, 1969; Hendricks et al., 1970; Schams et al., 1977).

The concentration of LH during the estrous cycle is normally low (1 < ng/ml), but during estrus rises to a

Schams et al., 1977). This surge of LH stimulates ovulation of the mature graffian follicle causing release of the ovum from the follicle. The LH surge peaks 26 hours before ovulation and with concentrations greater than 1 ng/ml occuring over 7-8 hours (Schams and King, 1969; Hansel and Snook, 1970; Hendricks et al., 1970; Schams et al., 1977). Ovulation usually occurs 10 to 20 hours after the end of estrus in the beef cow (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Marion et al., 1950). Following the LH surge, the concentration of LH in the plasma decreases to basal levels until the initiation of the next proestrus period. The cells of the ruptured graafian follicle undergo a differentiation process called luteinization, stimulated by LH (Hansel and Snook, 1970). The follicular cells develop into luteal cells of the functional corpus luteum. Under the stimulation of LH, the cells of the CL begin to produce and secrete the steroid hormone progesterone (P4) (Schams and Karg, 1969; Hansel and Snook, 1970; Hendricks et al., 1970; Garverick et al., 1971). Plasma progesterone concentration during metestrus is less than 1 ng/ml, gradually increasing to 2-3 ng/ml at the end of metestrus (Gomes and Erb, 1965; Hansel and Snook, 1970;Wettemann et al., 1972; Schams et al., 1977).

The longest period of the estrous cycle is diestrus, lasting from approximately Day 5 through Day 19 (Gomes and Erb, 1965; Salisbury et al, 1978). The CL increases P4

production from 1-2 ng/ml on Day 5 to 4-5 ng/ml by Day 10 with peak levels occuring on approximately Day 15. The function of P4 at this stage is to prepare the uterus for pregnancy by causing thickening of the uterine endometrium and development of secretory glands (Gomes and Erb, 1965; Salisbury et al., 1978).

During the late luteal phase, the uterine endometrium secretes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the uterine lumen (Bartol et el., 1981) and the uterine veinous drainage (Shemesh and Hansel, 1975).If the ovum has not been fertilized and/or pregnancy not established by Day 17, release of $PGF_{2\alpha}$ by the uterus into uterine-ovarian vascular system causes CL regression with P4 levels subsequently declining to basal levels (Glencross et al., 1973; Schams et al., 1977; Ireland et al., 1979).

The means by which luteolysis is stimulated is thought to be controlled by the release of $PGF_{2\alpha}$ from the uterus. Typical spike-like releases of $PGF_{2\alpha}$ into the uterine venous blood, and the appearance of 13, 14 dihydro-15-keto prostaglandin F metabolite (PGFM) in peripherial plasma, occur at the time of luteolysis in the cow (Rowson et al., 1972; Hixon and Hansel, 1974; McCracken et al., 1984). Corpus luteum regression can be induced earlier in diestrus by exogenous $PGF_2$ administration (Louis et al., 1974; Watts and Fuquay, 1985). Along with $PGF_{2\alpha}$, oxytocin (mainly of ovarian origin) could possibly contribute to luteolysis

through the release of uterine $PGF_{2\alpha}$. In the ewe, oxytocin concentrations are high in late diestrus luteal tissue (Flint and Sheldrick, 1982) and immunization aginst oxytocin has been demonstrated to delay luteal regression (Sheldrick et al., 1980). Production of estradiol-17B from the ovarian follicles is also essential for luteolysis since removal of follicles and inhibition of follicular growth of both ovaries during diestrus results in extended luteal function (Fogwell et al., 1985; Villa-Godoy et al., 1985). This observation is supported by Brunner et al. (1969) who demonstrated that exogenous E2 caused premature luteal regression in cattle. McCracken et al. (1984) proposed the hypothesis that luteolysis is caused by follicular production of E2, stimulating the formation of endometrial oxytocin receptors, which when stimulated by oxytocin induces $PGF_{2\alpha}$ release from the uterine endometrium. This, in turn stimulates further luteal oxytocin release and so a positive feedback loop is established. The uterine $PGF_{2\alpha}$ then continues to be released into the uterine vascular network where it travels to the ovary through the counter-current exchange at the ovarian pedicle causing CL regression at approximately Day 17. This hypothesis is supported by Hixon et al. (1983) who demonstrated that injections of $PGF_{2\alpha}$ and E2 act synergisticly to cause luteolysis as evidenced by shortened cycle length. The observation that increased secretion of E2 during diestrus

of ewes is associated with secretion of uterine $PGF_{2\alpha}$ (Ford et al., 1975) also supports the hypothesis of an estrogen-$PGF_{2\alpha}$ involvement in luteal regression.

Regression of the CL results in a dramatic decline in P4 concentration starting on Day 17-18 (Glencross et al., 1973; Chenault et al., 1976; Schams et al., 1977; Ireland et al., 1979). While the concentration of LH begins to gradually increase on Day 17, FSH maintains a wavelike pattern with peaks on Day 8, 12 to 13, and 17 to 18, resulting in an increase in follicular growth. Production and secretion of E2 increases slightly in late diestrus due to follicular growth, increasing from about 2 pg/ml on Day 17 to 4 pg/ml on Day 19 (Chenault et al., 1976; Schams et al., 1977).

**Pregnancy**

If the ovum is fertilized and the blastocyst continues to develop normally, the plasma progesterone levels in the cow change from a cyclic pattern to one that is continuous and uninterupted throughout most of the 280 day gestation. The reason for the extended maintenance of the plasma progesterone levels is that the corpus luteum has been maintained through production of a blastocyst factor which is luteostatic and/or luteotropic.

Beginning with estrus and through the first 17 days, endocrine patterns during pregnancy are similar to those that occur during the estrous cycle. Lutenizing hormone

surges normally just prior to estrus and declines rapidly to 1 ng/ml by Day 1 postestrus and remains at this low level throughout pregnancy (Wettemann and Hafs, 1973).

Estradiol-17B also follows similar levels in early pregnancy compared to cyclic cows. After the increase at estrus, E2 decreases from approximately 12 pg/ml to 8 pg/ml by Day 4 and ranges between 3-9 pg/ml during the first 30 days of pregnancy (Hendricks et al., 1972; Wettemann and Hafs, 1973; Lukaszewska and Hansel, 1980) but tends to increase by Day 17 postestrus, indicating either an increase in ovarian production and/or a contribution of E2 coming from the conceptus (Shemesh et al., 1979; Lukaszewska and Hansel, 1980).

Progesterone profiles in the early pregnant cow are similar to those of the cyclic cow in that P4 is less than 1 ng/ml at estrus and increase to 2 ng/ml by Day 3. Concentrations of progesterone increases to 7 ng/ml on Day 11 and reaches 8-12 ng/ml by Day 12 to 13. However, since the CL is maintained after Day 17 in the pregnant cow, P4 concentrations remain elevated and are maintained between 8-14 ng/ml from Day 18 to Day 75 (Hendricks et al., 1972; Glencross et al., 1973; Wettemann and Hafs, 1973; Bartol et al., 1981). Lukaszewska and Hansel (1980) have suggested that plasma P4 concentration is greater from Day 10 to 18 postestrus in the pregnant compared to nonpregnant cows, suggesting a possible luteotrophic effect of the conceptus.

## UTERINE FUNCTION

During early pregnancy, the bovine embryo spends a relatively long period of time unattached to the uterine epithelium with attachment not occuring until considerable growth and development of the embryo has taken place. Since the embryo develops in and is nourished by the histotroph found in the luminal fluids during this time (McLaren, 1980), any alteration of this environment may contribute to retarded embryonic development and/or increase the incidence of embryonic death.

While the embryo is undergoing cleavage and blastocyst formation (Day 2 to 8), the uterus is also undergoing developmental and secretory changes, preparing for placental attachment. During this progestational stage, the muscular activity of the uterus decreases helping to retain the blastocyst in the uterine lumen (Foley and Reece, 1953.). At the same time, blood flow to the uterine horn containing the conceptus increases 2 to 3 fold compared to the nongravid horn, indicating a local control of uterine blood flow by the conceptus.This change in uterine blood flow may function to create optimal conditions within the uterus for the continuation of pregnancy (Ford et al., 1979).

Associated with the increased progesterone levels from the CL, changes occur in the secretory activity of the glandular and surface epithelium of the uterine endometrium (Bartol et al., 1985b). High molecular weight compounds

(proteins, glycoproteins and mucopolysaccharides) accumulate in the uterine lumen along with cellular debris and plasma constituants which pass into the uterine lumen, forming the histotroph which provides nourishment for the embryo prior to establishment of placental attachment (McLaren, 1980).

Considerable research and speculation has been published on the proteins which make up uterine histotroph. Marinov and Lovel (1968) observed that an apocrine type secretion occured along with the release of lipids into the uterine lumen. However, the amount of secretion from the uterine glands reportedly did not change throughout the estrous cycle. It has since been shown that the uterine fluids undergo qualitative changes during the estrous cycle and pregnancy (Bartol et al., 1981, 1985b). The change occurs because of the biological requirement to establish a intrauterine environment capable of sustaining the bovine conceptus (Sreenan, 1978). Roberts and Parker (1974a) reported the appearance of two unique proteins of uterine origin on Day 13 or 14 of pregnancy. Since this is at the time of rapid blastocyst elongation and also the time of maximal P4 production, it is thought that these proteins are P4 dependent and may influence the embryonic development at this stage. Roberts and Parker (1976) and Bartol et al. (1981, 1985b) demonstrated that there is a marked change in the electrophoretic protein patterns on Day 14 of pregnancy. These observations can be compared to uterine specific

proteins detected in the pig at a similar stage of development (Murray et al., 1972) which are also undergoing qualitative changes at this time (Squire et al., 1972). Several of the uterine proteins in the pig have been shown to be P4 dependant (Knight et al., 1973). Changes in the protein concentrations within the bovine uterine lumen change later in pregnancy as Roberts and Parker (1976) observed more qualitative and quantitative differences in the uterine protein profile after Day 17 of pregnancy. More recent data from Bartol et al. (1981, 1985b) indicates that total protein within the uterine lumen is not altered between Days 8 to 19 of pregnancy however, a greater array of proteins of varying molecular weights are present on Day 19 of pregnancy compared to Day 19 of the estrous cycle. These differences probably occur through contribution of both endometrial and conceptus secretion.

Besides the qualitative changes in luminal proteins, the activities of several glycosidases are elevated in the uterine lumen when compared with serum. Since glycosidases have the capability of modifying glycoprotein and glycolipid structures, these enzymes have been suggested to be involved with dissolution of the zona pellucida during hatching, alteration of cell structure during the rapid growth phase of the blastocyst, and possibly in the initiation of the adhesive phase of implantation (Roberts and Parker, 1974b).

Also included in histotroph is a variety of ions,

including potassium, calcium, sodium, zinc, phosphorous, and iron (Olds et al., 1965; Biggers and Borland, 1976; McLaren, 1982). Little work has been done with these ions in the bovine uterine secretions and their role(s) in blastocyst formation is not known. However, potassium and calcium were the only ions found to be essential for development in the blastocyst stage of the mouse (Wales, 1970) whereas both calcium and ferrous iron are necessary for development of the preimplantation rabbit embryo (Biggers and Borland, 1976). Potassium ions are present in higher concentrations in uterine secretions than in serum while zinc and calcium concentrations vary before, during, and after implantation in the rabbit. Any or all of these factors may influence blastocyst growth and placental formation (McLaren, 1982). The result of all the constituents of the uterine fluid results in a uterine luminal pH of approximately 7.1 (Olds et al., 1956).

Thus, the uterine fluid which provides the environment for the developing embryo is constantly changing (Olds et al., 1956) in an attempt to provide the ideal setting in which the embryo may be nourished, attach to the uterine luminal epithelium and continue with it's growth and development. Shifts in the uterine environment may easily result in hostility to the development of the embryo and result in pregnancy termination.

## EMBRYONIC DEVELOPMENT: FERTILIZATION TO PLACENTATION

### Fertilization and Oviductal Transport

For pregnancy to be established, the ovulated ovum must be fertilized by a capicated sperm cell, which usually occurs in the lower isthmus of the oviduct. The fertile life span of the sperm in the genital tract of the cow is approximately 30 to 48 hours, after which they lose their viability to fertilize ova or produce viable embryos. The bovine ovum is capable of being fertilized up to 20 to 24 hours postovulation. Insemination of the cow between 6 and 24 hours before ovulation results in the greatest conception rates (Polge and Baker, 1976; Iritani and Niwa, 1977).

In the cow, sperm must undergo a biological change called capacitation before they can undergo the acrosome reaction necessary for penetration through the zona pellucida of the ovum (Lauderdale and Ericsson, 1970; Iritani and Niwa, 1977; Bedford, 1983). Because the process of fertilization is so biochemically oriented (Anderson, 1977; Bedford, 1983), changes in the oviductal environment in which fertilization takes place can interfere with fertilization and thus reduce conception rates.

It takes approximately 3 to 4 days before the fertilized bovine ovum enters the uterus from the site of fertilization, during which it is undergoing the process of cleavage (Hamilton and Laing, 1946). By approximately 24 hours after fertilization, the first cleavage has taken

place, the result of which is the 2-cell stage embryo. The 8-cell stage is reached approximately three days postovulation, while it is a 16-cell stage embryo that enters the uterus on Day 4. During this peroid of development, the cleaving embryo is still contained within the zona pellucida and retains its origional spherical shape (Winters et al., 1942; Hamilton and Laing, 1946; Perry, 1982). The rate of transport through the oviduct is timed as the embryo must enter the uterus at a precise time of the estrous cycle so that the uterine environment is not hostile to the embryo (Hafez, 1980).

Transport of the ovum through the oviduct is hormonally controlled as estrogen and progesterone control oviductal smooth muscle contractions while an increase in oviductal secretions from the oviductal epithelial lining increases under progesterone stimulation (Hafez, 1980). Chang (1966) concluded that exogenous or endogenous estrogen causes rapid egg transport through the oviduct while endogenous or exogenous progesterone counteracts the estrogenic effect and slows egg transport in the rabbit. Administration of estrogen after ovulation speeds the transport of eggs and causes their degeneration or expulsion from the uterus (Chang, 1966). Oviductal fluid is rich in substrates and cofactors such as amino acids, oxygen, carbohydrates, lipids and steroids which are involved with stimulating embryonic development (Olds et al., 1956; Hafez, 1980). However,

dioxide production of rabbit embryos increased drasticly between 4 and 5 days postfertilization, indicating an increasing metabolic turnover and a changing nutritional requirement for those embryos of more advanced cellar stages (Wales, 1975). Thus, oviductal and uterine secretions must be in synchrony with the rapidly developing embryo in order for proper conceptus development to occur.

**Early Conceptus Development**

The embryo is a "free floating" entity within the uterine lumen, sustained by uterine histotroph from the time that it enters the uterus on Day 3 to 4 postovulation until it begins attachment to the endometrium on approximately Day 20. During its early development within the uterine environment, the bovine blastocyst undergoes a series of expansions and contractions on Day 8-9, causing a split in the zona pellucida for its escape (McLaren, 1970). The process in which the blastocyst frees itself from the zona pellucida is called the hatching (McLaren, 1970; Sugie et al., 1980; Perry, 1982).

The blastocyst, now freed from the zona pellucida, remains spherical in shape and is composed of different layers of dividing cells. It is from these newly formed germ layers that the hatched blastocyst will develop into an embryo with its surrounding extraembryonic membranes (Greenstein and Foley, 1958). The conceptus retains a spherical morphology from the time of hatching until about

Day 13. After Day 14, the blastocyst undergoes a period of rapid growth and elongation. The bovine blastocyst measures 15-20 mm in length on Day 15 and 40-100 mm on Day 16, with the blastocyst extending through the ipsilateral horn by Day 17 and entering the contralateral horn by Day 18 (Chang, 1952; Greenstein et al., 1958; Greenstein and Foley, 1958; Betteridge et al., 1980). The conceptus, just prior to the period of maternal recognition of pregnancy (Day 17), is comprised of the embryonic disc and trophoblast (trophectoderm and extraembryonic endoderm). At this time, the trophoblast is approximately 50 times longer than the embryonic disc (Thatcher et al., 1985). The third germ layer, the mesoderm, has developed and may appear between the extraembryonic ectoderm and endoderm by Day 17.

**Placentation**

Placental attachment to the epithelial surface of the uterine endometrium provides a method of communication and a route for transport of nutrient and waste products between the developing conceptus and maternal vascular system. Placentation occurs first by apposition followed by adhesion and attachment between the trophoblast and uterine luminal surface epithelium.

After hatching through the zona pellucida, the blastocyst begins to align and orient itself in preparation for attachment. Apposition of the trophoblast to the uterine luminal epithelium is initiated on Day 17 to 18 of pregnancy

(King et al., 1981).

The apposition phase is characterized by a reduction in the maternal epithelial cell height, which may be in response to the presence of the trophoblast (King et al., 1981). The ultrastructure of the uterine epithelium of the Day 7-16 pregnant cow is similar to that observed in cyclic animals. Apical cytoplasmic projections, which are simply modifications of the uterine cell surface, are present on Days 12 to 16 in both cyclic and pregnant cows, indicating that progesterone may be involved in their formation. However, on Day 18 to 21 of pregnancy, these projections are still present on the caruncular and intercaruncular areas in both uterine horns while these protrusions have disappeared in cyclic animals by Day 18-21, suggesting an effect of the conceptus on maintenance of CL function. These cytoplasmic projections have been suggested to be involved with either an apocrine or merocrine type of secretion (King et al., 1981; Guillomot and Guay, 1982).

On Days 10 to 16, the trophoblastic cells of the conceptus have a rounded appearance with a surface covered by an abundant network of slender microvilli 1-2 um long. These microvilli become reduced in number by Day 18. Microvilli covering the trophoblastic cell surface during the early stages of gestation may be involved in the absorption of extracellular products by the conceptus during the time prior to uterine attachment (Guillomot and Guay,

1982).

At Day 20, placentomes can be recognized as discrete oval structures on the surface of the uterine lumen. The first firm contact between the trophoblast and the uterine epithelium is observed on Days 20 to 21 as some of the microvilli present on the maternal cells begin indenting the apical borders of the adjacent trophoblast cells. The trophoblast cells are now spindle-shaped and the cellular surface is irregularly ridged and completely devoid of microvilli. The portion of the conceptus located in the horn contralateral to the corpus luteum is still not adhered. Microvilli still cover the nonadherent trophoblastic cell surfaces but are absent from the adherent portion in the ipsilateral horn (King et al., 1980; Guillomot and Guay, 1982). By Day 24, the microvilli are present on some trophoblastic cells and, while their presence is not uniform, those present are interdigitated with the maternal surface epithelium, progressing to intimate attachment by Day 27 (King et al., 1980, 1981).

In addition to the development of trophoblastic adhesion and attachment to the maternal epithelium in the intercaruncular region between Days 22 and 27, the implantation process involves the simultaneous formation of definitive placentomes. The size of the placentomes and intimacy of attachment increase by Day 29 but no villi or crypts have formed at this time. Although the caruncular

surfaces are smooth during this period, there is apposition and tenuous adhesion between the chorion and maternal epithelium between Day 20 to 29 (King et al., 1980).

Besides the cellular contact established between the trophoblast and uterine epithelium, the portion of the trophoblast overlying the uterine glandular openings develops conical papillae about 50 um in lenght at Day 20-21. These papillae could be involved in absorption of the glandular secretions and act as a means of immobilizing the conceptus in the uterine cavity during attachment (Guillomot and Guay, 1982).

Actual firm attachment of the chorion to the maternal cotyledons lining the uterus begins on Day 30 and continues through Day 35. This is the beginning of the cotyledonary epitheliochorial placenta typical of ruminant species. The placentomes of the chorionic tissue covering the knob shaped caruncle of the endometrium first form in the vicinity of the embryo (Melton et al., 1951; Foley and Reece, 1953; Perry, 1982).

A few binucleate cells, which are observed in the trophoblast as early as Day 17 to 19, are found in contact with the maternal epithelium between Day 20 to 29 (Greenstein et al., 1958; King et al., 1980; Wathes and Wooding, 1980; Wooding, 1984). Binucleate cells, which form from unicellular chorion cells (a doubling of DNA without cell division) at the basement membrane of the chorion,

migrate toward the apical surface of the chorion. As the binucleate cell migrates toward the uterine epithelial interface, it increases the formation of cytoplasmic granules thought to contain placental lactogen (Flint et al., 1979).

By Day 20, a number of multinuclear cells are present within the maternal epithelium, of which the most common is the binucleate giant cell (King et al., 1980). Because the chorion maintains a close association with the maternal epithelium from Day 20 to parturition, the migrating binucleate cells are able to migrate from the chorion to the maternal epithelium.These migrating binucleate cells fuse with the uterine epithelial cells to form the multinucleate cells of a typical syncytium (King et al., 1981; Wooding, 1984). Binucleate cell migration and mulitnucleate cell formation is accompanied by a degeneration of the maternal epithelial cells, possibly through an alteration of the local environment caused by the binucleate cells. However, this loss of surface epithilium is only temporary in the cow (Wooding, 1984). By Day 24, the multinucleate giant cells account for nearly 50% of the epithelial area (Wathes and Wooding, 1980).

Binucleate cell migration continues throughout gestation in the cow but multinucleate cell formation ceases on about Day 28. Syncytial formation occurs at Day 20 to 30 in the cow in the caruncular and intercaruncular areas.

Therefore, after Day 28, binucleate cell fusion to uterine epithelium is only a transitory process which allows release of its product (placental lactogen and other protein material) followed by regression (Flint et al., 1979; Wooding, 1984).

The migration of the binucleate cell may function to transport blastocyst products to the maternal circulation without degradation. Syncytial formation is a method of reducing the distance between the fetal and maternal capillary beds, with the resulting fusion allowing for the delivery of the binucleate cell granules to the maternal circulation while still maintaining the trophectodermal barrier for other feto-maternal exchange. Also, the syncytium represents the formation of a feto-maternal hybrid tissue, which may also serve as an essential immunological barrier (Wooding, 1984). Because of the timing and intricate nature of the establishment of pregnancy in the cow, any alteration in this relationship could cause a decrease in placental development, failure of attachment, and increase in embryonic death.

## MATERNAL RECOGNITION OF PREGNANCY

During the time that the embryo is going through a rapid growth phase, it must also extend the life span of the corpus luteum by signaling the maternal system, referred to as "maternal recognition of pregnancy".

In order for pregnancy to be continued, the corpus

luteum must be rescued from luteolysis and the production of progesterone maintained. Rowson et al. (1972) indicated that successful embryo transfer in cattle requires that estrous cycles of donor and receipient cows be in synchrony by ± 24 hours. These data indicate that uterine development closely follows that of the developing embryo. Removal of the bovine embryo from the uterine horn before Day 17 of pregnancy results in normal cyclicity of cows whereas embryo removal on Day 17, 18 or 19 resulted in extended luteal lifespan (Northey and French, 1980). Daily intrauterine infusions of 17 and 18 day embryo homogenates to cows on Day 14 through 18 of the estrous cycle lengthened the interestrous interval by delaying regression of the corpus luteum (Northey and French, 1980). Similarly, embryo transfer is successful between Day 10-16 but not after Day 17, demonstrating that the embryo must be present by Day 16 in order to prevent luteolysis. Thus, maternal recognition of pregnancy in the bovine occurs approximately on Day 17 of gestation (Betteridge et al., 1980).

The actual mechanism(s) and substance(s) by which the bovine embryo signals the maternal system to maintain pregnancy is not known. Placental lactogen, produced by the binucleate cells of the blastocyst, is present at this time (Flint et al., 1979). However, it is generally felt that its apperance is merely coincidental and probably not involved in the supression of luteolysis (Flint et al., 1979;

Betteridge et al., 1980). Placental lactogen synthesis and release is possibly involved in placentation and/or the prevention of maternal immuno-rejection (King et al., 1980).

The blastocyst has considerable steroidogenic capability as progesterone, testosterone and estradiol-17B are synthesized and released byblastocysts curtured in vitro (Shemesh et al., 1979). Androstenedione and P4 can be metabolized by conceptus tissues to 5B-reduced steroids and E2 beginning on Day 15 of pregnancy (Eley et al., 1979b, 1983). Bovine conceptus synthesis of estradiol-17B and estrone is greatest on Day 16, which corresponds to the time when uterine blood flow to the gravid horn increases significantly compared to that to the nongravid horn (Ford et al., 1979; Gadsby et al., 1980; Ford and Chenault, 1981). However, E2 production by the bovine blastocyst is minimal in comparison with the amounts of E2 produced by the pig conceptus during early pregnancy (Gadsby et al., 1980). Estradiol administration is luteotropic and/or luteostatic in the pig (Frank et al., 1977) but luteolytic in the cow (Eley et al., 1979a). Therefore, if blastocyst E2 production is active in the cow, its effect is local (with uterine endometrium) rather than a systemic signal to induce CL maintenance (Gadsby et al., 1980). The production of prostaglandins by the conceptus during early pregnancy (Lewis et al., 1979b, 1980, 1984) may function in bovine blastocyst steroidogenesis (Shemesh et al., 1979) and/or

blastocyst development. The synthesis and maintenance of uterine secretion may be stimulated by the steroids and prostaglandins produced by the blastocyst (Lewis et al., 1980; Bartol et al., 1981).

Both the bovine blastocyst and endometrium are capable of prostaglandin ($PGF_{2\alpha}$ and $PGE_2$) production in vitro. When incubated seperately, bovine blastocysts recovered on Day 19 of pregnancy produce more prostaglandin and have a higher specific activity per milligram of tissue than endometrial slices (Lewis, 1984). However, it appears that the conceptus regulates the endometrial accumulation of $PGF_{2\alpha}$ and $PGE_2$ as the quantity of these two lipid molecules is lower when blastocyst tissue is incubated with endometrial tissue (Thatcher, 1985). This may be due to either metabolism of $PGF_{2\alpha}$ and $PGE_2$ by the endometrial tissue and/or suppression of endometrial synthesis by blastocyst tissue. Thus, in vivo the blastocyst may play a role in regulating the rate of prostaglandin synthesis and release from the uterine endometrium, which may be important for maintaining the synchrony between the blastocyst and the uterus.

Many researchers believe that one or more of the proteins synthesized and secreted by the bovine conceptus during early pregnancy may be the signal for maternal recognition of pregnancy (Lewis et al., 1979b; Bartol et al., 1981, 1985a; Beal et al., 1981; Knickerbocker et al., 1984; Thatcher et al., 1985 Helmer et al., 1985). Lewis et

al. (1979b) indicated that bovine blastocysts are capable of de novo protein synthesis. Bartol et al. (1981, 1985a) reported that four families of polypeptides were released by the bovine blastocyst in vitro. Knickerbocker et al. (1984) demonstrated that intrauterine administration of bovine conceptus secretory proteins (bCSP) to cyclic cows on Day 15 extended CL function 8 days beyond control cows. In addition, bCSP production/mg wet weight of blastocyst tissue appears to be elevated during the time of the rapid expansion between Days 16 to 18, corresponding to the time that the embryo signals maternal recognition.

It has not been established whether these proteins act as a luteotropic or antiluteolytic signal for the maintenance of the activity and lifespan of the CL. Beal et al. (1981) found that homogenates and extracts of Day 18 bovine blastocysts are luteotropic in that they stimulate P4 synthesis by luteal cells in vitro. However, conceptus secretory proteins infused into the uterine lumen did not appear to stimulate luteal synthesis of P4 in vivo but exerted an antiluteolytic effect that extended the life span of the CL (Knickerbocker et al., 1984; Thatcher et al., 1985). The contention of an antiluteolytic effect for conceptus secretory proteins is supported by the presence of 10 times more $PGF_{2\alpha}$ in the uterine lumen and endometrium of pregnant compared to cyclic cows. This suggests that secretion of $PGF_{2\alpha}$ into the blood is suppressed, therefore

not allowing its luteolytic action on the CL to occur (Bartol et al., 1981).

The characteristic nature of the protein(s) produced and secreted by the blastocyst during the time of maternal recognition has been the subject of recent work. The patterns of proteins produced by Day 19 and later stage conceptus tissues are more complex than that of a Day 16 conceptus and have a quantitative increase in protein production (Bartol et al., 1985a). As previously stated, both homogenates and extracts of Day 18 bovine blastocysts are luteotropic in that they stimulate P4 synthesis by luteal cells in vitro (Beal et al., 1981). Luteotropic activity of the blastocysts appears to be due to one or more heat-labile substances with a molecular weight of less than 12,000 daltons. Thus, Day 18 blastocysts contain small molecular weight polypeptides that may stimulate P4 synthesis, resulting in prolongation of pregnancy (Beal et al., 1981). Helmer et al. (1985) characterized bovine trophoblastic protein (bTP-1) and found that is immunologically and molecularly similar to oTP-1, which is a trophoblastic protein produced by the ovine conceptus trophoblast that extends luteal function in the ewe (Godkin et al., 1984). Bovine trophoblastic protein-1 is produced and secreted by the conceptus between Day 16-24 of pregnancy and may have an essential antiluteolytic role during pregnancy. Alteration in the quantitative or qualitative

aspects of protein production by the conceptus before and during the time of maternal recognition could result in the inability of the conceptus to signal the maternal system to maintain the pregnancy.

## HEAT STRESS

As illustrated in the previous sections, the processes of estrus, fertilization, maternal recognition and placentation are highly complex and are easily altered if changes occur in the hormonal balance of the maternal system. The result of such an alteration in the endocrine system may result in failure of the cow to maintain pregnancy.

Unfavorable environmental factors can stress the cow during pregnancy, possibly leading to an alteration of endocrine function. One possible contributor of external environmental stress is that stimulated by elevated ambient temperature.

Exposure to high environmental temperatures causes cattle to absorb heat from the surrounding environment. If the cow absorbs heat at a greater rate than can be dissipated through conduction, convection, radiation and evaporation, a rise in body core temperature as evidenced by a rise in rectal temperature will result (Fallon, 1962; Bond and McDowell, 1972; Mills et al., 1972; El-Nouty et al., 1976; Gwazdauskas et al., 1981). Cattle have a variety of

mechanisms to minimize rises in body temperature due to high environmental temperature. Cattle are considered to be homeothermic in that they maintain a constant body temperature over a wide range of environmental extremes (Schmidt-Neilsen, 1981). Changes in the rate of sweating, respiration rate, body metabolism, blood flow and hormone profile can be utilized by the cow in an attempt to maintain a constant set point temperature.

**Sweating**

Cattle respond to heat stress by actively secreting sweat onto the skin surface by way of sweat glands that are associated with the hair follicle unit. Sweating utilizes the physics of evaporatory heat loss to cool the skin surface. Because the bovine has many sweat glands present in the skin, the process of sweating can be used as the major means by which the body is cooled (Curtis, 1983). Due to the insulatory nature of the hair coat (in which the air between the hair strands is virtually still), the air immediately surrounding the skin becomes highly saturated with water. The amount of evaporation that occurs off of the skin is effected by the wind speed of the environment the animal experiences. Wind will move the saturated hair within the air coat replacing it with unsaturated air. Removal of water by evaporation results in the loss of heat energy, thus cooling the skin surface. It is in this way that convection facilitates the process of evaporative cooling (Fuquay,

1981; Schmidt-Nielsen, 1981; Robertshaw, 1985).

**Respiration**

The increased rate of sweating by a cow is often difficult to visualize. If an animal is heat stressed, it is easier to visualize an increase in the respiratory rate. An early response of cattle to a hyperthermic environment is to increase ventilation rate and thus the rate of respiratory-evaporative heat loss (Curtis, 1983). Heat dissipation through increased respiration involves vaporization of water within the nasal mucosa and the lungs where unsaturated air is saturated and exhaled into the external environment. The first respiratory response to hyperthermic surroundings is a progressive increase in breathing frequency and a decrease in breathing depth resulting in an increase in minute volume (product of number of the breaths per minute times the volume per breath). Rapid, shallow breathing, called thermal polypnea, is effective in increasing the amount of air passage through the upper respiratory passages. This type of breathing does not effect the rate of ventilation of the respiratory-exchange surface in the alveoli of the lungs (Curtis, 1983). Numerous investigators have reported an increase in the respiration of cattle that are in a hyperthermic environment (Arrillaga et al., 1952; Bond and McDowell, 1972; Brown and Harrison, 1984). Both sweating and panting attempt to take advantage of evaporation, conduction and convection in order to dissipate the heat buildup in the

cow (Robertshaw, 1985).

After peak thermal polypnea has been invoked in response to a progressively hotter environment, cattle shift to a second phase of respiration. Second phase breathing is characterized by a slight drop in breathing frequency from that at peak thermal polypnea accompanied by an increase in breathing depth and minute volume. Breathing becomes slower and deeper compared to that at peak thermal polypnea. The marked increase in minute volume occurs due to increased alveolar ventilation as well as increased upper-respiratory dead-space ventilation (Curtis, 1983).

The higher alveolar-ventilation rate can lead to an excessive loss of carbon dioxide through the lungs, resulting in respiratory alkalosis. The loss of carbon dioxide alters the normal blood pH buffering system which results in an increase in blood pH and alteration in the body electrolyte balance (Curtis, 1983; Brown and Harrison, 1984). Alteration of the blood pH and electrolyte supply to the uterus may have a detrimental effect on embryonic survival.

**Metabolism**

Heat is also generated from the basal metabolic activity of the cow. Body maintenance, exercise, growth, lactation, gestation and digestion are all sources of metabolic heat which contribute to the thermal load of the animal. When an animal's core temperature rises above

normal, the animal must either increase the rate of heat loss and/or reduce metabolic heat production. Metabolic heat can be reduced by minimizing daily muscular activity (Fuquay, 1981; Schmidt-Neilsen, 1981) or reducing feed intake so that the heat produced from the digestion of feedstuffs will decrease (Thatcher and Collier, 1980). Cattle will often not eat during the hottest part of the day but rather wait till the cool of the night to consume their rations (El-Nouty et al., 1976; Bond and McDowell, 1972; Fuquay, 1981). Although feed consumption is decreased during heat stress, water intake increases to prevent dehydration due to losses of water through sweating, respiration and urination (Bond and McDowell, 1972; Fuquay, 1981; Schmidt-Neilsen, 1981).

**Blood Flow**

In order to provide the water necessary for evaporatory heat loss at the skin, blood is preferentially shunted away from the internal organs and body core to the skin surface (Rubsamen and Hales, 1985). This also provides a means of moving the heat from the body core more rapidly to the surface via the shunted blood (Schmidt-Neilsen, 1981). Increased blood flow to the skin is facilitated by an increase in the cardiac output in heat stressed cows (Arrillaga et al., 1952; Brown and Harrison, 1984; Rubsamen and Hales, 1985). Shunting the blood toward the periphery and away from the body core may have an adverse affect on

the reproductive process, especially during early and late pregnancy. Brown and Harrison (1984) observed decreased uterine blood flow in heat stressed ewes during late pregnancy. A decrease in uterine blood flow can lead to increased uterine temperature (Canton et al., 1974a) as less core heat is shunted toward the surface. It is the blood supply to the uterus that provides the necessary nutrients for the developing embryo. Ferrell and Ford (1980) observed an increased uptake of glucose, $\alpha$-amino nitrogen and urea nitrogen by the bovine uterus during the course of gestation that was a result of increased blood flow to the uterus. A decrease in blood supply could decrease the available substrates necessary for development. This could be most critical at the time of rapid elongation and maternal recognition. The embryo may be exposed to an environment low in oxygen during heat stress if blood flow directed away from the uterus is substantial. Fernandez-Cano (1958) observed embryonic degeneration after implantation in rats at high altitudes, indicating that hypoxia may effect fetal development.

Exogenous P4 to both ovariectomized and Day 30-35 pregnant ewes decreased uterine blood flow and oxygen utilization by the uterus (Canton et al., 1974a,b). It has been demonstrated that blood flow to the uterus is also under estrogen influence as uterine blood flow during the estrous cycle of nonpregnant cows was positively correlated

with systemic concentrations of E2 and the ratio of estradiol-17B to progesterone (Ford et al., 1979). Roman-Ponce et al. (1978) demonstrated that exogenous E2 increased uterine blood flow in heat stressed cattle. However, the uterine blood flow was greater in E2 treated cows having shade compared the E2 treated cows having to no shade, indicating a reduced response in the cows exposed to a more severe environment. Huckabee et al. (1968) suggested that an increased concentration of the P4 in the blood, or a reduced estrogen concentration, may bring about an increase in the tone of the smooth muscle of the uterine arterioles and a reduction in blood flow. In contrast, elevated plasma levels of estrogen would result in a decrease in the tone of the uterine arterioles and increased blood flow. Blood flow to the uterus, as measured through the uterine artery, was increased over Day 8 levels by 88% to 221% in both heat stressed and control pregnant gilts during Days 11.5 to 12.5. Increased uterine blood flow in the pregnant gilts may be associated with increasing amounts of estrogens produced by the pig blastocyst at this time (Wettemann et al., 1984). Although a similar increase of blood flow in the pregnant cow occurs during Day 14 to 17, the role of blastocyst estrogen in this increase is not known. The amount of estrogen produced by the bovine blastocyst is considerably less than that produced by the pig blastocyst (Gadsby et al., 1980).

## Endocrine Function

Mammals can respond to changes in the thermal environment through hormonal regulation. Endocrine glands play an important role in regulating hormonal secretion in the animal's attempt to maintain a set point temperature and adapt to thermal stress. Thus, hormonal responses to a thermal environment is a vital mechanism for maintenance of homeostasis within the animal.

**Thyroid Hormones:** Thyroid hormones, thyroxine and triiodothyronine (T4 and T3) are involved with growth and metabolism as thyroidectomy reduces the basal metabolic rate of cattle (Thompson, 1973). As previously stated, cattle in a hyperthermic environment attempt to decrease the heat production from metabolic sources. By reducing the secretion of T3 and T4 from the thyroid gland, cattle decrease their metabolic output during heat stress (Thompson, 1973; El-Nouty et al., 1976; Thatcher and Collier, 1980; Johnson, 1985; Ross et al., 1985; Yousef and Johnson, 1985). El-Nouty et al. (1976) suggested that plasma T4 concentration may determine the heat adaptability of cattle. Heat tolerant animals had lower T4 values in both a thermoneutral and heat stressed environment and were able to decrease T4 by a larger percent between the two temperature ranges. Stott et al. (1975) found that elevated ambient temperature reduced plasma thyroxine in dry Holstein cows. Thermal stress decreased T4 concentrations of dairy cows during late

gestation which indicated an alteration in thyroid hormone secretion during heat stress in an attempt to lower basal metabolic rate (Collier et al., 1982).

**Progesterone:** Studies indicate that a hyperthermic environment tends to elevate P4 concentrations above normal during the estrous cycle (Gwazdawskas et al., 1973; Abilay et al., 1975; Johnson, 1985; Yousef and Johnson, 1985), early pregnancy (Mills et al., 1972; Thatcher, 1974), and late pregnancy (Collier et al., 1982). Kreider et al., (1978) reported that plasma P4 concentrations were elevated in heat stressed gilts on day 9 to 13 after estrus. Even though the ovary may be increasing P4 production and secretion, another possible source of increased circulating P4 in heat stressed cattle is from the adrenal cortex (Yousef and Johnson, 1985), as heat stress may also alter adrenal steroid secretion (Madan and Johnson, 1973; Thatcher, 1974; Wettemann et al., 1984).

**Estradiol:** Limited work has been done on the levels of estradiol-17B in the bovine during heat stress. Folman et al. (1983) indicated cows which had calved during the summer had higher E2 levels before, during, and after estrus than cows which had calved during the winter. Elevation of E2 may serve to increase the blood flow to the uterus during heat stress and provide a mechanism by which excess heat would be dissipated from the reproductive organs (Gwazdawskas et al., 1974; Roman-Ponce et al., 1978; Ford and Chenault, 1981).

Heat stress elevated levels of estrone-sulfate during the last third of gestation, but plasma concentration of estrone or estradiol-17B were uneffected (Collier et al., 1982).

**Luteinizing Hormone:** Fuquay et al. (1970) reported that cyclic cows exposed to high ambient temperatures had shorter overt estrus expression and erratic estrous cycle lengths. Estrous cycles exceeding 26 days were observed (Stott and Williams, 1962) during summer months of high humidity and temperature. This is consistant with results of Madan and Johnson (1973) which indicated that cows under high environmental conditions, where body temperature remained elevated by 1 to 1.5 C, had lower baseline as well as surge levels of LH during the estrous cycle compared to cows at lower environmental temperatures. Sustained elevated body temperatures resulted in a shorter duration of estrus while interestrous interval was lengthened. It was suggested that the increased plasma progesterone normally occuring during heat stress, from both ovarian and adrenal origin, may act together at the hypothalamic nuclei via a negative feedback mechanism to inhibit tonic production of LH (Madan and Johnson, 1973).

It is probable that a similar response to heat stress also occurs in swine, as gilts exposed to heat stress during the estrous cycle had extended cycle lengths (Tompkins et al., 1967). However, exposure to heat stress at 3 to 5 days prior to breeding had no significant effect on cycle length

in gilts (Edwards et al., 1968), indicating that the timing of heat stress during the estrous cycle may be a factor in altering LH levels, duration of estrus and estrous cycle lengths. It has been suggested that heat stress in gilts had no effect on ovulation rate but some gilts that ovulated during heat stress did not express signs of overt estrus (Warnick et al., 1965).

**Plasma Protein**

Cows in the last trimester of pregnancy had reduced plasma protein concentrations during heat stress (Collier et al., 1982). This may be the result of an expanded plasma volume as heat stressed cows have a higher water requirement for evaporative heat loss. An increase in ambient temperature, as reported by Bond and McDowell (1972), may cause metabolic changes as evidenced by qualitative fluctuations in serum proteins of heat stressed cyclic cows. However, this does not agree with data by Gwazdauskas et al. (1975) where elevated ambient temperature did not alter plasma protein concentration in cyclic heifers. Thus, increased ambient temperature can alter plasma protein concentration but may be dependant on the level of heat stress.

**Hematocrit**

Data concerning changes in hematocrit values with rising environmental temperatures are inconclusive. No changes in hematocrit were observed during late pregnancy

(Collier et al., 1982) or postpartum (Lewis et al., 1984). However, cyclic cows exposed to elevated temperatures and humidity were found to have decreased hemoglobin concentrations (Arrillaga et al., 1952; Gutierrez-De La R. et al., 1969). Again, this may be the result of increased water movement that is characteristic of animals in heat stress.

**Embryonic Death**

Heat stress not only alters the blood characteristics but can also effect the developing embryo. Exposure of females to elevated ambient temperatures prior to breeding and after breeding results in embryonic death and a subsequent decrease in pregnancy rates (Ulberg et al., 1967; Dunlap and Vincent, 1971; Gwazdawskas et al., 1973; Wetteman et al., 1984). Cows exposed to elevated ambient temperature during late pregnancy had reduced calf birth weights (Collier et al, 1982).

Pregnancy rates of lactating cows decreased sharply when exposed to high temperatures at the time of breeding (Ingraham et al., 1974). This observation agrees with other work by Dunlap and Vincent (1971), Gwazdawskas et al. (1973), and Badinga et al. (1985). However, thermal stress at this time may exert an effect directly on the spermatozoa and/or the ova which results in poor quality gametes at the time of fertilization.

Work done on the effect of heat stress during early

pregnancy in the rat (Fernandez-Cano, 1958) indicated that an increase of body temperature of rats from 37.2$^{0}$C to 40$^{0}$C in 5 hours for 2 consecutive days during early pregnancy induced embryonic degeneration before implantation. Studies conducted in the ewe indicated that the zygote is very sensitive to thermal stress (Alliston et al.,1961; Dutt, 1963). After exposing ewes in early pregnancy to elevated ambient temperatures, Alliston et al. (1961) observed that embryos were showing evidence of zona pellucida fragmentation and degeneration of individual blastomeres on Day 7 postbreeding. Also, growth and development was retarded, indicating that a loss of potential young occurs in heat stressed ewes due to a cessation or arresting of the embryos during the time of cleavage. Dutt (1963) indicated that the sensitivity of the ewe zygote to heat stress was greater on Day 0-1 of pregnancy than in Day 3 to 5 old embryos, indicating a more harmful effect of high ambient temperatures during the initial stages of development which would result in a normal estrous cycle length in the female. However, if embryo mortality occurs after it has entered the uterus for a period of time, a cycle of abnormal duration may result. Heat stress of Day 3-5 pregnant ewes resulted in some ewes having interestrus intervals of 41 to 66 days (Dutt, 1963).

Exposing gilts to an elevated thermal environment during early pregnancy decreased the number of live embryos

at Day 25 of pregnancy (Warnick, 1965). Edwards et al. (1968) observed that heat stress during the first 15 days postbreeding was more detrimental to productivity of gilts than when applied 15 to 30 days postbreeding. Heat stress the first 15 days after breeding tended to result in lower conception rates, fewer viable embryos, and lower survival rates. Embryos tended to be smaller at 30 days of gestation in gilts that were heat stressed from 15 to 30 days postbreeding. Decreased conception rates were also reported by Tompkins et al. (1967), who indicated that heat stress decreased the number of viable embryos during early pregnancy, with the greatest impact on those gilts treated 1-5 days postbreeding compared to heat stress later in gestation. This agrees with data of Omtvedt et al. (1971) in which heat stress during early pregnancy was more effective in reducing gilt fertility than in the later stages of pregnancy. Heat stress during Day 0-8 or 8-16 postbreeding decreased conception rates and reduced the number of viable embryos. However, heat stress had its greatest effect on reducing viable embryo numbers during Day 8-16 of pregnancy, indicating that the implantation period is a critical time of concern for the detrimental effect of heat stress. Gilts exposed to heat stress late in pregnancy tended to have smaller piglets (Omtvedt et al., 1971). Wildt et al. (1975) also indicated that heat stress applied during early pregnancy (Days 2-13 postbreeding) will increase embryonic

mortality. However, gilts exposed to high ambient temperatures during days 14-25 of gestation resulted in fetal degeneration on Day 42 of gestation, suggesting a continuous or delayed effect of thermal stress on embryo survival. Wettemann et al. (1984) recovered Day 16 embryos from gilts that were heat stressed during days 8-16 after estrus. Conceptus tissue flushed from the uterine horns of heat stressed gilts was fragmented with reduced tissue wet weights compared to gilts maintained in a thermal neutral environment. The total amount of $^{3}$H-leucine incorporated in embryonic tissue was lower in heat stressed embryos but $^{3}$H-leucine incorporated per mg of dry weight of embryonic tissue was not significant. These data indicate that although heat stress of gilts during Day 8-16 after estrus reduces the amount of embryonic tissue present at Day 16, the protein synthetic ability of the tissue present is not altered. These data from the pig suggest that heat stress during early pregnancy in swine is detrimental to embryo development and survival. In summary, heat stress prior to breeding has little effect, swine are susceptable to high ambient temperature at breeding, the first few days after breeding and at the time of placentation (Edwards et al., 1968).

In cattle, heat stress can also affect early embryonic development and pregnancy rates (Stott and Williams, 1962; Ulberg et al., 1967; Dunlap and Vincent, 1971; Thatcher,

1974). Stott and Williams observed low seasonal breeding efficiency associated with high ambient temperature and humidity in lactating dairy cows. The survival rates of pre-implantation embryos is reduced when cows are exposed to elevated environmental temperature (Ulberg et al., 1967) indicating that embryonic death can occur after the embryo enters the uterus. Dunlap and Vincent (1971) reported that high ambient temperature and humidity applied to heifers for 3 days immediately following breeding resulted in 0% conception rates with significant negative correlations between conception rate and rectal temperature or conception rate and respiration rate. These correlations agree with work by Thatcher (1974) who reported an association between uterine temperature and fertility maybe related to optimal timing of insemination to achieve maximal fertility. A deviation of $0.5^{0}C$ above the normal mean uterine temperature the day of insemination and the day after insemination results in decreased conception rates (Gwazdawskas et al., 1973). Thus, it was found that fertility is inversely related to the maximum environmental temperature the day after insemination and to the uterine temperature both at insemination and the day after insemination.

Effects of thermal stress during early pregnancy in the bovine has been mainly confined to the time of insemination. It appears that heat stress in all species is more detrimental in the early stages of pregnancy when

compared with late pregnancy as illustrated by the lower survival rates of embryos prior to implantation (Wettemann et al., 1984). This period in early pregnancy may be of special significance in the bovine because of the relatively greater length of time between fertilization and implantation compared with the sow and ewe. An important period of embryonic development is the time of rapid growth and maternal recognition where alterations in hormone patterns or uterine environment might be detrimental to embryo survival. Due to the lack of information on the effects of heat stress during this time period in the cow, the objective of this proposal was to determine the effects of hyperthermic conditions during early pregnancy on endocrine patterns, embryonic development and embryonic survival.

# CHAPTER III

# EFFECTS OF HEAT STRESS ON EARLY EMBRYONIC DEVELOPMENT AND SURVIVAL IN THE BEEF COW

## SUMMARY

Thirty-two cyclic multiparous Hereford and Hereford X Angus cows were utilized to determine the effects of heat stress on early embryonic development and survival in the beef cow. Prior to treatment, cows were acclimated to metabolism stalls in a temperature controlled building ($20^{0}$C). Estrous cycles were synchronized (Lutalyse) for group mating (4/group) to fertile bulls. On Day 7, the group of cows were cannulated via the jugular vein and assigned to one of the following treatments: Control (C: $22^{o}$C, 43%RH), mild heat stress (MHS: $36^{o}$C, 27%RH) and severe heat stress (SHS: $37^{o}$C, 38%RH). On days 8 to 16, daily measurements for respiration rate (RES), rectal temperature (REC) and water intake (WI) were taken along with jugular blood samples, which were analyzed for hematocrit (HEM), plasma protein (PP), progesterone (P4), estradiol-17B (E2), thyroxine (T4) and glucose (GLU). The uterus was recovered at slaughter on Day 17 and each horn flushed seperately with .9% saline to determine pregnancy status. Corpus luteum wet weight (CL)

was measured along with conceptus wet weight, if a conceptus was present. Compared to Control cows, MHS and SHS had increased (P<.01) RES (C 55, MHS 99, SHS 102 B/min). Severe heat stress cows had elevated (P<.05) REC (C 38.9, SHS 39.8$^0$C) compared to controls and decreased HEM (C 30.6, MHS 30.2, SHS 26.7 %) compared to C (P<.05) and MHS (P<.10). Thyroxine was decreased (P<.05) in SHS compared to MHS and C (C 46.98, MHS 47.70, SHS 37.62 ng/ml) while GLU was decreased in MHS vs C and SHS (C 72.0, MHS 67.0, SHS 72.3 mg%). Conceptus wet weights were decreased (P<.01) in MHS (0.1106 g) and SHS (0.0728 g) compared to C (0.1579 g). Corpus luteum wet weights were also reduced (P<.10) in SHS (2.8g) and MHS (2.77g) vs C (3.39g). Although heat stress did not significantly alter pregnancy rates, there was a trend for greater embryonic mortality in heat stressed cows (C 82, MHS 67, SHS 50%). Compared to nonpregnant cows (NP), pregnant cows (P) had reduced (P<.05) plasma P4 (P 6.4 vs NP 8.0 ng/ml) and elevated (P<.10) E2 (P 4.1 vs NP 3.4 pg/ml). Results indicate that heat stress during early pregnancy (D 8-16) in the bovine reduces conceptus weights and may increase embryonic mortality.

## INTRODUCTION

Environmental changes in ambient temperature, solar radiation and humidity are closely correlated with seasonal depressions of pregnancy rate in domestic cattle (Gwazdauskas et al., 1973; Ingraham et al., 1974; Thatcher,

1974; Badinga et al., 1985). The detrimental effect of elevated environmental temperatures and relative humidity upon semen quality on the bull is well documented (Johnson and Branton, 1953; Myerhoffer et al., 1985). In addition to its deleterious effect in male fertility, the use of artificial insemination has also implicated the dam as a significant contributor to suboptimal fertility experienced during the summer months (Stott, 1961).

A number of studies have demonstrated that high ambient temperature at the time of insemination or during the first few days after breeding has the greatest effect on reducing pregnancy rate (Ulberg and Burfening, 1967; Gwazdauskas et al., 1981; Badinga et al., 1985). Depression of reproductive efficiency through reduced pregnancy rate caused by an unfavorable thermal environment could be related to a direct effect of increased uterine temperature on embryo development. Thatcher and Roman-Ponce (1980) have previously indicated that elevated uterine temperatures are related to decreased uterine blood flow under thermal stress conditions in the cyclic cow. Fertilization and early embryo development are highly sensitive to elevated temperatures (Ulberg and Burfening, 1967).

Alternatively, pregnancy rate may be compromised indirectly through a modification in the endocrine status of the dam (for review, see Johnson, 1985). Several studies conducted during the estrous cycle and early pregnancy have

indicated that plasma progesterone concentrations are increased after exposure to hyperthermic environmental temperatures and high relative humidity (Abilay et al., 1975; Vaught et al., 1977; Thatcher and Roman-Ponce, 1980). Since progesterone and estrogen are involved with the regulation of uterine blood flow during the estrous cycle and early pregnancy in the cow (Thatcher and Roman-Ponce, 1980; Ford and Chenault, 1981), alteration in the levels of progesterone could affect uterine blood flow as well as influencing the local uterine environment in which the conceptus is developing.

Effects of elevated ambient temperature during the peri-implantation period in the cow have not been reported. However, short term exposure to thermal stress during the period of placentation and pregnancy recognition (maintenance of a functional corpus luteum) has been shown to affect early embryonic development and survival in other domestic ungulates such as the pig (Omtvedt et al., 1971) and sheep (Dutt and Jabara, 1976). With the exception of late pregnancy (for review, see Wettemann et al., 1984), heat stress has little effect after placentation is completed in the gilt, ewe and cow. The susceptible period to environmental heat stress may extend even longer during early pregnancy in the cow, since conceptus attachment to the uterine epithelium is not complete until Day 30 (King et al., 1980). Information on the effects of a hyperthermal

stress during the period of rapid blastocyst development and maternal recognition of pregnancy in the bovine is not available. Therefore, the present study was undertaken to determine the effect of elevated ambient temperature during the second and third week of pregnancy on the maternal endocrine profiles and embryonic development and survival in the beef cow.

## MATERIALS AND METHODS

Thirty-two cyclic multiparous Hereford and Hereford X Angus cows between 4 and 11 years of age were assigned randomly to either a control (C, n=11), mild heat stress (MHS, n=9) or severe heat stress (SHS, n=11) treatment. Prior to placement in the environmental chamber which had capacity to maintain four cows, animals were placed in metabolism stalls and acclimated to handling in a temperature controlled building ($20^0$C, 50% RH) for one week. Estrus was synchronized with Lutalyse[1] to group animals for placement in the environmental chamber. The first day of estrous expression was designated as Day 0 of pregnancy. After pen mating to Angus and Hereford bulls of proven fertility, cows were returned to the metabolism stalls. Following the insertion of an indwelling jugular cannula on Day 7, the synchronized group of four cows were transported to the environmental chamber where the assigned treatment was applied from Day 8 through 16. Control groups

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[1]Upjohn Veterinary Products, Kalamazoo, Michigan.

were placed in a thermal neutral environment of 21±1°C and 35±10% relative humidity. Both MHS and SHS treatment groups were maintained at 37±1°C between 0700-1900 h which was decreased to 33±1°C between 1900-0700 h to allow cows to dissipate some of the heat load during the evening hours. Relative humidity was maintained at 27±2% in the MHS environment while relative humidity was increased to 38±2% in the environment of SHS groups. All cows were individually fed a 20% crude protein maintenance diet (17.6 $kg \cdot cow^{-1} \cdot day^{-1}$) consisting mainly of cotton seed hulls and soybean meal.

Rectal temperature, respiration rate and water intake were monitored twice daily at 0700 and 1900 h from Day 8 through 16. Twenty ml of blood, with 0.2 ml of a 6.25% oxalate solution added to prevent clotting, was collected at 1900 h for hormonal analysis. An additional 10 ml of blood, recieving 0.1 ml of a 16% sodium fluoride solution to prevent glucose metabolism, was collected for plasma glucose analysis. All samples were placed immediately on ice until centrifugation at 2,000 Xg for 30 minutes. Plasma samples were stored at -20°C until analyzed. Hematocrit was immediately analyzed[2] from the collected samples and the resulting plasma was used to measure for plasma protein

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[2]International Microcapillary Reader, International Equiptment Company, Needham, Mass.

(g/100ml) with a refractometer[3]. Rectal temperature was monitored with a digital rectal thermometer[4] while respiration rate was measured by counting flank movements• $min^{-1}$. Water intake was monitored with a calibrated automatic drinking device. All cows were fed at 0700 h with feed consumption measured at 1900 h.

On Day 17, cows were transported to a local abattoir (15 km) where the uterus was recovered within 15 minutes after exsanguination. The uterus was immediately placed on ice and transported to the laboratory (15 km). The uterus was trimmed free of the mesometrium and the contralateral and ipsilateral horn to the CL flushed separately with 20 ml of 0.9% saline. Pregnancy was confirmed by the presence of conceptus tissue which was immediately weighed (wet weight). The corpus luteum was dissected from the ovary and trimmed of surrounding connective tissue for measurement of tissue wet weight.

**Assay Procedures:** Plasma samples were randomized in all analysis procedures so that each treatment was represented in each individual assay. Samples were analyzed for glucose, progesterone (P4), estradiol-17B (E2) and thyroxine (T4).

Plasma glucose was measured by colormetric procedure utilizing glucose oxidase and peroxidase with O-diaisidine

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[3]American Optical, New York, NY.

[4]Model 211, Agricultural Electronics Division, Montclair, Calif.

as a chromagen[5].

Progesterone concentration was quantified by radioimmunoassay as previously validated and described in our laboratory by Wettemann et al., (1978). Recovery of labeled tracer after hexane extraction was 89%. The minimum sensitivity of the assay was 25 pg/ml. Intra- and interassay coefficients of variation were 13.2 and 23.0%, respectively.

Plasma E2 concentration was determined by radioimmunoassay as previously reported in our laboratory by Hallford et al. (1979). The antiserum was prepared in sheep against 17B-estradiol-6B-bovine serum albumin. Samples were extracted with freshly distilled benzene with 85% of the labeled tracer recovered. The minimum sensitivity of the assay was 1 pg estradiol-17B/ml. Using an plasma sample with 5 pg/ml of E2, the intra- and interassay coefficients of variation were 34.6 and 43.8%, respectively.

Plasma thyroxine concentrations were quantified by radioimmunioassay as previously described in our laboratory by Pratt and Wettemann (1986). Thyroxine was displaced from thyroxine binding globulin by the addition of 8-anilino-1-napthalene sulfonic acid, while binding to prealbumin was inhibited by using barbitol buffer. The T4 antibody used had a cross-reactivity of less than 7% with triiodothyronine. Minimum sensitivity of the assay for T4 was 0.10 ng/ml. Intra- and interassay coefficients of variation were 21.4

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[5]Tech. Bull. No. 510, Sigma Chemical Co., St. Louis, Mo.

and 4.3%, respectively.

**Statistical Analysis:** Physiological responses and plasma hormone trends of treatments and pregnant vs nonpregnant were analyzed by least-squares analysis of variance. Day trends were characterized by polynomial regression curves, and individual treatment, pregnancy, treatment X day and pregnancy X day least-squares means were estimated. Data were analyzed with time used as a continuous independant variable. Analyses were evaluated with a split-plot design with cows nested in treatment and cross-classified across time. When significant effects were detected, means were compared by least significant difference. Tests of heterogeneity of regression were used to detect differences between treatments for day trends.

## RESULTS

Least-squares means and standard errors for C, MHS and SHS treatments are presented in table 1. Elevation of chamber temperatures stimulated an increase in animal respiration rate ($P<.01$) in both MHS and SHS groups while rectal temperatures were increased in the SHS cows compared to C ($P<.05$) and MHS ($P<.10$). Rectal temperature of SHS cows increased from Day 8 until approximately Day 12, remaining relatively stable to Day 16. Although a trend toward increased daily water consumption was observed in SHS cows ($P<.12$), no significant differences in water intake were

**TABLE 1. LEAST-SQUARES MEANS AND STANDARD ERRORS OF ENVIRONMENTAL AND PHYSIOLOGICAL RESPONSES**

| | Control | Mild Heat Stress | Severe Heat Stress | SE |
|---|---|---|---|---|
| Dry Bulb Temp, $^{o}C$ | $21.7^{a}$ | $36.1^{b}$ | $36.6^{b}$ | 1.0 |
| Relative Humidity, % | $43^{b}$ | $27^{a,c}$ | $38^{d}$ | 2.0 |
| Respiration Rate, /min | $55.1^{a}$ | $99.0^{b}$ | $102.1^{b}$ | 3.8 |
| Rectal Temp, $^{o}C$ | $38.9^{c}$ | $39.2^{e}$ | $39.8^{d,f}$ | .1 |
| Daily $H_2O$ Intake, L | 69.3 | 89.7 | 113.6 | 22.0 |
| Hematocrit, % | $30.6^{c}$ | $30.2^{e}$ | $26.7^{d,f}$ | 1.3 |
| Plasma Protein, g/100ml | 7.29 | 7.06 | 7.03 | .1 |
| Plasma Glucose, mg/100ml | $72.0^{e}$ | $67.0^{f}$ | $72.3^{e}$ | 2.2 |

$^{a,b}$Means not having a common superscript differ ($P<.01$).
$^{c,d}$Means not having a common superscript differ ($P<.05$).
$^{e,f}$Means not having a common superscript differ ($P<.10$).

detected in response to treatment. Hematocrit percentage declined in the SHS group compared to C ($P<.05$) and MHS ($P<.10$) while there was a tendancy for heat stress to decrease plasma protein in MHS ($P<.18$) and SHS ($P<.11$). Plasma glucose concentration decreased in MHS compared to SHS and C treatments ($P<.10$).

Least-square means for hormonal responses to treatments are listed in table 2. Analyses of plasma hormone measurements for treatments are presented in table 3. Plasma progesterone was not significantly affected by treatment and there was no evidence of heterogeneity of regression of the three treatments for P4 concentration. Day (X) effect on progesterone (Y) concentrations for all three treatments was best described by first order regression equation ($Y = 4.878 + 0.140X$: $r^2 = .63$ for C; $Y = 4.843 + 0.161X$: $r^2 = .54$ for MHS: $Y = 5.263 + 0.177X$: $r^2 = .52$ for SHS). Concentrations of E2 (table 2) were similar between treatment groups, while plasma concentrations of thyroxine were lower ($P<.05$) in SHS cows compared to MHS and C treatments. There was no evidence of heterogeneity of regression in T4 concentrations for the three treatments. Day (X) trends for peripheral plasma T4 (Y) concentrations were best described by a second order polynomial regression equation (figure 2) for control ($Y = 90.723 - 6.783X + 0.274X^2$: $r^2 = .54$), MHS ($Y = 109.109 - 10.458X + 0.439X^2$: $r^2 = .34$) and SHS ($Y = 114.608 - 10.967 + 0.416X^2$: $r^2 = .48$). SHS cows responded to the thermal stress

**TABLE 2. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR PLASMA HORMONE CONCENTRATIONS IN RESPONSE TO TREATMENT**

| | Control | Mild Heat Stress | Severe Heat Stress | SE |
|---|---|---|---|---|
| Progesterone, ng/ml | 6.59 | 6.81 | 7.48 | .56 |
| Estradiol-17B, pg/ml | 3.79 | 3.80 | 3.98 | .33 |
| Thyroxine, ng/ml | 46.98[a] | 47.70[a] | 37.62[b] | 3.48 |

[a,b]Means not having a common superscript differ (P<.05).

**TABLE 3. LEAST-SQUARES ANALYSIS OF HORMONAL VARIABLES IN RESPONSE TO TREATMENT**

| Source | Progesterone df | Progesterone MS | Estradiol-17B df | Estradiol-17B MS | Thyroxine df | Thyroxine MS |
|---|---|---|---|---|---|---|
| Treatment | 2 | 16.67 | 2 | 4.59 | 2 | 2028.26$^{+}$ |
| Cow(Treatment) | 28 | 20.92$^{**}$ | 28 | 198.35$^{**}$ | 29 | 770.26$^{**}$ |
| Days | 1 | 26.96$^{**}$ | 3 | 5.78$^{+}$ | 3 | 741.15$^{**}$ |
| Heterogeneity | 3 | .04 | 6 | 1.73 | 6 | 184.59 |
| Residual | 221 | 2.22 | 218 | 1.97 | 232 | 164.83 |

$^{**}P<.01$
$^{+}P<.10$

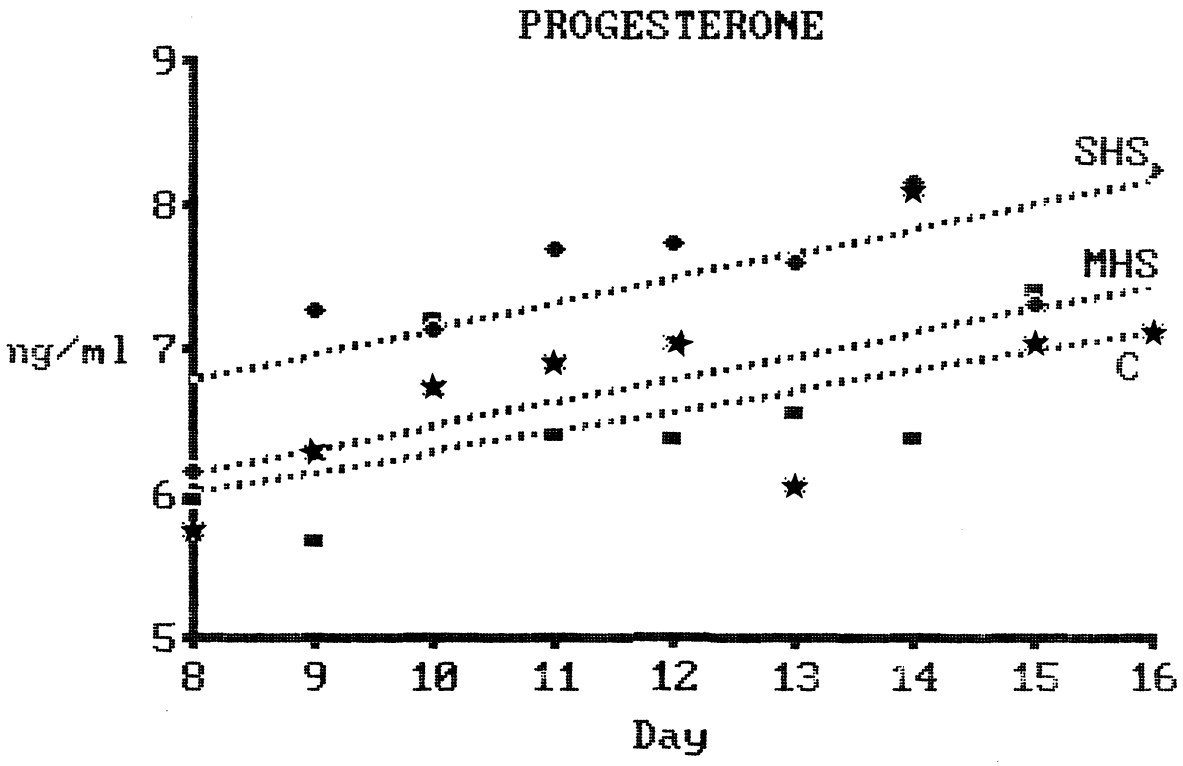


Figure 1. Least-squares regressions and least-squares means of progesterone concentration (ng/ml) for control (C, ■), mild heat stress (MHS, ★) and severe heat stress (SHS, ◆) cows.

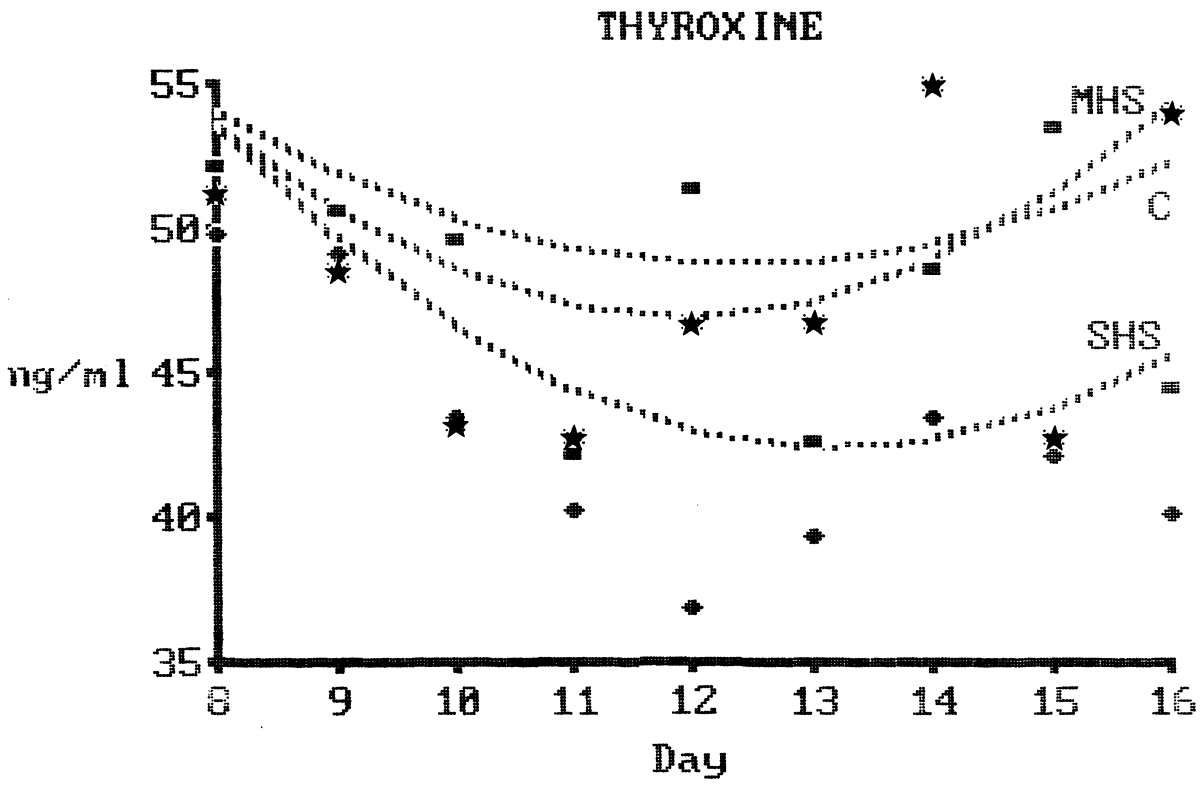


Figure 2. Least-squares regressions and least-squares means of thyroxine concentration (ng/ml) for control (C, ■ ), mild heat stress (MHS, ★ ) and severe heat stress (SHS, ◆ ) cows.

by reducing T4 concentration by approximately 12 ng/ml by Day 16 of pregnancy compared to MHS and C treatments.

Pregnancy rate, conceptus and CL wet weight in response to treatments are presented as least-squares means in table 4. Although differences were not significant, a trend for lower pregnancy rate was observed for SHS and MHS treatments compared to control (50 and 64 vs 83, %). Heat stress did significantly reduce conceptus wet weight as both pregnant MHS and SHS had smaller amounts of conceptus tissue present compared to control cows on Day 17 of pregnancy ($P<.01$). Corpus luteum wet weight was also reduced ($P<.10$) in MHS and SHS cows compared to control animals.

Since no pregnancy status (pregnant vs nonpregnant) by treatment interaction was detected for hormone and glucose concentrations, analysis of variance was conducted on effect of pregnancy status using day as a continuous independant variable. Least-squares means of hormonal concentrations between pregnant and nonpregnant cows are shown in table 5. Mean plasma P4 concentration was approximately 1.5 ng/ml greater ($P<.05$) in nonpregnant cows. There was evidence for a trend ($P<.10$) towards heterogenity of regression in P4 concentrations in the two groups (table 6). Day (X) effect on progesterone (Y) concentration for pregnant and nonpregnant cows was best described by first order regression equations ($Y = 4.007 + 0.204X$: $r^2 = .50$ for pregnant; $Y = 7.186 + 0.060X$: $r^2 = .61$ for nonpregnant)

**TABLE 4. LEAST-SQUARES MEANS AND STANDARD ERRORS OF PREGNANCY RATE, EMBRYO WEIGHT AND CORPUS LUTEUM WEIGHT IN RESPONSE TO TREATMENT**

| | Control (n=11) | Mild Heat Stress (n=9) | Severe Heat Stress (n=12) | SE |
|---|---|---|---|---|
| Pregnancy Rate, % | 82 | 67 | 50 | 14.5 |
| Conceptus Wet Weight, g | 0.1579[a] | 0.1106[b] | 0.0728[b] | .0213 |
| CL Weight, g | 3.39[c] | 2.77[d] | 2.80[d] | .29 |

[a,b]Means not sharing a common superscript differ (P<.01)
[c,d]Means not sharing a common superscript differ (P<.10)

**TABLE 5. LEAST-SQUARES MEANS OF HORMONE AND GLUCOSE CONCENTRATIONS AS AFFECTED BY PREGNANCY STATUS**

| | Pregnant | Nonpregnant | SE |
|---|---|---|---|
| Progesterone, ng/ml | 6.4[a] | 8.0[b] | .53 |
| Estradiol-17B, pg/ml | 4.2[c] | 3.5[d] | .33 |
| Thyroxine, ng/ml | 46.7 | 40.5 | 3.3 |
| Plasma Glucose, mg/l | 69.4 | 73.5 | 2.1 |

[a,b]Means not having a common superscript differ (P<.05).
[c,d]Means not having a common superscript differ (P<.10).

**TABLE 6. LEAST-SQUARES ANALYSIS OF HORMONAL VARIABLES VS PREGNANCY STATUS**

| Source | Progesterone df | Progesterone MS | Estradiol-17B df | Estradiol-17B MS | Thyroxine df | Thyroxine MS |
|---|---|---|---|---|---|---|
| Status | 1 | 127.89* | 1 | 29.24+ | 1 | 2705.45+ |
| Cow(Status) | 29 | 19.94** | 29 | 6.39** | 30 | 789.62** |
| Days | 1 | 46.24** | 3 | 5.08 | 3 | 741.15** |
| Heterogeneity | 1 | 8.04+ | 3 | .67 | 3 | 57.24 |
| Residual | 245 | 2.21 | 242 | 1.97 | 223 | 164.83 |

**P<.01
+P<.10

which is presented in figure 3. Nonpregnant cows consistently maintained a P4 concentration between 7.5 and 8.0 ng/ml throughout the experimental period. Plasma P4 concentration in pregnant cows was approximately 5.5 ng/ml on Day 8, gradually increasing to 7.0 ng/ml by Day 16.

Estradiol-17B in plasma was lower in nonpregnant cows compared to pregnant cows ($P<.10$) with no evidence of heterogenity of regression of day trends (table 6). Day (X) trends for peripheral plasma E2 (Y) concentration were best described by a third order polynomial regression equation (figure 4) for pregnant ($Y = 20.661 - 4.603X + 0.413X^2 - 0.012X^3$: $r^2 = .27$) and nonpregnant cows ($Y = 33.012 - 7.647X + 0.646X^2 - 0.018X^3$: $r^2 = .38$). Beginning on Day 10, pregnant cows maintained a peripheral E2 concentration approximately 0.5 pg/ml greater than nonpregnant cows throughout the experimental period.

Mean plasma T4 concentration tended to be lower ($P<.14$) in nonpregnant cows compared to pregnant. However, there was no evidence of heterogenity of regression of day trends for T4 concentration between the two groups. Plasma glucose had a tendancy to be lower ($P<.12$) in the pregnant animals compared to nonpregnant (69.4 and 73.5, mg/100ml).

## DISCUSSION

The physiological responses to heat stress in the present experiment indicate that the cows in the SHS group

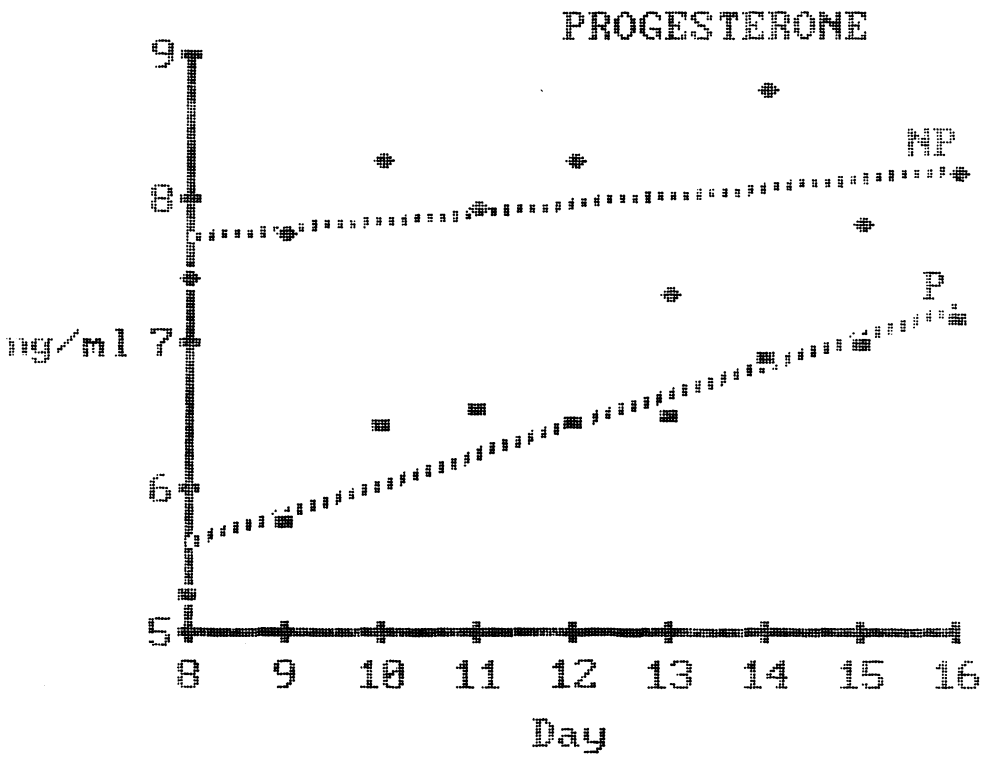


**Figure 3. Least-squares regressions and least-squares means of progesterone concentration (ng/ml) for pregnant (P, ■ ) and nonpregnant (NP, ◆) cows.**

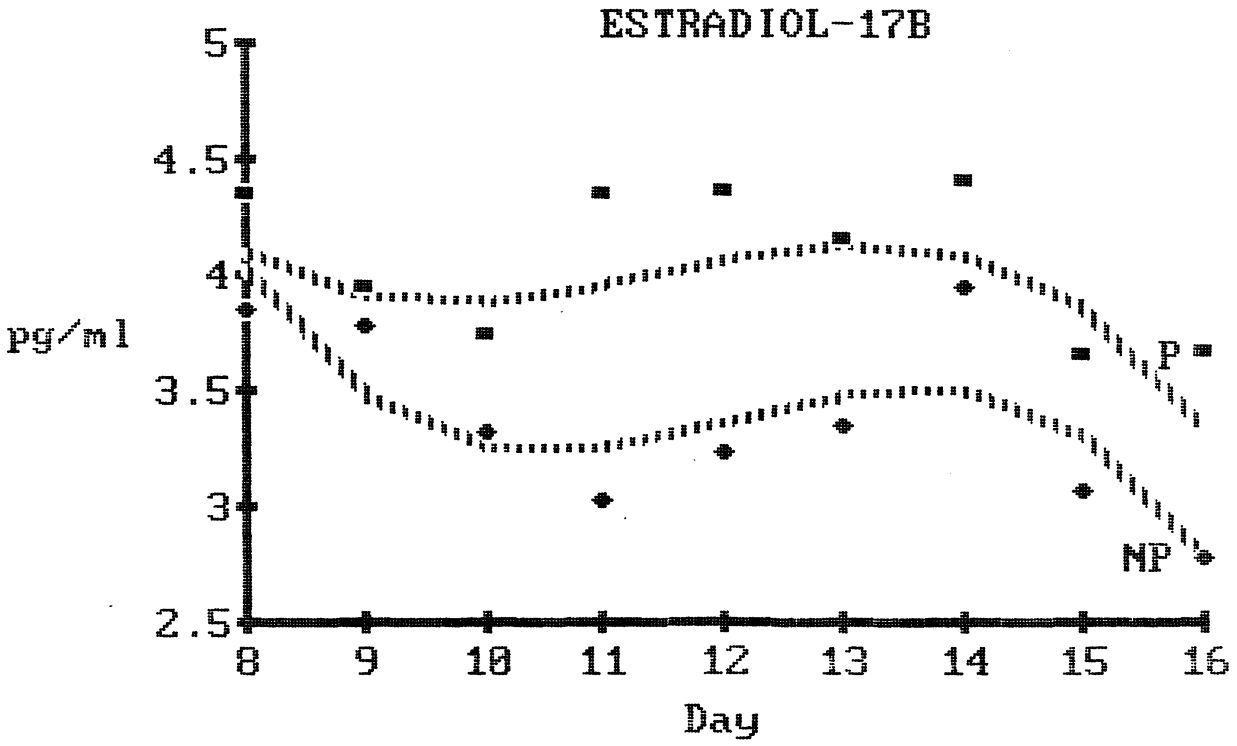


Figure 4. Least-squares regressions and least-squares means of estradiol-17B concentration (pg/ml) for pregnant (P, ■ ) and nonpregnant (NP, ◆ ) cows.

were exposed to a higher effective heat load when compared to Control cows. The increase in respiration rate and rectal temperature of the severe heat stressed cows in our study is similar to that reported during heat stress of cyclic cows (Bond and McDowell, 1972), late pregnant cows (Collier et al., 1982) and steers (Pratt and Wettemann, 1980). Elevated rectal temperatures indicated that the severe heat stressed group was absorbing heat at a greater rate than could be dissipated which stimulated an increase in respiration rate as cows attempted to decrease body core temperature by respiratory evaporation (Curtis, 1983).

Plasma protein concentrations during heat stress in the present study agree with the changes which occur during thermal stress of cows in the last trimester of pregnancy (Collier et al., 1982). Pratt and Wettemann (1980) reported similar alteration of hematocrit percentages in steers exposed to high ambient temperatures. Lower hematocrit and plasma protein concentration in SHS cows may have resulted from an increase in plasma volume (Collier et al., 1982). Water consumption tended to increased in SHS cows as a higher water intake requirement to meet the needs for evaporatory water loss would have been invoked in response to the increased thermal load.

Although plasma progesterone concentrations were not significantly affected by thermal stress in the present study, concentrations tended to be increased in both MHS and

SHS cows, which is consistant with previous reports of increased plasma progesterone concentration during thermal stress in the bovine (Abilay et al., 1975; Vaught et al., 1977; Thatcher and Roman-Ponce, 1980).

Thermal stress did not alter plasma estradiol-17B concentrations in our study. Ford et al. (1979) reported that blood flow to the gravid uterine horn increased by Day 14 of pregnancy. Since exogenous E2 will increase uterine blood flow in heat stressed cattle (Roman-Ponce et al., 1978), the estradiol-17B levels during heat stress may function to stimulate blood flow to the uterus, thereby increasing the amount of heat transfered from the body core to the skin via the blood supply in an attempt to prevent heat buildup and a rise in uterine temperature. Uterine blood flow is positively correlated with the ratio of estradiol-17B to progesterone in the peripheral plasma (Huckabee et al., 1968; Ford et al., 1979). Roman-Ponce et al (1978) demonstrated that heat stress reduces the ability of exogenous E2 to increase uterine blood flow in cattle. Thus, uterine blood flow may be reduced even though E2 concentrations remain unchanged. Gwazdauskas et al. (1973) and Thatcher (1974) indicated that fertility is inversely related to the uterine temperature both at the time of insemination and the day after insemination in cattle. Elevated uterine temperatures may result in increasing the metabolic rate of the conceptus, altering the

nutrient uptake and growth. Thus, decreased nutrient secretion by the uterus and/or the uptake by the blastocyst may result in retarded conceptus development and/or embryonic death as was observed in our study. The tendancy toward elevated P4 concentrations may also alter endometrial secretion, which results in advanced uterine development forming a hostile environment for conceptus growth. Should the conceptus survive up to the time of maternal recognition, the smaller blastocysts may not have the mature biosynthetic capabilities required to signal the maternal system to maintain CL function.

Plasma thyroxine concentrations in the present study were similar to those reported by Collier et al. (1982). Circulating levels of thyroxine in plasma were significantly lower in SHS cows while no effect on plasma thyroxine was observed in MHS treatment (table 2). The changes in thyroxine concentrations would suggest that SHS cows attempted to lower heat buildup from tissues by lowering metabolic rate (Thompson, 1973; El-Nouty et al., 1976; Johnson, 1985; Ross et al., 1985; Yousef and Johnson, 1985).

Even though the effect of heat stress on pregnancy rate was not significant (table 3), there is a strong trend towards lower conception rates with increasing thermal stress. This agrees with previous work in the rat (Fernandez-Cano, 1958), ewe (Alliston et al., 1961; Dutt, 1963) and gilt (Warnick, 1965; Tompkins et al., 1967;

Edwards et al., 1968; Omtvedt et al., 1971; Wildt et al., 1975; Wettemann et al., 1984) in which exposure to high ambient temperature during early pregnancy resulted in embryonic degeneration and decreased conception rates.

Along with the trend towards decreased conception rates, blastocyst wet weights were significantly lower in the heat stressed groups, with embryos from SHS cows weighing less than half the weight of embryos from Control cows. Similar reductions in conceptus size and weight due to heat stress during early pregnancy have been observed in the ewe (Alliston et al., 1961) and gilt (Edwards et al., 1968; Wettemann et al., 1984). Exposure of gilts to elevated ambient temperature during days 8-25 of gestation resulted in fragmented embryos and fetal degeneration (Wildt et al., 1975; Wettemann et al., 1984). Reduced conception rate and conceptus wet weight from the present study indicate that degeneration and death of the bovine conceptus due to heat stress during early pregnancy may be caused by similar mechanisms which affect sheep and swine. A decrease in the development of the blastocyst may lead to a reduced ability to signal the maternal system and maintain CL function as evidenced by reduced CL weights in the heat stressed groups (table 4).

Since there was a tendancy for heat stress to reduce pregnancy rates, the hormonal differences between the pregnant and nonpregnant cows across treatments were also

examined. Cows that did not maintain pregnancy had a greater P4 concentration compared to pregnant cows (table 4.) Pregnant cows showed a normal profile of plasma P4, increasing from Day 8 to 16 (Ford et al., 1979; Hansel, 1981); however, nonpregnant cows maintained a P4 concentration well above that of pregnant cows throughout the entire experiment (figure 3). The early elevation of P4 concentration, as observed in the nonpregnant cows, may represent adrenal progesterone release which could possibly effect uterine and/or secretory function (Adams, 1980; Bartol et al., 1981, 1985b) resulting in a hostile environment for conceptus development.

Plasma estradiol-17B concentrations were lower in the nonpregnant cows when compared to pregnant cows (table 4). Hansel (1981) previously reported that greater plasma estradiol-17B concentrations were present in pregnant compared to cyclic cows between Day 10 and 18. Figure 4 illustrates that E2 concentration for both groups were similar on Day 8, however, plasma E2 was elevated .5 to 1.0 pg/ml in pregnant cows from Day 9 to 16 compared to concentrations in nonpregnant females.

Plasma T4 concentrations were lower in nonpregnant cows when compared to pregnant cows (table 4). Since the majority of pregnant cows in this study were in the control group and exposed to a thermal neutral environment while most of the nonpregnant cows were in either the MHS or SHS groups and

had lower T4 levels than controls, it is not surprising that, overall, the pregnant cows had a higher thyroxine concentration than the nonpregnant cows throughout the experimental period.

In summary, heat stress during the time of rapid embryonic growth and development (Day 8-16) decreases conceptus wet weight, tends to decrease conception rate and tends to increase plasma progesterone concentration. The effects of heat stress during early pregnancy may lead to more dramatic results as gestation proceeds. Pregnancy may not be established due to incomplete conceptus attachment. Additional studies are needed to determine qualitative and quantitave changes in the uterine environment in response to heat stress during early pregnancy.

## CHAPTER IV

## GENERAL DISCUSSION

Previous research has indicated that exposure to elevated ambient temperature during early pregnancy could alter embryonic development and survival in the rat (Fernandez-Cano, 1958), ewe (Alliston et al., 1961; Dutt, 1963) and gilt (Warnick et al., 1965; Tompkins et al., 1967; Edwards et al., 1968; Omtvedt et al., 1971; Wildt et al., 1975; Wettemann et al., 1984). The effect of heat stress in the cow have been mainly confined to the time of insemination (Dunlap and Vincent, 1971; Gwazdawskas et al., 1973) and to late pregnancy (Collier et al., 1982; Lewis et al., 1984). These results suggested that hyperthermic conditions during early pregnancy in the cow may alter endocrine patterns, embryonic development and survival and encouraged us to design the present experiment.

Although it is granted that a criticism of this experiment is that placing cows in environmental chambers with a constant elevated ambient temperature would rarely be experienced in most natural environments, the purpose of this experiment was to determine if extreme thermal stress could affect bovine reproduction during early pregnancy. We did not exactly mimic normal environmental conditions by

gradually increasing the heat load of the cows, but rather desired to expose them to the most severe thermal stress possible. The heat stress did place the cows in an extreme situation, as one of the larger and heavier cows succumbed to the high ambient temperature and humidity after 6 days of treatment. This animal appeared to be in second phase breathing and maintained a rectal temperature of approximately 42.2$^{0}$C for 4 days before death.

In this experiment, severe thermal stress altered the bovine reproduction system during early pregnancy by lowering plasma thyroxine levels, decreasing embryo and corpus luteum wet weights, and tending to decrease embryo survival and increase progesterone concentration. The exact mechanism by which high environmental temperature affects early embryonic development is not known at this time but a number of possibilities can be suggested.

Blood flow to the uterus is correlated with the ratio of progesterone to estradiol-17B in the plasma (Huchabee et al., 1968; Ford et al., 1979). With a possible increase progesterone concentration in response to heat stress, there may be both a qualitative and quantitative alteration in the secretory pattern of proteins, glycoproteins, carbohydrates and ions from the uterine endometrium. The conceptus requires histotroph for growth and development, especially during the time of rapid growth, elongation, and maternal recognition. Any change in the secretion rate of the uterine

endometrium may act to retard conceptus development, resulting in a hostile uterine environment and blastocyst degeneration. If the conceptus development is slowed, there could also be a delay in the secretory pattern of protein and prostaglandins from the blastocyst which may in turn have an effect on uterine secretion, prostaglandin $F_{2\alpha}$ release, and maternal recognition of pregnancy.

Although the conceptus remains viable until Day 17 of pregnancy, its development may be inadequate to prevent luteolysis. An underdeveloped blastocyst may not have the capablities to qualitatively synthesize the polypeptides required to signal the maternal system to maintain the pregnancy. Even if the conceptus does have the ability to synthesize these polypeptides, the smaller mass of conceptus tissue could result in a qualitative decrease in the amount synthesized and released, resulting in insufficient supression of $PGF_{2\alpha}$ and CL regression. Elevated ambient temperature and humidity are also known to increase uterine temperature (Gwazdauskas et al., 1973; Thatcher, 1985). Reduction in blood flow to the uterus would cause less uterine heat to be shunted toward the surface. High uterine temperatures may have an adverse effect on the conceptus itself. It is possible that this increase in uterine temperature is harsh enough to increase the metabolic rate of the blastocyst. An increased metabolic rate could lead to the conceptus using and requiring more substrates for growth

rate in the MHS and SHS cows is probably a result of the exposure to the thermally stressful environment.

Pregnant cows had greater estradiol-17B concentrations when compared to nonpregnant animals. Higher estradiol-17B and lower progesterone concentrations in cows that were able to maintain a pregnancy may have influenced uterine blood flow as indicated by Roman-Ponce et al. (1978). Cows which were not able to adapt to the high ambient temperature, increased progesterone concentration and therefore the P4 to E2 ratio, possibly lowering blood flow to the uterus as well as altering endometrial secretion.

In conclusion, high ambient temperature and humidity during Day 8 to 16 of pregnancy is detrimental to bovine reproduction as evidenced by elevated progesterone concentration, lower corpus luteum and conceptus wet weights, and a tendancy towards lower conception rates. Further research in this area should include investigating the qualitative and quantitative changes in the fluid from the uterine lumen to determine the secretory and metabolic changes of the conceptus and uterine endometrium in response to high ambient temperature and humidity.

## LITERATURE CITED


Abilay, T. A., H. D. Johnson and M. Madan. 1975. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrus cycle. J. Dairy Sci. 58: 1836.

Adams, C. E. 1980. Retention and development of eggs transfered to the uterus at various times after ovulation in the rabbit. J. Reprod. Fertil. 60:309.

Alliston, C. W., G. E. Egli and L. C. Ulberg. 1961. Loss of potential young in the ewe due to high ambient temperature. J. Appl. Physiol. 16:253.

Anderson, G. B. 1977. Fertilization, early development, and embryo transfer. In: H. H. Cole and P. T. Cupps (Eds) Reproduction in domestic animals. pp 286-311. Academic Press, New York.

Anderson, L. L. 1966. Pituitary-ovarian-uterine relationships. In "Ovarian regulatory mechanisims". J. Reprod. Fertil. Suppl. 1:21.

Arrillaga, C. G., W. L. Henning and R. C. Miller. 1952. The effects of environmental temperature and relative humidity on the acclimation of cattle to the tropics. J. Anim. Sci. 11:50.

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

Badinga, L., R. J. Collier, W. W. Thatcher and C. J. Wilcox. 1985. Effects of climate and management factors on conception rate of dairy cattle in subtropical environment. J. Dairy Sci. 68:78.

Barrios, D. R., S. McConnell, M. J. Bowen and D. C. Kraemer. 1985. Effects of progesterone (P4) and estrogen (E2) on fertilization failure and early embryonic mortality in superovulated cows. Theriogenology. 23:176.

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

Reprod. 25:759.

Bartol, F. F., R. M. Roberts, F. W. Bazer, G. S. Lewis, J. D. Godkin and W. W. Thatcher. 1985a. Characterization of proteins produced in vitro by periattachment bovine conceptuses. Biol. Reprod. 32:681.

Bartol, F. F., R. M. Roberts, F. W. Bazer and W. W. Thatcher. 1985b. Characterization of proteins produced in vitro by bovine endometrial explants. Biol. Reprod. 33:745.

Basha, S. M., F. W. Bazer, R. D. Geisert and R. M. Roberts. 1980. Progesterone-induced uterine secretions in pigs. Recovery from pseudopregnant and unilaterally pregnant gilts. J. Anim. Sci. 50:113.

Bazer, F. W. 1975. Uterine protein secretions: relationship to development of the conceptus. J. Anim. Sci. 41:1376.

Beal, W. E., J. H. Lukaszewska and W. Hansel. 1981. Luteotropic effects of bovine blastocysts. J. Anim. Sci. 52:567.

Beardon, H. J., W. Hansel and R. W. Bratton. 1956. Fertilization and embryonic mortality rates of bulls with histories of either low or high fertility in artificial breeding. J. Dairy Sci. 39:312.

Bedford, J. M. 1982. Fertilization. In: C. R. Austin and R. V. Short (Ed) Reproduction in Mammals: 1. Germ Cells and Fertilization. pp 128-163. Cambridge University Press, Cambridge.

Bedford, J. M. 1983. Significance of the need for sperm capicitation before fertilization in eutherian mammals. Biol. Reprod. 28:108.

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

Biggers, J. D. and R. M. Borland. 1976. Physiological aspects of growth and development of the preimplantation mammalian embryo. Ann. Rev. Physiol. 38:95.

Bond, J. and R. E. McDowell. 1972. Reproductive performance and physiological responses of beef females as effected by a prolonged high environmental temperature. J. Anim. Sci. 35:820.

Brewster, J. E. and C.L. Cole. 1941. The time of ovulation in cattle. J. Dairy Sci. 24:111.

Britt, J. H., N. M. Cox and J. S. Stevenson. 1981. Advances in reproduction in dairy cattle. J. Dairy Sci. 64:1378.

Brown, D. E. and P. C. Harrison. 1984. Lack of peripheral sympathetic control of uterine blood flow during acute heat stress. J. Anim. Sci. 59:182.

Canton, D., R. M. Abrams, J. F. Clapp and D. H. Barron. 1974a. The effect exogenous progesterone on the rate of blood flow of the uterus of ovarectomized sheep. Quart. J. Exp. Physiol. 59:233.

Canton, D., R. M. Abrams, L. K. Lackore, G. James and D. H. Barron. 1974b. Effect of exogenous progesterone on the rate of uterine blood flow and oxygen consumption of sheep in early pregnancy. Quart. J. Exp. Physiol. 59:233.

Chang, M. C. 1952. Development of bovine blastocyst with a note on implantation. Anat. Rec. pp 143.

Chang, M. C. 1966. Effect of oral administration of Medroxyprogesterone Acetate and Ethinyl Estradiol on the transport and development of rabbit eggs. Endocrinology. 79:939.

Chenault, J. R., W. W. Thatcher, P. S. Kalra, R. M. Abrams and C. J. Wilcox. 1976. Plasma progestins, estradiol, and lutenizing hormone following prostaglandin F2a injection. J. Dairy Sci. 59:1342.

Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher and C. J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. J. Anim. Sci. 54:309.

Curtis, S. E. 1983. Determinants and partition of heat exchange In: Environmental management in animal agriculture. pp 47-57. Iowa State University Press, Ames.

Dale, B. 1983. Fertilization in Animals. pp. 26-46. The Camelot Press Ltd., Southampton.

Dunlap, S. E. and C. K. Vincent. 1971. Influence of postbreeding thermal stress on conception rates in beef cattle. J. Anim. Sci. 32:1216.

Dutt, R. H. 1963. Critical period for early embryo mortality in ewes exposed to high ambient temperature. J. Anim. Sci. 22:713.

Dutt, R. H. and C. K. Jabara. 1976. Gestation stage and embryo loss in ewes heat stressed during early placentogenesis. J. Animal Sci. 43:282.

Edwards, R. L., I. T. Omtvedt, E. J. Turman, D. F. Stephens and G. W. A. Mahoney. 1968. Reproductive performance of gilts following heat stress prior to breeding and in early gestation. J. Anim. Sci. 27:1634.

Eley, R. M., W. W. Thatcher and F. W. Bazer. 1979a. Luteolytic effect of oestrone sulfate on cyclic beef heifers. J. Reprod. Fertil. 55:191.

Eley, R. M., W. W. Thatcher, F. W. Bazer and M. J. Fields. 1979b. Metabolism of progesterone and androstenedione in vitro by bovine endometrium and conceptus. J. Anim. Sci. 49:294. (Abstr.).

Eley, R. M., W. W. Thatcher, F. W. Bazer and M. J. Fields. 1983. Steroid metabolism by the bovine uterine endometrium and conceptus. Biol. Reprod. 28:804.

Eley, D. S., W. W. Thatcher, H. H. Head, R. J. Collier and C. J. Wilcox. 1981. Periparturient endocrine changes of conceptus and maternal units in Jersey cows bred for milk yield. J. Dairy Sci. 64:296.

El-Nouty, F. D., H. D. Johnson and G. R. Hahn. 1976. Heat adaptability measures in Herefords. J. Animal Sci. 43:283.

Erb, R. E., R. D. Randel and C. J. Callahan. 1971. Female sex steroid changes during the reproductive cycle. J. Anim. Sci. Suppl. 1. 32:80

Fallon, G. R. 1962. Body temperature and fertilization in the cow. J. Reprod. Fertil. 3:116.

Ferdandez-Cano, L. 1958. Effect of increase or decrease of body temperature and hypoxia on pregnancy in the rat. Fert. Steril. 9:455.

Ferrell, C. L. and S. P. Ford. 1980. Blood flow, steroid secretion and nutrient uptake of the gravid bovine uterus. J. Anim. Sci. 50:1113.

Fogwell, R. L., J. L. Cowley, J. A. Wortman, N. K. Ames and

J. J. Ireland. 1985. Luteal function in cows following destruction of ovarian follicles at midcycle. Theriogenology. 23:389.

Foley, R. C. and R. P. Reece. 1953. Histological studies of the bovine uterus, placenta and corpus luteum. Massachusetts Agr. Expt. Sta. Bull. 468.

Folman, Y., M. Rosenberg, I. Ascarelli, M. Kaim and Z. Herz. 1983. The effect of dietary and climatic factors on fertility, and on plasma progesterone and oestradiol-17B levels in dairy cows. J. Steroid Biochem. 19:863.

Ford, S. P., C. W. Weems, R. E. Pitts, J. E. Pexton, R. L. Butcher and E. K. Inskeep. 1975. Effect of estradiol-17B and progesterone on prostaglandin F in sheep uteri and uterine venous plasma. J. Anim. Sci. 41:1407.

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

Ford, S. P. and J. R. Chenault. 1981. Blood flow to the corpus luteum-bearing ovary and ipsilateral uterine horn of cows during the oestrus cycle and early pregnancy. J. Reprod. Fertil. 62:555.

Frank, M., F. W. Bazer, W. W. Thatcher and C. J. Wilcox. 1977. A study of prostaglandin F2$\alpha$ as the luteolysin in swine. III. Effects of estradiol concentration in the utero-ovarian vein of nonpregnant gilts. Prostaglandins 14:1183.

Fridhandler, L. 1961. Pathways of glucose metabolism in fertilized rabbit ova at various preimplantation stages. Exp. Cell Res. 22:303.

Fuquay, J. W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 52:164.

Fuquay, J. W., W. H. McGee and F. M. Hutson. 1970. Seasonal effect on reproductive performance in dairy cattle. Mississippi Farm Res. 33:8.

Garverick, H. A., R. E. Erb, D. G. Niswender and C. J. Callahan. 1971. Reproductive steroids in the bovine. III. Changes during the estrous cycle. J. Anim. Sci. 32:946.

Gebremedhin, K. G. 1985. Heat exchange between livestock and

environment In: M. K. Yousef (Ed) Stress physiology in livestock. Volume I. Basic Priciples. pp 15-33. CRC Press, Florida.

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

Gerasimova, A. A. 1940. Duration of heat and time of ovulation in the cow. Anim. Breed. Abstr. 40:32.

Glencross, R. G., I. B. Munro, B. E. Senior, and G. S. Pope. 1973. Concentrations of oestradiol-17B, oestrone and Progestrone in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta Endocrinol. 73:374.

Godkin, J. D., F. W. Bazer and R. M. Roberts. 1984. Ovine trophoblast protein-1, an early secreated blastocyst protein, binds specificaly to uterine endometrium and affected protein synthesis. Endocrinology. 114:120.

Gomes, W. R. and R. E. Erb. 1965. Progesterone in bovine reproduction: a review. J. Dairy Sci. 53: 314.

Greenstein, J. S. and R. C. Foley. 1958. Early embryology of the cow. I. Gastrula and primitive streak changes. J. Dairy Sci. 41:409.

Greenstein, J. S., R. W. Murray and R. C. Foley. 1958. Observations on the morphogenesis and histochemistry of the bovine pre-attachment placenta between 16 and 33 days of gestation. Anat. Rec. p.321.

Guillomot, M. and P. Guay. 1982. Ultrastructure features of the cell surfaces of the uterine and trophoblastic epithelium during embryo attachment in the cow. Anat. Rec. 204:315.

Gutierrez-De La R., J. H., A. C. Warnick, J. J. Cowley and J. F. Hentges, Jr. 1969. Environmental physiology in the subtropics. I. Effects of continuous environmental stress on some hematological values of beef cattle. J. Anim. Sci. 32:968.

Gwazdauskas, F. C., R. M. Abrams, W. W. Thatcher, F. W. Bazer and D. Canton. 1974. Thermal changes of the bovine uterus following administration of oestradiol-17B. J. Anim. Sci. 87:87.

Gwazdauskas, F. C., W. W. Thatcher and C. J. Wilcox. 1973. Physical, environmental, and hormonal factors at

insemination which may affect conception. J. Dairy Sci. 56:873.

Gwazdauskas, F. C., W. W. Thatcher, M. J. Paape, C. A. Kiddy and C. J. Wilcox. 1975. Plasma characteristics and adrenal response of heifers to thermal stress. J. Dairy Sci. 58:776 (Abstr.).

Hackett, A. J. and H. D. Hafs. 1969. Pituitary and hypothalamic endocrine changes during the bovine estrous cycle. J. Dairy Sci. 28:531.

Hafez, E. S. E. 1980. Transport and survival of embryos. In: Reproduction in farm animals. pp 203-225. Lea and Fibiger, Philadelphia.

Hallford, D. M., E. J. Turman, R. P. Wetteman and C. E. Pope. 1979. Endocrine and reproductive response of beef cows to PMSG. J. Anim. Sci. 49:1030.

Hansel, W. 1981. Plasma hormone concentrations associated with early embryo mortality in heifers. J. Reprod. Fertil. Suppl. 30:231.

Hansel, W. and R. B. Snook. 1970. Pituitary ovarian relationships in the cow. J. Dairy Sci. 53:945.

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

Hawk, H. W., J. N. Wiltbank, H. E. Kidder and L. E. Casida. 1955. Embryonic Mortality between 16 and 34 days postbreeding in cows of low fertility. J. Dairy Sci. 38:673.

Hawk, H. W. and R. A. Bellows. 1980. Beef and dairy cattle. In: E. S. E. Hafez (Ed) Reproduction in farm animals. pp 337-345. Lea and Febiger, Philadelphia.

Helmer, S. D., P. J. Hansen, W. W. Thatcher, R. M. Roberts and F. W. Bazer. 1985. Similarities between ovine and bovine conceptus secreatory proteins associated with luteal maintenance. J. Anim. Sci. 61:400 (Abstr.).

Hendricks, D. M., J. F. Dickey and D. G. Niswender. 1970. Serum LH and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346.

Henricks, D. M., J. F. Dickey, J. R. Hill and W. E. Johnston. 1972. Plasma estrogen and progesterone levels

after mating, and during late pregnancy and postpartum in cows. Endocrinology. 90:1336.

Henricks, D. M. and D. T. Mayer. 1977. Gonadal Hormones and Uterine Factors. In: H. H. Cole and P. T. Cupps (Ed) Reproduction in Domestic Animals. pp 79-117. Academic Press, New York.

Hixon, J. E. and W. Hansel. 1974. Evidence for preferential transfer of prostaglandin F2$\alpha$ to the ovarian artery following intrauterine administration in cattle Biol. Reprod. 11:543.

Hixon, J. E., C. A. Pimentel, P. G. Weston, E. P. Chafetz, R. D. Shanks and W. Hansel. 1983. A luteotropic interaction between estradiol benzoate and prostaglandin F2$\alpha$ in cattle. J. Anim. Sci. 56:1190.

Huckabee, W. E., C. Crenshaw, L. B. Ciret and D. H. Barron. 1968. Blood flow and oxygen consumption of the uterus of the nonpregnant ewe. Quart. J. Exp. Physiol. 53:349.

Ingraham, R. M., D. D. Gillette and W. E. Wagner. 1974. Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate. J. Dairy Sci. 57:476.

Ireland, J. J., P. B. Coulson and R. L. Murphree. 1979. Follicular development during four stages of the estrous cycle of beef cattle. J. Anim. Sci. 49:1261.

Iritani, A. and K. Niwa. 1977. Capacitation of bull spermatozoa and fertilization **in vitro** of cattle follicular oocytes matured in culture. J. Reprod. Fertil. 50:119.

Johnson, H. D. 1985. Physiological responses and productivity of cattle In: M. K. Yousef (Ed) Stress physiology in livestock. Volume II. Undulates. pp 3-23. CRC Press, Florida.

King, G. J., B. A. Atkinson and H. A. Robertson. 1980. Development of the bovine placentome from Days 20 to 29 of gestation. J. Reprod. Fertil. 59:95.

King, G. J., B. A. Atkinson and H. A. Robertson. 1981. Development of the intercaruncular areas during early gestation and establishment of the bovine placenta. J. Reprod. Fertil. 61:469.

Knickerbocker, J. J., W. W. Thatcher, F. W. Bazer, M. Drost, D. H. Barron, K. B. Fincher and R. M. Roberts. 1984.

Proteins secreted by cultured day 17 bovine conceptuses extended luteal function in cattle. 10th Intern. Cong. Anim. Reprod. A.I. 2:88.

Knight, J. W., F. W. Bazer and H. D. Wallace. 1973. Hormonal regulation of porcine uterine protein secretion. J. Anim. Sci. 36:546.

Kreider, D. L., R. P. Wettemann, R. K. Johnson and E. J. Turman. 1978. Endocrine changes associated with heat stress of gilts after breeding. Okla. Agr. Exp. Stat. MP-103:219.

Lacroix, E., W. Eechaute and I. Leusen. 1974. The biosynthesis of estrogens by cow follicles. Steroids 23:337.

Lauderdale, J. W. and R. J. Ericsson. 1970. Biol. Reprod. 2:179.

Lewis, G. S., S. M. M. Basha, F. W. Bazer, R. M. Roberts and W. W. Thatcher. 1979a. Proteins originating from bovine and porcine blastocysts. J. Anim. Sci. 49:313. (Abstr.).

Lewis, G. S., W. W. Thatcher, F. W. Bazer and J. S. Curl. 1982. Metabolism of arachidonic acid in vitro by bovine blastocysts and endometrium. Biol. Reprod. 27:431.

Lewis, G. S., W. W. Thatcher, F. W. Bazer, R. M. Roberts and W. F. Williams. 1979b. Metabolism of arachidonic acid by bovine blostocysts and endometrium. J. Anim. Sci. 49:313 (Abstr.).

Lewis, G. S., W. W. Thatcher, L. A. Guibault, F. W. Bazer and F. F. Bartol. 1980. Prostaglandin production by bovine blastocysts and endometrium. J. Anim. Sci. 51:298 (Abstr.).

Lewis, G. S., W. W. Thatcher, E. L. Bliss, M. Drost and R. J. Collier. 1984. Effects of heat stress during pregnancy on postpartum reproductive changes in Holstein cows. J. Anim. Sci. 58:174.

Lewis, G. S. and R. A. Waterman. 1983. Effects of endometrium on metabolism of arachidonic acid by the bovine blastocysts in vitro. Prostaglandins 25:881.

Lewis, T. M., H. D. Hafs and D. A. Morrow. 1974. Intrauterine administration of prostaglandin F2 in cows: progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347.

Loeb, L. 1923. The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. Proc. Soc. Exp. Biol. Med. 20:441.

Lukaszewska, J. and W. Hansel. 1980. Corpus luteum maintenance during early pregnancy in the cow. J. Reprod. Fertil. 59:485.

Madan, M. L. and H. D. Johnson. 1973. Environmental heat effects on bovine lutenizing hormone. J. Dairy Sci. 56:1420.

Marion, G. B., V. R. Smith, T. E. Wiley and G. R. Barrett. 1950. The effect of sterile copulation on the time of ovulation in dairy heifers. J. Dairy Sci. 33:885.

Marinov, U. and J. E. Lovell. 1968. Cytology of the bovine uterine epithelium during the estrous cycle. Am. J. Vet. Res. 29:13.

Masters, R. A., R. M. Roberts, G. S. Lewis, W. W. Thatcher, F. W. Bazer and J. D. Godkin. 1982. High molecular weight glycoproteins released by expanding, preattachment sheep, pig and cow blastocysts in culture. J. Reprod. Fertil. 66:571.

Matton, P., V. Adelakoun, Y. Couture and J. J. Dufour. 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. J. Anim. Sci. 52:813.

McCracken, J. A., W. Schrann and W. C. Okuliez. 1984. Hormone receptor control of pulsitile secretion of PGF2 from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31.

McLaren, A. 1970. Fate of the zone pellucida in mice. J. Embryol. Exp. Morphol. 23:1

McLaren, A. 1980. Fertilization, cleavage and implantation In: E. S. E. Hafez (Ed) Reproduction in farm animals. pp 226-246. Lea and Febiger, Philadelphia.

McLaren, A. 1982. The embryo In: C. R. Austin and R. V. Short (Ed) Reproduction in mammals: 2. Embryonic and fetal development. pp 1-25. Cambridge University press, Cambridge.

Mellin, T. N. and R. E. Erb. 1965. Estrogens in the bovine-a review. J. Dairy Sci. 48:697.

Melton, A. A., R. O. Berry and O. D. Butler. 1951. The interval between the time of ovulation and attachment

of the bovine embryo. J. Anim. Sci. 10:993.

Merz, E. A., E. R. Hauser and B. G. England. 1981. Ovarian function in the cycling cow: relationship between gonadotropin binding to theca and granulosa and steriodogenesis in individual follicles. J. Anim. Sci. 52:1457.

Meizel, S. 1978. The Mammalian Sperm Acrosome Reaction, A Biochemical Approach In: M. H. Johnson (Ed) Development in Mammals. North-Holland Publishing Co., Amsterdam.

Mills, A. C., W. W. Thatcher, S. E. Dunlap and C. K. Vincent. 1972. Influence of postbreeding thermal stress on peripheral plasma progestin concentrations in heifers. J. Dairy Sci. 55:400.

Moor, R. M. and L. E. A. Rowson. 1966a. Local maintenance of the corpus luteum in sheep with embryos transfered to various isolated portions of the uterus. J. Reprod. Fertil. 12:539.

Moor, R. M. and L. E. A. Rowson. 1966b. The corpus luteum of sheep: Effect of the removal of embryos on luteal function. J. Endocrinol. 34:497.

Nalbandov, A. and L. E. Casida. 1942. Ovulation and its relation to estrus in cows. J. Anim. Sci. 1:189.

Northey, D. L. and L. R. French. 1980. Effect of embryo removal and intrauterine infusion of embryonic homogenates on the life span of the bovine corpus luteum. J. Anim. Sci. 50:298.

Olds, D. and N. L. Vandermark. 1957. Composition of luminal fluids in bovine female genitalia. Fert. Steril. 8:345.

Omtvedt, I. T., R. E. Nelson, R. L. Edwards, D. F. Stephens and E. J. Turman. 1971. Influence of heat stress during early, mid and late pregnancy gilts. J. Anim. Sci. 32:312.

Perry, J. S. 1982. The mammalian fetal membranes. J. Reprod. Fertil. 62:321.

Polge, C. and Baker, R. D. 1976. Fertilization in pigs and cattle. Canad. J. Anim. Sci. 56:105.

Pratt, B. R. and R. P. Wettemann. 1980. Physiological changes in steers after acute exposure to either cool or hot environmental temperatures. Okla. Agr. Exp. Stat. MP-107:170.

Pratt, B. R. and R. P. Wettemann. 1986. The Effect of Environmental Temperature on Concentrations of Thyroxine and Triiodothyronine After Thyrotropin Releasing Hormone in Steers. J. Anim. Sci. 62.

Renfree, M. B. 1982. Implantation and placentation In: C. R. Austin and R. V. Short (Ed.) Reproduction in mammals: 2. Embryonic and fetal development. pp 26-69. Cambridge University Press, Cambridge.

Roberts, G. P. and J. M. Parker. 1974a. Macromolecular components of the luminal fluid from the bovine uterus. J. Reprod. Fertil. 40:291.

Roberts, G. P. and J. M. Parker. 1974b. An investigation of enzymes and hormone binding proteins in the luminal fluid of the bovine uterus. J. Reprod. Fertil. 40:305.

Roberts, G. P. and J. M. Parker. 1976. Fractionation and comparison of proteins from uterine fluid and bovine allantoic fluid. Biochem. Biophys. Acta. 446:69.

Robertshaw, D. 1985. Heat loss in cattle In: M. K. Yousef (Ed.) Stress physiology in livestock. Volume I. Basic principles. pp 55-66. CRC Press, Florida.

Robertson, H. A. and G. J. King. 1979. Conjugated and unconjugated oestrogens in fetal and maternal fluids of the cow throught pregnancy. J. Reprod. Fertil. 55:463.

Roman-Ponce, H., W. W. Thatcher, D. Canton, D. H. Barron and C. J. Wilcox. 1978. Thermal stress effects on uterine blood flow in dairy cows. J. Anim. Sci. 46:175.

Ross, T. T., L. Goode and H. C. Linnerud. 1985. Effects of high ambient temperature on respiration rate, rectal temperature, fetal development and thyroid gland activity in tropical and temperate breeds of sheep. Theriogenology 24:259.

Rosenberg, M., Y. Folman, Z. Herz, I. Flamenbaum, A. Berman and M. Kaim. 1982. Effect of climatic conditions on peripheral concentrations of LH, progesterone and estradiol-17B in high milk-yielding cows. J. Reprod. Fertil. 66:139.

Rowson, L. E. A., R. Tervit and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J. Reprod. Fertil. 29:145.

Rowson, L. E. A., R. A. S. Lawson, R. M. Moor and A. A. Baker. 1972. Egg transfer in the cow: synchronization

requirements. J. Reprod. Fertil. 28:427.

Rubsamen, K. and J. R. S. Hales. 1985. Circulatory adjustments of heat-stressed livestock In: M. K. Yousef (Ed.) Stress physiology in livestock. Volume I. Basic principles. pp 143-154 CRC Press, Florida.

Salisbury, G. W., N. L. VanDemark and J. R. Lodge. 1978. The estrous cycle In: Physiology of reproduction and artificial insemination of cattle. pp 52-83. W.H. Freeman and Company, San Francisco.

Schams, D., E. Schallenberger, B. Hoffmann and H. Karg. 1977. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. Acta Endocrinol. 86:180.

Schams, D. and H. Karg. 1969. Radioimmunological estimation of LH in blood serum of cattle with special regard to the oestrus cycle. Acta. Endocrinol. 61:96.

Schmidt-Nielsen, K. 1981. Temperature regulation In: Animal physiology: Adaptation and environment. pp 228-281. Cambridge University Press, Cambridge.

Scott, I. M., H. D. Johnson and L. Hahn. 1975. Physiological response of Holstein cows to patterns of diurnally-imposed heat stress. J. Dairy Sci. 58:777. (Abstr.).

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

Shemesh, M., F. Malaguir, N. Ayalon and W. Hansel. 1979. Steroidogenesis and prostaglandin synthesis by the cultured bovine blastocysts. J. Reprod. Fertil. 56:181.

Sherwood, O. D. and W. H. McShane. 1977. Gonadotropins In: H.H. Cole and P.T. Cupps (Ed.) Reproduction in Domestic Animals. pp 17-47. Academic Press, New York.

Smith, V. G., L. A. Edgerton, H. D. Hafs and E. M. Convey. 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. J. Anim. Sci. 36:391.

Spicer, L. J., J. J. Ireland and J. F. Roche. 1981. Changes in serum LH, progesterone, and specific binding of 125-I-hCG to luteal cells during regression and development of bovine corpora lutea. Biol. Reprod. 25:832.

Squire, G. D., F. W. Bazer and F. A. Murray. 1972. Electrophoretic patterns of porcine uterine protein secretions during estrous cycle. Biol. Reprod. 7:321.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill Book Company. New York.

Stott, G. L. and R. L. Williams. 1962. Causes of low breeding efficiency in dairy cattle associated with seasonal high temperatures. J. Dairy Sci. 45:1369.

Sugie, T., G. E. Seidel, Jr., and E. S. E. Hafez. 1980. Embryo transfer In: E. S. E. Hafez (Ed.) Reproduction in farm animals. pp 569-594. Lea and Febiger, Philadelphia.

Thatcher, W. W. 1974. Effects of season, climate and temperature on reproduction and lactation. J. Dairy Sci. 57:360.

Thatcher, W. W. and R. J. Collier. 1980. Effect of heat stress on animal productivity In: M. Recheigl, Jr. (Ed) CRC handbook of agricultural productivity. pp 77-105. CRC Press, Inc., Boca Raton, Florida.

Thatcher, W. W., J. J. Knickerbocker, F. F. Bartol, F. W. Bazer, R. M. Roberts and M. Drost. 1985. Maternal recognition of pregnancy in relation to the survival of transfered embryos: endocrine aspects. Theriogenology. 23:129.

Tompkins, E. C., C. J. Heidenreich and M. Stob. 1967. Effect of postbreeding thermal stress on early embryonic mortality in swine. J. Anim. Sci. 26:377.

Thompson, G. E. 1973. Review of the progress of dairy science. Climatic physiology of cattle. J. Dairy Sci. 40:441.

Ulberg, L. C. and Burfening. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. J. Anim. Sci. 26:571.

Vaught, L. W., D. E. Monty, Jr. and W. C. Foote. 1977. Effect of summer heat stress on serum luteinizing hormone and progest erone values in Holstein Friesian cows in Arizona. Amer. J. Vet. Res. 38: 1027.

Villa-Godoy, A., J. J. Ireland, J. A. Worton, N. K. Ames, T. L. Hughes and R. L. Fogwell. 1985. Effect of ovarian follicles on luteal regression in heifers. J. Anim.

Sci. 60:519.

Wales. R. G. 1975. Maturation of the mammalian Embryo: Biochemical Aspects. Biol. Reprod. 12:66.

Warnick, A. C., H. D. Wallace, A. C. Palmer, E. Sosa, D. J. Duerre and V. E. Caldwell. 1965. Effect of temperature on early embryo survival in gilts. J. Anim. Sci. 24:89.

Wathes, D. C. and F. B. P. Wooding. 1980. An electron microscope study of implantation in the cow. Amer. J. Anat. 159:285.

Watts, T. L. and J. W. Fuquay. 1985. Response and fertility of dairy heifers following injection with prostaglandin F2$\alpha$ during early, middle and late diestrus. Theriogenology. 23:655.

Wettemann, R. P., F. W. Bazer, W. W. Thatcher and T. A. Hoagland. 1984. Environmental influences on embryonic mortality. 10th Intern. Cong. Anim. Reprod. A. I. 4:26.

Wettemann, R. P. and H. D. Hafs. 1973. LH, prolactin, estradiol and progesterone in bovine blood serum during early pregnancy. J. Anim. Sci. 36:51.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020.

Wildt, D. E., G. D. Riegle and W. E. Dukelow. 1975. Physiological temperature response and embryonic mortality in stressed swine. Amer. J. Physiol. 229: 1471.

Wintenberger-Torres, S. and J. E. Flechon. 1974. Ultrastructural evolution of the trophoblast cells of the pre-implantation sheep blastocyst from day 8 to day 16. J. Anat. 118:143.

Winters, L. M., W. W. Green and R. E. Comstock. 1942. Prenatal development of the bovine. Minnesota Agr. Expt. Sta. Tech. Bull. 151.

Wise, T. H., D. Caton, W. W. Thatcher, D. H. Barron and M.J. Fields. 1982. Ovarian function during the estrous cycle of the cow: ovarian blood flow and progesterone release rate. J. Anim. Sci.

Wooding, F. B. P. 1984. Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep.

Amer. J. Anat. 170:233.

Yanagimachi, R. 1981. Mechanisms of Fertilization in Mammals. In: L. Mastroianni and J. D. Biggers (Ed.) Fertilization and Embryonic Development in vitro. Plenum Press, New York.

Yousef, M. K. 1985. Heat production: Mechanisms and regulation In: Stress physiology in livestock. Volumme I. Basic principles. pp 47-54. CRC Press, Florida.

Yousef, M. K. and H. D. Johnson. 1985. Endocrine system and thermal environment In: M. K. Yousef (Ed.) Stress physiology in livestock. Volume I. Basic principles. pp133-141. CRC Press, Florida.

# APPENDIX

TABLE 7. ANALYSIS OF VARIANCE OF ENVIRONMENTAL TEMPERATURE FOR TREATMENTS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 36619.37 | 18309.69 | 746.90** |
| Cow(Treatment) | 29 | 710.92 | 24.51 | 4.53** |
| Day | 7 | 291.29 | 41.61 | 7.69** |
| Treatment*Day | 14 | 247.51 | 17.68 | 3.27** |
| Residual | 175 | 946.94 | 5.41 | |

**P<.01

TABLE 8. ANALYSIS OF VARIANCE OF RELATIVE HUMIDITY FOR TREATMENTS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 10866.18 | 5433.09 | 40.04** |
| Cow(Treatment) | 26 | 3527.70 | 135.68 | 17.11** |
| Day | 7 | 317.85 | 45.41 | 5.73** |
| Treatment*Day | 14 | 995.15 | 71.08 | 8.96** |
| Residual | 143 | 1133.84 | 7.93 | |

**P<.01

TABLE 9. ANALYSIS OF VARIANCE OF RECTAL TEMPERATURE IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 113.29 | 56.65 | 5.78** |
| Cow(Treatment) | 29 | 284.25 | 9.80 | 11.42** |
| Day | 7 | 25.32 | 3.62 | 4.21** |
| Treatment*Day | 14 | 14.73 | 1.05 | 1.23 |
| Residual | 159 | 136.42 | .86 | |

**P<.01

TABLE 10. ANALYSIS OF VARIANCE OF RESPIRATION RATE IN RESPONSE TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 110172.85 | 55086.43 | 67.92** |
| Cow(Treatment) | 29 | 23520.56 | 811.05 | 2.28 |
| Day | 7 | 12229.68 | 1747.10 | 4.91 |
| Treatment*Day | 14 | 4476.60 | 319.76 | .91 |
| Residual | 174 | 61905.38 | 355.78 | |

**P<.01

TABLE 11. ANALYSIS OF VARIANCE OF WATER INTAKE IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 3241.00 | 1620.50 | 2.10+ |
| Cow(Treatment) | 19 | 14635.94 | 770.31 | 21.55** |
| Day | 8 | 307.31 | 38.41 | 1.07 |
| Treatment*Day | 16 | 1184.55 | 74.03 | 2.07* |
| Residual | 104 | 3718.38 | 35.75 | |

**P<.01
*P<.05
+P<.10

TABLE 12. ANALYSIS OF VARIANCE OF HEMATOCRIT IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 678.20 | 339.10 | 3.28* |
| Cow(Treatment) | 28 | 2896.74 | 103.46 | 8.29** |
| Day | 7 | 74.67 | 10.67 | .85 |
| Treatment*Day | 14 | 169.76 | 12.13 | .97 |
| Residual | 150 | 1871.90 | 12.48 | |

**P<.01
*P<.05

TABLE 13. ANALYSIS OF VARIANCE OF PLASMA PROTEIN IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 2.56 | 1.28 | 1.63 |
| Cow(Treatment) | 28 | 22.03 | .79 | 12.77** |
| Day | 7 | 1.98 | .28 | 4.60** |
| Treatment*Day | 14 | 1.53 | .11 | 1.78* |
| Residual | 155 | 9.55 | .06 | |

**P<.01
*P<.05

TABLE 14. ANALYSIS OF VARIANCE OF PLASMA PROGESTERONE IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 33.33 | 16.67 | .80 |
| Cow(Treatment) | 28 | 585.98 | 20.93 | 9.40** |
| Day | 8 | 38.93 | 4.87 | 2.19* |
| Treatment*Day | 16 | 37.48 | 2.34 | 1.05 |
| Residual | 198 | 440.72 | 2.23 | |

**P<.01
*P<.05

TABLE 15. ANALYSIS OF VARIANCE OF PLASMA ESTRADIOL-17B CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 4.59 | 2.30 | .32 |
| Cow(Treatment) | 28 | 198.35 | 7.08 | 3.49** |
| Day | 8 | 26.10 | 3.26 | 1.61 |
| Treatment*Day | 16 | 21.23 | 1.33 | .65 |
| Residual | 197 | 400.11 | 2.03 | |

**P<.01

TABLE 16. ANALYSIS OF VARIANCE OF PLASMA THYROXINE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 4308.66 | 2154.33 | 2.74+ |
| Cow(Treatment) | 29 | 22773.23 | 785.28 | 5.29** |
| Day | 8 | 3221.87 | 402.73 | 2.71+ |
| Treatment*Day | 16 | 3268.15 | 204.26 | 1.37 |
| Residual | 201 | 29865.49 | 148.58 | |

**P<.01
+P<.10

TABLE 17. ANALYSIS OF VARIANCE OF PLASMA GLUCOSE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 1631.42 | 815.71 | 2.61+ |
| Cow(Treatment) | 28 | 8758.35 | 312.80 | 10.24** |
| Day | 8 | 437.73 | 54.72 | 1.79+ |
| Treatment*Day | 16 | 298.05 | 18.63 | .61 |
| Residual | 193 | 5895.67 | 30.55 | |

**P<.01
+P<.10

TABLE 18. ANALYSIS OF VARIANCE OF PLASMA PROGESTERONE CONCENTRATION FOR PREGNANCY STATUS

| Source | df | SS | SS | F |
|---|---|---|---|---|
| Status | 1 | 91.55 | 91.55 | 5.74* |
| Status*Treatment | 4 | 84.62 | 21.16 | 1.33 |
| Cow(Status*Treatment) | 25 | 399.02 | 15.96 | 7.31** |
| Day | 7 | 31.21 | 4.46 | 2.04* |
| Treatment*Day | 14 | 35.88 | 2.56 | 1.17 |
| Status*Day | 7 | 6.81 | .97 | .45 |
| Residual | 165 | 360.03 | 2.18 | |

**P<.01
*P<.05

TABLE 19. ANALYSIS OF VARIANCE OF ESTRADIOL-17B CONCENTRATION FOR PREGNANCY STATUS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Status | 1 | 22.46 | 22.46 | 3.45$^{+}$ |
| Status*Treatment | 4 | 21.47 | 5.37 | .83 |
| Cow(Status*Treatment) | 25 | 162.64 | 6.51 | 3.05$^{**}$ |
| Day | 7 | 16.90 | 2.41 | 1.13 |
| Treatment*Day | 14 | 14.80 | 1.06 | .50 |
| Status*Day | 7 | 11.63 | 1.66 | .75 |
| Residual | 164 | 349.24 | 2.13 | |

$^{**}P<.01$
$^{+}P<.10$

TABLE 20. ANALYSIS OF VARIANCE OF THYROXINE CONCENTRATION FOR PREGNANCY STATUS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Status | 1 | 2703.88 | 2703.88 | 4.09* |
| Status*Treatment | 4 | 2746.04 | 686.51 | 1.04 |
| Cow(Status*Treatment) | 26 | 17172.53 | 660.48 | 4.86** |
| Day | 7 | 3198.40 | 456.91 | 3.36** |
| Treatment*Day | 14 | 2307.76 | 164.84 | 1.21 |
| Status*Day | 7 | 644.85 | 92.12 | .68 |
| Residual | 168 | 22815.45 | 135.81 | |

**P<.01
*P<.05

TABLE 21. ANALYSIS OF VARIANCE OF GLUCOSE CONCENTRATION FOR PREGNANCY STATUS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Status | 1 | 494.26 | 494.26 | 1.94 |
| Status*Treatment | 4 | 2133.58 | 533.40 | 2.09 |
| Cow(Status*Treatment) | 25 | 6365.74 | 254.63 | 8.98** |
| Day | 7 | 412.91 | 58.99 | 2.08* |
| Treatment*Day | 14 | 241.57 | 17.26 | .61 |
| Status*Treatment | 7 | 353.21 | 50.46 | 1.78+ |
| Residual | 163 | 4623.72 | 28.37 | |

**P<.01
*P<.05
+P<.10

TABLE 22. HETEROGENEITY OF REGRESSION OF PROGESTERONE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F | |
|---|---|---|---|---|---|
| Control | 79 | 195.01 | | | |
| Mild Heat Stress | 65 | 107.66 | | | |
| Severe Heat Stress | 75 | 187.39 | | | |
| Total | 219 | 490.06 | 2.23 | | |
| Overall | 221 | 490.18 | | | |
| Heterogeneity | 3 | .12 | .04 | .02 | NS |

TABLE 23. ANALYSIS OF VARIANCE OF HETEROGENEITY OF REGRESSION FOR PROGESTERONE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 33.33 | 16.67 | .80 |
| Cow(Treatment) | 28 | 585.98 | 20.92 | 9.44** |
| Day | 1 | 26.96 | 26.96 | 12.16** |
| Heterogeneity | 3 | .12 | .04 | .02 |
| Residual | 221 | 490.18 | 2.22 | |

**P<.01

TABLE 24. HETEROGENEITY OF REGRESSION OF ESTRADIOL-17B CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Control | 77 | 138.72 | | |
| Mild Heat Stress | 63 | 100.84 | | |
| Severe Heat Stress | 72 | 180.17 | | |
| Total | 212 | 419.73 | 1.98 | |
| Overall | 218 | 430.09 | | |
| Heterogeneity | 6 | 10.36 | 1.73 | .87 NS |

TABLE 25. ANALYSIS OF VARIANCE OF HETEROGENEITY OF REGRESSION FOR ESTRADIOL-17B CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 4.59 | 2.30 | .32 |
| Cow(Treatment) | 28 | 198.35 | 7.08 | 3.59** |
| Day | 1 | 5.40 | 5.40 | 2.74+ |
| Day*Day | 1 | 2.85 | 2.85 | 1.45 |
| Day*Day*Day | 1 | 9.10 | 9.10 | 4.61* |
| Heterogeneity | 6 | 10.36 | 1.73 | .87 |
| Residual | 218 | 430.09 | 1.97 | |

**P<.01
*P<.05
+P<.10

TABLE 26. HETEROGENEITY OF REGRESSION OF THYROXINE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Control | 77 | 12210.68 | | |
| Mild Heat Stress | 63 | 7650.10 | | |
| Severe Heat Stress | 77 | 15788.57 | | |
| Total | 217 | 35649.35 | 164.28 | |
| Overall | 223 | 36756.86 | | |
| Heterogeneity | 6 | 1107.51 | 184.59 | 1.12 NS |

TABLE 27. ANALYSIS OF VARIANCE OF HETEROGENEITY OF REGRESSION FOR THYROXINE CONCENTRATION IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 4056.52 | 2028.26 | 2.63$^{+}$ |
| Cow(Treatment) | 29 | 22337.67 | 770.26 | 4.67$^{**}$ |
| Day | 1 | 327.15 | 327.15 | 1.98 |
| Day*Day | 1 | 429.85 | 429.85 | 2.61 |
| Day*Day*Day | 1 | 1466.44 | 1466.44 | 8.90$^{**}$ |
| Heterogeneity | 6 | 1107.51 | 184.59 | 1.12 |
| Residual | 232 | 36756.86 | 164.83 | |

$^{**}P<.01$
$^{+}P<.10$

TABLE 28. HETEROGENEITY OF REGRESSION FOR PROGESTERONE CONCENTRATION DURING PREGNANCY

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Pregnant | 167 | 350.85 | | |
| Nonpregnant | 77 | 181.93 | | |
| Total | 244 | 532.78 | 2.18 | |
| Overall | 245 | 540.82 | | |
| Heterogeneity | 1 | 8.04 | 8.04 | 3.68+ |

+P<.05

TABLE 29. HETEROGENEITY OF REGRESSION FOR ESTRADIOL-17B CONCENTRATION DURING PREGNANCY

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Pregnant | 164 | 369.95 | | |
| Nonpregnant | 75 | 104.80 | | |
| Total | 239 | 474.75 | 1.99 | |
| Overall | 242 | 476.77 | | |
| Heterogeneity | 3 | 2.02 | .67 | .34 NS |

TABLE 30. HETEROGENEITY OF REGRESSION FOR THYROXINE CONCENTRATION DURING PREGNANCY

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Pregnant | 149 | 23234.39 | | |
| Nonpregnant | 71 | 13350.75 | | |
| Total | 220 | 36585.14 | 166.30 | |
| Overall | 223 | 36756.86 | | |
| Heterogeneity | 3 | 171.72 | 57.24 | .34 NS |

TABLE 31. ANALYSIS OF VARIANCE OF PREGNANCY RATES IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | .58 | .29 | 1.27 |
| Residual | 29 | 6.64 | .22 | |

TABLE 32. ANALYSIS OF VARIANCE OF CORPUS LUTEUM WET WEIGHT IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | 2.02 | 1.01 | 1.52 |
| Residual | 29 | 19.22 | .66 | |

TABLE 33. ANALYSIS OF VARIANCE OF CONCEPTUS WET WEIGHT IN RESPONSE TO TREATMENT

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Treatment | 2 | .025 | .013 | 5.94** |
| Residual | 29 | .036 | .002 | |

**P<.01

# VITA

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Masters of Science

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