

Biotechnology and Reproductive Physiology at NDSU

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The most significant contribution toward improvement of overall efficiency of meat animal production can be made by improving reproductive efficiency (Trenkle and Willham, 1977). It has been shown that, in terms of economic value, improvements in reproductive performance would have great benefit for commercial producers. These improvements involve the ability to control various reproductive processes such as ovulation rates, pregnancy rates, number of offspring per animal per lifetime, growth and development of the fetus, etc. For example, to maximize efficiency of animal reproduction, techniques such as superovulation and embryo transfer have been utilized. However, these techniques produce highly variable results and are often unsuccessful. Therefore, present methods of controlling reproductive processes need to be "fine tuned" for more precise control.

Unfortunately, "fine tuning" of any biological process cannot be accomplished until one gains an in-depth understanding of the cellular and molecular events that control a given function. Understanding these cellular events leads to alternative methods by which a particular process can be controlled. For example, scientists studied regulation of the estrous cycle for many years, but it wasn't until the 1960s and 1970s that prostaglandins were found to exhibit a profound influence on the ovary. Understanding the basic mechanisms by which prostaglandins control ovarian function led to the development of compounds used for synchronizing estrus in cattle and sheep. The diligent efforts of scientists working in the area that often seemed fruitless to producers provided the basis for techniques commonly used today for reproductive management and treatment of reproductive disorders. This successful application of basic research provides an example of the importance of conducting research at cellular and molecular levels and using the tools of biotechnology for improving the reproductive performance and efficiency of meat animals.

OVARIAN FUNCTION

Two important components of ovarian function are follicular and luteal development. Follicles, which develop in the ovary throughout the estrous cycle, are the structures of the ovary that contain eggs. If follicles fail to grow at puberty, after parturition, after estrous synchronization, or during the estrous cycle, the animal will not exhibit estrus and will not ovulate. Failure of animals to develop follicles and ovulate is a major reproductive problem. Therefore, follicular development is an extremely important reproductive process

which culminates in the expulsion and delivery of eggs to the oviduct and eventually the uterus. Following ovulation, a corpus luteum forms (where the follicle was located) and secretes progesterone, an important hormone that regulates estrous cycle length and maintains pregnancy.

The mechanisms that control follicular and luteal development are not clearly understood. In order to more precisely control ovulation rate and the estrous cycle, factors that regulate follicular growth and luteal function must first be better understood. Our studies have focused on the development of the blood supply (angiogenesis) to local sites within the ovary and its importance to follicular and luteal development.

Regulation of blood vessel growth may be especially important in reproductive tissues (e.g., ovary, placenta) which represent normal tissues that exhibit dynamic, periodic growth and regression of vascular beds with attendant changes in rates of blood flow. The cyclic establishment and survival of structures in the ovary (i.e. follicles, corpora lutea) may be dependent on their ability to recruit a vascular supply. That rapidly growing tissues must recruit a blood supply was recognized by Folkman and Cotran (1976) and Folkman (1982) who demonstrated that growth of tumors progressed only if preceded by recruitment and establishment of a functional vascular supply.

The major cell type contained in small blood vessels and capillaries is the endothelial cell. We hypothesized that follicles and corpora lutea have the ability to recruit a vascular supply, and that they secrete a factor(s) that stimulates endothelial cells to grow, divide, and migrate toward them. We have used three different methods to evaluate the ability of follicles and corpora lutea to secrete angiogenic factors.

Figure 1a (Redmer et al., 1988) demonstrates the ability of luteal tissue from cows to stimulate vascular growth when placed on the chorioallantoic membrane of a developing chicken egg. Figure 1b is the response observed when a control tissue is placed on the chorioallantoic membrane. Figure 2 (Redmer et al., 1988) demonstrates the ability of secretions from luteal tissues to stimulate growth of vascular endothelial cells. In addition, Figure 2 shows that two hormones that normally regulate luteal function can affect the ability of luteal tissue to secrete angiogenic factor(s) and that secretion of luteal angiogenic factor(s) increases during the estrous cycle of cows.

Figure 3 (Redmer et al., 1988) shows the ability of luteal tissue to secrete a factor(s) that stimulates migration of endothelial cells *in vitro* as well as effects of hormones and stage of the estrous cycle. Table 1 (Taraska et al., 1988)

Redmer and Reynolds are associate professors and Tilton is professor, Department of Animal and Range Sciences.

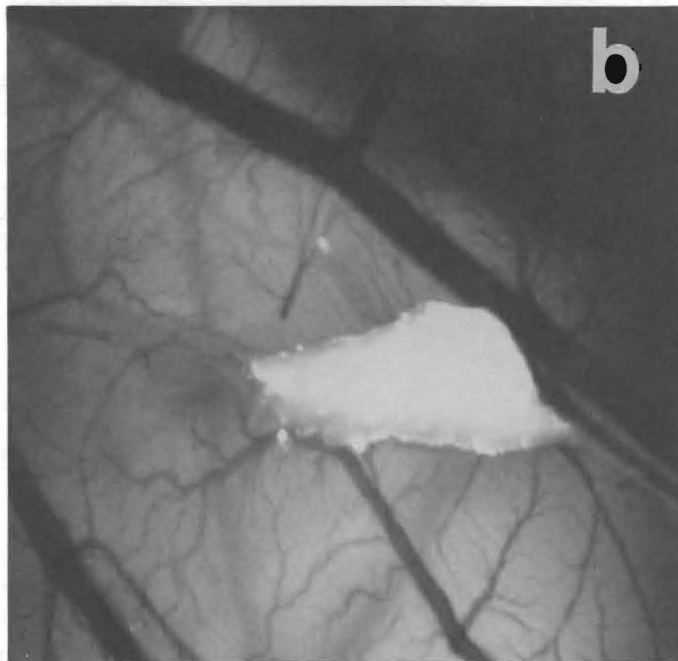
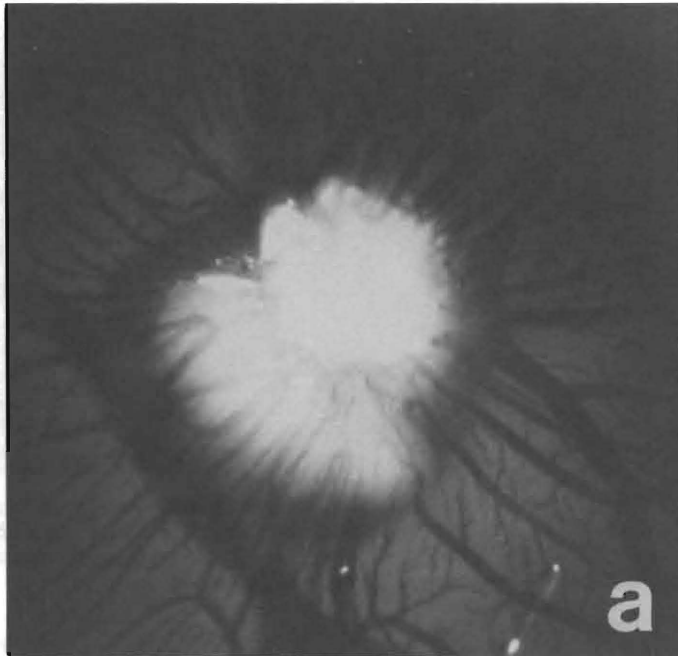


Figure 1. The chick chorioallantoic membrane assay is used commonly to study angiogenesis (growth of blood vessels). New blood vessels developed in a "spoke-wheel" pattern when angiogenic substances were placed on the membrane. Plate "a" shows a "spoke-wheel" pattern of vascular growth into luteal tissue from a cow. Plate "b" shows absence of a vascular response to fetal muscle (control) tissue.

shows that two cell types that make up the wall of the follicle secrete a factor that also stimulates endothelial cells to grow and that secretion of this angiogenic factor is associated with health of the follicle.

Our data demonstrate that specific tissues and cell types within the ovary secrete factor(s) that may directly influence

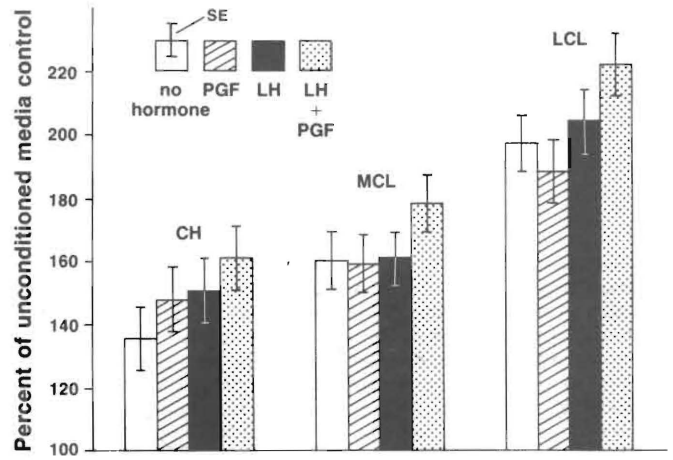


Figure 2. Proliferation (growth) of endothelial (blood vessel) cells in culture is another method for studying angiogenesis, since endothelial cells will proliferate if cultured in the presence of angiogenic factors. This figure shows the response of endothelial cells to secretions from early (CH), mid (MCL), and late (LCL) cycle corpora lutea of cows. In addition, response of endothelial cells to secretions from corpora lutea treated with prostaglandin F₂ (PGF) and/or luteinizing hormone (LH) are shown. Data are means \pm s.e.m. expressed as percent of control.

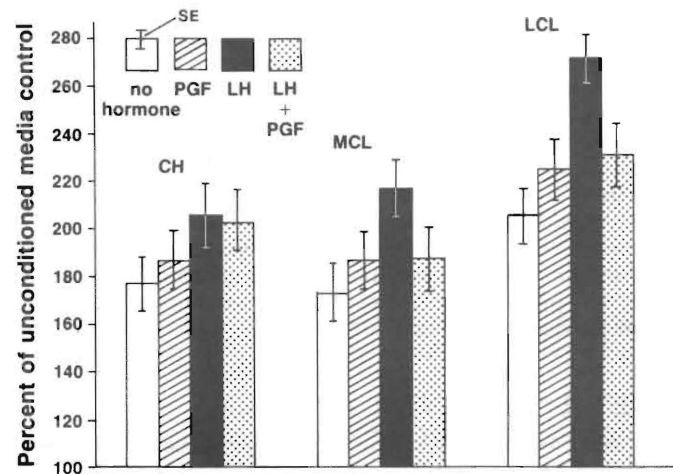


Figure 3. Migration of endothelial cells in culture is a method to study another component of the angiogenic process. Endothelial cells are known to migrate toward angiogenic factors. This figure shows the migratory response of endothelial cells to secretions from corpora lutea. Abbreviations as in Figure 2.

growth and development of vascular beds. Furthermore, these data show for the first time that secretion of angiogenic factor(s) changes with stage of the estrous cycle and can be influenced by reproductive hormones. These data indicate that ovarian tissues that are destined to grow and differentiate must first recruit the blood vessels needed to supply the developing tissues with oxygen and nutrients.

Table 1. Effects of secretions from follicular cells of ewes on proliferation of endothelial cells.

Follicle Type	Granulosa Cells	Thecal Cells
Healthy	113 ± 4 (17)	163 ± 10 (16)
Atretic	82 ± 8 (4)	118 ± 4 (4)

Granulosa and thecal cells are the major cell types of the follicle wall and were separated from each other and cultured. Secretions from these cells were analyzed for their ability to stimulate growth (proliferation) of endothelial cells. Data are means ± s.e.m. expressed as percent of control, where control is the response of endothelial cells in the absence of follicular cell secretions. Numbers in parenthesis indicate number of follicles examined.

Studies are now in progress to determine the identity of the angiogenic factor(s) that are secreted by these ovarian tissues. In addition, recent studies are designed to obtain a better understanding of how vascular development and associated follicular growth and luteal function are regulated by studying expression of genes and gene products that may regulate overall growth and development of follicles. Furthermore, laparoscopic techniques that we have recently developed allow us to observe ovaries frequently (Figures 4 and 5) and provide us with the ability to document changes in follicular populations, growth, development, and response to various treatments at repeated intervals in the same animals. These state-of-the-art techniques should significantly increase our understanding of ovarian function. A much improved understanding of regulation of ovarian function and follicular growth will lead to improved and precise methods of controlling reproductive function of livestock. With this in mind, increasing reproductive efficiency will provide greater economic benefits and opportunity for producers and consumers.

FETAL/PLACENTAL FUNCTION

A major expense to the livestock producer is maintenance of females of breeding age (Ferrell and Jenkins, 1985). Techniques for improving the number and/or quality of offspring produced by each female could have significant economic benefit in terms of reducing maintenance costs. It has long been recognized that embryo transfer could be used to increase the number and quality of offspring produced by each female (Willett et al., 1951). Recently, recombinant DNA techniques have been used to introduce specific genes into embryos to enhance the postnatal growth (Palmiter et al., 1983). The benefits of using these techniques to optimize efficiency of postnatal growth and development in meat animals are apparent. Before maximal benefit can be gained from such techniques, however, the resulting embryos must be capable of surviving and also of expressing their full genetic potential.

In cattle, sheep and pigs, only 60 percent to 75 percent of eggs that are fertilized result in birth of an offspring, with most of embryonic mortality occurring during the first quarter of gestation. Specific factors responsible for early embryonic death, however, have not been identified (Bazer and First,



Figure 4. Abdominal fistula implanted into the upper lumbar region of a cow provides easy, unpainful and frequent access to the ovaries. Ovaries are observed and manipulated with laparoscopic (fiber optic) instruments.



Figure 5. View of an ovary through a laparoscope. Large reddish tissue at the base of the ovary is a corpus luteum. A follicle can be seen near the top of the ovary. A blunt stainless steel probe (very top) is used to suspend the ovary for photographic documentation.

1983). A major factor in reduced survival and growth of newborns is reduced birth weight (Huffman et al., 1985). This seems reasonable since much of growth and differentiation occurs during fetal life. Factors which affect fetal growth will therefore have a significant effect on expression of genetic potential after birth.

The placenta (commonly referred to as the afterbirth) consists of both maternal and fetal tissues and is the organ by which respiratory gases, nutrients and wastes are exchanged between the maternal and fetal systems. The rate of blood flow to the placenta has been shown to be a primary determinant of the quantities of oxygen and nutrients available to the fetus (Reynolds et al., 1986). Thus, the ability of the placenta to support fetal growth and survival likely depends on adequate development of the placental blood supply. We are therefore actively investigating the ability of placental tissues to produce factors which stimulate angiogenesis (growth of blood vessels).

In studies to date, we have found that the maternal portion of the placenta is the primary source of angiogenic factor(s) in both cows and ewes (Figures 6 and 7; Reynolds and Redmer, 1988; Millaway et al., 1988). This observation may explain, in part, the profound influence of the maternal environment on fetal growth in livestock (Joubert and Hammond, 1958, and many others). Studies are in progress to determine the identity of the angiogenic factor(s) secreted by placental tissues. In addition, we are evaluating the developmental expression of genes which may be involved in growth of placental tissues. By understanding the process of placental growth, we hope to eventually achieve optimal conditions for fetal growth and development.

EMBRYONIC MORTALITY

The loss of potential young comprises one of the major factors depressing overall reproductive efficiency of farm animals. Embryonic loss in swine ranges from 30 percent to 40 percent before day 30 (Bazer et al., 1969; Weibel and Dziuk, 1974). Bovine embryo loss estimates at 25 days of gestation vary from 8 percent (Boyd et al., 1969) to 23 percent (Roche et al., 1981). Wastage of embryos in sheep are estimated to range from 11 percent to 40 percent with considerable variability between breeds due predominantly to differences in ovulation rate (Bolet, 1986). The losses are a result of a number of factors and appear to be associated with specific physiological events such as migration, elongation and attachment. Scofield et al. (1974) concluded that day 9 (21 percent loss) to day 13 (52 percent loss) post-breeding was the most critical period in the life of the prenatal pig.

Early pregnancy is a period of many dynamic endocrinological changes necessary for developing an optimal embryonic environment. The pig embryo receives nutrition from uterine secretions and is dependent on these secretions until placental attachment on day 18. The hormone produced by the corpus luteum, progesterone, is the primary regulator of the synthesis and/or secretion of uterine-specific proteins in the pig. Progesterone regulates the activities of endometrial enzymes and can either increase or decrease enzyme activity or act antagonistically to the action of estrogen. Progesterone has been known to stimulate endometrial protein secretions (Dickmann et al., 1976) which requires estrogen priming or estrogen acting synergistically with progesterone (Fazleabas et al., 1985). The very young pig blastocyst (day 11-12) begins to secrete estrogen which plays a role in the transformation process the conceptus must undergo. Estrogen synthesized by embryos promotes their migration and spacing (Pope et al., 1981) and modulates the effect of progesterone on uterine protein secretions (Geisert et al., 1982). The conceptus and endometrial tissues convert progesterone to estrogens (Fischer et al., 1985) and thus allow each tissue to effect uterine secretory activity, uterine blood flow, nutrient transport, conceptus elongation and placentation.

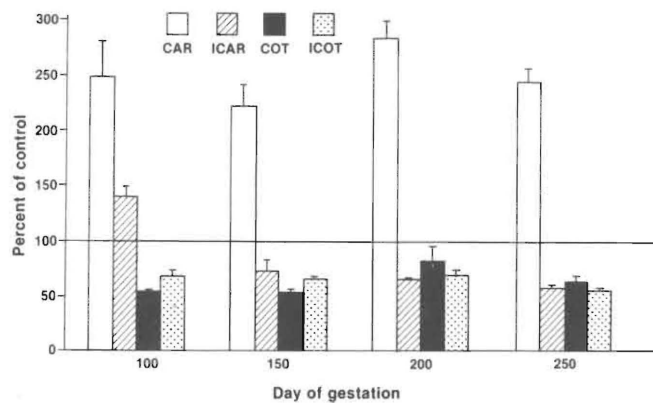


Figure 6. Proliferation of endothelial cells in response to secretions from maternal (CAR, caruncular; ICAR, intercaruncular) and fetal (COT, cotyledonary; ICOT, intercotyledonary) placental tissues of cows. Data are means \pm s.e.m. expressed as percent of control.

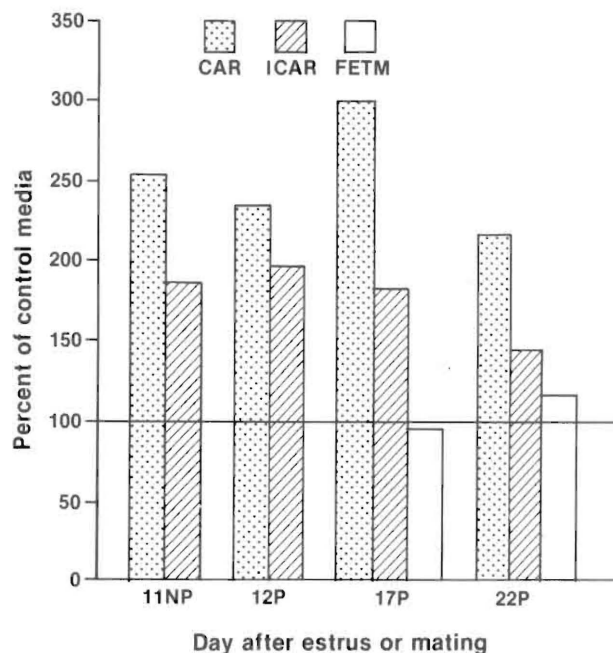


Figure 7. Proliferation of endothelial cells in response to secretions from maternal (CAR, caruncular; ICAR, intercaruncular) and fetal (FETM, fetal membrane) tissues of ewes. Data are means expressed as percent of control. Residual standard deviation = 39%.

Many attempts have been made to directly increase in vivo estrogen and progesterone concentrations by administering these compounds via various modes and at different intervals post-mating with results varying from detrimental to marginally successful. We proposed that administration of substances known to increase endogenous release of progesterone and estrogens could alter the fetal

endocrine environment and beneficially foster increased survivability. Subcutaneous injection of human chorionic gonadotropin (hCG) was found to modestly decrease ($P < .05$) the number of resorbed embryos (Table 2) and reduce the percentage of resorbed embryos (Schmidt et al., 1988). Treatments with hCG positively increased the circulatory concentrations of estrogen for three days (Figure 8). Progesterone concentrations were not evaluated in pregnant pigs (Schmidt et al., 1988; Garbel et al., 1989) but were increased in nonpregnant cycling gilts by hCG injections. These results suggest that the status of pregnancy has an effect on ovarian steroidogenesis and appears to down-regulate luteal function.

Our future goal is to continue study of hCG administration in early pregnancy to evaluate its potential role in reducing pregnancy wastage and alteration of endocrine secretory patterns.

Table 2. Uterine and ovarian morphological measurement from sows treated with hCG (least-squares means)

	Group I (saline)	Group II (hCG500)	Group III (hCG1000)	SE
No. corpora lutea	17.9	18.0	19.6	0.9
No. embryos (total)	15.1	14.4	15.8	0.8
No. viable embryos	12.1	12.9	13.3	0.8
No. resorbed embryos ^a	2.8	1.6	1.9	0.4
Resorbed embryos (%) ^{a,b}	20.5	10.3	11.6	2.9

^aGroup I vs others ($P < 0.05$).

^bRepresents % resorbed embryos of all observed embryos.

NEUROENDOCRINE REGULATION

The control of release of pituitary regulatory factors is not completely explained. The ability of the ovary to both positively and negatively control of its own activity has been an area of considerable study for the past 30 years. Recently new information has shown that various brain peptides play a role in the secretion of all hormones of the pituitary gland. The so-called "master gland" of the body has profound influences on reproduction of all species.

Endogenous opioid peptides (EOP) have been found to modulate gonadotropin and prolactin secretion in females. However, secretory profiles of the hormones after treatment with naloxone (NAL), an opioid antagonist, may differ between species and physiological states within the same species. Recent experiments have confirmed a stimulatory effect of NAL on LH and prolactin secretion during the luteal phase of the estrous cycle in sows (Barb et al., 1985; Barb et al., 1986). Barb et al. (1986) also tested the influence of NAL on LH and prolactin secretion during the early (days 15-17) and late (days 18-19) follicular phase in sows. In their experiment, serum LH concentrations were not affected by NAL administration at either phase. On the basis of the prolactin data, the authors could not conclude unequivocally that any relationship exists between EOP and prolactin secretion during the follicular phase in sows.

We have initiated experiments to determine changes in those hormones that have a regulatory effect on the ovary. The opioid antagonist, naloxone, was administered during the early and late phase of the estrous cycle. Naloxone altered ($P < .05$) the secretion of prolactin during the late follicular phase (day 20) as shown in Figure 9a. It was not found to influence the release of the other gonadotropins

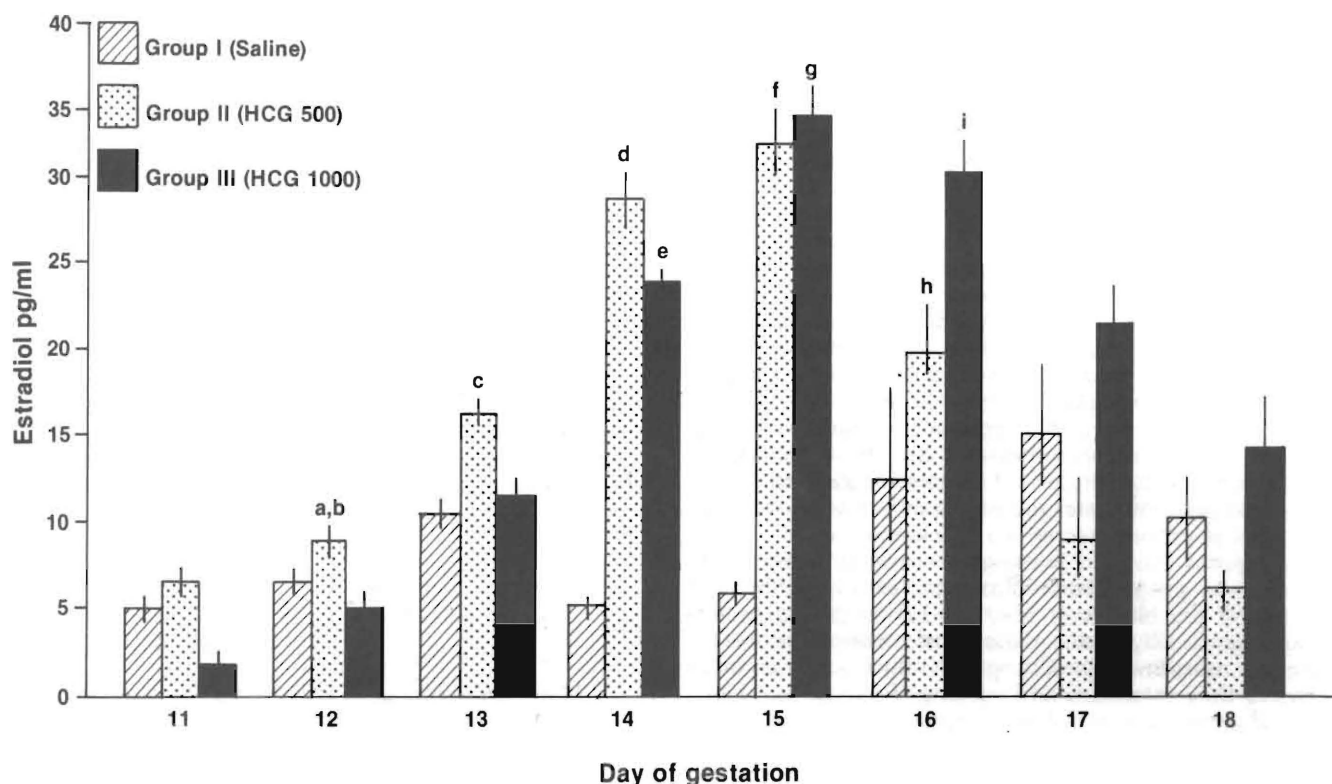


Figure 8. Estradiol concentrations following hCG administration at day 12. ^a $P < .06$, Group I vs II; ^b $P < .08$, Group II vs III; ^{c,d,i} $P < .01$, Group I vs II; ^{e,g,i} $P < .01$, Group I vs III, ^h $P < .02$ Group I vs. II.

(LH and FSH) at either day 16 or day 20 (Figures 9 and 10). The results suggest that endogenous opioid peptides are effective in modulating prolactin during the late phase of the estrous cycle (Okrasa et al., 1988).

CONCLUSION

It is clear that increasing reproductive efficiency of livestock will have tremendous benefits to the industry. However, we must first have a much better understanding of the complex mechanisms that control reproductive processes. Therefore, it is requisite that we pry open the secrets of various cellular and molecular events so that we can better understand the factors that regulate the whole. The cost for conducting research on the cutting edge of science is high but the returns to the producer are great. Advanced technology, state-of-the-art equipment, and development of technology centers on our campus are requisite for scientists to keep pace with the ever expanding needs of our livestock industry. As stated by Dr. Joanne Richards, who was recently reviewing National Science Foundation supported research programs at NDSU, "The development of biotechnology in North Dakota is essential for the development of new approaches to pest control, large animal breeding, and crop improvement."

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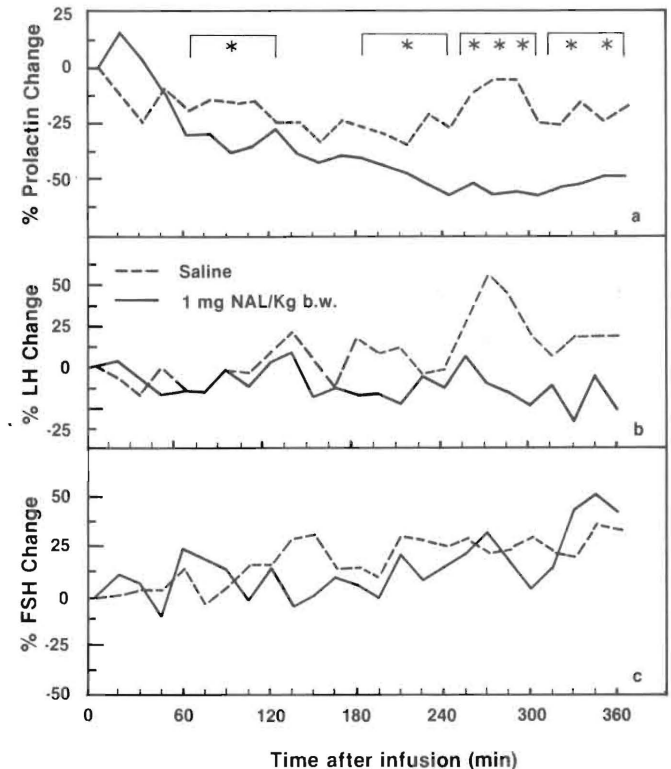


Figure 9. Changes in prolactin, LH and FSH concentrations in gilts after NAL or saline administration on day 20 of the estrous cycle. ***P < 0.01; **P < 0.03, *P < 0.07.

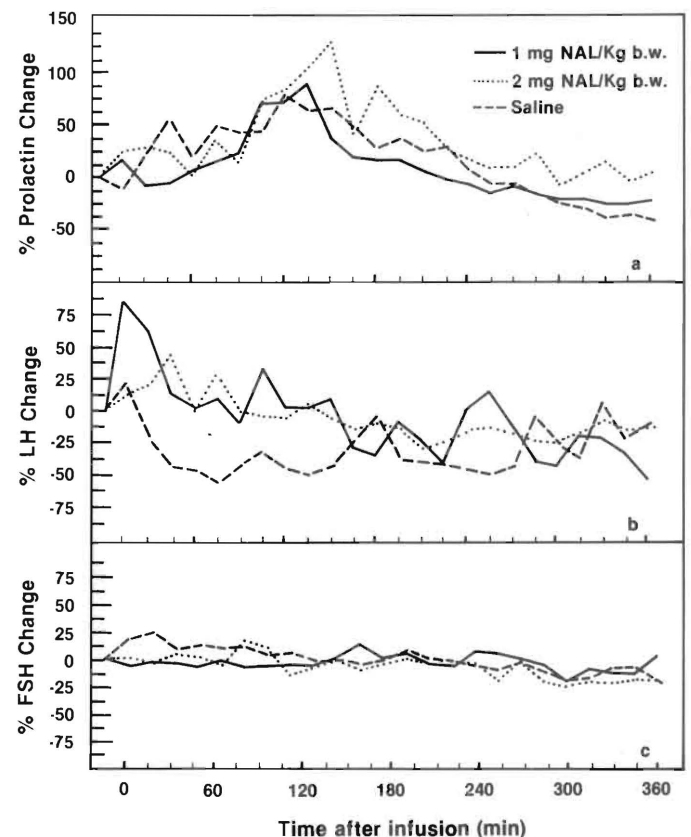


Figure 10. Changes in prolactin, LH and FSH concentrations in gilts after NAL or saline administration on day 16 of the estrous cycle.

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