INFLUENCE OF IMPLANTATION WITH ZERANOL ON SUBSEQUENT REPRODUCTIVE PERFORMANCE OF HEIFERS

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CHAPTER I

INTRODUCTION

Zeranol is a naturally occurring estrogenic compound which was originally isolated from moldy corn. It is produced by deep tank fermentation of grain followed by a chemical synthesis process. The implanting of small pellets containing zeranol, an exogenous source of estrogen, causes increased growth rate and increased feed efficiency in steers and heifers. Since zeranol is an estrogenic compound, it also may alter normal reproductive endocrine function.

Many heifer calves are implanted with estrogenic compounds at an early age to increase preweaning growth rate. However, the decision as to which heifers will be maintained in the breeding herd is usually not made until weaning and often not until a year of age. Therefore, some heifers that are implanted before weaning may be selected to be added to the herd at some later time.

There is limited information on reproductive development and functions of heifers that have been implanted with anabolic estrogens prior to one year of age. Estrogenic treatments could be the cause of reduced fertility often observed in beef heifers.

The purpose of this experiment was to determine the influence of single and multiple implantation with zeranol (Ralgro) before puberty on subsequent reproductive performance of heifers.

CHAPTER II

LITERATURE REVIEW

Zeranol

Zeranol is a derivative of zearalanone, a metabolite of the fungus Gibberella zeae which was found in moldy corn and isolated by Stob et al. (1962). Feeding moldy corn with zearalanone to swine results in enlargement of mammary glands in males and females, as well as swelling of the prepuce of males and hypertrophy of the vulva of females (Stob et al., 1962). In addition zearalanone is uterotrophic in ovariectomized mice and increases growth rate and feed efficiency in sheep. Urry et al. (1966) identified the chemical zearalanone and determined it was a β-resorcylate, a class of natural products.

Estrogenic Activity

Ewes exposed to estrogenic pastures for long periods of time exhibit decreased fertility which continues for several years after removal to non-estrogenic pastures (Schinckel, 1948). Impaired transport of spermatozoa through the cervix contributes to infertility observed in ewes on estrogenic pastures (Lightfoot et al., 1967). In addition, large numbers of developing follicles are present on the ovaries of

ewes grazing estrogenic pastures (Adams, 1977). This may be due to a lack of ovulation of follicles. Ewes grazed on estrogenic clover for three years, exhibited eleven percent lambing rate compared to seventy-six percent in controls on a non-estrogen diet (Adams et al., 1979). After removal of treated ewes to non-estrogenic pastures for six months, no significant difference in number of primordial follicles between treated and control ewes was observed. However, ovulation rate was increased significantly in treated ewes (Adams et al., 1979). Findlay et al. (1973) found the hypothalamus of phyto-estrogen treated ewes to be less sensitive to estrogen which may allow the increased number of follicles to develop. In general, estrogenic pasture treated ewes exhibit decreased fertility, which may be due to exogenous or increased endogenous estrogen production (Land et al., 1972; Wheeler et al., 1977).

Zeranol has estrogenic activity when given to animals. Rothenbacker et al. (1975) found a dose effect when zeranol was given to wethers. Greater hyperplasia and squamous transformation were observed in the prostrate and penile urethra, as well as more papillary proliferation in the seminal vesicles when wethers were given greater quantities of zeranol. Increased mammary alveolar growth and secretion in the gland occurred at dosages of 46 and 98 mg. of zeranol. Adrenal gland weight increased due to hypertrophy and hyperplasia of the adrenal cortex (zona fasiculata and zona reticularis). Thyroid gland weights and epithelial cell size decreased with increased amounts of zeranol. In addi-

tion, zeranol treatment increased eosinophilic activity in the blood and decreased the numbers of basophils in the anterior pituitary. Since growth hormone and prolactin are synthesized and stored in basophilic cells in the pituitary; this suggests that greater amounts of these hormones are synthesized and secreted and probably less thyrotropin and gonadotropins are secreted.

Zeranol has estrogenic activity in vitro since it will displace estradiol from uterine binding proteins (Peck and Chesworth, 1977). However, these workers found that zeranol had no estrogenic effect in vivo. Ewes treated with zeranol exhibited normal estrus and plasma LH concentrations near estrus and were not influenced by treatment. However, the zeranol treatment had been given for only five days after removal of progesterone pessaries. Estrogen would be expected to be elevated during this period. Therefore, the dose given may not have been a sufficient quantity or the length of time it was given may not have been long enough to elicit a response in vivo. Since the zeranol displacement of estradiol from uterine estrogen receptors in vitro was parallel to the estradiol curve, this suggests zeranol is a competitor of estradiol binding to uterine estrogen receptors.

Zeranol has estrogenic effects on human mammary cancer cells (Martin et al., 1978). Zeranol, as well as other phytoestrogens, competed with ^{3}H-estradiol for binding to unfilled cytoplasmic estrogen receptors or unfilled nuclear estrogen receptor sites. The cytoplasmic estrogen receptors

were translocated after binding with zeranol to the nucleus, similar to the cellular action of other estrogens. The receptor complexes in the nucleus exhibited a response similar to estradiol and stimulated growth of the cells. In addition, zeranol is not bound to serum proteins. This characteristic should make zeranol effective as an estrogen at low plasma concentrations.

Effects on Reproduction

Davis et al. (1977) observed that female mice treated with zeranol exhibited a decreased number of live litters when compared to controls. The number of live pups per litter was also decreased. Zearalanone treated rats exhibited decreased pregnancy rates and increased numbers of stillbirths and resorptions of young, when compared to controls (Bailey et al., 1976).

The influence of 12 mg. implants of zeranol in ram lambs implanted at 44 or 89 days of age was studied by Riesen et al. (1977). Animals were slaughtered at 139 days of age. Zeranol implantation at 89 days decreased pituitary concentrations of FSH, epididymal weight and seminiferous tubule diameter. Implantation at 44 days decreased serum LH concentrations when compared to controls. Testis weight at slaughter was significantly decreased by treatment at either 44 or 89 days. Since percentage of seminiferous tubule diameter within the testis was not different across treatments, it is suggested that tubular and intertubular tissues were both affected by treatment with zeranol.

Therefore, it appears that zeranol may be an inhibitor of gonadotropin synthesis and/or release.

Implantation of bulls with 36 mg. of zeranol at 28, 128, 228 and 328 days of age resulted in adverse effects on sexual development (Fink et al., 1979). Implanted bulls had decreased testis and penile length and weight, and decreased testicular and scrotal circumference when bulls were slaughtered at about fifteen months of age. Semen production was decreased in the implanted bulls and all were classified as infertile. In addition, libido scores, as measured by number of mounts, were decreased and pelvic area was greater in implanted bulls.

Similar effects in bulls implanted with DES at birth and three months of age were observed by Patton and Ralston (1968), and Ralston and Patton (1974). Implanted bulls had decreased development of secondary sex characteristics, testicular size, seminiferous tubule diameter, libido and semen production.

Bulls fed 10 mg. DES per head per day beginning at about two months of age had no gross or histological abnormalities when slaughtered at approximately twenty months of age (Jack Wagner, personal communication, 1977). Similar results were found by Reuber (1958). However, pregnancy rates for heifers bred to DES fed bulls was 88 percent compared to 100 percent for control bulls. This suggests that reduced fertility may occur when DES is fed to bulls (Jack Wagner, personal communication, 1977).

Ewes implanted with 12 mg. of zeranol had decreased

ovarian weight when compared to controls (Riesen et al., 1977). Control ewes had an average of 4.7 follicles greater than 3 mm in diameter and 1.3 corpora lutea, whereas implanted ewes had no follicles greater than 3 mm in diameter and no corpora lutea. The results of this preliminary study suggests zeranol may inhibit gonadotropins.

Heifers implanted with zeranol at approximately 200 kg. of body weight exhibited a greater incidence of anovulatory estrus and decreased pregnancy rates (Staigmiller et al., 1978). Implanting heifers increased pelvic area but had no effect on age at puberty. Decreased pregnancy rates were observed in heifers implanted with zeranol at 200 kg. (Nelson et al., 1972). However, heifers implanted at less than 75 days of age with zeranol and again 110 days later exhibited no decreased pregnancy rates when compared to controls (Sprott et al., 1979). Pregnancy rates were low for both control and implanted heifers suggesting possible bull infertility. Sharp and Dyer (1968) found no adverse effects on secondary sex characteristics, conformation or the estrus cycles in heifers implanted with zeranol at 285 kg. of body weight.

Ovaries of heifers treated with DES implants had fewer corpora lutea and contained large cystic follicles when compared to controls (Clegg and Cole, 1954). Some treated heifers suffered vaginal prolapse, and had lengthened teats and early udder development with the presence of milk. Similar effects on mammary development, plus swollen vulvas and irregular estrus and estrous cycle length, occurred in

DES implanted heifers (Dinusson et al., 1950). Wickersham and Schultz (1964) also found that DES caused early mammary development and swollen vulvas. But they found no effect on estrous regularity, occurrence of cystic follicles, size of ovaries and uterine horns, ovulation and pregnancy rates or reproductive performance following the first calf. However other workers found adverse effects on estrous cycle length and pregnancy rates in heifers implanted or fed DES (Reuber, 1958; Bond et al., 1971; Jack Wagner, personal communication, 1977). In general, all the DES treated heifers in the experiments reviewed were not treated prior to six months of age or 227 kg. of body weight. Therefore, attainment of puberty may have already begun and treatment with DES may not have had time to affect pregnancy rates.

Growth

Implanting sheep with zeranol results in increased daily gains (Wilson et al., 1972). Daily gains were greatest for wethers, moderate for rams and cryptorchid rams and less pronounced in ewes. The greatest effects on growth in wethers occurred in the period soon after implantation.

Zeranol treatment increases gains in suckling steers (Ward et al., 1978; Thomas et al., 1970; Nichols and Lesperance, 1973), as well as in growing and finishing animals. Steers implanted at 100 and 230 kg. of body weight exhibited increased gains of ten to fourteen percent and a nine percent increase in feed efficiency when compared to

controls (Bennett et al., 1974). Steers implanted with zeranol when placed in the feedlot had increased gains compared to controls of about 22 percent. Steers implanted once at 24 months of age and finished on grass pasture exhibited increased gains of approximately 35 percent compared to control animals (Bennett et al., 1974).

Borger et al. (1971) observed increased growth of 7.8 percent and increased feed efficiency of 7.1 percent in implanted steers on a growing-finishing ration. In close agreement, Thomas and Armitage (1970) found increased feed efficiency of six percent in implanted growing-finishing steers while Sharp and Dyer (1970) observed an increase in gain of 14 to 21 percent in finishing steers. The stimulatory affect of zeranol on body weight gain has been observed by others (Wilson and Wiggins, 1974; Ward et al., 1978; Sharp and Dyer, 1972, 1970; Koers et al., 1974; Hathaway et al., 1973).

Zeranol treatment of bulls results in increased gains compared to control bulls (Ralston, 1978; Fink et al., 1979), as well as increased gains and feed efficiency in heifers. Heifers fed on 70:30 concentrate-roughage ration and implanted with zeranol exhibited increased gains of 14 to 25 percent and increased feed efficiency of approximately fifteen percent when compared to controls (Sharp and Dyer, 1968). Heifers implanted with zeranol from nursing to finishing exhibited increased gains compared to controls (Ward et al., 1978). The most marked response occurred during the growing phase,

that period of growth associated with the occurrence of puberty, which agrees with Utley et al. (1976). Other investigators have found zeranol to increase gains in growing heifers (Perry et al., 1970; Wilson and Wiggins, 1974; Wilson and Burdett, 1973; Staigmiller et al., 1978).

In general, zeranol implantation in nursing calves improves gains by ten percent and feed efficiency by four percent. Implantation with zeranol during the growing phase increases gains by fifteen percent in steers and ten percent in heifers. Steers implanted during the finishing phase with zeranol have improved gains of ten percent and feed efficiency is increased by about seven percent. Gains in heifers are increased by seven percent and feed efficiency is about nine percent greater after zeranol treatment when compared to controls (Thomas, 1974).

Steers were implanted with zeranol at about 218 kg. of body weight and reimplanted at either 28, 56 or 112 day intervals in order to determine implant life length (Nicholson et al., 1973). There was no advantage to reimplanting at 28 or 56 day intervals. However, implanting at 112 day intervals revealed an advantage in increased growth. In another study, the greatest response to zeranol implantation occurred within the first 84 to 112 days after treatment (Perry et al., 1970). Therefore, it appears that by about 100 days after implantation with zeranol the effects have diminished.

Metabolism

The mechanism of action of growth stimulants is not clearly understood. The release of tritiated zeranol from implants in steers is slow and clearance from the blood is very rapid (Sharp and Dyer, 1972). About ten percent of the zeranol was excreted in the urine, ten percent remained as encapsulated implant and 45 percent was accounted for in the feces during a 22 day trial. Bile was proposed to be the primary excretory route and the liver responsible for its removal. In addition, no edible tissues contained measurable amounts of zeranol as detected by radiometric analysis.

The metabolism of labeled DES in ruminants appears to be similar to the metabolism of zeranol; the major portions of the labeled DES were found in the feces and urine (Aschbacher, 1972; Bories et al., 1977; Mitchell et al., 1959).

Zeranol increased the percent water and protein content of the whole empty body of steers (Sharp and Dyer, 1970). Although Borger et al. (1973) observed increased percent body water, the percent protein in the longissimus dorsi of steers implanted with zeranol was not altered. Zeranol implantation increased rib eye area in steers (Embry and Gates, 1976) and increased nitrogen retention in sheep (Sharp and Dyer, 1971).

Zeranol treatment increases plasma glucose and insulin concentrations (Sharp and Dyer, 1970). These investigators concluded increased growth hormone could cause blood glucose concentrations to increase, resulting in elevated insulin

secretion which may play a role in protein metabolism.

Blood concentrations of growth hormone, but not insulin, were increased in zeranol treated steers (Borger et al., 1973, 1971). They suggested the increased growth response to zeranol was due to the increased growth hormone secretion. Zeranol treatment increased plasma growth hormone and insulin but had no effect on blood glucose concentrations in sheep (Olsen et al., 1977). They concluded zeranol did not directly stimulate the release of insulin from the pancreas or growth hormone from the pituitary.

Treatment of ruminants with DES results in increased blood glucose and insulin, increased nitrogen retention (Preston and Burroughs, 1958, 1960; Davis et al., 1970; Struempler and Burroughs, 1959), greater water retention (Hathaway et al., 1973), and increased plasma growth hormone (Shroeder and Hansard, 1958; Clegg and Cole, 1954; Hutcheson and Preston, 1971).

Puberty

Puberty has been defined in several ways. McDonald (1969) defines puberty as the period in which the female becomes sexually mature and the secondary sex characteristics first become conspicuous. He defines sexual maturity as the capacity to reproduce. Hafez (1974) defines puberty as the period of adolescence when a male or female is first able to release gametes. In the female, the first ovulation or estrus with ovulation indicates puberty has been reached. For the purposes of this review, puberty in the bovine

female begins at the time of first ovulation coincident with an estrus, and ends at the time normal fertility and cyclicity, characteristic of the adult is reached.

The time at which puberty occurs in the bovine female is governed by several factors. Breed, age, body weight and plane of nutrition are all related to the attainment of puberty (Joubert, 1963; Lamond, 1970).

Age at first estrus varies between breeds and within breeds (Christian, 1957). Average age at first estrus for Angus, Hereford and Shorthorn, respectively, was 353, 378 and 383 days with a range for all breeds of 243 to 418 days of age. Both extremes of this range were observed in Angus heifers, demonstrating the variability within a breed. Average body weights were 238, 288 and 251 kg. for Angus, Hereford and Shorthorn heifers, respectively. Others also have found differences in age and weight at puberty within breed (Arijie and Wiltbank, 1971; Milagres et al., 1979; Wiltbank et al., 1959) and between breeds (Laster et al., 1972; Reynolds et al., 1963; Wiltbank et al., 1969; Joubert, 1954).

Plane of nutrition during the growth of heifers influences the age at puberty. Short and Bellows (1971) observed heifers grown on three different regimes beginning at 148 kg. of body weight. As the amount of energy and protein of the feed increased the age of puberty decreased. The influence of nutrition on age at puberty has been well documented

(Joubert, 1959; Crichton et al., 1959; Reid, 1960; Bellows et al., 1965; Wiltbank et al., 1966, 1969). In addition as daily feed intake and growth rate increased, the weight at attainment of puberty increased. It appears that greater nutrient intake increased body growth faster than physiological maturity (Short and Bellows, 1971). Thus, some factor other than weight may be important in determining age at puberty. When comparing energy intake, heifers on low energy intake have the greatest variability in age at puberty (Reid, 1960).

Turman et al. (1963) found that growth in heifers through the winter must be continuous for regular estrous cycles to occur. Some heifers that exhibited their first estrus at twelve to thirteen months of age did not cycle regularly during the following two to three months. Many of these heifers had lost weight during the winter. Heifers on a low plane of winter nutrition may have reduced fertility, delayed puberty and pregnancy may be delayed until late in the breeding period (Smithson et al., 1963; Turman et al., 1964; Pope, 1967). Age at first estrus for Hereford heifers on high, medium and low plane of nutrition was 353, 373 and 386 days, respectively (Turman et al., 1963).

Altered endocrine function is associated with the attainment of puberty in the heifer. Pituitary gonadotropic hormones are synthesized and released prior to puberty. Hypotholamic control of the gonadotropin secretion is also functional before the first ovulation since estrogen treatment will

induce LH release (Swanson and McCarthy, 1978).

Gonzalez-Padilla et al. (1975) observed no marked change in concentrations of serum gonadotropin releasing hormone (GnRH) as puberty approached or during the first estrous cycle. However, there was a slight positive correlation of GnRH concentration with serum progesterone and estradiol 17-β during the prepuberal period, suggesting that the ratio of these steroids may be involved in prepuberal regulation of GnRH release.

The average weight of the pituitary of Holstein heifers increased from birth to twelve months of age with 90 percent of this increase attributed to the anterior lobes (Desjardins and Hafs, 1968). Pituitary content of follicle stimulating hormone (FSH) was greatest at one month of age, declined at two months and was relatively constant from two to twelve months of age. If decreased pituitary FSH content indicates increased blood concentration of gonadotropins, then serum FSH may be increased after two months of age. However, Gonzalez-Padilla et al. (1975) found no association of serum FSH concentration with the onset of puberty in beef heifers observed from six to fourteen months of age.

Mean lutenizing hormone (LH) content of the pituitary gland increased from birth to three months of age, varied from three to seven months and declined from seven to twelve months of age in Holstein heifers (Desjardins and Hafs, 1968). In agreement, Swanson et al. (1972) observed that serum LH in prepuberal Holstein heifers increased near the onset of

puberty. In contrast, with more frequent sampling, Gonzalez-Padilla et al. (1975) found that serum LH decreased and fluctuated prior to the pubertal LH peak.

Average serum LH concentrations are greater and more variable prepuberal (Swanson et al., 1972; Gonzalez-Padilla et al., 1975), than during the luteal phase of the first cycle or during the luteal phase of the cycle of normal cows (Christensen et al., 1974; Henricks et al., 1970; Hansel and Snook, 1970).

Serum progesterone concentrations are less than one ng/ml during the prepuberal period (Gonzalez-Padilla et al., 1975). Transient increases in progesterone occurred in heifers prior to the first ovulation. The prepubertal increases in the preovulatory peak of LH occurred after the transient increase in serum progesterone had returned to baseline concentrations. Gonzalez-Padilla et al. (1975) concluded that LH secretion in prepuberal heifers increases gradually and may be a result of increasing plasma concentrations of progesterone, and that progesterone may be involved in establishing the phasic LH release in the cyclic female bovine.

Serum estradiol concentrations decline about 40 days before attainment of puberty (Gonzalez-Padilla et al., 1975), and remain at concentrations similar to the cycling cow (Henricks et al., 1971).

Estrous Cycle

The bovine estrous cycle may be divided into four

periods; proestrus, estrus, metestrus and diestrus.

Proestrus lasts between two to three days and is characterized by regression of the corpus luteum, resulting in a rapid decline in plasma progesterone concentration (Wettemann et al., 1972; Swanson et al., 1972; Christensen et al., 1974). Follicle stimulating hormone, released from the anterior pituitary, stimulates follicle growth and the plasma concentrations of estrogen increase (Wettemann et al., 1972; Henricks et al., 1971; Echternkamp and Hansel, 1971).

Estrus is the period in which standing heat or sexual receptivity occurs, and lasts for approximately 18 hours. Plasma concentration of estrogen begin to decline, the preovulatory surge of LH occurs and plasma progesterone concentrations are usually less than one ng/ml (Wettemann et al., 1972; Swanson et al., 1972; Stabenfeldt et al., 1969).

The duration of metestrus is approximately three days and encompasses ovulation. Plasma estrogen concentrations return to basal amounts and formation of the corpus luteum begins. Plasma progesterone begins to increase during metestrus (Wettemann et al., 1972; Swanson et al., 1972; Christensen et al., 1974).

Diestrus lasts for 14 to 15 days in the bovine and is characterized by increasing plasma progesterone as growth and maintenance of the corpus luteum continues and plasma progesterone becomes maximal late in this period. Plasma estrogen concentrations average two to four pg/ml (Wettemann

et al., 1972). As proestrus approaches, plasma progesterone concentrations decline as estrogen concentrations increase (Wettemann et al., 1972; Swanson et al., 1972; Stabenfeldt et al., 1969; Henricks et al., 1970).

Gonadotropins

Pituitary concentrations of FSH decline between day eighteen of the estrous cycle and the onset of estrus, indicating increased plasma FSH concentrations during rapid follicular growth and maturation of the Graafian follicle (Hackett and Hafs, 1969; Desjardins and Hafs, 1968).

Plasma concentrations of LH begin to increase from basal values about three days before estrus to a maximum near the onset of estrus. After the preovulatory surge of LH at estrus, plasma LH returns to less than 1 ng/ml by the day after estrus (Swanson et al., 1972; Christensen et al., 1974; Chenault et al., 1975). By two days after estrus, plasma LH concentrations are similar to those during early proestrus (Swanson et al., 1972). Duration of the preovulatory LH surge ranges from six to twelve hours (Swanson and Hafs, 1971; Christensen et al., 1974), and ovulation occurs 22 to 32 hours after the increase in LH (Swanson and Hafs, 1971; Chenault et al., 1975; Christensen et al., 1974; Henricks et al., 1970). Serum LH concentrations are less than one ng/ml after the preovulatory surge until the next estrus (Christensen et al., 1974; Henricks et al., 1970). In general serum LH in the heifer begins to increase one day

prior to estrus, exhibits a surge at estrus which lasts for approximately twelve hours and returns to less than one ng/ml one day after estrus with ovulation occurring 24 hours after the preovulatory surge of LH.

Estrogen

Plasma estradiol concentrations increase from less than five pg/ml three days before estrus to a maximum of about ten pg/ml about twelve hours before estrus (Wettemann et al., 1972; Ecternkamp and Hansel, 1971; Chenault et al., 1975). Plasma estradiol then decreases near the time of the LH surge to less than five pg/ml approximately fourteen to sixteen hours after the preovulatory surge of LH (Christensen et al., 1974; Chenault et al., 1975). The rise in plasma estradiol concentrations may be responsible for regulation of the preovulatory surge of LH in the bovine (Wettemann et al., 1972; Christensen et al., 1974; Chenault et al., 1975; Glencross et al., 1973; Lemon et al., 1975).

Progesterone

Plasma progesterone concentrations in the bovine female decrease rapidly from luteal phase concentrations to less than one ng/ml during the three days before estrus and remain low from day one prior to estrus until day two of the estrus cycle. Plasma progesterone increases rapidly, to greater than two ng/ml, from day four to day eleven (Wettemann et al., 1972; Swanson et al., 1972; Christensen et al., 1974; Henricks

et al., 1970; Chenault et al., 1975).

Effects of Estrogens on Gonadotropins

Rat

Serum concentrations of LH and FSH increase after ovariectomy in the prepuberal female rat (Caligaris et al., 1972; Caligaris et al., 1973). Both intact and ovariectomized rats exhibited a decrease in serum FSH concentrations after estradiol benzoate injection. A similar decrease in serum LH occurred after estrogen injection in prepuberal intact female rats, as well as advancement in the onset of puberty after estrogen injections (Ramirez and McCann, 1963; Ramirez and Sawyer, 1965). Therefore, plasma estrogens are involved in control of FSH and LH secretion in the prepuberal female rat via the negative feedback mechanism.

Ewe

In the anestrous ewe, injection of various amounts of estradiol 17-β induced an increase in serum LH and FSH concentrations approximately fifteen hours after injection (Reeves et al., 1974; Beck et al., 1973; Jonas et al., 1973). Bolt et al. (1971) observed an increase in serum LH after estradiol injection at day three of the estrous cycle in the ewe, but not day ten when the corpus luteum is producing maximal amounts of progesterone. Thus, estrogen elicits a positive effect on LH secretion in the anestrous and follicular phase ewes but does not in luteal phase ewes.

Heifer

A biphasic effect of estrogens on LH secretion has been described in ovariectomized heifers (Hobson and Hansel, 1972; Beck and Convey, 1977). Estradiol 17-β or diethylstilbestrol (DES) injection initially depressed serum LH for two to nine hours followed by a rapid increase in serum LH 14 to 22 hours after treatment. The response to DES was significantly greater than the response to estradiol injection (Hobson and Hansel, 1972). Intact prepuberal heifers exhibited a similar biphasic effect of estradiol injection on LH secretion (Swanson and McCarthy, 1978; Gonzalez-Padilla et al., 1975). In addition, pretreatment with progesterone had no effect on the LH response to estradiol injection in the prepuberal heifers. Estradiol injection on day two of the estrous cycle in the intact cow induced an LH surge (Martin et al., 1974) but not during midcycle (Hobson and Hansel, 1972). Thus, estradiol increases serum LH about 18 hours after treatment in the prepuberal heifer and follicular phase cow, but progesterone inhibits this effect during the luteal phase of the estrous cycle.

If heifers are treated with estradiol 17-β implants or progesterone pessaries, or both, and then ovariectomized, serum LH increases after removal of the treatments (Beck et al., 1976). During the 48 hours following ovariectomy, before treatment removal, serum LH gradually increased in the estradiol or progesterone treated heifers but not in the heifers given estradiol and progesterone. Convey et al.

(1977) observed that serum LH in heifers ovariectomized at estrus increased during the next 96 hours. Serum LH in intact heifers initially was similar to that in ovariectomized heifers, but began to decrease 48 hours after estrus. Ovariectomy during the diestrus period resulted in an increase in LH within 24 hours and then a gradual decrease to concentrations above pre-ovariectomy diestrus concentrations. These investigators suggested that the negative feedback control of estrogen and progesterone observed by Beck et al. (1976) is not operative from zero to 48 hours after estrus but becomes functional with the formation of the corpus luteum.

Serum FSH concentrations, in intact prepuberal heifers, were not altered following estradiol 17-β injection alone or in combination with progesterone (Gonzalez-Padilla et al., 1975).

An exogenous source of estrogens or estrogenic compounds may alter normal synthesis and/or release of gonadotropic hormones in the heifer.

CHAPTER III

MATERIALS AND METHODS

This study was conducted from April 1977 through May 1979 and involved seventy-five Hereford heifers maintained on tallgrass native range at Lake Carl Blackwell range area.

The zeranol implants used in this study were the commercial product known as Ralgro (IMC Chemical Group, Inc., Terre Haute, Indiana). Treated heifers were implanted with 36 mg of zeranol (three twelve mg pellets of zeranol at each implanting period). Implants were administered subcutaneously near the base of the ear, as recommended by the manufacturer.

Seventy-five Hereford heifers born in the spring of 1977 (February - April) were blocked by age and randomly assigned at 42 ± 17 days of age and 64 ± 26 kg body weight to treatments: control (no implant), single implant (36 mg zeranol at 42 days) and multiple implant (36 mg zeranol four times at 100 day intervals starting at 42 days). Implantation began on May 5, 1977, for the single and multiple implant groups and reimplanting occurred on August 11, 1977 and November 18, 1977, and March 2, 1978, for the multiple implant group.

The heifers were maintained on native range as one herd and weaned in October 1977 at approximately seven months of age. During the first winter (1977-78), dormant winter native grass range was supplemented with 2.27 kg per head per day of 20 percent natural protein range cubes composed of ground corn, cottonseed meal, di-calcium phosphate, molasses and vitamin A. Snow or ice covered the dry grass for 47 days during the winter and grass hay was offered ad libitum in addition to range cubes on those days. During the spring of 1978 (February - May) heifers were supplemented with 2.27 kg of ground corn per day to increase gain after the severe winter in an effort to achieve an acceptable breeding size by fifteen months of age. Heifers were exposed to fertile Angus bulls equipped with chinball markers at about 450 days of age for 55 days (June 9 through August 4, 1978). They were checked daily for marks indicating breeding activity. Pregnancy rates were determined by rectal palpation at 70 to 120 days after breeding (October 10, 1978). The experimental design is summarized in Table I.

Twenty ml blood samples were collected by tail vein puncture into vacutainers at seven day intervals for four weeks beginning the first day of exposure to bulls. Nineteen mg of oxalic acid in 0.3 ml water was added to the blood sample to prevent clotting. The blood samples were cooled in ice immediately after collection. Within three hours after collection, the samples were transferred to plastic tubes and centrifuged at 2500 RPM for fifteen

TABLE I

EXPERIMENTAL DESIGN

Item	Treatments		
	Control	Single Implant	Multiple Implant
No. of Heifers	24	26	25
Average Age (Days			
42		I*	I
140			I
240			I
340			I
450	Exposure to Fertile Bulls		
565	Rectal Palpation		
No. of Heifers	13	13	24
Average Age (Days)			
620	Exposure to Fertile Bulls		
760	Rectal Palpation		

I* -- 36 mg zeranol

minutes. The plasma was decanted into plastic vials and stored at -10°C until progesterone was quantified by radioimmunoassay.

At rectal palpation, non-pregnant heifers were separated and assigned to a second breeding period to begin when heifers were approximately 21 months of age to determine if implanting with zeranol had long term effects. The open heifers were maintained on native tallgrass range as one herd. Snow covered the ground for about 40 days during the winter of 1978-1979, and supplementation was provided as previously described for the winter of 1977-1978. Blood samples for plasma progesterone were collected at seven day intervals for four weeks beginning November 9, 1978, when heifers were about 20 months of age. Breeding activity was checked daily as described earlier from November 9, 1978, through January 30, 1979. Heifers were exposed to fertile Angus bulls equipped with chinball markers for 50 days (December 11, 1978 through January 30, 1979). Pregnancy rates were determined by rectal palpation 90 to 140 days after breeding (May 1, 1979).

Progesterone Quantification

Plasma progesterone was quantified by radioimmunoassay as described by Kittok et al. (1973) and validated in our lab by Wettemann et al. (1978). The specificity of the antisera has been reported in detail (Niswender, 1973). Charcoal separation of bound and free hormone was replaced

with a double antibody technique. Two hundred microliters of the second antibody (sheep anti-rabbit gamma globulin) was added at the desirable dilution after incubation of the first antibody, ^{3}H-progesterone and unknown or standard progesterone for 24 hours at 4°C. After an additional 48 hour incubation at 4°C, the tubes were centrifuged at 2500xg for fifteen minutes. The supernatant containing the unbound ^{3}H-progesterone was decanted and radioactivity was quantified in a liquid scintillation spectrometer (Packard Model C2425).

When two or five ng of progesterone were added to one ml samples of plasma from steers 106 ± 0.5% (n=28) and 111 ± 0.8% (n=6), respectively, were recovered. The between assay coefficient of variation was 12.5%.

Quantification of plasma progesterone was carried out in order to determine the presence of ovarian activity. Any heifer possessing greater than 1.5 ng/ml progesterone during two blood sampling periods or greater than 2.0 ng/ml progesterone during one sampling period, was considered to have ovarian activity.

Figure 1 depicts plasma progesterone concentrations for one heifer considered to exhibit ovarian activity (Heifer #5) and one heifer not exhibiting ovarian activity (Heifer #383) during the first breeding period. Figure 2 illustrates plasma progesterone concentrations for one heifer (Heifer #5051) not exhibiting ovarian activity during the first breeding period but exhibiting ovarian activity during the second breeding period. Figure 3 depicts breeding and

sampling schedules.

Statistical Analyses

Body weights were analyzed by analysis of variance using a completely randomized design. Pregnancy rates, estrus data and presence of ovarian activity were analyzed by Chi-square test.

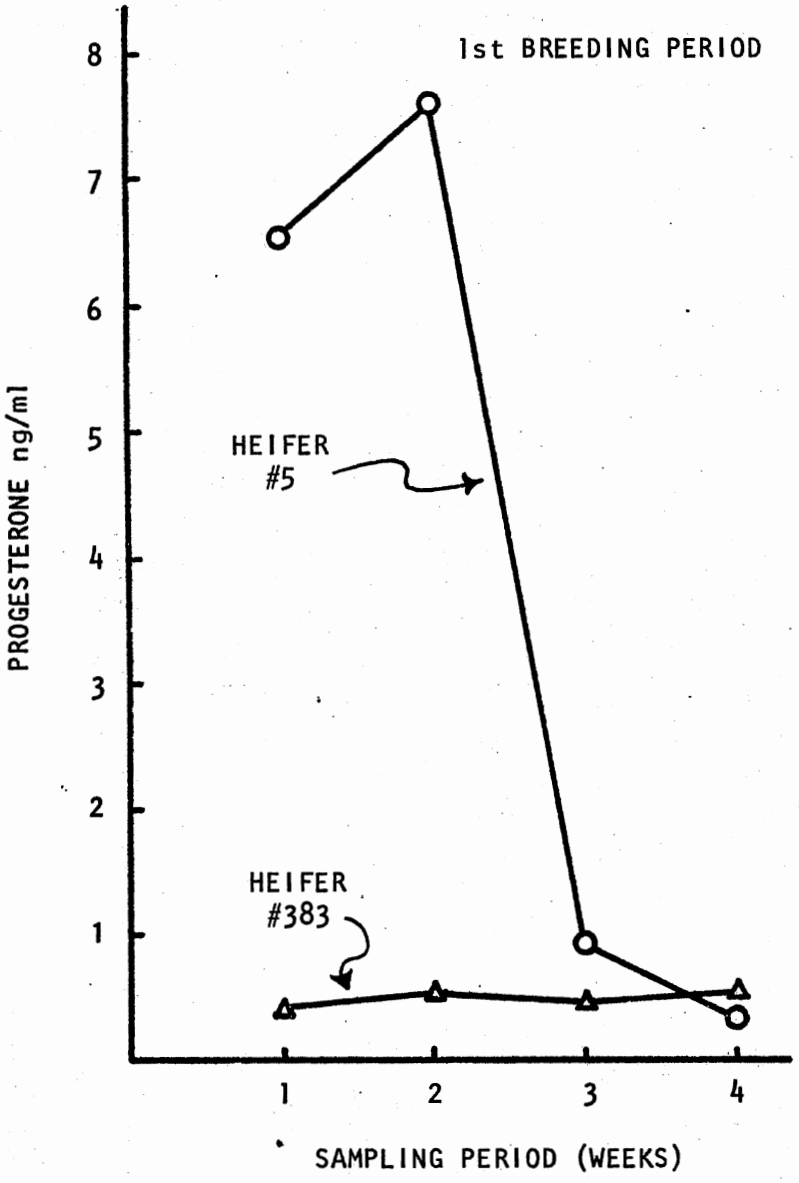

FIGURE I

Plasma Progesterone Concentrations of One Cycling and One Non-cycling Heifer During the First Breeding Period

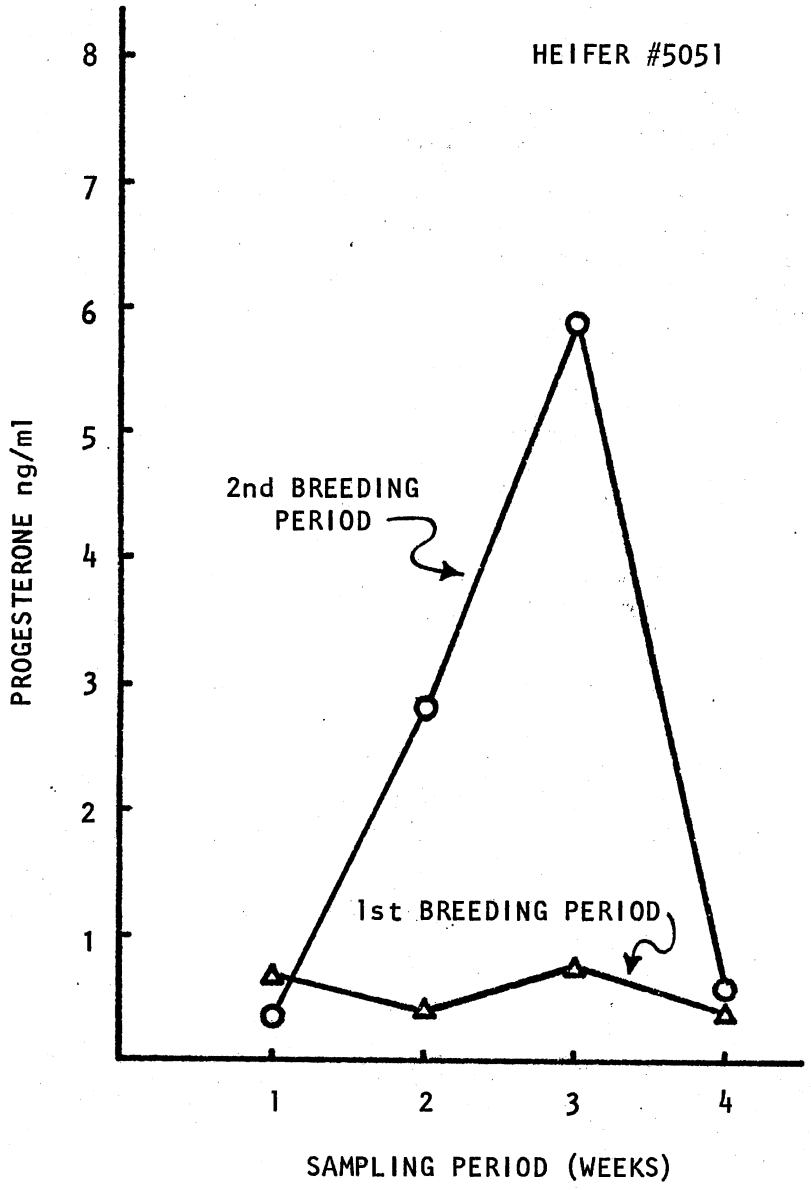

FIGURE 2

Plasma Progesterone Concentrations of One Heifer During the First and Before the Second Breeding Period

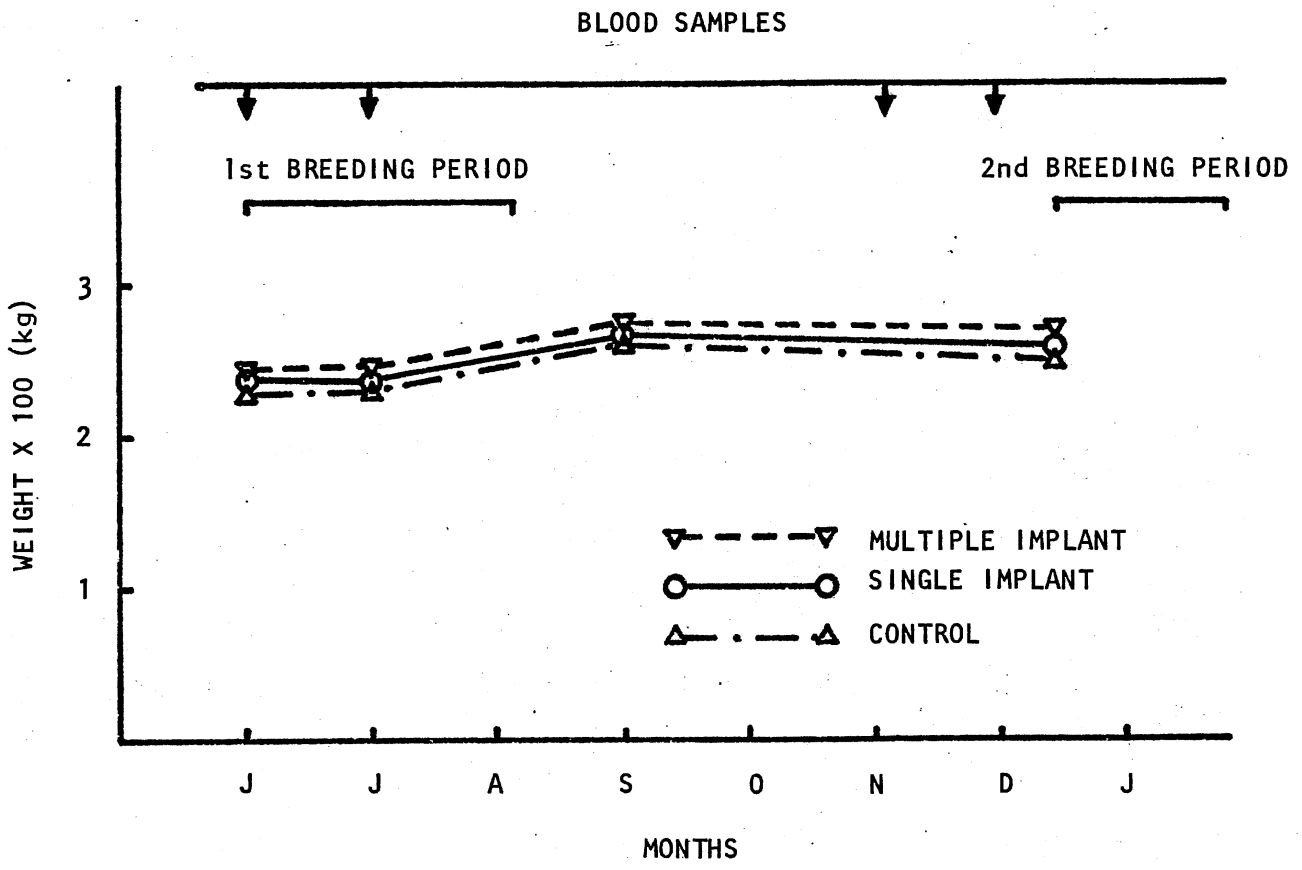

FIGURE 3

Breeding and Sampling Schedule

CHAPTER IV

RESULTS AND DISCUSSION

Growth

Body weights (Table II) were similar for all heifers when allotted to treatments (64 ± 13, 64 ± 13 and 61 ± 13 kg for control, single and multiple implant groups, respectively). The body weights for heifers at four months were not significantly different, although implanted heifers tended to be heavier (118 ± 24, 124 ± 20 and 120 ± 20 kg for control, single and multiple implanted heifers, respectively). Similar results were reported by Ward et al. (1978) who found heifers implanted with zeranol during the nursing phase did not exhibit increased weaning weights over controls. However, steers implanted with zeranol during the nursing period have demonstrated increased gains over controls (Ward et al., 1978; Thomas et al., 1970; Nichols and Lesperance, 1973).

Body weights were not significantly different at eight and twelve months of age, although both groups of implanted heifers tended to be heavier than the control heifers. Weights at eight months of age for control, single and multiple implant groups were 157 ± 24, 166 ± 25 and 164 ± 25 kg, respectively. Weights at twelve months of age were

TABLE II

CHARACTERISTICS OF HEREFORD HEIFERS
IMPLANTED WITH ZERANOL

Item	Treatment		
	Control	Single Implant	Multiple Implant
Number of Heifers	24	26	25
Age at 1st Implantation (da)	40	45	43
Weight at 1st Implantation (kg)	64 ± 13[a]	64 ± 13	61 ± 13
Weight at 4 mos. (kg)	118 ± 24	124 ± 20	120 ± 20
Weight at 8 mos. (kg)	157 ± 24	166 ± 25	164 ± 25
Weight at 12 mos. (kg)	173 ± 24	177 ± 25	180 ± 25
Weight at Start of Breeding 6/9/78 (kg)	222 ± 29[b]	227 ± 25[c]	237 ± 25[d]
Weight at End of Breeding 8/4/78 (kg)	264 ± 34	270 ± 25	276 ± 30
Age at Start of Breeding 6/9/78 (da)	442	447	444

[a]Mean ± standard deviation

[bd]Values with different superscripts differ ($P < .10$)

[cd]Values with different superscripts differ ($P < .25$)

173 ± 24, 177 ± 25 and 180 ± 25 kg, respectively, for control, single and multiple implant groups.

Similar results have been reported from other studies. When steers were implanted with zeranol and fed to gain 0.6 kg per head per day for 84 days during the winter, zeranol did not increase gains over controls during this period (Perry et al., 1970). Sharp and Dyer (1968) found heifers implanted with zeranol and fed either a 80:20 or 60:40 concentrate to roughage diet for 112 days did not exhibit increased gains over controls.

The small differences in weights among the treatments at twelve months could be attributed to the severe wintering conditions. The rate of gain for all heifers during the winter averaged 14 ± 3 kg and probably was reduced to the point that it did not permit the usual effects of the implant on growth to be expressed.

At the beginning of the first breeding period, when heifers were about fifteen months of age, body weights of the multiple implant group were slightly heavier than the control heifers (237 ± 25 vs. 222 ± 29; $P < .10$) and the single implant heifers (237 ± 25 vs. 227 ± 25 kg; $P < .25$). Although not significant, the trends observed in this study are in agreement with others (Ward et al., 1978; Utley et al., 1976).

Body weights were not significantly different among treatments at the end of the first breeding period when heifers were approximately 17 months of age. This indicates that the multiple implant heifers had lost any

advantage in increased gain as a result of zeranol implantation. Certainly, one would question whether the heifers could still be under zeranol stimulation since the last implant had been given approximately six months earlier. Small pieces of residual implant could be felt in some of the implanted heifers at about 100 days after implantation. Although the residual implants were not examined further, it is possible that these were encapsulated with fibrous tissue as observed by Sharp and Dyer (1972) and no longer effective.

Visual udder scores of the heifers, based on palpations by two individuals when the heifers were about 527 days of age (August 31, 1978), suggested that zeranol was still stimulatory in some heifers. These palpations revealed that one of 24, zero of 26 and four of 25 (control, single and multiple implanted heifers, respectively) had enlarged mammary glands. Although these differences are not significant, these data suggest either continued stimulation or that the mammary glands of these heifers had not recovered from the previous stimulatory effects of zeranol.

First Breeding Period

Data on reproductive performance of the heifers is presented in Table III. The percentages of animals exhibiting ovarian activity during the first four weeks of the breeding period (June 9, 1978 - June 30, 1978) as determined by plasma progesterone concentrations were 16,

TABLE III

CHARACTERISTICS OF HEREFORD HEIFERS IMPLANTED WITH ZERANOL (REPRODUCTIVE PERFORMANCE)

Item	Treatment		
	Control	Single Implant	Multiple Implant
Number of Heifers	24	26	25
Exhibited ovarian activity by 6/30/78 (%)	16	23	16
Exhibited estrus by 6/30/78 (%)	21	12	20
Exhibited estrus by 8/4/78 (%)	54	65	60
Pregnancy Rates 10/10/78 (%)	46[a]	50[a]	4[b]

[ab]Values with different superscripts differ ($P < .01$)

23 and 16% for control, single and multiple implant heifers, respectively, and were not influenced by zeranol implantation. During the same period the percentages of heifers observed in estrus were 21, 12 and 20%, respectively, for control, single and multiple implant groups and did not differ significantly. This indicates that few of the heifers on any treatment were exhibiting normal estrous cycles during the first four weeks of the breeding period.

The percentages of heifers observed in estrus during the total breeding period (June 9, 1978 - August 4, 1978) were 54, 65 and 60% for control, single and multiple implant groups, respectively. This low rate might suggest poor estrus detection with the result that many heifers actually in estrus were not observed. However, if such errors occurred in the observation of estrus, they should have been random across treatments. Thus, it is reasonable to assume there were no differences between treatment groups in the occurrence of estrus. In addition, the fact that the percentages of heifers observed in estrus in the control and single implant groups closely agree with the percentages determined to be pregnant suggests that estrus detection was not a problem in this study.

The first breeding period and estrus detection extended about 30 days after the last blood sample was taken to assess ovarian activity. It is apparent that normal estrus cycles commenced in many heifers in the control and single implant groups after the blood sampling period, as evidenced by a greater percentage of animals observed in

estrus and determined to be pregnant than animals exhibiting ovarian activity or observed in estrus the first 30 days of the breeding period.

Pregnancy rates during the first breeding period were greater for control and single implanted heifers ($P < .01$) than for the multiple implanted heifers. Only one of 25 (4%) in the multiple implant group became pregnant, while eleven of 24 (46%) and thirteen of 26 (50%) became pregnant in the control and single implant groups, respectively. Thus, the fact that sixty percent of the multiple implant heifers were observed in estrus but only four percent became pregnant suggests there was decreased fertility associated with multiple implants of zeranol, but not with a single implant.

Heifers implanted with zeranol at about 200 kg of body weight have been reported to exhibit decreased pregnancy rates (Staigmiller et al., 1978; Nelson et al., 1972). Likewise, decreased pregnancy rates have been observed in female mice treated with zeranol (Davis et al., 1977) and female rats treated with zearalanone (Bailey et al., 1976).

Several previous research studies have placed the weight at puberty in straightbred Hereford heifers at approximately 250 to 290 kg (Wiltbank et al., 1959; Wiltbank et al., 1969; Arijie and Wiltbank, 1971; Laster et al., 1972; Christian, 1957). At the start of the breeding season all heifers averaged about fifteen months of age, but weighed only 229 kg. The severe winter of 1977-78, when the pasture was covered with snow and/or ice for 47

days resulting in poor grazing conditions, was probably responsible for the light body weights of the heifers at the beginning of the breeding period. The mean body weight of the heifers determined to have ovarian activity was 228 ± 24 kg. The mean body weight of non-cycling heifers was 229 ± 29 kg. Therefore, it would not appear that the light body weights of the heifers were responsible for the small percentages of heifers cycling during the first month of the breeding season.

However, the mean body weights at the end of the breeding period of the control and single implant heifers that had conceived (Table IV) were significantly heavier (261 ± 26 vs. 230 ± 22 kg, for control pregnant vs. control non-pregnant; $P < .02$ and 266 ± 18 kg vs. 235 ± 25 kg, for single implanted pregnant vs. single implanted non-pregnant; $P < .02$) than those of heifers that did not conceive. Therefore, it appears from these data that the heavier heifers had reached puberty during the breeding period while the lighter heifers had not.

These data indicate that multiple implantation with zeranol at 100 day intervals starting prior to two months of age until about 100 days before breeding, has detrimental effects on pregnancy rates in heifers. However, a single implant prior to two months of age did not influence pregnancy rate.

Second Breeding Period

All heifers that failed to conceive during the first

TABLE IV

BODY WEIGHTS OF PREGNANT AND NON-PREGNANT HEIFERS AFTER 1ST BREEDING PERIOD

	Treatment			
	Control		Single Implant	
Item	Preg.	Non-preg.	Preg.	Non-preg.
Number of Heifers	11	13	13	13
Body Weights 8/4/78 (kg)	261 ± 26[ab]	230 ± 22[c]	266 ± 18[b]	235 ± 25[c]

[a]Mean ± standard deviation

[bc]Values with different superscripts differ ($P < .02$)

breeding period were assigned to a second breeding period on December 11, 1978. At this time the heifers were approximately 22 months of age, and the last zeranol implant had been given ten months previously. It was hoped that the performance of the heifers during this breeding period would determine whether zeranol implantation had long term detrimental effects on reproductive performance.

At the beginning of the second breeding period, the 24 multiple implant heifers were significantly heavier ($P < .025$) than the 13 control and 13 single implant heifers (Table V). It is not likely that these heavier weights were due to zeranol implantation, but were the result of the heavier heifers in the control and single implant groups having conceived during the first breeding period and, thus, being removed prior to the second breeding period.

Only 15 percent (two of 13) of each of the control and single implant heifers and four percent (one of 24) of the multiple implant heifers had blood progesterone levels that indicated they had ovarian activity during a one month period just prior to the start of the second breeding period (Table V).

As shown in Table V, only a limited number of heifers in any group were in estrus during the second breeding period of December 11, 1978 through January 30, 1979 (15, 15 and 16% for control, single and multiple implant groups, respectively). One of the two control heifers observed in estrus had also exhibited ovarian activity during the

TABLE V

CHARACTERISTICS OF HEREFORD HEIFERS IMPLANTED WITH ZERANOL (2ND BREEDING)

Item	Treatment		
	Control	Single Implant	Multiple Implant
Number of Heifers	13	13	24
Age at start of 2nd breeding 12/11/78 (da)	618	623	621
Weight at start of 2nd breeding 12/11/78 (kg)	250 ± 25[ab]	254 ± 29[b]	274 ± 29[c]
Exhibited ovarian activity between 11/9/78 and 11/30/78 (%)	15	15	4
Exhibited estrus between 12/11/78 and 1/30/79 (%)	15	15	16
Pregnancy Rates 5/1/79 (%)	7	7	0

[a]Mean ± standard deviation

[bc]Values with different superscripts differ ($P < .01$)

preceding month. Both of the single implant heifers observed in estrus had exhibited ovarian activity, while only one of the five multiple implant heifers had exhibited ovarian activity.

Pregnancy rates after the second breeding period were low for all treatment groups and were not significantly influenced by treatment (Table V). One of 13 heifers (17%) became pregnant in each of the control and single implant groups. This represents 50 percent of those heifers in these groups which exhibited ovarian activity. None of the 24 heifers that had received multiple implants became pregnant.

During the six month breeding period between breeding seasons, the heifers were on pasture yet gained on the average only 39 $\pm$ 10 kg. or about .22 kg. per head per day. Those heifers determined to exhibit ovarian activity gained 42 $\pm$ 11 kg. during this six month period, while those not exhibiting ovarian activity gained 39 $\pm$ 10 kg. In addition, the heifers exhibiting ovarian activity weighed 283 $\pm$ 11 kg. at the beginning of the second breeding period while those not exhibiting ovarian activity weighed 260 $\pm$ 28 kg. As previously mentioned, the weight at puberty in straightbred Hereford heifers, as determined by other studies, is 250 to 290 kg. Since the cycling heifers represent the upper end of this range and the non-cycling heifers represent the lower end, it may be that such poor gains from 15 to 21 months of age would account for the low percentages of heifers found to be cycling in each

group.

However, these data do not permit drawing any conclusions as to whether there is a long term detrimental effect of zeranol on reproductive performance. It is true that none of the twenty-four multiple implant heifers became pregnant during the second breeding period, although five were observed in estrus, and one heifer did become pregnant in each of the control and single implant groups. Thus, it is possible that zeranol did have long term detrimental effects on reproductive performance in the multiple implant heifers, but because the performance of the heifers in all treatment groups during the second breeding period was far less than normal, proper controls are not available for detecting this effect.

CHAPTER V

SUMMARY

Seventy-five Hereford heifers were used to study the effects of single and multiple implantation with zeranol on subsequent reproductive performance. Twenty-six heifers received a single implant of 36 mg zeranol at 42 days of age, 25 heifers received multiple implants, 36 mg at 42 days and were reimplanted three times with 36 mg zeranol at 100 day intervals and 24 heifers served as controls.

Body weights were similar for all heifers when allotted to treatments (64 ± 3, 64 ± 3 and 61 ± 2 kg for control, single and multiple implant groups, respectively). Control, single and multiple implant heifer weights at four months of age were 118 ± 5, 124 ± 4 and 120 ± 4 kg, respectively ($P < .10$). Body weights were not significantly different at eight and twelve months of age, although both groups of implanted heifers tended to be heavier than the control group. Weights for the multiple implant group were heavier than controls (237 ± 5 vs. 222 ± 6 kg, $P < .10$) and single implant heifers (237 ± 5 vs. 227 ± 5 kg, $P < .25$) at the beginning of the breeding period when heifers were about fifteen months of age. Body weights were similar for all heifers at the end of the breeding period.

Body weights for the multiple implant group at the beginning of the second breeding period, when heifers were about 21 months of age, were significantly heavier than the control and single implant groups. This difference is probably due to removal of the pregnant heifers from the treatment groups prior to the second breeding period.

Percentages of animals exhibiting ovarian activity at the beginning of the first breeding period (at 468 days of age) were 16, 23 and 16 for control, single implant and multiple implant groups, respectively. Percentages of heifers observed in estrus by 500 days of age were 54, 65 and 60 for control, single implant and multiple implant groups, respectively. Neither percent exhibiting ovarian activity nor percent observed in estrus during the first breeding period were influenced by treatment with zeranol. However, pregnancy rates were greater for the control and single implant heifers ($P < .10$) than for the multiple implanted heifers. Only one of 25 (4%) of the multiple implanted heifers became pregnant, while 11 of 24 (46%) and 13 of 26 (50%) became pregnant in the control and single implant groups, respectively.

Percentages of animals exhibiting ovarian activity prior to the second breeding period (11/6/78) when heifers were about 21 months of age were 15, 15 and four for control, single implant and multiple implant groups, respectively. Fifteen percent (two of 13) of the control and single implant groups and 16 percent (four of 24) of the multiple implant group heifers were observed in estrus during the second breeding

period. Pregnancy rates after the second breeding period were low for all treatment groups and were not significantly different. One heifer of 13 became pregnant in each of the control and single implant groups, while none of the 24 in the multiple implant group became pregnant.

These data suggest that multiple implantation with zeranol at 100 day intervals starting prior to two months of age until about 100 days before breeding, has detrimental effects on pregnancy rate in heifers. However a single implant prior to two months of age did not influence pregnancy rate.

Furthermore, these data do not conclusively answer the question whether implantation with zeranol beginning prior to two months of age in heifers has long term detrimental effects on reproductive performance. The heifers in this study experienced some degree of nutritional deprivation due to the severe winter of 1977-78 as evidenced by decreased growth during this period. Therefore, during the total experiment (until heifers were 22 months of age) 50% of the control, 54% of the single implanted and 4% of the multiple implanted heifers were pregnant. Although fertility of the control heifers was less than normal and the proper controls are not available for comparison of the pregnancy rate of the multiple implanted heifers for the second breeding period, these data suggest that multiple implantation with zeranol as performed in this experiment may have a long term detrimental effect on reproductive performance of heifers.

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